Leukemia Diagnostics and Therapy: On the Way to Personalized Medicine

Torsten Haferlach, Munich Leukemia Laboratory
Incidence of leukemia (SEER data)

Cytomorphology: Phenotypes
One Metaphase captured
Karyogram: 46,XY,t(9;22)(q34;q11)
The *BCR-ABL1* rearrangement

Chromosome 9 Chromosome 22  
Chromosome 9 Chromosome 22

**BCR-ABL1-**

Chromosome 9 Chromosome 22  
9q+  
22q-

translocation

**BCR-ABL1+**
Sanger Sequencing

R-> ABL_F:abl1 Q:0(100):22

S-> 06-10677_F:abl1 Q:0(100):30

S<-> 06-10677_F:abl1 Q:0(100):20

R<-> ABL_R: abl1 Q:0(100):15

1223t>a

ttc>atc

F359I / Phe359Iso
NGS read out
Why more Genomics is needed

- Improve Diagnosis
- Specify Classification
- Define Prognosis
- Allow individualized treatment
- Offer MRD strategies
- Reduce toxicity
- Increase cure rate
- Develop new drugs
THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

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

Daniel A. Arber,1 Attilio Orazi,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

THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

The 2016 revision of the World Health Organization classification of lymphoid neoplasms

Steven H. Swerdlow,1 Elias Campo,2 Stefano A. Pileri,3 Nancy Lee Harris,4 Harald Stein,5 Reiner Siebert,6 Ranjana Advani,7 Michele Ghielmini,8 Gilles A. Salles,9 Andrew D. Zelenetz,10 and Elaine S. Jaffe11
Genes in WHO 2016

n = 78
Molecular Markers

- Spliceosome
  - TET2
  - ASXL1
  - IDH2
  - PHF6
- Ser/Thr kinases
- RUNX1
  - KRAS/NRAS
  - TP53
- FLT3
- NPM1
- Cohesin
- PTPs
- IDH1
- DNMT3A
- MLL-X Fusions
- MYH11-CBF
- RUNX1-RUNX1T1
- PML-RARA
- KIT
- Other Tyk kinases

Unfavorable Cytogenetics
Intermediate Cytogenetics
Favorable Cytogenetics

TCGA, NEJM, 368, 2059-2074, 2013
Molecular Markers = Targets in AML

Unfavorable Cytogenetics

- ASXL1
- IDH2
- PHF6

Intermediate Cytogenetics

- RUNX1
- KRAS/NRAS
- TP53

Favorable Cytogenetics

- FLT3
- NPM1
- FLT3
- PML-RARA
- RUNX1-RUNX1T
- MYH11-CBF
- MLL-X Fusions

Spliceosome

- TET2
- Ser/Thr kinases

ACE-536

AG-120

AG-221

Sunitinib
Lonafarnib

Sorafenib
Midostaurin
Quizartinib
Lestaurtinib

Selinexor
LGH447
Idasanutlin
EPZ-5676
OTX015
CPX-315
Valproic Acid
Lenalidomid
Azacitidine
Oral Azacitidine
Decitabine
Guadecitabine
MEDI-4736
Everolimus
Lintuzumab
Gemtuzumab Ozogamicin
Ulocuplumab
Elacytarabine
Vosaroxin
Sapacitabine

CPX-315

MIDAS

ATO

ATRA

Midostaurin

Dasatinib

Palbociclib

Zolmitriptan
Mutations in MDS

Tyrosin Kinase Pathway
- JAK2
- NRAS
- CBL
- PTPN11
- KRAS
- BRAF
- RTK

Transcription Factors
- RUNX1
- ETV6
- GATA2
- EP300
- WT1
- PHF6

others
- TP53
- NPM1
- BCOR
- GNAS/GNB1
- RNA Helikase
- Cohesin

Epigenetic Regulation
- IDH 1 & 2
- DNMT3A
- EZH2
- DCA
- ASXL1
- TET2
- UTX
- ATRX
- SETBP1

Splicing
- SF3B1
- U2AF1
- ZRSF2
- SRSF2
- SF3A1
- SF1
- U2AF2
- PRPF40B
- PRPF8
- U2AF2

(adapted from G. Mufti)
Therapy Results in CML

Survival with CML over time
The German CML-Study Group experience 9/2014

![Graph showing survival with CML over time for different treatments and time periods.](image)

- Imatinib, 2002 – 2012 (CML IV)
  - 5-year survival 90%
  - 10-year survival 84%
- IFN or SCT, 1997 – 2003 (CML IIIA)
  - 5-year survival 71%
  - 10-year survival 61%
- IFN or SCT, 1995 – 2001 (CML III)
  - 5-year survival 63%
  - 10-year survival 48%
- IFN, ± HU, 1986 – 1994
  - 5-year survival
  - 5 year survival 44%
  - 10 yr surv. 18%
- Busulfan, 1983 – 1994
  - 5-year survival 38%
  - 10-year survival 11%

German CML Study Group, update 2014

Hehlmann et al., Ann Hematol, 2015
MLL Hem-Panel

myeloid
- ASXL1
- ASXL2
- BCOR
- BCORL1
- CALR
- CBL
- CSF3R
- CSNK1A1
- DNMT3A
- ETV6
- ETV6
- EZH2
- FLT3-TKD
- GATA1
- GATA2
- IDH1
- IDH2
- JAK2
- KIT
- KRAS
- MPL
- MYC

lymphatic
- ATM
- BCL2
- BIRC3
- BRAF
- BTK
- CXCR4
- EGR2
- FBXW7
- FOXO1
- ID3
- KLF2
- MAP2K1
- MYD88
- NFkBIE
- NOTCH1
- NOTCH2
- PHF6
- PLCG2

- NF1
- NPM1
- NRAS
- PIG-A
- PTPN11
- RAD21
- RUNX1
- SETBP1
- SF3B1
- SRSF2
- STAG2
- TET2
- U2AF1
- WT1
- ZRSR2

\[ \sum = 63 \text{ genes; } \sim 850 \text{ amplicons} \]
NGS Data Analysis pipeline (MLL)

1. LIMS
   - Plan Run Layout and prepare samples
     - DNA Isolation
     - DNA quantification
     - PCR

2. High Performance Compute Cluster

3. Data Analysis

4. Quality Management
   - Amplicon Check
   - Coverage Reports
   - Sequencing performance

5. Mutation Analysis

6. Final report

7. Web App / Database

8. Lab data
   - ClinVar
   - IARC
   - dbSNP
   - COSMIC
   - Mutation Taster
   - Polyphen
   - SIFT
Genome Aggregation Database, Broad Institute

- Aggregation of **123,126 exome data** and **15,496 genome data**. Coverage information as well as frequency of variants. Blend of database and computational approach.

**RUNX1**

<table>
<thead>
<tr>
<th>Project</th>
<th>Institution</th>
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<tbody>
<tr>
<td>1000 Genomes</td>
<td>EMBL, EU - 2008</td>
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<tr>
<td>ESP – Exome Sequencing project</td>
<td>NHLBI, USA - 2011</td>
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<tr>
<td>ExAC – Exome Aggregation Consortium</td>
<td>Broad Institute - 2014</td>
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</table>
Munich Leukemia Laboratory Selects IBM Watson and Illumina for Research Collaboration to Advance Diagnostics and Develop Personalized Treatment Tools for Leukemia & Lymphoma

Watson Takes on Hematology Research in First Collaboration in Germany; Effort Marks Illumina’s First European Adopter of NovaSeq™
• Pave cure in leukemia:
  - improve diagnostic accuracy
  - refine classification
  - guide targeted treatment
  - improve minimal residual disease testing
  - accelerate drug development

• Automize lab infrastructure via IoT:
  - define and combine best instruments
  - improve software and read out for results
  - reduce TAT
  - increase quality of reports, incl. treatment
<table>
<thead>
<tr>
<th>Camera</th>
<th>Megapixel</th>
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<tbody>
<tr>
<td>Cool Cube 1 C: MF</td>
<td>1,4</td>
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<tr>
<td>MKC-210HD Z: Microscope</td>
<td>2,1</td>
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<tr>
<td>Gryphax FISH</td>
<td>8</td>
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<tr>
<td>iPhone 7</td>
<td>12</td>
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Filter cards

Spots cutted for extraction of nucleic acid

Peripheral blood

Bone marrow

T. Haferlach et al., Leukemia, 30, 2123-2125, 2016
Comprehensive mutation screening

- 98.2% (213/217) concordance of results
- High correlation of mutation loads, e.g. *NPM1* (r=0.840, p<0.001)

T. Haferlach et al., *Leukemia*, 30, 2123-2125, 2016
# Sequencing Capabilities in MLL

<table>
<thead>
<tr>
<th>Key Methods</th>
<th>Miniseq</th>
<th>MiSeq</th>
<th>NextSeq</th>
<th>HiSeq</th>
<th>NovaSeq</th>
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</thead>
<tbody>
<tr>
<td>Amplicon and targeted gene</td>
<td>Amplicon and targeted gene panel sequencing</td>
<td>Whole exome and targeted resequencing</td>
<td>Population scale whole-genome sequencing</td>
<td>Population scale whole-genome sequencing</td>
<td>Population scale whole-genome sequencing</td>
</tr>
<tr>
<td>panel sequencing</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Maximum Output</td>
<td>7.5 Gb</td>
<td>15 Gb</td>
<td>120 Gb</td>
<td>1500 Gb</td>
<td>6000 Gb</td>
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<tr>
<td>Reads</td>
<td>25 million</td>
<td>25 million</td>
<td>400 million</td>
<td>5 billion</td>
<td>20 billion</td>
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<tr>
<td>Read Length</td>
<td>2 × 150 bp</td>
<td>2 × 300 bp</td>
<td>2 × 150 bp</td>
<td>2 × 150 bp</td>
<td>2x 150bp</td>
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<tr>
<td>Run Time</td>
<td>4–24 hours</td>
<td>4–55 hours</td>
<td>12–30 hours</td>
<td>7 hours–6 days</td>
<td>19 – 40 hours</td>
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<tr>
<td>Amplicon</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>WES (100x)</td>
<td>X</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>WGS (100x)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>✔</td>
<td>✔</td>
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<tr>
<td>RNA</td>
<td>Targeted</td>
<td>Targeted</td>
<td>Transcriptome</td>
<td>Transcriptome</td>
<td>Transcriptome</td>
</tr>
</tbody>
</table>
NGS in Large Scale Modus I
First HiSeqX run in MLL: 28.03.17
NGS in Large Scale Modus II

- 40h run time
- 6 Terabases data yield with 20 billion reads
- Scalable (4 flow cells)
- Any application:
  - Whole Genome
  - Whole Exome
  - Targeted (Amplicons, MRD)
  - Transcriptome
  - Targeted RNA
First Novaseq run in EU: 24.04.17
Landscape of Translational Genomics

Patient specific Dx + individual treatment + recommended MRD marker
Automated workflows = IoT for NGS

- Cell lyses
- Storage
- DNA/RNA extraction
- Library prep
- Storage
- Concentration
- Quality check
- Sequencing

- Processes linked to intranet, assembling data in LIMS
- Integrated reporting of all methods
- Sample tracking for internal use
- Sample tracking for external use
  - when will be reported
  - when to date the patient
  …
A Single Blood Test For All Cancers? Illumina, Bill Gates And Jeff Bezos Launch Startup* To Make It Happen