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LETTER TO THE EDITOR

Imatinib failure and response to dasatinib in a patient with chronic myeloid leukemia in blast crisis and a novel, nine-nucleotide BCR-ABL insertion mutation

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In chronic phase chronic myeloid leukemia (CML), the BCR-ABL kinase inhibitor imatinib leads to complete cytogenetic responses in the majority of cases. Resistance towards imatinib is associated with BCR-ABL kinase domain mutations, leading to structural changes that prevent imatinib from binding.[1]

In cases of failure towards imatinib treatment, second generation BCR-ABL kinase inhibitors such as dasatinib or nilotinib have demonstrated activity in CML.[2] Both drugs are capable of suppressing imatinib-resistant, mutant forms of BCR-ABL.[3] Most of the mutations in the BCR-ABL gene mediating inhibitor resistance are point mutations, replacing single nucleotides. Splice mutations in BCR-ABL leading to deletion or insertion of nucleotide stretches have rarely been described.[4]

Here, we report on a patient with CML in blast crisis after imatinib failure and second-line treatment with dasatinib harboring a so far undescribed p.K294S_insFPQ mutation (g.68009_68010ins GTTTCCCTC). A 37-year-old female patient initially presented with malaise, lymphadenopathy and splenomegaly. Her white blood cell count was 122.7/nl with 47% blasts. Bone marrow morphology showed 80% blast infiltration. Immunophenotyping revealed expression of CD34, HLA-DR, CD19, CD10, TdT and cyCD22. A Philadelphia chromosome-positive CML with fusion transcript-type M-BCR (p210, b2a2) in primary lymphoid blast crisis was diagnosed with a BCR-ABL/ABL ratio of 276%.

Chemotherapy with daunorubicine and cytarabine and treatment with imatinib at a daily dose of 800 mg was started. Imatinib was later reduced to 600 and 400 mg due to pancytopenia. The BCR-ABL/ABL ratio only decreased to 139.6 and 48.2% on day 50 and 89, respectively, with persistence of 10–15% bone marrow blasts and 17% blasts in the peripheral blood, indicating failure of treatment.

At day 99, BCR-ABL mutation analysis revealed a nine-nucleotide insertion mutation (K294S_insFPQ) in the ABL kinase domain. Imatinib was discontinued, dasatinib was started at a dose of 100 mg daily and three doses of vincristine and dexamethasone were given. On day 131, BCR-ABL/ABL ratio decreased to 13.8% and to 0.9% by day 173 under dasatinib treatment, indicating molecular response. Bone marrow analysis revealed $<5\%$ blast cells. The level of hemoglobin was 8.8 g/dl, leukocytes 0.9/nl with 0% blasts and platelet 30/nl, indicating complete remission with incomplete blood count recovery. BCR-ABL/ABL ratio had decreased to 13.8% under dasatinib treatment indicating molecular response. On day 173, the hemoglobin level was 8.6 g/dl, leukocytes 3.6/nl with 0% blasts and platelets 29/nl, indicating complete hematologic remission with incomplete recovery of platelets. BCR-ABL/ABL ratio was 0.9%.

Allogeneic HLA-(A*) antigen-mismatched peripheral blood stem cell transplantation (aSCT) was performed after conditioning with

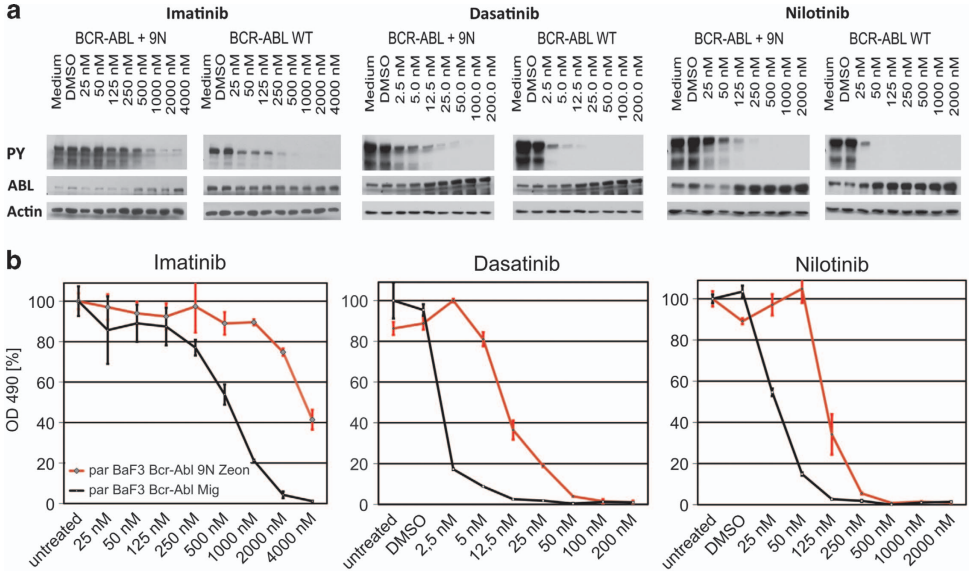

Figure 1. (**a**) Inhibition of autophosphorylation in BCR-ABL1 wild type and K294S_insFPQ demonstrated by western blot. Ba/F3 cells (BCR-ABL1 wild type and BCR-ABL1 K294S_insFPQ) were cultivated in the presence of imatinib, dasatinib and nilotinib for 2.5 h at the indicated concentrations. (**b**) Assessment of cell growth. Proliferation was measured using an MTT-based method. Measures were taken as triplicates after 48 h of culture without and in the presence of inhibitors at the indicated concentrations. Values are expressed as representatives of growth inhibition from three independent experiments. One representative experiment is shown. Bars indicate ± s.e. OD (optical density) ± s.e.m. (standard error of mean).

total body irradiation (at 12 Gy), cyclophosphamide and anti-thymocyte globulin on day 205 after admission.

On day 100 after aSCT, complete molecular response with a donor chimerism of 100% was achieved. Hemoglobin level was 7.9 g/dl, leukocytes 1.7/nl and platelets were 24/nl. One year after aSCT, graft versus host disease of the liver required the administration of steroids. CMV reactivation occurred, which was treated with ganciclovir. Fluorescence *in situ* hybridization analysis indicated complete cytogenetic response and BCR-ABL/ABL was 0% at different timepoints after aSCT (last result on day 757 after aSCT). Despite ongoing complete cytogenetic and molecular response, the bone marrow failed to completely recover. Transfusion-dependent thrombocytopenia and anemia persisted. On day 757 after aSCT, the bone marrow was still hypocellular with a donor chimerism of 97%. On day 916 after aSCT, the patient died of septic multiorgan failure.

As shown in Figure 1a–b, the p.K294S_insFPQ mutation identified in this patient mediated a strong imatinib resistance and moderate resistance towards nilotinib and dasatinib. Cellular IC50 (half-maximal inhibitory concentration) for imatinib was 4000 nM, which corresponds to plasma trough levels at a dose of 800 mg daily in humans.[5] Accordingly, imatinib was not able to completely abrogate autophosphorylation in BCR-ABL/K294S_insFPQ. In contrast, dasatinib and nilotinib were able to completely inhibit cell growth and BCR-ABL autophosphorylation in K294S_insFPQ-expressing cells at concentrations that correspond to plasma trough levels reported in treated patients (with dasatinib 70 mg twice daily 100 nM, nilotinib 400 mg twice daily 1700 nM).[6]

Our patient displayed primary hematologic failure towards imatinib with persistence of peripheral blood blasts, which in this case can be attributed to the identified BCR-ABL/p.K294S_insFPQ mutation. Dasatinib and nilotinib both show residual activity against this particular mutation. In accordance, dasatinib was able to overcome imatinib resistance and able to induce major hematologic response and a decrease of BCR-ABL/ABL ratio from 48.2 to 0.9% prior to aSCT. However, aSCT still bears the risk of nonrelapse mortality, even though the underlying disease is controlled.

Secondary mutations in BCR-ABL are mainly single-nucleotide point mutations of the kinase domain,[1] deletion or insertion mutations are rare. A K294R point mutation has been identified *in vitro* to mediate weak imatinib resistance.[7] A p.K294R_insGG mutation recently was identified in a patient with blast crisis CML and imatinib resistance.[8] Thus, more complex mutations such as insertion of several nucleotides adjacent to codon K294 that mediate strong resistance might require genetic instability as it is present in blast crisis. Mutations involving the ABL SH3-binding domain including K294 insertion mutations might retain sensitivity to dasatinib, as observed in our patient. This allows bridging to allogeneic stem cell transplantation, which to date still represents the only potential cure for advanced CML.

CONFLICT OF INTEREST

NvB and JD received honoraria from Novartis. SS declarespart ownership of the MLL Munich Leukemia Laboratory. All other authors declare no conflict of interest.

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