Recommendations and standardization of the management of CML

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QIAGEN would like to thank our speaker, Prof. Martin Müller, for his presentation.

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Monitoring of CML Patients: What Do We Measure, What Do We Treat?

- BCR-ABL mRNA Transcript
- Active protein
- Inhibition of TK by TKI

Quantitative determination of BCR-ABL transcripts using RQ-PCR

Monitoring Ph+ CML Disease

- **Hematologic response (HR)**
  - Measure of blood counts and differentials

- **Cytogenetic response (CyR)**
  - Chromosome banding analysis of marrow cell metaphases

- **Molecular response**
  - Measurement of BCR-ABL transcript levels relative to a control gene
  - Most sensitive measure of Ph+ CML disease burden

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Cytogenetic and Molecular Monitoring: European LeukemiaNet Recommendations

- Routine monitoring provides information necessary for clinical decision making

Correlation Between Response and Disease Burden: Molecular Response

Number of Leukemic Cells

- $10^{12}$
- $10^{11}$
- $10^{10}$
- $10^{9}$
- $10^{8}$
- $10^{7}$
- $10^{6}$

BCR-ABL Transcripts (log10)

- CHR (< 1-log)
- CCyR (2-log)
- MMR (3-log)

Hematologic

Cytogenetics

RQ-PCR

Dx

Months on Treatment

Time

~5 log reduction

Chromosome 22

Chromosome 9

Genetics of Chronic Myeloid Leukemia

Chromosome 9
Chromosome 22

5'

3'

5'

3'

BCR

ABL

m-bcr
M-bcr
μ-bcr

1b
1a
a2
a3
a11

e1

e1'
e2'
b1
b5

e19

e1a2

b2a2

b3a2

e19a2

e19

p190_{bcr-abl}
p210_{bcr-abl}
p230_{bcr-abl}
Multiplex PCR

Qualitative PCR before quantitative PCR!

Please ensure to know the transcript type of your patients! Only b3a2 and/or b2a2 transcripts can be compared according to the IS

Cross et al., Leukemia 1993.
## Molecular Analyses

- Quantitative Real-Time RT-PCR using LightCycler-technology according to the International Scale (IS; Hughes et al., Blood 2006).

Other housekeeping genes possible

→ most appropriate: ABL, GUS, B2M, BCR

Beillard et al., Leukemia 2003
Important for prognosis....

- Depth of response
- Time to achieve response
## ELN Recommendations 2009 on Treatment and Diagnostics of CML

<table>
<thead>
<tr>
<th></th>
<th>Optimal Response</th>
<th>Suboptimal Response</th>
<th>Failure</th>
<th>Warning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>3 Month</td>
<td>CHR + mCyR (Ph+ ≤ 65%)</td>
<td>No CyR (Ph+ &gt; 95%)</td>
<td>No CHR</td>
<td>High Risk CCA/Ph+</td>
</tr>
<tr>
<td>6 Month</td>
<td>PCyR (Ph+ ≤ 35%)</td>
<td>Less than PCyR (Ph+ &gt; 35%)</td>
<td>No CyR (Ph+ &gt; 95%)</td>
<td>N/A</td>
</tr>
<tr>
<td>12 Month</td>
<td>CCyR (Ph+ = 0%)</td>
<td>PCyR (Ph+ ≤ 35%)</td>
<td>Less than CCyR (Ph+ &gt; 35%)</td>
<td>N/A</td>
</tr>
<tr>
<td>18 Month</td>
<td>MMR</td>
<td>Less than MMR</td>
<td>Less than CCyR (Ph+ &gt; 0%)</td>
<td>Less than MMR</td>
</tr>
<tr>
<td>Anytime</td>
<td>Stable/Improving MMR</td>
<td>Loss of MMR Mutations*</td>
<td>Loss CHR Loss CCyR Mutations* CCA/Ph+</td>
<td></td>
</tr>
</tbody>
</table>

Molecular endpoints do not play a major role so far

Equivalence of cytogenetic with molecular endpoints?

- CCyR ≈ \( \leq 1\% \) BCR-ABL\(^{IS}\)

- PCyR ≈ \( \leq 10\% \) BCR-ABL\(^{IS}\)
Overall survival by CCyR at 6 months
MD Anderson Cancer Center

![Graph showing overall survival by CCyR at 6 months.]

- **6-mos CCyR**
  - Yes: 326, 14
  - No: 66, 15

*p < 0.001*
Overall survival by CCyR at 6 months
German CML-Study IV

Overall Survival (OS)

BCR-ABL$^I_S$ at 6 months ≤1% vs. 1-10% vs. >10%

Predictive Value of BCR-ABL Level at 3 months on Overall Survival (Marin 2012)

BCR-ABL/ABL < 9.8%  (n = 211)
OS = 93.3%
P < .001

BCR-ABL/ABL > 9.8%  (n = 68)
OS = 54%
Overall Survival (OS) by BCR-ABL IS at 3 months ≤10% vs. >10%


<table>
<thead>
<tr>
<th>BCR-ABL IS</th>
<th>n</th>
<th>5Y-OS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤10%</td>
<td>501</td>
<td>95%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>191</td>
<td>87%</td>
<td></td>
</tr>
</tbody>
</table>
Overall Survival (OS) by Ph+ at 3 months ≤35% vs. >35%

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>5Y-OS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤35%</td>
<td>336</td>
<td>95%</td>
<td>0.036</td>
</tr>
<tr>
<td>&gt;35%</td>
<td>124</td>
<td>87%</td>
<td></td>
</tr>
</tbody>
</table>

Overall Survival According to BCR-ABL Levels at 3 Months (DASISION Landmark Analysis)

**Dasatinib 100 mg QD**

- 84% had ≤10% BCR-ABL
- 95.5% 3-Year OS at ≤1% BCR-ABL, \( P = .7342 \)
- 96.5% 3-Year OS at >1-10% BCR-ABL, \( P = .0525 \)
- 85.9% 3-Year OS at >10% BCR-ABL
- \( \leq 10\% \) vs >10% BCR-ABL, \( P = .0348 \)

**Imatinib 400 mg QD**

- 64% had ≤10% BCR-ABL
- 100% 3-Year OS at ≤1% BCR-ABL, \( P = .3453 \)
- 95.0% 3-Year OS at >1-10% BCR-ABL
- 87.8% 3-Year OS at >10% BCR-ABL
- \( \leq 10\% \) vs >10% BCR-ABL, \( P = .0036 \)

Subjects at risk:

<table>
<thead>
<tr>
<th>Months</th>
<th>≤1%</th>
<th>&gt;1-10%</th>
<th>&gt;10%</th>
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<tr>
<td></td>
<td>112</td>
<td>86</td>
<td>37</td>
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<td>6</td>
<td>112</td>
<td>85</td>
<td>37</td>
</tr>
<tr>
<td>12</td>
<td>110</td>
<td>84</td>
<td>35</td>
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<td>18</td>
<td>109</td>
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<td>83</td>
<td>33</td>
</tr>
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<td>30</td>
<td>104</td>
<td>79</td>
<td>27</td>
</tr>
<tr>
<td>36</td>
<td>85</td>
<td>66</td>
<td>22</td>
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<tr>
<td>42</td>
<td>29</td>
<td>25</td>
<td>9</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Months</th>
<th>≤1%</th>
<th>&gt;1-10%</th>
<th>&gt;10%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32</td>
<td>122</td>
<td>85</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>121</td>
<td>85</td>
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<td>96</td>
<td>55</td>
</tr>
<tr>
<td>42</td>
<td>28</td>
<td>33</td>
<td>20</td>
</tr>
</tbody>
</table>

Overall Survival at 4 Years by BCR-ABL IS Levels at 3 Months (ENESTnd Landmark Analysis)

**nilotinib 300 mg BID**

- OS rates at 4 years for ≤10% vs >10%
  - BCR-ABL at 3 months: 96.7% vs 86.7%; \( P = .0116 \)

**imatinib 400 mg QD**

- OS rates at 4 years for ≤10% vs >10%
  - BCR-ABL at 3 months: 98.9% vs 83.6%; \( P < .0001 \)

**Evaluable patients**

<table>
<thead>
<tr>
<th>BCR-ABL at 3 months</th>
<th>Nilotinib 300 mg BID (N = 258)</th>
<th>Imatinib 400 mg QD (N = 264)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \leq 1% ) N=145</td>
<td>( &gt;1% ) ( \leq 10% ) N=89</td>
<td>( &gt;10% ) N=24</td>
</tr>
<tr>
<td>( \leq 1% ) N=43</td>
<td>( &gt;1% ) ( \leq 10% ) N=133</td>
<td>( &gt;10% ) N=88</td>
</tr>
<tr>
<td>% of patients</td>
<td>56%</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>34%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>9%</td>
<td>33%</td>
</tr>
</tbody>
</table>


## Evolving Recommendations of the ELN

<table>
<thead>
<tr>
<th>Time</th>
<th>Optimal Response 2009*</th>
<th>Optimal Response 2013**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>3 Month</td>
<td>CHR + mCyR (Ph+ ≤ 65%)</td>
<td>PCyR (Ph+ ≤ 35%) and/or ≤ 10% BCR-ABL&lt;sub&gt;IS&lt;/sub&gt;</td>
</tr>
<tr>
<td>6 Month</td>
<td>PCyR (Ph+ ≤ 35%)</td>
<td>CCyR (Ph+ 0%) and/or ≤ 1% BCR-ABL&lt;sub&gt;IS&lt;/sub&gt;</td>
</tr>
<tr>
<td>12 Month</td>
<td>CCyR (Ph+ = 0%)</td>
<td>≤ 0.1% BCR-ABL&lt;sub&gt;IS&lt;/sub&gt; (MMR)</td>
</tr>
<tr>
<td>18 Month</td>
<td>MMR</td>
<td></td>
</tr>
<tr>
<td>Anytime</td>
<td>Stable/ Improving MMR</td>
<td></td>
</tr>
</tbody>
</table>

Standardization - why?

- Standardized BCR-ABL monitoring has shown to predict for response
  
  Hughes et al., NEJM 2003; Hughes et al., Blood 2010
  Hanfstein et al., Leukemia 2012; Marin et al., JCO 2012

- Molecular endpoints in recent clinical studies
  
  Saglio et al., NEJM 2010; Kantarjian et al., NEJM 2010

- Huge variety of molecular methods leads to considerable variation of results
  
  → Noncomparability of results
  → Confusion of doctors and patients
  → Suboptimal treatment decisions
Historical (IRIS trial; 2000): The mean \textit{BCR-ABL} levels of 30 CML patients was defined as 100\% in each of the three participating laboratories using \textit{BCR} as a control.

The value corresponding to MMR in each laboratory defined as 0.1\% (reduction of 3 log from IRIS baseline, not from pre-treatment levels in the individual patient!)
Proposal of international CML experts for the expression of results in 2006

“We propose that the value corresponding to a MMR in each laboratory be mathematically converted to: “

0.10%

Therefore the International Scale will be fixed to an absolute value as defined in the IRIS trial

→ $\text{BCR-ABL}_{\text{local}} \times \text{CF}_{\text{local}} = \text{BCR-ABL}^\text{IS}$

Hughes et al., Blood 2006
Applying an International Scale Conversion Factor Can Significantly Alter Patient Results and Clinical Decisions

European LeukemiaNet guidelines define MMR as BCR-ABL ≤ 0.1% on the IS\(^1\)

Reporting MMR, therefore, requires application of a laboratory-specific CF aligned to the IS

As attainment of MMR is a critical milestone of CML therapy, errors in MMR determination may have an adverse impact on CML disease management

Without a CF, a laboratory cannot report if the patient achieved MMR or not

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MR\(^{4.5}\), molecular response ≥4.5-log reduction.
How to transmit the `International Scale´?

- Lab A (central lab, approved CF)
  Lab B (participating lab)

- Two major ways to align results between two labs:
  1) Send `reference material´ from lab A to lab B which has been adequately characterized in lab A
     → align results
  2) Send aliquots of patient samples from lab B to lab A
     → align results
Central distribution of dilution samples (round 1): Linearity of results compared to reference results

Linear results n=57

Non-linear results n=7

Calculation of CF
Calculation of CF

Formula: \[ \text{Log } y = (\text{slope} \times (\text{log MMR}^\text{IS})) + \text{intercept} \]
\[ \text{CF} = \frac{\text{MMR}^\text{IS}}{\text{antilog } y} \]

Example: \[ \text{Log } y = (0.9666 \times (-1)) - 0.3568 \]
\[ \text{CF} = \frac{0.1}{0.04749} = 2.1057 \]

Mean values after conversion to the international scale (IS)

<table>
<thead>
<tr>
<th>CF 0.878</th>
<th>CF 2.1057</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannheim mean</td>
<td>Lab X mean</td>
</tr>
<tr>
<td>Level 1</td>
<td>0.037</td>
</tr>
<tr>
<td>Level 2</td>
<td>0.26</td>
</tr>
<tr>
<td>Level 3</td>
<td>2.45</td>
</tr>
<tr>
<td>Level 4</td>
<td>23.81</td>
</tr>
</tbody>
</table>
BCR-ABL Levels in 64 Labs
Pre and Post Conversion

Validation of CFs using patient samples (round 2)

- **n=59** of the participating labs sent aliquots of 25-30 patient samples (median n=28; WBC in Trizol or GTC) to the central lab.

- BCR-ABL expression should cover a wide range (0.01%-10% BCR-ABL\textsuperscript{IS}).

- Comparison of results (Lab A vs. Lab B):
  - i) using the CFs from the dilution sample round
  - ii) using the CFs from the patient samples evaluation (Bland-Altman difference plots)
Problem: stability of CFs affects 2-fold concordance

- Stable CF: ns
- Unstable CF: p=0.0003

Graphs showing concordance for previous and refined CFs, with n=17 and n=21.
Changes in procedures

.... in either one or more components of the procedures (cDNA synthesis, PCR platform, RQ-PCR protocol)

- **Stable CFs:** 3 of 17 labs (18%)
- **Unstable CFs:** 9 of 21 labs (43%)
Standardization of BCR-ABL quantification in Europe – 2013
64 participating laboratories in 28 countries

Countries with a ref lab with validated CF
Countries using a ref lab in a different country
Countries with a ref lab – validation pending

EUTOS for CML
European Treatment and Outcome Study
How to standardize for deeper molecular responses?

Reference results

Müller et al., Leukemia 2009

pre conversion

post conversion
Do we need to qualify for deep molecular responses?

Isn´t it enough to get a negative PCR result?

→ BCR-ABL negative sample due to bad sample quality / low sensitivity?
Achieving complete molecular response (undetectable BCR-ABL)

Subsequent evaluation is available for 81 of the 95 (85%) patients who achieved undetectable levels of BCR-ABL, with a median of two follow-up tests (range, one to six). In 43 patients (53%), BCR-ABL/ABL became detectable again in subsequent testing, whereas 38 (47%) had sustained undetectable BCR-ABL levels over a median of 18 months (range, 3-27 months). Among 47 patients who lost a major

BUT: no sensitivity measure is given (e.g. ABL counts)
Importance of Reliable Measurement of Deep Molecular Responses

• Expected that more and more patients under first-line treatment with second-generation TKIs (Nilotinib, Dasatinib) will achieve deep molecular response

→ Justification for treatment discontinuation
Is Discontinuation Possible? STOp IMatinib (STIM) Trial - Results

- Successful discontinuation without molecular relapse: 39%
- Majority of molecular relapses (58/61) occurred early after discontinuation

Please ensure that your lab provides you with ABL (GUS) values as a measure for sensitivity!

**Definitions of Molecular Response**

- **MR**
  - 4.5 ($\geq 4.5 \log$ reduction; $\leq 0.0032\% IS$)
  - 4.0 ($\geq 4.0 \log$ reduction; $\leq 0.01\% IS$)
  - 5.0 ($\geq 5.0 \log$ reduction; $\leq 0.001\% IS$)

- **Minimum sample quality required**
  - $100\%$ [IRIS baseline]

- **International Scale**
  - $BCR-ABL$ undetectable
  - 100,000 ABL copies
  - 32,000 ABL copies
  - 10,000 ABL copies

- **log reduction** = reduction from IRIS baseline, not individual pretreatment levels

It's all about Sensitivity

- Improvement of sensitivity – main factors:
  - Number of leukocytes to use (10-20 Mio.)
  - RNA extraction method (Trizol vs kits)
  - cDNA synthesis protocol (use 5µg instead of 1µg RNA)
  - Optimize type and amount of RT enzyme
  - No dilution of cDNA
Current harmonization process is very laborious

Possible solutions?
Primary reference standards
WHO definition

- Ideally be as close as possible to real samples (should cover all steps of the process)
- Must be stable over several years (=freeze dried) and physically possible to prepare batches that last several years.
- Must be applicable to all or most existing methods

- Mixtures of K562 in HL60 to yield approx 10%, 1%, 0.1%, 0.01% BCR-ABL$^{IS}$.
- 3000 vials at each dilution freeze dried
- Assignment of IS values ($ABL$, $BCR$, $GUSB$)

Likely use of primary reference reagents

Available to routine testing laboratories
Standardization of Molecular Monitoring – Commercial Options in Development

- Ipsogen / QIAGEN Kits
- Nanogen Kits
- WHO International Standard
- Asuragen Standards
- Cepheid Platform (MMR, CMR)

Improved comparability using closed diagnostic systems?

- Some issues
  - Cost
  - Can sensitivity be improved?
  - Conversion of results to IS?
CML V (TIGER) Study

Induction

Nilotinib 2x300mg/d
Nilotinib 2x300mg/d
PEG-IFN 35(-50)µg/w

Maintenance

Cont. Nilotinib
PEG-IFN 50µg/w

Cure?

Confirmed MMR after ≥ 24 mo.
≥ 12 mo. MR^4

Discontinuation

Nilotinib Intoleranz → Imatinib
Nilotinib Resistenz → Transplantation/Dasatinib
Suboptimal Response: → Nilotinib 400 mg BID

>36 months therapy

R

≥36 months therapy
EURO-SKI Study Design

Inclusion criteria
TKI treatment at least 3 years

MR* at least 1 year

Study start

 Screening phase (Confirming MR*)

≤ 6 weeks

Informed consent

RQPCR
q4w

Year 1

Stop TKI

RQPCR
q6w

Year 2

Every 3rd month

Year 3

Follow-up

Courtesy of ELN
ENESTPath

Study Design

- **Induction phase**
- **Consolidation phase**

**N= 1058**
- Imatinib ≥24 months Response <MR^{4.0}
- Nilotinib 300 BID

- **12 Month**
- Stable MR^{4.0}
  - Unstable MR^{4.0} or <MR^{4.0}

- **12 Month**
  - Stable MR^{4.0}
  - Treatment-free remission phase 3 years (arm 1)

- **12 Month**
  - Treatment-free remission phase 2 years (arm 2)

**RND**
- Relapse during treatment-free remission will trigger re-start of Nilotinib 300 mg BID

**END Study**

Treatment-free remission phase
**In Case of Failure → Mutation Analysis → Most Relevant BCR-ABL Mutations**

<table>
<thead>
<tr>
<th>Specific mutations</th>
<th>Less sensitive to nilotinib</th>
<th>Less sensitive to dasatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y253H</td>
<td>Q252H</td>
</tr>
<tr>
<td></td>
<td>E255K/V</td>
<td>V299L</td>
</tr>
<tr>
<td></td>
<td>F359C/V</td>
<td>T315A</td>
</tr>
<tr>
<td></td>
<td>T315I</td>
<td>F317L</td>
</tr>
</tbody>
</table>

Technical sensitivity of Sanger sequencing ~20%

Conclusions (1/2)

• Ask for:
  – transcript type (once per patient) ✓
  – ABL (GUS) counts (sensitivity) ✓
  – lab is harmonized according to IS? ✓

• Early deep responses predict for PFS and OS (any TKI), determined by Molecular Monitoring (and/or Cytogenetics)

• New ELN recommendations take into account molecular milestones for optimal response and failure, e.g.
  – Optimal at 3 months: ≤10% BCR-ABL\textsuperscript{IS} and/or PCyR (Ph+ ≤35%)
  – Optimal at 6 months: ≤1% BCR-ABL\textsuperscript{IS} and/or CCyR (Ph+ 0%)

• Molecular Monitoring is more sensitive and allows an early detection of potential relapse (or non-compliance)
  – Can be performed using PB instead of BM
Conclusions (2/2)

• “New Goal”: Achievement of very deep molecular responses (MR^4, MR^5) → allowing to consider treatment interruptions within clinical trials (STIM, EUROSKI, ENEST Path)

→Cure?

• Standardization of molecular methods is the prerequisite for comparability of results and represents a challenge for interested laboratories due to the permanent need to validate their conversion factor and improve sensitivity

• TKI failure:
  – Disease phase? Blasts/precursors
  – Cytogenetics: additional chromosomal abnormalities
  – BCR-ABL mutation analysis
  – Compliance? Side effects? Comedication/interactions?
Thank you!

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