Mobilization of Autologous Hematopoietic Progenitors and Subsequent Transplantation is a Safe and Feasible Procedure in Chronic Phase Chronic Myelogenous Leukemia patients presenting a Cytogenetic Resistance to Imatinib

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Despite high rates of complete cytogenetic remissions (CCR), imatinib mesylate (IM) induces only few complete molecular responses.[1] Patients resist to IM through different mechanisms of which the onset of BCR-ABL mutations is the most frequent. Increased IM doses can overcome resistance, and, since PBSC collection under IM remains feasible in IM sensitive patients,[2-4] autologous hematopoietic PBSC harvest and transplant might be an attractive strategy to consider. Here, we report the case of an autologous PBSC harvest and subsequent transplantation for chronic phase chronic myelogenous leukemia (CP CML) in cytogenetic relapse and clonal evolution under IM, leading to a second disease response.

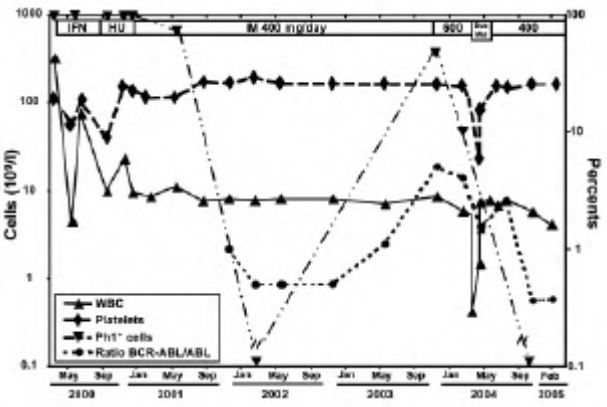

Figure 1. Summary of the clinical, biological, Ph1 chromosomal and molecular responses along the treatment course. IM states for: Imatinib Mesylate, HU for Hydroxy-Urea, WBC for White Blood Cells. Ph1⁺ cells: % of Ph1⁺ metaphases at karyotyping analysis. Molecular response corresponds to RQ-PCR values expressed as BCR-ABL/ABL ratios (%).

Case Report

In May 2000, a 29 year-old male without medical history was referred for abdominal pain and splenomegaly. Blood analysis revealed: WBC: 325 109/l (38% neutrophils, 5% eosinophils, 3% basophils, 1% lymphocytes, 13% promyelocytes, 16% myelocytes, 23% metamyelocytes, 1% myeloblasts, and 10% erythroblasts, hemoglobin: 87 g/L, platelets: 115 109G/l. BM analysis showed harmonious granulocytic and megakaryocytic hyperplasia, with no marrow fibrosis. BM karyotyping analysis was: 45, X,-Y, t(9;22)(q34;q11) [25]. RT-PCR demonstrated a M-Bcr transcript (b3a2). The diagnosis of CP CML was made. Sokal and European scores were high at 1.59 and 1308.8, respectively. No HLA-identical sibling donor was available. Interferon + cytarabine treatment resulted in transient CHR with no cytogenetic response. The patient entered the CSTI113 study[5] in January 2001. IM started at 400 mg/d, CHR was achieved at 1 month, minor cytogenetic response (74% Ph1+) at 6 months, and CCR at 12 months. In January 2004, the patient was in cytogenetic relapse with clonal evolution and Ph1 duplication: 47, X, -Y, +8, t(9;22), +der(22)t(9;22)[15]/46,XY[15]. FISH analysis, apart from Ph1 duplication, revealed no BCR-ABL amplification per se. No detectable ABL mutation was detected by PCR and sequencing. Increasing IM doses to 600 mg/d resulted 3 months later in a MCR (10% Ph1+), with no trisomy 8 detectable, RQ-PCR showed a 4% BCR-ABL/ABL ratio. Because cytogenetic responses after increasing IM doses might not be sustainable,[6,7] in the absence of an HLA histocompatible donor, autologous stem cell transplantation might be a useful strategy disease control. After 7 days of IM washout, the patient received subcutaneous rHuG-CSF (Lenograstim, Chugai Pharma France) 10 µg/kg/d, 5 days. Three aphereses were performed, collecting 16×10^8 MNC/Kg, 100×10^4 CFU-GM/Kg, and 4×10^6 CD34+ cells/Kg. Only 2% of the 500 nuclei analyzed by FISH were positive for BCR-ABL and 2.5% for trisomy 8 in the apheresis product. RQ-PCR was positive with 1.63% BCR-ABL/ABL in apheresis number 1. IM was restarted rapidly and pursued until conditioning with Busulfan + Melphalan. The whole graft was infused on the 27th of May 2004. Aplasia was short with ANC $>0.5\times10^9$/L at D10 and platelets $>20\times10^9$/L at D9. No RBC transfusion and only 2 packs of platelets were necessary. One FUO episode occurred which resolved with standard antibiotics. Patient was discharged at D11 post-transplant. At D30, the patient was in CHR, and IM was restarted (400 mg/d). At 3 months, RQ-PCR was positive (2.5% BCR-ABL/ABL), and at 6 months, FISH analyses for BCR-ABL and Cent 8 (over 300 nuclei) on PB CFCs were negative. RQ-PCR was positive at 0.363% BCR-ABL/ABL. At 9 months, the patient was in sustained CHR with stable 0.43% BCR-ABL/ABL ratio (Figure 1).

Discussion

In some patients, the reason for IM resistance remains obscure, with no detectable BCR-ABL mutation or amplification, or other phamacological explanation. In the absence of allogeneic donor, resistance might be (at least shortly) controlled by increasing doses of IM[6] which can induce cytogenetic responses.[6] However, long-term response might not be obtainable,[6,7] and the impact of high-dose IM on subsequent autologous PBSC transplant in CP is unknown. This strategy has been used in the past to restore normal hematopoiesis in patients in relapse under interferon.[8] We successfully used a G-CSF-based mobilization schedule after a short 7-days IM washout, as recommended by Drummond *et al.*[2] and Hui *et al.*[3] to avoid mobilization failures. Despite a long interval between CML diagnosis and collection (47 months), a long duration of IM treatment before collection (41 months), and 3 months of high-dose IM before collection, enough CD34⁺ cells with comparable yields with what has been reported so far in the literature [Median CD34+ cells collected: 2.1 106 (0.1-6.5) for Ref,[2] 3.7 106 (0.15-8.71) for interrupted IM group for Ref,[3] and <2-7.1 for Ref[4]] to allow safe autologous PBSC transplantation could be cryopreserved. Analysis of the Ph1 status of the PBSC product (FISH and RQ-PCR) was positive at low levels, as described.[3] G-CSF exposure did not induce any BCR-ABL transcript load progression as reported.[2,3,4] No particular side effects were noted after infusion, and hematopoietic recovery was fast and not different from what we described previously for interferon-resistant CML.[9] Once normal PB counts have been restored, IM was restarted, as for interferon post-transplant strategies.[10] At 6 and 9 months post-transplant, low BCR-ABL/ABL ratios, CCR with disappearance of clonal evolution were observed. There are several explanations to the elimination of Ph1+ hematopoiesis: i) exposure to G-

CSF followed by a sequence of IM before transplantation may have induce the proliferation of a fraction of Ph1+ quiescent stem cells[11] and its subsequent destruction by IM, ii) *in vivo* depletion of Ph1+ cells induced by the conditioning regimen, iii) IM treatment post-transplantation. There are a few reports of autologous PBSC transplants performed in CML patients treated with IM, one for a secondary CML occurring after chemotherapy for breast cancer, entering a second blastic phase and undergoing successful autologous PBSC transplant with cells previously harvested in CCR[12] and one for a patient in chronic phase recovering from a severe episode of pancytopenia under IM after reinfusion, without conditioning regimen, of apheresis products harvested at diagnosis. In our case, the imatinib-resistant patient remained in chronic phase (no other acceleration feature than clonal evolution), and increased doses of IM lead to an improvement of the cytogenetic response before transplant. To our knowledge, this is the sole example reported where a primary CML patient, in chronic phase, resistant to IM, harvested in MCR, was transplanted with a minimally contaminated graft (collected during IM treatment) after a conditioning regimen efficient on CML cells,[8] with a long-term re-sensitization of the disease to IM after all. In the context of IM resistance with no detectable BCR-ABL amplification or mutation, steady-state PBSC mobilization in patients achieving cytogenetic improvement with high doses of IM is feasible, and subsequent autologous PBSC transplantation followed by IM seems safe. A sustainable BCR-ABL response can be obtained after such an approach. However this benefit needs to be confirmed on series of CML patients exhibiting IM resistance with no Bcr-Abl mutation.

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