Cancer Immunometabolism: IDO Pathway and Its Therapeutic Correction

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President & CEO, Lankenau Institute for Medical Research (LIMR)
Editor in Chief, Cancer Research
LIMR at a glance

- **Faculty:**
  16 Resident Faculty at Institute
  60 Affiliate Clinical & Nursing Faculty at Lankenau Medical Center

- **Primary Research Programs:**
  Cancer, Diabetes, Cardiovascular Disease

- **Basic Science Theme:**
  Disease Modifier Pathways

- **Unique ‘Acapreneurial’ Research Model:**
  14 Non-Profit Laboratories + 10 Biotech Start-Up Companies

- **Biotech Incubator (managed by LIMR Development Inc.):**
  - LIMR Development Inc. (LDI) = Product Development & Business Affairs
  - ‘Spin-out’ and ‘Spin-in’ Biotech Companies = Drug & Device Development

Learn more at [www.limr.org](http://www.limr.org)
What is cancer?

- Cancer gene alterations
  Mainstream Theory
- Permissive microenvironment
  Established Trend
- Dysfunctional immunity
  Emerging Trend

Back to the future!
Virchow, Erlich, Coley (1800s-1900s)

‘Rogue’ Cells
OR
Mismanagement of ‘Rogue’ Cells?
Failure of cancer screening to extend survival of screened populations argues the presence of ‘rogue’ cells is not the same as the presence of cancer...?
Immunology in the Historical Mainstream of Cancer Research: Divorce, Remarriage and Elective Affinities of the Future

1800s
Roots of cancer immunology
Inflammation (Virchow)
Immune surveillance (Ehrlich)
Bacterial toxins (Coley)

1900s
Roots of cancer genetics
Animal tumor viruses (Rous)
Tissue culture (Eagle)
Cytogenetics (Nowell)

1970
‘Nude’ Immune Deficient Mouse (the Divorce)

1980-2000
Inflammation

2000s
‘Interferon Knockout’ Mutant Mice (the Remarriage)

2010s
Conceptual Synthesis

Cancer cell centric therapy
Host centric therapy
Immunocockchemotherapy

1970
Unchanged cancer risk despite disabled immunity (human tumor transplants OK)
Immunity unimportant for control ... ?

2000s
Elevated cancer risk caused by mutations in interferon pathway
Immunity essential for control !

Prendergast and Jaffee, Cancer Res. (2007)
Prendergast, OncolImmunology (2012)
Superior Cancer Staging by Memory T Cells

*CD45RO is a Key Marker*
Cancer as a problem of ‘rogue cell’ mismanagement by the immune system

Can one restore immune management?
What is Immunotherapy?

Drugs Which Recruit the Patient’s Natural Immune System to Fight Disease

Active Immunotherapy
- Adds New Capabilities
  - Adoptive Cell Therapy
  - Vaccines (Cells, Biologics)
  - e.g. CART therapy, Provenge®

Passive Immunotherapy
- Impedes or Promotes Existing Capabilities
  - Antibodies
  - Other Biologics
  - e.g. Herceptin®, Yervoy®

Immunomodulation
- Modifies Existing Capabilities
  - Inflammatory Modifiers
  - Immune Adjuvants
  - e.g. COX2i, Alum (vaccine adjuvant)

Movie provided courtesy of Dr. Pooja Jain (Drexel University College of Medicine)
Active Immunotherapy Has Mainly Failed Historically Because Immune Escape Was Not Understood

Immune Control

Cytotoxic Immune Cells
(T, NK, Innate)

Immune Escape

Supportive Inflammation
T Cell Suppression / Tolerance
1. Cancer cell-centric therapy is inherently flawed due to the selection problem (therapy resistance)
2. Tumor microenvironment must be reprogrammed
3. Immune suppression must be relieved
How do cancer cells escape immune control?

ONCOGENESIS

NORMAL → TRANSFORMED

Immune Surveillance → Select for Immune Resistance

ELIMINATION by immune system → EQUILIBRIUM with immune system (tumor dormancy) → ESCAPE from immune system (progressive disease)

IMMUNOEDITING

Inflammation & Immune Suppression

OCCULT → MALIGNANT

IDO

GENETICS & MODIFIERS & MICROENVIRONMENT

Prendergast, Oncogene (2008)
IDO may program an inflammatory state that supports several aspects of cancer progression.
What is IDO?

- Single-chain cytosolic enzyme that catabolizes tryptophan

- Implicated in T cell tolerance by evidence that IDO may protect allogeneic fetus against maternal immune attack

Munn, Mellor and colleagues, Science (1999)
IDO is one of four enzymes that catabolize Trp
All implicated in immune modulation

D-1MT = clinical lead indoximod

Many cells lack full enzyme cascade to make NAD

TDO  IDO2  IDO1  TPH

Tryptophan

Kynurenine

5-OH-Tryptophan

NAD

5-OH-Tryptamine (Serotonin)
IDO is widely deregulated in human cancer

Table 1: Expression of IDO in human tumors

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>IDO-positive tumor samples (no. positive per no. tested)</th>
<th>Proportion of IDO-positive tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostatic carcinomas</td>
<td>11/11</td>
<td>&gt;50% 7 10-50% 3 &lt;10% 1</td>
</tr>
<tr>
<td>Colorectal carcinomas</td>
<td>10/10</td>
<td>&gt;50% 5 10-50% 3 &lt;10% 2</td>
</tr>
<tr>
<td>Pancreatic carcinomas</td>
<td>10/10</td>
<td>&gt;50% 8 10-50% 2 &lt;10% 0</td>
</tr>
<tr>
<td>Cervical carcinomas</td>
<td>10/10</td>
<td>&gt;50% 0 10-50% 4 &lt;10% 6</td>
</tr>
<tr>
<td>Endometrial carcinomas</td>
<td>5/5</td>
<td>&gt;50% 0 10-50% 3 &lt;10% 2</td>
</tr>
<tr>
<td>Gastric carcinomas</td>
<td>9/10</td>
<td>&gt;50% 4 10-50% 3 &lt;10% 2</td>
</tr>
<tr>
<td>Glioblastomas</td>
<td>9/10</td>
<td>&gt;50% 6 10-50% 3 &lt;10% 0</td>
</tr>
<tr>
<td>Non-small-cell lung carcinomas</td>
<td>9/11</td>
<td>&gt;50% 1 10-50% 1 &lt;10% 7</td>
</tr>
<tr>
<td>Bladder carcinomas</td>
<td>8/10</td>
<td>&gt;50% 3 10-50% 1 &lt;10% 4</td>
</tr>
<tr>
<td>Ovarian carcinomas</td>
<td>8/10</td>
<td>&gt;50% 0 10-50% 3 &lt;10% 5</td>
</tr>
<tr>
<td>Head and neck carcinomas</td>
<td>7/11</td>
<td>&gt;50% 0 10-50% 3 &lt;10% 4</td>
</tr>
<tr>
<td>Esophageal carcinomas</td>
<td>7/10</td>
<td>&gt;50% 1 10-50% 2 &lt;10% 4</td>
</tr>
<tr>
<td>Mesotheliomas</td>
<td>6/10</td>
<td>&gt;50% 2 10-50% 1 &lt;10% 3</td>
</tr>
<tr>
<td>Renal cell carcinomas</td>
<td>5/10</td>
<td>&gt;50% 0 10-50% 1 &lt;10% 4</td>
</tr>
<tr>
<td>Melanomas</td>
<td>11/25</td>
<td>&gt;50% 0 10-50% 1 &lt;10% 11</td>
</tr>
<tr>
<td>Breast carcinomas</td>
<td>3/10</td>
<td>&gt;50% 2 10-50% 0 &lt;10% 1</td>
</tr>
<tr>
<td>Thyroid carcinomas</td>
<td>2/10</td>
<td>&gt;50% 0 10-50% 0 &lt;10% 2</td>
</tr>
<tr>
<td>Lymphomas</td>
<td>4/18</td>
<td>&gt;50% 0 10-50% 0 &lt;10% 4</td>
</tr>
<tr>
<td>Small-cell lung carcinomas</td>
<td>2/10</td>
<td>&gt;50% 0 10-50% 0 &lt;10% 2</td>
</tr>
<tr>
<td>Sarcomas</td>
<td>2/10</td>
<td>&gt;50% 0 10-50% 1 &lt;10% 1</td>
</tr>
<tr>
<td>Hepatocarcinomas</td>
<td>2/5</td>
<td>&gt;50% 0 10-50% 0 &lt;10% 2</td>
</tr>
<tr>
<td>Adrenal carcinomas</td>
<td>2/5</td>
<td>&gt;50% 1 10-50% 0 &lt;10% 1</td>
</tr>
<tr>
<td>Choriocarcinomas</td>
<td>1/5</td>
<td>&gt;50% 0 10-50% 0 &lt;10% 1</td>
</tr>
<tr>
<td>Cutaneous basocellular carcinomas</td>
<td>1/5</td>
<td>&gt;50% 0 10-50% 0 &lt;10% 1</td>
</tr>
<tr>
<td>Testicular seminomas</td>
<td>0/10</td>
<td>&gt;50% 0 10-50% 0 &lt;10% 0</td>
</tr>
</tbody>
</table>

*Expression of IDO protein was detected by immunohistochemistry using purified IDO-specific rabbit antibodies. Specificity of staining was controlled by blocking with a synthetic peptide corresponding to the C terminus of IDO (Fig. 2). *Number of tumor samples with the indicated proportion of IDO-positive tumor cells is given in each column. The proportion of positive tumor cells was estimated visually.

Van den Eynde and colleagues, Ludwig Institute for Cancer Research, Brussels

IDO overexpression in tumor cells is common

TDO and IDO2 also found upregulated in certain cancers (not as common)

Suppressor gene Bin1 which is widely attenuated in human cancer normally acts to limit IDO1 overexpression
IDO may act at multiple sites to blunt antitumor immunity.

TUMOR DRAINING LYMPH NODE

- APC
- CoReg
- B7
- Trp
- Kyn
- Effector T cells
- Treg

- IDO

TUMOR

- Cancer cell
- Cancer stem-like cells
- IDO
- Bin1
- TAMs
- Mesenchymal stem cells
- Endothelial cells
- MDSC

Munn, Mellor and colleagues
Fallarino, Grohmann, Orabona, Pucetti and colleagues
IDO is a modifier of inflammation and adaptive immunity

PAMPs

- Infection Signals e.g. LPS, CpG
- TLRs TNF, IL1

IFNs

- MyD88
- Jak/STAT IRFs

DAMPs

- TLR4 RAGE
- Sterile Inflam Signals e.g. HMGB1, ATP

(-)CoReg R

- CTLA-4
- CD200
- GITR

TGFβ R

IDO

- Kynurenine
- Glk1
- Gcn2

AhR
- IDO2
- Activate Kynurenine Signaling

mTOR
- S6K
- Block AA Sufficiency Signaling

eIF-2
- IL-6, CCL2
- Activate AA Insufficiency Signaling

Metz et al. Oncoimmunology (2012)
Our studies of the tumor suppressor gene Bin1 led us to identify IDO as a critical target for Bin1 control.
IDO inhibitors powerfully enhance the efficacy of ‘immunogenic’ chemotherapy

Response abolished by ablation of CD4+ or CD8+ T cells

New classes of orally bioavailable IDO inhibitors we discovered displayed similar in vivo properties

- Thiohydantoins
- Brassinins
  - Plant phytoalexin, chemopreventive
  - Some clinical experience
- Naphthoquinones
  - Known anticancer properties
  - Clinical experience
- Phenylimidazoles
- Hydroxylamines
- Enter Phase II 2013 (NewLink Genetics)

**B16 graft**
(IDO negative tumor)

**MMTV-neu BRCA model**

- J Med Chem (2008a)
- J Med Chem (2008b)
- Oncogene (2008)
Phase 1B trial: Taxotere Combination with Indoximod
*Intriguing responses in stage 4 BRCA patients*

Pre-treatment

One month treatment

Whole body PET scan

*SOC taxotere + 800 mg p.o. indoximod q.d. (28 day cycles)*

Courtesy of Hatem Soliman MD (Moffitt Cancer Center)
Is IDO critical for cancer development or progression?

If so, how does it contribute to cancer?

What is the basis for the anticancer effects of IDO inhibitors?

*Genetic investigations in IDO deficient mice*
IDO1 is essential for inflammatory carcinogenesis

- Classical model of inflammatory cancer: two-stage skin carcinogenesis
- No precocious autoimmunity or inflammation in IDO1−/− mouse
T cell immunity mediates the anticancer benefits of IDO loss

IDO1 double knockout mice
Is IDO critical for cancer *per se*? No.

Complete skin carcinogenesis (topical DMBA only)

Mammary carcinogenesis (i.p. DMBA + progesterone)

IDO programs a ‘cancerous inflammation’

No effect on growth of primary tumor grafts (e.g. 4T1 BRCA cells)

Where does IDO act to support inflammatory cancer?

**TUMOR DRAINING LYMPH NODE**

- **T cell**
- **Antigen presenting cell**
- **Trp**
- **Kyn**
- **CoReg**
- **B7**
- **Treg**

**Effector T cells**

**TUMOR**

- **Cancer cells**
- **Cancer stem-like cells**
- **IDO**
- **Bin1**
- **TAMs**
- **TANs**
- **Mesenchymal stem cells**
- **Endothelial cells**

**MDSC**
IDO function crucial mainly outside hematopoietic cells?

IDO function in cancer cells may be sufficient

Bin1−/− neoplastically transformed cells

Treat 1MT or placebo

Determine tumor mass

WT host

IDO−/− host

CD4+ T cell depletion abolishes response

“Immune escape” and “cancer-associated inflammation” are genetically synonymous?

-IDO programs inflammation to drive immune escape

Prendergast et al., Amer. J. Path. (2010)
How broadly relevant is this inflammatory connection?
K-Ras model of lung adenocarcinoma: IDO blockade blunts progression and promotes survival

Smith, Chang et al. Cancer Discovery (2012)

Defects in
Invasion & angiogenesis
IL-6 and CCL2 levels (myeloid attractants)
MDSC number & function
Lung angiogenic defect in IDO deficient mice

Tranverse microCAT

Effect accentuated in tumor-bearing animals

Smith, Chang et al. (2012) Cancer Discovery
Lung metastatic defect in IDO deficient mice

4T1 breast cancer metastasizes to lung from orthotopic graft

No difference in growth of primary tumor graft ...

... yet a survival benefit

------ Extravasion to blood unaffected ------

Defects in IL-6 and MDSC
Rescue MDSC and metastasis by restoring IL-6

Smith, Chang et al. (2012) Cancer Discovery
IDO programs an inflammatory state that supports several aspects of cancer progression.
Re-programming inflammation:

IDO inhibition vs IDO pathway blockade?
Block Expression -- Activity -- Effector signals

**Bin1**
**IDO1**
**T cells**

**Block Expression**
- NFκB blockade
- Ethyl Pyruvate
  - *Cancer Res (2010)*

**Activity**
- Enzyme inhibitors
  - *OncoImmunol (2012)*

**Effector signals**
- D-1MT (indoiximod)
  - *OncoImmunol (2012)*
  - IND application (2009)
    - Lankenau & Georgia (Preclinical)
    - NCI (Pharm/Tox)
    - New Link Genetics Corp. (GMP)
    - Moffitt CC (Phase I site)

**Kinase inhibitors**
- Imatinib (Gleevec®)
- Sorafinib
- JAK inhibitors
Indoximod safety findings

Hypophysitis
*Pituitary gland inflammation*

- Revealed by elevated TSH, ACTH
- Known side effect of anti-CTLA-4
  *(CTLA-4 upregulates IDO in mice)*
- Emerges in all patients at highest doses - DLT in Phase I trial
- Encouraging as autoimmunity may correlate with beneficial responses

Soliman, Antonia, Link, Vahanian and colleagues, Moffitt Cancer Center & NewLink Genetics Corp. ASCO abstracts 2011, 2012
PK: Clinical responses seen even at low exposure

- **AUC**<sub>(0-48)</sub> = 8118 (±2364) ng*hr/ml
- **AUC**<sub>(0-inf)</sub> = 8349 (±2389) ng*hr/ml
- **C<sub>max</sub>** = 1046.5 (±313.4) ng/ml (4.8μM)
- **C<sub>(24hr post)</sub>** = 40.8 (±24.3) ng/ml (0.2μM)
- **t<sub>1/2</sub>** = 9.2 (±3.3) hrs
- **T<sub>max</sub>** = 3.4 (±1.6) hrs
- Clearance = 25.9 (±7.9) L/hr

Detection by LS/MS/MS method
Recent findings suggest that D-1MT may act as a partial Trp mimetic to reverse mTOR blockade by IDO.
Trp depletion inhibits mTOR
D-1MT phenocopies Trp in relieving this effect

mTOR activity assay

Induce IDO

± W or L or 1MT

24 hr

Examine phospho-S6K 1-3 hr later

D-1MT a potent Trp mimetic (IC50 ~70 nM)

Metz et al. Oncoimmunology (2012)
IDO activates autophagy controlled by mTOR
D-1MT phenocopies Trp in relieving autophagy

Metz et al. Oncoimmunology (2012)
D-1MT a Trp mimetic in Trp sufficiency signaling to mTORC1

Suggests S6K phosphorylation by mTOR as clinical PD marker to monitor indoximod response (blood draw)
Implications

• D-1MT relieves mTOR inhibition by any Trp catabolic enzyme
  *Rationale for different, perhaps broader use than IDOi*

• D-1MT --> mTOR --> ICOS path
  *Provides a mechanistic rationale for Ipilimumab combination*

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Indoleamine 2,3-dioxygenase is a critical resistance mechanism in antitumor T cell immunotherapy targeting CTLA-4

Rikke B. Holmgaard,1,2 Dmitriy Zamarin,1,2,3 David H. Munn,4 Jedd D. Wolchok,2,3,5,6 and James P. Allison1,7

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5Weill Cornell Medical College and Graduate School of Medical Sciences of Cornell University, New York, NY 10065
6Ludwig Institute for Cancer Research, New York, NY 10065
7The University of Texas, MD Anderson Cancer Center, Department of Immunology, Houston, TX 77030
Safety concerns of enzymatic IDO\(\text{i}\) and indoximod may differ? *IDO\(^{-/-}\) mouse phenotypes not seen with indoximod*

**Heart calcification** (strain specific)

- WT BALB/c
- IDO\(^{-/-}\) BALB/c

Partially penetrant by 3 months of age
Not associated with lethality to 1 year

**Acute pancreatitis** after vaccination

- C57 peptide+CFA
- IDO\(\text{ko}\) peptide+CFA

Observed in all vaccinated mice examined which received complete Freund’s adjuvant

Chang et al., Cancer Biol. Ther. (2011)
Safety concerns of IDO blockade based on IDO−/− mice not seen with indoximod

Exacerbates hyperlipidemia

Heightens severity of colitis and Elevates incidence of inflammatory colon carcinogenesis

Blood serum from naive animals

Gross pathology of colon carcinomas induced by a classical two-stage inflammatory protocol (DMH + DSS)

Chang et al., Cancer Biol. Ther. (2011)
Deeper insights from thinking about D-1MT?

Can indoximod be conceptualized as an immune adjuvant principle? What is its target in the IDO-mTOR pathway?
Summary

IDO

- Programs inflammation to support cancer
- Immune escape derivative of general inflammatory role
- Blocks Trp sufficiency signaling to mTOR, an IDO target

IDOi

- May reprogram inflammation
- Different MOA of enzymatic IDOi versus indoximod
- Indoximod acts like a Trp mimetic for mTOR pathway

*Potently restores mTOR activity blocked by IDO*
*Treat cancers driven by any Trp catabolic enzyme?*
Cancer Immunochemotherapy of the Future

- Immunomodulators: Indoximod
- Checkpoint Pathway Inhibitors: Yervoy®, Anti-PD1, IDO inhibitors (e.g. 919)
- Reprogram Inflammation
- Correct Immune Escape
- Stimulate Adaptive Immunity
- Destroy Tumor Cells

Adoptive Cell Therapies & Vaccines: Provenge®, HyperAcute
Chemotherapy Radiotherapy
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Key Supporters

National Cancer Institute
DoD Research Programs
American Lung Association
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Lankenau Hospital Foundation
Main Line Health