Biomarker Directed Treatment Approaches for MDS

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Challenges to Targeted Drug Development in MDS

- The defining MDS diagnostic feature, dysplasia, is nonspecific
- Diverse pathogenetic drivers
- Empiric or prognostic driven treatment selection
- Selection by biological signature or surrogate vs. clinical risk stratum alone
Predictive Model for Response to rEPO + G-CSF
Nordic MDS Group [N=98]

<table>
<thead>
<tr>
<th>Score</th>
<th>Patients (n)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2</td>
<td>29</td>
<td>74%</td>
</tr>
<tr>
<td>±1</td>
<td>31</td>
<td>23%</td>
</tr>
<tr>
<td>&lt;-1</td>
<td>34</td>
<td>7%</td>
</tr>
</tbody>
</table>

Serum EPO mU/mL
- <100: Score +2
- 100–500: Score +1
- >500: Score -3

RBC transfusion
- <2 units/mo.: Score +2
- >2 units/mo.: Score -2
Myeloblast Antigen Aberrancy in MDS

Non-Aberrant

Aberrant

RCMD-RS  MDS-U  MDS/MPD

Westers TM, et al. *BLOOD* 2010; 115:1779
Anemia
Low/Int-1 IPSS
[n=46]

Aberrant Phenotype (aFC)

$\text{(CD5}^+, \text{CD7}^+, \text{CD56}^+, \text{CD33}^-)$

$\text{CD45}^{\text{dim}} \text{CD34}^+ \text{SSC}^\text{low}$

Responders
• n=18
• 15 (85%) sEpo < 100 mU/ml
• aFC 2 (18%)*

Epoetin alpha + GCSF

Non-Responders
• n=28
• 6 (21%) sEpo < 100 mU/ml
• aFC 21 (75%)

*HI-E 4 mos aFC vs. 12 mos nl FC.

Westers TM, et al. BLOOD 2010; 115:1779
Multivariate Logistic Regression Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC phenotype</td>
<td>0.035</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum Epo</td>
<td>0.0245</td>
<td>0.019</td>
</tr>
<tr>
<td>RBC transfusions</td>
<td>0.294</td>
<td>0.291</td>
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</table>
## Biomarker Model for ESA Response

### Serum Epo

<table>
<thead>
<tr>
<th>Serum Epo</th>
<th>Score</th>
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<tbody>
<tr>
<td>&lt;100</td>
<td>+2</td>
</tr>
<tr>
<td>100-500</td>
<td>+1</td>
</tr>
<tr>
<td>&gt;500</td>
<td>-3</td>
</tr>
</tbody>
</table>

### Flow Cytom

<table>
<thead>
<tr>
<th>Flow Cytom</th>
<th>Score</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Aberrant</td>
<td>-2</td>
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</table>

### Patients Response

<table>
<thead>
<tr>
<th>Score</th>
<th>Patients</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>14</td>
<td>94%</td>
</tr>
<tr>
<td>+1</td>
<td>23</td>
<td>17%</td>
</tr>
<tr>
<td>&lt;-1</td>
<td>9</td>
<td>11%</td>
</tr>
</tbody>
</table>

Westers TM, et al. *BLOOD* 2010; 115:1779
Advantage of Biomarker Modeling

- The low ESA response rate in MDS with aberrant blast phenotype may reflect greater intrinsic impairment in maturation potential.
- Myeloblast aberrancy identifies lower risk patients with more aggressive biological behavior and inferior survival.
- Biomarker modeling may foster early selection of disease altering therapy vs. ESA in lower risk MDS with greater AML potential.
Emerging Lenalidomide Response Biomarkers

Chromosome 5q deletion
- PP2Aca
- TP53 mu

Non-del(5q) MDS
- Erythroid gene signature
- RPS14 expression level
- immune signature
Allelic Haplodeficiency in Del(5q) MDS
Molecular Pathobiology & Selective Drug Sensitivity

Del(5q) MDS

Hematologic Phenotype

miR-145; -146a
↑Fli-1
↑TRAF6
↑IL-6
Thrombocytosis & dysplasia

RPS-14
↑TIRAP
Mild Neutropenia

Anemia

Lenalidomide Cytotoxicity

PP2A
↓p53
PP2A
Cdc25c
G2/M Arrest & Apoptosis

↑TRAF6
 ↑Fli-1
↑IL-6

↑TIRAP

↑p53

Mild Neutropenia

Anemia
Secondary Resistance to Lenalidomide is Associated with *PP2Acα* Over-Expression

*PP2Acα* over-expression abolishes LEN stabilization of MDM2 & p53 degradation

*PP2Acα* is up-regulated upon lenalidomide tx failure accompanied by p53 activation

Magnitude of *PP2Acα* suppression at response is directly associated with TI duration (P=0.021; HR =0.95).

- Median duration of TI not reached (1507+d) in pts with *PP2Acα* suppression vs. 679d in pts without (P=0.006, log rank).

- No correlation between change in Cdc25C or p53 and TI duration.
Median follow-up: 48 months (interquartile range 24-66).
Progression = blasts >10% or complex karyotype.

Probability of Disease Progression in Low/Int-1 del(5q) MDS by *TP53* mutation

**Karolinska Hospital [n=38]**

**AML-Free Survival by **

**TP53 Mutation**

**Progression-Free Survival by **

**TP53 Mutation**

### Response to Lenalidomide in Lower Risk Non-Del5q MDS

<table>
<thead>
<tr>
<th>Feature</th>
<th>Del(5q) (MDS-003)</th>
<th>Non-Del(5q) (MDS-002)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Patients</td>
<td>148</td>
<td>214</td>
</tr>
<tr>
<td>HI-E Major</td>
<td>99 (67%)</td>
<td>56 (26%)</td>
</tr>
<tr>
<td>HI-E Major+minor</td>
<td>112 (76%)</td>
<td>92 (43%)</td>
</tr>
<tr>
<td>Cytogenetic response</td>
<td>81/115 (71%)</td>
<td>9/47 (19%)</td>
</tr>
</tbody>
</table>
Gene Expression Signature for LEN Response

\[ \downarrow \text{STAT5 \& GATA-1 Gene Target Expression} \quad [N=16 \text{ Non-del5q}] \]

LEN Increases Erythroid CFC & Expression of Response Cassette Genes in CD34+ Cells

CD34+ cells were cultured in the presence 1 nM lenalidomide or an untreated control for 24 h before microarray analysis.


RPS14 haplodeficiency triggers ribosomal stress & p53 activation in del(5q) MDS erythroid precursors

Lenalidomide promotes G1 escape by stabilizing MDM2 & causing p53 degradation

Reduced expression of \textit{RPS14} was detected in 83 of 156 (53.2\%) non-del5q patients

Low expressors had higher platelet counts (p=0.012), higher erythroid apoptotic index (r=-0.54, p=0.013) & longer OS

Low RPS14 expression was associated with erythroid response to lenalidomide: 71.4\% (5/7) vs. 0/5 normal RPS14.
CD28 - an Immunocompetence Biomarker for Response to Lenalidomide

- CD28 is a member of the B7 family of T-cell co-stimulatory molecules that binds CD80/CD86 (B7-1/B7-2) to activate the CD3 TCR complex.
- CD28 expression is up-regulated by & is required for lenalidomide induced T-cell co-stimulation (p<0.001).
- Pre-treatment percentage of CD28+ directly correlated with hematologic response in non-del5q MDS, whereas non-responders had greater CD28- CD4+ (p=0.02) and CD8+ (p=0.03) T-cells within the total T-cell compartment vs. responders.

McDaniel JM, et al. ASH 2011, #1117a.
Immunosuppressive Therapy (IST)

Clinical Co-variates
- age, duration, HLA-DR15

Predictive Biomarkers
- immunologic profile
- LGL expansion
- STAT3 gene mutation
Lower CD4/CD8 Ratio in IST Responsive Patients

Zou et al. Leukemia 2009.

eATG-treated patients [n=20]
Homeostatic proliferation

- Distinct from antigen-driven proliferation
- Mediated by IL-2Rβγ common cytokines IL-7, IL-15, and IL-21
- 0.2-0.3% of naïve cells and 3% of memory cells
- Self-reactive and antigen-specific T cells have the same potential for proliferation under homeostatic conditions

Zou et al. Leukemia 2009.
Hematologic Response to Equine (e)ATG
Relation to Change in Homeostatic Proliferation

Zou et al. Leukemia 2009.
LGL TCR-Vβ Expansion in MDS with Terminal Effector Memory Phenotype

Healthy Control

MDS Patient

LGL Leukemia
LGL Associated MDS is Methotrexate Responsive

Transfusion-Dependent RCMD

RBC Transfusions

Methotrexate
Azanucleosides

Clinical Co-variates
- GFM (response/OS)

Predictive Biomarkers
- Somatic gene mutations
- Fas RFI in CD34+ cells
- Gene methylation profile
Prognostic Factors for Response & OS in Int-2/High Risk MDS Patients Treated With Azacitidine

GFM ATU Compassionate Study

n=282

OS Prognostic Score

<table>
<thead>
<tr>
<th>Variable</th>
<th>Score</th>
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<tbody>
<tr>
<td>PS &gt; 2</td>
<td>1</td>
</tr>
<tr>
<td>Circulating blasts</td>
<td>1</td>
</tr>
<tr>
<td>RBC-TD &gt;4U/8wks</td>
<td>1</td>
</tr>
<tr>
<td>Interm. karyotype</td>
<td>1</td>
</tr>
<tr>
<td>High risk karyotype</td>
<td>2</td>
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</table>

Azacitidine Response Score

<table>
<thead>
<tr>
<th>Variable</th>
<th>% RR (Y/N)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior LoDAC</td>
<td>24/46</td>
<td>0.009</td>
</tr>
<tr>
<td>NI karotype</td>
<td>51/39</td>
<td>0.003</td>
</tr>
<tr>
<td>Marrow blasts &gt;15%</td>
<td>35/50</td>
<td>0.004</td>
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Response Duration

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percentage</th>
<th>95% CI</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex karyotype</td>
<td>4.6 mos vs. 10.3</td>
<td>0.0003</td>
<td></td>
</tr>
</tbody>
</table>

*multivariate analysis

Itzykson R. et. al. BLOOD 2011; 117:403.
Epigenetic Program Mutations In MDS

DNMT3a
EVI-1
TET2
IDH1/2
EZH2
ASXL1
MLL
JAK2
# Response to Azanucleoside Treatment by Mutation Status


<table>
<thead>
<tr>
<th>Institution</th>
<th>No. Pts.</th>
<th>Gene(s)</th>
<th>Overall Mutant</th>
<th>Response WT (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFM</td>
<td>86</td>
<td>TET2</td>
<td>11/13 (85)*</td>
<td>34/73 (47)</td>
<td>0.01</td>
</tr>
<tr>
<td>Taussig (#3461a)</td>
<td>88</td>
<td>DNMT3A, TET2, IDH1/2</td>
<td>12/28 (64)</td>
<td>21/60 (35)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DNMT3A</td>
<td>6/7 (86)</td>
<td>33/81 (41)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TET2</td>
<td>12/18 (67)</td>
<td>27/70 (39)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ASXL1</td>
<td>11/13 (85)</td>
<td>14/37 (38)</td>
<td>0.003</td>
</tr>
<tr>
<td>OSU^ (#944a)</td>
<td>46</td>
<td>DNMT3A</td>
<td>6/8 (75)</td>
<td>13/38 (34)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

^AML pts treated with decitabine.

*includes mCR in ORR.
## Azanucleoside Response Mutation Analysis by Whole Exome Sequencing

Husseinzadeh HD, et. al. ASH 2012; 1698a.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>N=168, Aza/Decitabine treated</td>
</tr>
<tr>
<td>IWG 2006 Response</td>
<td>ORR=48% (CR 28%; HI 20%)</td>
</tr>
<tr>
<td>Non- Response mutations*</td>
<td>*TET2, SF3B1, PRPF8, LUCL71 (52%, p=.028)</td>
</tr>
<tr>
<td>Response mutations</td>
<td>*RUNX1, CBL, SRSF2, SETBP1, or PPFIA2 (86%, p=.0001)</td>
</tr>
</tbody>
</table>

*prioritized by recursive partitioning algorithm.
CD34+ Fas RFI* vs. Aza Response

CD34+ Fas RFI & Aza Response (n=66 HR-MDS/sAML)

*RFI denotes Ratio Fluorescence Intensity.

Emerging Actionable Biomarkers Guiding MDS Treatment

**CHARACTERIZATION**

MDS

- **Biomarker**
  - CD34 Fas RFI $\geq 1.8$, RUNX1, CBL, PPFIA2 mu
  - ESA Responsive [CD34 non-aberrant]
  - ESA Non-Responsive [CD34 aberrant; $>60\%$ CD28/CD3+ cells]
  - Del(5q) [PP2A-Cα-Lo, TP53 wt]
  - Auto-Immune [CD4:CD8 $<0.2$; LGL $>300/\mu l$ or STAT3 mu]

- **Treatment**
  - Azanucleoside
  - ESA
  - Azanucleoside
  - Lenalidomide
  - Lenalidomide
  - ATG+CsA
  - MTX, STAT3i
Thank you