

## Complete molecular remission in a patient with Philadelphia-chromosome positive acute myeloid leukemia after conventional therapy and Imatinib

**Philadelphia-chromosome (Ph)-positive acute myeloid leukaemia (AML) is rare and prognosis is poor with a median survival of six to seven months only. We report on a patient with Ph-positive AML (FAB M2, major BCR/ABL1 mRNA transcript, b2a2), who is in sustained complete cytogenetic and molecular remission for meanwhile 15 months. Cytarabine based chemotherapy was discontinued after two courses due to infectious complications. Since the b2a2 transcript was still detectable, Imatinib with quantitative RT-PCR monitoring was started. This result is promising and worth further evaluation to establish the role of Imatinib in patients with Ph-positive AML.**

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The t(9;22)(q34;q11) translocation results in fusion of the BCR and ABL1 genes, and plays an essential role in leukemic transformation. The Philadelphia chromosome (Ph) is the hallmark of chronic myeloid leukemia but is also found in patients with acute leukemia. Acute myeloid leukemia is a heterogeneous clonal disease often presenting with chromosomal aberrations, but t(9;22)(q34;q11) in *de novo* AML is an infrequent finding in 1-2% of newly diagnosed patients only.<sup>1,2</sup>

We report on a patient with Ph-positive AML with a major BCR/ABL1 mRNA transcript, who achieved complete cytogenetic and molecular remission after two courses of cytarabine based chemotherapy followed by Imatinib (STI571).

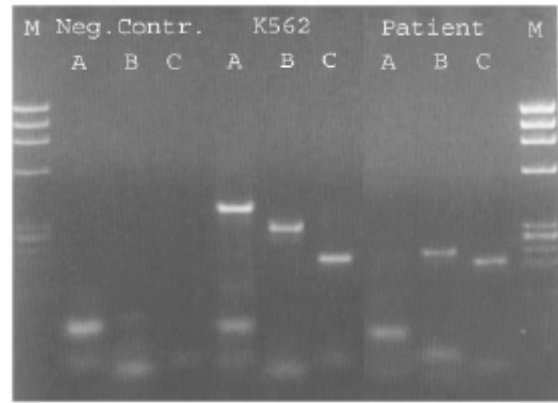
### Material and Methods

*Cytochemical assay, FACS and conventional cytogenetic analysis and FISH analysis*

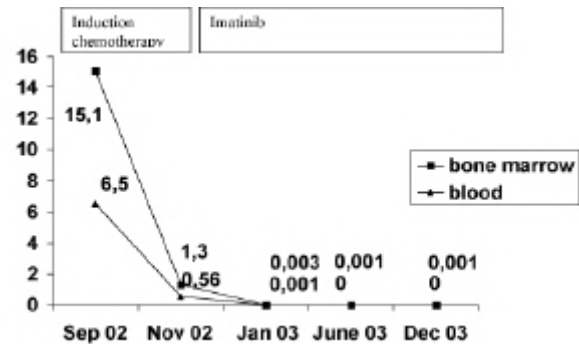
Cytochemical assays, FACS analysis, and conventional cytogenetic analyses were performed on bone marrow aspirates using standard methods. Double-colour Fluorescence in situ hybridisation (D-FISH) analysis was achieved according to supplier's protocol [Q BIOgene]. FISH signal patterns were evaluated as previously described.<sup>3</sup>

### Qualitative RT-PCR (reverse-transcription polymerase chain reaction)

After red blood cell lysis, total RNA was isolated from  $2 \times 10^6$  mononuclear cells using the Qiagen RNeasy Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was reversely transcribed as previously described using an ABL specific primer. Two sequential PCR-reactions were performed in order to amplify transcripts specific for M-bcr as well as m-bcr (breakpoint cluster region). In the first PCR-run one reverse primer specific for the a3-exon of the abl-gene was combined with two forward primers, for the e12 (b1)-exon and the e1-exon of the bcr-gene respectively. Thus, the first forward primer amplifies the M-bcr transcript (CML), whereas the e1-exon forward primer amplifies of m-bcr transcript (ALL). In the second PCR-run two separate nested PCR's were performed using 1  $\mu$ l of the first PCR-probe as target. M-bcr nested PCR was performed with primers from the a3 (ABL)- and the e13 (b2)-BCR-exon. The m-bcr nested PCR was performed with primers from the a3 (ABL)- and the e1 (BCR)-exon. The K562 cell line and a Ph negative probe served as positive and nega-



**Figure 1.** PCR for bcr. First PCR (A), nested PCR with primers specific for M-bcr (B), and m-bcr (C). The negative control is negative in all three PCR-reactions. K562 lane A: Only a 390 bp specific for M-bcr is amplified. K562 lane B: A 301 bp (b3a2) product is amplified in the M-bcr specific nested PCR. K562 lane C: A 195 bp product specific for the e1a2 transcript (m-bcr) is amplified. Patient lane B: A 226 bp product specific for b2a2 is amplified in the M-bcr specific nested PCR. Note the difference size as compared to the product in lane K562 B. The missing b3 exon has a length of 75 bp. Patient lane C: A PCR product of the same size as in lane K562 C was amplified. This 195 bp product represents the e1a2 transcript typical in Ph+ ALL and most likely resulted from alternative splicing of the b2a2 product. M:  $\lambda$ -X174/Hae III size Marker.



**Figure 2.** BCR-ABL/G6PD-ratios (LightCycler) – obtained between September 2002 and December 2003 from peripheral blood and bone marrow aspirates.

tive control respectively. Finally, PCR-products of all nine PCR-reactions were analysed on 1% agarose gel.

### Quantitative RT-PCR

RNA extraction and cDNA-synthesis for PCR-amplification were performed using the LightCycler-t(9;22) quantification Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. As a control for RT-PCR performance and as reference for relative quantification the housekeeping gene Glucose-6-phosphate dehydrogenase (G6PDH) was amplified in a separate reaction from an aliquot of patient's cDNA used for BCR/ABL1 amplification. The BCR/ABL1 and G6PDH crossing point (cp) were calculated by the LightCycler software as previously described.<sup>3</sup> Comparing these crossing points we calculated the relative number of bcr-abl transcripts per 100 G6PDH transcripts.

### Case Report

A 64-year old female patient was diagnosed with AML (FAB M2). Immunophenotyping showed myeloid blasts with strong coexpression of CD13/CD33/CD34/CD65/CD117/HLA-DR/MPO and aberrant expression of CD19.

Lymphoid markers were negative otherwise.

Conventional cytogenetic analyses (CCA) after GTG-banding detected the Ph translocation t(9;22)(q34;q11) in all metaphases and FISH showed the BCR/ABL1 rearrangement in 28 of 30 metaphases and all interphase nuclei. The complete karyotype at diagnosis was 46,XX,t(9;22)(q34;q11).ish 9q34(ABL1x3),22q11(BCRx3)(ABL con BCRx2)[28]/ish 9q34(ABL1x2), 22q11(BCRx2)[2]/nuc ish 9q34 (ABL1x3),22q11 (BCRx3) (ABL con BCRx2). RT-PCR analysis detected the major BCR/ABL mRNA transcript of the b2a2 CML-type (Figure 1).

The patient was treated with 2 courses of idarubicin 12 mg/m<sup>2</sup>/d 1 through 3 and cytarabine 200 mg/m<sup>2</sup> days 1 through 7 and achieved complete hematologic remission after the first course. However, BCR/ABL mRNA transcripts were still detectable. Chemotherapy was discontinued because of severe pulmonary infection (possibly consistent with aspergillus pneumonia), renal failure and repeatedly hypocellular bone marrow with trilineage dysplasia. Imatinib (STI571) in a dosage of 600 mg/d was started 3 weeks after end of chemotherapy.

For laboratory monitoring, quantitative BCR/ABL PCR was employed (Figure 2). Nine months after diagnosis BCR/ABL1 rearrangement was no more detectable in 250 interphases and 30 metaphases with D-FISH probe and not detected on CCA. The patient achieved complete hematologic and molecular remission within 4 months of therapy. Side effects of imatinib were mild with slight nausea at the beginning of treatment. The patient continues to be in complete molecular remission after 15 months of follow up.

### Discussion

The BCR-ABL1 translocation is rarely found in AML. No single clinical or hematologic feature distinguishes CML blast crisis from AML-Ph+. However, it is unlikely that our patient had CML for the following reasons: 1. Six weeks before AML was diagnosed the complete blood count (CBC) was normal.<sup>2</sup> The patient showed only minimal splenomegaly.<sup>3</sup> The CBC after the first chemotherapy course was consistent with CR rather than with CML in chronic phase.

Immunophenotyping of Ph-positive AML discloses CD34 coexpression in virtually all cases and a high incidence of aberrant expression of lymphoid markers (2 or more in 60% of cases). Patients with Ph-positive AML are genetically heterogeneous. Major and minor breakpoints with variable BCR/ABL1 mRNA transcripts such as e1a2, b2a2, b3a2, b2a3, e1a2, can be observed.<sup>1,2</sup> This concurs with the findings in our patient.

STI571 is a selective inhibitor of the ABL1 tyrosine kinase, induces high rates of complete cytogenetic remission in CML patients (74% in newly diagnosed CML) and has an excellent safety profile.<sup>6</sup> Preliminary results of STI571 in combination with chemotherapy in younger patients with *de novo* ALL show very good clinical results, however the rate of molecular remissions seems low (Ottmann OG *et al.*, Blood 2002; 100:85, abstract 213).

To our knowledge there exists only one report on treatment with STI571 in Ph+ AML: Yamaguchi *et al.*<sup>7</sup> reported on a 51-year-old man with refractory AML Ph positive (major BCR/ABL mRNA transcript), who achieved long term complete remission on STI571. In contrast to this report our patient had already achieved complete remission following cytarabine based chemotherapy before STI571 was started. The further clinical course with achievement of complete molecular remission, however, proves STI571 as a very effective treatment option in this otherwise fatal disease entity.

Myeloid blasts in AML express c-kit (CD117) in 65-90%<sup>8</sup> and excitingly STI571 has been recently reported to inhibit c-kit phosphorylation. However, Scappini B. *et al.*<sup>9</sup> failed to show cytotoxic effects of STI571 on c-kit-positive cell lines *in vitro*. Cortes *et al.*<sup>10</sup> did not observe any benefit in c-kit positive AML or MDS patients treated with STI571 400 mg daily. It is currently recognized that c-kit plays only a marginal role in pathogenesis of AML and therefore STI571 is inadequately active in this context. We therefore assume that inhibition of the ABL1 tyrosine kinase is the major therapeutic effect in Ph+ hematologic diseases.

Quantitative RT-PCR provides an excellent diagnostic tool for continuous sensitive monitoring of therapy with STI571.<sup>5</sup> We found no further BCR/ABL transcripts in neither blood nor bone marrow suggesting complete molecular remission in our patient within 4 months of treatment with ongoing molecular remission until now 15 months later.

### Conclusion

The Ph-translocation is a rare molecular abnormality in AML patients usually implying a poor prognosis. Treatment with the tyrosine kinase inhibitor STI571 may improve the outcome. Further trials with a larger patient number are worth exploring the role of STI571 in Ph-positive AML.

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