Molecular Minimal Residual Disease Detection in Acute Myeloid Leukemia

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Acute Myeloid Leukemia (AML)

Heterogeneous Clonal Disease

Morphology
Immunophenotype
Cytogenetics
Molecular Genetic Aberrations
Treatment Response
Treatment Outcome
**ELN recommendations AML 2017**

- **RUNX1-RUNX1T1**
- **CBFB-MYH11**
- **FLT3-ITD**
- **NPM1** mutation
- Bi-allelic **CEBPA** mutations

**Cytogenetics**

- **ASXL1** mutation
- **RUNX1** mutation
- **TP53** mutation

**HOVON-SAKK**

- **RUNX1-RUNX1T1**
- **CBFB-MYH11**
- **FLT3-ITD**
- **NPM1** mutation
- Bi-allelic **CEBPA** mutations
  
  **EVI1** overexpression

**Cytogenetics**

- **ASXL1** mutation
- **RUNX1** mutation
- **TP53** mutation
- **KIT** mutation
Is there a role for molecular MRD detection in risk stratification of AML?

Molecular minimal residual disease (MRD) monitoring based on single molecular targets provides powerful prognostic information.

Can we further improve risk stratification of AML by MRD detection based on next generation sequencing (NGS)?
Molecular Minimal Residual Disease Detection in Acute Myeloid Leukemia

Molecular minimal residual disease (MRD) monitoring recommended (ELN):

Acute Promyelocytic Leukemia (APL)

*PML-RARA*: Change from undetectable to detectable RT-PCR heralds disease relapse

Core-binding factor leukemias (AML)

*AML1-ETO*

*CBFB-MYH11*: Undetectable MRD by RT-PCR has better outcomes and lower risk of relapse (incl. concomitant mutations)
The presence of minimal residual disease, as determined by quantitation of mutant NPM1 transcripts, is a stable, reliable and independent prognostic factor for relapse and survival in AML.
Can we improve the predictive value of mutant \textit{NPM1} MRD by considering the \textit{FLT3}-ITD status at diagnosis?

\textbf{Induction cycle I}
- Ida 12 mg/m$^2$
- Ara-C 200 mg/m$^2$

\textbf{Induction cycle II}
- AMSA 120 mg/m$^2$
- Ara-C 1000 mg/m$^2$

\textbf{Mutant \textit{NPM1} RQ-PCR 104 ptn}

\textbf{Consolidation}
- Mitoxantrone Etoposide
- auto-HSCT
- \textit{allo}-HSCT
- \textbf{Poor /Very Poor}
- \textbf{Intermediate}
- \textbf{Good risk}

HOVON102 1st line AML \(\leq 65\) years
Mutant *NPM1* MRD is associated with a higher risk of relapse

Mutant *NPM1* MRD

Mutant *NPM1* MRD

**SHR 2.55** (95%CI 1.29-5.00)  
Gray’s test *p*=0.007


Mutant *NPM1* MRD and *FLT3*-ITD status define risk of relapse

Mutant *NPM1* MRD

- SHR 2.60 (95%CI 1.28-5.31)  
  Gray’s test p=0.009

*FLT3*-ITD

- SHR 2.31 (95%CI 1.17-4.57)  
  Gray’s test p=0.016

HOVON132 / SAKK 30/13 phase III study in AML/RAEB

**Ara-C 200mg/m2 d1-7c.i.**
Idarubicin 12 mg/m2 3-hr d1-3

**Ara-C 1000mg/m2 3-hr bid d1-6**
Daunorubicin 60 mg/m2 iv d1, 3, 5

**Ara-C 200mg/m2 d1-7c.i.**
Idarubicin 12 mg/m2 3-hr d1-3
Lenalidomide days 1-21

**Ara-C 1000mg/m2 3-hr bid d1-6**
Daunorubicin 60 mg/m2 iv d1, 3, 5
Lenalidomide days 1-21

MRD detection
mutant NPM1 and leukemia associated immunophenotype (LAIP)

**autoHSCT**
- Lenalidomide
+ Lenalidomide

**alloHSCT**
- Lenalidomide
+ Lenalidomide
Can we further improve risk stratification of AML by MRD detection based on next generation sequencing (NGS)?

Multiple molecular markers
### Targeted sequencing
#### Illumina Trusight Myeloid panel

<table>
<thead>
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<th>Gene 1</th>
<th>Gene 2</th>
<th>Gene 3</th>
<th>Gene 4</th>
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<tbody>
<tr>
<td>ABL1</td>
<td>DNMT3A</td>
<td>KDM6A</td>
<td>RAD21</td>
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<tr>
<td>ASXL1</td>
<td>ETV6/TEL</td>
<td>KIT</td>
<td>RUNX1</td>
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<td>EZH2</td>
<td>KRAS</td>
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<td>GNAS</td>
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<td>IDH1</td>
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<td>CUX1</td>
<td>JAK3</td>
<td>PTPN11</td>
<td>ZRSR2</td>
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</tbody>
</table>

Targets 54 mutations frequently present in myeloid malignancies (AML, MDS, MPN, CML, CMML and JMML)

Variant calling with in-house bioinformatic pipeline

Software including:
- Samtools
- Varscan
- Mutec
- Indelocator
- Pindel
Not all markers reliably detected with NGS (eg. FLT3-ITD and CEBPA mutations)

94% of all patients carry a molecular marker (incl. RUNX1-RUNX1T1 and CBFB-MYH11)

Average of 2.9 markers/ AML (min1/max8)
Can we further improve risk stratification of AML by NGS MRD detection with multiple markers?

HOVON102 1st line AML ≤ 65 years

**Induction cycle I**
- Ida 12 mg/m²
- Ara-C 200 mg/m²

**Induction cycle II**
- AMSA 120 mg/m²
- Ara-C 1000 mg/m²

**Consolidation**
- Good risk
  - Mitoxantrone Etoposide
  - auto-HSCT
  - allo-HSCT

- Intermediate
  - auto-HSCT
  - allo-HSCT

- Poor /Very Poor
  - allo-HSCT

**NGS**

- ABL1
- DNMT3A
- IDH1
- IDH2
- RAD21
- ASXL1
- ETV6/TEL
- KIT
- RUNX1
- ATRX
- E2F2
- Kras
- SETBP1
- BCR
- FBXW7
- MLK
- SF3B1
- BCR/ABL1
- FLI1
- MLL
- SMARCA
- BCL2
- GATA1
- MYB
- SMC3
- CEBPα
- GATA2
- NOTCH1
- SRSF2
- CEP51
- GNAS
- NPM1
- STAG2
- CEBPA
- IKZF1
- PHF6
- U2AF1
- CSF3R
- JAK2
- PTPN11
- ZRSR2
- BRAF
- GATA1
- MYD88
- SMC3
- CALR
- GATA1
- NOTCH1
- SRSF2
- CBL
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- PHF6
- U2AF1
- CSF3R
- JAK2
- PTPN11
- ZRSR2
- CBL
- GNAS
- NPM1
- STAG2
Methods NGS MRD detection

211 cases AML in complete hematological remission after induction

Mutations at diagnosis known per AML case

Mean coverage (all mutations, after induction): 3357x

Determine the distribution of VAFs in every base pair in all samples after induction (excluding those carrying a mutation at that position at diagnosis)

Statistical test to determine whether a mutation at diagnosis is present above noise after induction (p-value)
Number of mutations at diagnosis and MRD after induction

| DIAGNOSIS | 83 | 75 | 62 | 42 | 39 | 36 | 35 | 24 | 23 | 21 | 21 | 20 | 20 | 19 | 18 | 9 | 8 | 8 | 8 | 7 | 6 | 5 | 5 | 4 | 4 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| AFTER C2  | 3  | 57 | 2  | 5  | 20 | 9  | 10 | 0  | 11 | 2  | 2  | 6  | 2  | 1  | 9  | 1  | 0  | 0  | 3  | 3  | 1  | 0  | 1  | 1  | 1  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  |
**NPM1 mutations are cleared after induction**

- Mutations present at diagnosis
- Mutations present after induction

Mutant *NPM1* is still detectable in 30% of cases by RQ-PCR ($>10^{-4.5}$)
RAS-associated mutations are cleared after induction

- Mutations present at diagnosis
- Mutations present after induction
DNMT3A mutations persist after induction

- Mutations present at diagnosis
- Mutations present after induction

76% of DNMT3A mutations persist after induction at VAFs 0.002 - 0.51
Question

Is NGS MRD predictive for relapse?
Definition of NGS MRD

**DIAGNOSIS**

- Mutation present at diagnosis, present (above noise) after cycle 2: **MRD+**
- Mutation present at diagnosis, absent after cycle 2: **MRD-**

**Induction cycles I and II**

**AFTER CYCLE 2**

- MRD+
- MRD-
Preliminary results: NGS MRD detection all markers

Competing risk: relapse

Logrank P = 0.02

NGS MRD after cycle II
Failure - Comp. Risk: Rel/PD

Preliminary results: NGS MRD detection all markers

Competing risk: relapse

Logrank P = 0.02

NGS MRD after cycle II
Failure - Comp. Risk: Rel/PD
Number of mutations at diagnosis, after induction and after induction with VAF>2.5%

| Gene     | Diagnosis | After C2 | VAF>2.5%+
|----------|-----------|----------|----------
| NPM1     | 83        | 3        | 0        |
| DNM1    | 75        | 57       | 35       |
| NRAS     | 62        | 2        | 0        |
| WT1      | 42        | 9        | 1        |
| TET2     | 39        | 10       | 15       |
| IDH2     | 36        | 11       | 0        |
| RUNX1    | 24        | 2        | 0        |
| FLT3     | 23        | 2        | 1        |
| TKD      | 21        | 2        | 0        |
| ASXL1    | 21        | 9        | 0        |
| KRAS     | 20        | 1        | 0        |
| IDH1     | 19        | 1        | 0        |
| PHF6     | 18        | 0        | 0        |
| KIT      | 8         | 1        | 0        |
| SRSF2    | 8         | 0        | 0        |
| RAD21    | 7         | 2        | 0        |
| BCR      | 5         | 1        | 0        |
| BCOR1    | 5         | 1        | 0        |
| TP53     | 4         | 4        | 0        |
| GATA2    | 4         | 0        | 0        |
| SF3B1    | 3         | 7        | 0        |
| SFP3R    | 3         | 0        | 0        |
| CUX1     | 2         | 0        | 0        |
| CBL      | 2         | 0        | 0        |
| NOTCH1   | 2         | 0        | 0        |
| SETBP1   | 1         | 1        | 0        |
| SMCA3    | 1         | 1        | 0        |
| SMCC3    | 1         | 1        | 0        |

- **Mutations present at diagnosis**
- **Mutations present after induction**
- **Mutations present after induction VAF>2.5%**
Clonal Hematopoiesis of Indeterminate Potential (CHIP)

Features (in healthy individuals):

1. Absence of definitive morphological evidence of a hematological neoplasm

2. Does not meet diagnostic criteria of PNH, MGUS or MBL

3. Presence of a somatic mutation at a VAF > 2% (e.g. DNMT3A, TET2, ASXL1, JAK2, SF3B1, TP53, etc.)
DNMT3A mutations persist at VAF>2.5% after induction

- Mutations present at diagnosis
- Mutations present after induction
- Mutations present after induction VAF>2.5%

47% of DNMT3A mutations persist after induction at VAFs >2.5%

(Klco et al, JAMA 2015)
CHIP mutations frequently persist after induction (VAF>2.5%)

- Mutations present at diagnosis
- Mutations present after induction
- Mutations present after induction VAF>2.5%

![Bar chart showing the number of mutations for different genes.](chart.png)
Definition of NGS MRD excluding CHIP related mutations

**DIAGNOSIS**

- Mutation present at diagnosis, present (above noise) after cycle 2: MRD+
- Mutation present at diagnosis, absent after cycle 2 or CHIP mutation: MRD-

**AFTER CYCLE 2**

- MRD+
- MRD-

Induction cycles I and II

Pre-leukemic cells with CHIP mutation in *DNMT3A*, *TET2* or *ASXL1*
Preliminary results NGS MRD mutant \textit{DNMT3A}, \textit{TET2} or \textit{ASXL1} as single markers

Competing risk: relapse

Mutant \textit{DNMT3A} 

\begin{figure}[h]
\centering
\includegraphics[width=0.3\textwidth]{dnmt3a}
\end{figure}

\textbf{P=0.37}

Mutant \textit{TET2} 

\begin{figure}[h]
\centering
\includegraphics[width=0.3\textwidth]{tet2}
\end{figure}

\textbf{P=0.37}

Mutant \textit{ASXL1} 

\begin{figure}[h]
\centering
\includegraphics[width=0.3\textwidth]{asxl1}
\end{figure}

\textbf{P=0.19}

MRD +

MRD -
Preliminary results NGS MRD mutant *DNMT3A, TET2* and *ASXL1*

Competing risk: relapse

Logrank $P = 0.70$

At risk:

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<th></th>
<th>neg</th>
<th>pos</th>
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<tr>
<td></td>
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<tr>
<td></td>
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<td>14</td>
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<tr>
<td></td>
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</tr>
</tbody>
</table>
Preliminary results NGS MRD without mutant *DNMT3A, TET2* and *ASXL1*

Competing risk: relapse

P < 0.001
Pre-leukemic cells persist after treatment

Persisting pre-leukemic mutations do not predict for relapse, but do these CHIP-related mutations contribute to relapse?
Do CHIP-related pre-leukemic mutations contribute to relapse?

Whole exome sequencing of 31 diagnosis - relapse - T cell trios with variable time to relapse (mean coverage 150) → mutations known in (hematologic) malignancies selected

Targeted resequencing mutations (mean coverage 3800)
Diagnosis - relapse pair: time to relapse 161 days

**DIAGNOSIS**
(20% blasts)

- VAF 45%
  - DNMT3A (G543C)
  - SRSF2 (P95H)
  - TET2 (H1380Y)
  - ROS1 (G2248D)
  - ATP8A1 (R623X)
  - KIT (D816V)

- 46,XY,t(3;21)(q26;q22),del(12)(p12p13)[20]

**RELAPSE**
(27% blasts)

- VAF 39%
  - DNMT3A (G543C)
  - SRSF2 (P95H)
  - TET2 (H1380Y)
  - ROS1 (G2248D)
  - ATP8A1 (R623X)
  - KIT (D816V) (27%)

- 46,XY,t(3;21)(q26q22),del(12)(p12p13)[24]/46XY[3]

CHIP-related mutations at diagnosis and relapse
Mutations at diagnosis and relapse
Diagnosis - relapse pair: time to relapse 280 days

**Diagnosis**
- 78% blasts
- **Mutations**
  - DNMT3A (R882H)
  - SMC3 (Q719K)
  - ABCC5 (H517Q)
  - FLT3 ITD
  - NPM1(L287fs)
- 46,XX [21]

**Relapse**
- 57% blasts
- **Mutations**
  - DNMT3A (R882H)
  - SMC3 (Q719K)
  - ABCC5 (H517Q)
  - FLT3 ITD (72%)
  - NPM1(L287fs)
- 46,XX [21]

CHIP-related mutations at diagnosis and relapse
Diagnosis - relapse pair: time to relapse 2051 days

**VAF 38%**

- **DIAGNOSIS**
  - (45% blasts)
  - DNMT3A (A910V)
  - IDH1 (R132L)
  - BAP1 (I401E)

**46,XX [33]**

**VAF 7%**

- **RELAPSE**
  - (15% blasts)
  - DNMT3A (A910V)
  - IDH1 (R132L)
  - BAP1 (I401E)
  - NRAS (Q61K)

**46,XX,inv(12)(p1?2q1?4)[6]/46,XY(donor)[14]**

CHIP-related mutations at diagnosis and relapse

Mutations at diagnosis and relapse
Diagnosis - relapse pair: time to relapse 4004 days

CHIP-related mutations at diagnosis and relapse

Mutations at diagnosis and relapse

VAF 38%

DNMT3A (R882H)
NPM1 (L287fs)
IDH1 (R132H)
LZTR1 (A177T)
TSHZ1 (V181M)
ZCCHC17 (R74X)

46,XY [32]

VAF 44%

DNMT3A (R882H)
NPM1 (L287fs)
FLT3 (D835Y)
SMC3 (R661)
ATR (V1215I)

46,XY [22]
Pre-leukemic mutations persist and contribute to relapse even after long latency
Working model

Pre-leukemic cell → AML → Pre-leukemic cell

Time

CHIP

$DNMT3A, TET2, ASXL1, \text{ etc.}$ mutations

AML

Treatment

MRD with CHIP-related $DNMT3A$ mutations $TET2$ mutations $ASXL1$ mutations etc. persist

Pre-leukemic cell → AML

Time

Relapse
DNMT3A increases while mutant DNMT3A impairs mismatch repair activity of the DNA glycosylase TDG

AGCTGCGCGCAAT<sub>T</sub>GATGCGCCGGACGT
TCGACGCGCGTTA<sub>G</sub>GCTAGCGGCCTGCA

AGCTGCGCGCAAT<sub>T</sub>GATGCGCCGGACGT
TCGACGCGCGTTA<sub>G</sub>GCTAGCGGCCTGCA
Working model

Pre-leukemic cell → Mutant DNMT3A → AML → Treatment → Pre-leukemic cell

CHIP

DNMT3A, TET2, ASXL1, etc. mutations

C > T in CpG

Time

AML

MRD with CHIP-related
DNMT3A mutations
TET2 mutations
ASXL1 mutations etc. persist

C > T in CpG

Time

Relapse
Conclusion

Can we further improve risk stratification of AML by MRD detection based on next generation sequencing (NGS)?

- NGS MRD detection in the largest AML patient series reveals a systematic persistence of mutations associated with CHIP
- NGS MRD detection is a powerful predictor of relapse
- Persisting CHIP-related mutations contribute to relapse even after long latency
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