Caratterizzazione di cellule immunosoppressorie nel microambiente tumorale del mieloma multiplo

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4° WORKSHOP NAZIONALE della Società Italiana di Ematologia Sperimentale
14-15 Novembre 2013
MM disease: genetic alterations and microenvironment perturbation

Adapted from “Multiple myeloma: evolving genetic events and host interactions”. Kuehl WM, Bergsagel PL
Nat Rev Cancer 2002; 2(3):175-87
MM immune microenvironment: from surveillance to escape

Adapted from “Immunologic microenvironment and personalized treatment in multiple myeloma”, Rossi M et al
MM immune microenvironment: from surveillance to escape

MGUS

CD16
CD56
CD127
Foxp3
CD4
CD25
Treg

NKg2D
NK

αβ T cell

myeloma cell

MM immune microenvironment: from surveillance to escape

Myeloid-derived suppressor cells (MDSC): origin and definition

- heterogeneous population of cells of myeloid origin
- potent suppressors of various T-cell functions
- human phenotype: Lin^−^HLA-DR^{low/−}^CD33^+^ or CD11b^+^CD14^−^CD33^+^; CD15^+^ in PB
- accumulation in lymphoid organs and in tumors
- differentiation from tumor-associated macrophages (TAMs) in tumor tissues

MDSC in MM patients: what is known?

*Multiple myeloma induces the immunosuppressive capacity of distinct myeloid-derived suppressor cell subpopulations in the bone marrow.*


*Myeloid-derived suppressor cells regulate growth of multiple myeloma by inhibiting T cells in bone marrow.*


*Tumor-promoting immune-suppressive myeloid-derived suppressor cells in the multiple myeloma microenvironment in humans.*

Increased frequency of MDSC in PB and BM of MM patients

Ramachandran I.R. et al., JI 2013, 190:3815-3823
Increased frequency of MDSC in PB and BM of MM patients

Backgated on CD14-

<table>
<thead>
<tr>
<th></th>
<th>PB</th>
<th>BM</th>
<th>CTRL (n=19)</th>
<th>MM (n=47)</th>
<th>CTRL (n=10)</th>
<th>MM (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD33+CD11b+CD14-</td>
<td>5%</td>
<td>8%</td>
<td>65%</td>
<td>38%</td>
<td>85%</td>
<td>70%</td>
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<tr>
<td>CD11b+CD15+HLA-DRlow</td>
<td>3%</td>
<td>7%</td>
<td>5%</td>
<td>4%</td>
<td>5%</td>
<td>6%</td>
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</table>

* p<0.001
* p<0.001
^ p<0.001
Inhibition of conventional αβ T-cell proliferation by MM MDSC

Ramachandran I.R. et al., JI 2013, 190:3815-3823
Mechanisms of MDSC suppressive activity

- **MDSC:**
  - CD16
  - CD56
  - NKg2D
  - IL-10
  - Arginase
  - PDE5

- **NK:**
  - CD16
  - NKg2D

- **T cell stimulation:**
  - Arginase
  - TGFβ
  - IL-10

- **αβ T cell:**
  - Arginase
  - iNOS
  - ROS
  - CD14
  - CD68

- **γδ T cell:**
  - NKG2D
  - LFA1
  - TCR

- **Tregs:**
  - CD4
  - CD25
  - Foxp3
  - CD127

- **DC:**
  - CD16
  - CD56

- **TAMs:**
  - CD206

- **M2 polarization?**

Adapted from “Myeloid-derived suppressor cells: linking inflammation and cancer”, Ostrand-Rosenberg, *Journal of Immunology*, 2009
**Vγ9Vδ2 T cell-based immunotherapy in hematological malignancies: from bench to bedside**

Barbara Castella · Candida Vitale · Marta Coscia · Massimo Massaia

![Diagram of Vγ9Vδ2 T cell interaction with tumor cell](image-url)
Major determinants of intrinsic cancer cell susceptibility:

1. spontaneous IPP generation
2. abundance of KIR ligands
3. paucity of KAR ligands
4. lack of MHC class I molecules
5. costimulatory molecules

**Vγ9Vδ2 T cells in the peripheral blood:**
a rare subpopulation

![CD3 vs TCR gd plot](image)

3.6%

**Zoledronate**

**Mev pathway**

**IPP**

**APC**

**TCR**

**Vγ9Vδ2 T cell**

**proliferation**

**Tumor cell**

**Vγ9Vδ2**
Approx 50% of MM patients at diagnosis are refractory to ZA-induced monocyte-dependent IPP stimulation but DC ZA-treated allow to recover the proliferation of NR patients.


Impaired pAg-induced proliferation in BM Vγ9Vδ2 T cells of MM: BM-derived DCs fail to recover Vγ9Vδ2 T-cell proliferation
Bone marrow: MM tumor bed

- Osteoclast
- Osteoblasts
- Plasmacells
- NK cells
- CD56
- CD16
- NKg2D
- CD11b
- CD15
- MDSC
- CD33
- IL-4Ra
- Monocytes
- CD16
- CD14
- TAMs
- CD14
- CD68
- CD206
- Tregs
- Foxp3
- CD4
- CD25
- CD127
- TCR
- αβ T cell
- γδ T cell
- LFA1
- CD16
- NKG2D
- αβ T cell
- T EM
- T EM
- T EM
Inhibitory cells in the bone marrow: MDSC
Strategies of MDSC inhibition under investigation

**Deactivation of MDSC**
- NO inhibitors (PDE5 inhibitors, L-NAME)
- Arginase inhibitors (COX2 inhibitors, NOHA)
- ROS inhibitors
- MDSC migration inhibitors (anti-glican antibodies)

**Blocking development of MDSC**
- Bisphosphonates (Zoledronic acid)
- Modulators of cell signalling (JAK2/STAT3; VEGF inhibitors)

**Differentiation of MDSC into mature cells**
- Vitamins (ATRA, Vitamin A)
- Cytokynes (IL-12)
- Cytotoxic agents (gentamicine, cisplatin)
- HSP90 inhibitors
- IL-6 blockers

**Depletion of MDSC**

Wesolowski et al. J ImmunoTherapy of Cancer 2013
**In vitro inhibition of MDSC activity: PDE5 inhibitor**

- **MDSC**
- **PDE5**
- **γδ T cell**
- **Proliferation**

**BMMC culture (n=10)**

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<tr>
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<th>IL-2</th>
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<th>Sildenafil</th>
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<tbody>
<tr>
<td>Viable γδ T cells/well</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
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</table>
**In vitro** inhibition of MDSC activity: IDO inhibitor

MDSC

1-metiltriptophan

IDO

γδ T cell

Proliferation

γδ T cell

Viable γδ T cells/well

BMMC culture (n=6)

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<th>IL-2</th>
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<th>1-MT</th>
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<tr>
<td>+</td>
<td>+</td>
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MDSC *in vitro* depletion

**BMMC**

**MDSC-depleted BMMC**

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<td>+</td>
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</table>

Viable Vy9V62 T cells/well

- **BMMC**
- **MDSC-depleted BMMC**
Inhibitory pathways in tumor microenvironment
Inhibitory pathways in tumor microenvironment

![Graph showing % Vγ9Vδ2+ PD-1+ T cells in different conditions.](#)

- **CTRL (n=12)**
- **MM (n=64)**

- **PB (n=12)**: * p<0.001
- **BM (n=5)**: ° p=0.004
- **PB (n=35)**: *
- **BM (n=64)**: *
Inhibitory pathways in tumor microenvironment

- Tumor cell
- MDSC
- Vγ9δ2 T cell

Diagram showing interactions involving B7-2 (CD86), B7-1 (CD80), CD28, CTLA4, PD-L1, LAG-3, TCR, PD-L1 (B7-H1), PD-L2 (B7-DC), CD48, HVEM, CD244 (2B4), BTLA, CD160, TIM-3, and Galectin-9.
Inhibitory pathways in tumor microenvironment

% CD138+ positive cells

- BM MM (n=15)
- PB (n=4)
- BM (n=2)
- PB (n=12)
- BM (n=24)

CTRL

MM

% MDSC positive cells

% Vγ9Vδ2+ PD-1+ T cells

- PB (n=12)
- BM (n=5)
- PB (n=35)
- BM (n=64)

CTRL

MM

* p<0.001
° p=0.004
Partial recovery of BM Vγ9Vδ2 T cells reactivity upon PD-1 blockade

Clinical trial with blocking mAb:
- CT-011: bind to human PD1
- MDX-1106: blocks binding of PD1 to PDL1 and PDL2
The importance of PD-1/PD-L1 axis in BM microenvironment
The importance of PD-1/PD-L1 axis in BM microenvironment

Plasmacells

PDL-1

PD-1

γδ T cell

PI3K

Akt

Activation

CKs production

Proliferation

osteoblasts

Stromal cells

osteoclast

MDSC

MDSC

MDSC
The importance of PD-1/PD-L1 axis in BM microenvironment

- Osteoclasts
- Osteoblasts
- Stromal cells
- Plasmacells
- MDSC

Chemotherapy → Remission

- PDL-1
- PD-1

γδ T cell

PI3K
Akt
Activation
CKs production
Proliferation
Frequency and PD-L1 expression in BM MDSC of MM patients according to disease status

\[ p < 0.0001 \]

\[ p = 0.005 \]
PD-1 expression on MM BM Vγ9Vδ2 T cells and pAgs-reactivity according to disease status
Conclusions

MDSC are increased in the BM of MM patients at diagnosis and persist in high numbers after induction of clinical remission.

PD-L1 expression on MDSC could represent an additional suppressive mechanism in MM BM microenvironment.

The PD-1/PD-L1 axis is a potential target for the immunotherapy of multiple myeloma, even in complete remission after chemotherapy.
Thanks to all!

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Prof. Massaia Massimo
Myriam Foglietta
Patrizia Sciancalepore