Effective re-induction therapy with dasatinib and clofarabine in relapsed Philadelphia chromosome positive acute lymphoblastic leukemia

Anne Loes van den Boom,[1] H. Berna Beverloo,[2] Vincent H. J. van der Velden,[3] Arjan Lankester,[4] Rob Pieters,[1] C. Michel Zwaan[1]

[1]Department of Pediatric Oncology/Hematology, Sophia Children's Hospital, Erasmus University Medical Center, Rotterdam; [2]Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam; [3]Department of Immunology, Erasmus MC, Erasmus University Medical Center, Rotterdam; [4]Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands

Abstract

This case discusses a 10 year old female patient with a late relapse of Ph-chromosome positive B-cell precursor acute lymphoblastic leukaemias (ALL) who had previously been treated with chemotherapy and allogeneic stem-cell transplantation. Treatment for relapse consisted of single-agent dasatinib, followed by 2 blocks of a combination of dasatinib and clofarabine as consolidation therapy. Using this schedule both morphological and cytogenetic complete remission were obtained. This regimen was well tolerated, and no major toxicity concerns occurred. Subsequently, the patient received a 2nd stem cell transplantation from a matched unrelated donor. Unfortunately, the child died after complete molecular remission at day +104 post-transplantation, due to a disseminated adenoviral infection. We conclude that dasatinib and clofarabine combination therapy was safe and effective in this patient, and should be further explored as a salvage regimen in relapsed/refractory Philadelphia chromosome positive ALL patients.

Introduction

Approximately 2-5% of newly diagnosed acute lymphoblastic leukaemias (ALL) cases in children are Philadelphia chromosome positive (Ph^+) ALL. The Philadelphia chromosome, a fusion of the *ABL* gene on chromosome 9q34 to the *BCR* gene on chromosome 22q11, is a well-known poor prognostic factor in both pediatric and adult ALL.[1] In children, overall survival rates with chemotherapy only are in the 30-50% range.[2,3] The *BCR-ABL* fusion gene results in uncontrolled elevated tyrosine kinase activity.[4]

With the development of the tyrosine kinase inhibitor (TKI) imatinib, which inhibits the down-stream effects of the *BCR-ABL* fusion gene, outcome may improve.[5] Studies in adults may not provide a definite answer for the use of TKIs in children with Ph+ ALL, given the differences in breakpoints in *BCR* and *ABL* between children and adults, which are known to result in differences in kinase transforming activity.[6,7] Pediatric patients usually carry the minor breakpoint cluster region of *BCR* (m-*bcr* or p190), as opposed to adult patients where the breakpoint is usually found in the major breakpoint cluster region (M-*bcr* or p210). Data found in animal models suggest that the minor breakpoint induces leukemia with a greater transforming potential than the major breakpoint.[8] Imatinib activity in newly diagnosed pediatric Ph^+-ALL was recently demonstrated by the Children's Oncology Group (protocol AALL0031).[9] Their study showed a 3-year event free survival rate of 80% in patients receiving continuous imatinib combined with an intensive chemotherapy regimen, and was superior to treatment without imatinib using historical controls. Even though this was a non-randomized study, the results indicate that treatment for children with Ph^+ ALL should probably include a TKI, given that no other studies with chemotherapy alone have ever reached this level of efficacy. Recently, second generation TKIs became available, which are being given in case of imatinib resistance or intolerance due to the greater potency against BCR-ABL, their retained activity in case of imatinib-resistance *ABL* mutations, their ability to inhibit SRC which may be implied in resistance and, their ability to penetrate into the cerebrospinal fluid as has been shown specifically for dasatinib.[10,11] It is known that relapsed/refractory patients with Ph^+-ALL may respond to dasatinib, although generally, these responses are not durable,[12,13] and hence other therapy-elements need to be added. This case report presents a relapsed Ph^+-ALL patient treated by re-induction therapy with single-agent dasatinib, followed by consolidation with clofarabine and dasatinib and subsequent stem-cell transplantation (SCT). We conclude that this treatment schedule may be a successful strategy in relapsed/refractory Ph^+-ALL.

Correspondence: C. Michel Zwaan, Erasmus MC/Sophia Children's Hospital, Dr Molewaterplein 60, 3015 GJ Rotterdam, the Netherlands.
Tel. +31.10703.6691 - Fax: +31.10703.1134
E-mail: c.m.zwaan@erasmusmc.nl

Key words: dasatinib, clofarabine, Philadelphia chromosome positive acute lymphoblastic leukemia, children, relapse.

Contributions: AB wrote the paper and edited the manuscript, HB and VV performed essential diagnostics and experiments, AL and CM contributed clinical samples and patient data and RP revised the manuscript critically.

Conflict of interests: the authors report no potential conflict of interests.

Received for publication: 24 July 2011.
Revision received: 25 August 2012.
Accepted for publication: 30 August 2012.

This work is licensed under a Creative Commons Attribution NonCommercial 3.0 License (CC BY-NC 3.0).

©Copyright A.L. van den Boom et al., 2012
Licensee PAGEPress, Italy
Drugs and Therapy Studies 2012; 2:e13
doi:10.4081/dts.2012.e13

Case Report

A 6-year-old girl was diagnosed with Ph^+-B-cell precursor (BCP) ALL in November 2003. The karyotype showed the Philadelphia chromosome (molecular evaluation showed that it concerned the minor breakpoint) plus additional abnormalities: 52,XX,+X,+2,+4, t(9;22)(q34;q11),+16,+17,+20[10]/46,XX[10]. She was treated according to the high risk arm of the Dutch Childhood Oncology Group protocol ALL-9.[14] First complete remission (CR1) was achieved at the end of induction. However, approximately 2 months after obtaining CR1, 6% blasts were morphologically detected in the bone marrow. Therapy was switched from ALL-9 to the European EsPhALL protocol, which had just opened, and imatinib (300 mg/m^2) was added to the chemotherapy regimen. Morphological complete remission was again achieved, and after intensification, she received an allogeneic haplo-identical SCT. Imatinib (300 mg/m^2) was electively re-introduced on day +28 post-SCT, and was stopped 1 year later.

Three years after the transplantation, a relapse was diagnosed with 77% blasts in the bone marrow and 2% blasts in the peripheral blood. There was no central nervous system involvement, and white blood cell count was

Non-commercial use only

6.8x10^9/L. Minimal residual disease (MRD) analysis was performed using *BCR-ABL* levels with *ABL* as a household gene, as described before (Figure 1).[15,16] MRD results were set to 1.0 (100%) at diagnosis of relapse, and follow-up data were expressed as relative values according to the disease-load at diagnosis of relapse (Table 1).[17] Mutation analysis for *ABL* resistance mutations was carried out in the sample taken at diagnosis of relapse using direct sequencing, but no resistance mutations were detected.

The patient was referred to our center for inclusion in the CA180018 dasatinib study (protocol CA180018; NCT 00306202), and following the screening period which confirmed eligibility she started treatment with dasatinib orally at 80 mg/m^2 QD. No organ toxicity occurred during the first dasatinib course. After 3 weeks a re-evaluation was performed (Table 1). The bone marrow morphology showed a decrease of blasts to approximately 18%; however, both flow-cytometry and cytogenetics showed complete remission and MRD levels were decreased to 0.06. At day 42, six weeks following single-agent dasatinib, the same discrepancy was noted, with morphology showing approximately 20% blasts, and flowcytometry and cytogenetics indicative of complete remission. At this time, MRD levels were increased to 0.12. Because of the uncertainty regarding the achievement of CR, and in-line with the protocol, intra-patient dose-escalation of dasatinib to 100 mg/m^2 QD was initiated. Three weeks after this dose-increase the response was re-evaluated and now results were indicative of complete morphological and cytogenetic remission, although MRD levels increased. Because the protocol only allowed single-agent treatment with dasatinib, and we wanted to offer further treatment with chemotherapy in preparation for a stem-cell transplant, the patient came off-study.

The patient was then further treated with two blocks of clofarabine consisting of 52 mg/m^2 IV once daily, for 5 consecutive days. During clofarabine administration no dasatinib was given, but following each course, dasatinib was re-started two days after stopping clofarabine. Dasatinib was then given for 14 days, at a dose of 100 mg/m^2 QD. Following these two blocks she was in complete morphological and cytogenetic remission. Although MRD levels dropped to 0.013 at day +127 after relapse, she never obtained a major molecular remission (defined as a level of ≤0.001 compared to base-line).[18] During clofarabine and dasatinib administration the patient showed only some mild toxicity concerns and was therefore not hospitalized. At day +155 following relapse she was transplanted again, this time with a 9/10 matched unrelated stem cell donor. A reduced intensity conditioning schedule was used, because of the prior total body irradiation during the first transplant procedure. Conditioning consisted of Treosulphan (3×14 gr/m^2), Melphalan (2×70 mg/m^2) and ATG (total 10 mg/kg). Standard graft versus host disease prophylaxis with ciclosporin A (2 mg/kg IV) and methotrexate (10 mg/m^2 at day +1, +3 and +6) was given after SCT. Unfortunately, she died in complete molecular remission (no *BCR-ABL* transcript was detectable at day +31 post 2nd SCT) of a disseminated adenoviral infection at day + 104 post 2nd SCT. Dasatinib was not given after 2nd SCT. Until her death, our patient showed 100% donor chimerism in blood and bone marrow.

Discussion

Despite a major general improve of outcome in pediatric ALL, the prognosis in Ph$^+$-ALL patients is less favourable.[2,3] So far there has been no comprehensive strategy for relapsed Ph$^+$-ALL.

In this patient, our aim was to use dasatinib for remission induction, followed by clofarabine combined with dasatinib for consolidation, and a 2nd SCT, aiming at cure. After 9 weeks of treatment all results pointed towards a complete morphological and cytogenetic remission. To further reduce MRD-levels pre-SCT, we started sequential therapy with clofarabine and dasatinib. After two courses of this therapy our patient underwent SCT. The patient showed a complete molecular remission following SCT, which was not seen before.

The potential advantages of single-agent dasatinib as re-induction therapy include that it may be well-tolerated, has less organ-toxicity than regular re-induction schedules, and can be taken at home orally. Our patient experienced all of these benefits. A potential disadvantage may be the risk of developing resistance mutations during this phase of single-agent therapy. Our choice for adding clofarabine was based on the safety and activity profile of clofarabine for relapsed ALL, and its subsequent registration both in Europe and the United States.[19] Moreover, the patient had initially suffered from poor disease control on a regular ALL protocol, hence offering her a relapse protocol with similar medication was considered undesirable.

Current studies suggest that a combination of clofarabine with etoposide and cyclophosphamide is tolerable, although clofarabine is given at lower dose in such combination regimens.[20,21] However, at time of treatment, these data were not known yet and therefore we used single-agent clofarabine. In our patient, the clofarabine plus dasatinib combination was safe, and not associated with prolonged neutropenia or severe infections. Particularly, no cardiac, hepatic or skin toxicities were mentioned in this child while receiving clofarabine and dasatinib.

Three other pediatric case reports described the use of dasatinib for relapsed Ph+-ALL after therapy with imatinib.[22-24] Millot *et al.*[22] showed that a 4-year old child with Ph+ ALL in 1st relapse, after imatinib therapy and an allogeneic haplo-identical SCT, achieved a second complete cytologic and molecular response, and underwent a 2nd SCT. Their patient was still in complete molecular remission 18 months after the second SCT.[22] A case report by Ishida *et al.*[23] describes a 15-year old girl with Ph+ ALL with an early relapse after the first SCT. She achieved complete molecular remission with dasatinib at 140 mg/day and 12 months after the second SCT she still remains

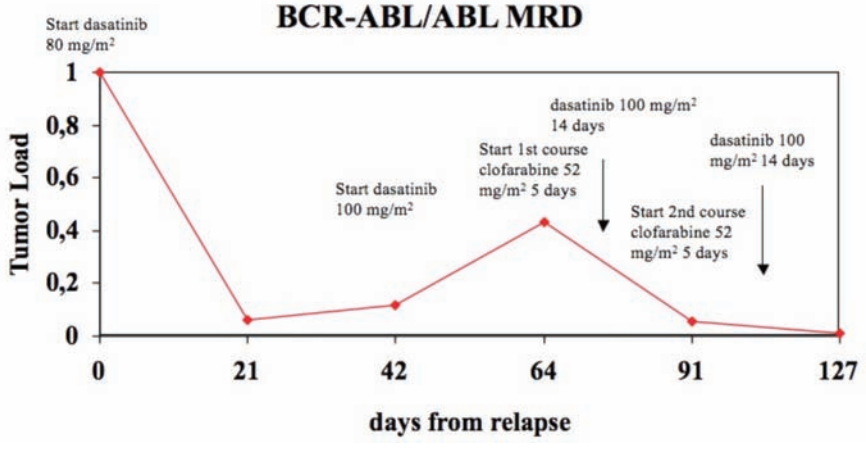

Figure 1. Minimal residual disease status versus treatment. Minimal residual disease was calculated using the BCR-ABL/ABL ratio. Results were normalized to base-line levels.

Non-commercial use only

Table 1. Results of morphology, cytogenetics, minimal residual disease and chimerism from diagnosis till death.

Time point	Morphology;*	Flow-cytometry°	Bone-marrow Cytogenetics#	Fish§	BCR-ABL MRD^	Chimerism	Peripheral blood Chimerism	Therapy given
Initial diagnosis	80%	77%	52,XX,+X,+2,+4,t(9;22)(q34;q11),+16, +17,+20[10]/46,XX[10]			NA	NA	ALL-9 and later EsPhALL protocol
At relapse	76.8%	68%	88~91,XXXX,+add(1)(p21),+i(1)(q10), +2,-3,add(3)(q13),-6,-7,+8,+8[4], t(9;22)(q34;q11)x2,-11[4],-12[5], -14[3],+15[5],-16[3],-17[4],-21[3], +1-3mar[cp8]/46,XX[13] (62%)	80%	1.0	NA	NA	Start dasatinib: 80 mg/m^2
Day +21	18%	1%	46,XX[22]	13%	0.06	NA	NA	
Day +42	20%	3%	46,XX[21]	33%	0.12	NA	NA	Dose-escalation of dasatinib: 100 mg/m^2
Day +64	0.4%	0.5%	46,XX[20]	7%	0.43	NA	NA	Start 1st block of clofarabine: 52 mg/m^2
Day +91	3.3%	2%	46,XX[20]	5.5%	0.058	NA	NA	Start 2nd block of clofarabine: 52 mg/m^2
Day +127	2%	0.4%	46,XX[25]	negative	0.013	NA	NA	
Day + 155 (SCT)	ND	ND	ND	ND	ND	NA	NA	
Day +186	<1%	ND	ND	ND	negative	100%	100%	
Day +259 (death)	ND	ND	ND	ND	ND	100%	100%	

BM, bone marrow; MRD, minimal residual disease; NA, not applicable; ND, not done. *Morphology (for bone marrow) was done using standard techniques. °Flow-cytometry (for bone marrow) was done using standard techniques as described previously.[17] #Cytogenetics (for bone marrow) was done using standard techniques. §FISH was used with probes specific for BCR/ABL using the LSI BCR/ABL ES probeset (Abott) since the patient was known to have the minor breakpoint. ^MRD was calculated using the BCR-ABL/ABL ratio. Results were normalized to base-line levels.[17]

in molecular remission. The use of dasatinib in this case was associated with myalgia, nausea, opportunistic infection and gastrointestinal bleeding which were not attributed to the SCT.[23] De Castro *et al.*[24] reported about the use of dasatinib in a multiple relapsed Ph+ ALL patient with a history of an allogenic haploidentical SCT. Dasatinib was used here in combination with other agents, such as etoposide, vincristine, prednisone and asparaginase. After this treatment, the patient achieved a bone marrow remission and could undergo a second SCT with the same donor as the first time. Unfortunately, two months after the second SCT the patient had a fourth bone marrow relapse and received palliative care. This child died four months after the second SCT.[24]

Conclusions

In conclusion, dasatinib could be considered as a useful and tolerable drug for remission-induction in heavily pre-treated Ph$^+$-ALL children. Following remission induction, clofarabine and sequential dasatinib could be given for consolidation therapy. Unfortunately our patient died in complete molecular CR from an infectious cause. Previous studies showed that adenovirus is a significant cause of morbidity in pediatric allogeneic SCT.[25,26] It can however not be included that clofarabine, which is known to be very immune-suppressive, also contributed to this. In this case report, we provide evidence that the chosen re-induction regimen was tolerable, and hence its use should be explored further in children with relapsed/refractory Ph$^+$-ALL who are in good physical condition and able to undergo a 2nd round of intensive therapy.

References

1. Radich JP. Philadelphia chromosome-positive acute lymphocytic leukemia. Hematol Oncol Clin North Am 2001;15:21-36.
2. Arico M, Valsecchi MG, Camitta B, et al. Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med 2000; 342:998-1006.
3. Arico M, Schrappe M, Hunger SP, et al. Clinical outcome of children with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia treated between 1995 and 2005. J Clin Oncol 2010; 28:4755-61.
4. Kurzrock R, Kantarjian HM, Druker BJ, Talpaz M. Philadelphia chromosome-positive leukemias: from basic mechanisms to

Non-commercial use only

molecular therapeutics. Ann Intern Med 2003;138:819-30.
5. Buchdunger E, Zimmermann J, Mett H, et al. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res 1996;56:100-4.
6. De Klein A, Hagemeijer A, Bartram CR, et al. Bcr rearrangement and translocation of the c-abl oncogene in Philadelphia positive acute lymphoblastic leukemia. Blood 1986;68:1369-75.
7. Suryanarayan K, Hunger SP, Kohler S, et al. Consistent involvement of the bcr gene by 9;22 breakpoints in pediatric acute leukemias. Blood 1991;77:324-30.
8. Voncken JW, Kaartinen V, Pattengale PK, et al. BCR/ABL P210 and P190 cause distinct leukemia in transgenic mice. Blood 1995;86:4603-11.
9. Schultz KR, Bowman WP, Aledo A, et al. Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. J Clin Oncol 2009;27:5175-81.
10. Shah NP, Tran C, Lee FY, et al. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science 2006;305:399-401.
11. Porkka K, Koskenvesa P, Lundan T, et al. Dasatinib crosses the blood-brain barrier and is an efficient therapy for central nervous system Philadelphia chromosome-positive leukemia. Blood 2008;112:1005-12.
12. Ottmann O, Dombret H, Martinelli G, et al. Dasatinib induces rapid hematologic and cytogenetic responses in adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia with resistance or intolerance to imatinib: interim results of a phase 2 study. Blood 2007; 110:2309-15.
13. Zwaan CM, Rizzari C, van der Velden, et al. Dasatinib in children and adolescents with relapsed or refractory leukemia: interim results of the CA180-018 phase I study from the ITCC consortium. Blood ASH Annual Meeting Abstracts 2008;112 (Abstract 3241).
14. Veerman AJ, Kamps WA, van den Berg H, et al. Dexamethasone-based therapy for childhood acute lymphoblastic leukaemia: results of the prospective Dutch Childhood Oncology Group (DCOG) protocol ALL-9 (1997-2004). Lancet Oncol 2009;10:957-66.
15. Beillard E, Pallisgaard N, van der Velden VH, et al. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using real-time quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) - a Europe against cancer program. Leukemia 2003;17:2474-86.
16. Gabert J, Beillard E, van der Velden VH, et al. Standardization and quality control studies of real-time quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer program. Leukemia 2003; 17:2318-57.
17. van Lochem EG, van der Velden VH, Wind HK, et al. Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: reference patterns for age-related changes and disease-induced shifts. Cytometry B Clin Cytom 2004;60:1-13.
18. Deininger MW. Milestones and monitoring in patients with CML treated with imatinib. Hematol Am Soc Hematol Educ Program 2008:419-426.
19. Jeha S, Gandhi V, Chan KW, et al. Clofarabine, a novel nucleoside analog, is active in pediatric patients with advanced leukemia. Blood 2004;103:784-9.
20. Vitale A, Grammatico S, Capria S, et al. Advanced Philadelphia chromosome positive acute lymphoblastic leukemia patients relapsed after treatment with tyrosine-kinase inhibitors: successful response to clofarabine and cyclophosphamide. Haematologica 2009;94:1471-3.
21. Breccia M, Capria S, Iori AP, et al. Clofarabine-based regimen as useful bridge therapy for allogeneic transplantation in myeloid blast crisis of Philadelphia-positive chronic myeloid leukemia resistant to imatinib and dasatinib. Acta Haematol 2010;124:150-2.
22. Millot F, Cividin M, Brizard F, et al. Successful second allogeneic stem cell transplantation in second remission induced by dasatinib in a child with Philadelphia chromosome positive acute lymphoblastic leukemia. Pediatr Blood Cancer 2009;52:891-2.
23. Ishida Y, Terasako K, Oshima K, et al. Dasatinib followed by second allogeneic hematopoietic stem cell transplantation for relapse of Philadelphia chromosome-positive acute lymphoblastic leukemia after the first transplantation. Int J Hematol 2010;92:542-6.
24. de Castro CG Jr, Gregianin LJ, Meneses CF, Brunetto AL. Dasatinib after allogeneic stem cell transplantation in a child with Philadelphia chromosome positive acute lymphoblastic leukemia. Pediatr Blood Cancer 2009;53:1161.
25. Flomenberg P, Babbitt J, Drobyski WR, et al. Increasing incidence of adenovirus disease in bone marrow transplant recipients. J Infect Dis 1994;169:775-81.
26. Ephros M, Friedman BC, Elhasid R, et al. Incidence and clinical manifestations of adenoviral infection among children undergoing allogeneic stem cell transplantation. Isr Med Assoc J 2009;11:744-8.

Non-commercial use only