Chapter

Research of the Philadelphia Chromosome in Chronic Myeloid Leukemia: Diagnostic and Prognostic Interests

Yahya Benbouchta, Ahmed Afailal Tribak and Khalid Sadki

Abstract

Myeloproliferative syndromes are cell proliferation involving one or more medullary lines without blocking maturation. Chronic myeloid leukemia (CML) is the most common of these syndromes, it corresponds to the monoclonal proliferation of a multipotent stem cell; the myeloblastic or lymphoblastic transformation of CM. has a poor prognosis. The Philadelphia chromosome t(9;22)(q34;q11) is the first cytogenetic abnormality that has been associated with a malignant process. It is found in 89 to 95% of CML. The search for the Philadelphia chromosome (Ph1) has multiple interests: Diagnostic, prognostic and in therapeutic monitoring. The search for the Philadelphia chromosome by molecular cytogenetics makes it possible to remedy the poverty of cell suspensions in metaphase to take up the inconclusive results of classic cytogenetics on nuclei in interphase and to detect residual disease during therapeutic monitoring. Through the literature review, we highlight the importance of the identification of the Philadelphia chromosome in Myeloproliferative Syndromes for the improvement of the quality healthcare of the affected patients.

Keywords: Philadelphia chromosome, myeloproliferative syndromes, karyotype, diagnostic and prognostic interest

1. Introduction

Leukemias are clonal and acquired diseases of the hematopoietic stem cell or a precursor already committed to lymphoid and /or myeloid lineages [1]. hyperplasia produced a tissue that results from cell proliferation as myeloid pathology. Chronic myeloid leukemia (CML) is a monoclonal pathology of the pluripotent stem cell characterized by neoplastic granulocytic overproduction. This myeloproliferative syndrome has two particular characteristics:

- Its evolutionary mode consists of a chronic chemosensitive phase, followed by an acceleration phase, then an acute (or blast) transformation, ineluctable and chemoresistant.

- A quasi-constant clonal cytogenetic marker which is the Philadelphia Ph1 chromosome or derived from chromosome 22. This chromosome abnormality

is generated from the reciprocal translocation involving the q34 band of chromosome 9 and the q11 band of chromosome 22.

The recent development of therapeutics targeted at the activity or stability of an oncogenic protein has recently been illustrated by the therapeutic successes obtained in the treatment of chronic myeloid leukemia and acute promyelocytic leukemia [1]. Until now cytogenetics has been the reference for structural abnormalities, in particular translocations, tools for precise diagnosis in certain disputed cases and the detection of residual diseases or possible relapses. However molecular cytogenetics can detect chromosomal abnormalities of small sizes not visible on metaphasic chromosomes (semi-cryptic). It is of particular interest in the analysis of acquired abnormalities and is involved in monitoring the persistence of an abnormal clone in order to detect predicted recurrent translocations and may also help characterize genes in the evolutionary process of carcinogenesis. The current recommendations are based on high-quality evidence reported in peer-reviewed journals, supplemented by expert group consensus. These recommendations apply to healthcare professionals who treat CML patients and CML patients to better understand their conditions and treatments [2].

2. Interest of chromosome Philadelphia in chronic myeloid leukemia

- The usual form or standard translocation.

It is the translocation of a distal fragment of the long arm of chromosome 22 (fragment 22q11.2) to the distal part of the long arm of chromosome 9 with recovery of a deleted part of the long arm of chromosome 9 on the long arm of chromosome 22. It is therefore a reciprocal translocation, without loss of chromosomal material (**Figure 1(a,b)**).

Since this date, we defined the standard Philadelphia chromosome as: **t(9;22) (q34;q11) or t(9; 22)(q34.1; q11.21).**

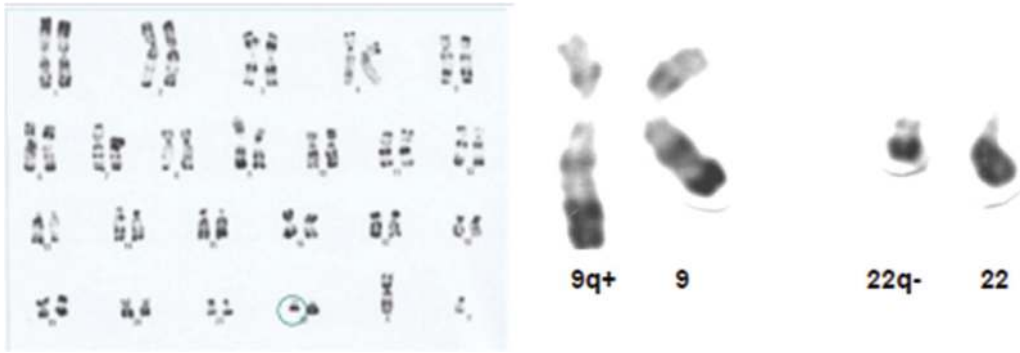


Figure 1.
(a) Result of a metaphase karyotype not classified in the R-band. (the circle indicates the Ph1 chromosome). (b) Partial RHG band karyotype of one of our patients: t(9;22)(q34;q11).

3. Diagnostic interest of chromosome Philadelphia in chronic myeloid leukemia

3.1 The chronic phase

The Philadelphia chromosome is the only element allowing a diagnosis in hyperleukocytosis. It is found in 89 to 95% of CML cells: In the granulomonocytic,

erythroblastic and B lymphocytic lines [3]. In most cases, CML is diagnosed on clinical and hematologic data alone. The differential diagnosis arises with all the pathologies that are accompanied by hyperleukocytosis with mild myelemia.

- The Ph1 chromosome: Diagnoctic key

The almost constant presence of this translocation in CML offers clinicians an additional diagnostic tool especially in myeloproliferative syndromes (MPS), chromosome 22 can be translocated to a chromosome other than chromosome 9 or else participates in a complex translocation of most interest, often three chromosomes of which the 22 and 9 one speaks then of Ph1 variant as opposed to the standard Ph1 chromosome. This same tanslocation t(9;22)(q34;q11) is found in a non-negligible percentage in ALL and AML.

3.2 Differential diagnosis in the acute phase

In acute leukemia, there is an accumulation of immature precursors of the hematopoietic lineage involved in the Bonne marrow, blood, or other tissue pathologies. The acutization phase CML disease there is a significant hyperleukocytosis with the presence of the Philadelphia chromosome on all mitoses. This acutization phase is preceded by the appearance of secondary anomalies: Trisomy 8, duplication of Ph1, and isochromosome 17, which conditions a poor prognosis.

We also find the Philadelphia chromosome:

- In 5% of acute lymphoblastic leukemia (ALL) in children and 20–30% of ALL in adults and also found in acute myeloid leukemia, In acute myeloid leukemia type 1 (LAM1) and LAL1 [4].

3.3 Other myeloproliferative syndromes

Essential thrombocythemia, myeloid splenomegaly, polycythemia vera or vaquez disease and chronic myelomonocyte leukemia (CMML) have the same phenotype as show in certain forms of CM. For this reason, it is important to confirm the diagnosis of chronic myelogenous leukemia by cytogenetic study or molecular biology [5]. Sometimes to give a right diagnosis is complicated so only the karyotype or molecular biology tests can help for that. The first test looks for the presence or not of the Ph1 while the other molecular biology tests investigate the *BCR-ABL* rearrangement.

3.4 Chronic myeloid leukemia in children

Chronic myeloid leukemia in children: There are two clinically and genetically distinct forms:

- The adult form occurring beyond the age of two years resembles in all respects a Ph1 + CML with the presence of the cytogenetic marker Ph1 + and break points in M-*BCR* especially in 5' [6, 7].

- The juvenile form before the age of two characterized by a peculiar clinical picture and a normal karyotype in most cases otherwise the most frequent chromosomal aberration is monosomy 7.

In some cases, the Ph1 chromosome may be masked due to the size of the fragment translocated which is submicroscopic, molecular cytogenetics are then used

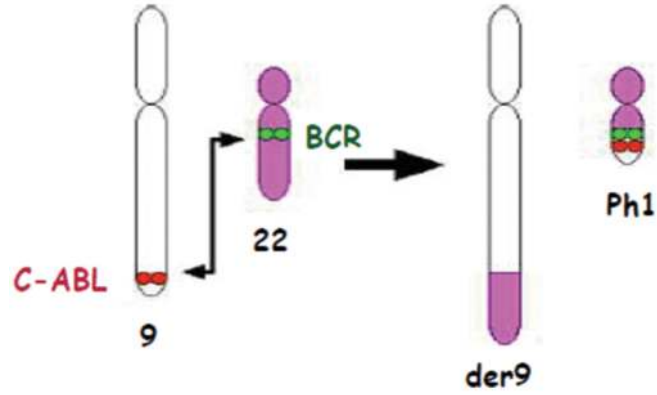


Figure 2.
The Philadelphia chromosome can be masked because of the size of the translocated fragment which is submicroscopic.

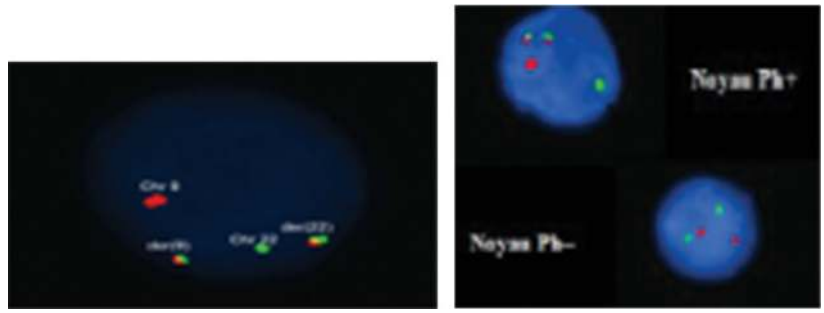


Figure 3.
FISH image of BCR/ABL *positive rearranged metaphase.*

(in situ hybridization: FISH) or real-time PCR search for the Philadelphia chromosome is necessary to confirm the diagnosis of CML and to monitor progress under certain anti-mitotic drugs (**Figure 2**).

In onco-hematology, FISH provides a decisive complement to the diagnosis, the prognosis and monitoring of targeted therapies. In leukemia chronic myeloid this technique highlights the fusion of genes *BCR* and *ABL* which characterize the Philadelphia chromosome (Ph). FISH is particularly interesting in the cytogenetic monitoring of CML. In due to culture problems (low mitotic index and the quality of the metaphases poor according to European Leukemia Net 2009. This service is currently offered to patients with CML as part of the cytogenetic monitoring of their disease (**Figure 3**).

3.5 Variant translocations

Variant translocations fall into two subgroups: Simple variants and complex variants; their definitions are based on the results of R, G banding and molecular biology. Although it is very common, it is quickly learned that the t(9;22) translocation is not pathognomonic for CML and it has several variants: the Ph1 (+) variants, the masked Ph1 chromosome and the Ph1 (−) variants. All chromosomes except Y are involved in the variant form of Ph1 especially chromosomes 3, 11, 12, 14 and 17 [8]. The variants can all be considered as complex translocations since the molecular genetic investigations of the supposed simple variants show that they involve at least three chromosomes and always the 9 and the 22 [9].

3.6 The blast transformation

In this phase, 65 to 80% of patients develop additional chromosomal aberrations not due to chance which precede clinical and hematological manifestations by several months and which can serve as indicators prognosis [10, 11]. Secondary anomalies appear: Double chromosome Philadelphia, trisomy 8, isochromosome 17 and trisomy 19. These four additional abnormalities are part of the clonal course in 70% of CML Other, more rarely encountered anomalies seem to be due to chance, thus taking the minor pathways. In more than 50% of cases, they are represented by:

- Monosomies: Y, 7, 17.
- Down's syndrome: 17 and 21.
- And the translocation t(3;21)(q26;q22) which has the characteristic of being accompanied by medullary fibrosis [12].

A quarter of patients [10, 11] will not develop any additional abnormalities and will keep Philadelphia alone for the duration of their survival.

3.7 Chronic myeloid leukemia with secondary abnormalities

The following partial karyotypes show the association of certain additional abnormalities to the Philadelphia chromosome (Ph1) in our patients. However, the therapeutic and prognostic approach is totally different. It is therefore necessary:

Make a positive diagnosis for CML.

- Correct the diagnosis of certain contentious cases.
- Specify the evolutionary stage.
- And make a differential diagnosis with myeloprolifirative and myelodysplastic syndromes.

During the blast phase of CML at Ph1 (+), analysis determines as a factor of poor prognosis [10]. As for the Philadelphia chromosome alone, it appears to have an independent prognostic value [13].

4. Prognostic interest of chromosome Philadelphia in CML

Evaluating the prognosis of CML using clinical-biological criteria can predict the probable date of onset of blast transformation which amounts to determining the probable duration of the chronic phase. As regards the cytogenetic criterion, it must be defined and homogeneous. The prognoses of Ph1 (+) CML and Ph1 (−) CML should be studied separately because we have seen the current difficulties of including the Ph1 (−) form in the nosological framework of CML.

In our medical genetics' laboratory. The suspected diagnosis was CML in 69 patients, unlabeled SMP in the remaining 22 patients (**Table 1**).

- Culture failure in 6 cases.
- Normal karyotype in 25 cases.
- Philadelphia chromosome or t(9;22)(q34;q11) in 60 cases.

The cytogenetic criterion is requested at two levels:

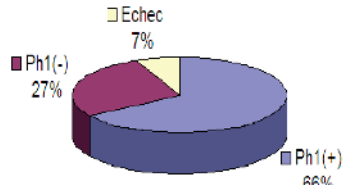
- For the initial assessment of prognosis at the time of diagnosis of CML in combination with baseline clinical and hematologic data.
- Then to assess the prognosis later during the blast transformation.

During the chronic phase of Ph1 (+) CML and without the knowledge of multi-parametric analyzes, it has been shown that the most significant prognostic factors which determine the duration of survival are [14]:

- The presence of additional clonal chromosomal abnormalities (relative risk "RR" = 4.5).
- Circulating blasts greater than 5% "RR = 1.8".
- A hemoglobin rate of less than 10 g / dl "RR = 1.30".

Results / Indications	Ph(+)	Ph(-)	Culture failure	Total
Chronic myeloid leukemia	54	12	03	69
Myeloprolofrative syndrome	06	13	03	22

(a)



(b)

Table 1.
(a) Cytogenetic analysis in myeloproliferative syndromes 91 patients. (b) Frequency of the Philadelphia chromosome (Ph1).

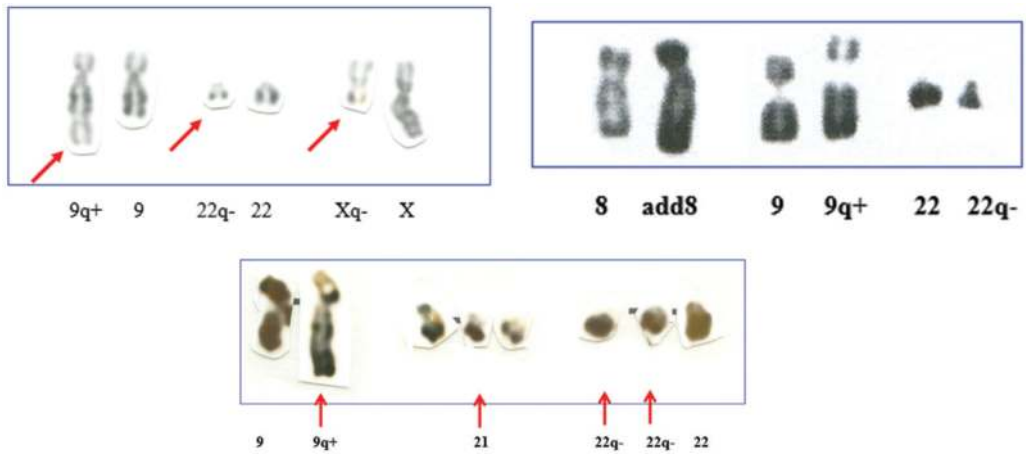


Figure 4.
(a) Presence of an extra Ph1 chromosome and trisomy 21. (b) Partial trisomy 8. (c) Partial band karyotype RHG: T(9;X;22).

- Thrombocytopenia.
- Leukocytosis>20.109 / l.
- Non-lymphoid blast cells.
- A clonal evolution a \ double chromosome Ph1, a trisomy 8, and typical aberrations of the acute phase (Ph1 (+), i(17q), hypodiploidy or hyperdiploidy).
- Lack of response to treatment.

It is interesting to note that the double chromosome Ph1 or trisomy 8 are more frequent in acute transformations of the AML, ALL type and that they respond poorly to treatment (**Figure 4**).

5. Surveillance of residual disease

Residual disease is defined as the number of malignant cells persisting after cytotoxic treatment, the eradicative action of which is intended to be as complete as possible: Chemotherapy, ionizing radiation, bone grafting. The residual malignant cells which escape this treatment can be the cause of a relapse hence the need to quantify them as precisely as possible. Before the introduction of molecular biology, hematologists had at their disposal various means of approach to define the biological remission of a hemopathy: Cytology, cytogenetics, immunology. In the best case, the sensitivity of these techniques did not make it possible to detect less than one residual cell in 100, a very insufficient sensitivity threshold to help clinician to decide for adequate treatment and to evaluate the quality control of the graft. The treatments envisaged must be carried out in order to obtain hematological remission and if possible a complete eradication of the Ph1 (+) cells (cytogenetic remission) with regard to chronic myeloid leukemia, the evolution takes place in two stages: A first chronic or myelocytic phase easily controlled by usual therapies then a second inconstant transition phase called acceleration with resistance to conventional chemotherapy, following which an acute transformation occurs, often of the terminal acute myelogenous leukemia type, constantly fatal, inevitable on average 3 to 4 years after diagnosis.

6. Contribution of oncocytogenetics in chronic myeloid leukemia

The molecular consequence is the formation of a *BCR-ABL* fusion gene, transcribed into 8.5 Kb mRNA and translated into 210 Kd protein with greater tyrosine kinase activity compared to the normal protein of the proto oncogene c-*ABL* from 145 Kb, this protein is involved in the pathological process of CML [15]. The molecular biology techniques applied to DNA, mRNA (RT-PCR) and encoded

Chromosomes	RNAm	Protein
9	ARNm c-abl	$P145^{c-abl}$
22	ARNm bcr	$P160^{bcr}$
22q-	ARNm bcr-abl	$P210^{bcr-abl}$

Table 2.
The chromosomes involved and their molecular consequences.

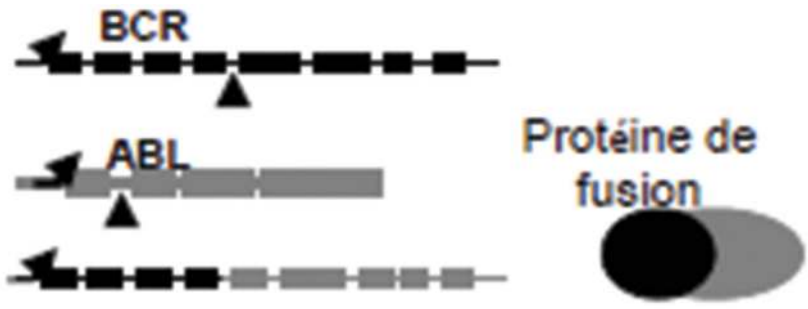


Figure 5.
Schema the Philadelphia chromosome t(9;22)(q34;q11) results in the fusion of BCR *genes on chromosome 22 and* ABL *on chromosome 22. The fusion protein has a strong activity tyrosine kinase responsible for tumor development.*

proteins have made it possible to specify the nature of the molecular events resulting from the rearrangement of *BCR-ABL* (**Table 2**).

Fluorescent in situ hybridization (FISH) using specific probes provides a useful tool for the detection of t(9;22)(q34;q11) and *BCR-ABL* rearrangement [16] (**Figure 3**). The fusion protein has a strong activity tyrosine kinase responsible for tumor development (**Figure 5**).

7. Conclusion

In summary, Philadelphia chromosome is an abnormal chromosome 22, resulting from a reciprocal translocation between chromosomes 9 and 22, a specific marker in chronic myeloid leukemia. His research in myeloproliferative syndromes has multiple interests: Diagnostic, prognostic and therapeutic follow-up which contributes to better patient care. Its demonstration in myeloproliferative syndromes makes it possible to confirm the nature of the disease and to distinguish between CM and other myeloproliferative syndromes.

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List of abbreviations

CML	chronic myeloid leukemia CML
Ph	Philadelphia
ALL	acute lymphoblastic leukemia
LAM	acute myeloid leukemia type 1
LAL1	acute lymphoblastic leukemia type 1
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
CMML	chronic myelomonocyte leukemia
MPS	myeloproliferative syndromes

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References

[1] Gisselbrecht, S., *[Oncogenes and leukemia: history and perspectives].* Med Sci (Paris), 2003. **19**(2): p. 201-210.

[2] Hochhaus, A., et al., *European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia.* Leukemia, 2020. **34**(4): p. 966-984.

[3] Shepherd, P.C., T.S. Ganesan, and D.A. Galton, *Haematological classification of the chronic myeloid leukaemias.* Baillieres Clin Haematol, 1987. **1**(4): p. 887-906.

[4] Médicale, G., *formelle, chromosomique, moléculaire, clinique. Par le collège National des Enseignants et Praticiens de Génétique Médicale. Coordonné par: Marc Jeanpiérre, Phlippe Jonveaux, Didier Lacombe, Nathalie Leporrier, Stanislas Lyonnet, Claude Mauraine*. 2004, Masson, Paris.

[5] Mahon, F.X. and J. Reiffers, *[Chronic myeloid leukemia. Diagnosis, course, prognosis].* Rev Prat, 1996. **46**(18): p. 2231-4.

[6] Arlin, Z.A., R.T. Silver, and J.M. Bennett, *Blastic phase of chronic myeloid leukemia (blCML): a proposal for standardization of diagnostic and response criteria.* Leukemia, 1990. **4**(11): p. 755-7.

[7] Fioretos, T., et al., *Molecular analysis of Philadelphia-positive childhood chronic myeloid leukemia.* Leukemia, 1992. **6**(7): p. 723-5.

[8] Fitzgerald, P.H. and C.M. Morris, *Complex chromosomal translocations in the Philadelphia chromosome leukemias. Serial translocations or a concerted genomic rearrangement?* Cancer Genet Cytogenet, 1991. **57**(2): p. 143-51.

[9] Dobrovic, A., et al., *Molecular diagnosis of Philadelphia negative CML using the polymerase chain reaction and DNA analysis: clinical features and course of M-bcr negative and M-bcr positive CML.* Leukemia, 1991. **5**(3): p. 187-90.

[10] Griesshammer, M., et al., *Chronic myelogenous leukemia in blast crisis: retrospective analysis of prognostic factors in 90 patients.* Ann Hematol, 1996. **73**(5): p. 225-30.

[11] Kantarjian, H.M., et al., *Chronic myelogenous leukemia in blast crisis. Analysis of 242 patients.* Am J Med, 1987. **83**(3): p. 445-54.

[12] Heim, S. and F. Mitelman, *Cancer cytogenetics: chromosomal and molecular genetic aberrations of tumor cells*. 2015: John Wiley & Sons.

[13] Tanaka, K., et al., *Influence of M-BCR breakpoint sites on the duration of chronic phase in 100 patients with chronic myelocytic leukemia.* Cancer Genet Cytogenet, 1993. **70**(1): p. 39-47.

[14] Ariad, S., et al., *Prognostic factors in chronic myeloid leukaemia--importance of staging or disease biology.* S Afr Med J, 1992. **81**(6): p. 299-303.

[15] Rowley, J.D., *Chromosome abnormalities in leukemia and lymphoma.* Ann Clin Lab Sci, 1983. **13**(2): p. 87-94.

[16] Martinet, D., D. Muhlematter, and M. Jotterand Bellomo, *[Fluorescent in-situ hybridization technique (FISH) in the diagnosis of Philadelphia translocation in chronic myeloid leukemia].* Schweiz Med Wochenschr, 1996. **126**(20): p. 855-63.