Table 1 Summary of *t*(9;14) cases

Case	Diagnosis	Age (years)	Cell line	Reference
1	T-cell neoplasia[a]	20	HD-MAR	4
2	T-ALL	32	HT-1	5
3	T-ALL	24	—	8
4	T-ALL	23	—	9
5	T-ALL	29	—	9
6	T-CLL	Unknown	—	10

Abbreviations: ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; HD, heterodimerization domain.
[a]After Hodgkin's lymphoma.

investigating the leukemic role of NOTCH1, a topic of pressing clinical and scientific interest.

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S Suzuki[1,2], S Nagel[1], B Schneider[1], S Chen[1], M Kaufmann[1], K Uozumi[2], N Arima[2], HG Drexler[1] and RAF MacLeod[1]
[1]*DSMZ, Department of Human and Animal Cell Cultures, Braunschweig, Germany and*
[2]*Center for Chronic Viral Diseases, Kagoshima Univ. Sch. Med. Dent. Sci., Kagoshima, Japan*
E-mail: rml@dsmz.de

References

1 Grabher C, von Boehmer H, Look AT. Notch 1 activation in the molecular pathogenesis of T-cell acute lymphoblastic leukaemia. *Nat Rev Cancer* 2006; **6**: 347–359.
2 Aster JC, Xu L, Karnell FG, Patriub V, Pui JC, Pear WS. Essential roles for ankyrin repeat and transactivation domains in induction of T-cell leukemia by notch1. *Mol Cell Biol* 2000; **20**: 7505–7515.
3 Weng AP, Ferrando AA, Lee W, Morris IV JP, Silverman LB, Sanchez-Irizarry C *et al*. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science* 2004; **306**: 269–271.
4 Ben-Bassat H, Mitrani-Rosenbaum S, Gamliel H, Naparstek E, Leizerowitz R, Korkesh A *et al*. Establishment in continuous culture of a T-lymphoid cell line (HD-Mar) from a patient with Hodgkin's lymphoma. *Int J Cancer* 1980; **25**: 583–590.
5 Abe M, Tasaki K, Nozawa Y, Tominaga K, Fukuhara S, Ohsato T *et al*. Establishment and characterization of a human T-cell lymphoblastic lymphoma cell line (HT-1) carrying an inversion of chromosome 14. *Cancer* 1992; **69**: 1235–1240.
6 Willis TG, Jadayel DM, Coignet LJ, Abdul-Rauf M, Treleaven JG, Catovsky D *et al*. Rapid molecular cloning of rearrangements of the IGHJ locus using long-distance inverse polymerase chain reaction. *Blood* 1997; **90**: 2456–2464.
7 Palomero T, Barnes KC, Real PJ, Bender JL, Sulis ML, Murty VV *et al*. CUTLL1, a novel human T-cell lymphoma cell line with t(7;9) rearrangement, aberrant NOTCH1 activation and high sensitivity to gamma-secretase inhibitors. *Leukemia* 2006; **20**: 1279–1287.
8 Gesk S, Martin-Subero JI, Harder L, Luhmann B, Schlegelberger B, Calasanz MJ *et al*. Molecular detection of chromosomal breakpoints in T-cell receptor gene loci. *Leukemia* 2003; **17**: 738–745.
9 Clare N, Boldt D, Messerschmidt G, Zeltzer P, Hansen K, Manhoff L. Lymphocyte malignancy and chromosome 14: structural aberrations involving band q11. *Blood* 1986; **67**: 704–709.
10 Zech L, Godal T, Hammarstrom L, Mellstedt H, Smith CI, Totterman T *et al*. Specific chromosome markers involved with chronic T lymphocyte tumors. *Cancer Genet Cytogenet* 1986; **21**: 67–77.

Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

Nuclear entrapment of BCR–ABL by combining imatinib mesylate with leptomycin B does not eliminate CD34$^+$ chronic myeloid leukaemia cells

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Chronic myeloid leukaemia (CML) arises from the formation of the Philadelphia (Ph$^+$) chromosome in haematopoietic stem cells. The translated fusion oncoprotein, BCR–ABL (p210$^{BCR-ABL}$), is a constitutively active tyrosine kinase (TK), which activates multiple proliferative and anti-apoptotic signalling pathways, causing deregulated cell growth.[1] Despite impressive rates of complete cytogenetic response (CCyR) in the majority of patients treated with the targeted TK inhibitor (TKI), imatinib mesylate (IM; Glivec, Novartis, Basle, Switzerland),[2] few patients achieve sustained molecular remission and a significant proportion develops resistance to IM.[3] The presence of pre-existing or acquired BCR–ABL kinase domain mutations, which decrease IM binding,[3] and the innate insensitivity of primitive quiescent CML stem cells to IM[4] are two contributing resistance mechanisms. Consequently, many strategies have been investigated to overcome IM resistance, including the development of second-generation TKIs, nilotinib (Tasigna, Novartis) and dasatinib (Sprycel, Bristol–Myers Squibb, Princeton, NJ, USA), although these remain ineffective against the T315I mutation.

In search of alternative strategies for the elimination of Ph$^+$ cells, Vigneri and Wang [5]showed an intriguing strategy of tricking BCR-ABL$^+$ cells into committing cell death. This mechanism was based on the observation that on treatment with IM, inactive BCR-ABL translocates into the cell nucleus. Entrapment of BCR–ABL there, coupled with re-activation of its TK activity, reversed its role to that of an activator of apoptosis. On the basis of the mounting evidence that BCR–ABL$^+$ stem cells are not eliminated by novel targeted therapies, such as IM and the second-generation TKIs, we investigated whether combination treatment of IM, with the nuclear export inhibitor leptomycin B (LMB Calbiochem), could drive CML CD34$^+$ cells into apoptosis.

Our initial experiments, over a period of 16 days, on the effects of IM, LMB and the combination of these drugs on the Ph$^+$ cell line K562, showed that treatment with either drug alone for the first 72 h was at least cytostatic. However, 5–8 days after drug washout, cells were able to recover, an effect not seen with the drug combination,in which irreversible growth

inhibition was observed (day 16, untreated: $9.4 \pm 3.7 \times 10^9$; 10 μM IM: $1.6 \pm 0.4 \times 10^5$; 10 nM LMB: $2.3 \pm 2.2 \times 10^7$; 10 μM IM + 10 nM LMB: $0.4 \pm 0.3 \times 10^3$ cells; (Figure 1a). This is consistent with the findings of Wang and Vigneri,[5] although we observed complete loss of viable cells at a later time point (day 14) than that reported earlier (day 9). K562 cells, exposed to the combination of IM and LMB, showed evidence of BCR–ABL localization within the nucleus. Western blot analysis confirmed that levels of nuclear BCR–ABL were significantly higher in cells treated with IM and LMB, compared with the untreated control ($P<0.05$) (Figure 1bi). Significant reduction of cytoplasmic BCR–ABL after exposure to IM and LMB was not detectected by western blot (Figure 1bii).

Earlier, the efficacy of the two-drug treatment was compared in myeloid progenitors from the bone marrow of CML patients versus normal CD34$^+$ samples.[6] These data showed that this combination preferentially targeted BCR–ABL-expressing cells, by producing the greatest decrease in the number of Ph$^+$ compared with Ph$^-$ colonies. In this study, we report the effect of IM and LMB directly on CML CD34$^+$ cells by evaluating the toxicity of this drug regime on these cells rather than the cytostatic effect studied in colony-forming assays by Aloisi *et al*.[6] Exposure of total CD34$^+$ cells (obtained from leukapheresis material (>95% CD34$^+$) from chronic phase (CP) CML patients with written informed consent) to the combination of IM with LMB failed to eliminate these cells (Figure 1c), despite the fact that nuclear localization of BCR–ABL was detected (Figure 1d). However, a 1-log reduction in total cell number, compared with input, resulted 3 days after drug washout (Figure 1c). In accordance with the findings of Aloisi *et al*., LMB alone

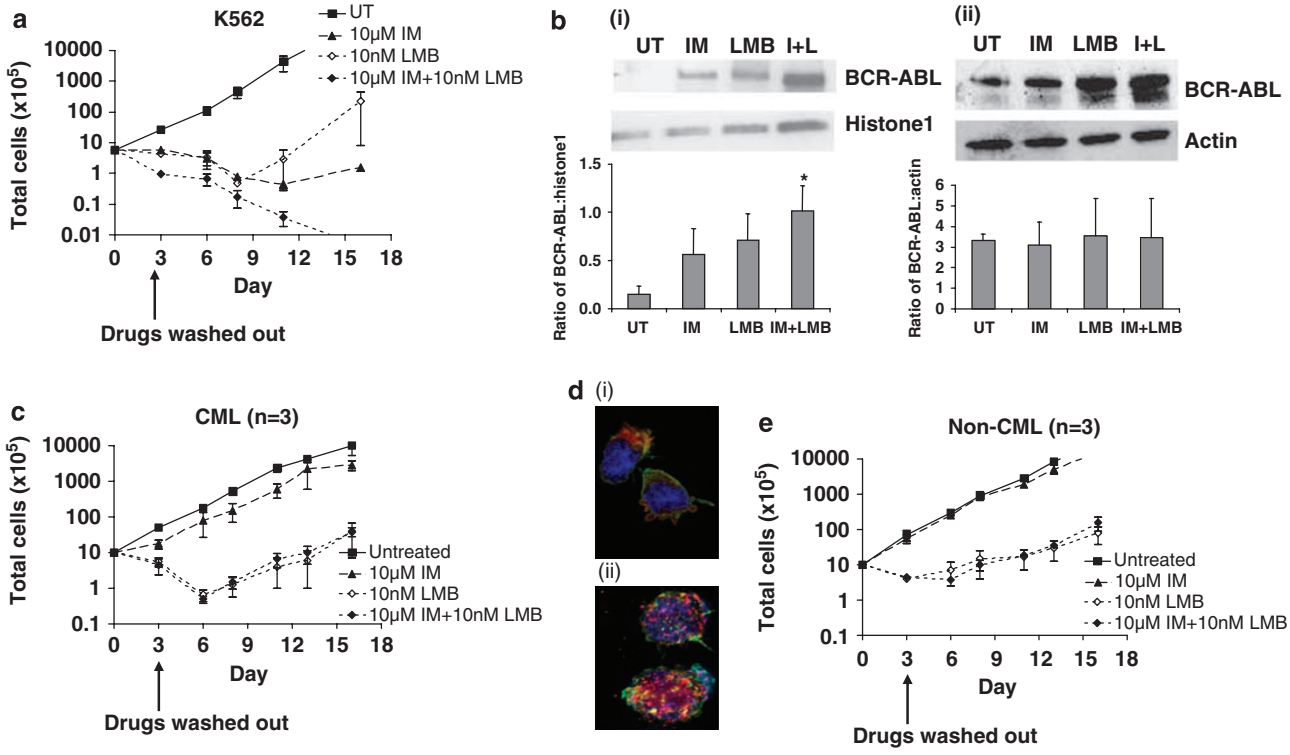

Figure 1 Effect of imatinib mesylate and Leptomycin B treatment on cell growth and localization of BCR–ABL in CML cell lines and primary cells. (**a**) The Ph$^+$ cell line K562 recovers from exposure to IM and LMB as single agents but not when used in combination. K562 cells seeded at 2×10^5/ml in 3-ml cultures were treated with IM (10 μM), LMB (10 nM), or both drugs simultaneously, in duplicate wells for 72 h before drug washout with phosphate buffer saline (PBS). Cells were re-seeded in the absence of further drug addition and counted, by trypan blue dye exclusion, at 2–3-day intervals, for a total of 16 days. Results were analysed by paired Student's *t*-test and are presented as the mean of three experiments ± s.e.m. (**b**) BCR–ABL protein is present in the nuclear fraction of K562 cells after IM and LMB exposure. 5×10^6 K562 cells were pre-treated with 5 μM IM, 5 nM LMB, or both, for 4 h and sub-fractionated using the ProteoExtract Subcellular Proteome Extraction Kit (Calbiochem, Merck Biosciences Ltd, Hull, UK) into (i) nuclear and (ii) cytoplasmic fractions. Extracts were analysed by western blotting. Nuclear and cytoplasmic BCR–ABL (anti-ABL 8E9 antibody (BD Ltd, Oxford, UK)) protein levels were expressed as the ratio of ABL to the loading controls, histone1 (anti-Histone H1, clone AE-4 (Upstate Millipore Ltd, UK)) and pan-actin antibody (Cell-Signalling, Hertfordshire, UK), for nuclear and cytoplasmic fractions, respectively, as determined by densitometry. Each blot is representative of three independent experiments. Results were analysed by paired Student's *t*-test and are presented as the mean of three experiments ± s.e.m. The asterisk (*) indicates significantly different protein ratios from the untreated control ($P< 0.05$) ,I,: IM; L: LMB. (**c**) Growth of CML CD34$^+$ cells is not irreversibly inhibited by simultaneous exposure to IM and LMB. Identical time-course experiments to those in (**a**) were set up for three CML patient-derived CD34$^+$ cells over a total of 16 days. Cells seeded at 5×10^5/ml in 2-ml cultures were treated with no drug, 10 μM IM, 10 nM LMB, or both, for 72 h before drug washout with PBS and counted at 2–3-day intervals thereafter. Results were analysed by paired Student's *t*-test and are presented as the mean of three experiments ± s.e.m. (**d**) BCR–ABL protein is present in the nucleus of CML CD34$^+$ cells after IM and LMB exposure. CML CD34$^+$ cells before (i) and after exposure to 24 h IM (10 μM) with LMB (10 nM) added for the last 12 h (ii). This shows the nuclear import of BCR–ABL (anti-ABL 8E9, BD, as above) (red) after combined treatment with IM and LMB in comparison with the cytoplasmic localization in the untreated cells. The green signals correspond to Phalloidin-conjugated Alexa Fluor 488 staining of F-actin (Molecular Probes, Invitrogen, Paisley, UK) with blue (DAPI) staining for DNA. (**e**) LMB induces cytostasis in non-CML CD34$^+$ cells. Duplicate wells seeded at 5×10^5 cells/ml of CD34$^+$ cells from normal autologous donors were cultured with no drug, 10 μM IM, 10 nM LMB, or both drugs, for 72 h, as for CML CD34$^+$ cells, before drug washout. Results were analysed by paired Student's *t*-test and are presented as the mean of three experiments ± s.e.m.

produced a similar inhibitory effect to that of the drug combination on CML $CD34^+$ cell viability.[6] However, LMB also inhibited the proliferation of $BCR–ABL^-$ $CD34^+$ cells by three-to fivefold, showing a cytostatic effect with recovery 3 days after drug washout, demonstrating the non-specific toxicity of this drug (Figure 1e). This result suggests that CML $CD34^+$ cells seem to be more sensitive to inhibition of nuclear export than $BCR–ABL^-$ $CD34^+$ cells. The recovery of both CML and non-CML cells after drug washout could be explained by the *de novo* synthesis of CRM1, which reversed the LMB effect.[7] As anticipated, Ph^+ $CD34^+$ cells were less sensitive than K562 cells to IM exposure, showing the persistence/resistance of these stem and progenitor cells in the presence of IM. IM led to a non-significant reduction in the viable Ph^- $CD34^+$ population (20%) ($P>0.05$) after both 3-day exposure and 13 days after drug washout (40%) ($P>0.05$), corroborating an earlier report in which exposure (3 days) to 10 μM IM did not significantly reduce the expansion of Ph^- $CD34^+$ cells.[8] Furthermore, as the combination treatment failed to eliminate the total CML $CD34^+$ cells, its effect on the more primitive quiescent leukaemic stem cells ($CD34^+38^-$ or equivalent) was not investigated.

In conclusion, our results indicate that even the manipulation of the cellular localization of BCR–ABL is not sufficient to induce cell death of all the Ph^+ $CD34^+$ cell population. It is possible that the combination of IM and LMB would be more effective if a selective inhibitor of the ABL nuclear export signal were available.

EK Allan[1,2], A Hamilton[1], S Hatziieremia[1], P Zhou[1], HG Jørgensen[1], P Vigneri[3] and TL Holyoake[1]
[1]*Paul O'Gorman Leukaemia Research Centre, Section of Experimental Haematology, Division of Cancer Sciences and Molecular Pathology, Faculty of Medicine, University of Glasgow, Glasgow, UK;*
[2]*Academic Transfusion Medicine Unit, Scottish National Blood Transfusion Service, Glasgow, UK;*
[3]*Department of Biomedical Sciences, Section of General Pathology, University of Catania, Catania, Italy*
E-mail: tlh1g@clinmed.gla.ac.uk

References

1 Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nat Rev Cancer* 2005; **5**: 172–183.
2 Druker BJ, Guilhot F, O'Brien SJ, Gathmann I, Kantarjian H, Gattermann N *et al.* Five-year follow up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med* 2006; **355**: 2408–2417.
3 Hochhaus A, Kreil S, Corbin AS, La Rosée P, Muller MC, Lahaye T *et al.* Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia* 2002; **16**: 2190–2196.
4 Graham SM, Jørgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L *et al.* Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 *in vitro*. *Blood* 2002; **99**: 319–325.
5 Vigneri P, Wang JY. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. *Nat Med* 2001; **7**: 228–234.
6 Aloisi A, Di Gregorio S, Stagno F, Guglielmo P, Mannino F, Sormani MP *et al.* BCR-ABL nuclear entrapment kills human CML cells: *ex vivo* study on 35 patients with the combination of imatinib mesylate and leptomycin B. *Blood* 2006; **107**: 1591–1598.
7 Kudo N, Matsumori N, Taoka H, Fujiwara D, Schreiner EP, Wolff B *et al.* Leptomycin B inactivates CRM1/exportin 1 by covalent modification at a cysteine residue in the central conserved region. *Proc Natl Acad Sci USA* 1999; **96**: 9112–9117.
8 Bartolovic K, Balabanov S, Hartmann U, Komor M, Boehmier AM, Bühring HJ *et al.* Inhibitory effect of imatinib on normal progenitor cells *in vitro*. *Blood* 2004; **103**: 523–529.

No evidence of frequent association of the *JAK2* V617F mutation with acute myocardial infarction in young patients

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Myeloproliferative neoplasms (MPNs) have been associated with high incidence of thrombosis and bleeding episodes, which significantly contribute to disease-related morbidity and mortality.[1] Notably, clinical data indicate an association of the *JAK2* V617F mutation, seen in nearly all polycythemia vera cases and half of those with essential thrombocythemia with erythrocytosis, leukocytosis and thrombotic complications. Particularly for essential thrombocythemia, a recent overview has suggested that the *JAK2* V617F positivity may be associated with an increased risk of thrombosis, an effect mediated by the leukocytosis conferred by the mutation.[2] Several studies have indicated that a substantial proportion of patients with idiopathic Budd–Chiari syndrome, portal vein or mesenteric vein thrombosis carry the *JAK2* V617F mutation, even in the absence of overt clinical or hematological features of an MPN.[3] Detection of *JAK2* V617F mutation in this setting is a marker of a 'latent' MPN as positive patients appear to have bone marrow histological features characteristic of MPN.[3,4]

Recently, Mercier *et al.* screened 27 patients aged <40 years, who had experienced an acute myocardial infarction (AMI). Three out of 27 (11.1%) patients carried the *JAK2* V617F mutation, leading the authors to conclude that a latent MPN may often be present in this group of patients, warranting screening for the V617F mutation.[5] In contrast, in a study of 28 patients with AMI at a young age (defined as <50 years), none was found to harbour the *JAK2* V617F mutation. That group of patients was part of a larger cohort of patients with arterial or venous thrombosis outside the splanchnic circulation. The study suggested that screening for *JAK2* V617F mutation is not warranted as part of the hypercoagulable work-up in patients with non-splanchnic thrombosis.[6]

We investigated the prevalence of the *JAK2* V617F mutation in a prospectively assembled cohort of AMI patients aged <35 years. We hypothesized that *JAK2* V617F may be further enriched in this group of patients as it represents a good model because the mainly genetic factors, and to a lesser extent the environmental factors, have an impact on the pathogenesis of the thrombosis. We studied consecutive patients admitted to our institution between January 1998 and December 2005 who had suffered their first AMI under the age of 35 years. DNA was extracted from peripheral blood