Chronic myeloid leukaemia (CML) arises from the formation of patients achieve sustained molecular remission and a significant mesylate (IM; Glivec, Novartis, Basle, Switzerland), few patients treated with the targeted TK inhibitor (TKI), imatinib (p210BCRABL), is a constitutively active tyrosine kinase (TK), which activates multiple proliferative and anti-apoptotic signalling pathways, causing deregulated cell growth. 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), few patients achieve sustained molecular remission and a significant proportion develops resistance to IM. The presence of pre-existing or acquired BCR–ABL kinase domain mutations, which decrease IM binding, and the innate insensitivity of primitive quiescent CML stem cells to IM 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 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 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 (p210BCR–ABL), is a constitutively active tyrosine kinase (TK), which activates multiple proliferative and anti-apoptotic signalling pathways, causing deregulated cell growth. 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), few patients achieve sustained molecular remission and a significant proportion develops resistance to IM. The presence of pre-existing or acquired BCR–ABL kinase domain mutations, which decrease IM binding, and the innate insensitivity of primitive quiescent CML stem cells to IM 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 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

<p>| Table 1 Summary of t(9;14) cases |
|-----------------|--------|------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Age (years)</th>
<th>Cell line</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T-cell neoplasia</td>
<td>20</td>
<td>HD-MAR</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>T-ALL</td>
<td>32</td>
<td>HT-1</td>
<td>5</td>
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<tr>
<td>3</td>
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<td>24</td>
<td>—</td>
<td>8</td>
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<tr>
<td>4</td>
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<td>23</td>
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<td>9</td>
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<td>29</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>T-ALL</td>
<td>Unknown</td>
<td>—</td>
<td>10</td>
</tr>
</tbody>
</table>

Abbreviations: ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; HD, heterodimerization domain.

After Hodgkin’s lymphoma.

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

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)
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) BCR–ABL protein is present in the nuclear fraction of K562 cells after IM and LMB exposure. (ii) 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 2 × 10⁵/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. Results were analysed by paired Student’s t-test and are presented as the mean of three experiments ± s.e.m. BCR–ABL protein is present in the nuclear fraction of K562 cells after IM and LMB exposure. 5 × 10⁴ 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, histone 1 (anti-Histone H1, clone AE-4 (Upstate Millipore Ltd, UK)) and pan-actin antibody (Cell-Signalling, Hertfordshire, UK), for nuclear and cytoplasmic BCR–ABL protein levels were expressed as the ratio of ABL to the loading controls, histone 1 (anti-Histone H1, clone AE-4 (Upstate Millipore Ltd, UK)) and pan-actin antibody (Cell-Signalling, Hertfordshire, UK), for nuclear and cytoplasmic BCR–ABL fractions, respectively, as determined by densitometry. Each blot is representative of three independent experiments.

Earlier, the efficacy of the two-drug treatment was compared in myeloid progenitors from the bone marrow of CML patients versus normal CD34+ samples. 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. 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. 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
produced a similar inhibitory effect to that of the drug combination on CML CD34+ cell viability. 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 1c). 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. 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 10µM IM did not significantly reduce the expansion of Ph+ CD34+ cells. Furthermore, as the combination treatment failed to eliminate the total CML CD34+ cells, its effect on the more primitive quiescent leukemic stem cells (CD34+38- 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 Allan1,2, A Hamilton1, S Hatzieremia1, P Zhou1, HG Jørgensen1, P Vigneri2 and TL Holyoake1

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References


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. 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. 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. 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.

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. 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.

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