Published in final edited form as:
Leuk Lymphoma. 2013 January ; 54(1): 138–144. doi:10.3109/10428194.2012.701739.

Molecular characterization of *de novo* Ph+ Acute Myeloid Leukemia

Sergej Konoplev[1], **C. Cameron Yin**[1], **Steven M. Kornblau**[2], **Hagop M. Kantarjian**[2], **Marina Konopleva**[2], **Michael Andreeff**[2], **Gary Lu**[1], **Zhuang Zuo**[1], **Rajyalakshmi Luthra**[1], **L. Jeffrey Medeiros**[1], and **Carlos E. Bueso-Ramos**[1]

[1]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

[2]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Abstract

Philadelphia chromosome-positive (Ph+) AML is a controversial diagnosis, as others propose it represents CML in blast phase (CML-BP). *NPM1* mutations occur in 25-35% of AML but are absent in CML patients. Conversely, *ABL1* mutations occur in 25% of Imatinib-naïve CML-BP but are not described in AML patients. We analyzed for *NPM1* and *ABL1* mutations in 9 Ph+ AML and 5 CML-BP patients initially presented in BP. In 6 Ph+ AML cases, we screened for a panel of gene mutations using Sequenome®-based methods including *AKT1*, *AKT2*, *AKT3*, *BRAF*, *EGFR*, *GNAQ*, *GNAS*, *IDH1*, *IDH2*, *KRAS*, *MET*, *NRAS*, *PIK3CA*, and *RET*. Two of 9 (22%) Ph+ AML patients had *NPM1* mutations and were alive 36 and 71 months after diagnosis. All Ph+ AML were negative for *ABL1* and other gene mutations. One (20%) CML-BP patients had *ABL1* mutation; no patients had *NPM1* mutations. These data suggest that Ph+ AML is distinct from CML-BP.

Keywords

acute myeloid leukemia; *BCR-ABL1*; blast phase; chronic myelogenous leukemia; *NPM1*; Philadelphia chromosome

INTRODUCTION

Patients with Ph+ acute myeloid leukemia (AML) account for 0.5-3.0% of all AML cases and, by definition, have no evidence of chronic myelogenous leukemia (CML)either before the onset of AML or after successful therapy for AML. [1-5] Nevertheless, the pathogenesis of Ph+ AML remains controversial as many investigators believe these neoplasms represent de no myeloid blast phase of CML. The most recent version of the World Health Organization (WHO) classification does not recognize AML associated with t(9;22)(q34;q11.2)/*BCR-ABL1*.[6] In a review article discussing the rationale for the WHO classification, Vardiman and colleagues wrote, “Although *BCR-ABL1*–positive AML has been reported, criteria for its distinction from CML initially manifesting in a blast phase are not entirely convincing.”[7]

Correspondence: Sergej Konoplev, MD, PhD, Department of Hematopathology, Unit 72, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA. Phone: 713-792-4117; Fax: 713-792-4821; skonople@mdanderson.org.

DECLARATION OF INTERESTS
The authors declare no conflict of interest.

Therefore, other means of distinguishing Ph+ AML from CML-BP, if they truly are different entities, would be helpful.

Gene mutations are known to occur with various types of AML and are involved in pathogenesis. *Nucleophosmin (NPM1*), mapped to chromosome 5q35, is one of the most commonly mutated genes in AML patients..[8-10]*NPM1* is mutated in 25-35% of all AML cases, with a higher frequency of 45-64% in AML patients with normal karyotype.[10]*NPM1* mutations, if not counterbalanced by other gene mutations (e.g. *FLT3*), are associated with favorable prognosis.[8,9] In contrast, others have shown that *NPM1* consistently is wild type in patients with CML, including CML-BP.[8-12]

ABL1 mutations occur in a proportion of patients with CML as well as Ph+ acute lymphoblastic leukemia.[13] Furthermore, *ABL1* mutations are more common in CML-BP than in CML in chronic phase, with a frequency as high as 80% in CML-BP patients.[14-16] Of note, *ABL1* mutations are not restricted to patients with prior Imatinib exposure. In a study of unselected Imatinib-naïve CML-BP patients, 5 of 19 patients had *ABL1* mutations.[15]*ABL1* mutations have not been reported Ph+ AML patients .

In this study, we hypothesized that analysis of *NPM1* and *ABL1* genes, often mutated in AML and CML-BP patients, respectively, might yield insights into the relationship between Ph+ AML and CML-BP. We also screened 6 cases of Ph+ AML for a number of other gene mutations that our laboratory screens for in the workup of malignant neoplasms of all types. Our results suggest that Ph+ AML is a clinicopathologic entity distinct from CML-BP.

MATERIAL AND METHODS

Study Group

Following approval by the Institutional Review Board, the database of the Department of Hematopathology at The University of Texas MD Anderson Cancer Center was searched for Ph+ AML patients seen from January 1998 until the time of writing. Cases were excluded from this study if there was: a clinical history of an antecedent hematologic disorder suggestive of CML in chronic or accelerated phase; evidence of a CML-like picture following therapy for AML; a history of chemotherapy and/or radiation therapy; presence of splenomegaly or basophilia (defined as >2% of basophils in peripheral blood) suggestive of a myeloproliferative neoplasm; and evidence of biphenotypic or bilineage leukemia as defined by the 2008 WHO classification.[6] Ph+ AML patients formed a study group.

For comparison, we searched for patients who were diagnosed with CML BC at initial presentation during the same period of time and had no clinical history of an antecedent hematologic disorder suggestive of CML in chronic or accelerated phase. The comparison group included patients with confirmed t(9;22)(q34;q11.2) and peripheral blood basophilia combined with either splenomegaly or additional cytogenetic abnormalities considered to be typical for CML (trisomy 8, isochromosome of the long arm of chromosome 17, trisomy 19, or an extra copy of Ph). Cases with lymphoid subtype of CML BP and cases fulfilling diagnostic criteria of the 2008 WHO classification for acute biphenotypic or bilineage leukemia[6] were excluded.

In addition, we also identified a group of patients who was diagnosed with CML-BP during the same period of time at our institution and had a well documented antecedent chronic phase during the same study period. Cases with lymphoid subtype of CML BP and cases fulfilling diagnostic criteria of the 2008 WHO classification for acute biphenotypic or bilineage leukemia[6] were excluded.

Morphologic, Cytochemical, and Immunophenotypic, and Cytogenetic Analysis

Bone marrow aspirate smears were stained with Wright-Giemsa and blast counts were performed manually. Bone marrow aspirate smears were also analyzed cytochemically for myeloperoxidase (MPO) and alpha-naphthyl butyrate esterase using previously reported methods.[17]

Four-color flow cytometric immunophenotypic immunophenotypic analysis was performed using a FACScalibur cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) and analyzed using the CellQuest software package (Becton Dickinson Immunocytometry Systems) as previously described.[17] Antibodies specific for following antigens were used: CD3, CD7, CD10, CD13, CD19, CD20, CD33, CD34, CD45, CD56, CD64, CD117, HLA-DR, MPO, and TdT. All antibodies were obtained from Becton-Dickinson Biosciences (San Jose, CA). Blasts were gated for analysis using CD45 expression and light side-scatter characteristics. Blasts were considered positive for antigens using an arbitrary but standard cutoff level of at least 20% blasts that expressed the antigen compared with an isotype control.

Conventional cytogenetic analysis was performed on bone marrow aspirate specimens at the time of initial presentation using standard GTG-banding as described previously.[18]

NPM1 and ABL1 Gene Mutation Analysis

Exon 12 of *NPM1* was amplified using PCR and two primers 5'-GATGTTGAACTATGCAAAGAGACA-3' (forward) and 5'-AACCAAGCAAAGGGTGGAGTT-3' (reverse). PCR products were purified using the MinElute PCR purification Kit (QIAGEN, Valencia, CA) and directly sequenced using the reverse primer GGCATTTTGGACAACACA and fluorescent dye chain-terminator chemistry (Sanger sequencing) with an ABI PRISM 3100 or 3130 genetic analyzer (Applied Biosystems, Foster City, CA). The ABI GeneMapper software program (Applied Biosystems) was used to analyze the raw *NPM1* gene mutation analysis data.

Mutational analysis of the *ABL1* kinase domain was performed using nested PCR followed by Sanger sequencing as described previously. [19] In the first round, the *BCR-ABL1* fusion transcripts b2a2, b3a2, and e1a2 were amplified. In the second round, the analysis consisted of two separate PCRs that covered codons 221-380 and codons 350-500 of the *ABL1* kinase domain, respectively. PCR products were sequenced using standard dideoxy chain-termination DNA sequencing with an ABI PRISM 3700 genetic analyzer (Applied Biosystems). The results were analyzed using the SeqScape and sequencing analysis software programs (Applied Biosystems). All *ABL1* mutations were confirmed by sequencing of forward and reverse strands, with a sensitivity rate of 20% mutation-bearing transcripts in the analyzed population established in periodic dilution studies.

Analysis of Other Genes in Ph+ AML

BCR-ABL1 fusion transcripts were assessed using real-time quantitative reverse transcriptase (RT)-PCR analysis of peripheral blood and/or bone marrow aspirate specimens as described previously.[20]

The *FLT3* gene was assessed for internal tandem duplication (ITD) and the codon 835/836 point mutation using polymerase chain reaction (PCR)-based methods also as described previously.[21] PCR-based DNA high-resolution melting curve analysis was used to screen for IDH gene mutations as described previously.[22] Mutations in codons 87-138 of exon 4 of *IDH1* and exon 4 of *IDH2* were specifically assessed.

A PCR-based DNA primer extension analysis with a MassARRAY system (Sequenom, San Diego, CA, USA) was used to screen for a number of gene mutations as described previously.[23] The genes that were assessed are part of a routine screening panel applied to the study of all newly diagnosed tumors. The panel is focused on known "hot spots" for gene mutation and includes: mutations in codons 17, 49, 173, 517, 179, and 536 of the *AKT1* gene; codons 17, 49, 175, and 523 of the *AKT2* gene; codons 17, 49, 171, and 511 of the *AKT3* gene; codons 464, 466, 469, 594, 597, 600, and 601 of the *BRAF* gene; codon 858 of the *EGFR* gene; codon 209 of the *GNAQ* gene; codons 201 and 227 of the *GNAS* gene; codon 132 of the *IDH1* gene; codon 172 of the *IDH2* gene; codons 12, 13, 61, and 146 of the *KRAS* gene; codons 375, 848, 988, 1010, 1112, 1124, 1248, 1253, and 1268 of the *MET* gene; codons 12, 13, and 61 of the *NRAS* gene; codons 60, 88, 110, 111, 345, 405, 418, 420, 453, 539, 542, 545, 546, 909, 1021, 1025, 1043, 1046, 1047, and 1049 of the *PIK3CA* gene; and codon 918 of the *RET* gene were detected

RESULTS

Clinical data

The clinical data of the study group and the comparison group are listed in Table 1. Among 2241 AML patients at our institution during the study period, we identified 12 (0.54%) patients Ph+ AML patients (0.54%). DNA extracted from bone marrow samples at presentation was available for 9 patients, the study group. The Ph+ AML patients were five men and four women, ages 22 to 76 years (median, 57). During the study period, we also identified 18 patients with CML who presented in myeloid blast phase and had no documented chronic phase or accelerated phase (CML-BP group). A total of 5 patients with readily available DNA extracted from bone marrow samples obtained at the time of CML-BP diagnosis were included in this study. CML-BP group had four men and one woman, ages 26 to 81 years (median, 44). All patients received intensive chemotherapy regimens; 4 of 9 Ph+ AML patients and all CML-BP patients also received Imatinib. The median follow-up was 19 months (range, <1 month – 113 months). Six Ph+ AML patients and three CML-BP patients achieved complete remission (CR), including two Ph+ AML patients who did not receive Imatinib; one Ph+ AML patient and one CML-BP patient relapsed. Five Ph+ AML patients and 3 CML-BP patients died, including one Ph+ AML patient in CR.

Laboratory data

The laboratory data at presentation are listed in Table 1. Compared to CML-BP patients, Ph + AML patients had higher percentage of peripheral blasts (median, 47% vs. 14%, p=0.03) and by definition, lower percentage of basophils (median, 0% vs. 10%, p=0.001). There was no statistically significant difference in white blood count, hemoglobin, or platelet count.

RT-PCR analysis for *BCR-ABL1* fusion transcript

The results of RT-PCR studies for *BCR-ABL1* fusion transcript at presentation and in CR are listed in Table 1. At presentation, p210 protein product was detected in 5 of 8 Ph+ AML patients tested and all CML-BP patients; p190 BCR ABL1 protein product was detected in 3 Ph+ AML patients and was not detected in any CML-BP patients. At the time of CR, no *BCR-ABL1* fusion transcript was detected in all 5 Ph+ AML who achieved CR, while *BCRABL1* fusion transcript persisted in 2 of 3 CML-BP patients who achieved CR. Third CMLBP patient (patient#11) received allogeneic stem cell transplantation in CR and was tested for *BCR-ABL1* fusion transcript after a successful engraftment, when the bone marrow chimerism studies showed 100% donor cells; no *BCR-ABL1* fusion transcripts studies were performed in CR

Other gene mutation testing

Two of 9 (22%) Ph+ AML patients had *NPM1* mutations (Figure 1). Both patients had a four-base-pair (TCTG) insertion consistent with a type A mutation. Of note, both patients were alive and in molecular remission 36 and 71 months after diagnosis, respectively. No *NPM1* mutations were detected in all CML-BP patients

One Ph+ AML patient had *FLT3* ITD, whereas the other 8 Ph+ AML patients and all CMLBP patients had wild-type *FLT3*.

All 9 Ph+ AML patients and all but one CML-BP had no evidence of *ABL1* gene mutations. One CML-BP patient has E459K *ABL1* gene mutation at presentation.

In 6 Ph+ AML patients tested analyzed by Sequenome-based methods no mutations were identified in *AKT1*, *AKT2*, *AKT3*, *BRAF*, *EGFR*, *GNAQ*, *GNAS*, *IDH1*, *IDH2*, *KRAS*, *MET*, *NRAS*, *PIK3CA*, or *RET*.

The bone marrow findings

The bone marrow findings are listed in Table 2. The only features discriminating CML-BP patients from Ph+ AML patients was presence of dwarf megakaryocytes, which were observed in 4 of 5 CML-BP patients, but were not detected in all but one Ph+ AML patient (p= .0319). There was no statistically significant difference in bone marrow cellularity, number of megakaryocytes, or blast percentage.

Cytogenetic data

The results of conventional cytogenetic studies at presentation are listed in Table 2. The translocation t(9;22)(q34;q11.2) was detected in all patients and was the sole cytogenetic abnormality in 6 Ph+ AML patients and in one CML-BP patient. Cytogenetically normal metaphases were observed, in addition to the abnormal ones, in 3 Ph+ AML patients and in 4 CML-BP patients. Trisomy 8 was detected as an additional abnormality in two CML-BP patients.

CML-BP with preceding CP group

During the study period we also identified 138 patients with CML who subsequently developed myeloid blast phase (CML-BP). A total of 37 patients with readily available DNA extracted from bone marrow samples obtained at the time of CML-BP diagnosis were available for this study. These patients included 23 men and 14 women, ages 23 to 81 years (median, 50). There was no evidence of *NPM1* mutation in all 37 CML-BP with preceding CP patients. Eighteen of 37 (49%) patients had *ABL1* mutations. The most common mutation was T315I (detected in five patients) followed by Y253H (detected in four patients). Other mutations identified included: E255K, E255V, E279K, F317L, G250E, N331S, Q252H, H396R, and V299L. Three patients had two *ABL1* mutations simultaneously: Q252H and E255K, T315I and E255K, and T315I and H396R.

DISCUSSION

The frequency of adult Ph+ AML in our study (0.54%) is at the low end of the range of 0.5-3.0% in reported the literature.[1,3-5] We believe the stringent exclusion criteria used in this study may explain the lower frequency.

Comparing bone marrow findings in Ph+ AML and CML-BP, we found that the only discriminating feature was the presence of dwarf megakaryocytes, which were found in all but one CML-BP patients and only one Ph+ AML. In contrast to the previous study by

Soupir and colleagues,[3] we did not find a statistically significant difference in bone marrow cellularity.

Six of nine (67%) Ph+ AML patients in our study had t(9;22)(q34;q11.2) as a sole cytogenetic abnormality, which is similar to patients with Ph+ AML in previous studies[1,3] but markedly different from patients with CML-BP who demonstrate additional cytogenetic abnormalities in up to 60-65% of patients.[11,24,25] Of three Ph+ AML patients with cytogenetic abnormalities other than Ph in our study, two had abnormalities involving chromosome 7. This finding is in accord with a report by Paietta et al who described a high frequency of abnormal chromosome 7 in Ph+ AML patients.[26] No cytogenetic abnormalities known to occur frequently in CML-BP patients, such as an isochromosome of the long arm of chromosome 17, gain of an extra copy of Ph, trisomy 19, and trisomy 8, were present in our Ph+ AML patients.[3,4,11,20,27] Of interest, normal metaphases were detected in both groups and therefore could not be regarded as a reliable way to discriminate Ph+ AML from CML-BP..

The frequency of *NPM1* mutations in the Ph+ AML patients (2/9; 22%) was similar to that reported in the general AML population,25-35%.[8,9] No *NPM1* mutations were identified in the CML-BP group. Conversely, frequency of *ABL1* mutations in the CML-BP patients (1/5; 20%) was similar to that reported in for CML-BP patients in the literature.[15] No Ph+ AML patients had *ABL1* mutations. We suggest that these data support the interpretation that Ph+ AML is a clinicopathologic entity distinct from CML-BP. Admittedly, the number of Ph+ AML patients is small, but this is a truly rare entity and 9 cases is an appreciable number of patients in this context.

We suggest that the findings we present add to other evidence suggesting that Ph+ AML is a distinct entity. Others have shown that Ph+ AML patients are less likely to have splenomegaly or peripheral basophilia, and show lower bone marrow cellularity, and have a lower myeloid to erythroid ratio than CML-BP patients.[3] At the cytogenetic level, the most frequent abnormalities observed in CML-BP patients, including gain of an extra Ph, trisomy 8, trisomy 19, and an isochromosome 17q, are consistently absent in cases of Ph+ AML reported.[3,11,20,27]

Although the “two-hit theory” of leukemogenesis suggests leukemia cells require class I and II mutations,[28] this is theoretically a minimum requirement. The co-existence of *BCR-ABL1* and *NPM1* mutations in 2 Ph+ AML patients in this study raises the possibility of synergistic effect between two class I mutations. Bacher and colleagues recently identified an AML patient with leukemic subclones carrying *BCR-ABL1* and *NPM1* mutations, supporting this concept of synergistic class I mutation in leukemia.[2]

Isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* genes encode cytoplasmic/peroxisomal *IDH1* and mitochondrial *IDH2* enzymes, respectively. Investigators have identified somatic heterozygous mutations of *IDH1* and *IDH2* in a subset of AML cases.[22,29,30]*IDH* mutations are most common in AML patients with normal karyotype and *NPM1* mutations.[30] Data regarding *IDH1* and *IDH2* mutations in CML-BP patients are scarce with minor discordance. One study did not detect any *IDH1* or *IDH2* mutations in 91 CML patients, including 20 patients with CML-BP.[31] Another study detected *IDH2* mutations in 3 of 75 patients with CML-BP[32] whereas all 75 patients had wild-type *IDH1*.[32] Overall, the frequency of IDH mutations is clearly low in CML and CML-BP and our data support these findings as all 37 patients lacked evidence of either *IDH1* or *IDH2* mutation. No data regarding *IDH1* and *IDH2* mutations in Ph+ AML patients was available in the literature until this study; all 9 Ph+ AML cases were negative.

In summary, we have presented molecular evidence to support the concept that Ph+ AML is distinct from CML-BP. Ph+ AML cases carried *NPM1* at a similar to that in AML patients in general, and lacked *ABL1* mutations. The important potential clinical implication of discriminating of Ph+ AML from CML-BC is that the current practice for CML patients requires indefinite therapy with imatinib,[33] but there is no data to support the need of indefinite therapy with imatinib for patients with Ph+ AML. However, a biger study of Ph+ AML patients with a long BCR-ABL1 fusion transcript surveliance is required to establish a standard of care for Ph+ AML patients.

Acknowledgments

We thank Sherry Pierce for her critical help in our database search; Yujia Huo, Michael Fernandez, and Hongbo Lu for their help with sequence analysis; Geneva Williams for manuscript preparation, and Don Norwood for editing the manuscript.

This work was supported in part by National Institutes of Health grants CA016672 and 2P50 CA100632-06.

REFERENCES

1. Keung YK, Beaty M, Powell BL, et al. Philadephia chromosome positive myelodysplastic syhndrome and acute myeloid leukemia – retrospective study and review of the literature. Leuk Res. 2004; 28:579–586. [PubMed: 15120934]
2. Bacher U, Haferlach T, Alpermann T, et al. Subclones with the t(9;22)/BCL-AML1 rearrangement occur in AML and seem to cooperate with distinct genetic alterations. Br J Haematol. 2011; 152:713–720. [PubMed: 21275954]
3. Soupir CP, Vergilio J, Dal Cin P, et al. Philadelphia chromosome-positive acute myeloid leukemia. A rare aggressive leukemia with clinicopatholgical features distinct from chronic myeloid leukemia in myeloid blast crisis. Am J Clin Pathol. 2007; 127:642–650. [PubMed: 17369142]
4. Bloomfield CD, Lindquist LL, Brunning RD, et al. The Philadelphia chromosome in acute leukemia. Virchows Arch B Cell Pathol. 1978; 29:81–91. [PubMed: 103315]
5. Cuneo A, Ferrant A, Michaux JL, et al. Philadelphia chromosome–positive acute myeloid leukemia: cytoimmunologic and cytogenetic features. Haematologica. 1996; 81:423–427. [PubMed: 8952155]
6. Swerdlow, SH.; Campo, E.; Harris, NL., et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC; Lyon, France: 2008.
7. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009; 114:937–951. [PubMed: 19357394]
8. Schnittger S, Schoch C, Kern W, et al. Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. Blood. 2005; 106:3733–3739. [PubMed: 16076867]
9. Boissel N, Renneville A, Biggio V, et al. Prevalence, clinical profile, and prognosis of NPM mutations in AML with normal karyotype. Blood. 2005; 106:3618–3620. [PubMed: 16046528]
10. Falini B, Mecucci C, Tiacci E, et al. GIMEMA Acute Leukemia Working Party. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med. 2005; 352:254–266. [PubMed: 15659725]
11. Grossmann V, Kohlmann A, Zenger M, et al. A deep-sequencing study of chronic myeloid leukemia patients in blast crisis (BC-CML) detects mutations in 76.9% of cases. Leukemia. 2011; 25:557–560. [PubMed: 21274004]
12. Piccaluga PP, Sabattini E, Bacci F, et al. Cytoplasmic mutated nucleophosmin (NPM1) in blast crisis of chronic myeloid leukemia. Leukemia. 2009; 23:1370–1371. [PubMed: 19421226]
13. Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, et al. Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. Blood. 2002; 100:1014–1018. [PubMed: 12130516]

14. Soverini S, Hochhaus A, Nicolini FE, et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011; 1185:1208–1215. [PubMed: 21562040]
15. Shah NP, Nicoll JM, Nagar B, et al. Multiple BCRABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor Imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell. 2002; 2:117–125. [PubMed: 12204532]
16. Willis SG, Lange T, Demehri S, et al. High-sensitivity detection of BCR-ABL kinase domain mutations in Imatinib-naive patients: correlation with clonal cytogenetic evolution but not response to therapy. Blood. 2005; 106:2128–2137. [PubMed: 15914554]
17. Oyarzo MP, Lin P, Glassman A, et al. Acute myeloid leukemia with t(6;9)(p23;q34) is associated with dysplasia and a high frequency of flt3 gene mutations. Am J Clin Pathol. 2004; 122:348–358. [PubMed: 15362364]
18. Onciu M, Schlette E, Medeiros LJ, et al. Cytogenetic findings in mantle cell lymphoma cases with a high level of peripheral blood involvement have a distinct pattern of abnormalities. Am J Clin Pathol. 2001; 116:886–892. [PubMed: 11764078]
19. Yin CC, Cortes J, Galbincea J, et al. Rapid clonal shifts in response to kinase inhibitor therapy in chronic myelogenous leukemia are identified by quantitation mutation assays. Cancer Sci. 2010; 101:2005–2010. [PubMed: 20557306]
20. Cortes J, Talpaz M, O'Brien S, et al. Molecular responses in patients with chronic myelogenous leukemia in chronic phase treated with Imatinib mesylate. Clin Cancer Res. 2005; 11:3425–3432. [PubMed: 15867244]
21. Chen W, Jones D, Medeiros LJ, Luthra R, et al. Acute myeloid leukaemia with FLT3 gene mutations of both internal tandem duplication and point mutation type. Br J Haematol. 2005; 130:726–728. [PubMed: 16115128]
22. Patel KP, Barkoh BA, Chen Z, et al. Diagnostic testing for IDH1 and IDH2 variants in acute myeloid leukemia an algorithmic approach using high-resolution melting curve analysis. J Mol Diagn. 2011; 13:678–86. [PubMed: 21889610]
23. Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. Curr Protoc Hum Genet. 2009 chapter 2:unit 2.12.
24. Kantarjian HM, Keating MJ, Talpaz M, et al. Chronic myelogenous leukemia in blast crisis. Analysis of 242 patients. Am J Med. 1987; 83:445–454. [PubMed: 3477958]
25. Griesshammer M, Heinze B, Hellmann A, et al. Chronic myelogenous leukemia in blast crisis: retrospective analysis of prognostic factors in 90 patients. Ann Hematol. 1996; 73:225–230. [PubMed: 8959940]
26. Paietta E, Racevskis J, Bennett JM, et al. Biologic heterogeneity in Philadelphia chromosome-positive acute leukemia with myeloid morphology: the Eastern Cooperative Oncology Group experience. Leukemia. 1998; 12:1881–1885. [PubMed: 9844918]
27. Berger R. Differences between blastic chronic myeloid leukemia and Ph-positive acute leukemia. Leuk Lymphoma. 1993; 11(suppl 1):235–237. [PubMed: 8251902]
28. Gilliland DG. Hematologic malignancies. Current Opinion in Hematology. 2001; 8:189–191. [PubMed: 11561153]
29. Paschka P, Schlenk RF, Gaidzik VI, et al. IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. J Clin Oncol. 2010; 28:3636–3643. [PubMed: 20567020]
30. Marcucci G, Maharry K, Wu YZ, et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol. 2010; 28:2348–2355. [PubMed: 20368543]
31. Roche-Lestienne C, Marceau A, Labis E, et al. Mutation analysis of TET2, IDH1, IDH2 and ASXL1 in chronic myeloid leukemia. Leukemia. 2011 [Epub ahead of print].
32. Soverini S, Score J, Iacobucci I, et al. IDH2 somatic mutations in chronic myeloid leukemia patients in blast crisis. Leukemia. 2011; 25:178–181. [PubMed: 20962862]

33. Barton MK. Discontinuation of imatinib may be possible in chronic myelogenous leukemia. CA Cancer J Clin. Mar-Apr. 2011; 61(2):65–6. [PubMed: 21372196]

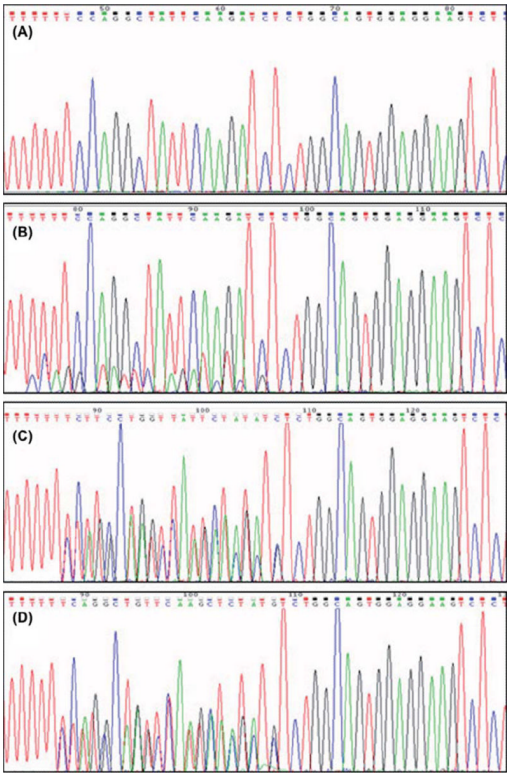

Figure 1.
Polymerase chain reaction-capillary electrophoresis for NPM1 mutational analysis. A (case 1) shows wild type NPM1 sequence. B (case 5) and C (case 6) show mutant NPM1 sequence. A positive control is shown in D.

Leuk Lymphoma. Author manuscript; available in PMC 2014 February 17.

Table 1

Pt #	Dx	Age	Sex	CR	relapse	FU (months)	Death	WBC	Hb	Plt	PB blasts	PB basos	Transcript		*NPM1*	*FLT3*	*ABL1*
													Dx	CR			
1	Ph AML	22	f	yes	yes	8	dead	84.7	6.6	28	72%	0%	ND	no	wild	wild	wild
2	Ph AML	76	m	no	no	1	dead	16.1	9	41	64%	0.5%	b3a2	NA	wild	wild	wild
3	Ph AML	71	m	no	no	1	dead	49.8	10.8	164	40%	0%	e1a2	NA	wild	wild	wild
4	Ph AML	70	m	no	no	6	dead	47.7	13.6	99	47%	0%	b2a2	NA	wild	wild	wild
5	Ph AML	50	m	yes	no	71	alive	120.6	12.3	43	68%	1%	b3a2	no	mutant	wild	wild
6	Ph AML	48	f	yes	no	36	alive	108.9	9.4	306	40%	1%	b3a2 b2a2	no	mutant	wild	wild
7	Ph AML	66	f	yes	no	32	alive	38.3	10.2	266	36%	0%	e1a2	no	wild	wild	wild
8	Ph AML	49	f	yes	no	28	alive	7.4	12.1	319	20%	1%	b3a2 b2a2	no	wild	wild	wild
9	Ph AML	57	m	no	NA	<1	dead	99.7	8.4	18	92%	0%	e1a2	NA	wild	*ITD*	wild
10	CML BP	46	m	yes	yes	14	dead	60.3	6.5	33	46%	6%	b2a2	yes	wild	wild	E459K
11	CML BP	43	f	yes	no	113	alive	60.3	7.4	165	14%	13%	b3a2	no	wild	wild	wild
12	CML BP	81	m	no	NA	9	dead	16.7	9.7	21	0%	10%	b3a2	NA	wild	wild	wild
13	CML BP	44	m	no	NA	30	dead	41	10.6	270	9%	11%	b3a2	NA	wild	wild	wild
14	CML BP	26	m	yes	no	23	alive	212	5.9	377	43%	8%	b3a2	yes	wild	wild	wild

Tabe 2

Pt #	Dx	Cellularity %	Megs	Druf Megs	BM blasts	MPO	Esterase	FAB	Cytogenetics at diagnosis
1	Ph AML	70-80	decreased	no	97	6%	neg	M1	46,XX,t(9;22)(q34;q11.2)[20]
2	Ph AML	50-60	decreased	no	65	neg	neg	M0	46,XY,t(9;22)(q34;q11.2)[19]
3	Ph AML	40-50	usual	no	63	68%	neg	M4	46,XY,der(7)inv(7)(q11.2q36)del(7)(q22q31), t(9;22)(q34;q11.2)[19]/46,XY[1]
4	Ph AML	90-100	decreased	no	82	36%	2%	M2	46,XY,t(9;22)(q34;q11.2)[1]/45,XY, -7,t(9;22)(q34;q11.2)[18]/46,XY[1]
5	Ph AML	95-100	decreased	no	87	70%	5%	M1	46,XY,t(9;22)(q34;q11.2)[20]
6	Ph AML	90-100	usual	no	60	40%	neg	M2	46,XX,t(9;22)(q34;q11.2)[20]
7	Ph AML	70-80	usual	no	69	70	40%	M4	46,XX,t(9;22)(q34;q11.2)[18]/46,XX[1]
8	Ph AML	50-60	increased	yes	33	1	2%	M7	46,XX,t(9;22)(q34;q11.2)[20]
9	Ph AML	80-90	absent	NA	88	1	neg	M0	46,XY,der(9)inv(9)(p12q13)t(9;22)(q34;q11.2)[3] /46,XY, inv(9)[7]
10	CML BP	95-100	decreased	yes	76	6	neg	M2	46,XY,t(9;22)(q34;q11.2)[17]/46,XY[3]
11	CML BP	70-80	decreased	yes	36	40	neg	M2	46,XX,t(8;11)(p11.2;p15),t(9;22)(q34;q11.2)[20]
12	CML BP	95-100	decreased	no	64	1	neg	M0	55,XY,+8,t(9;22)(q34;q11.2),+13,+14,+14,+16, +18,+19,+20,+der(22)t(9;22)[6]/46,XY[14]
13	CML BP	30-40	increased	yes	65	neg	neg	M2	46,X,-Y,+8,t(9;22)(q34;q11.2)[6]/46,X, - Y,+8,t(9;22)(q34;q11.2),inv(16)(p13q22)[7]/ 47,XY,+Y[2]/46,XY[5]
14	CML BP	95-100	increased	yes	38	20	neg	M7	46,XY,t(3;21)(q26.2;q22),del(7)(q22), t(9;22)(q34;q11.2) [2]/46,XY,t(3;21)(q26.2;q22), t(4;15)(p16;q22),del(7)(q22),t(9;22)(q34;q11.2) [5]/ 46,XY,t(3;14)(q27;q24),t(3;21)(q26.2;q22),del(7) (q22),t(9;22)(q34;q11.2)[5]/46,XX[11]