Mutational Profiling to Improve Outcomes in Acute Myeloid Leukemia

June 2014

Ross Levine
Human Oncology and Pathogenesis Program
Leukemia Service, Department of Medicine
Memorial Sloan Kettering Cancer Center
Weill Cornell School of Medicine
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 \(\rightarrow\) 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

• 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
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>Normal karyotype or inter-</td>
<td>Wild-type ASXL1, MLL-PTD, PHF6, and TET2</td>
<td>Intermediate</td>
</tr>
<tr>
<td>mediate-risk cyogenetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesions</td>
<td>Mutant CEBPA</td>
<td></td>
</tr>
<tr>
<td>FLT3-ITD-positive</td>
<td>Wild-type MLL-PTD, TET2, and DNMT3A and trisomy 8–negative</td>
<td></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></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%)

1 <1% 211 (99%) 100 Positive 111 Negative 2 IDH1 exon 4 negative by Sanger, positive by NGS (4%, 26%)

2 <1%

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)

TET2, ASXL1, DNMT3A, MLL mutations
Mutations in *IDH1/2* and *TET2* lead to impaired DNA Hydroxymethylation and Increased DNA Methylation

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
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?
- 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$_{0-10 hr}$
Efficacy of IDH2 Inhibition in Relapsed/Refractory AML

Cohort 1
30 mg BID

Cohort 2
50 mg BID

- On Study
- Off Study
- Response
- Bone Marrow

Cohort 1

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

Cohort 2

7. CR
8. CR
9. PR
10. CRp
Look to Future: Genotype Based Trials for AML

AML Patients → Genetic Profiling

Abnormal Karyotype P53-MT
1. BET
2. LSD1

Abnormal Karyotype P53-WT
1. MDM2/HDM2 (Amgen)
2. BET
3. Crenolanib

MLL fusion MLL-PTD
1. DOT1L
2. BET
3. LSD1

IDH2
1. AG221
2. Decitabine
3. BET

MLL-PTD
1. DOT1L
2. BET
3. LSD1

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

FLT3-TKD
1. ASP2215
2. Crenolanib
3. AUY922

JAK2
1. Jakafi/DAC

C-KIT
1. AUY922
2. Crenolanib

RAS
1. RAF/MEK
2. MEK/PI3K

C-Kit
1. AUY922
2. Crenolanib

IDH1
1. IDH1
2. Decitabine
3. BET

IDH1
1. IDH1
2. Decitabine
3. BET

BRAF (t-AML)
1. Vemurafenib

Spliceosome
1. Telomerase

MDM2/HDM2 (Amgen)
1. BET
2. LSD1
3. PD1/Immune therapies

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

RAS
1. Vemurafenib
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.
Acknowledgements

Levine Lab
• Alan Shih
• Kaitlyn Shank
• Jay Patel
• Lindsay LaFave
• Franck Rappaport
• Olga Guryanova
• Raajit Rampal
• Ria Kleppe
• Todd Hricik
• Sophie McKenney
• Matt Keller
• Gila Spitzer
• Lennart Bastian
• Brittany Woods
• Aaron Viny
• Efi Papalexı
• Elodie Pronier

MSKCC
• Omar Abdel-Wahab
• Craig Thompson
• Eytan Stein
• Kristina Knapp
• Marcel van den Brink
• Scott Armstrong
• Marty Tallman
• Scott Lowe
• Greg Raskin
• Ahmet Dogan

Agios
• Kate Yen
• Sam Agresta
• David Scheikein

Foundation Medicine
• Vince Miller
• Phil Stephens
• Geoff Otto
• Doron Lipson
• Jie He
• Michele Nahas

Cornell
• Ari Melnick
• Yanwen Jiang
• Cem Maydan
• Chris Mason