Abnormalities of the Immune System and Inflammation in MDS Pathogenesis

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NHS Foundation Trust
What is the role of immune system in MDS?

- Oligoclonal expansion of CD8+ T-cells that resolve following IST in responsive patients.
- Persistent oligoclonality in relapsing and non-responsive patients


Elaine M Sloand (1953 - 2010)

CD4\(^+\) T cells in BMFs

CD4\(^+\)CD25\(^{high}\) Foxp3\(^+\) regulatory T cells in myelodysplastic syndrome (MDS)

Shahram Y. Kordasti, Wendy Ingram, Janet Hayden, David Darling, Linda Barber, Behdad Afzali, Giovanna Lombardi, Marcin W. Wlodarski, Jaroslaw P. Maciejewski, Farzin Farzaneh, and Ghulam J. Mufti

- 52 patients (30 men, 22 women) median age of 64.5 years
- Low risk n=18, Intermediate risk n=25, High risk n=9
- Cytogenetic: Normal 49%, 5q- 17%, Complex 16%, Other 18%

Kordasti, et al
© 2009 Blackwell Publishing Ltd, British Journal of Haematology, 145, 64–72

Carlsten, M et al, Leukemia 2010
Tregs and MDS prognosis

Regulatory T cells and progenitor B cells are independent prognostic predictors in lower risk myelodysplastic syndromes

Higher number of Tregs correlates with poorer prognosis in lower risk MDS

Josephine D. Kahn, et al
Haematologica 2015
Immunogenic cell death (ICD)

Early events

- DC activation
- ATP release
- Immature DC
- P2RX7

Late cellular response

- Immuneological memory
- Memory T cells
- Clonal expansion
- T cell priming
- IL-1β production
- IL-17 production
- T cell recruitment
- Memory T cells
- IFN-γ release
- T cell lysis
- HMGB1 release
- TLR4
- HMG81
- Type 1 IFN release
- αβ T cell
- γδ T cell

Antigen uptake

DC maturation

DC homing

ANXA1 release

P2RX7

CALR exposure

DC recruitment

ATP

Immature DC
Immunogenic mutations (IMs)

Absence of Neoantigen: Smouldering inflammation which may contribute into genomic instability and promote malignant transformation.

Combination of ICD and IMs is likely to form an effective antigen specific immune response

Effects of chronic inflammation

42 pre-treatment MDS patients (median age 69.5 y) was analysed.
Twenty-three patients also had bone marrow samples available for analysis.
Somatic mutation related neoantigens

Can be predicted based on the type of mutation and HLA-type.

Daniel S. Chen & Ira Mellman
**Impact of neoantigens on survival in MDS**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients with neoantigens</th>
<th>Patients with no neoantigens</th>
<th>P value</th>
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<tbody>
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<td>Number</td>
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<td>51</td>
<td></td>
</tr>
<tr>
<td>Age (median in years)</td>
<td>68</td>
<td>68</td>
<td>NS</td>
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<tr>
<td>Sex (Male / Female)</td>
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<td>31 / 20</td>
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<td>Type of MDS</td>
<td></td>
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<tr>
<td>RARS</td>
<td>10</td>
<td>6</td>
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<tr>
<td>RCUD</td>
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<td>RCMD</td>
<td>42</td>
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<td>Isolated 5q-</td>
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<td>IPSS Categories</td>
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<td>Low</td>
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<td>Intermediate-1</td>
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<td>Intermediate-2</td>
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<td>High</td>
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<td>4</td>
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<td>Number of mutations (median)</td>
<td>2.1</td>
<td>1.3</td>
<td>P&lt;0.001</td>
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<td>Progression to AML</td>
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<td>Yes</td>
<td>26/129 (20.1%)</td>
<td>8/51 (15.7%)</td>
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<td>Vital Status</td>
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<tr>
<td>Dead</td>
<td>28/129</td>
<td>15/51</td>
<td>NS</td>
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</table>

**Combined UK & German cohort**

![Graph showing percent survival over follow-up with comparison between neoantigen and no neoantigen groups.](image)

*P<0.05. HR (0.033-0.991)*

Collaboration with Prof Uwe Platzbecker

Tom Coats, et al, ASH 2017,
Multivariate factors influence tolerance and immunity

Patient A: Myeloid-derived suppressor cells, TGF-β, IL-10, IL-4, PD-L1, CAFs
Patient B: Regulatory T cells, IL-1α, IL-1β, IL-12, IL-23, CD4⁺ T cells, CD8⁺ T cells

Cancer-immune set point

Genetics: Repression, Activation
Age: Older, Younger
Microbiome: Tolerogenic, Inflammatory
Viral infection: No infection, Infection
Exposure to sunlight: More sunlight, Less sunlight
Immune-modifying drugs: Immune suppression, Immune stimulation

Daniel S. Chen & Ira Mellman
Nature 541, 321–330, 2017
Summary (1)

• Immune response in MDS consists of two main components:
  o Immunogenic cell death and subsequent cellular response.
• There is an immune response “switch” from AA/LR MDS to HR MDS and AML.
• CD4⁺ T cells are regulated by the inflammatory environment.
• CD4⁺ T cells could orchestrate the overall immune response in MDS and Tregs play an important role in defining immune set point.
CD4\(^+\) T cells plasticity

Manual gating (Expert gating)

- **Tr I**: CD45-RA
- **Tr II**: FOXP3
- **Tr III**: IL-12, IFNy

**TCF3**

**IL-6**

**IL-12, IFNy**

**FOXP3**

**CD45-RA**
Cytome (CyTOF; mass cytometry)
Identification of Treg subset by automated clustering.

Kordasti et al. Blood 2016;128:1193 1205
Function and ontogeny of Treg subpopulations.

Table 3. Genes that are upregulated in the Treg population B compared with population A

<table>
<thead>
<tr>
<th>Gene</th>
<th>FDR q-value</th>
<th>Normalized enrichment score</th>
<th>Significant genes</th>
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<td>G2M checkpoint</td>
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<td>CASCL1, NUSAP1, CENPE, TP53, KIF11,</td>
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<td>BAP1, E2F1, SLCY14, HHV1, CHS52, CHS2,</td>
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<td>Mitosis</td>
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<td>M phase of mitotic cell</td>
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<td>IL2-STAT5 signaling</td>
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<td>SYT11, CCR4, TNFRSF9, CST7, ADAM19,</td>
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<td>FGF2, TNFRSF18, TNFRSF4, GALM, CXCL10,</td>
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<td>Immune response genes</td>
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<td>CDS1, IL10A, NCP4, CD89, GZMB, IL32,</td>
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<td>TNFRSF4, LAX1, DEF6MA, FGFR3B, TLR7, CD74,</td>
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<td>APOC4, CCL5, AP0E2C2G, CD79B, CTLA4</td>
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</table>
Tregs in MDS

Coats et al., unpublished
Abundance of Tregs – HD vs MDS

TotalTreg

% CD4+ unstim

HD vs MDS

TregA

% CD4+ unstim

**

Treg B

% CD4+ unstim

NS

HD vs MDS

CD4+ CD127lo CD25hi FOXP3hi Helios hi, CTLA4hi, CD95 Lo, CCR4 lo, CD45RO lo, (Treg A)

CD4+ CD127lo CD25hi FOXP3hi Helios hi, CTLA4hi, CD95hi, CCR4hi, CD45ROhi (Treg B)
Why Treg B are low?

After adding FAS-L (5 µg/ml) for 5 h, Treg B (and CD4+) have a higher rate of apoptotic and dead cells than Treg A.

Benedetta Costantini, ASH 2017
Immune check point

Patient 1

Patient 2

PD-1+

PD-1++

Coats et al, ASH 2017
Coats et al, unpublished
Treg signature in the presence of neoantigens

Diggins et al., "At the Bench: Precision Medicine with Single Cell Mass Cytometry," under review

Coats et al, unpublished
Treg subset identification in diagnostic lab

- Treg A: CD45, CD3, CD4, CD25, CD127, CD46RA, CCR4, CD95
- Treg B: CD45, CD3, CD4, CD25, CD127, CD46RA, CCR4, CD95
Treg Subsets in MDS v Controls (Bone Marrow)

31 suspected MDS patients: 20 MDS and 11 non-diagnostic
Function of *ex-vivo* expanded Tregs

<table>
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<tr>
<th>Bone</th>
<th>Spleen</th>
<th>Liver</th>
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<tr>
<td>T cells</td>
<td></td>
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<tr>
<td>T cells + Tregs</td>
<td></td>
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<tr>
<td>Tregs</td>
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</table>
DNMTi Induce Interferon Responses

Aza-Upregulated Viral Defense Genes Are Significantly Correlated with ERVs in Primary Tumors and Correlate with Sensitivity to Immune Therapy

Chiapinelli et al. Cell 2015

Costantini B et al. Haematologica 2013;98:1196-1205
Summary (2)

• Using a less biased and unsupervised method, we have identified 2 distinct subpopulation of Tregs.
• The frequency of these subpopulation could predict response to IST in AA and potentially disease progression in MDS.
• Inflammatory environment (particularly FAS mediated) affects the composition of Tregs.
• Predictor for response to immunotherapy (i.e. CPI)?
The importance of patient profiling

Clinical and Omics data
NGS, Cytom, Proteomics, Antigen prediction, RNA seq etc

Smouldering inflammation
Targeting myelodysplastic inflammation (S100A9, Inflammasomes, Caspase act vation and TLR pathways)

AI dominant and lack of regulation
Reconstitution immune regulation (Treg, overall dose IL-2) +IAT

Immunogenic mutations and IS dominant
Revving immune response (CPI, HMA), Pept de vac ccnes

Non-immunogenic mutations and IS dominant (HR features)
CAR, HSCT, HMA
THANK YOU