Myelodysplastic Syndromes: Is It Time to Incorporate NGS and What Is New in Terms of Therapy?

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COI

• Relevant financial relationships in the past twelve months by presenter or spouse/partner.

Consultant: Incyte, Celgene
Speakers Bureau: Novartis

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Myelodysplastic Syndromes (MDS)

- A group of malignant hematopoietic neoplasms characterized by:
  - Bone marrow failure with resultant cytopenia and related complications
  - Evidence of clonality by cytogenetic abnormalities or somatic gene mutations.
  - Dysplastic cytologic morphology is the hallmark of the disease
  - Tendency to progress to AML
- Overall incidence 3.7-4.8/100,000
  - In US (true estimates ≈37,000-48,000)
- Median age: 70 yrs; incidence: 34-47/100,000 >75 yrs

AML = acute myeloid leukemia.
Minimal Diagnostic Criteria

**Cytopenia(s):**
- Hb <11 g/dL, or
- ANC <1500/μL, or
- Platelets <100 x 10⁹/L

**MDS “decisive” criteria:**
- >10% dysplastic cells in 1 or more lineages, or
- 5-19% blasts, or
- Abnormal karyotype typical for MDS, or
- Evidence of clonality

**EXCLUDE other causes of cytopenias and morphological changes:**
- Vitamin B12/folate deficiency
- HIV or other viral infection
- Copper deficiency
- Alcohol abuse
- Medications (esp. methotrexate, azathioprine, recent chemotherapy)
- Autoimmune conditions (ITP, Felty syndrome, SLE etc.)
- Congenital syndromes (Fanconi anemia etc.)
- Other hematological disorders (aplastic anemia, LGL disorders, MPN etc.)
MDS pathogenesis model

1a) Genetic/epigenetic changes owing to: aging, oxidative stress, genotoxic stress, etc
1b) Damage by inflammation derived from autoimmune disorders or unresolved inflammation

1) Origin of the MDS clone

2) Secretion of inflammatory cytokines and chemokines

3a) Loss of quiescence
3b) Expression of death receptors
3c) Recruitment of immune cells

3) LOW-RISK MDS

4) Apoptosis

5a) Reduced number of differentiated cells
5b) Impaired/skewed differentiation

5) HPC → Blast

6a) Reprogramming of the BM niche
6b) Recruitment of suppressor cells

6) MSC → TGF-β, IL-10

7) Normal effects of aging on hematopoiesis
- Increased HSC proliferation rates
- Loss of progenitor functionality and long/short-term colony formation ability
- Myeloid skewing
- Decreased lymphopoiesis
- Decreased output of mature myeloid cells
- Large environmental contribution

7a) Accumulation of additional genetic/epigenetic aberrations
7b) Switch in gene expression

7) HIGH-RISK MDS

8) Abnormal proliferation

9) Recruitment of immunomodulatory cells
# Genetic Abnormalities in MDS

<table>
<thead>
<tr>
<th>Translocations/Rearrangements</th>
<th>Uniparental Disomy/Microdeletions</th>
<th>Copy Number Change</th>
<th>Point Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare in MDS</td>
<td>Rare – often at sites of point mutations</td>
<td>About 50% of cases</td>
<td>Most common</td>
</tr>
<tr>
<td>t(6;9)</td>
<td>4q - TET2</td>
<td>del(5q)</td>
<td>Likely in all cases</td>
</tr>
<tr>
<td>i(17q)</td>
<td>7q - EZH2</td>
<td>-7/del(7q)</td>
<td>~80% of cases have mutations in a known gene</td>
</tr>
<tr>
<td>t(1;7)</td>
<td>11q - CBL</td>
<td>del(20q)</td>
<td></td>
</tr>
<tr>
<td>t(3;?)</td>
<td>17p - TP53</td>
<td>del(17p)</td>
<td></td>
</tr>
<tr>
<td>t(11;?)</td>
<td></td>
<td>del(11q)</td>
<td></td>
</tr>
<tr>
<td>inv(3)</td>
<td></td>
<td>+8</td>
<td></td>
</tr>
<tr>
<td>idic(X)(q13)</td>
<td></td>
<td>-Y</td>
<td></td>
</tr>
</tbody>
</table>

**Karyotype**

**Array CGH SNP Array**

**Karyotype/FISH**

**Genotyping Sequencing**

**Observed Frequency in MDS**

What is a mutation?

- Germline or somatic
- Synonymous versus non-synonymous
- SNV/polymorphism vs pathological
- Driver vs passenger

Type of mutations
- Missense
- Non-sense
- Insertion
- Deletion
- Frame shift
- duplication
Genes Recurrently Mutated in MDS

Tyrosine Kinase Pathway
- JAK2
- KRAS
- BRAF
- NRAS
- RTKs
- PTPN11
- CBL

Transcription Factors
- RUNX1
- ETV6
- GATA2
- WT1
- PHF6

Epigenetic Dysregulation
- IDH 1 & 2
- DNMT3A
- EZH2
- TET2
- UTX
- ASXL1
- ATRX
- SETBP1

Others
- TP53
- NPM1
- NOTCH?
- MAML?
- ZSWIM4?
- UMODL1?
- BCOR

Splicing Factors
- SF3B1
- U2AF1
- SRSF2
- SF1
- U2AF2
- PRPF8
- SF3A1

Courtesy of Bejar R.
Recurrent Genetic Mutations in MDS

~89% of patients had a mutation by NGS

MDS DIAGNOSIS
Myelodysplastic Syndromes (MDS)

- Aplastic Anemia
- Acute Myeloid Leukemia (AML)
- Paroxysmal Nocturnal Hematuria
- T-LGL
- Fanconi Anemia
- Myeloproliferative Neoplasms

Vitamin Deficiency
- Copper Deficiency
- Iron Deficiency

HIV
EBV
Hepatitis

Autoimmune Disorders
Hepatic or Renal Disease
Alcohol Abuse
Vitamin Deficiency
Copper Deficiency
Iron Deficiency

HIV
EBV
Hepatitis

Non-Clonal

Clonal

Diagnostic Overlap
Mutations in MDS

- MDS-associated gene mutations can establish the presence of clonal hematopoiesis, which can help exclude benign causes of cytopenias in cases with non-diagnostic morphology.
- Mutations may not establish a diagnosis of MDS in the absence of clinical diagnostic criteria.
- In the appropriate context (e.g., cytopenias present without AML defining criteria, no evidence of other malignancy), they could aid in the determination of diagnosis.
NGS Myeloid Panels can efficiently identify clonality

97% Specificity for CMML

Meggendorfer et al Blood 2013

Malcovati L Blood 2014
Mutations in certain genes may favor related myeloid neoplasms or possible mimics of MDS

MDS CLASSIFICATION
SOMATIC MUTATIONS INDICATIVE OF CLONAL HEMATOPOIESIS ARE PRESENT IN A LARGE FRACTION OF CYTOPENIC PATIENTS WHO LACK DIAGNOSTIC EVIDENCE OF MDS

Jeff M Hall¹, Jenan Al Hafidh¹, Emily Balmert¹, Bashar Dabbas¹, Christine Vaupel¹, Carlos El Hader¹, Matthew McGinniss¹, Shareef Nahas¹, Julie Kines¹, Sue Beruti¹, and Rafael Bejar²
¹Genoptix, Inc., a Novartis company; ²UC San Diego, San Diego, CA

Hall J, et al. ASH 2014, Abstract 3272
ANALYSIS

Age-related mutations associated with clonal hematopoietic expansion and malignancies


Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Giulio Genovese, Ph.D., Anna K. Köhler, Ph.D., Robert E. Handsaker, B.S., Johan Lindberg, Ph.D., Samuel A. Rose, B.S., Samuel F. Bakhoun, M.D., Ph.D., Kimberly Chambert, M.S., Eran Mick, B.S., Benjamin M. Neale, Ph.D., Menachem Fromer, Ph.D., Shaun M. Purcell, Ph.D., Oscar Svanstossen, M.S., Mikael Landén, Ph.D., Martin Höglund, M.D., Ph.D., Sören Lehmann, M.D., Ph.D., Stacey B. Gabriel, Ph.D., Jennifer L. Moran, Ph.D., Eric S. Lander, Ph.D., Patrick F. Sullivan, M.D., Pamela Sklar, M.D., Ph.D., Henrik Grönborg, M.D., Ph.D., Christina M. Hultman, Ph.D., and Steven A. McCarroll, Ph.D.

Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes

Siddhartha Jaiswal, M.D., Ph.D., Pierre Fontanillas, Ph.D., Jason Flannick, Ph.D., Alisa Manning, Ph.D., Peter V. Grauman, B.A., Brenton G. Mar, M.D., Ph.D., R. Coleman Lindsey, M.D., Ph.D., Craig H. Mermel, M.D., Ph.D., Noel Burkitt, B.S., Alejandro Chavez, M.D., Ph.D., John M. Higgins, M.D., Vladislav Moltchanov, Ph.D., Frank C. Kuo, M.D., Ph.D., Michael J. Kluk, M.D., Ph.D., Brian Henderson, M.D., Leena Kinnunen M.Sc., Heikki A. Koistinen, M.D., Ph.D., Claes Lidenwall, Ph.D., Gad Getz, Ph.D., Adolfo Correa, M.D., Ph.D., Benjamin F. Banahan, Ph.D., Stacey Gabriel, Ph.D., Sekar Kathiresan, M.D., Heather M. Stringham, Ph.D., Mark I. McCarthy, M.D., Michael Boehnke, Ph.D., Jaakko Tuomilehto, M.D., Ph.D., Christopher Haiman, Sc.D., Leif Groop, M.D., Ph.D., Gil Atzmon, Ph.D., James G. Wilson, M.D., Donna Neuberg, Sc.D., David Altshuler, M.D., Ph.D., and Benjamin L. Ebert, M.D., Ph.D.
Risk of acquiring mutations increases with age
Allele frequency is rarely over 20%
Acquisition of somatic clones is not benign.
**How do we classify these patients?**

<table>
<thead>
<tr>
<th></th>
<th>Traditional ICUS</th>
<th>MDS by WHO 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHIP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Non-clonal ICUS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CCUS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LR-MDS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HR-MDS</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>CHIP</th>
<th>Non-clonal ICUS</th>
<th>CCUS</th>
<th>LR-MDS</th>
<th>HR-MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonality</td>
<td>+</td>
<td>–</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>–/+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Cytopenias</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>BM Blast %</td>
<td>&lt; 5%</td>
<td>&lt; 5%</td>
<td>&lt; 5%</td>
<td>&lt; 5%</td>
<td>5-19%</td>
</tr>
<tr>
<td>Overall Risk</td>
<td>Very Low</td>
<td>Very Low</td>
<td>Low (?)</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

Are these two the same? _Does morphologic dysplasia matter?_

CCUS = clonal cytopenias of undetermined significance; ICUS = idiopathic cytopenias of undetermined significance; CHIP = clonal hematopoiesis of indeterminate potential; LR = lower risk, HR = higher risk
New Proposed WHO classification

Table 1. Proposed nomenclature changes for MDS categories

<table>
<thead>
<tr>
<th>Proposed Category</th>
<th>Current or prior WHO category</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS with single lineage dysplasia (MDS-SLD)</td>
<td>Refractory cytopenia with unilineage dysplasia (RCUD; encompassing refractory anemia, refractory thrombocytopenia, and refractory neutropenia)</td>
</tr>
<tr>
<td>MDS with multilineage dysplasia (MDS-MLD)</td>
<td>Refractory cytopenia with multilineage dysplasia (RCMD)</td>
</tr>
<tr>
<td>MDS with single lineage dysplasia and ring sideroblasts (MD-RSSL)</td>
<td>Refractory anemia with ring sideroblasts (RARS)</td>
</tr>
<tr>
<td>MDS with multilineage dysplasia and ring sideroblasts (MDS-RSMLD)</td>
<td>Refractory cytopenia with multilineage dysplasia and ring sideroblasts* (RCMD-RS)</td>
</tr>
<tr>
<td>MDS with excess blasts-1 (MDS-EB1)</td>
<td>Refractory anemia with excess blasts-1 (RAEB1)</td>
</tr>
<tr>
<td>MDS with excess blasts-2 (MDS-EB2)</td>
<td>Refractory anemia with excess blasts-2 (RAEB2)</td>
</tr>
</tbody>
</table>

* RCMD-RS was an entity in the 2001 WHO Classification, but was merged with RCMD in the 2008 Classification.

- Eliminate non-erythroid blast count if erythroid cells > 50%
- RS > 5% and SF3B1 mutation MDS-RS-SLD

ASH 2015 Educational book
MDS RISK STRATIFICATION
Impact of Mutations by IPSS Group

- **TP53**
- **ETV6**
- **ASXL1**
- **EZH2**
- **RUNX1**

Overall Survival vs Years for:
- IPSS Low (n=110)
- IPSS Int1 (n=185)
- IPSS Int2 (n=101)
- IPSS High (n=32)

- **IPSS Low Mut Absent (n=87)**
- **IPSS Low Mut Present (n=23)**
  - $p < 0.001$
- **IPSS Int1 (n=185)**

- **IPSS Int1 Mut Absent (n=128)**
- **IPSS Int1 Mut Present (n=57)**
  - $p < 0.001$
- **IPSS Int2 (n=101)**

- **IPSS Int2 Mut Absent (n=61)**
- **IPSS Int2 Mut Present (n=40)**
  - $p = 0.02$
- **IPSS High (n=32)**

---

**RUNX1**

Impact of Mutations by IPSS Group

- **ETV6**
- **ASXL1**
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- **IPSS Int2 Mut Present (n=40)**
  - $p = 0.02$
- **IPSS High (n=32)**
Somatic Gene Mutations Improve Precision of the IPSS-R

# IWG Molecular analysis

## IPSS-R Risk Groups (vs. Very Low)

<table>
<thead>
<tr>
<th>Group</th>
<th>HR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>1.08</td>
<td>0.542</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1.97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>High</td>
<td>2.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Very High</td>
<td>4.36</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

## Mutated Genes (vs. Unmutated)

<table>
<thead>
<tr>
<th>Gene</th>
<th>HR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>2.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RUNX1</td>
<td>1.51</td>
<td>0.0002</td>
</tr>
<tr>
<td>EZH2</td>
<td>1.58</td>
<td>0.0006</td>
</tr>
<tr>
<td>NRAS</td>
<td>1.44</td>
<td>0.019</td>
</tr>
<tr>
<td>SF3B1</td>
<td>0.82</td>
<td>0.041</td>
</tr>
<tr>
<td>CBL</td>
<td>1.35</td>
<td>0.056</td>
</tr>
<tr>
<td>U2AF1</td>
<td>1.22</td>
<td>0.069</td>
</tr>
<tr>
<td>ASXL1</td>
<td>1.17</td>
<td>0.090</td>
</tr>
<tr>
<td>TET2</td>
<td>0.88</td>
<td>0.104</td>
</tr>
<tr>
<td>IDH2</td>
<td>1.31</td>
<td>0.111</td>
</tr>
<tr>
<td>KRAS</td>
<td>1.22</td>
<td>0.362</td>
</tr>
<tr>
<td>NPM1</td>
<td>1.2</td>
<td>0.546</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
</tr>
<tr>
<td><img src="image-url" alt="Graph" /></td>
</tr>
</tbody>
</table>
The adverse prognostic impact of the complex karyotype is entirely driven by its frequent association with mutations of **TP53**
Clonal burden of *TP53* Mutation Predicts for Inferior Survival

**Sallman et al., Leukemia journal**
Number of Mutations and Prognosis

THERAPEUTIC IMPLICATIONS
Gene (n) | VAF ≥ 0.1 | Unadjusted OR (95% CI) | p-value | Adjusted OR (95% CI) | p-value
---|---|---|---|---|---
*TET2* (50) | | 1.99 (1.05, 3.80) | 0.036 | 1.98 (1.02, 3.85) | 0.044
*ASXL1* wt (23) | 3.65 (1.38, 9.67) | 0.009 | 3.64 (1.35, 9.79) | 0.011

**Response by Variant Abundance**

![Variant Allele Frequencies by Mutated Gene](image-url)
Can we tailor therapy accordingly?

A. HMA Overall Response Rate

B. Duration of HMA Treatment

C. DNA Methylation Mutations and HMA

Sallman, et al. ASH 2016
MDS with Founder TP53 Mutations are Highly Responsive to Decitabine

- **Welch JS, et. al. *NEJM* 2016; 375:2023.**
  - 116 MDS/AML treated with decitabine 20 mg/m²/d x 10d q 28d
  - exome sequencing pretreatment & serially
  - Higher ORR in TP53 mutant vs. Wt (21/21 [100%] vs. 32/78 [41%], \( P <0.001 \))
  - CR/Cri higher in TP53 mutant vs. Wt (13/21 [62%] vs. 26/78 [33%], \( P=0.04 \))

- **Chang CK, et. al. *Brit J Haematol* 2016; Epub.**
  - 109 MDS treated with decitabine 20 mg/m²/d x 5d q 28d
  - exome sequencing pretreatment
  - CR rate higher in TP53 mutant vs. Wt (10/15 [66.7%] vs. 20/94 [21%], \( P =0.001 \))
  - No difference in ORR (TP53 mutant, 11/15 [73%] vs. 63/94 [67%] Wt)
  - Poor OS in TP53_{mu} MDS (median, 14 vs. 39 mos; \( P=0.012 \))
Probability of AML Progression in Low/Int-1 del(5q) MDS by TP53 mutation

Median follow-up: 40 months
Progression = blasts >10% or complex karyotype.

TP53 with median clone size of 11% was detected in 18% of pts.

5 out of 12 patients who progressed to AML had TP53 mutation.

8 out of 10 mutated Tp53 patients received lenalidomide where a trend toward AML progression was noted.

no complete CCR observed among p53 mutated pts.
Somatic Gene Mutations (SGM) as Biomarkers for Response to Immunosuppressive Therapy (IST)

- Independent clinical covariates for response to ATG + CsA include age, HLA-DR15+ & duration of transfusion dependence in the NIH model
- 66 IPSS Low/Int risk MDS pts treated with ATG + CsA with 42% (n=28) ORR
- No SGM in detected 50% of patients.
- Absence of SGM associated with higher IST ORR (70% vs 40%, P=0.16) with a mean response duration of 12 mos in SGM- vs 9 mos in SGM+pts (P=0.09).
- SF3B1 mutation was associated with IST nonresponse (11% SF3B1Mu+ vs 68% WT, p=0.01)
- Rate of AML transformation in pts with non-SF3B1 SGM > SGM-, p=0.023 with reduced OS.

Impact of *TP53* Mutation & Age on AlloHCT

**OS by TP53 Mutation Status & Age**

![Graph showing survival rates by TP53 mutation and age](image)

**OS by TP53 Mutation**

![Graph showing survival rates by TP53 mutation](image)

<table>
<thead>
<tr>
<th>No. at Risk</th>
<th>No TP53 mutation</th>
<th>TP53 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40 yr of age</td>
<td>214 159 133 115 100 78 42 23 13</td>
<td>27 14 7 5 5 4 4 3</td>
</tr>
<tr>
<td>≥40 yr of age</td>
<td>1010 598 396 255 161 105 67 30 19</td>
<td>262 95 59 34 21 15 10 3 2</td>
</tr>
</tbody>
</table>

Would you use lenalidomide or HMAs?

**Lenalidomide**

![Bar chart showing patients (%)](chart)

**Azacitidine**

<table>
<thead>
<tr>
<th>Lineage HI in Evaluable Pts, n/N (%)</th>
<th>5-2-2 (n = 50)</th>
<th>5-2-5 (n = 51)</th>
<th>5d (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythroid Ma</td>
<td>19/43 (44)</td>
<td>19/43 (44)</td>
<td>20/44 (46)</td>
</tr>
<tr>
<td>RBC-TI</td>
<td>12/24 (50)</td>
<td>12/22 (55)</td>
<td>15/25 (64)</td>
</tr>
<tr>
<td>Platelet Ma</td>
<td>12/28 (43)</td>
<td>8/30 (27)</td>
<td>11/22 (50)</td>
</tr>
<tr>
<td>Any HI</td>
<td>22/50 (44)</td>
<td>23/51 (45)</td>
<td>28/50 (56)</td>
</tr>
<tr>
<td>Neutrophil I Ma</td>
<td>4/23 (17)</td>
<td>4/23 (17)</td>
<td>9/24 (38)</td>
</tr>
</tbody>
</table>

Median duration of response 32.9 weeks (95%CI, 20.7–71.1) among RBC-TI ≥ 8 weeks

Phase III Intergroup Study of Lenalidomide + Epoetin Alpha After ESA Failure [ECOG 2905]

- **Eligibility:** Low/Int-1 IPSS, ESA failure or low response profile, Hgb <9.5 g/dL
- **Stratification:** serum EPO (> vs. <500mU/ml), prior ESA (EA vs. DA vs. None)
- **Epoetin alfa 60,000 units SC weekly**
- **Primary Endpoint (EP):** MER
- **Secondary EP:** Time to MER, MER duration, LEN cross-over response, candidate response biomarkers (CD45 isoform profile)

Randomize [n=250]

1. **Lenalidomide**
   - 10 mg/day x 21d

2. **Lenalidomide + Epoetin α**

- **NR Cross-over LEN Arm only**
- **IWG MER Continue**

List A, et. al. ASH 2016; #223a.
### Response Analysis

<table>
<thead>
<tr>
<th>Response &amp; Cohort</th>
<th>Arm A (%) LEN</th>
<th>Arm B (%) LEN+Epo</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ITT Analysis [n=163]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MER</td>
<td>9 (11.1)</td>
<td>21 (25.6)</td>
<td>P=0.025</td>
</tr>
<tr>
<td>Minor ER</td>
<td>15 (18.5)</td>
<td>13 (15.9)</td>
<td>P=0.68</td>
</tr>
<tr>
<td>Overall ER</td>
<td>24 (29.6)</td>
<td>34 (41.5)</td>
<td>P=0.14</td>
</tr>
<tr>
<td>Arm A Crossover MER</td>
<td>N=34</td>
<td>7 (21%)</td>
<td></td>
</tr>
<tr>
<td><strong>Week 16 Evaluable [n=117]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MER</td>
<td>8 (14.3)</td>
<td>20 (32.8)</td>
<td>P=0.029</td>
</tr>
<tr>
<td>Minor ER</td>
<td>13 (23.1)</td>
<td>13 (21.3)</td>
<td>P=0.83</td>
</tr>
<tr>
<td>Overall ER</td>
<td>21 (37.5)</td>
<td>33 (54.1)</td>
<td>P=0.09</td>
</tr>
</tbody>
</table>

List A, et. al. ASH 2016; #223a.
Duration of MER

Log Rank Test p=0.37

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TOTAL</th>
<th>FAIL</th>
<th>CNSR</th>
<th>MEDIAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEN</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>13.0</td>
</tr>
<tr>
<td>LEN+EPO</td>
<td>21</td>
<td>9</td>
<td>12</td>
<td>25.4</td>
</tr>
</tbody>
</table>

List A, et. al. ASH 2016; #223a.
Low-Dose HMAs in LR MDS: Response Rates

<table>
<thead>
<tr>
<th>Response,* %</th>
<th>Decitabine (n = 70)</th>
<th>Azacitidine (n = 39)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR</td>
<td>70</td>
<td>49</td>
<td>.03</td>
</tr>
<tr>
<td>CR</td>
<td>37</td>
<td>36</td>
<td>.90</td>
</tr>
<tr>
<td>mCR</td>
<td>9</td>
<td>5</td>
<td>NR</td>
</tr>
<tr>
<td>HI</td>
<td>24</td>
<td>8</td>
<td>NR</td>
</tr>
<tr>
<td>SD</td>
<td>26</td>
<td>44</td>
<td>NR</td>
</tr>
<tr>
<td>PD</td>
<td>4</td>
<td>8</td>
<td>NR</td>
</tr>
<tr>
<td>CCyR</td>
<td>25</td>
<td>6</td>
<td>.12</td>
</tr>
<tr>
<td>PCyR</td>
<td>36</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td><strong>CCyR + PCyR</strong></td>
<td>61</td>
<td>25</td>
<td>.02</td>
</tr>
</tbody>
</table>

*Median treatment cycles (range): 9 (1-41).

- Strongest predictors of response included BM blasts ≥ 5%, MDS/MPN or CMML diagnosis, high MDA LR MDS score, and IPSS intermediate-1 risk

Type of IST used (N=217) and responses

Prednisone only: 150 (41%) patients -> Excluded from analysis

ATG + CysA + Etanercept 8%
ATG + CysA 21%
ATG + Tacrolimus 4%
Tacrolimus 4%
CysA 13%
ATG + Prednisor 43%
Others 7%

Rabbit 62%
Horse 38%

Response | % | 95%CI
---|---|---
CR | 11.2 | 6.5-18.4
PR | 5.6 | 2.5-11.6
HI | 32.0 | 24.1-41.0
SD | 39.2 | 30.7-48.4
PD | 12.0 | 7.1-19.3
ORR | 48.8 | 39.8-57.9

Stahl M et al. ASH 2017 [Abstract # 422]
Transfusion independence (TI)

- **TI achieved**: 30%
- **TI not achieved**: 70%

**Time to TI (weeks)**
- 9.4 weeks (95% CI 6.3-12.6)

**Duration of TI (months)**
- 19.9 months (95% CI 12.8-27)

Stahl M et al. ASH 2017 [Abstract # 422]
Excess Smad2/3 Signaling Suppresses Late-Stage RBC Maturation in MDS

TGF-β ligands (e.g. GDF15, GDF11, BMP6, activin A) negatively regulate late erythropoiesis

Bone marrow microenvironment

Luspatercept releases maturation block

• Mobilizes cells from precursor pools into blood
• Effect relies on continuous formation of late-stage precursors from earlier progenitors
ACE-011 (Sotatercept) and ACE-536 (Luspatercept)

Novel Ligand Traps for TGFβ Superfamily Ligands

<table>
<thead>
<tr>
<th></th>
<th>ACE-011 (Sotatercept)</th>
<th>ACE-536 (Luspatercept)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with ligand</td>
<td>Extracellular</td>
<td>Modified Extracellular</td>
</tr>
<tr>
<td>trap activity</td>
<td>Domain of ActRIIA</td>
<td>ActRIIB Domain</td>
</tr>
<tr>
<td>Receptor ligand</td>
<td>GDF11, Activin-A</td>
<td>GDF11, GDF8, Activin-B,</td>
</tr>
<tr>
<td>interaction</td>
<td></td>
<td>BMP6, BMP10</td>
</tr>
<tr>
<td>Heme effect</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bone effect</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

Luspatercept PACE-MDS Phase 2 Clinical Trials Overview

A Phase 2, multicenter, open-label, 3-month dose-escalation study in adults with lower-risk MDS followed by a 5-year extension study

### Base Study (N=106)
- 3 months
- NCT01749514

### Extension Study (N=70)
- 5 years (ongoing)
- NCT02268383

<table>
<thead>
<tr>
<th>Patient Population</th>
<th>Efficacy Endpoints</th>
</tr>
</thead>
</table>
| Multiple cohorts enrolling low/intermediate-1 risk (IPSS) MDS patients including:  
  • Non-transfusion dependent and transfusion dependent patients  
  • ESA-naïve and ESA-experienced patients  
  • Patients with a range of baseline EPO levels  
  • RS+ and non-RS patients | **IWG (2006) HI-E:**  
  • Hb increase ≥ 1.5 g/dL for all values over 8 weeks for patients with < 4 units/8 wk and Hb < 10 g/dL  
  • ≥ 4 RBC unit decrease over 8 weeks for patients with ≥ 4 units/8 wk |

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Other Efficacy Endpoints</th>
</tr>
</thead>
</table>
| Luspatercept 0.125 – 1.75 mg/kg (base study); 1.0 – 1.75 mg/kg (extension) SC q3 weeks  
All patients followed up for 2 months post last dose or early discontinuation | RBC-TI: RBC-transfusion independence ≥ 8 weeks (RBC evaluable patients, ≥2U/8 weeks)  
Time to/duration of HI-E response |

Platzbecker U et al. ASH 2017 [Abstract # 2982]
IWG HI-E and RBC-TI Response Rates by ESA, EPO, RS Status

*Patients Treated at Dose Levels ≥ 0.75 mg/kg*

<table>
<thead>
<tr>
<th>Response Rates</th>
<th>IWG-HI-E, n/N (%) (N=99)</th>
<th>RBC-TI, n/N (%) (N=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>52/99 (53%)</td>
<td>29/67 (43%)</td>
</tr>
<tr>
<td>ESA-naïve</td>
<td>28/53 (53%)</td>
<td>17/31 (55%)</td>
</tr>
<tr>
<td>Prior ESA</td>
<td>24/46 (52%)</td>
<td>12/36 (33%)</td>
</tr>
<tr>
<td>Baseline EPO &lt;200 U/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS+</td>
<td>25/39 (64%)</td>
<td>16/24 (67%)</td>
</tr>
<tr>
<td>Non-RS</td>
<td>7/13 (54%)</td>
<td>3/7 (43%)</td>
</tr>
<tr>
<td>Baseline EPO 200-500 U/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS+</td>
<td>10/14 (71%)</td>
<td>4/9 (44%)</td>
</tr>
<tr>
<td>Non-RS</td>
<td>4/8 (50%)</td>
<td>3/5 (60%)</td>
</tr>
<tr>
<td>RS Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS+</td>
<td>40/62 (65%)</td>
<td>22/42 (52%)</td>
</tr>
<tr>
<td>Non-RS</td>
<td>12/35 (34%)</td>
<td>7/23 (30%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0/2 (0%)</td>
<td>0/2 (0%)</td>
</tr>
</tbody>
</table>

Platzbecker U et al. ASH 2017 [Abstract # 2982]
Durability of Response in RBC-TI Responders

Patients Treated at ≥ 0.75 mg/kg with Baseline RBC ≥2U/8 weeks

Platzbecker U et al. ASH 2017 [Abstract # 2982]

Data as of 08 Sept 2017
Eligibility: Non-del(5q) MDS with ≥15% RS, VL-Int. IPSS-R, ≥ 2 U PRBC/8 wks, prior ESA

Key Exclusions: Prior treatment with IMiDs, azanucleosides or IST; ANC < 500, plat<50K

Stratification: RBC transfusion burden (< 6 vs ≥6 U/8wk), IPSS-R VL/Low vs. Int.

Primary end-point: Transfusion Independence x ≥ 8 weeks
Anemia Management Algorithm in LR-MDS

1. **Epo<200mU/ml, <2U RBC/mo**
   - **ESA**
     - Del5q
       - **Lenalidomide**
         - **Non-del5q**
           - Wt, Mu VAF<20%
             - LEN
           - Mu VAF>40%
             - Daco

2. **Del (5q) Iso- or +1**
   - **Lenalidomide**
     - TP53
       - **Epo>200mU/ml, >2U RBC/mo**
         - Age
           - >60
             - SF3B1 Mu+
               - MDS >6 mos
                 - IST
               - Mu-
                 - HLA-DR15+, +8
           - ≤60
             - No SGM or SF3B1 Mu-
               - Non-del5q pathway

3. **<1.5**
   - LEN+Epo

4. **>1.5**
   - AZA 5 day

5. **HSCT**

*SGM, somatic gene mutation*.
HR-MDS
Allogeneic Hematopoietic Stem Cell Transplantation remains the only curative option for MDS patients.

Impact of *TP53* Mutation & Age on AlloHCT

**OS by TP53 Mutation Status & Age**

<table>
<thead>
<tr>
<th>Years since Transplantation</th>
<th>No. at Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>TP53</em> mutation</td>
</tr>
<tr>
<td>0</td>
<td>289</td>
</tr>
<tr>
<td>1</td>
<td>109</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

*P* < 0.001

**OS by TP53 Mutation**

<table>
<thead>
<tr>
<th>Years since Transplantation</th>
<th>No. at Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>TP53</em> mutation</td>
</tr>
<tr>
<td></td>
<td>&lt; 40 yr of age</td>
</tr>
<tr>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

*P* < 0.001

|                              | < 40 yr of age | ≥ 40 yr of age |
| 0                           | 262           | 1010          |
| 1                           | 95            | 598           |
| 2                           | 59            | 396           |
| 3                           | 34            | 255           |
| 4                           | 21            | 161           |
| 5                           | 15            | 105           |
| 6                           | 10            | 67            |
| 7                           | 3             | 30            |
| 8                           | 2             | 19            |

*P* = 0.50

Lindsley RC, et. al. NEJM 2017; 376: 536.
AZA-001 Trial: Azacitididine Significantly Improves Overall Survival

HR: 0.58 (95% CI: 0.43-0.77; log-rank $P = .0001$)

Treatment With AZA OR ICT Prior AHSCT

![Survival Curves]

**OS (Probability)**
- AZA alone: HR = 1
- ICT alone v AZA alone: HR = 1.41 (95% CI: 0.83-2.42; \(P = \text{NS}\))
- ICT-AZA v AZA alone: HR = 3.08 (95% CI: 1.38-6.85; \(P = .006\))

**Event-Free Survival (Probability)**
- AZA alone: HR = 1
- ICT alone v AZA alone: HR = 1.48 (95% CI: 0.90-2.44; \(P = \text{NS}\))
- ICT-AZA v AZA alone: HR = 2.72 (95% CI: 1.38-5.34; \(P = .01\))

**3-Year Relapse (Probability)**
- AZA alone: HR = 1
- ICT alone v AZA alone: HR = 1.35 (95% CI: 0.73-2.46; \(P = \text{NS}\))
- ICT-AZA v AZA alone: HR = 1.87 (95% CI: 0.69-5.06; \(P = \text{NS}\))

**3-Yr NRM Survival (Probability)**
- AZA alone: HR = 1
- ICT alone v AZA alone: HR = 1.23 (95% CI: 0.55-2.76; \(P = \text{NS}\))
- ICT-AZA v AZA alone: HR = 2.50 (95% CI: 0.89-7.05; \(P = .08\))

Azacitidine Maintenance after AHSCT

  - N= 45, majority AML patients (n=37).
  - Excluded active disease, active GVHD, active infections.
  - MTD AZA 32mg/m² SQ for 5 days SQ X 4 cycles.
  - Median EFS 18.2 mo (95% CI: 11.9-NR), One year EFS and OS 58% and 77%

- Mishra et al. Leukemia Research, vol 55, S1, April 2017, Page S48

![Survival Functions Graph](image)
How do HMAs perform in the real-life setting?

- A retrospective analysis of 636 HR-MDS in the MDS Clinical Research Consortium database (6 tertiary centers, no single center accounted for > 39%).
- 69.6% INT-2, 30.4% high IPSS
- Median follow-up 15.7 months (95% CI: 14.6, 16.8).
- Median time from diagnosis to HMA initiation 0.95 months (95%CI: 0.86, 1.06).
- 67.9% azacitidine, 32.1% decitabine.
- Median number of cycles 5.0 (IQR: 3.0, 8.0)
- 72.2% received ≥ 4 cycles.

Median OS from diagnosis 17.0 months (95%CI: 15.8, 18.4).
MDS with Founder TP53 Mutations are Highly Responsive to Decitabine

- 116 MDS/AML treated with decitabine 20 mg/m²/d x 10d q 28d
- exome sequencing pretreatment & serially
- ORR higher in fav/int cytogenetic risk vs. unfavorable (29/43 [67%] vs. 24/71 [34%], \( P < 0.001 \))
- Higher ORR in *TP53* mutant vs. Wt (21/21 [100%] vs. 32/78 [41%], \( P < 0.001 \))
- CR/Cri higher in *TP53* mutant vs. Wt (13/21 [62%] vs. 26/78 [33%], \( P = 0.04 \))
- No relation between response & change in cytosine methylation or subclonal *TP53* mutation

- 109 MDS treated with decitabine 20 mg/m²/d x 5d q 28d
- exome sequencing pretreatment
- CR rate higher in *TP53* mutant vs. Wt (10/15 [66.7%] vs. 20/94 [21%], \( P = 0.001 \))
- No difference in ORR (*TP53* mutant, 11/15 [73%] vs. 63/94 [67%] Wt)
- Poor OS in *TP53* _mu_ MDS (median, 14 vs. 39 mos; \( P = 0.012 \))
Rate of Clearance of Somatic Gene Mutations in Decitabine Treated Patients

Clearance of $TP53_{mu}$ Clones

Change in VAF by Somatic Mutation

Overall Survival by TP53 Mutation Status

OS in $TP53_{mu}$ vs. Wt

OS with HSCT by TP53

APR-246 Restores Wild-type p53 Function

Enasidenib in mIDH2 MDS: Response

<table>
<thead>
<tr>
<th>Response, n/N (%)</th>
<th>MDS Pts (N = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR*</td>
<td>10/17 (59)</td>
</tr>
<tr>
<td>CR†</td>
<td>1/11 (9)</td>
</tr>
<tr>
<td>PR†</td>
<td>1/11 (9)</td>
</tr>
<tr>
<td>mCR†</td>
<td>3/11 (27)</td>
</tr>
<tr>
<td>Any HI</td>
<td></td>
</tr>
<tr>
<td>▪ Erythrocytes</td>
<td>5/17 (29)</td>
</tr>
<tr>
<td>▪ Platelets</td>
<td>3/15 (20)</td>
</tr>
<tr>
<td>▪ Neutrophils</td>
<td>4/12 (33)</td>
</tr>
<tr>
<td>▪ Trilineage</td>
<td>4/10 (40)</td>
</tr>
<tr>
<td>improvement</td>
<td>2/5 (40)</td>
</tr>
<tr>
<td>▪ Bilineage</td>
<td>2/5 (40)</td>
</tr>
<tr>
<td>improvement</td>
<td></td>
</tr>
</tbody>
</table>

- 7 of 13 pts (54%) with prior HMA responded to enasidenib
- Median time to response: 21 days (range: 10-87)

*CR + PR + mCR + HI.
†Investigator-assessed; pts had ≥ 5% BM blasts at BL.

Proposal for HR-MDS Treatment Algorithm

P53 VAF > 40%
- Clinical trial
  - Decitabine
    - P53 clearance
      - AHSCT
      - ? AHSCT at time of HMA failure

P53 VAF < 20%
- TET-2 MT VAF > 10%/ASXL-1 WT
  - YES
  - HMA
    - Cytopenia/Myeloblasts > 10%
      - NO
        - HMA
          - NO
            - Observe prior to AHSCT
            - Post AHSCT HMA
              - Prior response or no prior HMA
              - Loss of CD33 donor chimerism
        - YES
          - HMA prior to AHSCT
  - NO
    - AHSCT candidate
only perfect counts

Moffitt MDS Team