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Isolated Central Nervous System Relapse in Two Patients with BCR–ABL-positive Acute Leukemia While Receiving a Next-generation Tyrosine Kinase Inhibitor

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Abstract. *We describe two patients with break point cluster region-Abelsen (BCR–ABL)-positive acute leukemia who had an isolated relapse in the central nervous system (CNS) while receiving a next-generation BCR–ABL inhibitor. The first had B-cell acute lymphoblastic leukemia which relapsed in the CNS while maintaining molecular remission in the bone marrow on nilotinib. The second patient had an isolated CNS myeloid blast crisis of chronic myeloid leukemia while maintaining complete cytogenetic remission in the bone marrow on dasatinib. Mutation analysis of the kinase domain revealed 35 base pair insertion (35INT) between exon 8 and 9 in both cases.*

The central nervous system (CNS) is a sanctuary site for acute lymphoblastic leukemia (ALL). Amongst patients with break point cluster region-Abelson (*BCR-ABL*)-positive ALL, the incidence of CNS involvement is between 8% and 17% (1, 2). CNS involvement is rare in CML during the chronic phase, however the risk increases during blast crisis.

Imatinib was the first tyrosine kinase inhibitor (TKI) approved for treating *BCR-ABL*-positive leukemia. However, it achieves low concentrations in the CNS and is unable to prevent CNS relapses (3, 4). The next-generation BCR–ABL inhibitors include nilotinib, dasatinib, bosutinib and ponatinib. The first three are considered second-generation while ponatinib is considered a third-generation TKI. They are exponentially more potent than imatinib in inhibiting BCR–ABL and some, like dasatinib, are reported to achieve levels in the cerebro spinal fluid (CSF) which may be therapeutic (5).

We report on two patients with BCR–ABL-positive acute leukemia who had an isolated CNS relapse while maintaining remission in the bone marrow while receiving next-generation TKIs. Intermittent non-compliance with consistent TKI use was felt to contribute to the relapse in both cases. Both patients were found to have 35 nuclotide insertion (35 INT) mutation in the ABL kinase domain. Cranio-spinal irradiation, intrathecal cytotoxic chemotherapy and continued BCR–ABL inhibition led to resolution of symptoms and eradication of leukemia in both cases.

Case Reports

Case 1. The first patient is a 26-year-old male who was diagnosed with BCR–ABL-positive, B-ALL in 2012. Cytogenetic studies on bone marrow aspirate at diagnosis had shown t (9;22) (q34; q11.2) and del(6)(q21) in all metaphases. Fluorescent *in situ* hybridization studies confirmed *BCR-ABL* translocation. CSF was not involved. He was treated per CALGB 8811 protocol and also received daily dasatinib (6). After three months of therapy, the patient became non-compliant with chemotherapy but continued taking dasatinib. He experienced relapse in 2013. Dasatinib was discontinued and he was started on ponatinib (45 mg daily). In addition, the patient received multiple cycles of hyper-fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone alternating with high-dose methotrexate and cytarabine. He also received intrathecal prophylaxis with methotrexate and cytarabine. He achieved a complete molecular remission and was transitioned to maintenance therapy with monthly pulses of vincristine and prednisone, and daily ponatinib. Ponatinib therapy was interrupted in November 2013 due to the drug not being commercially available and the patient was started on nilotinib (300 mg twice a day).

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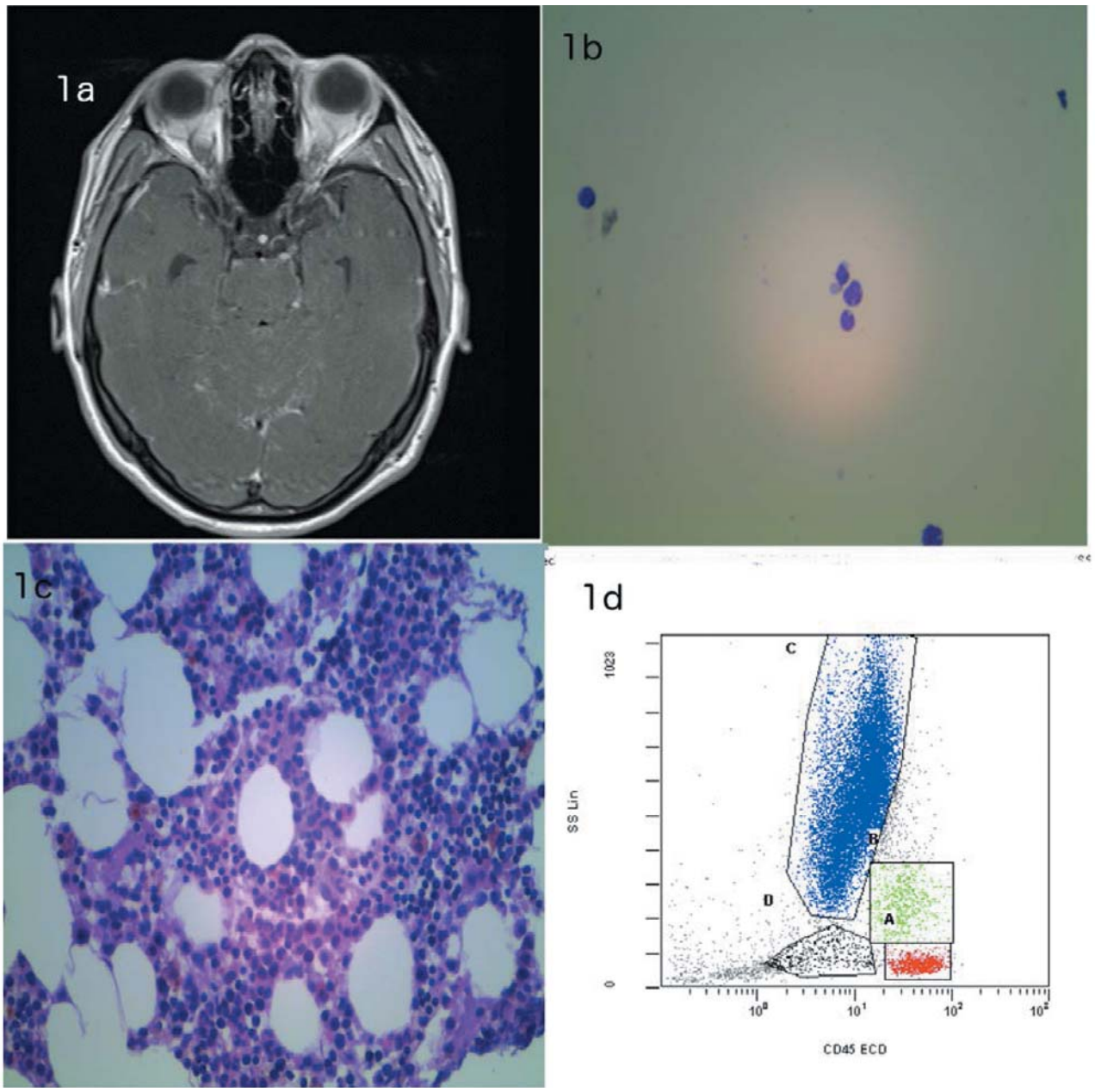

Figure 1. *a: Brain magnetic resonance imaging showing leptomeningeal enhancement. b: Cerebrospinal fluid cytology showing leukemia blasts. c and d: Normal bone marrow with no increase in blasts.*

In January 2014, the patient presented with headaches, tinnitus, left facial droop and difficulty walking. Physical examination showed left facial droop and decreased muscle strength in the left thigh. Complete blood counts, renal function and liver function were normal.

Magnetic resonance imaging (MRI) of the brain showed a pattern of diffuse leptomeningeal enhancement (Figure 1a). CSF cytology showed atypical lymphocytes (Figure 1b). Bone marrow biopsy was normocellular with no increase in blasts (Figure 1c and d). Cytogenetic studies were normal. Qualitative polymerase chain reaction (PCR) for *BCR-ABL* was negative in the bone marrow aspirate, but positive in the CSF. In addition, *ABL* kinase domain mutation analysis showed the presence of 35INT splice variant at a level of 12.3%.

The patient received 24 Gy of craniospinal irradiation, and twice weekly intrathecal chemotherapy with alternating

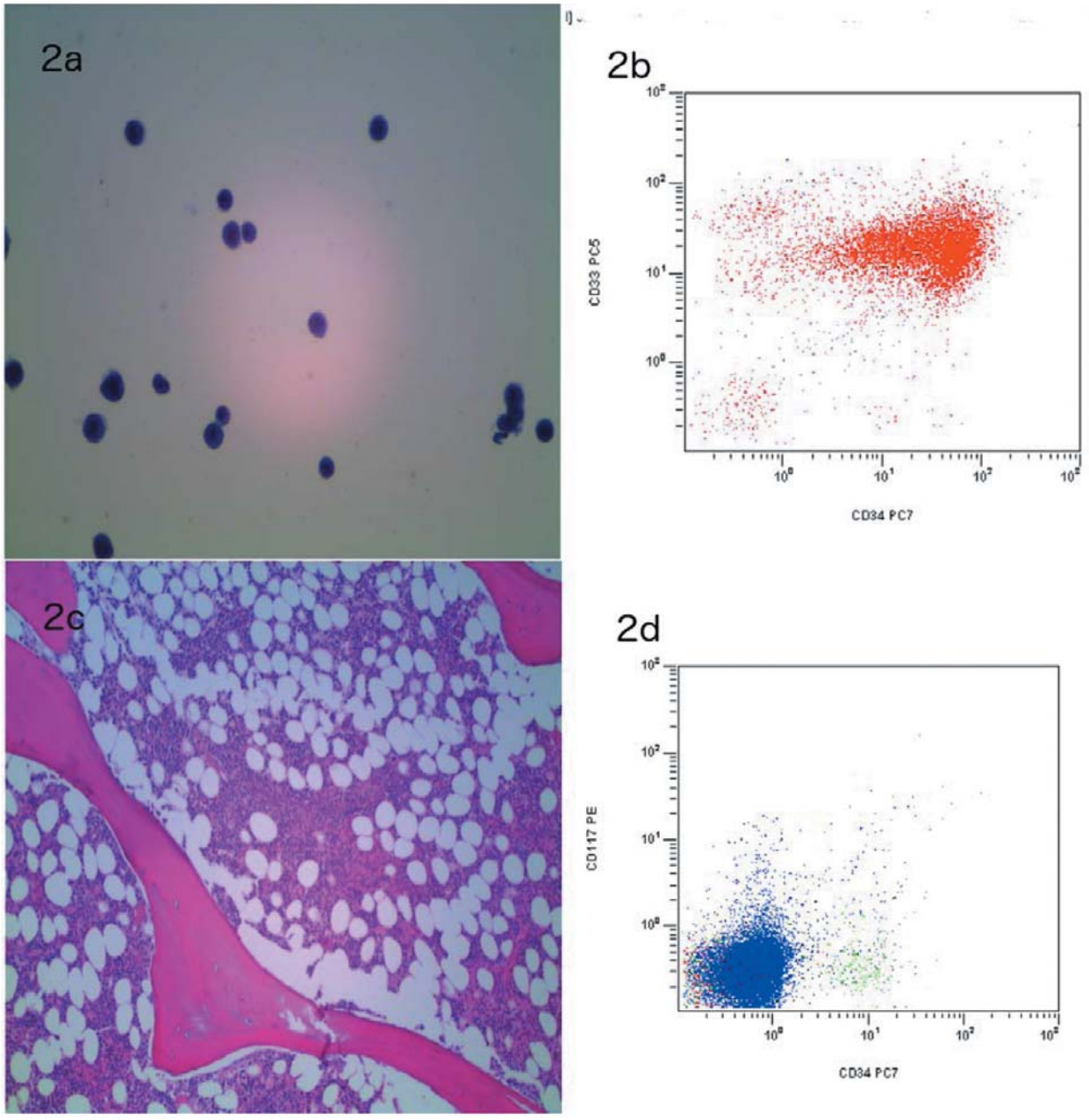

Figure 2. *a: Cerebrospinal fluid cytology showing leukemia blasts. b: CSF flow cytometry showing expression of CD33 and CD34 on blasts. c and d: Normal bone marrow with no increase in blasts.*

methotrexate and cytarabine. Nilotinib was discontinued and he was re-started on ponatinib. The patient's symptoms improved rapidly. No matched donors were identified among his siblings or the national marrow donor registry. The patient continues on daily pontanib, monthly pulse-dose prednisone and monthly intrathecal methotrexate. Vincristine was discontinued in view of on-going moderate thrombocytopenia secondary to radiotherapy.

Six months after CNS relapse, the patient remains in molecular remission with normal CSF analysis.

Case 2. The second patient is a 30-year-old male diagnosed with CML in 2004. He had been on imatinib and nilotinib, with intermittent non-compliance. In 2011, the patient developed accelerated phase of CML with a white blood cell count of 287,000/μl, hemoglobin of 8 g/dl and a platelet count of 95,000/μl. Cytogenetic studies were performed on

20 metaphase nuclei in the bone marrow. All had t(9;22) (q34; q11.2). In addition, 12 metaphases had an extra copy of Philadelphia chromosome, suggesting clonal evolution. No mutations were detected in the *ABL* kinase domain.

The patient was started on dasatinib at a dose of 100 mg daily. Bone marrow biopsy after six months of dasatinib therapy showed normal cellularity, however, 35% of the cells continued to exhibit t (9;22), with 15% having an extra copy of Philadelphia chromosome. The patient was felt to have a sub-optimal response to therapy. Dasatinib was discontinued and he was started on ponatinib at a dose of 45 mg daily. Six months after ponatinib therapy, bone marrow assay showed a complete cytogenetic response. Ponatinib was continued for an additional four months (total 10 months of therapy) at which point it was stopped due to the drug becoming temporarily unavailable in the United States. The patient was restarted on dasatinib and was being evaluated for allogeneic transplantation.

Two months after stopping ponatinib, the patient presented with visual loss, headaches, and weakness of his lower extremities. Brain MRI showed diffuse supratentorial and infratentoria lepto-meningeal enhancement. A CSF analysis showed a white blood cell count of 1,770/μl, the majority of which were myeloblasts (Figure 2a and b). Bone marrow biopsy and aspirate were normal (2c and d). Cytogenetic studies showed ongoing complete cytogenetic response. However, PCR analysis showed *BCR-ABL* transcripts at a level of 8.1%. In addition, *ABL* kinase domain mutation analysis showed 35 nucleotide insertion mutation between exons 8 and 9.

Twice weekly intrathecal therapy with cytarabine and 24 Gy of craniospinal irradiation were administered. Dasatinib was continued. Bone marrow biopsy and CNS analysis performed two months after CNS relapse showed no signs of CNS leukemia and ongoing complete cytogenetic response in the bone marrow. The patient is awaiting an allogeneic stem cell transplant.

Discussion

Intermittent non-compliance with therapy in the preceding years was felt to contribute to relapse in both patients while receiving a next-generation TKI. Both had a normal bone marrow examination at the time of CNS relapse. This attests to the remarkable potency of the next-generation TKIs in cases of imatinib failure, however, it also suggests that they may not be able to achieve levels in the CSF to prevent CNS relapses. Although dasatinib has been shown to penetrate the blood–brain barrier, the levels achieved in CSF may not be enough to effectively prevent CNS relapses (5).

In both the above cases, CNS relapse occurred within a few weeks of discontinuing ponatinib, suggesting the two might be causally related. Of note, the phase II PACE trial which led to the approval of ponatinib excluded patients with active CNS disease and there is a lack of data regarding its CNS penetrance. Further research regarding its activity in patients with BCR-ABL-positive CNS leukemia is needed.

We did not identify any point-mutations in the *ABL* kinase domain in either patient, however, both were noted to have the 35 base pair insertion mutation at the junction of exon 8 and 9 at the time of CNS relapse. This mutation introduces a stop codon after 10 intron coded residues leading to an alternatively spliced *BCR-ABL* variant (7). It is reported to be present in 1-2% of patients with imatinib resistance. The clinical relevance of this mutation is unclear. Case reports and evidence from molecular dynamics simulations suggest that it can lead to TKI resistance (8-10). On the other hand, cell-based and biochemical evidence suggests that it is kinase inactive and does not contribute to TKI resistance (11). The above two cases suggest this mutation might predispose patients to CNS relapse. Further studies are required to clarify its clinical relevance.

Conclusion

Isolated CNS relapses can occur in patients with *BCR-ABL*-positive acute leukemia while receiving a next-generation tyrosine kinase inhibitor. Achievement of complete cytogenetic responses and molecular responses in the bone marrow may not be enough to prevent risk of CNS relapse. Clinicians should have a low threshold in obtaining CSF analysis and brain MRI in such patients to investigate vague CNS symptoms. Aggressive intrathecal chemotherapy and craniospinal irradiation can effectively control CNS disease and prevent significant morbidity and mortality.

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