All patients with FLT3 mutant AML should receive midostaurin-based induction therapy

Not so fast!

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Disclosures

• **Consultancy**
  Agios, Amgen, Celator/Jazz, Daiichi Sankyo, ImmunoGen, Incyte, Millennium/Takeda, Novartis, Ono, Pfizer, Seattle Genetics, Sunesis

• **Research Funding**
  Agios, Amgen, Astellas, Celator, Daiichi Sankyo, ImmunoGen, Janssen, Juno, Millennium/Takeda, Seattle Genetics

• **Speakers Bureau:**
  Celgene, Incyte, Novartis

• **Other:**
  Registry Chair, Celgene; DSMB, Glycomimetics, Inc
FLT3 ITD Mutation PCR Assay

Intron

327 bp

> 327 bp

PCR

Electrophoresis

Expanded exon 14
372 bp product

327 bp fragment = wt
Interpreting Allelic Ratios (AR) and Variant Allelic Frequencies (VAF)

• If all cells in sample are leukemic, and one allele is mutated and the other normal, then VAF = 50%.
• If all cells in sample are leukemic, and one allele is mutated and the other deleted (LOH), then VAF = 100%.
• If all cells in sample are leukemic, and both alleles have same mutation, then VAF = 100%.
• If majority of cells in sample are leukemic, then low VAF suggests mutation is only in subset of cells.
Variant allelic frequencies (VAF)

- Gene mutations at low allelic frequencies suggest that these mutations are only present in a subpopulation.
- 5 of the 6 genes with the lowest median VAF (FLT3, KIT, NRAS, KRAS, PTPN11) are involved in growth-factor signaling.
- Therefore, alterations in these pathways are often acquired relatively late during the evolution of the leukemic clone.

Interpretation of AR / VAF: Challenges

- Not all cells in the sample are leukemic
  - A relapse sample may only have 5% blasts

- The leukemic cell population may have arisen from myelodysplastic syndrome.
  - If a mutation is present in a myeloid progenitor cell capable of differentiation, and this clone then progresses to AML, the AR / VAF may be high, even though the number of blasts in the sample is low.

- The size of an insertion affects the efficiency of PCR amplification as well as ability to detect insertion mutation by Next Gen Sequencing.

- Different definitions of high vs low AR / VAF are found in the literature.
Absence of FLT3 Wild Type Allele Predicts Poor Survival in Adult De Novo NK-AML with FLT3 ITD Mutation

<table>
<thead>
<tr>
<th></th>
<th>WT/WT (N = 59)</th>
<th>ITD/WT (N = 15)</th>
<th>ITD/- (N = 8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR (%)</td>
<td>86</td>
<td>79</td>
<td>75</td>
<td>0.70</td>
</tr>
<tr>
<td>mDFS (mo)</td>
<td>52</td>
<td>24</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>12 mo DFS</td>
<td>71%</td>
<td>51%</td>
<td>17%</td>
<td>0.0017</td>
</tr>
<tr>
<td>mOS (mo)</td>
<td>46</td>
<td>46</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>12 mo OS</td>
<td>74%</td>
<td>65%</td>
<td>13%</td>
<td>0.0014</td>
</tr>
</tbody>
</table>

Whitman et al. (CALGB 9621) *Cancer Res.* 2001; 61; 7233
Clinical Implications of FLT3 ITD in Pediatric Acute Myeloid Leukemia

- 988 pediatric de novo AML, CCG 2941 and CCG 2961, 1995-2001
- 630 (64%) available marrow samples
- 77 (12%) had FLT3 ITD, 42 (6.7%) FLT3 ALM, mutually exclusive
- Allelic ratio varied from 0.01 to 7.5 (median 0.53)
- Increasing FLT3 ITD AR associated with worse PFS and OS
- Validated in German/Dutch pediatric cohort (panel B)

Outcome of Young AML Patients with Intermediate Risk Karyotype and FLT3 ITD with NPMc (N=65)

303 de novo AML patients
Less than age 60
Intermediate Risk
31% FLT3 ITD+, 53% NPMc+

Pratcorona et al. (CETLAM, Spain) *Blood* 2013; 121: 2734.
Higher FLT3 ITD allelic ratio (> 0.5) correlated with higher WBC, % BM or PB blasts, and LDH

Rate of CR after induction decreased with increasing FLT3 ITD allelic ratio

*Intermediate-risk karyotype only

Impact of FLT3 ITD Allelic Ratio on Outcome following Allo HSCT


FLT3 ITD:WT ≥0.51

FLT3 ITD:WT <0.51
FLT3 TKD Mutations Have More Favorable Prognosis than FLT3 ITD in Younger AML Patients

Mutational Complementation and Complexity in AML (E1900)

Influence of FLT3 Mutation on Outcomes in APL with ATRA/chemotherapy

No Effect of FLT3 Mutation Status on Outcome of APL with APML4 Protocol

Induction: ATRA, idarubicin x 4 doses, arsenic trioxide day 9-36
Consolidation: ATRA/ATO x 2 cycles
Maintenance: ATRA, MP and MTX x 8 - 90 day cycles

Mutational Profiling of Core Binding Factor AML

Mutations in chromatin modifiers and cohesin are more common in t(8;21) than inv(16)

Variant Allelic Frequencies of TK Gene Mutations Affect Cumulative Risk of Relapse in t(8;21) AML

- **KIT mutation VAF affect t(8;21) prognosis**
  - VAF $\geq$ 35%: 69.4% 5 year CIR
  - VAF < 35%: 30.7% 5 year CIR
  - No KIT mutation: 31.9% 5 year CIR

- **FLT3 TKD VAF affect t(8;21) prognosis**
  - VAF $\geq$ 10%: 58.8% 5 year CIR
  - VAF < 10%: 20.0% 5 year CIR
  - No TKD mutation: 31.5% 5 year CIR

Impact of Other Mutations on Relapse Risk in CBF AML

## Selected FLT3 Inhibitors in Pre-Clinical Studies

### Inhibition of FLT3-ITD Autophosphorylation

<table>
<thead>
<tr>
<th>Agent</th>
<th>IC$_{50}$ (medium)*</th>
<th>IC$_{50}$ (plasma)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lestaurtinib</td>
<td>2 nM</td>
<td>700 nM</td>
</tr>
<tr>
<td>Midostaurin</td>
<td>3 nM</td>
<td>1700 nM</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>3 nM</td>
<td>484 nM</td>
</tr>
<tr>
<td>Quizartinib</td>
<td>1 nM</td>
<td>18 nM</td>
</tr>
</tbody>
</table>

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*Lestaurtinib (CEP-701)*

*Midostaurin (PKC-412)*

*Sorafenib*

*Quizartinib (AC220)*

*Molm-14 cells incubated in RPMI/10% FBS. †Molm-14 cells incubated in plasma.*

AML Blast Reduction (BR*) with Midostaurin**

<table>
<thead>
<tr>
<th></th>
<th>FLT3 Mutant</th>
<th>FLT3 WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>35</td>
<td>57</td>
</tr>
<tr>
<td>Previously treated</td>
<td>22/32 (69%)</td>
<td>13/35 (37%)</td>
</tr>
<tr>
<td>Treatment naïve</td>
<td>3/3 (100%)</td>
<td>11/22 (50%)</td>
</tr>
<tr>
<td>FLT3 ITD</td>
<td>19/26 (73%)</td>
<td>N/A</td>
</tr>
<tr>
<td>FLT3 D835Y</td>
<td>6/9 (67%)</td>
<td>N/A</td>
</tr>
<tr>
<td>AML</td>
<td>24/32 (75%)</td>
<td>22/51 (43%)</td>
</tr>
</tbody>
</table>

* BR = > 50% reduction in BM blast % or absolute peripheral blood blast count
** Randomly assigned to 50 mg or 100 mg twice daily

SORAML: AML, treatment naive, < 60 years

**Induction I**
- DA I
- SORA

**Induction II**
- DA II (HAM)
- SORA

**Consolidation**
- 3 x HiDAC

**Maintenance**
- SORA
- 12 months

Favorable risk (FR): t(8;21), inv(16)
High risk (HR): ≥3 aberrations, monosomy 7 or 5, t(6;9), t(6;11), t(11;19) or insufficient response on day 16 after induction #1 (in this case second induction with HAM)
Intermediate risk (IR): all cytogenetics not FR or HR

SORAML Primary Endpoint:
Event-Free Survival (ITT, SCT censored)

3-year EFS
Placebo vs Sorafenib: 22% vs 40%

Median EFS
Placebo vs Sorafenib: 9 month vs 21 month

p=0.013

Median follow-up 36 months

SORAML: Relapse-Free Survival (ITT, no SCT censoring)

3-year RFS
Placebo vs Sorafenib: 38% vs 56%

Median RFS
Placebo vs Sorafenib: 23 mo vs not reached

Median follow-up 36 months

**FLT3 Plasma Inhibitory Assay and Response to Chemo + Lestaurtinib in FLT3 Mut + Relapsed/Refractory AML**

![Image of western blot analysis]

<table>
<thead>
<tr>
<th>P-FLT3</th>
<th>Number of subjects</th>
<th>Number with CR/CRp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples available</td>
<td>79 (of 111 total)</td>
<td>21 (27%)</td>
</tr>
<tr>
<td>&gt; 85% inhibition at aplasia assessment (A)</td>
<td>46</td>
<td>18 (39%)</td>
</tr>
<tr>
<td>NOT inhibited at aplasia assessment (A)</td>
<td>33</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>Inhibited at both aplasia and outcome assessments (A, O)</td>
<td>21</td>
<td>12 (57%)</td>
</tr>
</tbody>
</table>

ECOG E1900: Overall Survival

All Patients (N = 647)

Induction Treatment
- DNR 45 mg/m²/day CR 57%
- DNR 90 mg/m²/day CR 71%

Favorable and Intermediate Cytogenetics
- N = 178
- Log Rank P = 0.004

Unfavorable Cytogenetics
- N = 180
- Log Rank P = 0.45

All Patients (N = 647)
- N = 327
- N = 330

Log Rank P = 0.003

Benefit of High Dose Daunorubicin during Induction Therapy for Adults < Age 60 Years with FLT3 ITD + AML

<table>
<thead>
<tr>
<th></th>
<th>DNR 90</th>
<th>DNR 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR rate</td>
<td>70%</td>
<td>48%</td>
</tr>
<tr>
<td>Median OS</td>
<td>15.2 mo</td>
<td>10.1 mo</td>
</tr>
<tr>
<td>OS at 4 years</td>
<td>28%</td>
<td>17%</td>
</tr>
</tbody>
</table>

Luskin MR et al. (Update ECOG 1900). *Blood* 2016
No Benefit of Daunorubicin 90 vs 60 mg/m² during Induction Therapy for Younger AML Patients

Burnett AK et al. *Blood* 2015
Higher Daunorubicin Exposure Benefits FLT3 Mutated AML (MRC AML 17)

<table>
<thead>
<tr>
<th>At 3 years</th>
<th>DNR 90</th>
<th>DNR 60</th>
<th>HR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIR</td>
<td>44%</td>
<td>60%</td>
<td>0.58</td>
<td>0.01</td>
</tr>
<tr>
<td>RFS</td>
<td>45%</td>
<td>33%</td>
<td>0.63</td>
<td>0.02</td>
</tr>
<tr>
<td>OS</td>
<td>54%</td>
<td>34%</td>
<td>0.65</td>
<td>0.03</td>
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All patients with FLT3 mutant AML should receive midostaurin-based induction therapy

• All patients?
  • APL, CBF translocations, t(6;9)...
  • 20% of FLT3 Mutated subjects not treated. Why?

• Is mutant FLT3 the target??
  • Does inhibition of WT FLT3 explain the effect of midostaurin?
  • Midostaurin targets multiple tyrosine kinases

• Which induction regimen???
  • Idarubicin
  • Daunorubicin 90 versus 60 mg/m2 for three days

• Just during induction????

• Is the statement too restrictive or not restrictive enough?
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Design of clinical trials of next generation FLT3 inhibitors

- Which induction chemotherapy?
- What will be the experimental arm?
  - Chemo + midostaurin vs Chemo + next generation inhibitor OR
  - Chemo / midostaurin plus next generation inhibitor versus placebo
- Wait for FLT3 assay or begin chemo and add FLT3 inhibitors?