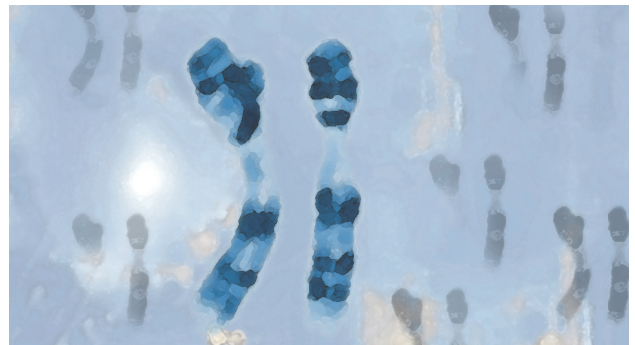




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## Chronic myeloid leukemia (CML)

*diagnostics and  
therapeutic monitoring*

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## ■ Introduction

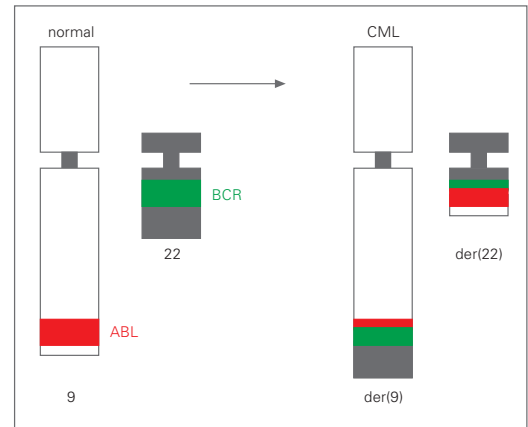
With the recent development of fundamentally new forms of therapy, new treatment strategies have opened up for patients with chronic myeloid leukemia that may lead to a significantly improved prognosis. Imatinib (Gleevec™, STI571), for example, is a highly effective drug that is available in oral form. It is the first tyrosine kinase inhibitor that has been officially approved for clinical treatment. It acts specifically at the molecular level of CML and with it, permanent remission has now become a realistic possibility. Treatment with Imatinib causes relatively few side effects, but prolongs survival times.

## ■ Molecular background

In about 90 % of CML patients the affected bone marrow cells exhibit a chromosome 22 with a shortened long arm. Chromosomes 22 that are modified in this way are designated 22q or called the Philadelphia chromosome (Ph). This structural abnormality is caused by a rearrangement between chromosomes 9 and 22 and is therefore also called the Philadelphia translocation (Figure 1).

A Philadelphia translocation can also be found in about 2 % of patients with acute myeloid leukemia (AML) and in about 25 % of adult patients and 5 % of children affected with acute lymphoblastic leukemia (ALL) (1).

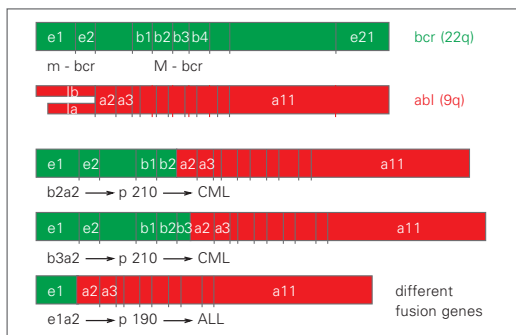
The balanced exchange of chromosomal fragments that occurs during a Philadelphia translocation is called a reciprocal translocation. In cytogenetic terms, such an event is described as  $t(9;22)(q34;q11)$ . At the molecular level this corresponds to the fusion of two genes. Here, the oncogene *c-ABL* is translocated from chromosome 9 into the *bcr* (breakpoint cluster region) of chromosome 22 (Figure 1). The fusion of coding sequences of the *ABL* gene and the *BCR* gene results in a chimeric gene, whose transcription product (*BCR-ABL*-mRNA) will lead to the formation of a modified protein with increased tyrosine kinase activity in the affected cells. It is this particular molecular rearrangement that leads to the development of this form of leukemia.



**Figure 1:**

The Philadelphia chromosome is the derivative 22 [der(22)] that is caused by the balanced reciprocal translocation 9;22 [t(9;22)]. This causes coding segments of the cellular oncogene *c-ABL* to be translocated into the breakpoint cluster region (*BCR*).

Individual translocation breakpoints can be divided up into different types. While the translocation breakpoint on chromosome 9 is always localized within a coding segment (exon) of the *c-ABL* gene, there are different breakpoints within two distinct regions of the *bcr* gene on chromosome 22 – the major (*M-bcr*) and the minor (*m-bcr*) breakpoint cluster region. Expression of this fusion gene results in the generation of different transcription products, in the case of *M-bcr* mainly *b2a2* and *b3a2* (210 kD protein) and in the case of *m-bcr* *e1a2* (190 kD protein). Almost all breakpoints of CML patients are of the *M-bcr* type, while in case of Ph-positive ALL patients 50 % are either of the *M-bcr* or of the *m-bcr* type (Figure 2).



**Figure 2:** While Philadelphia chromosomes appear quite homogenous at the cytogenetic level, they are very different at the molecular level. Shown are the most frequent detectable fusion products with their usual nomenclature and assignment to the various forms of leukemia.

## ■ Diagnostic strategies

The Philadelphia chromosome-positive CML is a genetically defined disease entity. If the existence of a Philadelphia chromosome cannot be detected with any of the methods described here, the CML diagnosis should be re-evaluated. Nowadays, the detection of a Philadelphia chromosome and/or the BCR-ABL rearrangement defines a CML and/or an ALL, respectively, as Philadelphia chromosome-positive. This is all the more relevant clinically since the response to a specific treatment with Imatinib can now be predicted (2).

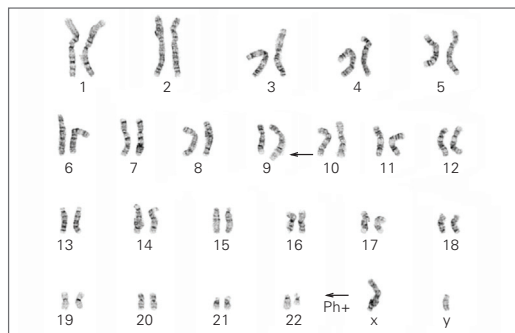
For the routine diagnosis of a Philadelphia translocation and the resulting gene rearrangements, three different detection methods are currently available, each with its own specific clinical relevance:

- Conventional cytogenetics (chromosome analysis)
- Molecular cytogenetic detection of gene rearrangements applying fluorescence in-situ hybridisation (FISH)
- Molecular genetic analysis of fusion products using RT-PCR (qualitative), with the special application of quantitative PCR (qPCR)

## 1. Conventional cytogenetic analysis

Using conventional chromosome analysis, the so-called classical Philadelphia translocation can be detected in about 90 % of the CML patients. Around 5-10 % of the patients either do not show a translocation or the translocations are such that another chromosome other than 9 is involved in addition to chromosome 22. These translocations are called "variant" Philadelphia chromosomes (3).

Conventional cytogenetic analysis is useful for both first diagnosis as well as for future monitoring of the disease, because unlike other detection methods, this technique can detect atypical translocation variants, as well as additional chromosomal rearrangements. Some of these rearrangements act as an indicator for the progression of the disease and can show whether the disease has progressed from a chronic phase into a more acute phase. The detection of an additional third chromosome 8 (trisomy 8), a third chromosome 19 (trisomy 19), an additional Philadelphia chromosome (+ Ph<sup>1</sup>), or of a so-called isochromosome 17 (+i17q) are all characteristic for the transition of the disease into a blast crisis (1).



**Figure 3:** Conventional chromosome analysis of a classical Philadelphia-Translocation t(9;22)(q34;q11).

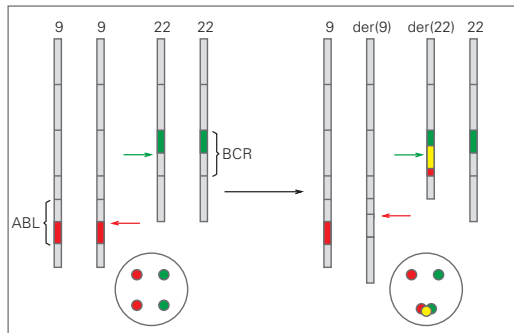
For conventional cytogenetic chromosome analysis to be applied, dividing cells need to be present. Since the mitotic ability of the cells to divide can be negatively affected by the progression of the disease

or by the use of anti-mitotic chemotherapeutic agents, conventional chromosome analysis may not always be successful. For this and other reasons – which will be elaborated later on – conventional chromosome analysis may be complemented with two further detection methods.

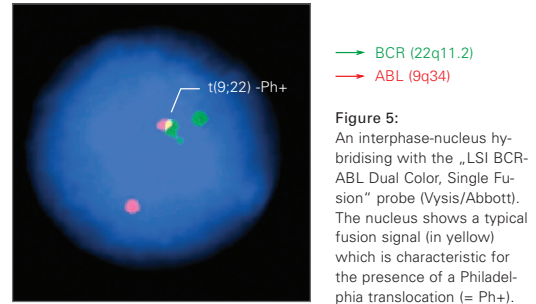
## 2. Fluorescence in-situ hybridisation (FISH)

Fluorescence in-situ hybridisation (FISH) can be used on metaphase chromosomes or interphase nuclei to specifically and quantitatively detect the BCR-ABL translocation.

An advantage of the FISH technique over conventional chromosome analysis is that it can also be successfully performed on interphase nuclei, i.e. on non self-dividing cells. In contrast to a qualitative, PCR-based detection method, it is also possible using FISH to monitor the proportion of blast cells during the course of the disease. Such a quantification of a Philadelphia translocation result is particularly important when quantitative PCR is not available, say, for logistic reasons.



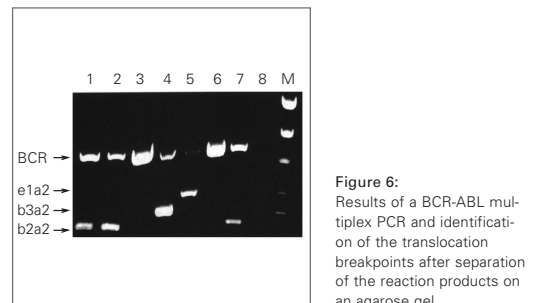
**Figure 4:**  
Schematic drawing of the used FISH probe („LSI BCR-ABL, Dual color, single fusion“, Abbott/Vysis) and the resulting image bound to an interphase nucleus.  
Left image: normal signal pattern  
Right image: Philadelphia-translocation (picture modified according to Abbott/Vysis).



**Figure 5:**  
An interphase-nucleus hybridising with the „LSI BCR-ABL Dual Color, Single Fusion“ probe (Vysis/Abbott). The nucleus shows a typical fusion signal (in yellow) which is characteristic for the presence of a Philadelphia translocation (= Ph+).

## 3. PCR-detection of the BCR-ABL gene rearrangement

For an initial diagnosis, the molecular genetic analysis of a BCR-ABL gene rearrangement is a very sensitive method, with which the translocation breakpoint and the fusion product can be characterized very precisely at the RNA level. Using qualitative RT-PCR, the expression of the chimeric BCR-ABL fusion transcripts in peripheral blood cells can be detected in a single reaction following RNA isolation and subsequent transcription into cDNA through amplification with BCR-ABL specific primers (4). The three major breakpoints (b2a2, b3a2,



**Figure 6:**  
Results of a BCR-ABL multiplex PCR and identification of the translocation breakpoints after separation of the reaction products on an agarose gel.

Lane 1 and 2: CML with b2a2 BCR/ABL translocation  
Lane 3: BCR-ABL negative CML  
Lane 4: CML with b3a2 BCR-ABL translocation  
Lane 5 and 7: ALL with e1a2 and b2a2 BCR-ABL translocation, respectively  
Lane 6: BCR-ABL negative ALL  
Lane 8: negative control  
Lane M: pEMBL/TaqI marker  
Figure modified according to (5)

e1a2) are identified according to their fragment size and by comparison with defined control fragments. Fragments not showing the expected size are analysed by sequence analysis.

If BCR-ABL transcripts are detected, a quantification of the residual tumorigenic cells for future monitoring as well as for specific drug treatment is recommended using quantitative PCR (realtime-PCR). This method quantifies the expression of the BCR-ABL gene characteristic for leukemia cells, as well as the expression of a reference gene (e.g. G6PD, beta actin, ABL etc.) (6). The expression of the reference gene is a measure for the number of leucocytes, while BCR-ABL expression is specific for leukemia cells. The ratio of both reflects the residual tumor load.

Fluorescence in-situ hybridisation and quantitative PCR are the most suitable methods for therapeutic monitoring of Philadelphia-positive CML. However, as outlined above, conventional chromosome analysis may also be a useful complementary tool in estimating the acceleration of an open acute leukemia into blast crisis because it reveals additional chromosomal aberrations.

#### 4. Comparison of the various diagnostic methods

Owing to differences in resolution, sensitivity, limitation of clinical significance during chemotherapy and the presentation of individual (single) clones side by side at the cellular level, these techniques can be seen as complementary to each other. And, depending on their clinical relevance, they should therefore be used in parallel both at the first diagnosis and for future monitoring (Fig.7).

	Initial Diagnostics	After 3 Months	After 6 Months	then semi-annually
Cytogenetics	+		+	+
FISH	+		+	+
PCR / qRT PCR	+	+	+	+

**Figure 7:**  
Recommendations for a multi-modal diagnostic therapeutic monitoring of CML  
Table modified according to (8)

## ■ Sample requirements and results

For conventional chromosome analysis and FISH, heparin blood or heparinised bone marrow aspirate are required (min. 2-4 ml). Samples should be taken on the same day as shipping. Fixed cell suspensions may also be sent, provided that they show sufficiently high cell or metaphase densities, respectively.

Cell analysis is carried out strictly according to national and international guidelines (7, 8). The results of chromosomal and FISH analysis will be described and interpreted in a detailed tumor cytogenetic report. Reports are usually issued within 6 to 8 days after receipt of sample material.

For molecular genetic analysis using RT-PCR peripheral blood (10ml EDTA blood, Vacutainer or Mono-vette) will be required. The sample should be shipped within 24-48 hrs at room temperature or at 4°C.

Requests for quantitative PCR can only be processed after consultation with our client service (Center for Human Genetics) by phone. Quantitative PCR will be performed at one of our cooperating partner laboratories.

Turn-around times for analysis and reporting are approximately 1 week for qualitative PCR. The final report will contain particular information on whether or not BCR-ABL transcripts were detected in the sample. In case a BCR-ABL transcript is detected, the exact type of the identified BCR-ABL transcript (e.g. b2a2) will be specified.

The report and interpretation of a quantitative PCR result will refer to former expression data (if available) and the status of the therapy applied. For the interpretation, it is of particularly importance whether BCR-ABL expression has increased, stagnated or declined in comparison to the previous measurements. A prerequisite for the use of quantitative PCR is the indication of the specific type of BCR-ABL transcript that was detected for the respective patient. Otherwise qualitative PCR has to be carried out first in order to determine the exact type of the BCR-ABL transcript present.

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