THERAPEUTIC IMPLICATIONS IN PATIENTS WITH PHILADELPHIA-POSITIVE CHRONIC MYELOID LEUKAEMIA, EXPRESSING P190 BCR-ABL+

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INTRODUCTION: The discovery of the Philadelphia (Ph) chromosome t(9;22) in 1960 by Nowell & Hungerford was the first consistent chromosomal aberration associated with a specific type of leukemia-Chronic myeloid leukemia (CML) was a breakthrough in cancer biology. The breakpoints within the ABL gene at q34 can occur anywhere over a large area at its 5’ end. Regardless of the exact location, BCR sequences are fused to ABL exon a2. In contrast to ABL, breakpoints within BCR localize to 1 of 3 so-called breakpoint cluster regions (bcr). In most patients with CML the break occurs within BCR exons 13-16 defined as M-bcr: Alternatively, following splitting, fusion transcripts with either 5′ or 3′ truncated bcr exons can be formed leading to a 210-216 kDa chimeric protein (P210BCR-ABL). In the remaining patients the breakpoints are located between the alternative BCR exons 6′ and 62′, and termed the minor breakpoint cluster region (m-bcr). The resultant e12-A2 mRNAs is translated into a 190-kd protein (P190BCR-ABL). Recently, a third breakpoint cluster region (μ-bcr) was identified downstream of exon 19, giving rise to a 230-kd fusion protein (P230BCR-ABL).

Figure 1. Locations of the breakpoints in the ABL and BCR genes and structure of the chimeric mRNAs derived from the various breaks.

CORRELATION OF BCR BREAKPOINT WITH LEUKAEMIC PHENOTYPE

<table>
<thead>
<tr>
<th>BCR breakpoint</th>
<th>Junction BCR-ABL mRNA</th>
<th>Protein</th>
<th>Diseases</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor (m)</td>
<td>e12/a2</td>
<td>P190</td>
<td>CML</td>
<td>Very rare</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AML</td>
<td>ALL</td>
<td>85-90% of adult Ph+ ALL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myeloma</td>
<td>N.H.</td>
<td>Very rare</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-2 case</td>
</tr>
<tr>
<td>Major (M)</td>
<td>b2/a2 or b3/a2</td>
<td>P210</td>
<td>CML</td>
<td>100% of all CML patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AML</td>
<td>ALL</td>
<td>50-50% of adult Ph+ ALL</td>
</tr>
<tr>
<td>Micro (μ)</td>
<td>c3/a2</td>
<td>P230</td>
<td>CML</td>
<td>Rarely evidenced WBC &gt;2x10^9/l, mild anaemia</td>
</tr>
</tbody>
</table>

CASE 1: A 67 years old female patient was admitted to the University Clinic of Hematology and enlarged spleen -2 cm below costal margin.

Findings at the diagnosis:

- Laboratory tests:
  - Hematological: ER 4.0-4.4;4.5 to 1.21;X11120;WBC 145.4 to 10^9/L; Hb: Mysob 5-2%; Promy 62-2%; Eos 6%; Bas 1PLT 287-485x10^9/L
  - Biochemical: total protein 6-8.5 g/dL; uric acid 4-8 mg/dL; ura: creatinine within referent ranges. LDH 1251

- Myelogram:
  - Hypocellular bone marrow. Neutrophils 1% to 3%, promyelocytes 10-20%.

- Molecular analysis:
  - Philadelphia chromosome e12 transcripts A1 protein and no p120: According to these data a diagnosis of CML (Ph) + p190 BCR-ABL - chronic phase was accepted. Following initial standard cytoreductive treatment with Ara-C and Hydro, from August 2006 treatment with Imatinib (Glivec) 400mg/d was started. Soon afterwards the dose was reduced to 300mg/daily because of recurrent neutropenia and anemia, as well as dermatitis, manifested as generalized skin rash.

Therapeutic outcome:

- On the 6th month - hematomal response was achieved with WBC 7.0x10^9/L, Pt 293x10^9/L, DBC: 594%, Sg 56%, Mysob 2%, Lt 27%, Eos 1%; normocellular bone marrow. Neutrophils 1%, Myeloblast 1%, Promyelocytes 1%, No increase of the Es and Ba. Minor cytogenetic response: 46XX/46XX(I-1)2(22)(q14)1(14%) = 81-98%.
- On 30th month the patient has not achieved a complete Gy response yet. At that time the serum level of immatinib was tested in The University of Bordeaux (EUTOS), because of insufficient response. The result showed serum level of immatinib of 53 ng/ml. On month 30 the patient has not achieved a complete Gype response yet. At that time the serum level of immatinib was tested in The University of Bordeaux (EUTOS), because of insufficient response. The result showed serum level of immatinib of 53 ng/ml. Currently the patient is in hematological remission, with Major CypG, on 360mg imatinib daily. The next assessment is planned for 2009.

CASE 2: In July 2006, a 78- year old male patient was admitted to the University Clinic of Haematology, Plovdiv because of elevated WBC 30.1x10^9/L, anemia Hb 86g/l, and thrombocytopoenia 25x10^9/L. He complained of exhaustion, weight loss, bone pain in both calves and substernal thickness. On physical examination liver 3 cm below costal margin, spleen on the umbilical line He had history of anemia since Feb 2000 (Hgb 11g/l), 3 normal WBC 4.3x10^9/L with left shift, myeloid cells 1.8%, 25% myeloblasts, low platelets (40x10^9/L), normal electrolytes, serum glucose 176mg/dL, creatinine 0.8mg/dL, uric acid 5.5mg/dL, ura 0.1mg/dL, creatinine within referent ranges. LDH 1035 UI

- Myelogram: Hypocellular bone marrow. Neutrophils 1%, Myeloblast 17%, Promyelocytes 5%, Eos 4%

- Cytogenetic analysis: 46XY/9(22)(q13)/t(9;22)(q34;q11)

- Molecular analysis: presence of molecular equivalents of Philadelphia chromosome e12 transcripts p190 protein and no p210: According to these data a diagnosis of CML (Ph) (P190 BCR-ABL) - blast crisis was accepted. The patient was in group with high risk according to Sokal and Hashford. Treatment with Ara-C was initiated, after cytoreduction to WBC 4.3x10^9/L, Imatinib (Glivec) 600mg/d was started. Improvement in his general condition along with reduction of blasts in peripheral blood to 3%, increase of the Hg level >100g/l without haemotransfusions and PLT >100 x10^9/L was registered for about 2 months. This short-term stabilization was followed by an acceleration of the disease and death occurred 5 months after the diagnosis.

CASE 3: Already published:


The patient was 77 years old woman who had been referred to the Hematology clinic in the National Center of Hematology and Transfusionistry in 1998 for evaluation of leukocytosis and thrombocytosis. Physical examination revealed enlarged liver and spleen 3-4 cm below the respective costal margins. Laboratory tests (hematological) Hb 85g/L, WBC 100x10^9/L, normal electrolytes, biochemical: total protein 6-8.5 g/dL; uric acid 4-8 mg/dL; ura: creatinine within referent ranges. LDH 1033 UI

- Myelogram: Hypocellular bone marrow. Neutrophils 1%, Myeloblast 17%, Promyelocytes 5%, Eos 4%

- Cytogenetic analysis: 48XX/8(22)(q13)/t(9;22)(q14)

- Molecular analysis: presence of molecular equivalents of Philadelphia chromosome e12 transcripts p190 protein and no p210: Accordingly to these data a diagnosis of CML (Ph) (P190 BCR-ABL) - blast crisis was accepted. The patient was in group with high risk according to Sokal and Hashford. Treatment with Ara-C was initiated, after cytoreduction to WBC 4.3x10^9/L, Imatinib (Glivec) 600mg/d was started. Improvement in his general condition along with reduction of blasts in peripheral blood to 3%, increase of the Hg level >100g/l without haemotransfusions and PLT >100 x10^9/L was registered for about 2 months. This short-term stabilization was followed by an acceleration of the disease and death occurred 5 months after the diagnosis.

DISCUSSION: Progress in understanding the molecular basis of signal transduction has contributed substantially to clarifying the mechanisms of leukemogenesis and of leukemia progression and has led to the identification of a number of specific molecular targets for treatment. The identification of the leukemia-specific hybrid tyrosine kinase BCR-ABL has led to the identification of the successful therapeutic application of the powerful tyrosine kinase inhibitor, imatinib. The resulting chimeric proteins from the bcr - abl fusion P210, P190 and P250 can retain a constitutively activated tyrosine kinase activity. Thus Ph + p190BCR-ABL patients reasonably benefit from the application of tyrosine kinase inhibitors. There are less than 30 cases of Ph + p190BCR-ABL patients described in literature so far. While the molecular findings and the clinical features of these patients are largely described, in single cases only the therapeutic approach is mentioned. Moreover the majority of the patients were diagnosed in the pre-imatinib era thus leaving clinicians making decisions on their personal judgement and theoretical knowledge. Recently a molecular remission in CML-chronic phase patient with p190BCR-ABL expression with imatinib treatment was reported. Our patient (case 1) achieved a major CypG response. Both have outcomes which are typical for the chronic phase of CML patients. Blast crisis is a condition in which CML patients fail to induce durable responses irrespectively of the molecular pattern. The patient we describe ( case 2) had survival of 5 months, which is the mean survival of the CML-blast crisis in general. It will hardly be possible to conduct a large clinical trial for this extremely rare clinical entity. Accumulation of cases is a possibility to elucidate the fate of these patients and the best treatment choice.

CONCLUSION: We believe that in Ph + P190BCR-ABL the tyrosine kinase inhibitors are a reasonable choice of treatment. Molecular pattern can modulate the clinical phenotype, but the therapeutic outcome depends mainly on the phase of the disease.