Molecular Genetic Testing to Predict Response to Therapy in MDS

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Overview

• Response Criteria

• Lenalidomide – del(5q) vs. non-del(5q)

• Hypomethylating Agents

• Other Agents

• Bottom Line
MDS Response Criteria
Key response criteria:
- Reduction of blasts
- Improvement of cytopenias
- Cytogenetic response

Clinically meaningful endpoints:
- Prolonged survival
- Reduced transformation
- Transfusion independence
- Improved Quality of Life

Formal response and clinical benefit are not synonymous as they can occur independently.

No Mention of Molecular Response
Somatic Mutations in MDS

When to Predict Response?

MDS therapies often take several cycles to generate a clinical response. Patients may experience side-effects yet never receive clinical benefit. Biomarkers that predict response before treatment OR early in the course of therapy would have great clinical utility.
Lenalidomide
# Lenalidomide in del(5q)

<table>
<thead>
<tr>
<th>Measure</th>
<th>MDS-003</th>
<th>MDS-004</th>
<th>MDS-002</th>
<th>MDS-005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfusion Independence</td>
<td>67%</td>
<td>56%</td>
<td>26%</td>
<td>27%</td>
</tr>
<tr>
<td>Complete Cytogenetic Response</td>
<td>45%</td>
<td>29%</td>
<td>9%</td>
<td>?</td>
</tr>
<tr>
<td>Median Response Duration</td>
<td>&gt; 2 years</td>
<td>83 weeks</td>
<td>41 weeks</td>
<td>33 weeks*</td>
</tr>
</tbody>
</table>

Del(5q) is the strongest genetic predictor of response we have for MDS

Del(5q) has a high rate of *TP53* mutation, especially in complex karyotypes

*TP53* mutations are markers of shorter OS even in complex karyotype patients

*TP53* mutations may be markers of relapse in del(5q) MDS
Lenalidomide in del(5q)

**By TP53 mutation**

- **B**
  - Probability of Freedom From AML
  - Time (years)
  - TP53 not mutated
  - TP53 mutated
  - $P = .045$

- **C**
  - Probability of Freedom From AML
  - Time (years)
  - p53++ < 2%
  - p53++ ≥ 2%
  - $P = .012$

**By 2% p53++***

**B**

- p53++ (%)
- Time (months)
- FISH del(5q)
- TP53 mutation
- Mutated/del(5q)

*Progression

Martin Jädersten et al. JCO 2011;29:1971-1979
Lenalidomide in NON-del(5q)

Phase III Randomized Trial:
- LEN vs. LEN+ESA
- ESA refractory MDS patients without del(5q)
- Response rates: 23% for LEN vs 39% for LEN+ESA

Genetic Predictors of Response:
- $CRBN$ haplotype at rs1672753
- 70% with A/A response rate of 33%
- 30% with G/A or G/G response rate of 57%
- $DNMT3A/SF3B1$ mutant patients may have higher RR

Effect on Clonal Architecture:
- Responders saw a drop in VAF more often than non-responders
  75% of cases vs. 45%

Lenalidomide in NON-del(5q)

Proposed gene expression response signature

Could not be validated in MDS-005

• MDS with del(5q) alone (or +1 abnormality) is a strong predictor of response to lenalidomide and is the basis for selecting this therapy in lower risk patients.

• Mutations do not appear to greatly affect response rate, but TP53 abnormalities might predict shorter duration of response and progression to AML in del(5q) MDS.

• Response rates in non-del(5q) lower risk MDS are more modest and do not appear to be strongly influenced by somatic genotype or published gene expression signatures.

• Better biomarkers are still needed in this population!
Hypomethylating Agents
**TET2 Mutations and HM Response**

N = 86 patients

Sanger sequencing for TET2 only

13 TET2 mutants

ORR 85% vs. 47%

No difference in OS

<table>
<thead>
<tr>
<th></th>
<th>Including SD with HI</th>
<th></th>
<th>Excluding SD with HI</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutated TET2</td>
<td>5.92 (1.05–33.33)</td>
<td>0.044</td>
<td>5.92 (1.43–24.39)</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Cytogenetic risk<sup>a</sup>

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>P</th>
<th></th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate</td>
<td>0.24 (0.06–0.98)</td>
<td>0.048</td>
<td>Poor</td>
<td>0.33 (0.11–0.95)</td>
<td>0.040</td>
</tr>
<tr>
<td>Preceviously therapy</td>
<td>1.56 (0.47–5.15)</td>
<td>0.47</td>
<td></td>
<td>0.47 (0.13–1.65)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

*Itzykson et al. Leukemia. 2011*
N = 92 patients

Sanger sequencing of several genes:

\[ \text{TET2, DNMT3A, IDH1/2, ASXL1, CBL, NRAS, KRAS, SF3B1, TP53} \]

No single mutated gene was predictive of response
213 MDS patients Treated with Hypomethylating Agents

Exclusions: AML before treatment - no DNA sample available - other heme malignancy

Demographics: 73% were male - 48% were 70 years old or more
No single mutated gene was associated with response
ASXL1 and TET2 Mutations

Two Gene Analysis: ASXL1 and TET2

<table>
<thead>
<tr>
<th>Gene Condition</th>
<th>Unadjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TET2 mutant + ASXL1 wt</td>
<td>2.37 (1.00, 5.58)</td>
<td>0.049</td>
</tr>
</tbody>
</table>

Bejar et al., Blood. 2014
### Response by Variant Abundance

#### Variant Allele Frequencies by Mutated Gene

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant Read Fraction</th>
<th>Unadjusted OR (95% CI)</th>
<th>p-value</th>
<th>Adjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TET2</td>
<td>1.99 (1.05, 3.80)</td>
<td>0.036</td>
<td></td>
<td>1.98 (1.02, 3.85)</td>
<td>0.044</td>
</tr>
</tbody>
</table>

#### Literature Reference

Bejar et al., *Blood*. 2014

Krisitn Stevenson and Donna Neuberg
Prognosis in Treated Patients

63 patients with Low or Intermediate-1 risk and adverse prognostic mutations

83 patients with Intermediate-2 or High risk and adverse prognostic mutations

Bejar et al., *Blood*. 2014
Prognosis in Treated Patients

Survival data available for 146 patients – 116 deaths recorded

Multivariable Analysis

- Age Group (< 70 vs. ≥ 70)
- IPSS Risk (Low/Int-1 vs. Int-2/High)
- 30 Frequently Mutated Genes

<table>
<thead>
<tr>
<th>Gene (n)</th>
<th>Unadjusted HR (95% CI)</th>
<th>p-value</th>
<th>Final Model Adjusted HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53 (31)</td>
<td>2.01 (1.29-3.14)</td>
<td>0.007</td>
<td>1.98 (1.26, 3.09)</td>
<td>0.003</td>
</tr>
<tr>
<td>PTPN11 (6)</td>
<td>3.26 (1.41-7.58)</td>
<td>0.056</td>
<td>3.11 (1.34, 7.22)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Kristen Stevenson and Donna Neuberg
Prognosis in Treated Patients

Karolinska and King’s College AZA Treated MDS Cohorts:

- 134 sequential AZA-treated higher risk MDS patients
- **No mutated gene significantly associated with response**
- Trend for \( TET2 \) mutated: 69% vs. 53% ORR, \( p = 0.19 \)
- Trend for \( ASXL1/EZH2 \) mutated: 69% vs. 51% ORR, \( p = 0.09 \)

Survival advantage for \( ASXL1 \) or \( EZH2 \) mutated patients:

![Graphs showing survival advantage for \( ASXL1 \) and \( EZH2 \) mutations](image)

Lessons from CMML?

Cohort of 79 CMML patients treated with DEC or AZA:

- ORR for AZA 61% and DEC 55%
- No mutated gene predicted response
- Somatic mutations carried prognostic information

Lessons from CMML?

Cohort of 40 CMML patients treated with DEC in first line:

- No difference in response by mutated gene.
- A predictive DNA methylation signature was identified.
- Validated in a small, independent cohort with 15/16 correct calls.

Tracking Allele Burden

Cohort of 49 WES and 17 WGS patients with CMML:

No decrease in VAF with treatment and comparable rates of clonal evolution!

Tracking Allele Burden

Cohort of 49 WES and 17 WGS patients with CMML:

Methylation as a Biomarker

Cohort of 49 WES and 17 WGS patients with CMML:

Methylation as a Biomarker

DNA Methylation after 4 cycles of DEC treatment in MDS:

Decitabine and TP53 Mutation

Dynamic Changes in Clonal Clearance with Decitabine Therapy in AML and MDS Patients

- 45 elderly AML and 24 higher risk MDS
- Decitabine 20 mg/m2 x 10 days
- WES at diagnosis, cycle 1-d10, cycle 1-d28, cycle 2-d28, ...
- 6/6 TP53 mutant patients saw clone clearance by cycle 2
- No other gene associated with consisted mutation clearance
- Complete remissions often occurred with persistent mutations
- Responders had decreased methylation at cycle 1-d28

Decitabine and TP53 Mutation

*A Primary Study of the Gene Mutations in Predicting Treatment Response to Decitabine in Patients with MDS*

- 106 MDS patient samples collected prior to treatment
- Decitabine treatment (ORR 67%, CR 26%)
- 30 recurrently mutated genes sequenced
- 10/14 TP53 mutant patients achieved CR
- 5/7 TP53 mutant patients eradicated the TP53 clone
- No mutated genes predicted ORR
- TP53 mutant patients still had inferior overall survival

Bottom Line

• Somatic mutations are not robust biomarkers of response to hypomethylating agents overall.

• Genetic profiles do NOT justify withholding AZA or DEC therapy as some patients in every genetically defined group will respond.

• Mutation tracking may not help predict eventual responses, but might be used to track early relapses in responding patients.
Other Agents
Response Markers to Other Tx

Biomarkers of Ineffective Erythropoiesis Predict Response to Luspatercept in Patients with Low or Intermediate-1 Risk Myelodysplastic Syndromes (MDS): Final Results from the Phase 2 PACE-MDS Study

Subgroup Analyses of a Phase 3 Study in Patients With MDS Failing HMA Treatment: Identification of a Homogeneous Population Who Benefit From Rigosertib Therapy
- monosomy 7 or trisomy 8; trend for TP53, ASXL1, or SRSF2

Besides del(5q) for len, genetic biomarkers are not strong predictors of response to current therapies.

Mutations may better serve as prognostic markers in the context of treatment.

Monitoring mutation burden is challenging as VAFs may not correlate with response.

Newer targeted therapies may have more predictive companion diagnostics.
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