Genomic Profiling in Haematological Malignancies

Molecular Diagnosis Centre
Department of Laboratory Medicine
National University Hospital
Singapore

Benedict Yan, Head and Consultant
Molecular Diagnosis Centre

- Founded in 1998 (19 year history)
- CAP-accredited
- Offer ~100 tests
  - Infectious diseases
  - Oncology
  - Inherited diseases
  - Prenatal diagnostics
Outline

- Clinical NGS in Singapore
- Genomic Profiling in Hematological Malignancies
- Acute Myeloid Leukemia (AML)
- Clinical NGS – Technical Considerations
- Genomic Case Studies & Insights
Clinical NGS in Singapore
In 2013, Singapore:

- Ranked 6th globally for healthcare outcomes
- Spends less on healthcare than any high-income country
  - Half that of Europe
  - A fourth of the U.S.
Next-generation...???!
Thoughts

- NGS is ‘expensive’
- What is the evidence that more information (i.e. more biomarkers) = better patient outcomes?
- What is the evidence that “standard-of-care biomarkers” are relevant to our local population?
- How can we minimize inter-laboratory variation in variant interpretation?
Genomic Profiling in Hematological Malignancies
Milestones in Molecular Hematology – Pre-Genomic Era

1960
- Philadelphia chromosome in CML (Nowell & Hungerford)

1972
- Identification of t(9;22) (Rowley)

1972
- Identification of bcr-abl fusion (Ben-Neriah)

1986
- Identification of PML-RARα (de The; Kakizuka)

1990/1991
- Identification of PML-RARα (de The; Kakizuka)

1996
- FLT3 ITD in AML (Nakao)
- JAK2 V617F in MPD (Baxter; James; Kralovics; Levine)

1996
- CEBPA mutations in AML (Pabst)

1996
- NPM1 mutations in AML (Falini)

2001
- Jan 2005
- JAK2 Exon 12 Mutations in PV (Scott)

2001
- NPM1 mutations in AML (Falini)

2006
- MPL W515L in MPD (Pikman)

2007
- MPL W515L in MPD (Pikman)
Milestones in Molecular Hematology - Genomic Era

- 2010
  - Feb: AML - first cancer genome sequenced (Ley)

- 2011
  - Mar: MM genome sequenced (Chapman)
  - Jun: MDS exome sequenced (Yoshida)
  - Aug: MYD88 L265P in WM (Treon)
  - Sep/Oct: SF3B1 mutations in MDS (Papaemmanuil)

- 2012
  - Jan: ALL genome sequenced (Zhang)
  - Mar: MM genome sequenced (Chapman)
  - Aug: ALL genome sequenced (Zhang)
  - Sep/Oct: SF3B1 mutations in MDS (Papaemmanuil)

- 2013
  - Jun: EZH2 mutation in FL & DLBCL (Morin)

- 2014
  - Sep/Oct: SF3B1 Mutations in MDS (Papaemmanuil)

- 2015
  - Jun: AML genomic classification (Papaemmanuil)

- 2016
  - Jun: AML genomic classification (Papaemmanuil)
  - Dec: Juno Hematopoiesis and Blood-Cancer Mutations in Hematopoiesis (Genovese)
Introduction to Genomics in Hematologic Malignancy

Benjamin L. Ebert, Brigham and Women’s Hospital and Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA
Jonathan W. Friedberg, University of Rochester Wilmot Cancer Institute, Rochester, NY

The growing field of genomic medicine has had a profound impact on our understanding of malignancy. For many cancers, we are now in an era of precision medicine, where therapy is individualized based on the genetic and molecular features of the tumor. In this special series issue of Journal of Clinical Oncology, we focus on the power of genomics in defining the biology of hematologic malignancies. Common themes emerge from the set of articles. Each hematologic malignancy discussed has complex genetics caused by the combinatorial diversity of somatic genetic mutations. However, many malignancies are associated with frequent disruption of a single biologic process, such as Janus kinase 2 signaling in myeloproliferative neoplasms, the mRNA spliceosome in myelodysplastic syndrome, and mitogen-activated protein kinase signaling in multiple myeloma. Clinical genetics offers the potential for improved disease classification, prognostication, identification of predictors of therapeutic response, and evaluation of the depth of disease remission. The articles in this issue, written by international authorities in the field, emphasize the translation of this increased genomic understanding to the clinic, with the practicing hematologist and oncologist in mind.

We begin with an article by Shekine et al. that presents key strategic decisions required to optimize a clinical genomic testing program. This article discusses the distinct features of major technologies available for next-generation sequencing and the computational biology expertise required for data analysis. With this important background, we then have nine articles focused on genomics of specific diseases within the hematologic oncology space.

Our understanding of the molecular pathogenesis of acute myeloid leukemia (AML) has been greatly advanced by genomic medicine. Bullinger et al. describe key mutations that affect genes of different functional categories within a complex clonal architecture. These data are now increasingly being used to inform disease classification, risk stratification, and clinical care of patients. For example, two new provisional entities, AML with mutated RUNX1 and AML with BCR-ABL1, have been included in the current update of the WHO classification of myeloid neoplasms and acute leukemia, and mutations in three genes, RUNXI, ASXL1, and TP53, have been added in the risk stratification of the 2017 European LeukemiaNet recommendations for AML. Integrated evaluation of baseline genetics and assessment of minimal residual disease is expected to improve risk stratification and selection of postremission therapy for many patients. Genomic analysis now provides a platform for disease definition, prognosis, and therapeutic choice in myeloproliferative diseases. Zoi and Cross outline the unexpected genomic complexity, including inherited factors, phenotype driver mutations, and additional abnormalities associated with disease progression. Deregulated Janus kinase 2 signaling is the central driver of BCR-ABL1-negative myeloproliferative disease and represents a unifying therapeutic target.

Moffat and Dave examine the genomic landscapes of aggressive B-cell and T-cell lymphomas. They focus on broad genomic features that characterize the heterogeneous clinical entities and new therapeutic opportunities that arise from these findings, emphasizing the state of the art for the practicing clinician.

Robiou du Pont et al. focus on exciting recent data on the basis of whole-exome sequencing in multiple myeloma that confirms disease heterogeneity and demonstrate that alteration of the mitogen-activated protein kinase pathway is common. In addition, the demonstration of subclonality in all the patients, including subclonal mutations of established driver genes such as KRAS, NRAS, or BRAF, has increased our understanding of variability of clinical behavior and disease resistance to treatment.

We then move to myelodysplastic syndrome (MDS), where Kennedy and Ebert focus on the clinical implications of genetic mutations in MDS. Genetic studies have enabled the identification of a set of recurrently mutated genes central to the pathogenesis of MDS, which can be organized into a limited number of cellular processes, including RNA splicing, epigenetic and traditional transcriptional regulation, and signal transduction. The sequential accumulation of mutations drives disease evolution from asymptomatic clonal hematopoiesis to frank MDS and, ultimately, to secondary AML. This detailed understanding of the molecular landscape of MDS has led to the introduction of genetic-focused therapeutic studies.

Iacobacci and Mullighan focus on the genetic basis of acute lymphoblastic leukemia (ALL). Both B-ALL and T-ALL comprise multiple subtypes harboring distinct constellations of somatic structural DNA rearrangements and sequence mutations that commonly perturb lymphoid development, cytokine receptors, kinase and Ras signaling, tumor suppression, and chromatin modification. Recent studies have helped to understand the genetic basis of clonal evolution and relapse and the role of inherited genetic variants in leukemia. In addition to refining diagnosis and prognosis in ALL, genomics is currently being used clinically to monitor residual disease and detect early relapse, with the ultimate goal of targeted relapse prevention strategies.

Chronic lymphocytic leukemia (CLL) has a remarkably heterogeneous course, ranging from indolent disease with no need of
Genetic Basis of Acute Lymphoblastic Leukemia
Baris Iacobucci and Charles G. Munlligan

Clinical Implications of Novel Genomic Discoveries in Chronic Lymphocytic Leukemia
Gregory Lazenby, Roman Gutze, and Catherine J. Wu

Clinical Implications of Genetic Mutations in Myelodysplastic Syndrome
James A. Kennedy and Benjamin L. Ebert

Genomics of Myeloproliferative Neoplasms
Katerina Zei and Nicholas C.F. Cren

Germline Genetic Predisposition to Hematologic Malignancy
Elisa Furumoto and Akiko Shikuma

Genomics of Acute Myeloid Leukemia Diagnosis and Pathways
Lars Bulinger, Konstanze Döhner, and Hartmut Döhner

Genomics of Hairy Cell Leukemia
Enrico Tacci, Valentina Pettini, Gianluca Schiavoni, and Brunangelo Falini

Genomics of Multiple Myeloma
Sebastian Rebou de Pont, Alice Clayson, Charlotte Forsman, Michel Aud, Nikhil Murishi, Jill Corne, and Hervé Aver-Loesche

Genomics, Signaling, and Treatment of Waldenström Macroglobulinemia
Zachary R. Hunter, Guang Song, Lian Xu, Xia Liu, Jorge J. Castilla, and Steven P. Tron

Clinical Applications of the Genomic Landscape of Aggressive Non-Hodgkin Lymphoma
Andrew R. Moffett and Sandeep S. Dave
AML Genomics
Acute Myeloid Leukemia

- Blood cancer
- Abnormal cells in the blood and bone marrow
- Patients are grouped into different categories (risk profiles) based on the AML genetics
- Treatment is based on risk profile

AML Genome: 
~13 mutations
somes in 2 groups that were replaced by chromosomes in 2 other groups. When I used banding to analyze the chromosomes in these patients they both had the 8;21 translocation [t(8;21)(q22;q22)] (Figure 1). It was the first recurrent translocation in leukemia described. I wrote a brief letter and sent it to the New England Journal of Medicine in the summer of 1972, which rejected it. Being impatient, I then sent the paper to a journal (Annales de
AML Genetics/Genomics

1. Classification (Taxonomy)
2. Clinical management
### Table 1. WHO classification of acute myeloid leukemia

- Acute myeloid leukemia with recurrent genetic abnormalities
  - Acute myeloid leukemia with t(8;21)(q22;q22), \((AML1/ETO)\)
  - Acute myeloid leukemia with abnormal bone marrow eosinophils and
    \(\text{inv}(16)(p13q22)\) or \(t(16;16)(p13;q22)\), \((CBFβ/MYH11)\)
  - Acute promyelocytic leukemia with \(t(15;17)(q22;q12)\), \((PML/RARα)\) and variants
  - Acute myeloid leukemia with \(11q23\) \((MLL)\) abnormalities
### Table 1. WHO classification of acute myeloid leukemia with recurrent genetic abnormalities

<table>
<thead>
<tr>
<th>Acute myeloid leukemia with t(8;21)(q22;q22) or inv(16)(p13q22) or t(16;16)(p13;q22)</th>
<th>RUNX1-RUNX1T1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myeloid leukemia with t(15;17)(q22;q12)</td>
<td>PML-RARA</td>
</tr>
<tr>
<td>Acute promyelocytic leukemia</td>
<td></td>
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<tr>
<td>Acute myeloid leukemia with 11q23.1 rearrangement</td>
<td></td>
</tr>
<tr>
<td>Acute myeloid leukemia with 11q23.1 rearrangement and mutated NPM1</td>
<td>Provisional entity: AML with mutated NPM1</td>
</tr>
<tr>
<td>Acute myeloid leukemia with 11q23.1 rearrangement and mutated CEBPA</td>
<td>Provisional entity: AML with mutated CEBPA</td>
</tr>
</tbody>
</table>

### Table 2. WHO classification of myeloid neoplasms and acute leukemia

<table>
<thead>
<tr>
<th>Acute myeloid leukemia with recurrent genetic abnormalities</th>
</tr>
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<tbody>
<tr>
<td>AML with t(8;21)(q22;q22); RUNX1-RUNX1T1</td>
</tr>
<tr>
<td>AML with inv(16)(p13.q22) or t(16;16)(p13.q22); CBF-MYH11</td>
</tr>
<tr>
<td>AML with t(15;17)(q22;q12); PML-RARA</td>
</tr>
<tr>
<td>AML with t(9;11)(p22;q23); MLLT3-MLL</td>
</tr>
<tr>
<td>AML with t(6;9)(p23;q34); DEK-NUP214</td>
</tr>
<tr>
<td>AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1</td>
</tr>
<tr>
<td>AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1</td>
</tr>
</tbody>
</table>

Provisional entity: AML with mutated NPM1

Provisional entity: AML with mutated CEBPA
The World Health Organization (WHO) classification of the myeloid neoplasms

James W. Vardiman, Nancy Lee Harris, and Richard D. Brunning

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</tr>
<tr>
<td>Acute promyelocytic leukemia with MLL-ENL</td>
</tr>
<tr>
<td>Acute myeloid leukemia with 11q23 rearrangements</td>
</tr>
</tbody>
</table>

The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia

Daniel A. Arber,1 Attilio Orlandi,2 Robert Hasserjian,3 Jürgen Thiele,4 Michael J. Borowitz,5 Michelle M. Le Beau,6 Clara D. Bloomfield,7 Mario Cazzola,8 and James W. Vardiman9

Table 1. WHO classification of myeloid neoplasms and acute leukemia

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<tr>
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<tr>
<td>AML with t(9;11)(p22;q23); MLL-AF4</td>
</tr>
<tr>
<td>AML with t(6;9)(p23;q34); DEK-CAN</td>
</tr>
<tr>
<td>AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); GATA2; MECOM</td>
</tr>
<tr>
<td>AML (megakaryoblastic) with t(3;31)(p14;q25); BCR-ABL1</td>
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<td>AML with mutated NPM1</td>
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<td>AML with biallelic mutations of CEBPA</td>
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</table>

The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes

James W. Vardiman,1 Jürgen Thiele,2 Daniel A. Arber,3 Richard D. Brunning,4 Michael J. Borowitz,5 Anna Porwit,6 Nancy Lee Harris,7 Michelle M. Le Beau,8 Eva Hellström-Lindberg9 Ayalew Tefferi,10 and Clara D. Bloomfield11

Table 2. WHO classification of myeloid neoplasms and acute leukemia

<table>
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<tr>
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<td>AML with biallelic mutations of CEBPA</td>
</tr>
<tr>
<td>Provisional entity: AML with mutated RUNX1</td>
</tr>
</tbody>
</table>
Genomic Classification and Prognosis in Acute Myeloid Leukemia

Papaemmanuil et al. NEJM June 2016
<table>
<thead>
<tr>
<th>Genomic Subgroup</th>
<th>Frequency in the Study Cohort (N = 1540)</th>
<th>Most Frequently Mutated Genes*&lt;br&gt;gene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML with NPM1 mutation</td>
<td>418 (27)</td>
<td>NPM1 (100), DNMT3A (54), FLT3&lt;sup&gt;ITD&lt;/sup&gt; (39), NRAS (19), TET2 (16), PTPN11 (15)</td>
</tr>
<tr>
<td>AML with mutated chromatin, RNA-splicing genes, or both†</td>
<td>275 (18)</td>
<td>RUNX1 (39), MLL&lt;sup&gt;PTD&lt;/sup&gt; (25), SRSF2 (22), DNMT3A (20), ASXL1 (17), STAG2 (16), NRAS (16), TET2 (15), FLT3&lt;sup&gt;ITD&lt;/sup&gt; (15)</td>
</tr>
<tr>
<td>AML with TP53 mutations, chromosomal aneuploidy, or both‡</td>
<td>199 (13)</td>
<td>Complex karyotype (68), −5/5q (47), −7/7q (44), TP53 (44), −17/17p (31), −12/12p (17), +8/8q (16)</td>
</tr>
<tr>
<td>AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB–MYH11</td>
<td>81 (5)</td>
<td>inv(16) (100), NRAS (53), +8/8q (16), +22 (16), KIT (15), FLT3&lt;sup&gt;ITD,KD&lt;/sup&gt; (15)</td>
</tr>
<tr>
<td>AML with biallelic CEBPA mutations</td>
<td>66 (4)</td>
<td>CEBPA&lt;sup&gt;biallelic&lt;/sup&gt; (100), NRAS (30), WT1 (21), GATA2 (20)</td>
</tr>
<tr>
<td>AML with t(15;17)(q22;q12); PML–RARα</td>
<td>60 (4)</td>
<td>t(15;17) (100), FLT3&lt;sup&gt;ITD&lt;/sup&gt; (35), WT1 (17)</td>
</tr>
<tr>
<td>AML with t(8;21)(q22;q22); RUNX1–RUNX1T1</td>
<td>60 (4)</td>
<td>t(8;21) (100), KIT (38), −Y (33), −9q (18)</td>
</tr>
<tr>
<td>AML with MLL fusion genes; t(x;11)(x;q23)‡</td>
<td>44 (3)</td>
<td>t(x;11q23) (100), NRAS (23)</td>
</tr>
<tr>
<td>AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); GATA2, MECOM(EVI1)</td>
<td>20 (1)</td>
<td>inv(3) (100), −7 (85), KRAS (30), NRAS (30), PTPN11 (30), ETV6 (15), PHF6 (15), SF3B1 (15)</td>
</tr>
<tr>
<td>AML with IDH2&lt;sup&gt;R172&lt;/sup&gt; mutations and no other class-defining lesions</td>
<td>18 (1)</td>
<td>IDH2&lt;sup&gt;R172&lt;/sup&gt; (100), DNMT3A (67), +8/8q (17)</td>
</tr>
<tr>
<td>AML with t(6;9)(p23;q34); DEK–NUP214</td>
<td>15 (1)</td>
<td>t(6;9) (100), FLT3&lt;sup&gt;ITD&lt;/sup&gt; (80), KRAS (20)</td>
</tr>
<tr>
<td>AML with driver mutations but no detected class-defining lesions</td>
<td>166 (11)</td>
<td>FLT3&lt;sup&gt;ITD&lt;/sup&gt; (39), DNMT3A (16)</td>
</tr>
<tr>
<td>AML with no detected driver mutations</td>
<td>62 (4)</td>
<td></td>
</tr>
<tr>
<td>AML meeting criteria for ≥2 genomic subgroups</td>
<td>56 (4)</td>
<td></td>
</tr>
</tbody>
</table>
AML Patients

Mutated NPM1 without FLT3-ITD (normal karyotype)
Biallelic mutated CEBPA (normal karyotype)

Favorable Risk Profile:
- Chemotherapy

Intermediate Risk Profile:
- Hematopoietic stem cell transplant
- Chemotherapy

Mutated NPM1 and FLT3-ITD (normal karyotype)
Wild-type NPM1 and FLT3-ITD (normal karyotype)
Wild-type NPM1 without FLT3-ITD (normal karyotype)
AML Patients

### Favorable Risk Profile
- Mutated NPM1 without FLT3-ITD (normal karyotype)
- Biallelic mutated CEBPA (normal karyotype)
- Wild-type NPM1 and FLT3-ITD (normal karyotype)
- Wild-type NPM1 without FLT3-ITD (normal karyotype)

#### Additional markers:
- ASXL1, RUNX1, TP53

### Intermediate/Adverse Risk Profile
- Mutated NPM1 and FLT3-ITD (normal karyotype)
- Wild-type NPM1 and FLT3-ITD (normal karyotype)
- Wild-type NPM1 without FLT3-ITD (normal karyotype)

#### Intermediate/Adverse Risk Profile:
- Hematopoietic stem cell transplant
- Chemotherapy

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**Table 5. 2017 ELN risk stratification by genetics**

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Genetic abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>t(8;21)(q22;q22.1); RUNX1-RUNX1T1; inv(16)(p13.1;q22) or (16;16)(p13.1;q22); CBFB-MYH11; Mutated NPM1 without FLT3-ITD or with FLT3-ITD; Biallelic mutated CEBPA</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Mutated NPM1 and FLT3-ITD (normal karyotype)</td>
</tr>
<tr>
<td>Adverse</td>
<td>Wild-type NPM1 without FLT3-ITD or with FLT3-ITD; Cyto genetic abnormalities not classified as favorable or adverse</td>
</tr>
</tbody>
</table>

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Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel

Hartmut Döhner,1 Elihu Estey,2 David Grimwade,3 Sergio Amadori,4 Frederick R. Appelbaum,2 Thomas Büchner,5 Hervé Dombret,6 Benjamin L. Ebert,7 Pierre Fenaux,6 Richard A. Larson,9 Ross L. Levine,10 Francesco Lo-Coco,4 Tomoki Naoki,11 Dietger Niederwieser,12 Gert J. Ossenkoppele,13 Miguel Sanz,14 Jorge Sierra,15 Martin S. Tallman,10 Hwei-Fang Tien,16 Andrew H. Wei,17,18 Bob Löwenberg,19 and Clara D. Bloomfield20

Table 2. WHO classification of myeloid neoplasms with germ line predisposition and guide for molecular genetic diagnostics

<table>
<thead>
<tr>
<th>WHO classification</th>
<th>Guide for molecular genetic diagnostics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid neoplasms with germ line predisposition without a preexisting disorder or organ dysfunction</td>
<td>Myelodysplastic predisposition/acute leukemia predisposition syndromes&lt;br&gt;Cancer predisposition syndromes§&lt;br&gt;Li Fraumeni syndrome (TP53)&lt;br&gt;Germ line BRCA1/BRCA2 mutations&lt;br&gt;Bone marrow failure syndromes&lt;br&gt;Dyskeratosis congenita (TERC, TERT)&lt;br&gt;Fanconi anemia</td>
</tr>
<tr>
<td>AML with germ line CEBPA mutation</td>
<td>CEBPA, DDX41, RUNX1, ANKR266, ET6, GATA2, SRP72, 14q32.2 genomic duplication (ATG2B/GSK3P)</td>
</tr>
<tr>
<td>Myeloid neoplasms with germ line DDX41 mutation†</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms with germ line predisposition and preexisting platelet disorders</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms with germ line RUNX1 mutation†</td>
<td></td>
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<tr>
<td>Myeloid neoplasms with germ line ANKRD26 mutation†</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms with germ line ET6 mutation†</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms with germ line predisposition and organ dysfunction</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms with germ line GATA2 mutation</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms associated with bone marrow failure syndromes</td>
<td></td>
</tr>
<tr>
<td>Juvenile myelomonocytic leukemia associated with neurofibromatosis, Noonan syndrome, or Noonan syndrome-like disorders</td>
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<tr>
<td>Myeloid neoplasms associated with Noonan syndrome</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms associated with Down syndrome†</td>
<td></td>
</tr>
</tbody>
</table>

Döhner et al. Blood January 2017
Clinical NGS: Technical Considerations
Test Performance/Quality

MM09-A2
Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline—Second Edition

Metrics

- Accuracy
- Precision
- Limit of Detection
- Analytical specificity
- **Coverage**
  - Read depth
  - Completeness
TruSight ® Myeloid Sequencing Panel (Illumina)

54 genes: ABL1, ASXL1, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CSF3R, CUX1, DNMT3A, ETV6/TEL, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2, JAK3, KDM6A, KIT, KRAS, MLL, MPL, MYD88, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2
THE ROLE OF FLT3 IN NEOPLASTIC MALIGNANCIES

Denis L. Stennikoff and Jared M. Riddell

Normal hematopoietic cells are complex systems to control proliferation, differentiation, and cell death. The control of proliferation in particular, is accomplished through the ligand-induced stimulation of receptor tyrosine kinases, which signal downstream effector mechanisms through the Ras/Erk pathway. Recently, mutations in the FLT3 androgen receptor tyrosine kinase (FLT3) gene, which encodes a receptor tyrosine kinase, have been found to be the most common genetic lesion in acute myeloid leukemia (AML), occurring in 50% of cases. Exploiting the mechanism by which FLT3 mutations can cause uncontrolled proliferation of hematopoietic stem cells has been an active area of research for the development of new drugs.

The FLT3 receptor kinase is a cytokine receptor tyrosine kinase (RTK) that has a crucial role in normal hematopoiesis. Recently, FLT3 mutations have been found in patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and some myelodysplastic syndromes (MDS), making FLT3 an attractive target for the treatment of hematopoietic malignancies. A flurry of research has been conducted over the last few years to understand the structure and function of FLT3 and how they affect clinical outcomes, but many questions remain unanswered. The absence of effective therapy from clinical trials directed at FLT3 has led to the development of FLT3 inhibitors as potential therapeutic agents. One such promising agent, midostaurin (midostaurin),

2006

Nucleophosmin and cancer

Sylvia Gerstung, Cristino Meurell, Bhumanghol Faiyaz and Pietro Lisanti

Altunç (NPM-1) is a crucial gene to consider in the context of the genetics and biology of cancer. NPM-1 is frequently mutated in neuroendocrine tumors, small cell lung cancer, and prostate cancer. NPM-1 has been also associated with tumour-suppressive functions. Therefore, NPM-1 could contribute to tumour suppression in many tumour types. The goal of this study is to analyze the role of NPM-1 in cancer and examine how deregulated NPM activity (either gain or loss of function) can contribute to tumourigenesis.

Nucleophosmin (NPM), also known as BCL2, is a nuclear phosphoprotein that is expressed at high levels in the normal myeloid lineage. Significant evidence has been gathered to suggest that NPM-1 is involved in nuclear transport and localization, as well as in the regulation of cell cycle progression and apoptosis. NPM-1 is known to interact with several proteins, including p53, a critical regulator of cell cycle arrest and apoptosis. The aberrant expression of NPM-1 has been associated with poor clinical outcomes in various cancers, suggesting a role in tumourigenesis.

2004

C/EBPα MUTATIONS IN ACUTE MYELOID LEUKAEMIAS

Claud Nef

Specific mutations in the DNA-binding domain of the CCAAT enhancer binding protein alpha (C/EBPα) are frequently associated with acute myeloid leukaemias. Several alternative subtypes of the factor C/EBPα are transcribed in haematopoietic cells.

C/EBPα in normal physiology

C/EBPα mutations are characterized by a block in differentiation and/or maturation of myeloid cells. The role of C/EBPα in hematopoiesis is complex, involving the regulation of numerous genes that are critical for the development and function of different hematopoietic lineages. The disruption of C/EBPα function can lead to myeloid leukemia, which is characterized by the overexpression or underexpression of specific genes. C/EBPα mutations are associated with increased leukemia risk and are often observed in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). C/EBPα mutations are also implicated in the development of myelodysplastic syndromes (MDS), which can progress to acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL).

C/EBPα mutations in hematopoietic malignancies

C/EBPα mutations are common in hematopoietic malignancies, including acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). These mutations are associated with increased risk of relapse and decreased survival. C/EBPα mutations are often found in combination with other genetic alterations, such as rearrangements of the FLT3 gene, which encodes a receptor tyrosine kinase. The FLT3 gene is frequently mutated in AML, and these mutations are associated with a poor prognosis. C/EBPα mutations are also found in other hematopoietic malignancies, including chronic myeloid leukemia (CML) and myelodysplastic syndromes (MDS). C/EBPα mutations are also associated with the development of secondary leukemia following myeloid malignancies.

C/EBPα in hematopoietic malignancies

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Peculiarities of the Genes & Mutations

- FLT3 mutations:
  - Long insertions (internal tandem duplications/ITDs) - >100 base pairs (bp)
  - Point mutations (D835)

- NPM1 mutations: 4 base pairs (bp) insertions

- CEBPA: GC-rich (~66%)
Peculiarities of the Genes & Mutations

- FLT3 mutations:
  - Long insertions (internal tandem duplications/ITDs) - >100 base pairs (bp)
  - Point mutations (D835)
- NPM1 mutations: 4 base pairs (bp) insertions
- CEBPA: GC-rich (~66%)
Case

- 33 year old male patient
- Diagnosed with AML, subtype M2 (with maturation)
- Cytogenetics: del(9)(q13q22)
- Flow cytometry: AML, suggestive of clonal evolution from a myelodysplastic syndrome
- Conventional mutational analysis:
  - FLT3 – no mutation
  - NPM1 – no mutation
  - CEBPA – double mutations
Diagnosed with AML-M2 (Vietnam)

Mar15

Induction Chemotherapy D + A

1Apr15 10Apr15

#1 HiDAC

21May15

MRD 0.8%

#2 HiDAC

19Jun15 6Aug15

#3 HiDAC

7Aug15 28Sep15

MRD 0.2%

Buccal Sample NGS

23Nov15

Sanger sequencing + Targeted NGS (BM; AD594c)

MRD 0.06%

Sanger sequencing + Targeted NGS (BM; AD594a)
Pelgeroid neutrophils observed (BMA; Dr Grace Moshi)
CEBPA Mutations: Pre-induction

c.134dupC

c.937_938ins (33bp)
CEBPA Mutation(s): Post-induction

c.134dupC

c.937_938ins (33bp)
Targeted NGS

- Targeted 54 gene NGS performed on AML and germline sample
- CEBPA mutations were not identified
Corresponding genomic coordinate for CEBPA c.134dupC mutation
Coverage is a problem
The ‘GC Effect’

Summarizing and correcting the GC content bias in high-throughput sequencing

Yuval Benjamin* and Terence P. Speed

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Received August 1, 2011; Revised November 16, 2011; Accepted December 20, 2011

ABSTRACT

GC content bias describes the dependence between fragment count (read coverage) and GC content found in Illumina sequencing data. This bias can dominate the signal of interest for analyses that focus on measuring fragment abundance within a genome, such as copy number estimation (DNA-seq). The bias is not consistent between samples; and there is no consensus as to the best methods to remove it in a single sample. We analyze regularities in the GC bias patterns, and find a consistent description for this unimodal curve family. It is the GC content of the full DNA fragment, not the sequenced read, that most influences fragment count. This GC effect is unbiased both GC-rich fragments and AT-rich fragments are underestimated in the sequencing results. This empirical evidence strengthens the hypothesis that PCR is the most important cause of the GC bias. We propose a model that produces predictions at the base pair level, allowing systematic GC-effect correction regardless of the downstream mapping or filtering. These modeling considerations can inform other high-throughput sequencing analyses such as ChiP-seq and RNA-seq.

INTRODUCTION

Since it was introduced, Illumina Genome Analyzer high-throughput sequencing has become an increasingly popular technology for determining relative abundance of DNA. As a result, the DNA of interest is fragmented and one or both of the fragments sequenced. These sequencing short ends, or paired ends, are aligned to a reference genome. Counts of aligned fragments may be used to measure DNA copy number (DNA-seq), protein expression (ChIP-seq) or

METHOD

Analyzing and minimizing PCR amplification bias in Illumina sequencing libraries

Daniel And, Michael G. Ross, Wei-Sheng Chen, Maxwell Danielson, Timothy Ferrer, Carsten Raus, David J. Attie, Chad Noltebaum, and Andreas Grotke

Background

The Illumina sequencing platform [1], like other massively parallel sequencing platforms, continues to produce ever-increasing amounts of data, yet suffers from under-representation and reduced quality at loci with extreme base compositions that are resistant to the technology [1-6]. Uneven coverage due to base composition nonsequencing to excessively high coverage for GC-rich genomic sequences [7-10] for sensitive polymerase discovery [8,9]. Although loci with extreme base composition constitute only a small fraction of the human genome, they include biologically and medically relevant sequence targets. For example, 10% of the first 135 coding bases of the retinoblastoma tumor suppressor gene RB1 are GC-rich.

Traditional Sanger sequencing has long been known to suffer from problems related to the base composition of sequencing templates. GC-rich stretches led to compromised reads. Polarity error in poly(A) reads and AT dinucleotide repeats caused missing sequencing ladders and poor read quality. Presence of the actual sequencing read blocks, or “donut” sequence, against inverted repeats, extreme base compositions, or genes not tolerant of the bacterial cloning host. Gaps due to undetected sequences had to be recovered and finished by PCR [10], or, in some cases, by resorbing to alternative host [11]. Cloning bias hindered efforts to sequence the AT-rich genomic DNA and Plasmodium [12] and excluded exons of about 10% of protein-coding genes [13] in the next generation sequencing technology (NGS). To overcome the reduced performance of NGS, the use of long read sequencing strategies has been proposed.

Annotating GC content of amplicon (%)
**TruSight Myeloid Panel**
- 54 genes
- 568 amplicons
- CEBPA – 6 amplicons

**CEBPA**
- Tumor suppressor
- Very GC rich (~66%)
“How Much Is Enough? is a delightful book. It addresses a Big Question without the jargon and obfuscation that pollutes so much philosophy. The prose is lucid, and all the relevant issues are raised and addressed.”
—Wall Street Journal

HOW MUCH IS ENOUGH?
Money and the good life

WITH A NEW AFTERWORD BY THE AUTHORS

ROBERT SKIDELSKY
and
EDWARD SKIDELSKY
Oncology – Molecular and Cellular Tumor Markers

“Next Generation” Sequencing (NGS) guidelines for somatic genetic variant detection

The following describes requirements for the development of procedures and the establishment of performance (validation) of assays for the detection of somatic genetic variants by Next Generation massively parallel sequencing (NGS) technologies. These requirements should be used in conjunction with and not in lieu of the existing molecular oncology guidelines (http://www.wadsworth.org/labcert/TestApproval/forms/Oncology_Molecular_Checklist.pdf). Overall, clinical validation of NGS assays follows the same basic principles for validating most other complex molecular diagnostic procedures. It is anticipated that these guidelines will evolve as the field matures and gains experience. Please make sure you use the most up-to-date version of these guidelines. Issues that must specifically be addressed include:

SOP:
- must include step-by-step description of all steps involved, from template to library preparation to data analysis and interpretation
- must include indication for the specific procedure(s) used for confirmation testing, including criteria for when confirmation must be performed

QC:
- Quality of the base scoring must meet a minimum of Q20 or equivalent per base.
- Establish minimum criteria for depth and uniformity of coverage, i.e. number of reads, across all target areas.
  A minimum average of 500 reads or greater is strongly recommended.
- Define the minimum coverage required for a target area (such as amplicon, exon) below which you
Definition of Lower-performance amplicons:

Average read depth < 500x across 158 samples

16 amplicons meet this criteria
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<th>Chr</th>
<th>Gene</th>
<th>Genomic coordinates</th>
<th>Amplicon</th>
<th>GC content</th>
<th>Mean read depth</th>
<th>Samples with zero coverage (%)</th>
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</table>
Amplicon read depths for the lower-performance amplicons

Versus

Mean amplicon read depth per sample

Individual patients (n=158)

Lowest mean amplicon read depth: 2250x

Mean read depth per amplicon against GC content for 568 amplicons

\[ y = -23467x + 20820 \]

\[ R^2 = 0.2214 \]
TruSight Myeloid Sequencing Panel

- Hybridization of Oligo Pools
- Extension-Ligation of Bound Oligos
- PCR Amplification
- PCR Clean-up + Library Normalization
- Library Pooling for MiSeq Sequencing

**CEBPA-specific Nextera XT Sample Prep**

- Tagmentation of *CEBPA* Amplicons
- Reduced-cycle PCR Amplification

57 TruSight Myeloid libraries (8 samples) : 1 CEBPA-specific Nextera XT libraries (8 samples)
Genomic Case Studies
Case 1

- 27 year old female
- Known case of hereditary thrombocytopenia (germline RUNX1 mutation)
- Family history of thrombocytopenia
<table>
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<tr>
<th>Genomic Coordinates</th>
<th>Gene</th>
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<th>HGVS p</th>
<th>Variant Allele Frequency (%)</th>
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Germline *RUNX1*-mutant AML
PRELEUKEMIC ACUTE HUMAN LEUKEMIA

Matthew Block, M.D., Ph.D., Leon O. Jacobson, M.D.
and William F. Betiard, M.D., Chicago

During the past three years we have had the unusual opportunity to observe 12 patients for as long as 27 months prior to the development of acute myelogenous or stem cell leukemia. During the preleukemic phase, the transitional phase to acute leukemia, as well as thereafter until death, their clinical courses were recorded, frequent complete peripheral blood cell counts were made, and serial biopsies of the bone marrow and, in some cases, biopsies of liver and spleen were obtained.

Interest in the preleukemic phase of human acute leukemia has been heightened by recent studies in induced mouse leukemia. In both humans and mice, the disease is usually rapidly fatal, with only short remissions sometimes produced by steroid or antilulcer acid therapy. In the mouse, however, treatment with cortisone in the preleukemic phase of the disease has been shown to decrease the incidence of leukemia induced by X-ray and possibly that induced by methylcholanthrene.

This study is presented because it concerns the preleukemic phase of human acute leukemia, knowledge of which is at best fragmentary, and because studies on mouse leukemia have pointed to the possibility of a therapeutic approach to what is still a rapidly and uniformly fatal disease. This paper emphasizes the clinical course of various patients. In a companion publication, emphasis will be placed on the histogenetic of human acute leukemia.

It was sometimes difficult to determine the exact time at which a particular patient might be classified as being leukemic. One might object to a concept that differentiates a preleukemic from a leukemic phase in a patient who dies of leukemia on the grounds that the patient should be considered as having leukemia from the onset of symptoms closely related to his final illness. As already mentioned, the recent studies on mouse leukemia point to a possible difference between what we choose to call the preleukemic and the leukemic phase. Furthermore, there are clinical grounds on which this differentiation may be made. In the preleukemic phase, the characteristic enlargement of any of the hematopoietic tissues, except in two patients, was not demonstrable. In our experience, as well as in that of others, one may encounter middle-aged women with neutrophilia, other patients with refractory anemia or thrombocytopenia, and even those with large numbers of immature cells (including stem cells) in the peripheral blood, in whom leukemia does not develop but who recover from or die of some other disease. Here's to a 

Leukemia 2017

Leukemia: one name, many meanings

HP Koehne, LM and G. Leu definition of preleukemia has evolved. It was first used to describe the myelodysplastic syndrome (MDS) with a propensity to progress to acute myeloid leukemia (AML). Individuals with generalized mutations of either RUNX1, CEBPA, or CALR2 may be so labeled as preleukemic because they have a markedly increased incidence of evolution into AML. Also, alkylating chemotherapy or radiation can cause MDS/preleukemia, which may always progress to AML. More recently, investigators noted that AML patients who achieved complete morphologic remission after chemotherapy often become hematopoietic abnormalities predominantly marked by either DNMT3A, TET2, or RBX1 mutations, which were also present at diagnosis of AML. This preleukemic clone represents involvement of an early hematopoietic stem cells, which is resistant to standard therapy. The same clonal hematopoietic mutations have been identified in older normal individuals who have a modest increased risk of developing frank AML. These individuals have occasionally been described, probably inappropriately, to have a preleukemic clone. Our evolving understanding of the term preleukemia has been acquired by advancing technology, including studies of K chromosome inactivation, cytogenetics, and more recently deep nucleotide sequencing.


PRELEUKEMIA ASSOCIATED WITH MYELODYSPLASTIC SYNDROME

The term ‘preleukemia’ has undergone a major evolution since it was first used in 1953 by Jibol et al. At that time, the term referred to the heterogenous group of hematopoietic disorders associated with a block in myelodysplastic and chronic myeloproliferative neoplasms. We now call these disorders myelodysplastic syndromes (MDS). Prevalence of MDS in all developed countries is approximately 1.5 per 10,000 persons per year. Most of these individuals do not progress to acute leukemia (AML) and, thus, these individuals might justifiably be known as leukaemias.

In the early 1960s and 1970s, one of the prominent advances in our understanding of MDS/AML was the concept that the MDS/AML leukemic event occurred in stem cell, which had a hematopoietic growth advantage causing a clonal population of MDS/AML leukemic cells. This insight was based on the observation of X-chromosome inactivation in females. Females have two X chromosomes: between the 4th and 16th cell stage of blasts, one of the X chromosomes is inactivated and the other remains active. A number of methods have been used to elucidate the inactivation of the X chromosome. This technique allows the identification of all females as opposed to a small population of predominant AML cells with G6PD deficiency. This preleukemia group showed clonal hematopoietic mucosa, occurring in a number of hematopoietic disorders including chronic myelogenous leukemia, acute lymphocytic leukemia and AML. Allo, Pajonk noted that in many cases of AML the clonality occur in the myeloid compartment and not the lymphoid compartment. MDS, in contrast, appeared to have an identical clonality of both the myeloid and lymphoid compartments, albeit the myeloid compartment appeared to have a major growth advantage. This suggests that the MDS clone initiated in a very early hematopoietic stem cell.

With the use of exome parallel nucleotide sequencing, we have a clear idea of the major mutations that affect patients with MDS, who evolve from MDS to AML (secondary AML MDS) and those with de novo AML (Table 1). In the latter cases, more occur frequently in MDS than in de novo AML. However, the inactivation of these cases is frequently in de novo AML. These mutations are involved in ineffective myelodysplastic hematopathy, not frank AML. Mutations that are often observed in de novo AML are known as Driver or non AML mutations including mutations of NPM, TET2, DNMT3A, IDH1, IDH2, U2AF, U2AF1 and MLL. These Driver mutations produce a major block in differentiation providing a proliferative advantage to these clones.

Leukemia 2017

JAMA 1953
Preleukemia

- Oligoclonal hematopoiesis
- Germline preleukemia
- Therapy-related preleukemia
- Preleukemia in morphologic CR after AML chemotherapy

Koeffler & Leong; Leukemia 2017
Case 2

- 35 year old female
- Diagnosed with AML (M4/5)
  - FLT3 – Mutation (108 bp insertion) present
  - NPM1 – Mutation present
- Complete morphological remission after induction chemotherapy
- Underwent allogeneic stem cell transplant
- Relapsed two months after transplant
- Targeted NGS performed on both the primary and relapsed AML samples:
  - FLT3 ITD present
  - NPM1 insertion present
  - DNMT3A R882C present
Prognostic impact of DNMT3A status in local cohort

- Studied DNMT3A mutations in 157 patients
- Correlated DNMT3A status with clinical outcome in 114 patients
DNMT3A mutations

- 33/157 (21%) patients had DNMT3A mutations
- 22 different mutations were identified
  - 15 missense (R882H and R882C most common)
  - 1 nonsense
  - 6 frameshift
‘Triple-positive’ AMLs

Figure 2: Kaplan-Meier curves for overall survival by mutational status.

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<th>Number at risk</th>
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Papaemmanuil et al. NEJM 2016

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