Monitoring Minimal Residual Disease in AML with molecular markers

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23 genes recurrently mutated in AML

Challenges to molecular targeting

- AML is genetically heterogeneous
- Inhibitors against one target will not suppress all leukemogenic clones
- Clearing all mutations increases overall survival

### 2017 ELN risk stratification by genetics

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Genetic abnormality</th>
</tr>
</thead>
</table>
| **Favorable** | t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*  
inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*  
Mutated *NPM1* without *FLT3-ITD* or with *FLT3-ITD*\(^{\text{low}}\)  
Biallelic mutated *CEBPA* |
| **Intermediate** | Mutated *NPM1* and *FLT3-ITD*\(^{\text{high}}\)  
Wild-type *NPM1* without *FLT3-ITD* or with *FLT3-ITD*\(^{\text{low}}\) (without adverse-risk genetic lesions)  
t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*\(^{\pm}\)  
Cytogenetic abnormalities not classified as favorable or adverse |
| **Adverse** | t(6;9)(p23;q34.1); *DEK-NUP214*  
t(v;11q23.3); *KMT2A* rearranged  
t(9;22)(q34.1;q11.2); *BCR-ABL1*  
inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVII)*  
−5 or del(5q); −7; −17/abn(17p)  
Complex karyotype;\(^{\S}\) monosomal karyotype\(^{\Pi}\)  
Wild-type *NPM1* and *FLT3-ITD*\(^{\text{high}}\)  
Mutated *RUNXI*\(^{\S}\)  
Mutated *ASXL1*\(^{\S}\)  
Mutated *TP53*\(^{\S}\) |

\(^{\S}\) Some cases with monosomy 5 are classified as intermediate. **\(^{\Pi}\)** Some cases with monosomy 7 are classified as intermediate.
Can MRD improve outcome determination?

- a) capture differences in treatment response that reflect an underlying molecular heterogeneity
- b) capture inter-patient variability in drug availability and metabolism, which may significantly influence outcome
Qualitative PCR analysis

Gene A                    Gene B

20 cycles

Sensitivity potentially achievable: $10^{-4}/10^{-5}$

Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease

Report of the BIOMED-1 Concerted Action:
Investigation of minimal residual disease in acute leukemia

JMJ van Dongen¹, EA Macintyre², JA Gabert³, E Delabesse², V Rossi⁴, G Saglio⁵, E Gottardi⁵, A Rambaldi⁶, G Dotti⁶, F Griesinger⁷, A Parreira⁸, P Gameiro⁹, M González Diáz⁹, M Malec¹⁰, AW Langerak¹¹, JF San Miguel¹² and A Biondi⁴
2.11. Molecular remission in the bone marrow should be assessed at completion of consolidation by RT-PCR assay affording a sensitivity of at least 1 in $10^4$.

2.12. Patients with confirmed molecular persistence should be considered for allogeneic HSCT.

2.13. For patients with molecular persistence who are not candidates for allogeneic HSCT, ATO or gemtuzumab ozogamicin may be considered.

2.15. Because early treatment intervention in patients with evidence of MRD affords a better outcome than treatment in full-blown relapse, every 3 months MRD monitoring of bone marrow should be offered to all patients for up to 3 years after completion of consolidation therapy.

2.16. Bone marrow generally affords greater sensitivity for detection of MRD than blood and therefore is the sample type of choice for MRD monitoring to guide therapy.

2.17. For patients testing PCR-positive at any stage after completion of consolidation, it is recommended that a bone marrow is repeated for MRD assessment within 2 weeks and that samples are sent to the local laboratory, as well as to a reference laboratory for independent confirmation.
Survival of APL patients treated for haematologic
Vs. molecular relapse (GIMEMA)

Molecular relapses

Haematological relapses

P = 0.01

32  20  7  2
94  47  26  14
Most patients with CBFs leukemias remain RT PCR positive after completion of therapy, independently from the final outcome.

Guerrasio A et al., Leukemia 2002
Real Time PCR in CBFb-MYH11 positive AML patients

CBFb/MYH11 x 10^4 copies

Guerrasio et al., Leukemia 2002
# Real Time PCR in CBFb-MYH11 positive AML patients

**Post-induction**

<table>
<thead>
<tr>
<th>&lt;100 copies</th>
<th>&gt;100 copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/12 relapses</td>
<td>6/7 relapses</td>
</tr>
<tr>
<td><strong>P=0.003</strong></td>
<td><strong>P=0.006</strong></td>
</tr>
</tbody>
</table>

**Post-consolidation**

<table>
<thead>
<tr>
<th>&lt;10 copies</th>
<th>&gt;10 copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/12 relapses</td>
<td>8/11 relapses</td>
</tr>
</tbody>
</table>

**Relapses /total cases**

Guerrasio et al., Leukemia 200
Several studies confirm the value for prognostication of MRD quantification in CBFs AMLs:

- Schnittger S et al., Blood 2003
- Yoo SJ et al., Haematologica 2005
- Perea G et al., Leukemia 2006
- Stentoft J et al., Leuk Res 2006
- etc..........................
FLIT3 ITD and TKD as markers for MRD in AML

• FLIT3 ITD and FLIT-TDK are suitable markers for MRD detection and quantification in AML
  – Stirewalt DL et al., Leuk Res 2001
  – Schnittger S et al., Acta Haematol 2004
  – Scholl S et al., J Lab Clin Med 2005

• Need for a patient-specific probe

• Unstable marker?
NPM1 as a marker for MRD in AML

Falini B., NEJM 2005
Non responders

Relapses 3/3

Relapses 1/4

Paolo Gorello et al., Leukeima 2006
Early assessment of MRD status in *NPM1* mutant AML provides independent prognostic information

Adam Ivey, Neesa Bhudia, Mandy Gilkes, Rosemary Gale & Robert Hills
Prognostic value of MRD assessment is independent of FLT3-ITD status in NPM1 mutant AML

NPM1 mut/FLT3-ITD neg

NPM1 mut/FLT3-ITD +ve

Adam Ivey, Neesa Bhudia, Mandy Gilkes, Rosemary Gale & Robert Hills
Search for a universal marker
## WT1 expression

<table>
<thead>
<tr>
<th></th>
<th>mean value (WT1 copies/10000 ABL copies)</th>
<th>range</th>
<th>percentage of cases with WT1 overexpression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal BM</td>
<td>35</td>
<td>0-90</td>
<td></td>
</tr>
<tr>
<td>Normal PB</td>
<td>5</td>
<td>0-20</td>
<td></td>
</tr>
<tr>
<td><strong>Conditions associated with WT1 overexpression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute myeloid leukemia (AML)</td>
<td>27669</td>
<td>1081-121806</td>
<td>100%</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia (ALL)</td>
<td>13807</td>
<td>318-94682</td>
<td>100%</td>
</tr>
<tr>
<td>CML chronic phase and blastic phase</td>
<td>3262</td>
<td>191-54171</td>
<td>100%</td>
</tr>
<tr>
<td>Chronic Myelomonocytic leukemia (CMML)</td>
<td>4667</td>
<td>1070-23674</td>
<td>100%</td>
</tr>
<tr>
<td>Ph negative CML like diseases</td>
<td>9731</td>
<td>890-70980</td>
<td>100%</td>
</tr>
<tr>
<td>Primitive Hypereosinophilic Syndromes</td>
<td>280</td>
<td>102-7800</td>
<td>95%</td>
</tr>
<tr>
<td>Refractory anaemias</td>
<td>366</td>
<td>100-1289</td>
<td>65%</td>
</tr>
<tr>
<td>RAEB</td>
<td>2262</td>
<td>227-11006</td>
<td>100%</td>
</tr>
<tr>
<td>RAEB-T</td>
<td>14033</td>
<td>3757-51700</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Conditions associated with normal WT1 expression

regenerating BM (immature but normal cells)
G-CSF stimulated cells
policlonal anaemias
inflammatory diseases
reactive thrombocytosis
WT1 Standardisation
ELN WP12

Normalized WT1 expression (WT1 copies/10^4 ABL copies)

Cell source

Cilloni et al., JCO 2009
Follow-up of a patient with inv(16) AML
CN AML patients

Graph a

Graph b
23 CR patients with WT1 above the normal upper limit relapsed after a median of 7 months from diagnosis (range 6-44)
27 pts with WT1 within the normal range after induction chemotherapy persisted in CR

Cilloni et al. Haematologica 2008; 93:921
21 patients with WT1 within the normal range after induction chemotherapy relapsed after a median of 15 months.
Until few years ago there were contrasting data in literature
Reasons for discrepancies

The vast majority of the published studies are retrospective.

Different populations of patients.

Different methods and procedures.
Important steps in WT1 monitoring

- RT-PCR
- Quantitative RT-PCR
- Standardization of the methods
Standardization of Real Time procedure for WT1 detection

- 9 published and in house $WT1$ sets of primers and probe were tested
- Plasmid containing the full length WT1 sequence was provided by Ipsogen (Marseille, France)
- Standard curves: plasmid dilutions
- Normal and diagnostic BM and PB samples
- The influence of different instruments and reagents was established
- $ABL$ used as housekeeping gene
Assays were excluded basing on:

- demonstrated lack of RNA-specificity
- location within the 3’ region of the gene which has been shown to be subject to deletion or mutations in AML

Selected assay: located within the 5’ region

(J.P. Van Dijk, Br J Haematol 118:1027-1033, 2002)

Forward  ccgctattcgcaatcagggtta  High level of RNA specificity
Reverse  gggcggtgtgaccgtagct  High sensitivity
Probe:  cagcagggtcaccttcgacgg
WT1 expression in 204 normal samples
(61 BM, 118 PB, 25 PBSC)

WT1 copies/10^4 ABL copies

Median      19,8                0,01                  6,1
Range       0-200             0,01-47            0-39
Open issues

- Can WT1 levels after chemotherapy predict the outcome of AML patients?
- If yes at which time point during/after chemotherapy?
- Does WT1 value at diagnosis impact on prognosis?
- Is WT1 useful to predict relapse after allogeneic stem cell transplant?
- BM or PB?
WT1 expression in 729 samples from AML at diagnosis (collected by the European Leukemia Net) (588 BM, 141 PB)

11% 12%
• No significant difference in WT1 expression at diagnosis by stratifying the patients according to:
  – cytogenetic risk groups (except for APL patients who show significantly higher WT1 values)
  – mutations of NPM1 or FLT3

Cilloni et al. JCO 2009
### AML patients during follow-up (ELN study)

- 114 patients evaluated at diagnosis and during follow-up
  - All the patients included have been previously characterized by cytogenetic and molecular analysis
  - Clinical data available
  - All the patients were treated with intensive anthracycline and ARA-C
  - 91/114 (80%) showed WT1 copies > $20,000/10^4$ ABL at diagnosis (2 logs higher than normal controls)
The achievement of normal WT1 values after induction chemotherapy is predictive of relapse

Relapse risk by achievement of normal levels post induction

<table>
<thead>
<tr>
<th></th>
<th>No. Patients</th>
<th>No. Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not normal</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>Normal</td>
<td>78</td>
<td>26</td>
</tr>
</tbody>
</table>

Cilloni et al. JCO 2009
The achievement of normal WT1 values after consolidation chemotherapy is predictive of relapse.
Kinetics of WT1 response following induction therapy predicts risk of subsequent relapse

Analysis in 91/114 cases with baseline WT1 > 2 x 10^4 copies/ 10^4 ABL copies

Greater reduction in WT1 decreases risk of relapse
HR 0.54 (0.36-0.83) p=0.004

Cilloni et al. JCO 2009
Does log reduction add to the risk score?

- Regression analysis showed that “log reduction” is an independent predictor of relapse
  
  - adjusted for age: HR 0.54 (0.35-0.83) p=0.05
  - adjusted for WBC: HR 0.54 (0.35-0.81) p=0.003
  - adjusted for cytogenetics: HR 0.63 (0.41-0.98) p=0.04

- Log reduction remains prognostic even when adjusted for age, WBC, cytogenetics individually
Kinetics of WT1 transcript reduction post-induction correlate with MRC risk index

Cilloni et al. JCO 2009
Prospectic study

WT1 at diagnosis and during follow up in AML patients (18-60 years) enrolled in the GIMEMA study treated with the same chemotherapeutical scheme
PB or BM for MRD detection?

The vast majority of published papers showed that PB is as sensitive as BM.

PB even better?
Strong correlation between the MRD detected in BM and PB.

Conclusions

Detection of WT1 in PB may be even more sensitive than in BM because of the lower level of expression in normal PB samples
Need for a higher level of standardization

Two different WT1 qRT-PCR assay kits are mainly used in published papers

- Otsuka Pharmaceutical Co., Ltd. (Approved in Japan)
- Ipsogen (France) (Qiagen) (Cilloni et al. JCO 2009)
- Additional standardized assays: Willasch et al. Leukemia 2009. (pediatric AML)

Potential of next generation sequencing approaches to track treatment response

- Diagnosis
- Remission
- Relapse

**NPM1 mut**

- Allelic ratio NPM1/mut (NGS)
- Days after diagnosis

**FLT3-ITD**

- Mutated alleles
- Proportion of different clones

**DNMT3A mut**

- Detection of DNMT3A mutation by next generation sequencing

Ivey A, Simpson MA, Burnett AK & Grimwade D, unpubl.
Thank you David!