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

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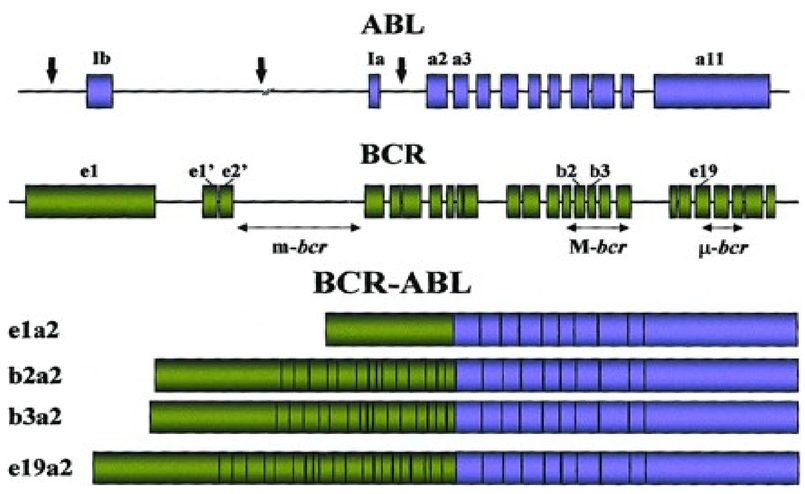
INTRODUCTION : The discovery of the Philadelphia (Ph) chromosome t(9;22) in 1960 by Nowell P& Hungerford D as the first consistent chromosomal abnormality associated with a specific type of leukemia- Chronic myeloid leukaemia (CML) was a breakthrough in cancer biology. The breakpoints within the *ABL* gene at 9q34 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 12-16 defined as M-*bcr*. Because of alternative splicing, fusion transcripts with either b2a2 or b3a2 junctions can be formed deriving a 210-kd chimeric protein (P210*BCR-ABL*). In the remaining patients the breakpoints are between the alternative *BCR* exons e2' and e2, termed the minor breakpoint cluster region (m-*bcr*). The resultant e1a2 mRNA is translated into a 190-kd protein (P190*BCR-ABL*). Recently, a third breakpoint cluster region (μ-*bcr*) was identified downstream of exon 19, giving rise to a 230-kd fusion protein (P230*BCR-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

BCR breakpoint	Junction BCR-ABLmRNA	Protein	Diseases	Frequency
Minor (m)	e1/a2	P190	CML ALL AML Myeloma NHL	Very rare 85% of childhood Ph+ ALL 50-70% of adult Ph+ ALL Very rare 1 case 1 case
Major (M)	b2/a2 or b3/a2	P210	CML ALL	100% 15% of childhood Ph+ALL 30-50% of adult Ph+ALL
Micro (μ)	c3/a2	P230	AML CML-N	Very rare Rare

CASE 1. A 67 years old female patient was admitted to the University Clinic of Hematology in Plovdiv in June 2006 because of intermittent pain in the sternal bone, elevated WBC= 80,2x109/l, mild anemia and enlarged spleen -2 cm below costal margin.

Findings at the diagnosis:

Laboratory tests(hematology): Er 4.09-4.3.45x1012/l, Xr112-92g/l, WBC 145-4,5x109/l, DBC: Mylobl 2-5%, Promc 6-2%; Eo4%; Ba1% PLT 287-485x109/l

Laboratory tests (biochemistry): total protein,albumin, serum glucose, billirubine, ASAT, ALAT, electrolytes, uric acid, urea , creatinine - within referent ranges. **LDH 1251**

Myelogram: Hypercellular bone marrow, Gr/Er 12-14/1; Myeloblasts 1%, Promc3%; Eo4%

Cytogenetic analysis: 46XX/46XX t(9;22);(q34;q11) = 14%/86%

Mollecular analysis: presence of molecular equivalents of Phiadelphia chromosome e1a2 transcripts/ p190 protein and no p210:

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 Hydrea, 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 rush.

Therapeutic outcome:

On the 6th month - hematological response was achieved with WBC 7.0x9/l , Plt 239x109/l , DBC: St4%, Sg 56%, Mo2%, Ly27%, Eo1%; normocellular bone marrow, Gr/Er=4/1; Myeloblastst 1%, Promyelocyes 2%. No increase of the Eo and Ba. ***Minor cytogenetic response : 46XX/46XX t(9;22);(q34;q11) = 36%/64%***

On the 12th month: sustained hematological response, minor CyG response

On the18th *month*: sustained hematological response, major CyG response: 46XX/46XX t(9;22);(q34;q11) = 84%/16%.

On month 30th the patient has not achieved a complete CyG reponse yet. At that time the serum level of imatinib was tested in The University of Bordeaux (EUTOS), because of insufficient response. The result showed serum level of Imatinib of 853 ng/ml. ***On month 30th*** the patient has not achieved a complete CyG reponse yet. At that time the serum level of imatinib was tested in The University of Bordeaux (EUTOS), because of insufficient response.Theresult showed serum level of Imatinib of 853 ng/ml..Currently the patient is in hematological remission, with Major CyGR, on 300mg Imatinib /daily. The next assessment is planne for 2009

C **ASE 2**. In July 2008, a 76 - year old male patient was admitted to the University Clinic of Haematology, Plovdiv because of elevated WBC 30.1x109/l, anemia Hg 86g/l, and thrombocytopenia 25x109/l. He complained of exhaustion, weight loss, bone pain in both calfs and subfebrile temperature. On physical examination liver 3 cm bellow costal margin, spleen on the umbilical line He had history of anemia since February 2006 (Hg112 g/l), normal WBC 4,3x109/l but with left shift in DBC – ProMc1%, Mc3% and slightly enlarged spleen. He did not consult a hematologist, although referred by his general practicioner.

Findings at the diagnosis:

Laboratory tests(hematology): Er 3.14-3.58x1012/l, Hg 86-102/l, WBC 30.1-4.2x109/l, DBC: Mylebl 17%, Promc 6%, Mc 15%; J 11%; St 13%; Sg 27%, Ly7%, Eo3%; Ba1% PLT 25x109/l

Laboratory tests (biochemistry): total protein,albumin, serum glucose, billirubine, ASAT, ALAT, electrolytes, uric acid, urea , creatinine -within referent ranges. **LDH 1033 U/l**

Myelogram: Hypercellular bone marrow, Gr/Er 12-13/1; Myeloblasts 17%, Promc5%; Eo4%

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

Mollecular analysis: presence of molecular equivalents of of Phiadelphia chromosome e1a2 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 Hasford. Treatment with Ara-C was initiated , after cytoreduction to WBC 4.3 x109/l, Imatinib (Glvek) 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 x109/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:

Balatzenko G et al. Philadephia chromosome – positive chronic myeloid leukemia with p190BCR-ABL+ rearrangement, overexpression of EV11 gene and extreme thrombocytosis: a case report. Cancer genetics and cytogenetics 2008;181:75-7

The patient was 77 year-old woman referred to the Hematology clinic in the National Center of Hematology and Transfusiology in 1998 for evaluation of leucocytosis and thrombocytosis. Physical examination revealed enlarged liver and spleen 3-4 cm below the respective costal margins.

Laboratory tests(hematology) Hg 85g/l;WBC 100x109/l with left shift: myeloblasts <3%; 10 nucleated erythroid cells /100 WBC and extreme thrombocytosis Plt 2 974x109/l

Bone marrow aspirate: Extremely hypercellular bone marrow, because of increased number of myeloid cell with left shift maturation, < 8% myeloblasts, dysplasia in >50% of the maturating myeloid cells, increased number of small to medium-sized megacaryocytes with abundant platelets. Ba 5%, Mo and Macrophages 4%, Ly 2%

Flowcytometry of the peripheral blood showed aberrant myeloid phenotype: CD13+, CD33+, CD34+, HLA-DR+, CD38+, CD117+, CD 133/2+, CD 56dim+coexpression

Cytogenetic analysis: 46XX t(9;22);(q34;q11.2) – G banding technique, on cultured bone marrow cells

Molecular analysis:using reverse transcription polymerase chain reaction RT-PCR t(9;22) /M-BCR-ABL(-) negative;t(9;22)/m-BCR-ABL (+) positive. In addition: overexpression of EVI1 gene and overexpression of MDR1 gene were detected using semi-quantitative RT-PCR analyses with coamplification of β-actin m RNA and β2-microglobulin m RNA incorporated as internal controls.

A diagnosis of **CML (Ph+) p190 BCR-ABL** was accepted and treatment wit Ara-C + Hydrea was started (this is the pre-imanitinib era). One month later the patient died of brain haemorrhage.

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 and the successful therapeutic application of the powerful tyrosine kinase inhibitor, imatinib. The resulting chimeric proteins from the bcr –abl fusion gene P210, P190 and P230 retain a constitutively activated tyrosine kinase activity. Thus Ph + p190BCR-ABL+ CML patients reasonably will benefit from the application of tyrosine kinase inhibitors. There are less than 30 cases of Ph + p190BCR-ABL+ CML 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 clinitians 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 CyG response. Both have outcomes which are typical for the chronic phase of CML patients. Blast crisis is a condition in which tyrosine kinase inhibitors 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 possible way to elucidate the faith of these patients and the best treatment choice.

CONCLUSION: We believe that the in Ph + p190BCR-ABL+ CML patients 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.