Acknowledgments: we thank Linda Wellik, David Lewallen, Franklyn Sim and Deb Strauss for assistance and Judith S. Sebolt-Leopold (Pfizer Global Research & Development, Ann Arbor, MI, USA) for providing the CI-1040.
DPS is currently a Mayo Foundation Research Scholar at the Weatherall Institute of Molecular Medicine, University of Oxford, Headington, Oxford, United Kingdom.

Key words: MAPK, MEK inhibitors.

Funding: this study was supported in part by NCI Grant K23 CA96780 (to R.A.M.) and a grant from the Jack Taylor Family Foundation.

Correspondence: Scott H. Kaufmann, MD, PhD, Division of Oncology Research, Guggenheim 1342 C, Mayo Clinic 200 First St., S.W., Rochester, MN, 55905 USA. Phone: international +1.507.2848950. Fax: international +1.507.2843906. E-mail: kaufmann.scott@mayo.edu

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received June 10, 2003; accepted August 4, 2003.

References

1. Towatari M, Iida H, Tanimoto M, Iwata H, Hamaguchi M, Saito H. Constitutive activation of mitogen-activated protein kinase pathway in acute leukemia cells. Leukemia 1997;11:479-84.
2. Kim SC, Hahn JS, Min YH, Yoo NC, Ko YW, Lee WJ. Constitutive activation of extracellular signal-regulated kinase in human acute leukemias: combined role of activation of MEK, hyperexpression of extracellular signal-regulated kinase, and downregulation of a phosphatase, PAC1. Blood 1999;93:3893-9.
3. Milella M, Kornblau SM, Estrov Z, Carter BZ, Lapillonne H, Harris D, et al. Therapeutic targeting of the MEK/MAPK signal transduction module in acute myeloid leukemia. J Clin Invest 2001;108:851-9.
4. Sebolt-Leopold JS, Dudley DT, Herrera R, Van Becelaere K, Wiland A, Gowan RC, et al. Blockade of the MAP kinase pathway suppresses growth of colon tumors in vivo. Nat Med 1999;5:810-6.
5. Lo Russo PM, Adjei AA, Meyer MB, Wozniak A, Gadgeel SM, Hanson LJ, et al. A phase 1 clinical and pharmacokinetic evaluation of the oral MEK inhibitor, CI-1040, administered for 21 consecutive days, repeated every 4 weeks in patients with advanced cancer. Proc Am Soc Clin Oncol 2002;21:81a[abstract].
6. Eaves C, Lambie K. In: Atlas of human hematopoietic colonies. StemCell Technologies Inc., Vancouver. 1995.
7. Claessens YE, Bouscary D, Dupont JM, Picard F, Melle J, Gisselbrecht S, et al. In vitro proliferation and differentiation of erythroid progenitors from patients with myelodysplastic syndromes: evidence for Fas-dependent apoptosis. Blood 2002; 99:1594-601.
8. Theodosiou A, Ashworth A. MAP kinase phosphatases. Genome Biol 2002;3:reviews3009.
9. Meighan-Mantha RL, Wellstein A, Riegel AT. Differential regulation of extracellular signal-regulated kinase 1 and 2 activity during 12-O-tetradecanoylphorbol 13-acetate induced differentiation of HL-60 cells. Exp Cell Res 1997;234:321-8.

Methodologic and biological variability of quantitative real-time polymerase chain reaction analysis of Bcr-Abl expression in Philadelphia chromosome-positive acute lymphoblastic leukemia

We examined to what extent technical and biological factors may affect the validity of Bcr-Abl polymerase chain reaction quantification in Philadelphia chromosome-positive acute lymphocytic leukemia and found that technical variance is the predominant limitation of the method and was not exceeded by biological variance. Interestingly, the number of $p210^{Bcr-Abl}$ (b2a2 or b3a2) transcripts per blast was more then 10-fold higher than the number of $p190^{Bcr-Abl}$ copies per blast.

haematologica 2003; 88:1074-1076
(*http://www.haematologica.org/2003_09/1074.htm*)

Bcr-Abl RNA transcripts[1] are suitable molecular markers for minimal residual disease (MRD) analysis in Philadelphia-positive acute lymphoblastic leukemia (Ph^+ALL) and may be used to guide therapy. However, MRD analysis by real time polymerase chain reaction (RT-PCR) may be affected by intra- and inter-assay variability as well as potentially by changes in Bcr-Abl copies per blast and differences between bone marrow (BM) and peripheral blood (PB).

PB and BM samples were obtained from 56 patients with relapsed or refractory Ph^+ ALL who were treated in phase II studies with imatinib (CSTI571 109 and CSTI571 114) as described previously.[2,3] Total RNA was extracted from mononuclear cells (Ambion) and cDNA synthesized from 1-5 μg RNA (RNA at 70°C for 10 min, 4°C until addition of core mix including 500 U SuperScript II RNase H- RT (Invitrogen), 50 U RNAguard (Amersham), 6.25 μM pDN_6 (Amersham), 1 mM dNTP and 10 mM DTT in 50 μL; then thermocycled at 25°C for 10 min, 42°C for 45 min and 99°C for 3 min) before undergoing column purification (Qiagen). Ten-fold plasmid standard dilutions with 10 to 10^6 copy numbers of Bcr-Abl and GAPDH (housekeeping gene) were analyzed simultaneously with patients' samples in duplicate reactions employing an ABI PRISM 7700 (Applied Biosystems) and Taqman PCR under standard conditions already described.[3] Bcr-Abl levels were calculated as the logarithm of the ratio between Bcr-Abl and GAPDH copies and, if appropriate, normalized to 100% leukemic blasts by cytology and flow cytometry at successive relapses. Statistical analysis included t-tests, ANOVA tests and F tests. Ranges including 90% of Gaussian distribution of intra- and inter-assay variability were calculated by the formula $\times$ +/- $t_{p,n-1}$. s ($\times$: mean; $t_{p,n-1}$: specific factor for n data points from table for t-values; s: standard deviation).[4]

The method permitted detection of 10^{-5} SD1/Nalm6 cells, 10^{-6} K562/Nalm6 and 10^{-4} BV173/Nalm6 cells. Leukemic blasts from patients (n=3, respectively) expressing e1a2, b2a2 or b3a2 were titrated into normal PB and BM cells. The average sensitivity of detection was 4×10^{-5}, 8×10^{-6} and 2×10^{-6} in BM samples and 6×10^{-6}, 7×10^{-7} and 4×10^{-6} in PB samples.

Intra-assay variability of SD1 cells (diluted 1:500) was ±0.20 log for RNA extraction plus cDNA synthesis plus PCR (n=5), ±0.17 log for separate cDNA synthesis plus PCR and ± 0.11 log for PCR only (differences not significant).

The inter-assay variability of patients' samples ranged from ±0.3 log at high MRD (log Bcr-Abl/GAPDH: -1.5) to ± 0.9 log at low levels (-5) and ± 1.4 log at a MRD level at the detection limit (-7) (Figure 1).

The correlation coefficient of paired PB and BM levels of e1a2, b2a2, or b3a2 was high (0.9, 0.85 and 0.93, respectively). Levels of Bcr-Abl were higher in BM than in PB: by 0.8 log for e1a2 ($p<0.001$), 0.3 log for b2a2 ($p=0.20$) and 0.7 log for b3a2 ($p=0.004$). Since Bcr-Abl levels normalized to 100%

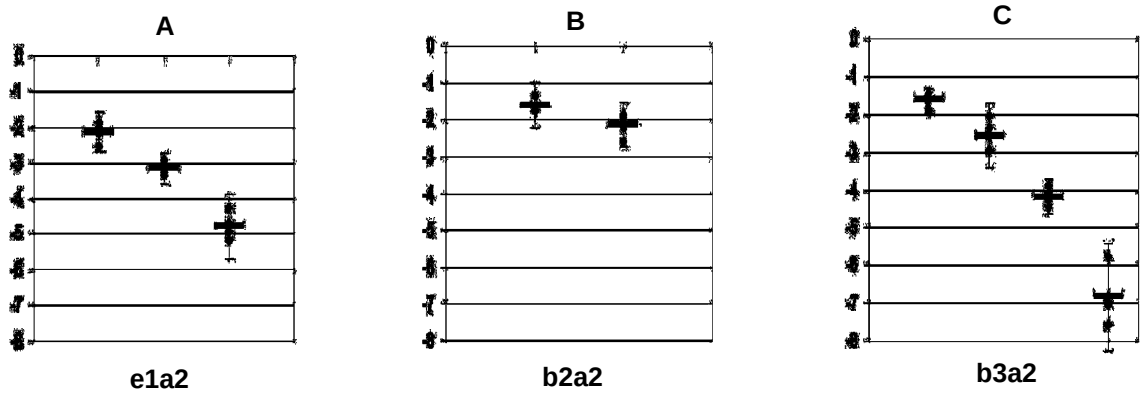

Figure 1. Technical inter-assay variability of Bcr-Abl quantification in aliquots (n=5) of one patient's sample is depicted for transcripts e1a2 (a), b2a2 (b) and b3a2 (c) at various MRD levels. Technical variability reflects inter-assay variability of RNA extraction, cDNA synthesis and Taqman PCR for Bcr-Abl and GAPDH. Logarithmic values of Bcr-Abl/GAPDH ratios are shown with means and calculated range including 90% of the Gaussian distribution. A change of the Bcr-Abl level to a value outside this range in a subsequent sample would indicate an actual biological increase or decrease of Bcr-Abl expression (5% probability of error).

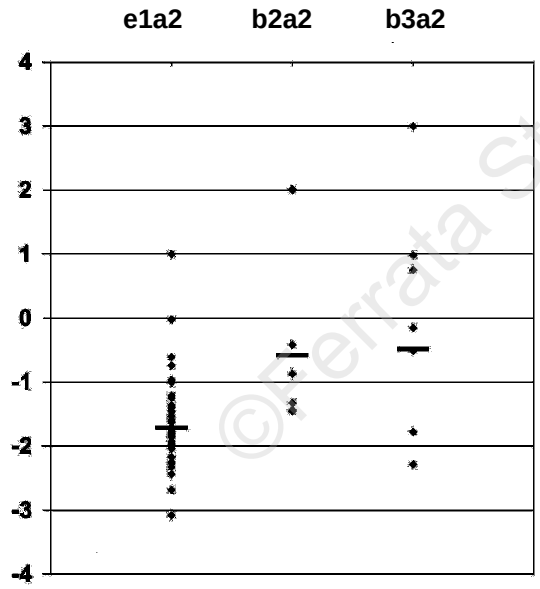

Figure 2. Logarithmic Bcr-Abl/GAPDH values normalized to 100% leukemic blasts in patients with $p210^{Bcr-Abl}$ (b2a2 or b3a2) were 1.1 log higher than those in patients with $p190^{Bcr-Abl}$ (e1a2) (p=0.0006). Individual statistical subtesting revealed a difference between both e1a2 and b2a2 (p=0.014) as well as betwenn e1a2 and b3a2 (p=0.008), but not between b2a2 and b3a2 (p=0.98). Within a group of patients with the same fusion transcript, inter-individual variance did not exceed intra-individual variance significantly.

blasts did not differ significantly between BM (–1.31) and PB (–1.10), differences in Bcr-Abl levels between PB and BM in individuals appear to reflect different proportions of leukemic blast cells.

The numbers of Bcr-Abl transcripts per blast in 25 sequential BM and 8 sequential PB sample pairs did not change significantly during evolution of Ph^+ ALL. Furthermore, intra-individual variance did not differ from technical variance.

The mean number of p210 copies per blast was more than 1.1 log higher than that for p190 (p=0.0006) (Figure 2).

We found a sensitivity between 10^{-5} and 10^{-6} with patients' blasts, which is similar to the 10^{-5} sensitivity in other reports.[5] The technical variability increased with decreasing MRD levels. At high ($>10^{-4}$), low ($<10^{-4}$ to $>10^{-6}$) and very low (<10-6) Bcr-Abl/GAPDH levels, changes by more than 0.6 log, 1.0 log and 1.5 log, respectively, are considered significant. However, clinical therapeutic decisions should be based on more than one sample.

Our data confirm previous reports that BM samples offer a greater sensitivity in B-lineage ALL monitoring.[5-8] However, the degree of divergence between PB and BM levels may vary according to treatment phase.[3]

The fact that we found higher copy numbers of $p210^{Bcr-Abl}$ per blast than $p190^{Bcr-Abl}$, confirming observations of others who found a 6- to 7- fold difference,[5] suggests that specific prognostic criteria may be required for these subgroups.

Urban J. Scheuring, Heike Pfeifer, Barbara Wassmann, Patrick Brück, Dieter Hoelzer, Oliver G. Ottmann

Department of Hematology and Oncology, Johann Wolfgang Goethe University Hospital, Frankfurt, Germany

Funding: the present study was supported by a grant from the Adolf Messer Stiftung, Germany; Competence Network "Acute Leukemias" Grant No. 01G19971.

Key words: acute lymphoblastic leukemia (ALL), Philadelphia chromosome, Bcr-Abl tyrosine kinase, real-time PCR, minimal residual disease (MRD).

Correspondence: Urban J. Scheuring, MD, Medizinische Klinik III, Abteilung für Hämatologie und Onkologie der Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany. Phone: international +49.69.630183044. Fax: international + 49.69.63016131. E-mail: scheuring@em.uni-frankfurt.de

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Francesco Lo Coco, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received June 30, 2003; accepted July 15, 2003.

References

1. van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia 1999; 13:1901-28.
2. Ottmann OG, Druker BJ, Sawyers CL, Goldman JM, Reiffers J, Silver RT, et al. A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. Blood 2002;100:1965-71.
3. Scheuring UJ, Pfeifer H, Wassmann B, Bruck P, Atta J, Petershofen EK, et al. Early minimal residual disease (MRD) analysis during treatment of Philadelphia chromosome/Bcr-Abl-positive acute lymphoblastic leukemia with the Abl-tyrosine kinase inhibitor imatinib (STI571). Blood 2003;101:85-90.
4. Fisher RA, Yates F. Statistical tables for biological, agricultural and medical research. Edinburgh, United Kingdom: Oliver and Boyd Ltd.; 1963. p. 46, Table 3.
5. Mitterbauer G, Nemeth P, Wacha S, Cross NC, Schwarzinger I, Jaeger U, et al. Quantification of minimal residual disease in patients with BCR-ABL-positive acute lymphoblastic leukaemia using quantitative competitive polymerase chain reaction. Br J Haematol 1999;106:634-43.
6. Brisco MJ, Sykes PJ, Hughes E, Dolman G, Neoh SH, Peng LM, et al. Monitoring minimal residual disease in peripheral blood in B-lineage acute lymphoblastic leukaemia. Br J Haematol 1997;99:314-9.
7. Martin H, Atta J, Bruecher J, Elsner S, Schardt C, Stadler M, et al. In patients with BCR-ABL-positive ALL in CR peripheral blood contains less residual disease than bone marrow: implications for autologous BMT. Ann Hematol 1994; 68:85-7.
8. van der Velden VH, Jacobs DC, Wijkhuijs AJ, Comans-Bitter WM, Willemse MJ, Hahlen K, et al. Minimal residual disease levels in bone marrow and peripheral blood are comparable in children with T cell acute lymphoblastic leukemia (ALL), but not in precursor-B-ALL. Leukemia 2002;16:1432-6.

A novel translocation t(14;15)(q32;q24) bearing deletion on der(14) in Philadelphia-positive chronic myeloid leukemia

We report the molecular cytogenetic characterization of a case of Philadelphia positive chronic myeloid leukemia (CML) with a t(14;15)(q32;q24) at onset, showing deletions on der(14) in addition to the loss of chromosome 22 sequences on der(9). To our knowledge, the presence of deletions on chromosomes involved in distinct and concomitant rearrangements other than t(9;22) has never been observed in CML patients.

haematologica 2003; 88:1076-1077
(*http://www.haematologica.org/2003_09/1076.htm*)

The Philadelphia chromosome (Ph) is the cytogenetic hallmark of chronic myeloid leukemia (CML) and is present in approximately 90–95% of patients.[1] The loss of genomic sequences on der(9) chromosome has been described in approximately 9% of CML patients. These deletions identify a subgroup with a worse prognosis.[2] Albano *et al.*[3] recently reported the occurrence of deletions on the third chromosome involved in variant complex t(9;22) translocations. In the present paper, we report the molecular cytogenetic characterization of a Ph+ CML case with a t(14;15)(q32;q24) at onset, showing deletions on der(14) in addition to the loss of chromosome 22 sequences on der(9). Conventional cytogenetic analysis of a 24-48 hour culture was performed on bone marrow cells at diagnosis by giemsa-trypsin-giemsa (GTG) banding. The patient was tested by reverse transcription polymerase chain reaction (RT-PCR) and fluorescence *in situ* hybridization (FISH).

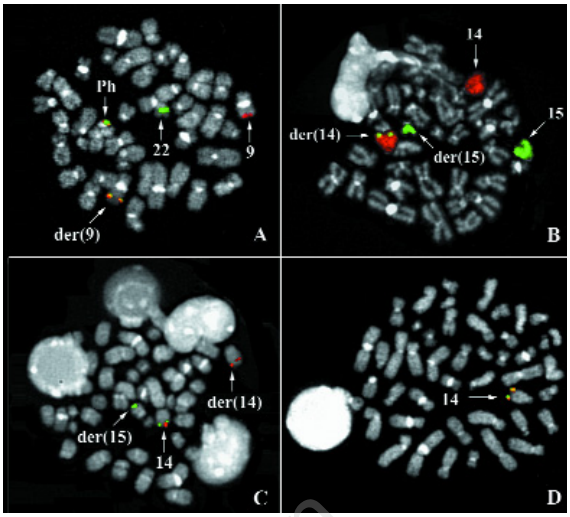

Figure 1. A) FISH co-hybridization with clones specific for ABL (red) and BCR (green) genes showed a fusion signal on both Ph and der(9) chromosomes. A faint BCR signal was observed on the der(9) chromosome, suggesting a partial deletion. B) WCP#14 (red) and WCP#15 (green) revealed the t(14;15) translocation. C) FISH experiments with clones RP11-796G6 (red) and RP11-356L8 (green) allowed us to map the breakpoint in 14q32.31. D) Clones RP11-350L3 (red) and RP11-114H15 (green), both mapping in 14q32.31, gave hybridization signals only on the normal 14 chromosome, indicating deletion of the chromosomal region encompassed by the two clones.

BAC RPCI-11 164N13 and a mixture of PACs RPCI-5 835J22 and 1132H12 were used to identify BCR and ABL genes, respectively.[3] Whole-chromosome paints (WCPs) for chromosomes 14 and 15, derived from flow-sorted chromosomes, were DOP-amplified. A series of 15 PAC/BAC clones specific for chromosomes 14 and 15 was used to define the t(14;15) breakpoints. Probe locations and gene mapping were derived from the University of California Santa Cruz database (November 2002 release). Primers specific for *WDR20* (exons 2: 5'TCTGCTGTGGCTGTTAGGTG3'; exon 3: 5'TTCCTCGTGGATTTGCTCTT3') and *PML*[4] (PML-C2: 5'AGCGCGACTACGAGGAGAT3') genes were selected and employed in the RT-PCR experiments.

In March 2002 a 64-year old female was admitted to our Institution with leukocytosis and splenomegaly. The diagnosis of chronic phase-CML was made. After initial cytoreduction obtained with hydroxyurea, the patient started Glivec therapy. She is now doing well in hematologic remission.

The t(14;15) translocation was detected in all analyzed metaphases, in addition to the classical t(9;22) rearrangement, providing the following karyotype: 46, XX, t(9;22)(q34;q11), t(14;15)(q32;q24)[25]. FISH experiments with probes specific