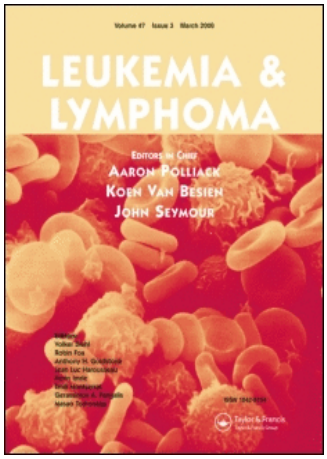

Leukemia and Lymphoma
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713643806

Philadelphia chromosome mosaicism at diagnosis in chronic myeloid leukemia: Clinical correlates and effect on imatinib mesylate treatment outcome

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First Published on: 06 October 2007
To cite this Article: Landstrom, Andrew P., Knudson, Ryan A., Dewald, Gordon W., Ketterling, Rhett P. and Tefferi, Ayalew (2007) 'Philadelphia chromosome mosaicism at diagnosis in chronic myeloid leukemia: Clinical correlates and effect on imatinib mesylate treatment outcome', Leukemia and Lymphoma, 48:11, 2137 - 2140
To link to this article: DOI: 10.1080/10428190701618284
URL: http://dx.doi.org/10.1080/10428190701618284

Leukemia & Lymphoma, November 2007; 48(11): 2137 – 2140

informa
healthcare

ORIGINAL ARTICLE: CLINICAL

Philadelphia chromosome mosaicism at diagnosis in chronic myeloid leukemia: Clinical correlates and effect on imatinib mesylate treatment outcome

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(Received 29 May 2007; revised 5 August 2007; accepted 7 August 2007)

Abstract
In chemotherapy-treated patients with chronic myeloid leukemia (CML), the karyotypic detection of Philadelphia chromosome (Ph)-negative metaphases at diagnosis (i.e. Ph mosaicism) is not considered significant as a prognostic factor for survival. In the current retrospective study, clinical correlates and prognostic relevance of Ph mosaicism were evaluated in 63 Ph-positive patients with CML, including 59 in chronic phase and 4 in accelerated phase, receiving imatinib mesylate as either first ($n = 46$) or second ($n = 17$) line therapy. Thirteen patients (21%) displayed Ph-negative metaphases at diagnosis and, compared to the other 50 patients with 100% Ph-positive metaphases, presented with significantly lower leukocyte count ($p = 0.0004$), circulating blast percentage ($p = 0.02$), and incidence of palpable splenomegaly ($p = 0.02$). Ph mosaicism did not correlate with other CML-pertinent prognostic factors including Sokal score ($p = 0.4$) or the presence of additional chromosome changes ($p = 0.96$) found in 10 patients (16%). Neither Ph mosaicism nor the presence of additional chromosome changes affected complete or partial cytogenetic remission rates to IM. Multivariable analysis identified Ph mosaicism as a risk factor for shortened survival. Due to the small sample size, the current preliminary observations require validation in a larger group of patients.

Keywords: *Myelogenous, prognosis, BCR-ABL, cytogenetics, karyotype, imatinib*

Introduction

In approximately 95% of chronic myeloid leukemia (CML) patients in chronic phase (CP-CML), the *BCR-ABL* disease-causing mutation is marked by the karyotypically evident Philadelphia chromosome (Ph), t(9;22)(q34;q11). In the remaining 5%, *BCR-ABL* is detected by fluorescent in situ hybridization (FISH) or PCR-based molecular tests [1,2]. In Ph-positive CML, the majority of patients display the Ph in 100% of metaphases analyzed by conventional cytogenetic studies. Despite this, ~20% of cases feature Ph mosaicism (i.e. the presence of both Ph positive and negative metaphases) at the time of diagnosis and/or before specific treatment is instituted [3]. During the pre-imatinib mesylate (IM) era of CML therapy, Ph mosaicism at diagnosis, in CP-CML, was shown to be prognostically neutral [4 – 7], whereas the presence of additional chromosomal changes was believed to be detrimental to survival [8]. However, these observations might not be applicable to patients receiving IM or interferon alpha (IFN-α) therapy. For example, investigators in one study demonstrated that the presence of additional cytogenetic abnormalities at the time of diagnosis was not significantly associated with worse survival in patients receiving IFN-α-based treatment [9]. In the current study, we evaluated both clinical correlates and the prognostic relevance of Ph mosaicism at diagnosis in IM-treated patients with CP-CML.

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ISSN 1042-8194 print/ISSN 1029-2403 online
DOI: 10.1080/10428190701618284

Methods

After approval from the Mayo Foundation Institutional Review Board, and in accordance with federal regulations, the Mayo Clinic patient database was used to identify newly diagnosed patients with Ph-positive CP-CML and accelerated phase CML (AP-CML) without prior treatment. Diagnosis was made according to the World Health Organization criteria [10] and only patients receiving IM either as initial or second line therapy were considered. In each case, demographic and pertinent clinical information, including disease stage, treatment details and outcome, as well as survival, was obtained. Some clinical parameters including age, hemoglobin level, leukocyte count, and circulating blast percentage were treated strictly as continuous variables, while others were treated both as continuous and categorical variables: percent Ph-positive metaphases, spleen size, platelet count, percentage of circulating eosinophils or basophils, Sokal Score, and Sokal risk category designation [11]. Relative risk (RR) estimate with a 95% confidence interval was calculated for appropriate categorical variables. Conventional cytogenetic and D-FISH studies were performed as previously described [1,12]. All statistical analysis was completed using SAS software (SAS, Cary, NC, USA), and statistical significance was set at the level of $p \leq 0.05$.

Results

A total of 63 patients with Ph-positive CML were retrospectively evaluated. Among this cohort, 5 subjects (8%) were in AP-CML while the remaining were in CP-CML at diagnosis. Clinical and laboratory features at diagnosis are outlined in Table I; 13 patients (21%) displayed Ph-negative metaphases at diagnosis and 10 patients (16%) carried other cytogenetic abnormalities in addition to the Ph (Table II). Initial treatment consisted of either IM ($n = 46$) or IFN-α ($n = 17$) with no difference in treatment allocation between patients with or without Ph mosaicism ($p = 0.7$). All 17 patients initially treated with IFN-α subsequently received IM therapy.

At diagnosis, the presence of Ph mosaicism correlated significantly with lower leukocyte count ($p = 0.0004$), lower circulating blast percentage ($p = 0.02$), and reduced incidence of palpable splenomegaly ($p = 0.02$), but not with age, gender, hemoglobin level, platelet count (both as a continuous and categorical variable, i.e. greater than 700×10^9/L or not), or marrow blasts greater than or equal to 5%, circulating basophil or eosinophil percentage (both as continuous and categorical variable, i.e. greater than 15% basophil + eosinophil

Table I. Clinical and laboratory features of 63 newly diagnosed patients with Philadelphia chromosome-positive chronic myeloid leukemia.

Median age in years (range)	58 (18–84)
Number of males/females	33/30
Median hemoglobin level in g/dL (range)	12 (5.5–14.9)
Median leukocyte count $\times 10^9$/L (range)	74.6 (2.0–531.5)
Median platelet count $\times 10^9$/L (range)	355 (29–1940)
Number of patients with platelet count $> 700 \times 10^9$/L (%)	8 (13%)
Median circulating blast percentage (range)	1 (0–18)
Median circulating basophil percentage (range)	4 (0–16)
Median circulating eosinophil percentage (range)	2 (0–16)
Number of patients with basophil + eosinophil percentage $\geq 15\%$ ($n = 52$ available) (%)	4 (8%)
Number of patients with bone marrow blasts $\geq 5\%$ (%)	5 (8%)
Median palpable spleen size in cm past costal margin (range)	0 (0–25)
Median Sokal score (range)	0.87 (0.52–4.01)
Number of patient with Sokal risk category of low/intermediate/high	21/24/18
Median Ph-positive metaphase percentage by karyotype (range)	100 (6.7–100.0)
Number of patients with $<100\%$ Ph-positive metaphases by karyotype (i.e. Ph mosaics) (%)	13 (21%)
Median percentage of *BCR-ABL*-positive interphase bone marrow cells by FISH (range)	97.4 (13.0–99.4)
Number of patients with additional chromosomal changes (%)	10 (16%)
Number of patients initially treated with IM/IFN-α	46/17
Median follow-up in years (range)	3.3 (0.3–9.8)
Status at last contact, alive/dead	54/9

FISH, fluorescent in situ hybridization.

Table II. Chromosomal abnormalities in addition to the Philadelphia chromosome in 10 patients with Ph-positive chronic myeloid leukemia.

Additional cytogenetic abnormality	Percent Ph-positive metaphases
del(20q)	13%
del(7p13)	100%
del(7q)	100%
ins(9q10)	7%
Multiple Philadelphia chromosomes	100%
t(1;2)(q23;q21), del(3q), t(1;5)(q25;q21)	100%
t(8;9;22)(q22;q34;q11.2)	100%
t(9;22;14)(q34;q11.2;q24)	100%
Trisomy 21	100%
Trisomy 8	100%

del, deletion; ins, insertion; t, translocation.

percentage or not). There was no correlation between Ph mosaicism and the presence of additional chromosome changes; the latter were documented in 8 of 50 (16%) patients without and 2 of 13 (15%) with Ph mosaicism ($p = 0.96$).

At a median follow-up of 3.3 years, death was documented in 9 patients, including 5 (10%) among the 50 patients with 100% Ph-positive metaphases at diagnosis and 4 (31%) among the 13 Ph mosaics. Univariate analysis, which included other CML-relevant prognostic parameters considered by Sokal et al. [11], identified the following as being significantly associated with shortened survival: Ph mosaicism both as a categorical and continuous variable, lower hemoglobin level, and lower platelet count. The following were found to not be significant: presence of additional cytogenetic abnormalities, circulating blast percentage, bone marrow blast percentage greater than or equal to 5%, advanced age, leukocyte count, circulating basophil percentage, circulating eosinophil percentage, as well as Sokal score. Multivariable analysis identified only Ph mosaicism as predictor of inferior survival. These results are summarized in Table III. Importantly, Sokal score and Sokal risk category were not found to be correlated with Ph mosaicism ($p = 0.4$ and $p = 0.5$ respectively). The number of patients with either a platelet count greater than 700×10^9/L or combined eosinophil/basophil percentage greater than 15% was too small to allow for valid statistical comparisons.

In order to address the possible issue of treatment heterogeneity in our cohort (i.e. primary therapy of IM vs. IFN-α), we explored the relevance of both treatments on survival and Ph mosaicism. We determined that initial treatment was not correlated with survival by Kaplan-Meier analysis ($p = 0.9$), and that there is no statistical difference between the incidence of Ph mosaicism in those patients treated with IM vs. IFN-α initially ($p = 0.7$). Furthermore, upon exclusion of the 17 patients treated initially with IFN-α, we demonstrated that Ph mosaicism by the categorical variable had increased prognostic significance by univariate analysis ($p = 0.008$) with an estimated relative risk of 0.05 (0.005, 0.461). This association was maintained by multivariate analysis. Finally, Ph mosaicism was found to be significantly correlated to inferior survival through Kaplan Meier analysis ($p = 0.006$) as demonstrated in Figure 1.

Table III. Association of CML-relevant clinical parameters with length of survival by univariate analysis, multivariate analysis, and appropriate relative risk estimates in the complete cohort of 63 patients.

Clinical parameter	*p* value
Ph mosaicism, categorical (RR; CI)	0.02* (0.16; 0.04, 0.72)
Ph mosaicism, continuous	0.0003*
Lower hemoglobin level	0.05
Lower platelet count	0.04
Presence of additional cytogenetic abnormalities	0.4
Circulating blast percentage	0.2
Bone marrow blast percentage $\geq 5\%$	0.1
Advanced age	0.2
Leukocyte count	0.7
Circulating basophil percentage	0.3
Circulating eosinophil percentage	0.2
Sokal score	0.9
Initial therapy (IM vs. IFN-α)	0.9

*Denotes significance by multivariate analysis.
RR, relative risk estimate; CI, relative risk estimate 95% confidence interval.

Discussion and limitations

The reason behind the above-mentioned CML survival effect, from Ph mosaicism, is currently unexplained because complete or partial cytogenetic remission to IM was similar between patients with 100% or less than 100% Ph-positive metaphases at diagnosis ($p = 0.9$). Furthermore, while details were not available for analysis, the cause of death in the majority of the cases was not due to blast crisis. Therefore, the results of the current study, in this regard, should be treated as being preliminary and subject to validation by a larger study. The possibility exists that the observed relationship between Ph mosaicism and survival is actually due to Type I error and a false relationship due to chance. On the other hand, the lesser degree of myeloproliferation (i.e. lower leukocyte and circulating blast counts, lower incidence of palpable splenomegaly) in patients with Ph mosaicism might indicate the presence of a background Ph-negative clone that is active enough to enter mitosis as readily as Ph-positive cells but with reduced overall proliferate potential. The existence of Ph-negative clonal cells in Ph-positive CML has previously been suggested [13,14] and could be partly responsible for the emergence of Ph-negative cytogenetic abnormalities in IM-treated patients with BCR-ABL molecular remissions [15]. Such a scenario does not necessarily result in differences in initial response to IM treatment since the bulk of the tumor burden, in most Ph mosaic patients, is represented by the Ph-positive clone. Regardless of these potential possibilities, more studies are needed to clarify the nature of the Ph-negative metaphase in newly diagnosed CML patients and its impact on overall outcome in IM-treated patients.

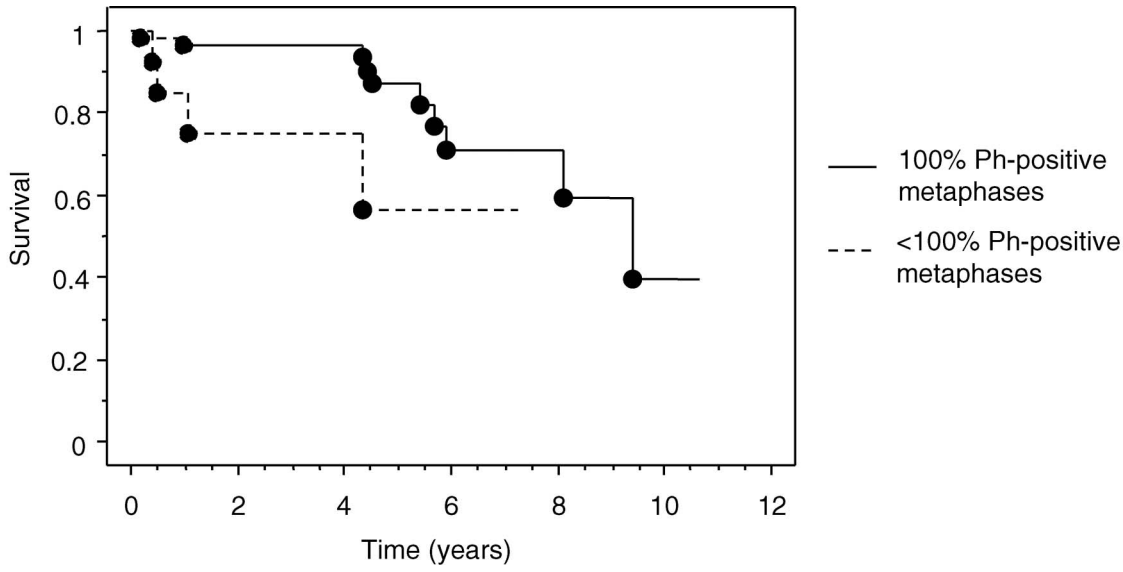

Figure 1. Kaplan Meier survival analysis of the presence of Ph-negative metaphases at diagnosis (<100% Ph-positive metaphases) vs. absence of Ph-negative metaphases (100% Ph-positive). $p = 0.006$ by log-rank analysis.

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