Molecular Biology:
Measuring and Reporting BCR-ABL Transcripts Level

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1st Question to be addressed

• Why is it so important to measure BCR-ABL transcript levels in the follow-up of CML patients treated with imatinib?
RESIDUAL DISEASE IN CML

CML Chronic Phase
First line Imatinib 400 mg

98%

CHR

86%

CCyR

~80%

PCR-

5-7%

~80% of the cases are in CCyR but PCR-positive!

Leukemic Burden

1 log reduction

2 log reduction

<3 log

4 log reduction

>3 log

MMR

~80%

90% PFS

98% PFS
2nd Question to be addressed

• Which is the best way to measure the BCR-ABL transcript levels?
Real time quantitative RT-PCR (RQ PCR) is the method of choice!

I. Hydrolysis Probes
Release from quenching by hydrolysis

II. Hybridization Probes
Increased resonance energy transfer by hybridization

Both are valid, but specific rules must be followed!
RQ-PCR measures the copy number of BCR-ABL transcript in a given amount of RNA obtained from blood, but we need to know its absolute concentration!
To assess the amount of an appropriate control gene it is essential to compensate for variations due to:

- Sample degradation
- Efficiency of the RT step, etc.

......and to assess the sensitivity reached in each degraded sample
An ideal control gene should satisfy the following criteria:

- it should have an expression level broadly similar in all types of blood cells, normal and leukemic;

- it should have an expression level broadly similar to that of \textit{BCR-ABL} at diagnosis of CML;

- it should have stability similar to \textit{BCR-ABL}. 
Which are the best control genes?

ABL is probably the best, but also BCR and GUS are acceptable.
3rd Question to be addressed
What is the better way to express the results?
- log reduction (as in the IRIS study)?
- BCR-ABL/control gene ratio (as by most European groups)?
To be considered!

In the IRIS study, the “log reduction” definition expresses an absolute amount of residual disease.

(It’s not a log reduction with respect to the pre-treatment value of the patient, but with respect to an artificial “reference” sample, obtained by pooling together the pretreatment samples of 30 patients.)
The evidence obtained with the IRIS study is that the absolute and not the relative amount is important!
In order to avoid further confusion.....

it would be better to express the results as a percentage....... 

...but a percentage of what, as different control genes are acceptable?
Monitoring CML patients responding to treatment with tyrosine kinase inhibitors – review and recommendations for ‘harmonizing’ current methodology for detecting BCR-ABL and kinase domain mutations and for expressing results

Timothy Hughes, Michael Deininger, Andreas Hochhaus, Susan Branford, Jerald Radich, Jaspal Kaeda, Michele Baccarani, Jorge Cortes, Nicholas C P Cross, Brian J Druker, Jean Gabert, David Grimwade, Rüdiger Hehlmann, Suzanne Kamel-Reid, Jeffrey H Lipton, Janina Longtine, Giovanni Martinelli, Giuseppe Saglio, Simona Soverini, Wendy Stock, John M Goldman

Bethesda Meeting, October 25 – 2005

paper on Blood 2006
Considerations

• A number of different and valid RQ-PCR methods for monitoring patients with CML already exist.

• The alternative to a single ‘global’ protocol would be:
  - to select a limited number of RQ-PCR assays that are already widely adopted;
  - to establish a set of agreed principles to be applied in each analysis (listed in the paper);
  - to express the results in a common and comparable way with an INTERNATIONAL SCALE.
The BCR-ABL transcript levels mirror the number of the residual leukemic cells.

Mean value observed at diagnosis

- Complete Hematologic Response
- Complete Cytogenetic Response
- Major Mol Response
- Complete Molecular Response (undetectable transcripts)

BCR-ABL% (according to the International Scale):
- 10^12: 100%
- 10^11: 10%
- 10^10: 1%
- 10^9: 0.1%
- 10^8: 0.01%
- 10^7: 0.001%
- 10^6: 0.0001%

Number of leukemic cells:
- 10^12
- 10^11
- 10^10
- 10^9
- 10^8
- 10^7
- 10^6
$4^{th}$ Question to be addressed

How can we make the results obtained in different labs, with different methods, with different control genes, really comparable?

In the same way that was used to establish the INR for the PT (Prothrombin Time)
Reference samples, (centrally prepared and distributed) corresponding to 100%, 1%, 0.1%, 0.01% BCR-ABL/control gene.

Analysing the reference samples, all the labs will know which BCR-ABL/control gene values in their hands correspond to 100%, 1%, 0.1%, 0.01% BCR-ABL according to the International Scale and they can calculate a Conversion Factor.
The formula is:

$$BCR-ABL \text{ (local value)} \times \text{conversion factor} = BCR-ABL \text{ (Int.Scale)}$$

Example:

- in Turin, thanks to effort of the Adelaide Lab, I know that 0.1% BCR-ABL (MMR threshold) corresponds to our BCR-ABL/ABL → 0.045%
- therefore our Conversion Factor is 0.1/0.045 = 2.22
- and I have to multiply all my BCR-ABL/ABL% values for 2.22 to express them according to the International Scale
| Laboratory | $\text{MMR}^\text{Eq}$ | $0.1\%/\text{MMR}^\text{Eq}$ (%) = | Conversion Factor | Formula for conversion of a given result to the international scale $(BCR-ABL^L \times CF = BCR-ABL^\text{IS})$

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<thead>
<tr>
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<th>$BCR-ABL^L \times \text{CF}$</th>
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</thead>
<tbody>
<tr>
<td>Adelaide</td>
<td>0.08%</td>
<td>$0.1/0.08 = 1.25$</td>
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<td>$BCR-ABL^L \times 1.25$</td>
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<tr>
<td>Mannheim</td>
<td>0.12%</td>
<td>$0.1/0.12 = 0.83$</td>
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<td>$BCR-ABL^L \times 0.83$</td>
</tr>
<tr>
<td>London</td>
<td>0.045%</td>
<td>$0.1/0.045 = 2.22$</td>
<td></td>
<td>$BCR-ABL^L \times 2.22$</td>
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BCR-ABL% International Scale

Pretreatment or diagnosis 3rd month 6th month 9th month 12th month

No CCyR

CCyR limit

MMoIR

PCR neg
Reference samples

• How will they be prepared?
• Who will prepare them?
• Who will be in charge of the quality control rounds?
• How many times per year must a lab analyse them?