Mutational Profiling to Improve Outcomes in Acute Myeloid Leukemia

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Disclosures

• Consultant: Foundation Medicine

• Advisory Board: Agios
Acute Myeloid Leukemia Still Associated with Poor Overall Survival

Even with intensive induction chemotherapy/transplantation most patients die of their disease → new insights are needed

Issa, Kantarjian et al, Cancer 2008
Two-hit model of AML Pathogenesis

Class I Mutations (FLT3, JAK2, RAS)
- Enhance proliferation and survival
- No effect on differentiation

Class II Mutations (RUNX1, CEBPA)
- Impair differentiation
- No effect on proliferation/survival

MPN

Class II Mutation

AML

MDS

Class I Mutation

Gilliland and Griffin Blood 2002

• But not all patients have mutations in class I and class II genes

• Does not reflect role of novel AML mutations in leukemogenesis
Discovery of novel mutations in myeloid leukemia patients

- Whole genome sequencing has identified novel recurrent disease alleles in AML
  - IDH1 mutations (Mardis et al. NEJM 2009)
  - DNMT3A mutations (Ley et al. NEJM 2010)

- Candidate gene/array based studies have identified novel disease alleles in AML, MDS, MPN
  - ASXL1 (Birnbaum et al. BJM 2009)
  - PHF6 (Van Vlierberge et al. Leukemia 2011)
  - Spliceosome component mutations (Ogawa et al. Nature 2011)

- Biologic and prognostic relevance of these novel disease alleles has not been fully delineated->but some of these mutations are thought to have a role in regulating the epigenetic state of leukemic cells
Barriers to improving molecular prognostication in the clinic

- Many studies have identified additional mutations, expression changes, micro-RNA profiles but few have been adopted into clinical practice

- What are the limitations to bringing these markers into the clinic?
  
  - Sufficient data in homogeneously treated patient cohorts to demonstrate robust relevance of specific biomarkers
  
  - Multivariate analyses showing that new markers add value to existing classification/prognostication
  
  - Clinical-grade assays to test for these markers in the clinic including for mutation, expression, miRNA
  
  - Clear evidence that specific biomarkers should impact therapeutic decisions including transplantation, chemotherapy, targeted therapies
Mutational Profiling of ECOG 1900 Cohort*

We performed mutational profiling of the 18 genes known to be mutated in AML in the E1900 phase III trial cohort
- identify novel genes with prognostic relevance
- integrate mutational data with epigenetic analysis of cohort
- make novel insights about AML biology
- determine if specific genetically defined subsets benefit from high dose induction chemotherapy

*Patel, Gonen, Abdel-Wahab et al. NEJM 2012
# Mutational Profiling in AML

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT3 (ITD, TKD)</td>
<td>37 (30, 7)</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>24</td>
</tr>
<tr>
<td>NPM1</td>
<td>24</td>
</tr>
<tr>
<td>KIT</td>
<td>14</td>
</tr>
<tr>
<td>TET2</td>
<td>10</td>
</tr>
<tr>
<td>WT1</td>
<td>10</td>
</tr>
<tr>
<td>CEBPA</td>
<td>10</td>
</tr>
<tr>
<td>NRAS</td>
<td>10</td>
</tr>
<tr>
<td>IDH2</td>
<td>8</td>
</tr>
<tr>
<td>IDH1</td>
<td>6</td>
</tr>
<tr>
<td>ASXL1</td>
<td>4</td>
</tr>
<tr>
<td>KRAS</td>
<td>2.5</td>
</tr>
<tr>
<td>PHF6</td>
<td>2.5</td>
</tr>
<tr>
<td>RUNX1</td>
<td>5</td>
</tr>
<tr>
<td>PTEN</td>
<td>1.5</td>
</tr>
<tr>
<td>TP53</td>
<td>2</td>
</tr>
<tr>
<td>MLL</td>
<td>10</td>
</tr>
</tbody>
</table>

Patel et al. NEJM 2012
### Revised AML Risk Stratification Based on Integrated Mutational Profiling

<table>
<thead>
<tr>
<th>Cytogenetic Classification</th>
<th>Mutations</th>
<th>Overall Risk Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>Any</td>
<td>Favorable</td>
</tr>
<tr>
<td>FLT3-ITD-negative</td>
<td>Mutant NPM1 and IDH1 or IDH2</td>
<td></td>
</tr>
<tr>
<td>FLT3-ITD-negative</td>
<td>Wild-type ASXL1, MLL-PTD, PHF6, and TET2</td>
<td>Intermediate</td>
</tr>
<tr>
<td>FLT3-ITD-negative or positive</td>
<td>Mutant CEBPA</td>
<td>Intermediate</td>
</tr>
<tr>
<td>FLT3-ITD-positive</td>
<td>Wild-type MLL-PTD, TET2, and DNMT3A and trisomy 8–negative</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>FLT3-ITD-negative</td>
<td>Mutant TET2, MLL-PTD, ASXL1, or PHF6</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>FLT3-ITD-positive</td>
<td>Mutant TET2, MLL-PTD, DNMT3A, or trisomy 8, without mutant CEBPA</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>Any</td>
<td></td>
</tr>
</tbody>
</table>
Revised AML Risk Stratification Based on Integrated Mutational Profiling

Green and red curves represent patients whose risk-classification changes using more extensive mutational profiling.

Validation Cohort (p<0.01)

Outcome not improved with allogeneic transplant in this cohort.
How Do We Identify High Risk Genotypes in the Clinical Setting

• Need robust sequencing platforms for rapid, accurate mutational profiling

• A subset of these genes are large tumor suppressors in which nonsense/frameshift mutations are clinically relevant
  • Call of mutation/wild-type has profound prognostic relevance
  • Ability to get high quality coverage for entire coding region is as important as cost/throughput

• Rapid, accurate analysis is as important as sequencing technology

• Sensitivity is an issue: not clear if rare (1–5%) subclones with good/poor prognosis mutations have prognostic relevance
Hematologic Malignancy Assay to Query All Known Leukemia, Lymphoma, and Myeloma Genes

- Robust, clinically tractable platform for detection of mutations, amplifications/deletions, and fusion genes
- Available Dec 2013 as CLIA certified test

- DNA: 405 genes (exons only + tiled intron coverage of 23 genes for fusions) ~4,000 SNP (CNA analysis)
- RNA: ~300 gene fusions (exons only)

- Additional advantages of an RNA-seq component
  - Improved sensitivity as intronic repeats not a problem
  - Single exon deletions/tandem duplications difficult to characterize in DNA
  - Lack of expression of a TSG may be surrogate for promoter methylation and other mechanisms that cannot be characterized from DNA
  - Leveraging combined DNA/RNA diagnostics in a single test maximizes sensitivity and capacity
Hematologic Malignancy Assay to Query All Known Leukemia, Lymphoma, and Myeloma Genes

- High pass rate (97%) on retrospective FFPE lymphoma samples mirrors clinical success achieved with solid tumors tested with FoundationOne
- Similar success with blood and bone marrow aspirate samples with pass rate of >95%
High Concordance Observed Between NGS and Current Employed Clinical Assays

1 FLT3 ITD positive by PCR, negative by NGS (<5%)

2 IDH1 exon 4 negative by Sanger, positive by NGS (4%, 26%)

Compared to current CLIA assays
- 99% sensitivity to detect mutations/indels
- 100% sensitivity to detect fusion genes
- Identify lesions missed by conventional CLIA assays

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Clinical Assay</th>
<th># Positive</th>
<th># Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT3 ITD + D835</td>
<td>PCR → Fragment Analysis</td>
<td>20*</td>
<td>33</td>
</tr>
<tr>
<td>JAK2 V617F</td>
<td>RT-PCR</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>CEBPA</td>
<td>PCR → Fragment Analysis / Sanger</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>NPM1</td>
<td>Allele-specific PCR</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>IDH1/2</td>
<td>Sequenom</td>
<td>6</td>
<td>23*</td>
</tr>
<tr>
<td>KIT exon 17</td>
<td>Sequenom</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>MPL 505/515</td>
<td>Allele Specific PCR</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>BCR-ABL1, PML-RARA, MLL fusions</td>
<td>RT-PCR &amp; FISH</td>
<td>36</td>
<td>0</td>
</tr>
</tbody>
</table>
Combined DNA/RNA Capture/Sequencing Markedly Increases Ability to Detect Fusion genes

- DNA-seq
  - 31 genes – recurrent fusion hotspots (introns)
  - IGH/IGL/IGK regions – recurrent rearrangement hotspots
- RNA-seq
  - 265 genes – coverage of entire coding sequence

Detected 56 fusion/rearrangement events:

**Common isoforms:**
- BCR-ABL1; PML-RARA; MLL-PTD

**Extra-gene rearrangements:**
- IGH-MYC; IGH-BCL2
- IGH-BCL6

**Uncommon fusions/isoforms:**
- BCR-ABL1; ETV6-ABL1
- MYST3-CREBBP; P2RY8-CRLF2
- PAX5-FLI1; ETV6-EVI1; CBFB-MYH11
- NUP214-DEK; TCF3-PBX1
Hematologic Malignancy Assay to Query All Known Leukemia, Lymphoma, and Myeloma Genes

- Identification of somatic alterations with clinical relevance—prognostic and therapeutic value
Profiling of ALL/AML

- Can reliably identify mutations, small insertions/deletions, homozygous deletions, amplifications, fusions/translocations in AML/ALL

- All samples here have been xenografted → high correlation of genomic lesions between primary sample and leukemias engrafted in mice

- Can be used for preclinical therapeutic studies and in “co-clinical” studies matched with therapeutic trials
How do we improve outcomes for AML patients with mutations in epigenetic modifiers?

(p<0.001)
Mutations in *IDH1/2* and *TET2* lead to impaired DNA Hydroxymethylation and Increased DNA Methylation.

Figueroa, Abdel-Wahab, Lu et al, *Cancer Cell* 2010

How do these alleles contribute to AML pathogenesis?
Development of Models of High Risk AML With Mutations in Epigenetic Modifiers

- Lack of faithful models of adverse rise subsets of AML AML based on cooperation between known co-occurring disease alleles other than in MLL positive AML (FLT3-ITD + MLL fusions/MLL-PTD)

- Few murine or xenograft models of poor-risk, multiple-hit genotypes of AML seen commonly in the clinic

- Development of such models is of biologic and therapeutic relevance, including to test novel therapies and to understand mechanisms of resistance
AML in FLT3-ITD/TET2 KO mice

- Similar disease in FLT3-ITD mice with biallelic loss of TET2 or with TET2 haploinsufficiency, consistent with human genetic data

- Resistant to araC/daunorubicin, FLT3 inhibitor (AC220) therapy

- Similar data in mice expressing FLT3-ITD + IDH1/2 mutant disease alleles (Lowe, Pandolfi, Mak labs) consistent with similar mechanism of transformation

- What are the molecular pathways required for leukemic transformation/maintenance? >used RNA-seq/methylation analysis to identify core pathways
GATA1/2 Epigenetic Silencing in FLT3-TET2-mutant AML

Reduced GATA signature in AML Stem Cells

GATA2 Methylation in AML Stem Cells

GATA gene expression signature also altered in FLT3-ITD/IDH2-mutant mice (Kats et al. Cell Stem Cell 2014)
Can re-expression of silenced GATA genes restore differentiation in FLT3 + TET2/IDH mutant AML?
Rexpression of GATA1/2 abrogates in vivo transformation of FLT3/TET2-mutant AML cells

- Agents which restore differentiation in AML driven by mutations in epigenetic modifiers may offer significant efficacy alone or in combination with other AML therapies
  - We do not have the optimal agents for TET2 mutant AML (hypomethylating agents->more specific therapies)
  - IDH1/2 mutations result in an aberrant gain of function->can this lead to alterations in epigenetic state and to therapeutic efficacy?
Development of Specific inhibitors of IDH1/2*

- Small molecule inhibitors of IDH2 and IDH1 have been developed with potent, specific on target effects

- In vitro and in vivo assays show significant efficacy alone and in combination with chemotherapy

- Led to first-in-man clinical trials of AG-221, IDH2-specific inhibitor in relapsed/refractory IDH2-mutant AML (Eytan Stein, PI)

*Kate Yen/Agios, Alan Shih
Preliminary Plasma Mean AG-221 Exposure and 2-HG Inhibition in Patients with IDH2R140Q Mutation (Agios, E. Stein PI)

- High AG-221 accumulation after multiple doses
- Greater than 90% plasma 2-HG inhibition after multiple doses

*2-HG baseline was taken at Day-3 pre-treatment; 2-HG inhibition is estimated based on 2-HG AUC_{0-10hr}
Efficacy of IDH2 Inhibition in Relapsed/Refractory AML

Cohort 1
30 mg BID

1. NE
2. NE
3. NE
4. CR
5. CR
6. PD

Cohort 2
50 mg BID

7. CR
8. CR
9. PR
10. CRp

Response:
- CR: Complete Response
- CRp: Complete Response with platelet recovery
- PR: Partial Response
- PD: Progression of Disease

Status:
- On Study
- Off Study
- Bone Marrow

To transplant
Look to Future: Genotype Based Trials for AML

AML Patients → Genetic Profiling

- **MLL Fusion**
  - 1. DOT1L
  - 2. BET
  - 3. LSD1

- **IDH2**
  - 1. IDH1
  - 2. Decitabine
  - 3. BET

- **FLT3**
  - 1. ASP2215
  - 2. Crenolanib
  - 3. AC220

- **C-KIT**
  - 1. AUY922
  - 2. Crenolanib

- **RAS**
  - 1. RAF/MEK
  - 2. MEK/PI3K

- **Abnormal Karyotype**
  - P53-WT
    - 1. MDM2/HDM2 (Amgen)
    - 2. BET
  - P53-MT
    - 1. BET
    - 2. LSD1

- **BRAF** (t-AML)
  - 1. Vemurafenib

- **JAK2**
  - 1. Jakafi/DAC

- **Spliceosome**
  - 1. Telomerase

- **No molecular lesion to guide rx**
  - 1. BET
  - 2. LSD1
  - 3. PD1/Immune therapies

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AML Patients

- **Abnormal Karyotype**
  - PS3-MT

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**Genetic Profiling**
Summary

• Genetic studies of leukemia patients can identify mutations which point to novel pathways involved in the pathogenesis of hematologic malignancies.

• Novel disease alleles can be used to improve prognostic and therapeutic decisions in cancer patients.
  - Targeted, focused DNA sequencing approach can help many patients, right now.
  - Will lead to exome/genome sequencing of all cancer patients, but we need to demonstrate this actually can help patients in specific ways.

• In many cases, this may guide the use of existing therapies, assignment/interpretation of clinical trials, and lead to development of novel therapies.

• Agents which restore differentiation in AML driven by mutations in epigenetic modifiers may offer significant efficacy alone or in combination with other AML therapies.
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