Metaphase chromosomes, interphase FISH, and RQ-PCR for early monitoring of CML treatment response.

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Analysis options for monitoring post treatment CML

• Marrow (BM)
  – Conventional cytogenetic metaphase analysis (until CCyR)
  – RQ-PCR for BCR/ABL

• Blood (PB)
  – RQ-PCR for BCR/ABL
  – Dividing cells obtained: metaphase G-banded analysis
  – No dividing cells: Interphase FISH
Concerns

• Observations from monitoring treatment response in CML
  – Serial results from > 400 patients
  – Integrated approach – chromosomes, FISH, RQ-PCR

• Concerns over accuracy of early treatment monitoring….
  – Discrepant results between:
    • Ph +ve metaphase cells
    • Interphase FISH for BCR/ABL
    • RQ-PCR for BCR/ABL
    • Blood (PB) vs marrow (BM)
Patient A

- Presented April 1999
- Age 57
- 46,XX,t(9;22)[20]
- IFN:
  - Clinical remission but all cells remained Ph+ve cytogenetically. IFN stopped in Feb 2000
- Started on Glivec in August 2001
- Clinician requested interphase FISH analysis in addition to metaphase G-banded analysis

<table>
<thead>
<tr>
<th>Date of BM</th>
<th>Metaphase % Ph+ve</th>
<th>Interphase FISH % BCR/ABL +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug 2001</td>
<td>100 [30]</td>
<td>78</td>
</tr>
<tr>
<td>Oct 2001</td>
<td>100 [40]</td>
<td>67</td>
</tr>
<tr>
<td>Feb 2002</td>
<td>100 [45]</td>
<td>56</td>
</tr>
<tr>
<td>May 2002</td>
<td>100 [30]</td>
<td>95</td>
</tr>
<tr>
<td>Sept 2002</td>
<td>100 [30]</td>
<td>73</td>
</tr>
<tr>
<td>Dec 2002</td>
<td>95 [19/20]</td>
<td>49</td>
</tr>
<tr>
<td>Mar 2003</td>
<td>100 [25]</td>
<td>35</td>
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</tbody>
</table>
Why were the marrow metaphase and interphase results discrepant?

• ? Haemo-dilution:
  – Increases proportion of non-CML cells (lymphocytes)
  – Extent varies between samples
    • Sampling procedure effect
    • Treatment effect

• ? Glivec induced neutropenia:
  – Under-representation of myeloid cells
  – Increases likelihood of haemo-dilution
  – Transient?

• ? FISH scoring artefacts
  – Score only non-overlapping nucleii
  – Ph clone primarily granulocytes (neutrophils, basophils, eosinophils) which have lobed/multi-lobed nuclei and precursors

• Re-scored slides examining ‘lobed’ nuclei alone
FISH scoring: based on nuclear morphology

Round
BCR/ABL -ve

Lobed
BCR/ABL -ve

Round
BCR/ABL +ve

Lobed
BCR/ABL +ve
Patient A. Review of cytogenetic results

- Metaphase analysis:
  - No response

- Whole marrow interphase analysis:
  - ? Response

- Lobed nuclei interphase analysis:
  - No response
  - gIF correlates better with Ph metaphase cells than wIF

<table>
<thead>
<tr>
<th>Date of marrow</th>
<th>Metaphase % Ph +ve</th>
<th>Interphase analysis Whole marrow wIF % BCR/ABL +ve</th>
<th>Interphase analysis Lobed nuclei gIF %BCR/ABL +ve</th>
</tr>
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<td>95 [19/20]</td>
<td>49</td>
<td>71</td>
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</table>

- Are these discrepancies reproducible? Also in PB?
  - Significant no. of PBs received for treatment monitoring.
  - Analysis often performed using interphase FISH.

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Serial interphase FISH results

- Prospective study comparing results from whole PB and selected lobed nuclei analysis
- Scored 50-100 interphases from whole PB and 50 selected lobed interphase cells

<table>
<thead>
<tr>
<th></th>
<th>Patient B</th>
<th>Patient C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Sample</td>
<td>% BCR/ABL+ve</td>
<td>% BCR/ABL+ve</td>
</tr>
<tr>
<td></td>
<td>Whole Blood</td>
<td>Lobed nuclei</td>
</tr>
<tr>
<td>Jan 03</td>
<td>75</td>
<td>95</td>
</tr>
<tr>
<td>May 03</td>
<td>36</td>
<td>100</td>
</tr>
<tr>
<td>Aug 03</td>
<td>66</td>
<td>100</td>
</tr>
<tr>
<td>Jan 04</td>
<td>65</td>
<td>98</td>
</tr>
</tbody>
</table>

Potentially misleading results from whole sample interphase FISH (wIF)
- Due to neutropaenia & proportion of uninvolved lymphocytes in sample
- Seen consistently since.

Are there implications for RQ-PCR on PB?
Patient D. RQ-PCR on PB

Level of BCR/ABL normalised to ABL

Follow up at 3/12. Pattern typical – seen in many patients.

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Factors to consider

• What cells are expected in the BM?
• What cells are expected in PB?
• Which cells represent the leukaemia?

• How do CML cells behave?
Typical Marrow and Blood cells

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Marrow %</th>
<th>Blood %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils (segmented)</td>
<td>10-30</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (band)</td>
<td>10-30</td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0-6</td>
<td>4</td>
</tr>
<tr>
<td>Basophils</td>
<td>0-2</td>
<td>1</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0-3</td>
<td>6</td>
</tr>
<tr>
<td>Myeloid Precursors</td>
<td>11-48</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>5-15</td>
<td>20-40</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0-2</td>
<td></td>
</tr>
<tr>
<td>Megakaryocytes</td>
<td>0-2</td>
<td></td>
</tr>
<tr>
<td>Nucleated rbc</td>
<td>18-34</td>
<td></td>
</tr>
</tbody>
</table>

Data combined from various sources

Is treatment induced neutropenia due to reduction in Ph cells? Or a generalised response? What are the kinetics of response? Do blood mature Ph + grans clear most rapidly? If neutropenic - blood virtually all lymphocytes - ? Minimal involvement in CML

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Marrow cells mitotic, blood cells are not.

- How do CML cells behave?
  - No differentiation block
  - Early release from marrow
  - Delayed maturation
    - i.e. continue dividing after enter blood

- Normal blood does not contain mitotic cells
Factors to consider - and consequences

- What cells are expected in the BM?
  - Mostly immature myeloid cells

- What cells are expected in PB?
  - Mostly mature granulocytes & lymphocytes

- How do CML cells behave?
  - No differentiation block
  - Early release from marrow
  - Delayed maturation
    - i.e. continue dividing after enter blood

- Ph +ve metaphase cells
  - Gold standard for level of disease
  - (if there is enough leukaemia)

- BCR/ABL +ve interphase cells
- RQ-PCR BCR/ABL:ABL ratios
  - If neutropaenic - potential to under-represent level of disease

- Ph +ve metaphase cells in blood
  - Over-represent level of disease
More Prospective data

• Data extracted from diagnostic cases where parallel tests have been performed.
  – Chronic phase CML, within first 2 years of treatment with Glivec
  – Diagnosed since 2002
  – Ph percentage on at least 20 cells (max 60)
  – Interphase FISH on at least 50 nuclei
  – Samples within 2 weeks considered concurrent
  – Prior to any subsequent complete loss of response
  – BCR/ABL to ABL percentage ratio adjusted to % of patient specific diagnostic ratio, or % of mean diagnostic ratio (Diagnosis = 100%).

• Not comprehensive data as resource limited diagnostic series.
Mean wIF/gIF = 0.61
Excludes gIF < 15%

Mean PB RQ/gIF = 0.72
Excludes gIF < 15%
Correlations 2

- Correlation between PB wIF and PB RQ
- Correlation between BM Ph Mets and BM RQ
Summary – Prior to achieving CCyR

True level of leukaemia

- BM metaphase
- BM Gran iFISH
- PB Gran iFISH

BM RQ-PCR
- BM whole iFISH

PB RQ-PCR
- PB whole iFISH

PB metaphase
Summary – Implications for early treatment monitoring

Our view:

- BM samples every 3/12 until CCyR preferred (then PB every 3/12).
- If PB when not in CCyR:
  - Caution with respect to RQ-PCR & whole interphase FISH
  - Caution with respect to metaphase chromosome analysis
  - Granulocyte iFISH preferred
    - Selective scoring by morphology
    - or selection prior to test (MACS, FACS, lymphoprep)
      - See Reinhold et al. Leukaemia 2003;17:1925-1929
- RQ-PCR at 3/12 on PB likely to be misleading.
  - If no HR at 3/12 then BM preferred to PB
  - If PB:
    - If RQ-PCR shows no response – plausible
    - If RQ-PCR shows response – NOT reliable – confirm granulocyte iFISH