A novel BCR-ABL1 fusion gene with genetic heterogeneity indicates a good prognosis in a chronic myeloid leukemia case

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Abstract

Background: Chronic myelogenous leukemia (CML) is a pluripotent hematopoietic stem cell disorder caused by the fusion of the BCR and ABL1 genes. Quantitative RT-PCR (qRT-PCR) is a routinely performed screening technique to identify BCR-ABL1 fusion genes, but a limitation of this method is its inability to recognize novel fusions that have not been previously characterized. Next-generation sequencing (NGS) is an effective and sensitive detection method for the determination of novel BCR-ABL1 fusion genes as well as previously characterized ones. The oncoprotein tyrosine kinase BCR-ABL1 is a constitutively active kinase involved in the activation of a number of signaling pathways, and it has been the therapeutic target for tyrosine kinase inhibitors (TKIs) such as imatinib. Reports have presented opposing viewpoints about the effect of the disrupted Src homology 3 (SH3) domain on TKI efficacy.

Findings: We here report that using NGS we identified a novel BCR-ABL1 fusion gene with breakpoints in the BCR intron 14 and the ABL1 intron 2, leading to partial deletion of its SH3 domain. In the present case, the patient received targeted therapy with the TKI imatinib at 400 mg/day and no adverse reaction was reported. The patient eventually entered remission with decreased proliferation of karyocytes and granulocytes. We also identified mutations in genes, including TP53, FLT3, ASXL1, SETBP1, CEBPA and CBL, that seemed to have an influence on the outcome of TKI therapy targeting the BCR-ABL1 protein.

Conclusions: Together with previously reported results, it is clear that the genetic heterogeneity of CML patients significantly affects the presentation of the disease and its progression and therefore should inform the design of the therapeutic strategy.

Keywords: CML, BCR-ABL1, NGS, SH3 domain, Genetic heterogeneity
myeloid leukemia” has been published recently. Here, we report that we have also identified this novel BCR-ABL1 fusion gene in another patient using NGS technology. We also report that this patient carries a different set of genetic mutations than those that impacted the outcome of TKI imatinib treatment in the Lyu et al. report [9]. Comparison of these studies demonstrates that genetic heterogeneity can be a key influencing factor in the therapeutic resolution of CML.

Results
Our patient is a 62-year-old male who presented at our hospital in February 2016 with intermittent nasal bleeding that had exceed 1 month in duration. After hospitalization, we determined the patient had a significantly elevated platelet level that increased the risk of bleeding and thrombosis to a life-threatening level. No superficial lymph nodes were detected anywhere in the body. The patient was diagnosed with CML through blood and bone marrow examinations. Peripheral blood smear analysis indicated elevated levels of total white blood cells (WBCs, 55.24 g/L), neutrophils (34.58 g/L), thrombocytes (2597 g/L), and a normal level of hemoglobin (103 g/L). Bone marrow aspiration analysis revealed the active proliferation of bone marrow nucleated cells (BMNCs) and elevated proportions of eosinophils and basophils. Granulocytes accounted for 88% of WBCs due to the excessive proliferation of band granulocytes and segmented granulocytes (Table 1 and Fig. 1a). We also observed a decreased level of lymphocytes with normal morphology.

Bone marrow karyotype analysis showed a phenotype of 46,XY,t(9;22)(q34;q11.2) (Data not shown). Fluorescence in situ hybridization (FISH) analysis was then used to detect the fusion between BCR and ABL1 genes, which were demonstrated as dots of yellowish fluorescent signals formed from the colocalization of the green (BCR) and red (ABL1) fluorescent signals. We found at least one yellowish fluorescent dot per cell in 44% of cells, representing the tumor cells with BCR-ABL1 fusions (Fig. 1b). However, fluorescent qRT-PCR failed to detect the previously characterized BCR-ABL1 fusion transcripts p190 (e1-a2), p210 (e13-a2 and e14-a2) and p230 (e19-a2). To further clarify the existence of a BCR-ABL1 fusion in this patient, we conducted whole genome sequencing (WGS) analysis on a NGS platform. We detected a BCR-ABL1 fusion gene with novel breakpoints.

| Cell type                         | Cell count (%) | Reference (Mean ± sem) | Before therapy | After therapy |
|-----------------------------------|----------------|------------------------|----------------|--------------|
| Myeloblast                        | 0.64 ± 0.33    | 1.5                    | 0              |
| Promyelocyte                      | 1.57 ± 0.60    | 1.0                    | 0              |
| Neutrophil                        |                |                        |                |
| N.myelocyte                       | 6.49 ± 2.04    | 7.0                    | 1              |
| N.metamyelocyte                   | 7.90 ± 1.97    | 6.5                    | 1              |
| N. band                           | 23.72 ± 3.50   | 33.0                   | 20             |
| Neutrophil                        | 9.44 ± 2.92    | 28.0                   | 45             |
| Eosinophil                        | 0.86 ± 0.61    | 10.5                   | 7              |
| Basophil                          | 0.03 ± 0.05    | 3.5                    | 0              |
| Lymphocyte                        | 22.78 ± 7.04   | 4.0                    | 20             |
| Monocyte                          | 3.00 ± 0.88    | 0                      | 3              |

Table 1 Comparison of bone marrow aspiration analyses before and after therapy
in BCR intron 14 and ABL1 intron 2 (Fig. 1c), confirming the fusion of BCR exon 14 (e14) and ABL1 exon 3 (a3). The corresponding BCR-ABL1 hybrid mRNA was eventually identified by RT-PCR with a pair of custom primers targeting e14 and a3, followed by Sanger sequencing (Fig. 1d). After 7 days of imatinib treatment, the disease was under control with an improved platelet count and the patient discharged. As an outpatient, he then continued treatment with imatinib at 400 mg/day, accompanied by sodium bicarbonate tablets at 3.0 g/day and allopurinol tablets at 0.3 g/day, with regular follow-up visits. After 4 months, we measured a significant decrease in bone marrow karyocyte proliferation with the reduced number of granulocytes now accounting for 67% of WBCs in this CML patient (Table 1, Fig. 1e). We saw a great improvement in disease progression – the patient achieved both hematologic and molecular remission (Fig. 1e–f).

**Discussion**

Philadelphia translocation, formed by the junction of BCR and ABL1 genes, has been proven to be involved in the carcinogenesis of CML. In this work, we have identified a novel BCR-ABL1 fusion gene by NGS, together with other co-existing mutations, indicating that genetic heterogeneity is associated with the response to imatinib treatment for this CML case and may require optimization of the personalized therapeutic schedule targeting CML.

The introduction of small molecule TKIs has contributed to marked improvements in the therapeutic outcomes of CML by forcefully blocking phosphorylation by the BCR-ABL1 oncoprotein and inhibiting its cell signal transduction activity [10–12]. Imatinib is a tyrosine-kinase inhibitor used in the treatment of multiple cancers and was the first TKI to receive approval by the Food and Drug Administration for the treatment of patients with Philadelphia chromosome-positive (Ph+) CML [13, 14]. After detecting the novel BCR-ABL1 fusion gene in our patient, imatinib was used at 400 mg/day as a targeted treatment. It has been reported that the SH3-SH2 (Src homology 3-Src homology 2) domain in the ABL protein plays a crucial role in regulating its tyrosine kinase activity [15]. The function of partial deletions of the SH3 domain, which is encoded by ABL1 exons 2 and 3, remains controversial. The report from Lyu et al. showed that their patient was intolerant to a normal dosage of imatinib, indicating an interaction between this unusual therapy outcome and the incomplete SH3 domain caused by the deletion of ABL1 exon 2 [9]. Our case differed from that of Lyu et al. in that our patient with the e14-a3 BCR-ABL1 fusion gene was not refractory to or intolerant of imatinib treatment. The patient achieved both hematologic and molecular remission after 4 months of imatinib treatment. A previous study [16] reported that the STAT5 signaling pathway induced by the ABL1 SH3 domain plays a critical role in the anti-apoptotic activity and cell cycle progression involved in BCR-ABL1 leukemogenesis. Thus the truncation of the SH3 domain caused by deletion of ABL exon 2 may result in the reduction of leukemogenesis. CML patients with an e13a3 fusion gene were found to have a good response to imatinib, and could achieve complete hematologic and cytogenetic remission [17].

Similar to previous results, we also detected nonsynonymous mutations in leukemic genes such as TPS3 (c.C215G; p.P72R) and FLT3 (c.C680T; p.T227M) through NGS. Furthermore, we also found mutations in ASXL1 (c.T2444Cp.L815P), SETBP1 (c.G664A: p.A222T) (c.G3301A: p.V1101I) (c.C3388A: p.P1130T), CEBPA (c.570_571insCACCCCG: p.H191delinsPH) and CBL (c.C1858T:p.L620F) that co-existed with the BCR-ABL1 fusion in our patient. ASXL1 mutations are common in myeloid neoplasms, including myelodysplastic syndrome (MDS) [18, 19], chronic myelomonocytic leukemia (CMML) [20, 21], primary myelofibrosis [18, 22], and acute myeloid leukemia (AML) [19, 23]. SETBP1 mutations have been identified in atypical chronic myeloid leukemia (aCML), which is a rare disorder of hematopoietic stem cells and shares clinical and laboratory features with CML but lacks the BCR-ABL1 fusion gene [24]. Other strongly linked hematological malignancies, such as chronic neutrophilic leukemia (CNL), CMML, unclassified MDS, myeloproliferative neoplasms (MPNs), and secondary acute myelocytic leukemia (AML) evolving from MDS [25–29], are also related to SETBP1. Despite the fact that mutations in both ASXL1 and SETBP1 are generally associated with an adverse prognosis [20, 21, 26, 30], our patient’s symptoms seemed not to be related to his mutations in these genes. CCAAT enhancer binding protein α (C/EBPα), a general inhibitor of cell proliferation and a tumor suppressor [31] plays a pivotal role in early granulocyte development. C/EBPα is one of the crucial transcription factors for myeloid cell development and has been found to be involved in hematopoietic differentiation. The mutation of its coding gene CEBPA results in dysregulation of transcription, translation or post-translational modifications. These disruptions cause disorders of differentiation and over proliferation of immature hematopoietic cells [32, 33]. In our patient, the outcome of imatinib therapy for CML suggests that his disease was not significantly affected by what we can consider to be ancillary mutations. Considering the results reported by Lyu et al. [9], it can be concluded that the variety of genetic mutations among individual CML patients may lead to different treatment outcomes of TKI therapies targeted for BCR-ABL1. More research is needed to illuminate the interactions between these uncommon mutations and the variety of BCR-ABL1 fusion genes in CML.

pathway induced by the ABL1 SH3 domain plays a critical role in the anti-apoptotic activity and cell cycle progression involved in BCR-ABL1 leukemogenesis. Thus the truncation of the SH3 domain caused by deletion of ABL exon 2 may result in the reduction of leukemogenesis. CML patients with an e13a3 fusion gene were found to have a good response to imatinib, and could achieve complete hematologic and cytogenetic remission [17].
Conclusions
We report this case to demonstrate that by NGS we have detected the same BCR-ABL1 fusion that disrupts the SH3 domain, as Lyu et al. [9]. Meanwhile, we also found numerous other mutations in genes such as TPS3, FLT3, ASXL1, SETBP1, CEBPA and CBL, suggesting that CML may be more highly heterogeneous than previously appreciated. Our findings show that such genetic heterogeneity may significantly affect treatment outcomes and should therefore inform the therapeutic strategy. Since these conclusions remain speculative, more studies should be performed to characterize the various interactions between BCR-ABL1 gene rearrangements and mutations in other oncogenes.

Methods
Detection of BCR-ABL fusion by FISH analysis
To validate the presence of BCR-ABL1 fusion, we performed FISH analysis with dual color, single fusion probes on the patient’s bone marrow aspiration sample using the BCR-ABL FISH Probe kit (Jinpuijia Medical, Beijing, China) according to the manufacturer’s instructions. DNA probes targeting the BCR (chromosome 22q11.2) and ABL1 (chromosome 9q34) genes were labeled with green and red fluorescent dye, respectively. In normal cells, two green signals and two red signals were separated, representing that two probe-targeted sequences were located on different chromosomes. The presence of yellowish signal dots indicated the fusion events that resulted from the colocalization of BCR-targeting green fluorescent signals and ABL1-targeting red signals. The percent of cells with BCR-ABL1 fusions was counted and the cutoff value for the BCR-ABL1 fusion was set at 3% in our hospital.

Detection of BCR-ABL1 gene rearrangement by one-step RT-PCR
Routine fluorescence one-step RT-PCR was carried out to detect BCR-ABL1 fusion transcripts. RNA from patient bone marrow aspiration samples was extracted using an RNaseasy Kit (Qiagen, CA, USA), following the protocol provided by the manufacturer. RNA was purified by DNase I (Ambion, Applied Biosystems, TX, USA) digestion and was then subjected to one-step RT-PCR by a Leukemia Related Fusion Gene Detection Kit for BCR-ABL p210, p190, or p230 (Yuanqi Bio-Pharmaceutical, Shanghai, China). In each PCR process, a total volume of 25 μl reaction solution contains 3 μl template RNA, 2 μl multiplex Enzyme and 20 μl multiplex RT-PCR buffer. Amplification and detection were performed on a 7300 Real Time PCR System (ABI, USA). PCR procedure parameters were as follows: reverse transcription at 42 °C for 30 min, inactivation at 94 °C for 5 min, followed by 40 cycles of fluorescence detection at 94 °C for 15 s, and annealing at 60 °C for 60 s.

Whole genome sequencing in a NGS platform
A genomic DNA (gDNA) library was constructed for sequencing following protocols of the TruSeq Nano DNA Library Preparation Kit (Illumina, San Diego, CA). Adaptors were ligated to library fragments sheared by Covaris (Covaris, Woburn, MA, USA) and were then subjected to PCR amplification. The quantitation and abundance determination of PCR amplicons were performed on Qubit 3.0 Fluorometer (Life Technologies, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, USA), respectively. WGS was performed on HiSeq X (Illumina, San Diego, CA), with the use of Illumina bcl2fastq software version 2.15 for base calling analysis.

Abbreviations
AML: Acute myeloid leukemia; C/EBPα: CCAAT enhancer binding protein α; CML: Chronic myelogenous leukemia; CMML: Chronic myelomonocytic leukemia; CNL: Chronic neutrophilic leukemia; FISH: Fluorescence in situ hybridization; MDS: Myelodysplastic syndrome; MPNs: Myeloproliferative neoplasms; NGS: Next-generation sequencing; SH3: Src homology 3; TKI: Tyrosine kinase inhibitor; WBCs: White blood cells; WGS: Whole genome sequencing

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Availability of data and material
The datasets supporting the conclusions of this article are included within the article. More details are available upon request.

Authors’ contributions
FZ and HM collected the patient history and statistics, analyzed data, and were major contributors in writing the manuscript. RJ and YH collected the patient history and managed the clinical trials. HM managed the clinical trials, formulated the research program and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Written informed consent was obtained from the patient for publication of this Case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Ethics approval and consent to participate
This study was approved by the ethics committee of Union Hospital at the Tongji Medical College, Huazhong University of Science and Technology.

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