

A rapid and sensitive assay for detecting the BCR-ABL-T315I kinase domain mutation in chronic myeloid leukaemia

Cytogenetics & MRD Group

Department of Haematology / Hammersmith Hospital

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January 2008

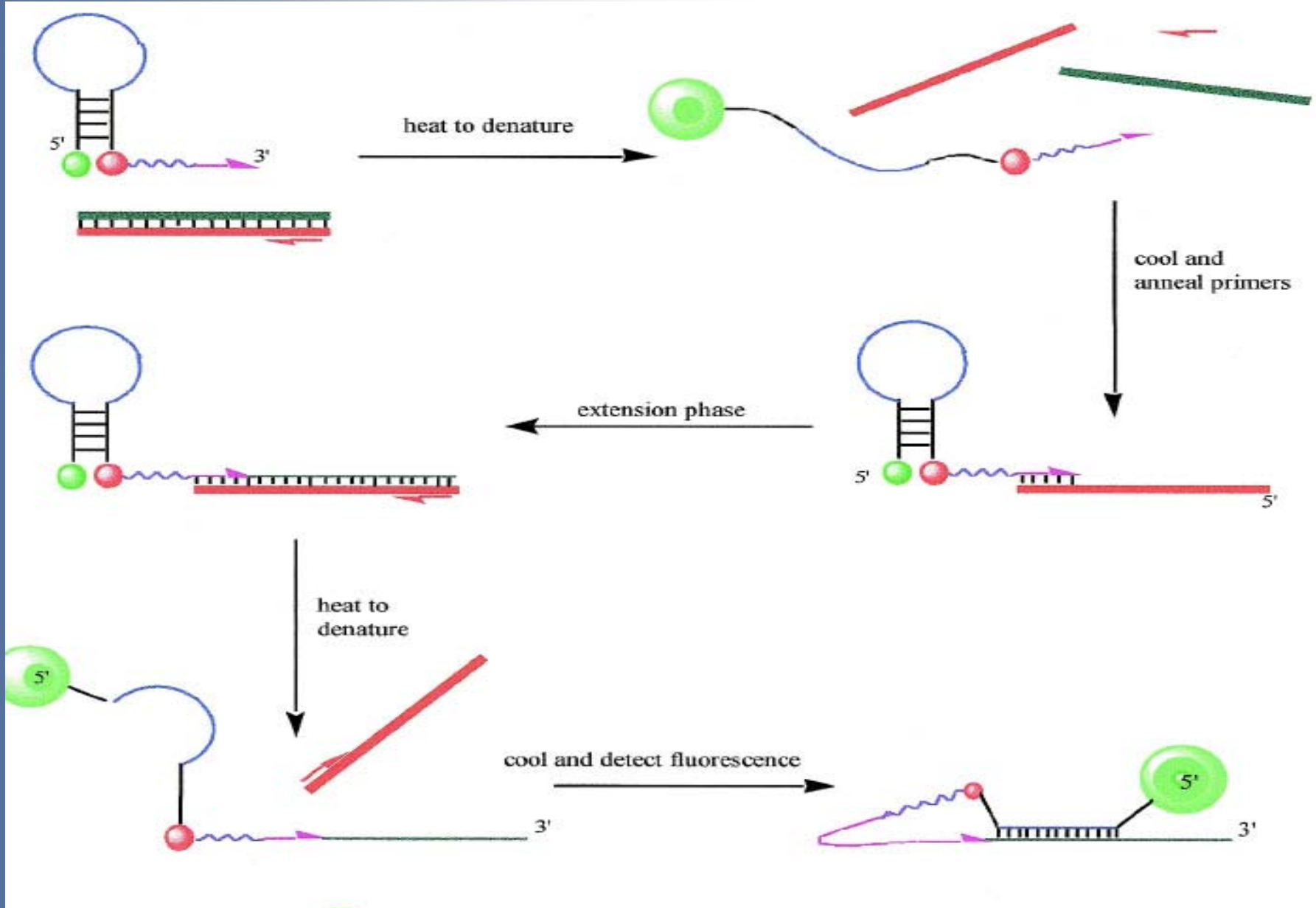
Importance of BCR-ABL-T315I KD Mutation Detection

- ⊙ Emergence of a BCR-ABL clone harbouring KD mutations: the best known mechanism of IM resistance
- ⊙ Over 45 different KD mutations reported from patients with resistance to IM
- ⊙ T315I is the most resistant mutation to IM and to all the current available Tyr kinase inhibitors

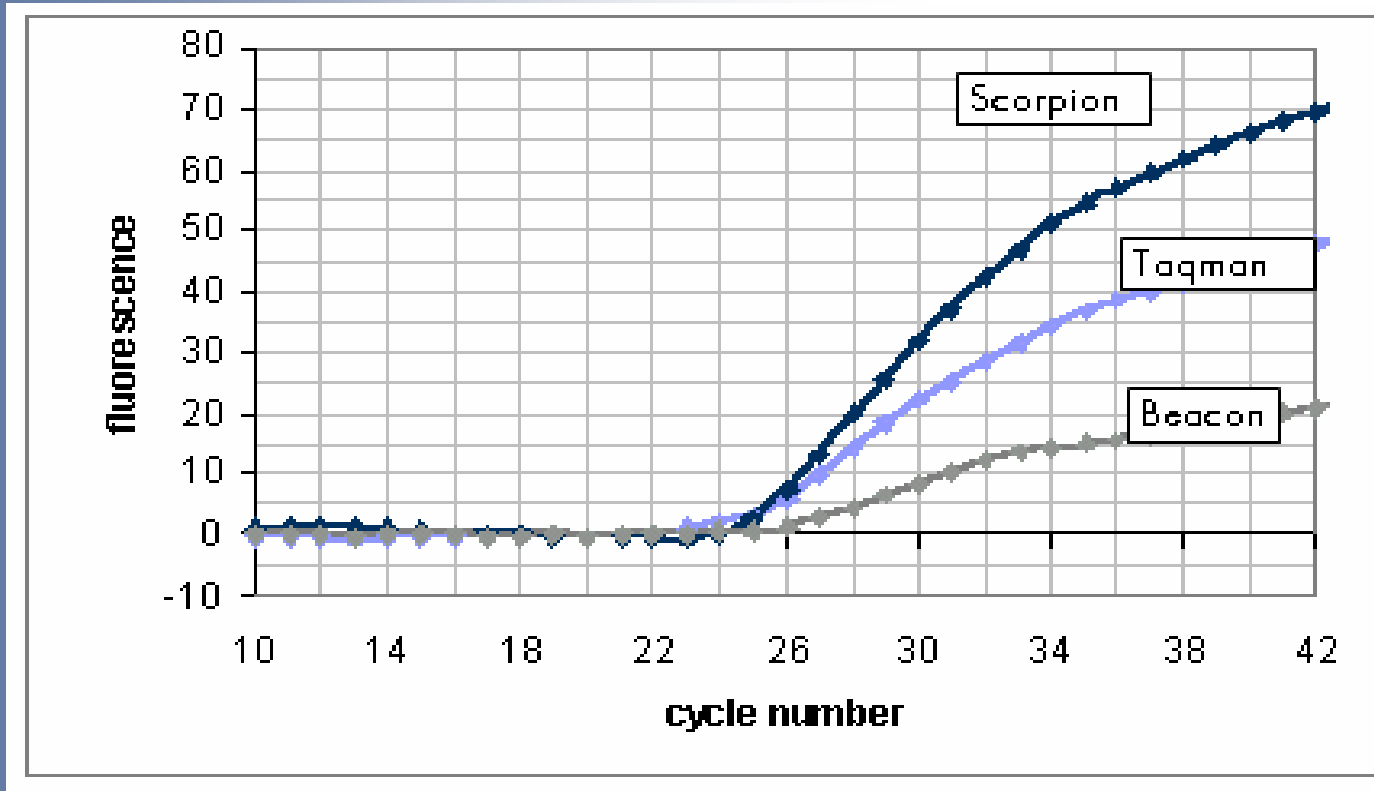
Different Methods for T315I Screening (sensitivity)

- ⊙ Direct Sequencing (>20-30%)
- ⊙ RFLP (>10%)
- ⊙ Enhanced PCR-RFLP (~0.1%)
- ⊙ Pyrosequencing (>5%)
- ⊙ DHPLC (1-5%)
- ⊙ MassARRAY genotyping
- ⊙ Ligation –PCR (0.1-0.05%)

Principle of Scorpion Assay (Unimolecular Format)



Comparison of Fluorescence Emission among Scorpion, Taqman and Molecular Beacon Probes



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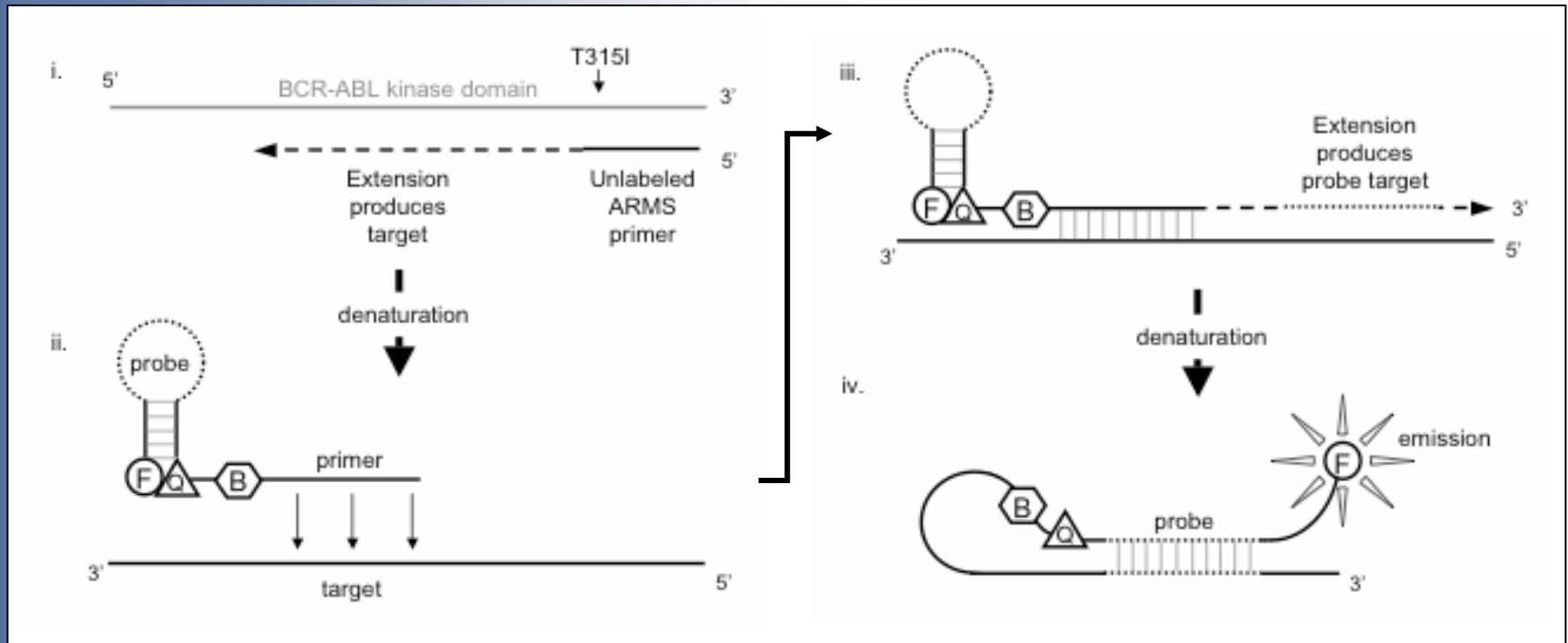
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Materials

- ⊙ cDNA (5 μ L)
- ⊙ T315I Assay Reaction mix (19.6 μ L)
- ⊙ Control Reaction mix (19.6 μ L)
- ⊙ *Taq* (0.4 μ L)
- ⊙ Standard

Principle of T315I Scorpion assay



2 reactions: 1) T315I specific primer 2) control primer

$\Delta C_t = T315I\ CT - Cont\ CT.$

Threshold for T315I positivity: $\Delta C_t < 11$

Study design

- ⊙ Serial dilution of BCR-ABL-T315I Ba/F3 cells in non mutated BCR-ABL Ba/F3 cells
- ⊙ 34 patient samples proved to be positive by either direct sequencing or pyrosequencing
- ⊙ 27 patient samples proved to be negative by either direct sequencing or pyrosequencing

Detection of BCR-ABL^{T315I} transcripts in limiting dilution experiments

BCR-ABL ^{T315I} Ba/F3 Cell Dilution	Δ CT (Scorpion)	Pyrosequencing	Direct Sequencing
0.1%	13.3	Undetected	Undetected
1%	10	Undetected	Undetected
10%	5.8	Positive	Undetected
20%	4.7	Positive	Undetected
40%	4.4	Positive	Positive
80%	2	Positive	Positive
100%	0.43	Positive	Positive

Summary of samples used to validate the T315I Scorpion assay

Patient material:	
Patients	25
Total samples	61
T315I status assessed by direct sequencing/pyrosequencing:	
T315I positive	34
T315I negative*	27
T315I status assessed by Scorpion assay:	
T315I positive	34
T315I negative*	27

* Including 3 samples with T315A and one sample with F317L

Summary

- ⊙ Mutation status was correctly assigned in all 61 samples
- ⊙ Absence of false positive/negative results
- ⊙ High sensitivity
- ⊙ Practicality of application in diagnostic lab

Acknowledgments

Hammersmith Hospital

- ⊙ Alistair Reid
- ⊙ Letizia Foroni
- ⊙ Jane Apperley
- ⊙ John Goldman
- ⊙ David Marin
- ⊙ Dragana Milojkovic
- ⊙ MRD Group

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- ⊙ Nicola Thelwell
- ⊙ Julie Watson