CASE REPORT

A rare BCR-ABL1 transcript in Philadelphia-positive acute myeloid leukemia: case report and literature review

Monica Piedimonte¹, Tiziana Ottone², Valentina Alfonso², Antonella Ferrari¹, Esmeralda Conte¹, Mariadomenica Divona², Maria Paola Bianchi¹, Maria Rosaria Ricciardi¹, Simone Mirabili¹, Roberto Licchetta¹, Alessia Campagna¹, Laura Cicconi², Giulia Galassi¹, Sabrina Pelliccia¹, Anna Paola Leporace¹, Francesco Lo Coco² and Agostino Tafuri*¹

Abstract

Background: Philadelphia (Ph) chromosome results from the reciprocal translocation t(9;22)(q34.1;q11.2) and is diagnostic for chronic myeloid leukemia (CML). However, this translocation is also found in acute lymphoid leukemia (ALL), as well as in rare cases of acute myeloid leukemias (AML). Most patients with CML harbor either the e13a2 or the e14a2 BCR-ABL fusion product, while a small subset of the cases expresses e1a2 or e19a2 transcripts. Moreover, several atypical BCR-ABL1 transcripts, beside the most common e1a2, e13a2 and e14a2, have been described, mainly in patients with CML. However, ALL and de novo AML may also carry BCR-ABL1 atypical transcripts which will confer a poor prognosis.

Case presentation: A 78-years old male was admitted at our hospital with clinical and laboratory features allowing to make the diagnosis of AML. No evidence of a preceding CML (splenomegaly or basophilia) was found. The karyotype on G-banded metaphases was 46,XY, t(9;22)(q34;q11). While the molecular analysis was ongoing, the patient started treatment based on hydroxyurea followed by 5-aza-2′-deoxycytidine. The molecular biology analysis revealed the simultaneous presence of the common p190 e1a2 and the rare e6a2 isoforms. Because of persistent pancytopenia and presence of blasts, according to the molecular data, he was then switched to tyrosine kinase inhibitors (TKIs) treatment. Nevertheless, after 2 months, the patient was still refractory to second line treatment dying because of a pulmonary infection.

Conclusion: The atypical p190 e6a2 transcript seems to be associated in AML with aggressive disease. TKI therapy alone does not seem to control the disease. Prompt observations on these patients carrying rare BCR-ABL1 transcripts may help to establish optimal treatment approaches on these aggressive BCR-ABL1 phenotypes in different setting of patients.

Keywords: Acute myeloid leukemia, Philadelphia chromosome, BCR-ABL1 e6a2, Atypical transcripts, TKI

* Correspondence: agostino.tafuri@uniroma1.it
¹Department of Clinical and Molecular Medicine, Hematology Sant’Andrea University Hospital, Sapienza University of Rome, Rome, Italy
Full list of author information is available at the end of the article

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Background

The t(9;22) chromosome translocation originating the BCR-ABL1 fusion oncoprotein is detected in more than 95% of patients with CML representing the hallmark of the disease [1]. However, this translocation is also found in 10 to 20% of adults and in 2 to 5% of children with ALL, as well as in rare cases (1%) approximately) of AML [2].

The breakpoint on chromosome 9 is localized between exons 1a, 1b and a2. In contrast, breakpoints on chromosome 22 occur within the BCR gene, consisting of 23 exons, thus resulting in several distinct fusion genes. Generally, the breakpoints location on BCR gene occur between b1-b5 exons, within a central region called major breakpoint cluster region (M-bcr), whereas rare cases occur within two other breakpoint cluster regions: minor (m-bcr), with the breakpoint between e1-e2 exons, and micro (µ-bcr) with rupture point between e19-e20 exons. Depending on the breakpoint of the ABL1 gene, the fusion protein has different molecular weight: p190, p210 and p230 proteins for m-bcr, M-bcr and µ-bcr, respectively [3]. Moreover, atypical BCR breakpoints outside the cluster regions have been described, involving splicing between whole exons, insertion of small sequences or genomic breakpoints within exons. For example, e8a2, e15a2 and e6a2 have been also described and their clinical significance is under investigation [2, 4, 5]. The atypical e6a2 BCR-ABL1 transcript produces a rare fusion protein of 185 kDa conferring a poor prognosis in CML due to its association with aggressive phenotype and early transformation, perhaps due to the lack of an important regulatory BCR sequence within the fusion proteins [6]. Here we report a rare case of de novo AML carrying the BCR-ABL1 transcript e6a2.

Case presentation

A 78-years old male was admitted to the Hematology of the University Hospital Sant'Andrea-Sapienza, because of worsening fatigue and abdominal pain. Written informed consent was obtained from the patient and the study was approved by our institutional review board.

The peripheral blood count showed hyperleucocytosis (WBC $118 \times 10^9/L$), anemia (hemoglobin 8.6 g/dl) and mild thrombocytopenia (98 $\times 10^9/L$), with no associated splenomegaly. Peripheral blood smear showed hypercellularity with 90% blast cells.

The morphological examination of bone marrow (BM) aspirate showed 90% agranular blast cells of medium and large size (Fig. 1) and the immunophenotypic analysis performed on a FACScalibur flow cytometer using standard protocol revealed that blast cells were CD34+, CD117+, CD33+, CD13+, HLA-DR+, CD2+ MPO+/−, CD7+/− [7].

A diagnosis of AML (M2) was established and the patient started cyto reduction with hydroxyurea obtaining after seven days of treatment a WBC count of $39 \times 10^9/L$.

Conventional karyotyping was performed on the BM diagnostic aspirate after short-term culture and analyzed after G-banding. The description of the karyotype was made according to the International System for Human Cytogenetic Nomenclature. The cytogenetic analysis on G-banded metaphases disclosed a 46,XY,t(9;22)(q34;q11) karyotype. Then, interphase FISH experiments were carried out using BCR-ABL1 probes (Vysis) and demonstrated the presence of BCR-ABL1 fusion gene.

At the resolution of a pulmonary aspergillus infection treated with voriconazole, while the cytogenetic and molecular analyses were ongoing, the patient started treatment with 5-aza-2′-deoxycytidine (otherwise called decitabine, 20 mg/m² for 5 days) for a total of two cycles. Subsequently, nested RT-PCR revealed the simultaneous presence of the common p190 e1a2 and the rare e6a2 isoforms (Fig. 2). PCR products, corresponding to BCR-ABL1 p190 amplification, were purified from agarose gel (QIAquick PCR Purification Kit - Qiagen) and directly sequenced on ABI/Prism 3130 Sequencer (Thermo Fisher Scientific) using BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). RQ-PCR for e6a2 BCR-ABL1 transcript analysis was performed using the forward primer Q-BCR_ex6_F (GATCCAACGACCAAGAACTCTCT), the reverse primer ENR561 and the ENP541 probe reported elsewhere [8]. The p190 e6a2 values were normalized on the number of ABL1 transcripts and expressed as the number of p190 e6a2 copies every $10^4$ copies of ABL1. To assess molecular response after treatment, a real-time quantitative RT-PCR assay (RQ-PCR) for p190 e6a2 was designed by which we observed significant different kinetics through the e1a2 and e6a2 transcripts with consistent persistence of e6a2 [9].
After the first, BM aspirated showed 70% blast cells and two transcripts e1a2 and e6a2 were respectively 3.09 and 5805.47/10^4 ABL1, while after second cycles blast cells were 20% and e1a2 and e6a2 5.71 and 5747.52.

Because of persistent pancytopenia and presence of blasts after two cycles of decitabine and in light of molecular data, the patient was then switched to TKI treatment.

The initial therapy consisted of imatinib 600 mg/day for two weeks that was subsequently reduced to 400 mg/day due to febrile neutropenia. After one month of imatinib, bone marrow showed 60% blast cells with small improvement of thrombocytopenia. Therefore, treatment was switched to dasatinib 100 mg/day, but it was discontinued five days later because of pulmonary embolism. At 10 days of TKI discontinuation, e1a2 and e6a2 were 0.17 and 9477.16/10^4 ABL1, respectively. After two months of continuous therapy with TKIs, bone marrow infiltration was present and the two transcripts were e1a2 1.6 and e6a2 23727.06/10^4 ABL1 (Fig. 2).

The patient, still refractory to second-line treatment, died of pulmonary infection.

Discussion

The AML with BCR-ABL1 fusion is a rare entity and has been included in the 2016 revised World Health Organization (WHO) as a provisional entity of myeloid neoplasm and acute leukemia [10]. The 2017 European LeukemiaNet risk stratification allocated AML with BCR-ABL1 to the adverse risk group [11]. In fact, the patients with BCR-ABL1 positive AML are often refractory to chemotherapy while the responses to imatinib are of limited duration [12]. A recent paper has reported that allogenic stem cell transplantation (allo-SCT) may improve outcome of younger patients [13]; however, given the small number of cases, the optimal therapy for AML with BCR-ABL1 has not been established yet. Beside common BCR-ABL1 transcripts, unusual transcripts have been also described in CML and acute leukemias. We report a rare case of AML characterized by the co-expression of the atypical e6a2 BCR-ABL1 transcript with the common e1a2 one. The e6a2 transcript is mainly observed in CML and sporadically in acute leukemia. In particular, so far about 24 e6a2 cases, in addition to our, have been described in the literature: 17 in CML (1 developed after chronic myelomonocytic leukemia -CMML-), 1 in a case of acute basophilic leukemia, 5 in AML (3 de novo and 2 cases developed after CMML and Myelofibrosis respectively) and 1 in an ALL patient [14]. Table 1 is a summary of the clinical features of e6a2 BCRABL-positive leukemia patients described so far. Notably, co-expression of e6a2 and e1a2 transcripts was previously described in only few cases of CML [15, 16]. Here we reported the first evidence of coexpression in AML.
Data reported so far indicate that the course of disease associated with e6a2 is particularly aggressive both in chronic and acute leukemias. In fact, within CML cases harboring this transcript, 6/17 developed an accelerated or blastic phase [6, 14–20]. Furthermore, there was a prevalence of males on female (ratio 14:3) and the median age at diagnosis was 48 years old (range 18–76 years). Notably, the 3 de novo AML cases with e6a2 transcript were female and the age at diagnosis ranged from 53 to 55 years old [21–23]. These patients underwent allo-SCT in first CR after TKIs and chemotherapy. In these cases, dasatinib and nilotinib were effective treatments in inducing molecular remission after imatinib failure [22, 23] and against relapse after transplant maybe thanks to synergy between TKI and the graft versus-leukemia effect, reaching sustained response [21, 22]. They were alive at 24, 30 and 52 months from diagnosis [21–23]. On the contrary, the case here presented was older and not eligible to allo-SCT. The absence of prior clinical or laboratory evidences of CML (splenomegaly or basophilia) suggested a diagnosis of de novo AML with BCR-ABL1 [24]. He was resistant to decitabine and to TKIs and he died for disease shortly after the diagnosis.

Despite the clinical heterogeneity, all previously reported cases and the one here reported indicate that the atypical e6a2 transcript is associated with aggressive disease and underline the potential advantage of TKIs and allo-SCT in improving prognosis of these patients [6, 14, 15, 17–20, 25]. As suggested, the poor prognosis could be attributed to the partial loss of the Guanine Exchange Factor (GEF)/dbl-like domain [26]. This GEF/dbl-like domain is able to mediate the interaction with several Ras-like G proteins involved in proliferation, signalling

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**Table 1** Cases reported in literature showing the e6a2 transcript

| Diagnosis                        | Sex/age | Coexpression of other transcripts | Other molecular alterations | Treatment                  | References       |
|----------------------------------|---------|-----------------------------------|-----------------------------|----------------------------|------------------|
| CP - CML                         | M/41    |                                   |                             | BMT                        | Hochhaus et al. [2] |
| CP - CML                         | M/50    | IFN-Ara C-> BMT                   |                             |                            | Dupont et al. [30] |
| BC - CML                         | M/65    | Imatinib                          |                             |                            | Schultheis et al. [6] |
| CP - CML                         | M/76    | IFN                               |                             |                            | Colla et al. [31]  |
| CML secondary to CMML            | F/64    | t(11;16)                          |                             | Imatinib                  | Hayette et al. [32] |
| CP - CML                         | M/37    | e1a2                              |                             | Imatinib                  | Roti et al. [16]  |
| Acute basophilic leukemia        | M/71    |                                   |                             | Imatinib-Dauno-Ara C      | Gregoire et al. [33] |
| ALL                              | F/29    | G MALL 7/03-Imatinib-> BMT        |                             |                            | Burmeister et al. [34] |
| CP - CML                         | M/43    | Imatinib                          |                             |                            | Breccia et al. [35] |
| AML                              | F/53    | Imatinib-Ida-Ara C                | -- > BMT-Dasatinib         |                            | Ritchie et al. [22] |
| AML (M7)                         | F/53    | del(18)(p10)                      |                             | Imatinib-I da-Ara C       | Corm et al. [23]  |
| CP - CML                         | M/48    | Imatinib-> Dasatinib              |                             |                            | Schnittger et al. [36] |
| CP - CML                         | M/48    | t(7;9), ins(22;9)                 |                             | Imatinib-> BMT            | Vefring et al. [17] |
| AP - CML                         | M/42    | Imatinib                          | -- > BMT-Dasatinib         |                            | Vefring et al. [17] |
| CP - CML                         | M/67    | Imatinib                          |                             |                            | Popovic et al. [26] |
| CP - CML                         | F/18    | Imatinib                          |                             |                            | Torres et al. [37] |
| AP - CML                         | M/57    | trisomy 8                         |                             | Imatinib                  | Beel et al. [18]  |
| CP - CML                         | M/36    | e1a2                              |                             | Imatinib-Nilotinib        | Langabeer et al. [15] |
| AP - CML                         | M/51    | Imatinib-> BMT                    |                             |                            | Rohon et al. [19]  |
| BC – CML                         | M/48    | Dasatinib                         |                             |                            | Zagaria et al. [14] |
| AML (M4)                         | F/55    | Mitox-Ara C-Imatinib              | -- > BMT-Dasatinib-DLI     |                            | Harada et al. [21] |
| BC - CML                         | F/48    | trisomy and tetrasomy 8           | Dauno-Ara c-Imatinib       | -- > BMT                  | Crampe et al. [20] |
| AML secondary to CMML            | M/58    | DNM3TA RUNX1 SUZ12                | 3 + 7-Dasatinib-Nilotinib  |                            | Yao et al. [25]   |
| AML secondary to myelofibrosis    | M/80    | JAK2-V617F                        | Hydroxyurea-valproic acid  | Dasatinib-Imatinib        | Brattas et al. [38] |
and cell organization. Therefore, this truncation seems to determine an increased kinase activity with a greater transforming oncogenic potential. In our patient, the significant different kinetics of e1a2 and e6a2 transcripts quantified by RQ-PCR during the treatment further supports this hypothesis.

The case here described presents a diagnostic and therapeutic challenge since it shows the importance of combining conventional karyotype/FISH and appropriate genetic assays to detect rare BCR-ABL1 fusion transcripts. Furthermore, the different clearance of the e1a2 and e6a2 transcripts underlines the importance to design specific molecular assays for the evaluation of minimal residual disease and to choose the best therapeutic options.

**Conclusions**

In conclusion, hematological malignancies with the atypical e6a2 show an aggressive phenotype with a higher capacity of transformation to blast crisis in CML. Ph + AML is more rare, but with the same worse prognosis [15]. Therapeutic options include TKIs and allo-SCT. In fact, these patients can benefit from use of TKI, although this treatment seems to be more effective as salvage or maintenance therapy or even as bridging to transplant after a remission [15].

For patients not eligible to transplantation, the prognosis is even more unfavourable and a different approach should be considered. There is no standard of care and treatment of patients affected by Ph + AML, especially elderly, remains an area of unmet need. Notably, Shao et al. recently reported that dasatinib single agent may be an effective and tolerable induction therapy for AML patients with BCR-ABL1 and poor physical condition [27]. However, prognosis with low-intensity therapies is still poor and novel therapeutic strategies are needed. Future approaches may investigate role of Ras-Mek inhibitors, nowadays under investigation as targeted therapies in other diseases. Additional strategies can combine also novel agent: g Wendtumab ozogamicin, tipifarnib, volasertib [28]. Moreover, interesting data arise from the use of ibritinib [29] that inhibits AML blast proliferation, enhancing cytotoxic activities of cytarabine or daunorubicin.

Prompt observations of patients carrying rare BCR-ABL1 transcripts associated with unfavourable prognosis remain mandatory to include these patients in more aggressive treatment strategies. In vitro studies are necessary to clarify the transforming property and the mechanisms of resistance of e6a2 transcript and to design novel target therapeutic agents.

**Abbreviations**

ALL: Acute lymphoid leukemia; AML: Acute myeloid leukemia; BM: Bone marrow; BMT: Bone marrow transplantation; CM: Chronic myeloid leukemia; DAC: Dacogen; DAS: Dasatinib; FUO: Fever of unknown origin; GEF: Guanine exchange factor; IFI: Invasive fungal disease; M-bcr: Major breakpoint cluster region; m-bcr: Micro breakpoint cluster region; m-bcr: minor breakpoint cluster region; MRD: Minimal residual disease; Ph: Philadelphia; RQ-PCR: Real-time quantitative RT-PCR; TKIs: Tyrosine kinase inhibitors; WBC: White blood cells

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**Availability of data and materials**

The raw data supporting our findings can be requested from the corresponding author.

**Authors’ contributions**

MP analyzed the data and wrote the paper according to the revisions of MRR, AT and FLC; TO, MD, VA and LC performed the assays and reported results. TO wrote methods and drew the figure too. MP, AF, EC, MRB, AC, GG, SP, APL and AT treated the patient. AT, FLC, MRR, SM, RL, TO approved the final version. All authors read and approved the final manuscript.

**Consent for publication**

Written informed consent was obtained from the patient for the publication of this case report and the accompanying images.

**Ethics approval and consent to participate**

Not applicable.

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**Author details**

1.Department of Clinical and Molecular Medicine, Hematology Sant’Andrea University Hospital, Sapienza University of Rome, Rome, Italy. 2.Department of Biomedicine and Prevention, University of Tor Vergata, Rome, Italy.

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