Bcr-Abl Kinase Domain Mutations

Martin Müller
Frequency of imatinib resistance within 3 years

<table>
<thead>
<tr>
<th>Phase</th>
<th>Primary resistance</th>
<th>Relapse/progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early chronic phase</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Late chronic phase</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Accelerated phase (600 mg/d)</td>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td>Blastic phase (600 mg/d)</td>
<td>66</td>
<td>93</td>
</tr>
</tbody>
</table>

Hochhaus and La Rosée *Leukemia*. 2004
Observations associated with resistance

Genomic amplification / overexpression

BCR-ABL Mutations

Clonal evolution / aneuploidy

Pharmacologic mechanisms

- Association with AGP-levels?
- Low MRP-1 levels independent prognostic factor (Lange et al.)
- Decrease of intracellular imatinib-levels in PGP+ patients (Illmer et al.)
### Molecular - cytogenetic causes of resistance

<table>
<thead>
<tr>
<th>Patients With Hematologic Resistance/Relapse</th>
<th>Chronic phase (n=35)</th>
<th>Accelerated phase (n=33)</th>
<th>Blastic phase (n=66)</th>
<th>All (n=134)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCR-ABL mutations (%)</strong></td>
<td>10/20 (50)</td>
<td>13/21 (62)</td>
<td>10/33 (30)</td>
<td>33/74 (45)</td>
</tr>
<tr>
<td><strong>Clonal evolution (%)</strong></td>
<td>15/29 (52)</td>
<td>8/16 (50)</td>
<td>16/22 (73)</td>
<td>39/67 (58)</td>
</tr>
<tr>
<td><strong>Combination (%)</strong></td>
<td>5/17 (29)</td>
<td>2/9 (22)</td>
<td>4/17 (24)</td>
<td>11/43 (26)</td>
</tr>
</tbody>
</table>

Lahaye et al. Cancer. 2005
Mutant BCR-ABL have increased IC$_{50}$ values for imatinib mesylate

Resistance to imatinib: 31 BCR-ABL mutations in 25 amino acids (n=126)

Polymorphisms: K247R, T315T, E499E
Survival after imatinib failure
Initial Australian experience

P-Loop: Highly conserved area

n=27

P-loop, n=13

Non P-loop, n=14

p=0.0024

Survival after imatinib failure
Current experience

n=105

T315I, n=14
Others, n=53
P-Loop, n=38

p=0.031 for T315I
CML, chronic phase, IFN-resistant

% Mutated BCR-ABL

Y253H, 100%

Years after start of imatinib mesylate

Imatinib 400-600 mg/d

Hydroxyurea
Significance of P-loop mutations in CML

• 89 imatinib resistant pts in 5 French centers; 26 with P-loop mutations, 18 with T315I, 50 with other mutations → significantly worse survival from diagnosis for P-loop or T315I mutations (p=0.014)

• 159 pts from MDACC: 52 mutations in 49 pts; 19 P-loop mutations median F/U 6 mo: 1/19 P-loop vs 4/30 non P-loop died; 15 mo surv 95% vs 83%

Indications for mutation screening

- hematologic resistance / relapse
- cytogenetic resistance / relapse
- 5-10-fold increase of BCR-ABL load
- prior to therapy with alternative kinase inhibitors (nilotinib, dasatinib)
- 3-monthly intervals under therapy with nilotinib and dasatinib
<table>
<thead>
<tr>
<th>Method</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequencing</td>
<td>unspecific</td>
<td>10-20%</td>
</tr>
<tr>
<td>Restriction digest analysis</td>
<td>specific</td>
<td>~2-5%</td>
</tr>
<tr>
<td>D-HPLC</td>
<td>unspecific</td>
<td>0.1-10%</td>
</tr>
<tr>
<td>Allele specific PCR</td>
<td>specific</td>
<td>0.01%</td>
</tr>
<tr>
<td>Sequencing of clones</td>
<td>unspecific</td>
<td>1-5%</td>
</tr>
</tbody>
</table>
D-HPLC: nested RT-PCR

1st step

BCR

Fragment A: BCR (Exon b2 / e1) – ABL (Exon 10/11)

ABL Kinase Domain (AA 235-509)

2nd step

B (AA 207-324)

C (AA 279-414)

D (AA 382-517)
Sensitivity of D-HPLC

0.1-1%

mutant BaF3T315I

100%

50%

10%

1%

0.1%

normal BaF3BCR-ABL
Dasatinib inhibits growth of 14/15 imatinib-resistant BCR-ABL-expressing Ba/F3 cell lines in vitro

Shah et al., Science, 2004
Conclusions

• Frequency of imatinib resistance depends on the stage of CML.

• The major causes of resistance are BCR-ABL mutations and clonal evolution.

• The impact of early detection of mutated clones by sensitive assays requires prospective evaluation.

• Certain BCR-ABL mutations might especially impair prognosis, in particular on continuous imatinib therapy.

• Early elucidation of imminent resistance to kinase inhibitors might contribute to individualized therapy according to molecular data.