Myeloid-Derived Suppressor Cells & Altered Innate Immunity in MDS Pathogenesis

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Disclosures

- **Consultant**
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  - Amgen

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  - Cell Therapeutics Inc., Trillium, Amphimed, Amphivena
Traditional Model of MDS Pathogenesis

Ineffective Hematopoiesis

Stage 1
Intrinsic Increase in Apoptotic Response and Inflammation

- ↑ TNFα-induced apoptosis
- ↑ ROS
  - Induction of homeostatic mechanisms
  - Telomere erosion and senescence
- Bone Marrow
- Abnormal ribosomes
- Altered MP localization
- Stromal Cell Defects
- Altered T-cell homeostasis
- Inflammatory Microenvironment
- Stem Cell Depletion

High Risk for AML Transformation

Stage 2
Acquisition of anti-apoptotic molecules

- ↑ Bcl-2
- Expansion
- Emergence of mutant driver clones
- Impaired Immunosurveillance by NK and T-cells

Stage 3
Initiation of clonal evolution

- Abnormalities in DNA repair mechanisms with propagation of abnormal cells
Innate Immunity
An Emerging Pathogenetic Driver in MDS

- Chronic inflammation & activation of innate immunity are linked to hematopoietic senescence & MDS pathobiology
  
  - TLR-2, -4 & -9 are overexpressed in MDS HSPC, with TLR4 implicated in progenitor apoptosis & cytopenias (Hoffman W, Blood 2002; Wei Y, Leukemia 2013)
  
  - TRAF6 is up-regulated in MDS CD34+ cells, with amplification of the TLR4 signaling intermediates, TRAF6 & TIRAP* (Gondek LP; Starczynowski DT, Blood 2008)
  
  - TLR signaling is constitutively active in del5q MDS d/t miR-145 & miR-146 allelic deletion and TIRAP & TRAF6 de-repression (Starczynowski DT, Nat Med 2010;16:49)
  
  - The TLR4 adaptor kinase IRAK is overexpressed & hyperactive in MDS, whereas IRAK1 inhibition impairs MDS HPC expansion (Rhyasen, Cancer Cell 2013)
  
  - Our recent work implicates expansion of Myeloid Derived Suppressor Cells (MDSC) as key innate immune effectors in MDS pathogenesis (Wei S, JCI 2013)

*TRAF6: tumor necrosis factor receptor- associated factor-6;
TIRAP:Toll-interleukin-1 receptor domain-containing adaptor protein.
• TLR ligation drives GMP expansion in the absence of myeloid GFs, while reducing lymphocyte production by CLPs similar to normal senescence
• Chronic TLR activated HSC lose quiescence causing HSC depletion

Myeloid-Derived Suppressor Cells

- Immature myeloid cells with distinct function & phenotype
  - Mouse MDSC: CD11b⁺Gr⁻1⁺ (±B220, CD31)
  - Human MDSC: Lin⁻HLA-DR⁻CD33⁺
- MDSC expand with age, infection, inflammation, and neoplastic diseases.
- MDSC induce tumor immune tolerance & T-reg cell expansion.
- Mechanisms of inhibition: elaboration of ROS, NO, and Arginase, VEGF, TGF-β, IFN, IL-6, IL-10 & others
- MDSC expansion and activation driven by TLR ligands (e.g., DAMP signals)

*DAMP: danger-associated molecular pattern.*
MDSC Expand in the BM of Lower Risk MDS Patients

MDSC MDSC are Genetically Distinct from the MDS Clone

- MDSC lack both cytogenetic abnormalities & gene mutations intrinsic to the MDS clone
- Absence of genetic abnormalities indicates that MDS MDSC derive from non-neoplastic HSPC & precede emergence of MDS clones
MDS-MDSC Suppress T cell Proliferation & Interferon-γ Elaboration


- **3H-Thymidine incorporation (cpm)**
  - T cells
  - MDSC
  - beads + T cells:MDSCs 1:0.5
  - beads + T cells:MDSCs 1:0.25
  - T cells + beads

- **IFN-γ (pg/ml)**
  - T cells
  - MDSC
  - beads + T cells:MDSCs 1:0.5
  - beads + T cells:MDSCs 1:0.25
  - T cells + beads

- **Positive cells of BrdU (%)**
  - Control
  - MDS

- **Donor 1, Donor 2, Donor 3**
MDS-MDSC Generate Inflammatory Molecules

MDS-MDSC Suppress Autologous Hematopoiesis

Granzyme Mobilization

CD33 (red); granzyme B (green)

Apoptosis

BFU-E

p<0.001

Number of BFU-E

Unsorted MDSC+ MDSC-

The ITIM Signaling Receptor CD33-SIGLEC3 is Over-expressed in MDS-MDSC

**ITAM Signaling**

- Activatory receptor
- Adaptor protein
- Syk

**ITIM Signaling**

- Inhibitory receptor
- SHP1/2

**Promotes Myeloid Differentiation & Maturation**

**Blocks Differentiation & Maturation**

*Immunoreceptor tyrosine-based inhibition motif (ITIM); Sialic Acid-binding Ig-Type Lectin*

CD33-ITIM* Signaling Cooperates in MDSC Expansion & HPC Apoptosis

*Immunoreceptor tyrosine-based inhibition motif (ITIM)

S100A9 is the Native Ligand for CD33

CD33-IgG Fc Fusion

CD33 Binds S100A9

S100A9/CD33 Engagement Induces MDSC Activation

Normal donor BM-MNC’s RAGE, TLR4, CD33, or their combination were blocked prior to culturing cells by with or without 1 μg of S100A9 for 48 hours followed by assessment of IL-10 gene and protein expression (qPCR – top, ELISA on the bottom).
S100A9 Interacts with TLR4 & CD33 (SIGLEC-3)

Siglecs (Sialic acid-binding immunoglobulin-type lectins)
S100A9-Tg Mice Display Age-related MDSC Expansion & Ineffective Hematopoiesis

S100A9-Tg Mice Develop Trilineage Cytological Dysplasia Phenocopying MDS

A. Hypercellular marrow with megakaryocytic hyperplasia

B. Dysplastic megakaryocytes showing single or hypolobation and increased micro-megakaryocytes (dwarf megakaryocytes)

C. Hypogranulated and hyposegmented PMNs (pseudo-Pelger-Huet changes) and nuclear budding in erythroid precursors. (All cells are partially degenerated)

D. PAS stain highlights erythroid predominance
**S100A9-Tg Mice Develop Multilineage Cytopenias with Age**

All data are means ± SEM (n=3-5 mice). Peripheral blood samples were prepared from both S100A9Tg and control (wt) mice in ages of 6, 18 and 24 weeks and analyzed on a Hema True Hematology Analyzer (Heska). *p<0.05; **p<0.01; ***p<0.001 vs wt-mice

<table>
<thead>
<tr>
<th>Test</th>
<th>Units</th>
<th>6 weeks</th>
<th></th>
<th>18 weeks</th>
<th></th>
<th>24 weeks</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>WT</td>
<td>S100A9-Tg</td>
<td>WT</td>
<td>S100A9-Tg</td>
<td>WT</td>
<td>S100A9-Tg</td>
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<tr>
<td>WBC</td>
<td>$10^3/\mu l$</td>
<td>$5.4 \pm 0.5$</td>
<td>$4.2 \pm 1.5$</td>
<td>$6.0 \pm 1.4$</td>
<td>$2.9 \pm 0.2^*$</td>
<td>$6.3 \pm 0.8$</td>
<td>$3.0 \pm 0.4^*$</td>
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<td>LYM</td>
<td>$10^3/\mu l$</td>
<td>$3.9 \pm 0.5$</td>
<td>$2.6 \pm 1.2$</td>
<td>$4.7 \pm 1.1$</td>
<td>$2.4 \pm 0.1^*$</td>
<td>$4.8 \pm 0.7$</td>
<td>$2.5 \pm 0.3^*$</td>
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<td>MONO</td>
<td>$10^3/\mu l$</td>
<td>$0.4 \pm 0.1$</td>
<td>$0.3 \pm 0.2$</td>
<td>$0.4 \pm 0.1$</td>
<td>$0.2 \pm 0.1^*$</td>
<td>$0.4 \pm 0.1$</td>
<td>$0.2 \pm 0.1^*$</td>
</tr>
<tr>
<td>GRAN</td>
<td>$10^3/\mu l$</td>
<td>$1.1 \pm 0.6$</td>
<td>$1.2 \pm 1.2$</td>
<td>$0.9 \pm 0.3$</td>
<td>$0.3 \pm 0.2^*$</td>
<td>$1.1 \pm 0.4$</td>
<td>$0.3 \pm 0.1^{**}$</td>
</tr>
<tr>
<td>HCT</td>
<td>%</td>
<td>$48.1 \pm 3.2$</td>
<td>$42.4 \pm 2.1$</td>
<td>$45.7 \pm 0.7$</td>
<td>$35.5 \pm 3.0^{**}$</td>
<td>$45.4 \pm 3.5$</td>
<td>$32.1 \pm 2.7^{**}$</td>
</tr>
<tr>
<td>MCV</td>
<td>fl</td>
<td>$51.4 \pm 1.6$</td>
<td>$49.8 \pm 2.0$</td>
<td>$50.3 \pm 0.5$</td>
<td>$50.0 \pm 1.2$</td>
<td>$50.0 \pm 1.3$</td>
<td>$50.0 \pm 1.6$</td>
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<tr>
<td>RDWa</td>
<td>fl</td>
<td>$35 \pm 0.7$</td>
<td>$33.2 \pm 2.2$</td>
<td>$34.0 \pm 1.3$</td>
<td>$32.5 \pm 1.7$</td>
<td>$33.3 \pm 1.4$</td>
<td>$32.2 \pm 1.5$</td>
</tr>
<tr>
<td>RDW%</td>
<td>%</td>
<td>$16.3 \pm 0.7$</td>
<td>$15.9 \pm 0.1$</td>
<td>$16.3 \pm 0.5$</td>
<td>$15.5 \pm 0.5$</td>
<td>$16.0 \pm 0.5$</td>
<td>$15.4 \pm 0.6$</td>
</tr>
<tr>
<td>HGB</td>
<td>g/dl</td>
<td>$14.2 \pm 0.8$</td>
<td>$13.0 \pm 0.7$</td>
<td>$13.9 \pm 0.3$</td>
<td>$11.1 \pm 0.7^{**}$</td>
<td>$13.7 \pm 0.6$</td>
<td>$10.3 \pm 0.5^{**}$</td>
</tr>
<tr>
<td>MCHC</td>
<td>g/dl</td>
<td>$29.5 \pm 1.1$</td>
<td>$30.6 \pm 1.3$</td>
<td>$30.4 \pm 0.4$</td>
<td>$31.5 \pm 0.9$</td>
<td>$30.3 \pm 1.0$</td>
<td>$32.3 \pm 1.1$</td>
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<tr>
<td>MCH</td>
<td>pg</td>
<td>$15.1 \pm 0.3$</td>
<td>$15.2 \pm 0.1$</td>
<td>$15.3 \pm 0.1$</td>
<td>$15.8 \pm 0.3$</td>
<td>$15.2 \pm 0.2$</td>
<td>$16.2 \pm 0.4$</td>
</tr>
<tr>
<td>RBC</td>
<td>$10^6/\mu l$</td>
<td>$9.4 \pm 0.7$</td>
<td>$8.5 \pm 0.4$</td>
<td>$9.1 \pm 0.1$</td>
<td>$7.1 \pm 0.6^{**}$</td>
<td>$9.1 \pm 0.3$</td>
<td>$6.4 \pm 0.2^{***}$</td>
</tr>
<tr>
<td>PLT</td>
<td>$10^9/\mu l$</td>
<td>$555.7 \pm 96.6$</td>
<td>$412.0 \pm 124.0$</td>
<td>$431.3 \pm 33.9$</td>
<td>$95.7 \pm 35.0^{***}$</td>
<td>$437.0 \pm 41.9$</td>
<td>$61.0 \pm 23.5^{***}$</td>
</tr>
</tbody>
</table>

Candidate Therapeutics Targeting Innate Immune Activation in MDS

Sites of Target Inhibition

- CD33/IgG$_H$ fusion
- S100A8/9
- OPN-305
- CD33/IgG
- TGF$\beta$
- arginase
- TGF$\beta$
- MDSC & T-Regs
- TGF$\beta$
- TNF$\alpha$
- iNOS
- Anakinra
- IL-1$\beta$
- IL-1RI
- IL-1 family, including IL-18 and IL-33
- ND-2158
- ARQ-092
- Arry614
- Sotatercept
- ACE-536
- LY2157299
- BI-836858
- INCB24360
- ICTa

Figure modified from www.nimbusdiscovery.com
IL-1 Receptor-Associated Kinase [IRAK]  
A Candidate Therapeutic Target in MDS

IRAK4 Inhibition (ND-2158)

Selective Suppression of MDS CFC with IRAK Inhibition

Selective Apoptotic Response to IRAK Inhibition

Figure adopted from www.nimbusdiscovery.com

Rhyasen GW & Starczynowski D. Cancer Cell 2013.
Novel Strategies to Abrogate Aberrant Innate Immune Activation

Figure adapted from Chen X, et. al. J Clin Invest 2013; 123(11):4595-4611
Constitutive Activation of TGF-β Signaling Suppresses Hematopoiesis in MDS

- TGFβ Type I receptor kinase phosphorylates Smad2 & 3 forming transcriptional complexes, whereas the inhibitory Smad7 extinguishes TGFβ-R1 activity
- miR-21 upregulation significantly reduces Smad7 in MDS BM progenitors
- R1 kinase is constitutively activated in MDS with sustained Smad2 phosphorylation
- Suppression of R1 kinase improves MDS progenitor CFC \textit{in vitro}

Phase 2a Study of TGF-β Receptor I Kinase Inhibitor LY2157299 (galunisertib)

- Selective a novel oral TGF-βRI/II dual kinase inhibitor
- Dihydropyrolopyrazole ATP binding pocket binder with RI IC$_{50}$ 86 nM
- Phase I activity in GBM
- p-SMAD2/3 inhibition
- Eligibility: Low/Int-1 MDS, Hgb<9.5
- Dose: 300mg/d po x14d,q4wks
- Primary endpoint: HI$_E$@24 wks
- N=40
ACE-011 (sotatercept) Targets Stromal-Mediated Inhibition of Erythropoiesis

- High affinity Activin-A receptor (RIIA)/IgG1 fusion protein
- Sustained neutralization of activin-A & GDF11 ligands for up to 32 days
- Relieves GDF11 and activin-A suppression of erythropoiesis to restore differentiation
- Inhibits osteoclasts & promotes osteoblast survival
- MTD in normal volunteers: erythrocytosis

ACE-011 (Sotatercept) and ACE-536 Novel Ligand Traps for TGFβ Superfamily Ligands

<table>
<thead>
<tr>
<th>ACE-011 (Sotatercept)</th>
<th>ACE-536</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion protein with ligand trap activity toward the activin type 2 receptors</td>
<td>Modified Extracellular Domain of ActRIIB</td>
</tr>
<tr>
<td>Drug does not bind EPO receptors</td>
<td>Fc Domain of human IgG1 Antibody</td>
</tr>
<tr>
<td>Extracellular Domain of ActRIIA</td>
<td>Fc Domain of human IgG1 Antibody</td>
</tr>
<tr>
<td>Heme effect</td>
<td>+</td>
</tr>
<tr>
<td>Bone effect</td>
<td>+</td>
</tr>
</tbody>
</table>
Randomized Phase II Study of Sotatercept in Transfusion-Dependent LR-MDS Epo Failures

**Dose Finding Phase**

- **Sotatercept**
  - 0.1 mg/kg SC q21d
  - 0.3 mg/kg SC q21d
  - 0.5 mg/kg SC q21d
  - 1.0 mg/kg SC q21d
  - 2.0 mg/kg SC q21d

**Extension Phase**

- **Response Estimate**
  - **MED [n=15]**

**Eligibility**
- Low/Int-1
- WHO MDS
- MDS/MPN
- Hgb<9g/dl

**Endpoints:**
1. HI-E (IWG 2006 Cycle 5-8)
2. HI-E duration - Progression

MED, Maximally Effective Dose.
ARRAY-614-112 Phase 1 Study in LR-MDS
Enabled ARRY-614 Formulation

p38 MAPK Inhibitor

Dose Escalation Phase

- Daily dosing:
  - 200
  - 400
  - 600
  - 800
  - 1000

- BID dosing:
  - 100
  - 200

• 3 + 3 dose escalation design with expansion
• Cycle = 28 days

Expansion Phase

Primary Objective
- Safety, tolerability & MTD.
- PK

Secondary
- IWG 2006 response
- Explore PD profile

ARRY-614 Reduces BM-MNC phospho-p38

*Sample collected prior to the first dose of ARRY-614

†Number of pts for whom bone marrow samples available at screening and cycle 2

Paired t test, $P < 0.05$

Representative Image

Aperio whole slide scanning and scoring performed by Flagship Biosciences

### ARRY-614 Hematologic Response

<table>
<thead>
<tr>
<th>All responses (HI and platelet transfusion)</th>
<th>HI-E</th>
<th>HI-P-Any</th>
<th>HI-N</th>
<th>Total pts with HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 66</td>
<td>n = 42</td>
<td>n = 22</td>
<td>N = 71</td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>5 (7.6)</td>
<td>8 (19)</td>
<td>6 (27)</td>
<td>14 (20)</td>
</tr>
<tr>
<td>Median duration, weeks (range)</td>
<td>11 (9-29)</td>
<td>30.1 (10.4-91)</td>
<td>17.6 (8.7-67.4)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transfusion improvement</th>
<th>RBC</th>
<th>Median Duration (range)</th>
<th>platelets</th>
<th>Median Duration (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=41</td>
<td>n=16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfusion reduction n (%)</td>
<td>5 (12)</td>
<td>11 (9.0-28.6)</td>
<td>7 (44)</td>
<td>18 (10.4–91.1)</td>
</tr>
<tr>
<td>Transfusion indepnt n (%)</td>
<td>2 (5)</td>
<td>19.0 (9.3-28.6)</td>
<td>5 (31)</td>
<td>14.1 (10.4–90.7)</td>
</tr>
</tbody>
</table>

Conclusions

• MDSCs (LIN-HLA-DR-CD33+) are activated & profoundly expanded in the bone marrow of MDS patients.
• MDS-MDSCs are distinct from the MDS clone, display a CD33^{Hi}/lineage^{−} phenotype, produce inflammatory/suppressive molecules & serve as cellular effectors of ineffective hematopoiesis via direct cytotoxicity to autologous progenitors.
• S100A9 is a myeloid-derived peptide & TLR4/CD33 ligand that promotes both autocrine-reinforced MDSC activation, & paracrine mediated myeloid progenitor apoptosis.
• Strategies that neutralize S100A9, or inhibit TLR & CD33 ITIM signaling offer therapeutic potential in the treatment of patients with MDS.
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Eric Padron
Rami Komrokji
1. Which factors determine primarily the incidence of relapse after HCT for MDS?
   a. Transfusions given before HCT
   b. Marrow myeloblast count
   c. Cytogenetics
   d. Pre-transplant therapy
   e. b and c

2. Which would be your order of priority in selecting a transplant donor?
   a. HLA-matched (HLA=) sibling > HLA= unrelated donor (URD) > HLA haplo-identical relative > cord blood
   b. HLA= sibling > HLA= URD > cord blood > HLA haplo-identical relative
   c. HLA= sibling > HLA haplo-identical relative > HLA= URD > cord blood
   d. HLA= sibling > cord blood > HLA=URD > HLA haplo-identical relative
3. Iron overload in MDS is prognostic and:
   a. Correlates with a poor overall survival
   b. Correlates with certain comorbidities
   c. Should be corrected before stem cell transplantation
   d. a, b and c

4. In the context of MDS, fatigue is:
   a. Rarely seen
   b. Frequently recorded
   c. Often found in those who have comorbidities
   d. b and c
5. The presence of TET2 mutations predicts for:
   a. Worse survival in MDS patients
   b. A worse response to hypomethylating agents
   c. A lower than normal platelet count
   d. None of the above

6. DNA methylation patterns predict for:
   a. A worse survival in patients with RAEB-I
   b. Response to decitabine or 5-azacytidine
   c. The presence of specific mutations within the MDS genome
   d. Clonal diversity at diagnosis in MDS patients
7. Myeloid-derived suppressor cells (MDSC) are a phenotypically distinct innate immune effector cell that displays high expression of which of the following antigens?
   a. CD34
   b. CD33
   c. CD14

8. Bone marrow-MDSC are markedly expanded in MDS and are responsible for which of the following?
   a. Cell death of hematopoietic progenitors
   b. Suppression of anti-tumor immune response
   c. Elaboration of inflammatory cytokines
   d. All of the above
9. Somatic mutations in one of the following genes of RNA splicing machinery are associated with an MDS subtype with distinct phenotype and indolent clinical course. Which is the gene?
   a. SF3B1
   b. SRSF2
   c. U2AF1

10. More than 90% of patients with chronic myelomonocytic leukemia carry somatic mutations of genes of various biologic pathways. Many of them have concomitant mutations in 2 genes: which is the typical co-mutation of CMML?
   a. SF3B1-JAK2
   b. TET2-SRSF2
   c. CSF3R-SETBP1