Published in final edited form as:

Br J Haematol. 2012 May ; 157(4): 507–510. doi:10.1111/j.1365-2141.2012.09039.x.

Imatinib resistant *BCR-ABL1* mutations at relapse in children with Ph+ALL: a Children's Oncology Group (COG) study

Bill H Chang¹, Stephanie G Willis², Linda Stork¹, Stephen P Hunger³, William L Carroll⁴, Bruce M Camitta⁵, Naomi J Winick⁶, Brian J Druker^{2,8}, and Kirk R Schultz⁷

¹Division of Pediatric Hematology and Oncology, Department of Pediatrics and OHSU Knight Cancer Institute, Oregon Health & Science University, Portland Oregon

²Division of Hematology and Medical Oncology and OHSU Knight Cancer Institute, Oregon Health & Science University, Portland, Oregon

³Division of Pediatric Hematology, Oncology, BMT, Children's Hospital Colorado, University of Colorado School of Medicine, Aurora, Colorado, USA

⁴NYU Cancer Institute, New York University Medical Center, New York, New York, USA

⁵Midwest Center for Cancer and Blood Disorders, Children's Hospital and the Medical College of Wisconsin, Milwaukee, Wisconsin, USA

⁶Pediatric Hematology/Oncology, University of Texas Southwestern Medical Center, Dallas, Texas, USA

⁷Pediatric Hematology/Oncology/BMT University of British Columbia and BC Children's Hospital, Vancouver, British Columbia, Canada

⁸Investigator, Howard Hughes Medical Center

Keywords

Leukemia; *BCR-ABL1*; Mutations; Childhood

Dear Editor

Philadelphia chromosome positive acute lymphoblastic leukemia (Ph⁺ALL) occurs in 2-5% of pediatric ALL and is historically associated with a poor prognosis. Although 80-90% of children achieve remission, their event-free-survival (EFS) with conventional chemotherapy prior to tyrosine kinase inhibitors (TKI) was poor with a 32% 7-year event-free survival rate

Corresponding Author: Bill H. Chang, MD, PhD, Mailcode: CDRCP, 3181 SW Sam Jackson Park Rd, Portland, Oregon, changb@ohsu.edu, Tel.: 503-494-1543, Fax.: 503-494-0714.

Contributions:

BHC, SGW, LS, SPH, BJD, and KRS designed the research and analyzed the data. BHC, SGW, LS, WLC, BMC, NJW, SPH, BJD, and KRS wrote the manuscript.

Conflict of Interest:

BHC, SGW, LS, WLC, BMC, NJW and KRS have no competing financial interests. SPH is a member of the Bristol Myers Squibb Dasatinib Pediatric Advisory Committee (without compensation). His children own stock in Bristol Myers Squibb. B.J.D. has financial interest in MolecularMD and receives clinical trial funding from Novartis and Bristol Myers Squibb.

(Arico, *et al* 2010). The addition of imatinib as monotherapy appeared promising in initial treatment of adults with Ph⁺ALL, despite a high rate of relapse (Druker, *et al* 2001). Many relapsed adults on imatinib monotherapy were found to have a resistant mutation within the kinase domain of *BCR-ABL1* (Jones, *et al* 2008). Other studies have shown that TKI's like imatinib or dasatinib as monotherapy can select for TKI resistant clones which may then be overcome by the addition of cytotoxic chemotherapy in the mouse model (Boulos, *et al* 2011).

The Children's Oncology Group (COG) clinical trial, AALL0031, used imatinib (340mg/m²/day) in conjunction with intensive chemotherapy to treat children and adolescents with Ph⁺ALL (Schultz, *et al* 2009). This dosage is equivalent to approximately 600mg/day in adults and was well tolerated with minimal additional side effects as compared to the identical chemotherapy arm without imatinib. AALL0031 differed from adult protocols in several aspects: use of drug combinations not common in adult protocols, intensive dosing of imatinib that was given continuously for the majority of 2.5 years and no continuation of TKI after completion of therapy. Three-year EFS on this treatment was 84% (more than double those of patients treated in the pre-imatinib era). Thus far, it remains unknown whether patients that relapse following this treatment approach have recurred due to development of imatinib resistance.

A two year-old male with Ph⁺ALL and initial white blood cell count of 117×10^9 cells/L was initially treated with standard four-drug induction with vincristine, asparaginase, doxorubicin, and prednisone. At presentation he showed no evidence of extramedullary disease. He achieved complete morphologic and cytogenetic remission at the end of induction. He then received post-induction therapy according to COG AALL0031 cohort 5 (not on study). His therapy included the intensive systemic regimen with CNS-directed therapy without cranial radiation. Twenty-four months into treatment he presented with headaches and mental status changes caused by a CNS relapse. *BCR-ABL1* sequence analysis of his cerebrospinal fluid (CSF) blasts identified a guanine substitution for adenine producing the missense mutation methionine 244 to valine (M244V) (Figure 1a). Concomitant bone marrow aspiration showed no leukemia by morphology, flow cytometry or by fluorescent in situ hybridization. However, sequence analysis of the marrow sample identified the same mutation found in his CSF. *BCR-Abl* sequencing of the bone marrow specimen from initial diagnosis identified no mutation (Figure 1a).

A biologic correlate study to AALL0031 was developed to determine whether or not *BCR-ABL1* kinase domain mutations were present in medullary relapse samples from Ph⁺ALL patients. COG AALL0031 enrolled 93 patients with Ph⁺ALL aged 1 – 21 years from 2002 to 2006 (Schultz, *et al* 2009). From this study, nine relapsed bone marrow samples were available for sequence analysis (Table 1). Eight of the 9 samples from imatinib-treated patients showed no *BCR-ABL1* kinase domain mutation (Table 1). One sample from a patient who relapsed 15 months after diagnosis, carried the histidine 396 to proline (H396P) mutation (Figure 1b). A bone marrow sample from initial diagnosis of this child identified no mutation (Figure 1b).

These results further validate that *BCR-ABL1* kinase domain mutations can occur after treatment of Ph⁺ALL with imatinib and intensive multiple chemotherapeutic agents. From these ten samples we identified two resistant mutations from patients who received imatinib and combination chemotherapy for more than one year. This mutation rate appears to be less than previously published in adults treated with imatinib monotherapy (Jones, *et al* 2008) (15 of 17 relapsed patients) or with hyperCVAD combination therapy (Ravandi, *et al* 2010) where mutations were observed in 3 of 5 relapsed patients.

Neither mutation was detected in samples obtained at diagnosis suggesting that the vast majority of the leukemic cells did not have the mutation. This does not preclude the concept of a low level of mutations at diagnosis as previously shown (Pfeifer, *et al* 2007). M244V and H396 mutations have been shown to be more resistant to imatinib but both have been shown to be sensitive to second generation TKI's such as nilotinib and dasatinib (O'Hare, *et al* 2005). Treatment with dasatinib has been shown to overcome H396R resistance in CML (Talpaz, *et al* 2006).

Our results are the first to describe *BCR-ABL1* kinase domain mutations in pediatric patients with Ph⁺ALL treated with intensive chemotherapy and imatinib. We are also the first to report an imatinib resistant *BCR-ABL1* kinase mutation from a CNS recurrence in a pediatric patient. It has been previously shown that imatinib has low penetrance into the CNS, which implies that selective pressure occurred systemically followed by expansion in the sanctuary of the CNS.

The two mutations we identified are predicted to be sensitive to second generation TKIs, suggesting that these TKIs may be effective reinduction therapy for relapse following treatment of Ph⁺ALL with chemotherapy and imatinib. Importantly, dasatinib penetrates the CNS, a property that may help to decrease the risk of CNS recurrence in Ph⁺ALL. These next generation TKI's may further decrease relapse rates when used for initial therapy of Ph⁺ALL. Many patients who experience a relapse with combination chemotherapy and a TKI do not appear to carry a TKI resistant mutation, suggesting that other BCR-Abl independent pathways play critical roles in leukemia cell survival. Other tyrosine kinases such as HCK, FGR, and LYN are essential for Ph⁺ALL transformation (Hu, *et al* 2004). Therefore, less selective inhibitors like dasatinib may play an important role in salvage therapy for these patients. The current and planned COG Ph⁺ALL trials combine dasatinib rather than imatinib with intensive chemotherapy. Future studies will address whether dasatinib resistant BCR-Abl mutants develop in patients who relapse on these studies.

Acknowledgments

COG samples were obtained through the ALL Cell Bank (#2004-04) with local Institutional Review Board approval. Portions of this research was funded by the National Childhood Cancer Foundation (NCCF Laura and Greg Norman Research Fellowship); Children's Oncology Group grants CA098543 (Chair's Award), U10 CA98413 supporting the COG Statistical Center, and U24 CA114766 supporting Human Specimen Banking in NCI Supported Cancer Trials. B.H.C. is supported in part by the Oregon Child Health Research Center (National Institute of Child Health and Development K12) and the St. Baldrick's Foundation. SPH is the Ergen Family Chair in Pediatric Cancer.

References

- Arico M, Schrappe M, Hunger SP, Carroll WL, Conter V, Galimberti S, Manabe A, Saha V, Baruchel A, Vettenranta K, Horibe K, Benoit Y, Pieters R, Escherich G, Silverman LB, Pui CH, Valsecchi MG. Clinical outcome of children with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia treated between 1995 and 2005. *J Clin Oncol*. 2010; 28:4755–4761. [PubMed: 20876426]
- Boulos N, Mulder HL, Calabrese CR, Morrison JB, Reh JE, Relling MV, Sherr CJ, Williams RT. Chemotherapeutic agents circumvent emergence of dasatinib-resistant BCR-ABL kinase mutations in a precise mouse model of Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood*. 2011; 117:3585–3595. [PubMed: 21263154]
- Druker B, Sawyers C, Kantarjian H, Resta D, Reese S, Ford J, Capdeville R, Talpaz M. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *The New England Journal of Medicine*. 2001; 344:1038–1042. [PubMed: 11287973]
- Hu Y, Liu Y, Pelletier S. Requirement of Src kinases Lyn, Hck, and Fgr for BCR-ABL 1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. *Nat Genet*. 2004; 36:453–461. [PubMed: 15098032]
- Jones D, Thomas D, Yin CC, O'Brien S, Cortes JE, Jabbour E, Breeden M, Giles FJ, Zhao W, Kantarjian HM. Kinase domain point mutations in Philadelphia chromosome-positive acute lymphoblastic leukemia emerge after therapy with BCR-ABL kinase inhibitors. *Cancer*. 2008; 113:985–994. [PubMed: 18615627]
- O'Hare T, Walters D, Stoffregen E, Taipng J, Manley P, Mestan J, Cowan-Jacob S, Lee F, Heinrich M, Deininger M, Druker B. In vitro Activity of Bcr-Abl Inhibitors AMN107 and BMS-354825 against Clinically Relevant Imatinib-Resistant Abl Kinase Domain Mutants. *Cancer Res*. 2005; 65:4500–4505. [PubMed: 15930265]
- Pfeifer H, Wassmann B, Pavlova A, Wunderle L, Oldenburg J, Binckebanck A, Lange T, Hochhaus A, Wystub S, Bruck P, Hoelzer D, Ottmann OG. Kinase domain mutations of BCR-ABL frequently precede imatinib-based therapy and give rise to relapse in patients with de novo Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). *Blood*. 2007; 110:727–734. [PubMed: 17405907]
- Ravandi F, O'Brien S, Thomas D, Faderl S, Jones D, Garriss R, Dara S, Jorgensen J, Kebriaei P, Champlin R, Borthakur G, Burger J, Ferrajoli A, Garcia-Manero G, Wierda W, Cortes J, Kantarjian H. First report of phase 2 study of dasatinib with hyper-CVAD for the frontline treatment of patients with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia. *Blood*. 2010; 116:2070–2077. [PubMed: 20466853]
- Schultz KR, Bowman WP, Aledo A, Slayton WB, Sather H, Devidas M, Wang C, Davies SM, Gaynon PS, Trigg M, Rutledge R, Burden L, Jorstad D, Carroll A, Heerema NA, Winick N, Borowitz MJ, Hunger SP, Carroll WL, Camitta B. Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. *J Clin Oncol*. 2009; 27:5175–5181. [PubMed: 19805687]
- Talpaz M, Shah N, Kantarjian H, Donato N, Nicoll J, Paquette R, Cortes J, O'Brien S, Nicaise C, Bleickardt E, Blackwood-Chirchir M, Lyer V, Chen T, Huang F, Decillis A, Sawyers C. Dasatinib in Imatinib-Resistant Philadelphia Chromosome-Positive Leukemias. *N Engl J Med*. 2006; 354:2531–2541. [PubMed: 16775234]

Figure 1. Sequence chromatogram of case

a. Sequence results from the presented case at diagnosis and relapse. Briefly, patient total RNA was isolated from mononuclear cells and cDNA was synthesized with random hexamers using Superscript III (Invitrogen, Grand Island, NY, USA). Patient cDNA (1- μ l) was PCR amplified using B2A forward (TTCAGAAGCTTCTCCCTGACAT) and *ABL1* 4317 reverse (AGC TCT CCT GGA GGT CCT C) in a 20- μ l reaction (Table 1). PCR product (1- μ l) was used as template in the second round (nested) 50- μ l reaction with *BCR* F4 forward (ACAGCATTCCGCTGACCATCAATA) and *ABL1* 4307 reverse (GAGGTCCTCGTCTTGGTGG) primers. All PCR reactions were performed with Accuprime TAQ, (Invitrogen) and 20 pmol of each primer. Resulting PCR products were sequenced with 2 forward and 2 reverse sequencing primers. Sequences were aligned with Sequencher (Gene Codes, Ann Arbor, MI, USA) sequence analysis program. b. Sequence results from case #9 from bone marrow samples from diagnosis and relapse.

Table I

Patient Samples

#	time to relapse [*]	imatinib exposure	<i>BCR-ABL1</i>
Case ^{\$}	24 months	>1 yr	M244V
1	26 months	<1 yr (BMT) [†]	WT
2	10 months	<1 yr	WT
3	refractory	<1 yr	WT
4	12 months	<1 yr	WT
5	23 months	<1 yr	WT
6	19 months	<1 yr (BMT) [†]	WT
7	39 months	>1 yr	WT
8	45 months	>1 yr	WT
9	15 months	>1 yr	H396P

^{*} As defined from initiation of treatment

^{\$} Not on study, treated according to AALL0031 cohort 5

[†] patients who underwent BMT were scheduled to receive 6 months of imatinib after engraftment.