A minority of chronic myeloid leukemia patients (CML) express a variety of atypical BCR-ABL1 fusion variants and, of these, the e6a2 BCR-ABL1 fusion is generally associated with an aggressive disease course. Progression of CML to blast crisis is associated with acquisition of additional somatic mutations yet these events have not been elucidated in patients with the e6a2 BCR-ABL1 genotype. Moreover, molecular monitoring is only sporadically performed in CML patients with atypical BCR-ABL1 fusion transcripts due to lack of consensus approaches or standardization. A case of CML is described in which comprehensive molecular analysis, including targeted next-generation sequencing, revealed a single ASXL1 mutation cooperating with an e6a2 BCR-ABL1 fusion transcript at blast crisis. A quantitative molecular monitoring approach was devised and adopted that reflected the disease response from initial treatment through allogeneic stem cell transplantation which resulted in undetectable e6a2 BCR-ABL1 transcripts. This case emphasizes the requirement for molecular monitoring in CML patients with atypical BCR-ABL1 fusion transcripts and emphasizes that comprehensive sequencing has the potential to identify targets for novel therapies in CML patients with advanced disease.
Acquisition of further cytogenetic abnormalities and AML-associated, disease driving mutations are common findings in blast crisis of CML [17] but whether these additional events impact on prognosis is unclear [18]. Analogous to AML, identification of cooperating mutations may establish targets for novel therapeutic interventions in addition to TKI therapy [19]. Information on the incidence and pattern of these cooperating mutations in CML patients with the rare fusion transcript types is lacking.

Molecular monitoring of BCR-ABL1 transcripts by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is an integral part of the modern management of CML patients with achievement of molecular milestones according to internationally standardized practice incorporated into current management guidelines [20, 21]. However, standardization of these techniques applies only to the detection of BCR-ABL1 fusion transcripts but further analysis with a qualitative approach was employed to detect additional cooperating mutations as previously described [22]. At diagnosis the variant allele frequency (VAF) of the total cellularity, consistent with a diagnosis of blast crisis CML with marrow fibrosis.

In order to quantitate e6a2 BCR-ABL1 transcripts, a BCR exon 6 forward primer [4] was used with reverse primer ENR561 and probe ENP541 [22]. A standard curve was established by tenfold dilutions of patient presentation PB cDNA (slope $-3.538$, $r^2$ 0.996). Control ABL1 transcripts were detected as previously described [22]. At diagnosis the patients’ PB BCR-ABL1/ABL1 transcript level was 78.6%.

An NGS approach was employed to detect additional mutations cooperating with the e6a2 BCR-ABL1 fusion in driving blast crisis in this patient. Amplicon libraries covering 30 commonly mutated genes implicated in myeloid malignancies either covering the entire coding region (CALR, CEBPA, ETV6, EZH2, RUNXI, SH2B3, TET2, TP53, and ZRSR2) or mutational hotspots (ABL1, ASXL1, BRAF, CBL, CSF3R, DNMT3A, FLT3, GATA2, IDHI, IDH2, JAK2, KIT, KRAS, MPL, NPM1, NRAS, PTPN11, SETBP1, SF3B1, SRSF2, and U2AF1) were generated using 20 ng BM genomic DNA and sequencing performed using Ion AmpliSeq™ methodology (Thermo Fisher Scientific, Paisley, UK). Calling of somatic mutations was achieved using an algorithm that excluded synonymous mutations, variants located within intronic or untranslated regions, and those present at a variant allele frequency (VAF) of <5%. A minimum target depth of coverage for variant calls was set at 500x as previously described [24]. A single ASXL1 p.E1102D mutation (c.3306G $>$ T; reference sequence NM_015338.5) was detected with a variant allele frequency of 45.5%.

The patient received one cycle of daunorubicin and cytarabine (DA 3 + 10) with imatinib 400mg daily which was escalated to 600 mg daily achieving hematological and morphological remission and resulting in a PB BCR-ABL1/ABL1 transcript level of 0.06%. She was subsequently treated with a second cycle of chemotherapy (DA 3 + 8) which was
complicated by septicemia. Prior to ASCT the PB BCR-ABL1/ABL1 transcript level was 0.03% (Figure 2). Her imatinib was stopped prior to busulfan and cyclophosphamide conditioning for ASCT from a matched sibling donor that was complicated by mucositis and a coagulase-negative staphylococcus infection during her inpatient stay. After discharge she developed grade four skin graft versus host disease (GVHD) and was treated with high dose steroids subsequently developing CMV reactivation and steroid induced diabetes. Antiviral treatment with valganciclovir caused a pancytopenia requiring treatment with granulocyte colony-stimulating factor. BCR-ABL1 transcripts were not detected by RT-qPCR at day 38 after ASCT with full donor chimerism achieved (100% at day 43 post-ASCT). The patient remains off TKI and generally well at last follow-up. The CMV reactivation has resolved and she continues on steroid taper for GVHD. Continued close RT-qPCR monitoring is planned.

3. Discussion

The e6a2 BCR-ABL1 variant is rare with less than twenty CML patients of this genotype reported. Clinically, CML cases that express the variant e6a2 BCR-ABL1 fusion transcript often present in advanced stages and display an aggressive disease course, confirmed by this case report: it must be noted that occasional good responses to TKI have also been documented. In the case described herein, imatinib combined with AML induction therapy resulted in a considerable reduction in the BCR-ABL1 transcript level with the patient able to proceed with ASCT.

Little is known of the biological characteristics of this genetic subtype of CML and in those with other variant BCR-ABL1 fusion genes. NGS targeted for prognostically and clinically relevant mutations recurrently observed in myeloid malignancies revealed a mutation of ASXL1 in this patient. ASXL1 encodes an epigenetic regulator involved in posttranslational chromatin modification with aberrant histone modification being one of the important mechanisms underlying altered epigenetic regulation in other myeloid malignancies such as the myelodysplastic syndromes (MDS) [29], are potentially applicable to other ASXL1-mutated malignancies such as blast crisis CML, suggesting that individual molecular characterization of advanced phase CML patients may be increasingly necessary. It must be acknowledged that mutations of ASXL1 and other myeloid malignancy-associated genes have been detected at low levels by deep sequencing in both Ph-negative and Ph-positive clones of CML patients. These findings suggest that expression of BCR-ABL1 may be insufficient for the development of CML which has implications for treatment with both TKIs and other novel agents [18, 30]. Whether the ASXL1 mutation detected in this case was present in a BCR-ABL1-independent clone cannot be excluded or confirmed as NGS was not performed when the patient had achieved remission after chemotherapy; however, the high ASXL1 mutation VAF at presentation points to the ASXL1 mutation and BCR-ABL1 fusion coexisting in the same clone.

BM fibrosis, as evident in this case, is an infrequent but recurrent feature of CML. In the classical Philadelphia chromosome-negative myeloproliferative neoplasms, several lines of evidence link ASXL1 mutations with BM fibrosis: ASXL1 mutations are associated with a higher degree of BM fibrosis in primary myelofibrosis [31]; ASXL1 mutations are associated with an increased risk of myelofibrotic transformation in patients with essential thrombocythemia and polycythemia vera [32]; and ASXL1 mutations are more frequently detected in overt as opposed to prefibrotic primary myelofibrosis [33]. An association therefore potentially exists between ASXL1 mutations and BM fibrosis that requires further investigation in CML patients.

Despite the fact that there is no standardized approach to monitoring atypical BCR-ABL1 transcript types, RT-qPCR has been adopted in some e6a2 BCR-ABL1 cases of CML correlating well with clinical course as in the case described herein. An emerging, alternative methodology to RT-qPCR is digital droplet PCR that allows for more accurate quantitation of target molecules without the use of standard curves. This approach has recently been applied to molecular monitoring of a CML patient with an e6a2 BCR-ABL1 fusion, demonstrating an initial three-log reduction in transcripts with dasatinib monotherapy [34].

In conclusion, comprehensive molecular genetic characterization of the rare e6a2 BCR-ABL1 fusion and accompanying mutations facilitates prospective molecular monitoring and may also provide targets for potential therapeutic intervention in CML patients with this hostile genotype.
Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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