

Standardized Molecular Monitoring

EUTOS for CML



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Rationale for molecular monitoring

- Early recognition of relapse:
 - A rise in BCR-ABL transcript levels may be a warning sign of relapse due to imatinib-resistant BCR-ABL mutations, inappropriate imatinib dosing or lack of patient adherence with therapy
 - The ELN recommendations (2006) note that ‘...there is currently no consensus regarding the degree of increase which should cause concern’¹
 - The NCCN guidelines (V. 2.2009) suggest that in patients with a 1-log increase in BCR-ABL transcript levels, molecular testing should be repeated in 1–3* months and mutational analysis considered²
- Prognostic information:
 - In the IRIS trial all patients achieving both CCyR and MMR at 12 months of treatment remained free from disease progression to AP/BC at 5 years³

*In patients without a major molecular response, bone marrow cytogenetics is recommended

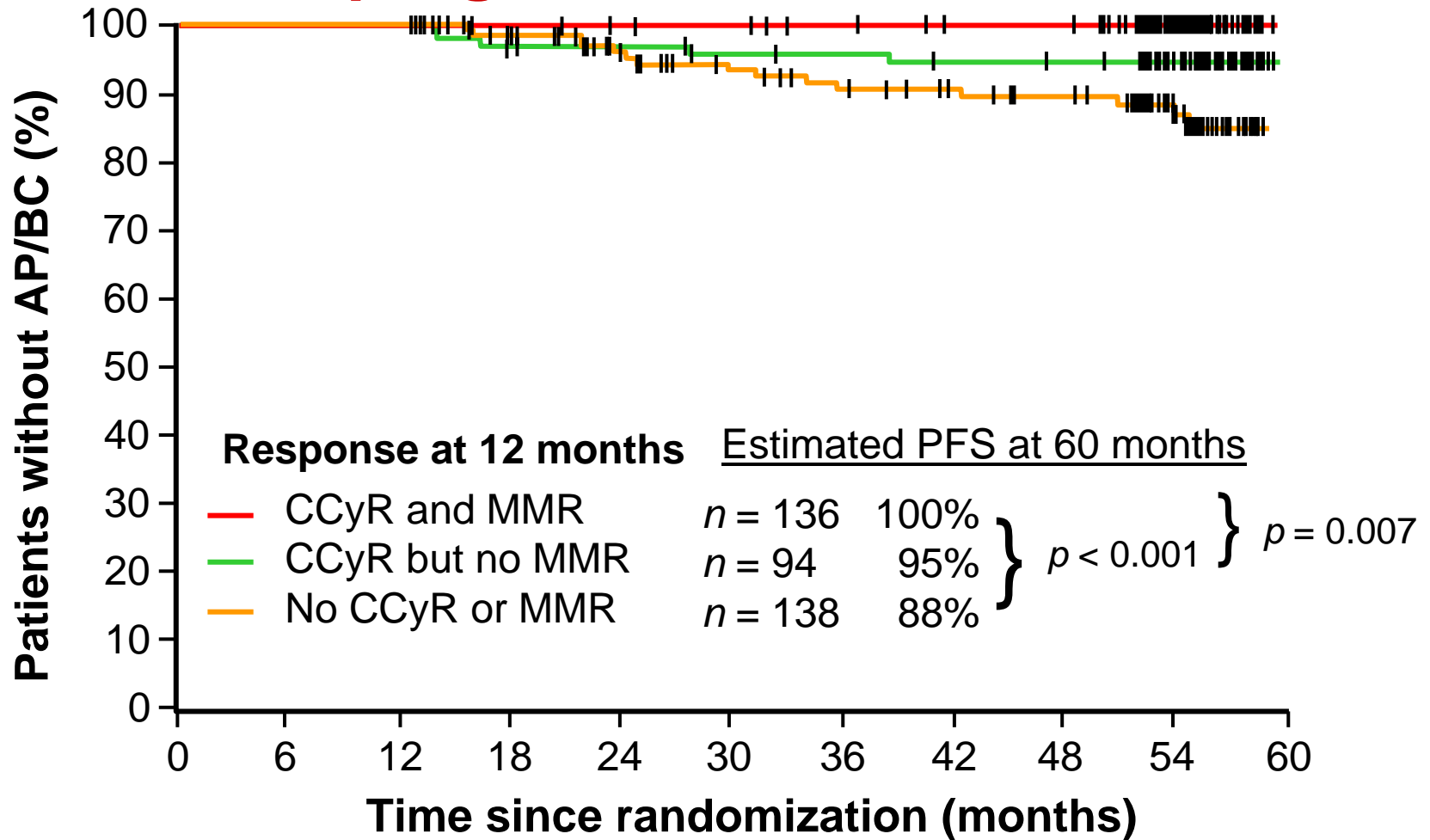
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1. Baccarani *et al.* *Blood* 2006;108:1809;
2. NCCN guidelines V.2.2009;
3. Druker *et al.* *N Engl J Med* 2006;355:2408.

Rationale for molecular monitoring: prognostic information



CCyR, complete cytogenetic response;
MMR, major molecular response

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Druker *et al.* *N Engl J Med* 2006;355:2408.

ELN recommendations 2006*: monitoring treatment response

	Hematologic response	Cytogenetic response	Molecular response
Frequency	<ul style="list-style-type: none"> ▪ Every 2 weeks until a complete response has been achieved and confirmed ▪ Every 3 months unless otherwise required 	<ul style="list-style-type: none"> ▪ Every 6 months until a complete response has been achieved and confirmed ▪ Then every 12 months 	<ul style="list-style-type: none"> ▪ Every 3 months
Methods	<ul style="list-style-type: none"> ▪ Complete blood count with differential 	<ul style="list-style-type: none"> ▪ Conventional cytogenetic examination ▪ FISH (only before treatment) 	<ul style="list-style-type: none"> ▪ RQ-PCR

*ELN recommendations due for update in 2009

FISH, fluorescence *in situ* hybridization

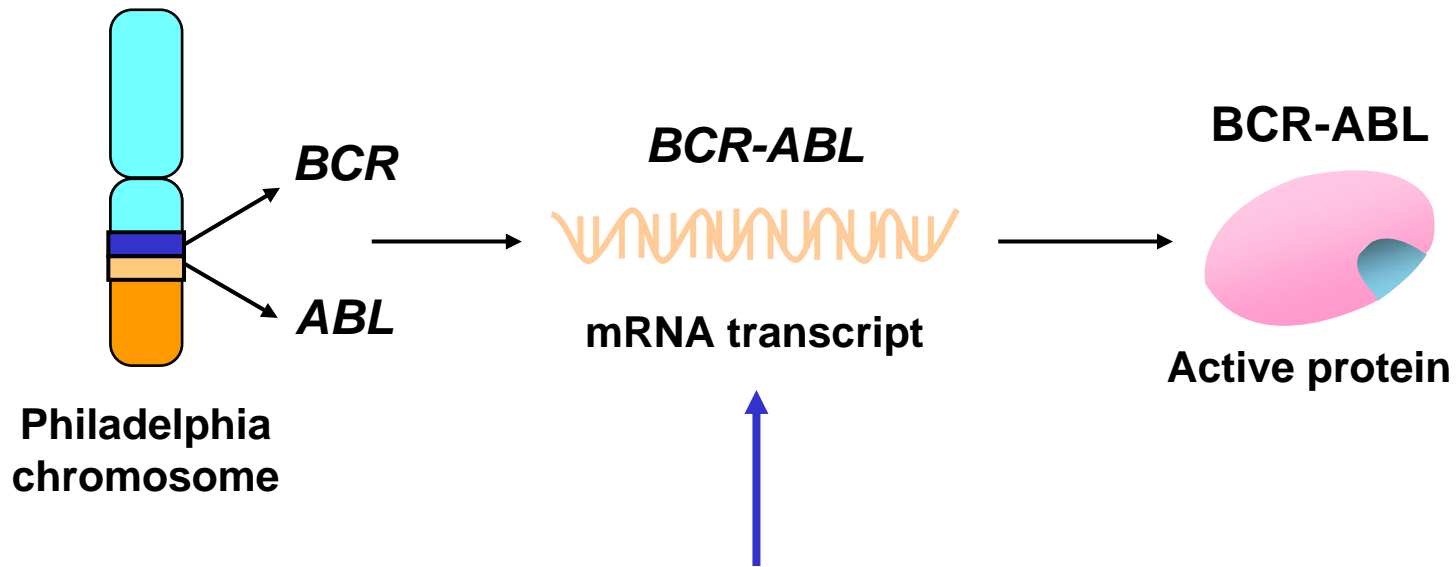
Baccarani *et al. Blood* 2006;108:1809.

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Molecular monitoring: measuring *BCR-ABL* transcript levels



Quantitative detection of *BCR-ABL* transcripts
by real-time quantitative PCR (RQ-PCR)

Adapted from Mensink *et al.*
Br J Haematol 1998;102:768.

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Objectives of the EUTOS for CML Molecular Monitoring project

- International standardization of RQ-PCR analysis for BCR-ABL quantification and for detection of BCR-ABL mutations
- Determine prognostic significance of BCR-ABL mutations
- Establish at least one ELN-certified lab per country that can serve as a national reference lab.
- An exchange program for scientists and technicians to allow spread of quality controlled, standardized RQ-PCR methodologies all over Europe
 - Regular control rounds using patient material (lysates of CML cells in normal cells) will ensure high-quality reliable results in the monitoring of CML patients in all countries

Final aim – European standardization

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Molecular Monitoring Working Group

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- Andreas Hochhaus (Germany)
- Giuseppe Saglio (Italy)
- Alfredo Covelli – Novartis

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Rationale for standardized molecular monitoring

- Differences in testing methods and control genes have caused variability between laboratories, making it difficult to
 - Compare results for the same patient measured in different laboratories
 - Compare the results seen in a patient with those observed in the IRIS trial
- Standardization of RQ-PCR methodology and reporting allows the results from different laboratories to be compared with one another, and also with the IRIS data
- The EUTOS for CML Molecular Monitoring project is facilitating the standardization of RQ-PCR monitoring across Europe

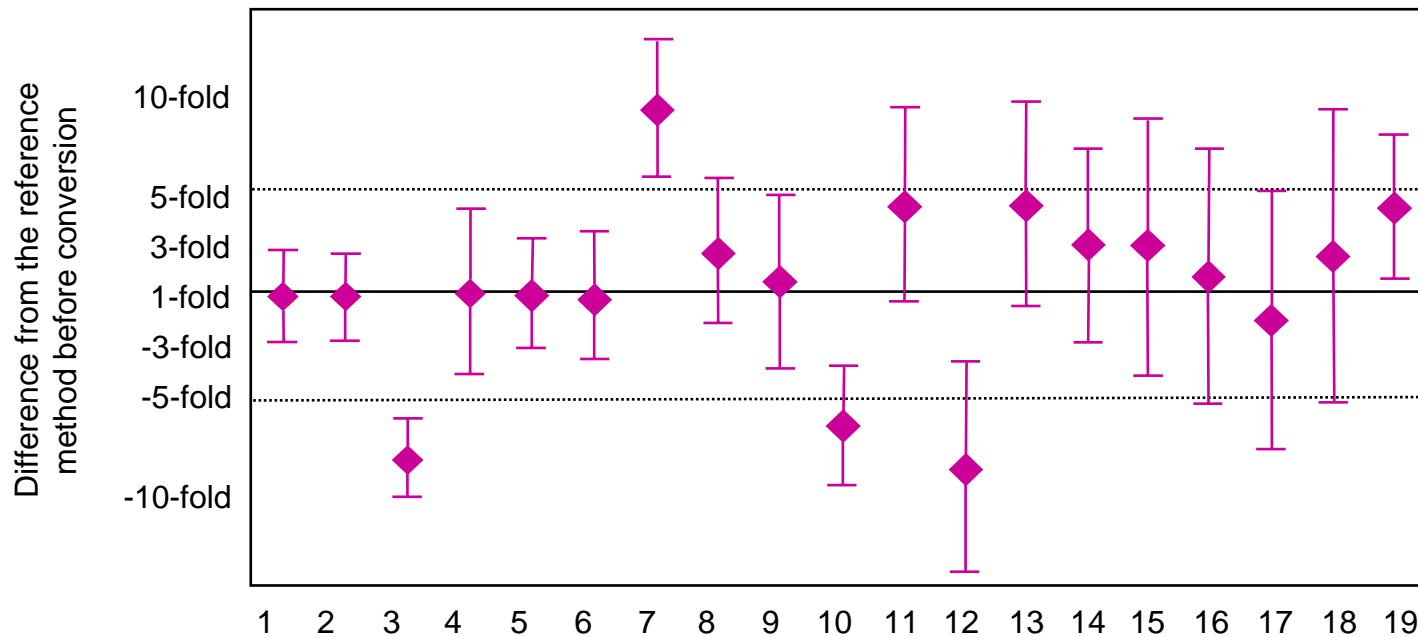
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19 BCR-ABL transcript quantification methods: results before conversion to IS

- Branford et al completed validation procedures for 19 BCR-ABL transcript quantification field-methods
- Before conversion the average difference between each field method ranged from 7.7-fold lower to 8.1-fold higher



Field method number
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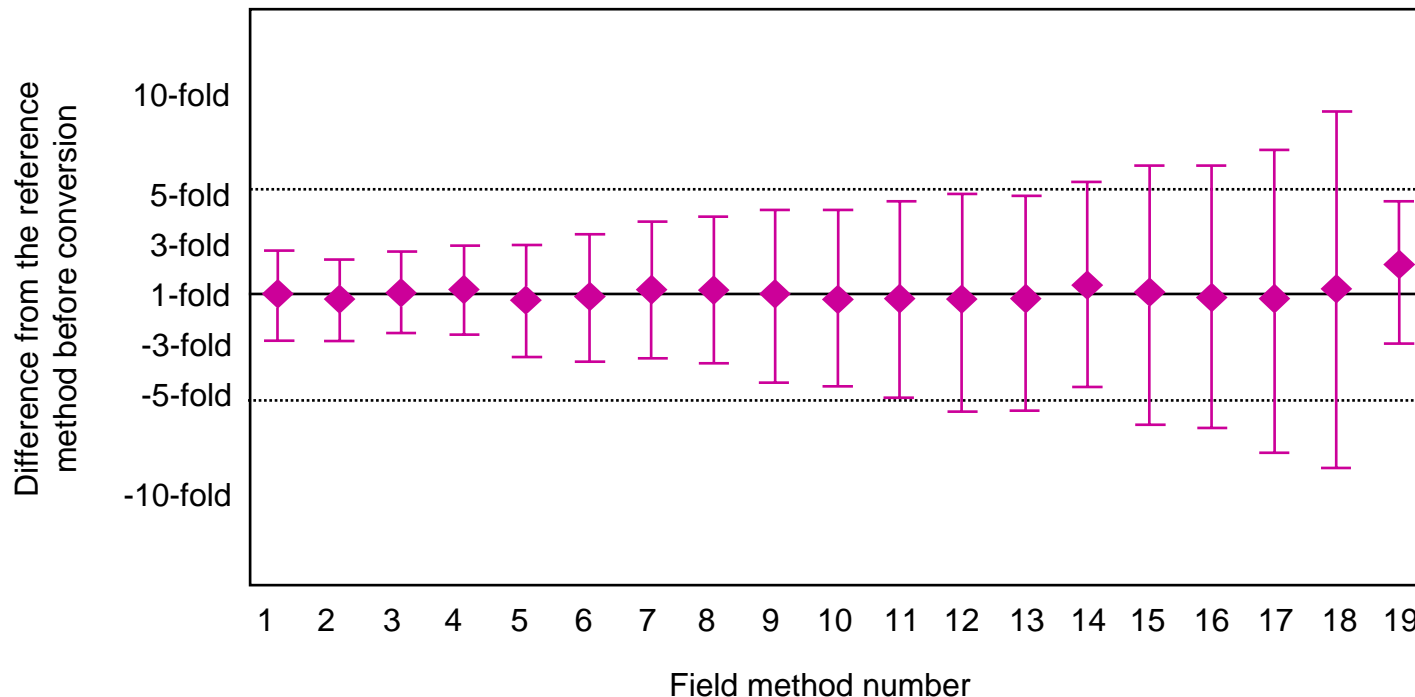
Branford *et al. Blood* 2008;112:3330–3338.



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19 BCR-ABL transcript quantification methods: results following conversion to IS

- Following conversion the average differences between each field method were within +/- 1.2-fold*



*95% limits of agreement varied

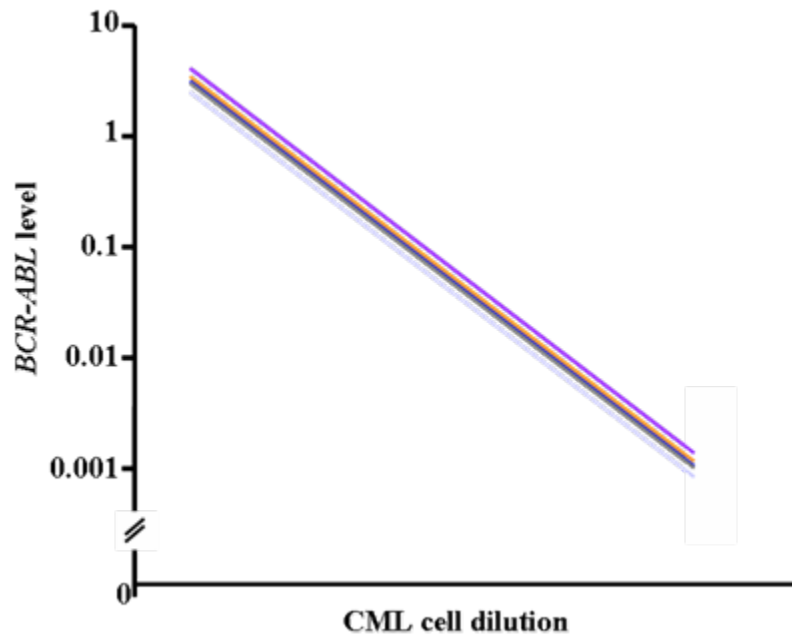
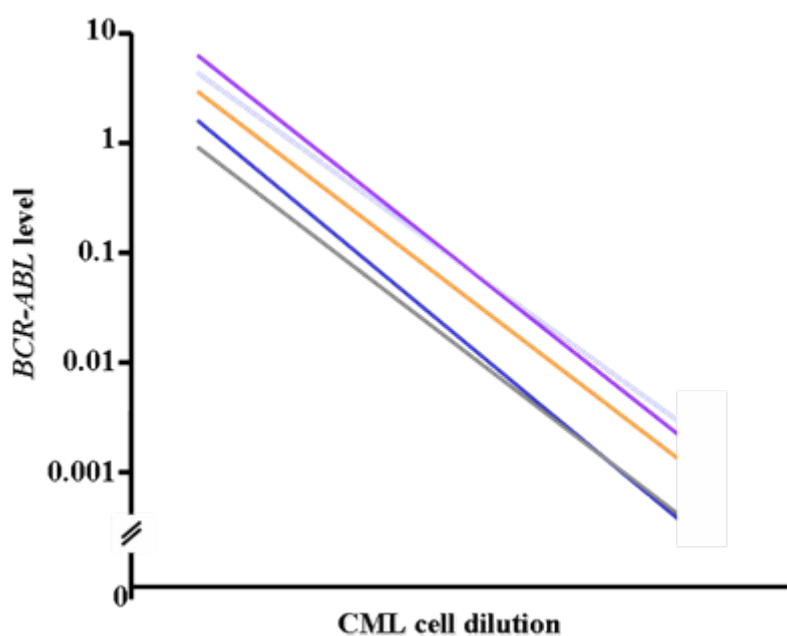
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Branford *et al. Blood* 2008;112:3330–3338.

Why do we need a conversion factor? Inter-laboratory results before and after conversion



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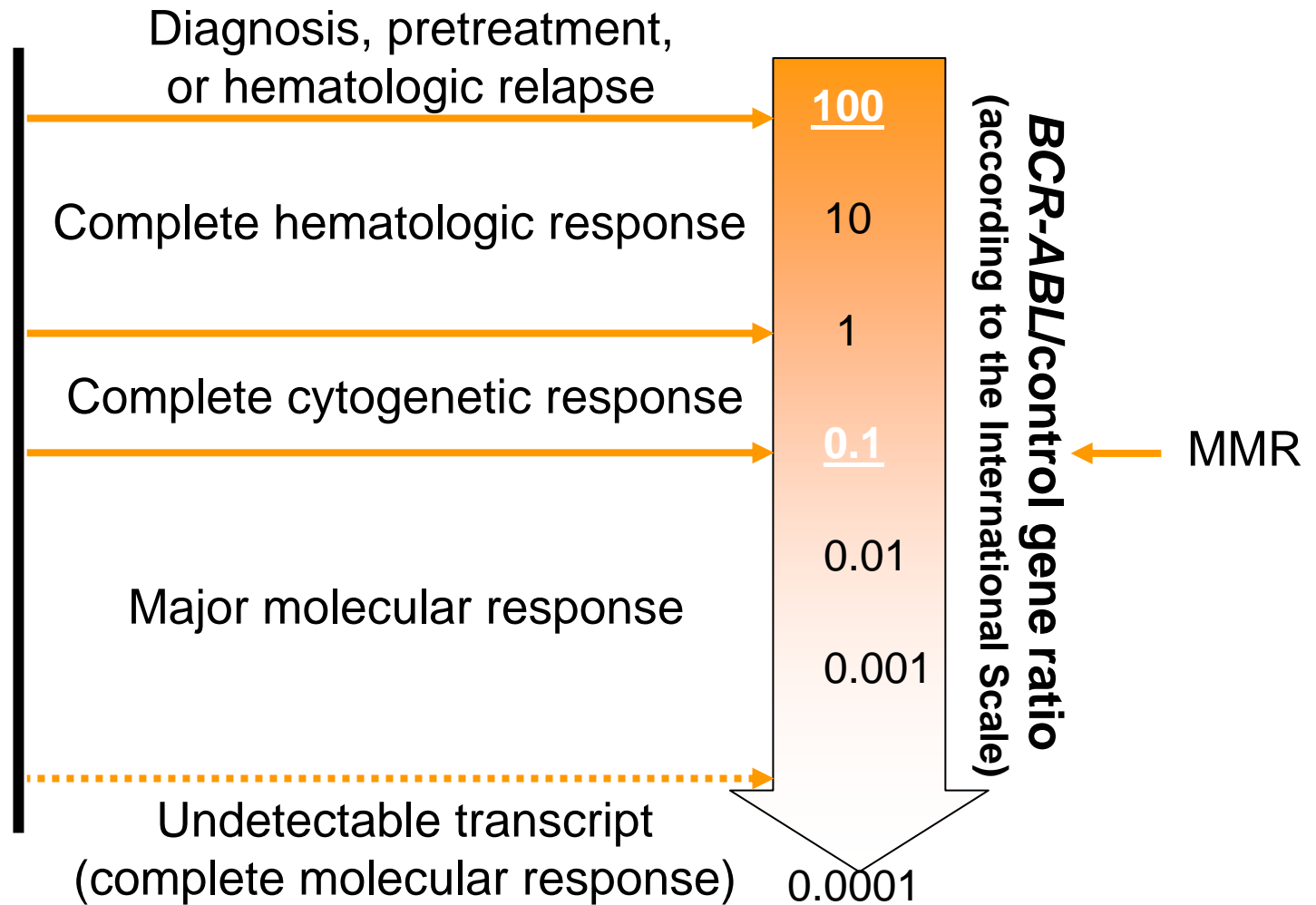
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International Scale

- The International Scale (IS) is based on the ratio of *BCR-ABL* copy numbers to control gene copy numbers
- It is dependent on two anchor values
 - A standardized baseline for the ratio of *BCR-ABL* to control gene (taken as 100% on the IS)
 - A 3-log reduction from this baseline (taken as 0.1% on the IS). This value is equivalent to MMR



Anchor values in the International Scale



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Hughes *et al.* *Blood*
2006;108:28–37.

Current status (Jan 2009)

- Fifty national reference centers to be included in the project
 - Phase I, 23 labs
 - Phase II, 27 additional labs
- Standardized lysates have been sent out to labs
- Educational documents describing project and providing lab guidelines have been circulated

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Summary

- Variability between testing laboratories has made it difficult to compare molecular monitoring data between labs
- The International Scale reduces variability and allows for standardized testing and reporting
- Through EUTOS for CML, 50 labs will be standardized around Europe

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