SH2017-0144:
Differential response to FLT3 inhibition (using quizartinib/AC220) in acute myeloid leukemia is affected by baseline molecular genetics and cytogenetics

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Introduction

- Cohort of 19 patients with relapsed/refractory acute myeloid leukemia, all known to be FLT3-ITD mutation positive
- Clinical trial of FLT3 inhibitor, Quizartinib (AC220) monotherapy
- Bone marrow studies pre- and post-therapy (interval 29 days)
- Highlight two prototypical cases illustrating different therapeutic responses to AC220 therapy
Patient A

- A 33-year-old woman with a history of relapsed acute myeloid leukemia (AML) with mutated $NPM1$
- Known to be $FLT3$-ITD positive
- Peripheral Blood:
  - WBC: 13.6 K/$\mu$L; Hgb: 11.9 g/dL; Plt: 96 K/$\mu$L; MCV: 93 fL
    - 53% neutrophils, 6% lymphocytes, 3% monocytes, 0% eosinophils, 0% basophils, **38% blasts**
Pre-Therapy Bone Marrow

Patient A
Pre-Therapy Laboratory Results

Flow Cytometry: Blasts (74%): CD13+ CD33+ CD34+ CD64(s)+ CD117+ HLA-DR+

Genetic Studies

- Cytogenetics: 46,XX[20]
- FLT3-ITD:
  - ITD size: 165 bp
  - Mutant-WT ratio: 0.45
- NGS studies:
  - DNMT3A (VAF: 51%)
  - NPM1 (VAF: 45%)
  - TET2 (VAF: 47%)
Post-Therapy Bone Marrow

Patient A
Post-Therapy Laboratory Results

**Flow Cytometry:** Blasts (<5%): Spectrum of myeloid differentiation

**Genetic Studies**

- **Cytogenetics:** 46,XX[20]
- **FLT3-ITD:**
  - ITD size: 165 bp
  - Mutant-WT ratio: 0.56
- **NGS studies:**
  - Previously detected variants still present
  - No new pathogenic variants

**Peripheral Blood**

- Normal CBC and differential count
Patient B (Contrast Case)

- 70-year-old man with history of relapsed AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1
- Known to be FLT3-ITD positive
- CBC: WBC 12.5 K/µL; Hgb 9.4 g/dL; Plt 50 K/µL
  - 6% neutrophils, 49% lymphocytes, 7% monocytes, 0% eosinophils, 0% basophils, 38% blasts
**Pre-Therapy Laboratory Results**

- **Flow Cytometry**: Blasts (95%): CD33+ CD34+ CD56+ CD117+ HLA-DR+
- **Cytogenetics**: 45,X,-Y,add(2)(p12),t(6;19)(p21;q11.2),t(8;21)(q22;q22),add(16)(q13),add(20)(q?13.1)[20]
- **FLT3-ITD**:
  - ITD size: 33 bp
  - Mutant-WT ratio: 0.43
- **NGS studies**: TET2*
Post-Therapy Laboratory Results

- Flow Cytometry: <5% Blasts
- Cytogenetics: similar
- **FLT3-ITD:**
  - ITD size: 33 bp
  - Mutant-WT ratio: 0.05
- NGS studies: No new variants
- Peripheral blood: pancytopenia, no circulating blasts
Summary of Findings

• **Case A** shows a **differentiation response**:
  – maintenance of marrow cellularity
  – return of trilineage hematopoiesis, with myeloid maturation
  – Sustained peripheral blood neutrophil recovery
  – essentially unchanged M:WT ratio

• In contrast, **Case B** shows a **cytotoxic response**:
  – marked reduction in marrow cellularity
  – marked decrease in hematopoiesis
  – decreased FLT3-ITD M:WT ratio
Morphology vs ITD Mutant Fraction?

• Relatively constant/increased $FLT3$-ITD mutation fraction suggests a drug-induced maturation of the leukemic clone

• $FLT3$-ITD fraction was similar in peripheral blood and bone marrow specimens

• This finding is similar to ATRA therapeutic response in APL with $PML$-$RARA$ and more recently with IDH2 inhibitors in AML[ 2-3]

• Bone marrow biopsies may be reported descriptively to address apparent discordance
FLT3 ITD Biology

- FLT3:FMS-like tyrosine kinase 3
  - Member of class III receptor tyrosine kinase family
- FLT3-ITD mutations leads to terminal block in myeloid differentiation by inhibition of CEBPα by phosphorylation [4-6].
- Pharmacologic inhibition of FLT3 (AC220 and CEP-701) overcomes differentiating block in leukemic cell lines [6].
- Concomitant CEBPα mutations may negate this effect [4].
Why the differential responses?

• AML with *FLT3*-ITD, *DNMT3A*, and *NPM1* mutations: patients tend to be younger and female, with high blast counts and perhaps an overall worse prognosis [7].
  – *DNMT3A* mutation $\rightarrow$ hypomethylation of hematopoietic enhancers
  – *NPM1* mutation $\rightarrow$ cytoplasmic localization of protein, suppression of ARF-p53 pathway [8]
• *RUNX1-RUNX1T1*: transcriptional repressor $\rightarrow$ repress microRNA miR-223 $\rightarrow$ block myeloid maturation [9-10].
Clinical Followup

• **Patient A:**
  – Rapid clearance of leukemic blasts in peripheral blood and bone marrow, with sustained neutrophilic recovery
  – Eventual withdrawal from study for allogeneic HSCT.
  – Post-transplant bone marrow biopsy *FLT3*-ITD negative (>99% donor)
  – At last account, patient is alive and well, with no relapse of AML.

• **Patient B:**
  – Withdrawal from study due to leukemia progression
Summary of Cohort Data

• Our analysis of a cohort of 19 patients shows that baseline genetic studies affect responses

• In particular, \textit{NPM1} and \textit{DNMT3A} mutational status and cytogenetics are useful in predicting the type of response

• Cases that undergo \textit{differentiation responses} tend to be cytogenetically normal and possess \textit{DNMT3A} and/or \textit{NPM1} mutations

• Those that undergo \textit{cytotoxic responses} tend to be cytogenetically abnormal and complex and tend to lack these mutations
## Summary of Cohort Data

<table>
<thead>
<tr>
<th>Subject</th>
<th>FLT3 genotype</th>
<th>Best marrow blast %</th>
<th>Baseline Karyotype</th>
<th>Karyotype at response</th>
<th>Cooperating mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1009-01</td>
<td>FLT3-ITD</td>
<td>10%</td>
<td>46, XY</td>
<td>46, XY</td>
<td>DNMT3a*,NPM1,ASXL1,IDH1</td>
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<td>1009-02</td>
<td>FLT3-ITD</td>
<td>15%</td>
<td>46, XX</td>
<td>46, XX</td>
<td>DNMT3a,NPM1,TET2</td>
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<tr>
<td>1009-14</td>
<td>FLT3-ITD</td>
<td>&lt;5%</td>
<td>46, XX</td>
<td>46, XX</td>
<td>DNMT3a,NPM1</td>
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<td>1009-04</td>
<td>FLT3-ITD</td>
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<td>46, XX</td>
<td>DNMT3a,NPM1,WT1,ATM*</td>
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<td>1009-07</td>
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<td>46, XX</td>
<td>DNMT3a,NPM1</td>
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<tr>
<td>1009-09</td>
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<td>&lt;5%</td>
<td>46, XX</td>
<td>46, XX</td>
<td>DNMT3a,NPM1,TET2</td>
</tr>
<tr>
<td>1009-11</td>
<td><strong>FLT3-ITD</strong></td>
<td><strong>&lt;5%</strong></td>
<td><strong>46, XX</strong></td>
<td><strong>46, XX</strong></td>
<td><strong>DNMT3a,NPM1,TET2</strong></td>
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<tr>
<td>1009-10</td>
<td>FLT3-WT</td>
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<td>46, XY</td>
<td>TET2</td>
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<td>1009-21</td>
<td>FLT3-ITD</td>
<td>10%</td>
<td>46, XY, +11</td>
<td>46, XY, +11</td>
<td>DNMT3a,ASXL1</td>
</tr>
</tbody>
</table>

### Patient A

<table>
<thead>
<tr>
<th>Subject</th>
<th>FLT3 genotype</th>
<th>Best marrow blast %</th>
<th>Baseline Karyotype</th>
<th>Karyotype at response</th>
<th>Cooperating mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1009-18</td>
<td>FLT3-ITD</td>
<td>&lt;5% with multiple additional abnormalities</td>
<td>46, XY, (t(8;21)(q22q22)</td>
<td>46, XY, (t(8;21)(q22q22) with multiple additional abnormalities</td>
<td>TET2*</td>
</tr>
<tr>
<td>1009-16</td>
<td>FLT3-ITD</td>
<td>15%</td>
<td>46, XY, del(5)(q23q33)</td>
<td>46, XY, del(5)(q23q33) with multiple additional abnormalities</td>
<td>ATM*</td>
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<tr>
<td>1009-15</td>
<td>FLT3-ITD</td>
<td>70%</td>
<td>47, XX, +8, del(16)(q13)</td>
<td>47, XX, +8, del(16)(q13)</td>
<td>DNMT3a,NPM1</td>
</tr>
</tbody>
</table>

### Patient B

<table>
<thead>
<tr>
<th>Subject</th>
<th>FLT3 genotype</th>
<th>Best marrow blast %</th>
<th>Baseline Karyotype</th>
<th>Karyotype at response</th>
<th>Cooperating mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1009-06</td>
<td>FLT3-ITD</td>
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<td>FLT3-ITD</td>
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<td>TP53, NOTCH1</td>
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<td>complex</td>
<td>46, XY</td>
<td>No sample available</td>
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<td>FLT3-WT</td>
<td>&lt;5%</td>
<td>complex</td>
<td>46, XY</td>
<td>TP53, JAK2</td>
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<td>no growth</td>
<td>No mutations</td>
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<td>RUNX1</td>
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<tr>
<td>1009-18</td>
<td><strong>FLT3-ITD</strong></td>
<td><strong>&lt;5%</strong></td>
<td><strong>46, XY, (t(8;21)(q22q22) with multiple additional abnormalities</strong></td>
<td><strong>46, XY, (t(8;21)(q22q22) with multiple additional abnormalities</strong></td>
<td><strong>TET2</strong></td>
</tr>
<tr>
<td>1009-16</td>
<td>FLT3-ITD</td>
<td>15%</td>
<td>46, XY, del(5)(q23q33)</td>
<td>46, XY, del(5)(q23q33)</td>
<td>ATM*</td>
</tr>
<tr>
<td>1009-15</td>
<td>FLT3-ITD</td>
<td>70%</td>
<td>47, XX, +8, del(16)(q13)</td>
<td>47, XX, +8, del(16)(q13)</td>
<td>DNMT3a,NPM1</td>
</tr>
</tbody>
</table>
References


Final/Panel Diagnosis

• **Patient A**: Acute myeloid leukemia with mutated \textit{NPM1}

• **Patient B**: Acute myeloid leukemia with t(8;21)(q22;q22:1);\textit{RUNX1-RUNX1T1}