Genetic Profiling of Leukemia Patients: Clinical and Translational Insights

October 15, 2012
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Leukemia Service, Department of Medicine
Acute Myeloid Leukemias Are Still Associated with Poor Overall Survival

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

Fernandez et al. NEJM 2009
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

MDS
Class I Mutation

AML

• But not all patients have mutations in class I and class II genes
• Does not reflect role of novel AML mutations in leukemogenesis

Gilliland and Griffin Blood 2002
Prognostication in AML

- Cytogenetic studies have separated AML into three distinct cytogenetic subgroups
  - Favorable risk: inv 16, 8:21, 15:17
  - Unfavorable risk: -5, -7, MLL translocations, complex karyotype
  - Intermediate: everyone else

- Has allowed risk stratification and has been used to guide therapeutic decisions in AML
  - Allogeneic transplant reserved for patients with unfavorable+/- intermediate risk disease

- Many studies have identified additional mutations, expression changes, micro-RNA profiles
  - Few, if any of these have been adopted into clinical practice
Intermediate risk AML*

- Analysis of >800 patients with intermediate risk disease
- Two favorable genotypes
  - CEBPA mutations
  - NPM1 mutations without FLT3-ITD
- Benefit of allograft in patients with “other genotypes”
- Has led to clinical testing for FLT3-ITD, NPM1, +/- CEBPA

*Schlenck et al. NEJM 2008
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)

• Biologic and prognostic relevance of these novel disease alleles has not been fully delineated
Single Gene Studies: Prognostic Relevance of TET2 mutations in AML*

• Analysis of 110 patients with de novo and secondary AML at MSKCC

• First suggestion that TET2 mutations associated with poor outcome

• Relatively small cohort, non-uniform treatment, no other genetic data

*Omar Abdel-Wahab, Cyrus Hedvat et al Blood 2009
Goals of Leukemia Profiling Efforts

- Identify novel genetic factors which predict relapse rate and outcome in AML
  - Robust analysis of large, homogeneously treated cohorts
  - Multivariate analysis including clinical, molecular data to determine which factors best predict outcome

- Use genetic data to make novel insights into the biology of myeloid malignancies

- Translate this to the clinical arena with prospective mutational testing
Large scale mutational studies in MDS have identified many new alleles of biologic and prognostic relevance.

- Mutations in epigenetic modifiers: TET2, ASXL1, DNMT3A
- Mutations in spliceosome components
- Informed molecular/pathologic classification of disease
- Led to development of robust prognostic schema with clinical utility

Bejar et al., *NEJM* 2011
Bejar et al., *JCO* 2012
# Risk Modeling – Multivariable Analysis IPSS

<table>
<thead>
<tr>
<th></th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥55 yrs vs. &lt;55 yrs</td>
<td>1.81 (1.20-2.73)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>IPSS Risk Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Int1 vs. Low</td>
<td>2.29 (1.69-3.11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Int2 vs. Low</td>
<td>3.45 (2.42-4.91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High vs. Low</td>
<td>5.85 (3.63-9.40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Mutational Status - Present vs. Absent</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>TP53</em> Mutation</td>
<td>2.48 (1.60-3.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>EZH2</em> Mutation</td>
<td>2.13 (1.36-3.33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>ETV6</em> Mutation</td>
<td>2.04 (1.08-3.86)</td>
<td>0.029</td>
</tr>
<tr>
<td><em>RUNX1</em> Mutation</td>
<td>1.47 (1.01-2.15)</td>
<td>0.047</td>
</tr>
<tr>
<td><em>ASXL1</em> Mutation</td>
<td>1.38 (1.00-1.89)</td>
<td>0.049</td>
</tr>
</tbody>
</table>

- Identified several biomarkers which inform prognosis, and add to existing risk schema
- Licensed test for mutational profiling of 5 genes (Genoptix)
  - Considers each gene as a distinct biomarker
  - Not clear how therapeutic decisions in MDS should be modified based on molecular testing (transplant)
  - Not tested in phase III trial cohorts or validated in other series
Muta-onal	
  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
Mutation Summary

- Including cytogenetic abnormalities, 591 somatic mutations in 398 samples:
  - With updated data 98.2 % have at least one clonal somatic abnormality
  - Number of mutations did not affect outcome
Mutational Profiling of ECOG 1900 Patient Cohort (502 patients)

<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>

DNMT3A-mutant AML
Relevance to Clinical Practice

- Do these novel mutations have prognostic value in AML?

- If so can integrated mutational analysis be used to refine our ability to risk-stratify AML patients

- E1900 tested standard dose vs. high dose daunorubicin->do any genetic factors influence effects of induction dose intensity?
FLT3-ITD, ASXL1, PHF6, MLL-PTD mutations associated with adverse outcome

- Specific Mutations Associated with adverse overall survival
  - FLT3-ITD (p=0.001)
  - ASXL1 (p=.002)
  - PHF6 (p=.02)
  - MLL PTD (P<0.001)
IDH2 R140Q Mutations Associated With Improved Overall Survival

- IDH2 R172K and IDH1 mutations no effect on overall survival
It is likely that >1 mutation impacts prognosis/response to therapy in AML in individual patients—however previous studies have largely looked at mutations as distinct variables.

We used classification trees to determine which mutations and cytogenetic lesions has the largest impact on outcome:
- Cytogenetic risk
- FLT3-ITD

We then performed multivariate analysis of different mutational combinations within different risk subsets:
- Prognosis of cytogenetically favorable or unfavorable outcome is not impacted by specific mutation
- Focused on 63% of AML patients with cytogenetic intermediate risk AML
Intermediate Risk AML

- Specific Mutations Associated with Adverse Outcome
  - FLT3-ITD ($p=0.002$)
  - TET2 ($p=0.04$)
  - ASXL1 ($p=0.02$)
  - PHF6 ($p<0.001$)
  - MLL PTD ($P<0.001$)

- Specific Mutations Associated with Favorable Outcome
  - IDH2 ($p<0.02$) but not IDH1
  - CEBPA
IDH/NPM1 mutations define favorable outcome in FLT3-negative, intermediate risk AML

- IDH/NPM1-mutant patients, but not NPM1-mutant/IDH-WT patients have a favorable outcome
Modified risk model for FLT3-ITD negative, intermediate risk AML

- Group 1 (favorable): IDH2/NPM1 mutant
- Group 3 (poor-risk): TET2, PHF6, ASXL1, MLL-PTD mutations
- Group 2: all others
Multivariate Model for FLT3-ITD-positive intermediate risk AML

- Group 1 (favorable): CEBPA
- Group 3 (poor-risk): TET2, trisomy 8, DNMT3A R882, or MLL-PTD
- Group 2: all others – similar to CEBPA-mutant
- Can discriminate a set of patients with FLT3-mutant disease with much poorer outcome than others
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>Normal karyotype or inter-</td>
<td>Mutant CEBPA</td>
<td></td>
</tr>
<tr>
<td>mediate-risk cyogenetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesions</td>
<td></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>
</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.

Outcome not improved with allogeneic transplant in this cohort

Validated cohort (p<0.001)
Revised AML Risk Stratification Based on Integrated Mutational Profiling

28% of AML Patients are reclassified into unfavorable/favorable risk based on mutational studies
How do we use this data to improve outcomes for leukemia patients?

- Elucidate novel insights into AML biology

- Identify specific, poor prognosis genotypes: develop laboratory models which can be used to test preclinical therapies

- Inform the development of novel clinical trials

- Guide the use of existing therapies
Human genetics is always right: using mutational studies to elucidate AML pathogenesis

- By profiling primary patient samples we can improve our understanding of AML biology

- We can identify lesions that commonly occur together (NPM1/IDH) to guide development of new models, pathways to transformation...

- But...we can also identify mutations which NEVER occur together and define novel complementation groups/mutational classes
  - Would suggest that specific genes function in a pathway
  - Or that specific genes have a “synthetic lethal” interaction

- We hypothesized that we could elucidate the function of IDH mutations in AML by identifying mutations exclusive of IDH mutations of AML
ECOG 1900 Cohort: IDH1/2 mutations mutually exclusive of TET2 mutations

<table>
<thead>
<tr>
<th></th>
<th>TET2 Wildtype</th>
<th>TET2 Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH1/2 Wildtype</td>
<td>300</td>
<td>28</td>
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<tr>
<td>IDH1/2 Mutant</td>
<td>57</td>
<td>0</td>
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</table>

*P-value = 0.009 (Left-tailed Fisher’s exact test)

Figueroa, Abdel-Wahab, Lu et al, *Cancer Cell* 2010
Conversion of 5-Methylcytosine to 5-Hydroxymethylcytosine in Mammalian DNA by MLL Partner TET1

Mamta Tahiliani,1 Kian Peng Koh,1 Yinhua Shen,2 William A. Pastor,1 Hozefa Bandukwala,1 Yevgeny Brudno,2 Suneet Agarwal,3 Lakshminarayan M. Iyer,4 David R. Liu,2a L. Aravind,4* Anjana Rao1*

Cytosine (unmethylated) → Methyl-Cytosine (hemimethylated)

Cytosine → α-KG → TET 1-3 → Hydroxy-methyl-Cytosine

Inactivating mutations of TET proteins result in hypermethylation of DNA and loss of DNA hydroxymethylation

TET family enzymatic function is α-KG dependent
AML patient samples -> decreased 5-OH-methylcytosine and increased cytosine methylation with IDH/TET2 mutations*

Methylcytosine

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IDH1\textsuperscript{mut}</th>
<th>IDH2\textsuperscript{mut}</th>
<th>TET2\textsuperscript{mut}</th>
</tr>
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<tbody>
<tr>
<td>%5mC</td>
<td>1.0</td>
<td>1.3</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>(normalized to control)</td>
<td>(normalized to control)</td>
<td>(normalized to control)</td>
<td>(normalized to control)</td>
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</table>

P = 0.0002

Hydroxy-Methylcytosine

<table>
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<tr>
<th></th>
<th>Control</th>
<th>IDH1\textsuperscript{mut}</th>
<th>IDH2\textsuperscript{mut}</th>
<th>TET2\textsuperscript{mut}</th>
</tr>
</thead>
<tbody>
<tr>
<td>%5hmC</td>
<td>1.0</td>
<td>0.6</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>(normalized to control)</td>
<td>(normalized to control)</td>
<td>(normalized to control)</td>
<td>(normalized to control)</td>
</tr>
</tbody>
</table>

P = 0.034

Done using LC/MS – critical as not all methods distinguish mC from HmC

Ken Figueroa, Raajit Rampal, Lucy Godley
IDH1/2 Mutations Inhibit TET2-mediated 5-OH-Me-Cytosine formation

Chao Lu, Pat Ward (Craig Thompson)
**IDH1/2 and TET2: convergent mechanism of transformation by mutations in metabolic enzymes and epigenetic modifiers**

How do these alleles contribute to hematopoietic transformation?
Acquired Skewing/Clonal Hematopoiesis in Elderly Patients*

- Increased incidence of nonrandom X-inactivation rations found in blood cells of the female elderly population

- Multiple Hypotheses for mechanism:
  - Caused by mutations conferring selective growth advantage in stem cells.
  - Stochastic clonal dominance secondary to significant stem cell depletion with age in normal females.
  - Genetic trait by a gene on one of the two X chromosomes.

- Hypothesized clonal hematopoiesis due to somatic mutations- >performed exome sequencing on paired granulocyte/normal tissue on elderly subjects with X allele skewing

*Busque, Patel, Abdel-Wahab et al. Nature Genetics AOP Sept 2012
Exome sequencing identifies somatic TET2 frameshift mutation in patient with clonal hematopoiesis
Recurrent somatic missense, nonsense, and frameshift TET2 mutations in elderly patients with clonal hematopoiesis

<table>
<thead>
<tr>
<th>Nucleotide substitution</th>
<th>Amino-acid substitution</th>
<th>Chromosome</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.286_298delCGCAC</td>
<td>p.Arg96Asnfs*12</td>
<td>4</td>
<td>106155385</td>
</tr>
<tr>
<td>AGTTAGTG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.1330delA</td>
<td>p.Thr444Hisfs*6</td>
<td>4</td>
<td>106156429</td>
</tr>
<tr>
<td>c.1348delA</td>
<td>p.Lys450Lysfs*2</td>
<td>4</td>
<td>106156447</td>
</tr>
<tr>
<td>c.1547delC</td>
<td>p.Pro516Hisfs*16</td>
<td>4</td>
<td>106156646</td>
</tr>
<tr>
<td>c.1630C&gt;T</td>
<td>p.Arg544*</td>
<td>4</td>
<td>106156729</td>
</tr>
<tr>
<td>c.3311_3312insAT</td>
<td>p.Phe1104Leufs*3</td>
<td>4</td>
<td>106158411</td>
</tr>
<tr>
<td>c.3991A&gt;C</td>
<td>p.Thr1331Pro</td>
<td>4</td>
<td>106182952</td>
</tr>
<tr>
<td>c.5200delA</td>
<td>p.Met1734Leufs*11</td>
<td>4</td>
<td>106196867</td>
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<tr>
<td>c.5575insT</td>
<td>p.Ile1859tyrfs*16</td>
<td>4</td>
<td>106197239</td>
</tr>
<tr>
<td>c.5725G&gt;T</td>
<td>p.Glu1909*</td>
<td>4</td>
<td>106197392</td>
</tr>
</tbody>
</table>
Decreased 5-hmC and increased 5mC in patients with clonal hematopoiesis
Increased methylation and decreased expression of loci which are altered in TET2-mutant leukemia patients
Implications

• Recurrent somatic mutations in TET2 are observed in patients with clonal hematopoiesis without overt disease
  - Consistent with a premalignant clonal state
  - Likely additional genes contribute to this process which may also contribute to pathogenesis of myeloid malignancies

• Prediction-> TET2 loss will increase competitive advantage of mutant stem cells over an extended period of time-> predisposing cells to acquire additional alterations which result in MPN (JAK2), MDS (SF3B1), or AML (FLT3-ITD) based on specific genotype
Conditional TET2 KO mouse (Vav, Mx-Cre)

Alan Shih (Levine), Linsey Reavie/Kelly Moran (Aifantis)
TET2 deletion leads to serial replating of cells with a myeloid progenitor phenotype
TET2 deletion leads to increased competitive transplantation in vivo
TET2 loss leads to myeloproliferation in vivo

- leukocytosis/monocytosis
- splenomegaly/extramedullary hematopoiesis with monocyte/neutrophil expansion
- Increased stem/progenitor numbers
- No features of AML
Loss of a single Tet2 allele sufficient to confer self-renewal and malignant phenotype

Enhanced serial replating

Outcompete normal HSCs in transplantation assays

Leukocytosis and monocytosis/splenomegaly
Benefit of High Dose Daunorubicin in AML

- First positive phase III trial in AML in more than 20 years
- Already validated in second patient cohort (Blood 2011)
- However the benefit of increased daunorubicin is modest
- Might specific subsets of AML patients benefit significantly, or not at all, from more intensive chemotherapy?
Newest Epigenetic Mutation: DNMT3A mutations in AML

- Frameshift mutations and recurrent mutations at R882 – limited functional data consistent with loss of function
- Occur in 25-30% of patients with AML
- Associated with poor outcome in two retrospective cohorts

Ley et al. NEJM 2010

Yan et al. Nature Genetics 2011
DNMT Mutational Status Does not Affect Outcome in E1900 Cohort

- Better outcome than in other trials, not different than DNMT3A-WT patients
- Patients were randomized to standard vs. high dose daunorubicin induction therapy -> might induction dose intensity improve outcome in DNMT3A mutant patients?
High Dose Daunorubicin Improves Outcome in DNMT3A Mutant Patients, but not DNMT-WT patients

- Are there other mutations whose impact on survival is affected by induction dose?
High Dose Daunorubicin Improves Outcome in Patients with DNMT3A mutations, MLL Fusions, or NPM1 mutations

DNMT3A/MLL/NPM1-WT pts

DNMT3A, MLL, NPM1 mutant

--- High Dose
--- Standard dose

P=0.674

P=0.001
Implementing this 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
MSKCC Hematologic Oncology Genomics Project

- Initiated in 2010 with the support of Solomon Fund

- Includes investigators from
  - Medicine: Marcel van den Brink, Ross Levine, Lia Palomba, Alan Hanash, Raajit Rampal, Omar Abdel-Wahab, Andy Intelkofer, Andy Zelenetz, Jay Patel, Virginia Klimek
  - Pathology: Cyrus Hedvat, Mike Berger, Chris Park
  - C Bio: Nick Socci, Franck Rapaport
  - Genomics: Agnes Viale
  - Pediatrics: Scott Armstrong
  - SKI: Mike Kharas

- Two goals
  - Develop platforms for genomic profiling of patients with hematologic malignancies
  - Transition these to the clinical setting and to mechanism-based clinical trials
Myeloid Leukemia Mutational Panel

- Robust, clinically tractable platform for rapid, accurate mutation detection
- First phase has 32 genes: 18 genes from the AML study plus 14 additional genes recently identified in AML, MPN, MDS
- Developed a platform for rapid, PCR amplification and sequencing using RainDance and HiSeq/MiSeq
- Rapid (15-20 minutes) analysis of raw sequence data for known/predicted pathogenic variants
- Goal is to achieve actionable information by hospital discharge for patients with myeloid malignancies
- Have now developed a similar panel for B-ALL being tested on 192 MSKCC/ECOG samples (ETC ALL Project)
High Coverage of Genes in Sequencing Panel

The image shows a series of bar charts representing the sequencing coverage of various genes. The x-axis lists gene names, and the y-axis indicates sequencing coverage levels (500x, 200x, 100x). Each bar chart corresponds to different samples or conditions, labeled as AML14, CHRF, EOL_1, and F36P.
Identification of DNMT3A mutation using Raindance/HiSeq*

*Franck Rapaport
Summary

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

• Novel disease alleles can be used to improve prognostic and therapeutic decisions in cancer patients
  - Even a 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 and lead to development of novel therapies