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IDO1 activity correlates with HGF levels and immune system impairment in multiple myeloma

Sergio Rutella, MD PhD
Department of Hematology
Catholic Univ. Medical School, Rome, Italy

Current affiliation: Department of Pediatric Hematology/Oncology
IRCCS Bambino Gesù Children’s Hospital
Rome, Italy
Presenter disclosure information

Sergio Rutella

The following relationships exist related to this presentation:

No relationships to disclose
**IDO1: Background**

- Constitutively expressed by tumor cells and/or tumor environmental cells
- Induced by IFN-γ, a prototypical pro-inflammatory mediator
- Mediates tumoral immune escape
- Confers an unfavorable prognosis to certain tumor types (i.e., acute myeloid leukemia, ovarian cancer)
- Can be targeted with selective inhibitors (i.e., 1-methyl-tryptophan; INCB024360)

IDO1-driven TRP catabolism

Serotonin

Protein ← L-TRP

IDO1

N-formyl-kynurenine

Kynurenine formamidase

KYN

Bloodstream

3-HK

3-HAA

Quinolinic acid

Effects on the immune system

+ > GCN2 stress kinase

- < mTOR

T-cell anergy

> Treg cells

< NK proliferation

Th1 apoptosis

Hepatocyte growth factor (HGF) induces *IDO1* in human DC

(a) 672 significant probe sets up-regulated by HGF (ANOVA)

(b) 29 probe sets up-regulated by HGF (Tukey post-hoc test)

(c) GM4 and HGF

(d) T-cell proliferation index

Rutella S et al, Blood 2006a
Rutella S et al, Blood 2006b
The immune defect in MM

- Infections are the leading cause of death
- DC are dysfunctional \((\text{IL-10}^+ \text{IL-12}^- \text{CD80/86}_{\text{low}})\)
- MM cells express CD28 and B7-coinhibitory molecules (PD-L1)
- Immune suppressive and angiogenic cytokines are increased \((\text{HGF, VEGF, IL-10, TGF-}\beta)\)
- Treg cells are abnormal, both quantitatively and qualitatively
- Sensitivity to the graft-versus-myeloma effect indicates that the immune system is crucial to control the disease

Study hypothesis

MM cells

IDO1

Trp → Kyn

Naïve T cell

Treg cells

Anti-myeloma CD8+ T cells

BMSC
Patients

- 34 consecutive patients with PC dyscrasia
  - 27 symptomatic MM
  - 4 SMM
  - 3 MGUS
- 26 (77%) at disease onset / relapse
- 23 (67%) were not taking any medication at time of sampling
- $\beta_2$-microglobulin averaged 3.1 mg/dl (range 1.4-33.0)
- M-component averaged 2.6 g/dl (range 0.9-7.6)
KYN are increased in MM patients

Median KYN in HC = 1.8 μM/L

IDO⁻ MM < 1.8 μM/L (8 pts, 23%)
IDO⁺ MM > 1.8 μM/L (26 pts, 77%)
IDO1 is constitutively expressed by PC

![Cell sorting diagram](image)

**a)** Pre-sort and Post-sort cell populations

**b)** Morphological image of MM cells

**c)** IDO1 mRNA expression levels before and after IFN-γ treatment

**d)** KYN/TRP ratio and β2-microglobulin levels in THP-1 and MM cells

- Pre-sort: CD138 -> CD38 > CD56 ->
- Post-sort: CD138 -> CD38 > CD56 ->

+ IFN-γ

<table>
<thead>
<tr>
<th>THP-1</th>
<th>MM</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDO1 mRNA (qPCR)</td>
<td>10^6</td>
</tr>
<tr>
<td>KYN/TRP ratio (μM/μM*100)</td>
<td>0.6406</td>
</tr>
</tbody>
</table>

β2-microglobulin (mg/dl)
MM BMSC do not constitutively express IDO1

![Graph showing KYN or tryptophan levels with bar charts for CM (t0), Ctr (t24h), and IFNγ (t24h). The levels are compared between Kyn and Tryptophan conditions.]

**Legend:**
- Red: Kyn
- Blue: Tryptophan

# of exp = 4

**b) Flow Cytometry Results:**
- CD4:
  - Kyn: 1.1
  - Tryptophan: 2.7
- CD4+allo-Mo + BMSC supernatant:
  - Kyn: 46
  - Tryptophan: 8
- CD4+allo-Mo + BMSC supernatant + TRP (100 µM):
  - Kyn: 89.3
  - Tryptophan: 1.5
- CD4+allo-Mo + BMSC supernatant + TRP (100 µM):
  - Kyn: 86.2
  - Tryptophan: 2.0
IDO1 may expand Treg cells *in vivo*

*By ANOVA*
IDO1 induces Treg cells *in vitro*

MTLC with IDO+ PC

± D,L-1MT

Allogeneic CD4+

CD4+PC (1:3) +1MT (200 µM)

CD4+ + + + +

αCD3/CD28 Ab - + + +

MM-induced Tregs (1:1) - - + -

MM-induced Tregs (2:1) - - - +

% CD4+FoxP3+ Treg cells

a) # of exp = 4

CD4 +PC

+1MT

CFSE

CFSE dim

CFSE bright

CD4

CD4+PC (1:3)

CD4+PC (1:3)

+1MT (200 µM)

CFSE (MFI)

b) # of exp = 4

c)

# of exp = 4
IDO1 restrains Th1/17 but not Th2 responses
The NY-ESO-1 CT antigen

- A tumor-specific antigen (not expressed in normal tissues) and potential target of the graft-versus-MM effect
- Detected in 10-60% of MM (and in 100% of MM with cytogenetic abnormalities)
- T cells reactive against NY-ESO-1 account for 0.2-0.6% of CD8⁺ T cells in MM patients and can be detected with tetramers / pentamers in HLA-A2⁺ subjects

IDO1 restrains MM-reactive T cells

a) 

% A2/NY-ESO-1\(^+\)CD8\(^+\) T cells

- IDO\(^+\) HLA-A2.1\(^+\) MM
- IDO\(^-\) HLA-A2.1\(^+\) MM
- A2\(^{neg}\) MM

b) 

- Pt#1
  - NY-ESO-1\(_{157-165}\)
  - Flu peptide\(_{58-66}\)
- Pt#2
  - NY-ESO-1\(_{157-165}\)
  - Flu peptide\(_{58-66}\)

n=14
n=6
n=6
IDO1 activity correlates with HGF

*By ANOVA
IDO1 in MM cell lines

a) Histograms showing IDO expression in MOLP-8, LP-1, and HuNS-1 cells.

b) Bar chart showing Kynurenine (µM) levels at T=24h, T=48h, T=72h for MOLP-8, LP-1, and HuNS-1 cells.

b) Graph showing HGF (ng/ml) levels at t=24h for A2780, LP-1, and MOLP-8 cells.

d) Western blot showing c-Met and GAPDH expression in MOLP-8 and LP-1 cells.
HGF induces IDO1 in HGF-sensitive MM cells

a) MOLP-8 (HGF\textsuperscript{low})

b) LP-1 (HGF\textsuperscript{high})

c) IDO protein (KS)

- MOLP-8
  - t0
  - t24

- LP-1
  - t0
  - t24
Conclusions

• IDO1 is expressed in MM
• IDO1 activity correlates with HGF release
• Bona fide Treg cells are increased and inversely correlate with myeloma-reactive T cells
• In vitro, IDO1 skews T-cell function towards a Th2/Treg cytokine secretion profile
• The HGF/IDO1 axis is a potential target of immune intervention in MM and other HGF-secreting tumors
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