Dasatinib and allogeneic stem cell transplantation enable sustained response in an elderly patient with *RCSD1-ABL1*-positive acute lymphoblastic leukemia

The addition of tyrosine kinase inhibitors (TKI) to treatment regimens of patients with Philadelphia chromosome-positive (Ph^+) acute lymphoblastic leukemia (ALL) has significantly improved overall survival.[1,60] However, allogeneic stem cell transplantation (allo-SCT) offers the only curative option in the established therapeutic armamentarium for patients with Ph^+ ALL. Ph^+ ALL is characterized by the translocation t(9;22)(q34;q11.2), leading to the expression of the fusion protein BCR-ABL1. Treatment with TKI, such as imatinib or dasatinib, targets the ABL1 tyrosine kinase portion of the fusion protein, thereby inhibiting proliferation and inducing apoptosis.[2] However, several genes other than *BCR* have been identified to form rearrangements with *ABL1* in B-cell ALL, including *RCSD1, ETV6, NUP214, RANBP2, FOXP1, SFPQ, SNX2* and *ZMIZ1*.[2-4] Here, the *RCSD1-ABL1* rearrangement is characterized by the fusion of the N-terminal portion of the *RCSD1* gene to *ABL1* exon 4, a feature unique for the *ABL1* fusion partner genes *SFPQ* and *RCSD1*.[4] This unusual breakpoint of the *ABL1* gene retains only a part of the *SRC* homology 2 (SH2) domain regulating autophosphorylation, and lacks the SH3 inhibitory module.[2,4] The *RCSD1* gene encodes the phosphoprotein CapZ-interacting protein (CapZIP) that contributes to regulation of actin filament assembly, and is found in immune cells, splenocytes and muscle tissue.[5] There is growing evidence that Ph-negative (Ph^-) patients showing *ABL1* translocations also might benefit from treatment with TKI. However, sustained molecular remissions, even with the incorporation of TKI to treatment protocols, have rarely been reported, and patients frequently die from early relapse or refractory disease.[3] In particular, the capacity of alloimmune effects complementing TKI-based targeted therapy to induce long-term remissions in patients with *RCSD1-ABL1* rearrangements is unclear. Moreover, the prognostic impact of minimal residual disease (MRD) levels preceding allogeneic stem cell transplantation (allo-SCT) remains elusive in this context. We here show sustained molecular remission in a highly pretreated elderly patient with a refractory *RCSD1-ABL1*-positive B-cell ALL;

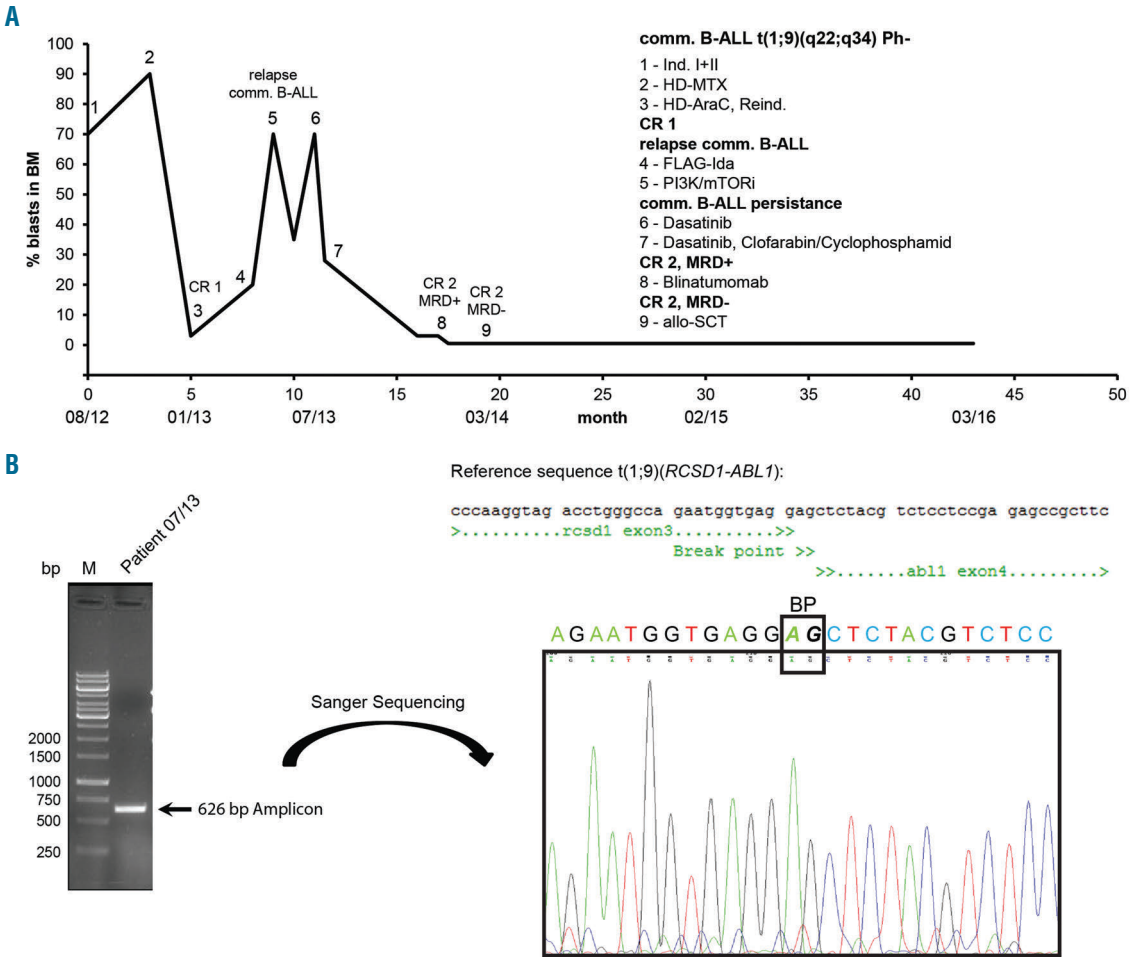

Figure 1. Treatment course and identification of the *RCSD1-ABL1* translocation. A. Treatment course of the patient with common Ph^- B-ALL with a translocation t(1;9)(q22;q34) in association with percentage of blasts in the bone marrow over 43 months. The box indicates the treatment regimens. B. RT-PCR of *RCSD1-ABL1* transcript performed with primers F1 (5'-GGGACAGCGGGGATCGTGAG-3') and R1 (5'-AGGAGCTGCACCAGGTTAGG-3'). A 626 bp long product was amplified. Product was used for Sanger sequencing. Chromatograms show the breakpoints of chromosomes 1 and 9 leading to the fusion of exon 3 of *RCSD1* and exon 4 of *ABL1*. Ind.: induction course; CR: complete remission; M: DNA marker; BP: break point.

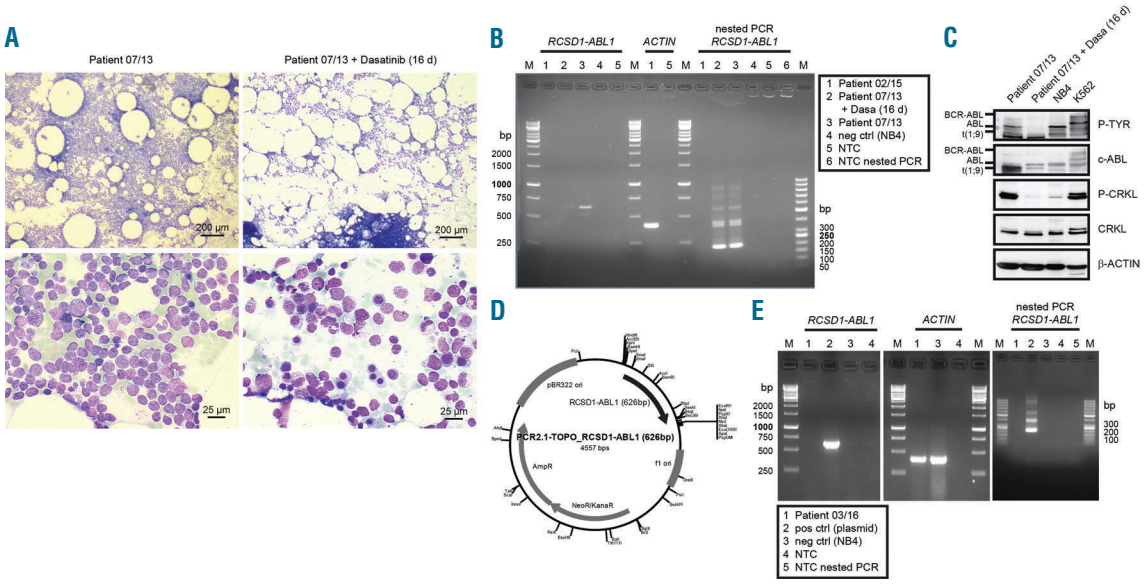

Figure 2. Dasatinib causes a decrease of *RCSD1-ABL1*-positive cells and leads, in combination with allo-SCT, to long-term remission. A. May-Grünwald-Giemsa staining of patient's bone marrow smears from 07/2013 before (left panels) and after (right panels) 16 d dasatinib treatment. Before treatment, the blastic population accounted for 70% of cells, while a partial response with 28% blasts was achieved after treatment with dasatinib. Pictures of the stained cells are shown as x100 (upper panels) and x600 (lower panels) original magnification. B. RT-PCR/nested PCR of the *RCSD1-ABL1* transcript was established for clinical monitoring of MRD or relapse. Nested PCR was performed with primers F1-nested (5'-AAACCAACCCGAAGGAAACC-3') and R1-nested (5'-ATAGACAGTGGGCTTGTTGC-3'). A 207 bp long product was amplified. Shown are the amplification products of the patient 11 months after first diagnosis, 16 days after dasatinib treatment (11.5 months), and 30 months after first diagnosis. *ACTIN* served as an internal control. C. Western analysis of P-Tyr-ABL1/ABL1 and its downstream target P-CRKL/CRKL in patient samples before (11 months after first diagnosis) and after 16 days of dasatinib treatment (11.5 months), corresponding to the samples in A and B. β-ACTIN served as loading control. NB4 cells served as negative control for ABL1 translocations and K562 served as positive control for BCR-ABL1 expression. D. Vector map of PCR2.1-TOPO_*RCSD1-ABL1*(626bp). The *RCSD1-ABL1* 626 bp amplicon was inserted in the vector backbone of PCR2.1-TOPO as a positive control for clinical monitoring via RT-PCR/nested PCR. E. Regular clinical monitoring of the RCSD1-ABL1 transcript using NB4 sample as negative control and the PCR2.1-TOPO_RCSD1-ABL1(626bp) plasmid as positive control. Shown here is a patient sample taken 43 months after first diagnosis. *ACTIN* served as an internal control. M, DNA marker; NTC, no template control.

molecular remission was achieved via multimodal treatment, including the sequential use of dasatinib and the bispecific T-cell engager blinatumomab followed by consolidation with allo-SCT. A 61-year old female presented in August 2012 with increasing fatigue and pallor. Peripheral blood counts revealed hemoglobin of 9.7 g/dL, white blood cell count of 2.5 × 10^9/L with 2% blasts and 66 × 109/L platelets. The bone marrow (BM) aspirate showed 70% blasts, and was consistent with acute leukemia (AL). Immunophenotyping revealed the blasts to be positive for CD10, CD19, CD22, CD79a, CD34 and HLA-DR. Of the blasts, 46% were positive for TdT, and the cells were negative for CD20. No myeloid or T-cell markers could be detected. Based on these results, a common-B-cell-ALL was diagnosed.

Cytogenetic analysis showed two coexisting clones with an abnormal karyotype of 47,XX,+17,t(1;9)(q22;q34)[3]/46,XX,der(6)t(6;17)(p23;q21),t(1;9)(q22;q34)[2] and, in the molecular analysis, no evidence for *BCR-ABL1* was found. Figure 1A illustrates the patient's course of disease and treatment. The patient was enrolled on GMALL elderly (01/2003) and treated accordingly. After two induction courses (Figure 1A, 1), her BM evaluation on day 36 revealed primary induction failure with progressive disease, and 90% of leukemic blasts. She therefore continued chemotherapy with high-dose methotrexate and asparaginase three months after induction therapy was initiated (Figure 1A, 2). Her disease achieved first complete remission (Figure 1A, CR 1) in January 2013, and high-dose cytarabine was administered (Figure 1A, 3). At the same time, an unrelated donor search was initiated. Donor search, however, was futile, and the patient experienced a BM relapse with 20% of leukemic blasts in April 2013. Re-induction chemotherapy with one cycle of FLAG-Ida comprising fludarabine, cytarabine, G-CSF and idarubicin was administered (Figure 1A, 4), but BM revealed residual disease with 70% of leukemic blasts one month later, in May 2013. The patient was included in the BEZ235 study and treated with an oral PI3K/mTOR inhibitor (Figure 1A, 5), but no remission could be achieved. The patient was transferred to our hospital. Due to the patients progressive disease and the suspicion of an *ABL1*-activating fusion gene based on the translocation t(1;9)(q22;q34), salvage therapy with single agent dasatinib was initiated at a dose of 100 mg once daily, in July 2013 (Figure 1A, 6). The *RCSD1-ABL1* t(1;9)(q22;q34) fusion between exon 3 of *RCSD1* and exon 4 of *ABL1* could be validated by RT-PCR and sequencing of the fusion transcript (Figure 1B). A stable disease with partial remission in the BM was achieved (Figure 2A). This was confirmed by a decrease of *RCSD1-ABL1* amplicon in RT-PCR (Figure 2B, lanes 2 and 3). Moreover, inhibition of *RCSD1-ABL1* activity by dasatinib was shown by Western analysis of phosphorylated *ABL1* and its downstream target CRKL (Figure 2C). Treatment with dasatinib was complemented with two cycles of chemotherapy, including clofarabine and cyclophosphamide (Figure 1A, 7). CR 2 was achieved in October 2013, whereas minimal residual disease (MRD) levels still remained positive. In this regard, treatment with blinatumomab was started within the MT103-203 study in January 2014 (Figure 1A, 8), and MRD levels became negative. Flow cytometric analysis revealed no further evidence of any $CD10^+/CD19^+$ BM cells. In March 2014, allo-SCT was performed successfully

(Figure 1A, 9). Since then, there is an ongoing remission that, to date, has lasted 30 months. During this period, the patient has not recieved any antileukemic treatment. Clinical monitoring of the patient is performed via RT-PCR of the *RCSD1-ABL1* fusion transcript, followed by nested PCR of the amplicon, to detect early relapse or MRD (Figure 2B, E). As positive control for clinical monitoring, the plasmid PCR2.1-TOPO_ *RCSD1-ABL1*(626bp) was synthesized encoding *RCSD1-ABL1* amplicon with the fusion site (Figure 2D, E).

For the management of elderly patients with refractory ALL and *ABL1*-activating fusion genes not involving *BCR*, the present case provides several important implications. In addition to this report, ten cases with B-cell precursor ALL and a molecular genetically confirmed *RCSD1-ABL1* gene rearrangement have been reported in literature to date.[2,3,6-12] To our knowledge, this is the first reported case of a patient with relapsed and refractory *RCSD1-ABL1*-positive B-cell ALL in whom salvage therapy with single-agent dasatinib induced a meaningful response and a durable molecular remission, which was consolidated with allo-SCT. TKI-based treatment of patients with RCSD1-ABL1-positive B-cell ALL has been described before. Thus, Perwein *et al.* (2016) described an imatinib-induced long-term remission in a pediatric patient with RCSD1-ABL1-positive B-cell precursor (BCP) ALL. In contrast to our case, imatinib complemented relapse chemotherapy, but its sole antileukemic efficacy could not be proved. Furthermore, no consolidation with allo-SCT was performed.[12] Moreover, Mustjoki *et al.* (2009) presented a 40-year old patient who achieved morphological remission after addition of dasatinib to a modified CVAD regimen and allo-SCT was performed. However, at 12 months from allo-SCT, their patient relapsed.[7] We here show that dasatinib monotherapy seems to be an effective treatment option in *RCSD1-ABL1*-positive cases to increase the number of patients who can proceed to allo-SCT. The single-agent antileukemic activity of dasatinib was further intensified with a clofarabine-cyclophosphamide combination, as this approach induced significant response in adult patients with advanced ALL, even in the presence of adverse prognostic factors like the *BCR-ABL1* rearrangement.[13] The administration of blinatumomab, resulting in negative MRD levels, might also have contributed to this long-term remission. In patients with Ph$^+$ ALL, the MRD status before allo-SCT is a strong predictor of relapse.[14] As described by Kolb *et al.* (1995), patients with Ph$^+$ disease benefit from adoptive immunotherapy.[15] Thus, contrary to Perwein *et al.* (2016), we consider the graft-*versus*-leukemia effect in our patient to be critical for curative therapy. Of note, dasatinib was not continued as maintenance due to concerns about its potential vascular toxicity, since our patient has a medical history of arterial hypertension, hypercholesterolemia, and coronary artery disease with stent implantation in 2009. In particular, the high non-relapse mortality (NRM) of allo-SCT in the group of elderly patients and preexisting comorbidities remains a significant problem. The uncertainty in the management of these patients often withholds them from definitive therapy. In conclusion, the present case demonstrates that monotherapy with TKI, such as dasatinib, is effective in refractory *RCSD1-ABL1* ALL. In addition, our case should encourage clinicians to consider allo-SCT even for elderly patients with uncommon cytogenetic abnormalities and preexisting comorbidities as the only curative treatment option.

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