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
DxS diagnostic innovations
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)

Thewell N. et al.  Nucleic Acid Research 2000
Comparison of Fluorescence Emission among Scorpion, Taqman and Molecular Beacon Probes

DxS diagnostic innovations
Materials

- cDNA (5µL)
- T315I Assay Reaction mix (19.6µL)
- Control Reaction mix (19.6µL)
- *Taq* (0.4µL)
- Standard
Principle of T315I Scorpion assay

2 reactions: 1) T315I specific primer  2) control primer
\[ \Delta C_T = T315I \text{ CT} - \text{ Cont CT}. \]
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

<table>
<thead>
<tr>
<th>BCR-ABL$^{T315I}$ Ba/F3 Cell Dilution</th>
<th>ΔCT (Scorpion)</th>
<th>Pyrosequencing</th>
<th>Direct Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1%</td>
<td>13.3</td>
<td>Undetected</td>
<td>Undetected</td>
</tr>
<tr>
<td>1%</td>
<td>10</td>
<td>Undetected</td>
<td>Undetected</td>
</tr>
<tr>
<td>10%</td>
<td>5.8</td>
<td>Positive</td>
<td>Undetected</td>
</tr>
<tr>
<td>20%</td>
<td>4.7</td>
<td>Positive</td>
<td>Undetected</td>
</tr>
<tr>
<td>40%</td>
<td>4.4</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>80%</td>
<td>2</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>100%</td>
<td>0.43</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Summary of samples used to validate the T315I Scorpion assay

<table>
<thead>
<tr>
<th>Patient material:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>25</td>
</tr>
<tr>
<td>Total samples</td>
<td>61</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T315I status assessed by direct sequencing/pyrosequencing:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>T315I positive</td>
<td>34</td>
</tr>
<tr>
<td>T315I negative*</td>
<td>27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T315I status assessed by Scorpion assay:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>T315I positive</td>
<td>34</td>
</tr>
<tr>
<td>T315I negative*</td>
<td>27</td>
</tr>
</tbody>
</table>

* 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

DxS diagnostic innovations
- Nicola Thelwell
- Julie Watson