In vitro activity of imatinib in cells from patients with adult acute lymphoblastic leukemia

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We evaluated the *in vitro* activity of imatinib on BCR–ABL-positive and -negative tumor cells from patients with adult acute lymphoblastic leukemia (ALL), and investigated *in vitro* interactions between imatinib and conventional agents. A non-clonogenic cytotoxicity assay was used to analyze p190 BCR–ABL-positive ($n=4$), p210 BCR–ABL-positive ($n=2$) and BCR–ABL-negative ($n=9$) tumor cells from adult ALL patients. The *in vitro* cytotoxic effect of imatinib was studied alone, and in combination with the cytotoxic agents cytarabine, prednisolone, vincristine, daunorubicin, asparaginase and mercaptopurine. The BCR–ABL-positive samples were significantly ($p<0.05$) more sensitive to imatinib than the BCR–ABL-negative at the concentrations 0.1, 1 and 10 μM. Interestingly, the two p210 samples were somewhat less sensitive to imatinib than the p190 samples. Daunorubicin, prednisolone and cytarabine showed the largest benefit from combination with imatinib compared to the most active single agent. The study confirms that drug sensitivity to imatinib is specific for BCR–ABL-positive samples. The results also suggest that combinations between imatinib and daunorubicin, predisolone or cytarabine may be advantageous for the treatment of Philadelphia-positive ALL. *Anti-Cancer Drugs* 16:631–634 © 2005 Lippincott Williams & Wilkins.

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Introduction

In adult acute lymphoblastic leukemia (ALL) the presence of the Philadelphia (Ph) translocation, t(9.22)(q34;q11), is an important adverse prognostic factor associated with long-term survival less than 10% when treated with conventional chemotherapy regimens [1]. The translocation results in either a p190 or a p210 Bcr–Abl fusion protein and is found in 20–30% of adult ALL cases [1,2]. Imatinib (imatinib mesylate, STI 571; Glivec), a competitive inhibitor of the Bcr–Abl protein tyrosine kinase [3,4], has been shown to be highly effective in the treatment of chronic myeloid leukemia (CML) in the chronic phase [5]. Imatinib has also shown substantial initial efficacy in the treatment of Ph-positive ALL and the blast phase of CML, but with rapid disease progression [6,7].

This study was performed to evaluate the *in vitro* activity of imatinib on tumor cells from adult ALL patients with p190 or p210 BCR–ABL compared to BCR–ABL-negative ALL tumor cells and, furthermore, to investigate if adding imatinib to conventional agents used in current ALL treatment could enhance or reduce activity of those agents *in vitro*.

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Methods

Patients and samples

Samples from bone marrow or peripheral blood from 25 adult patients with ALL were used in the study. Leukemic cells were prepared by Ficoll-Isopaque density-gradient centrifugation [8]. Cryopreserved cells were used throughout the study. Data concerning immunophenotype, Ph status and stage of disease were obtained from the patient's medical charts. Thereafter, the experiments were performed with coded samples. Peripheral blood mononuclear cells (PBMCs) from two healthy donors were used as controls. The local ethical committee approved the study.

Reagents and drugs

Cytotoxic agents were selected based on clinical usefulness in ALL and diversity in mechanism of action. The concentrations were chosen based on data from previous studies in primary ALL cells, to produce an intermediate drug effect (cytarabine 10 μM, prednisolone 30 μM, vincristine 0.5 μM, daunorubicin 0.2 μM, asparaginase 10 U/ml and mercaptopurine 300 μM; all drugs were obtained from commercial sources) [9]. Imatinib was supplied from Novartis Pharma (Basel, Switzerland) and

dissolved in DMSO to a concentration of 10 mM. The drug was tested at six 10-fold solutions ranging from 100 to 0.01 μM. The concentration of 1.0 μM of imatinib resulted in approximately 50% cell survival of Ph-positive cells and was chosen for the combination study.

Fluorometric microculture cytotoxic assay (FMCA)

The FMCA is based on the measurement of fluorescence generated from hydrolysis of fluorescein diacetate (FDA) to fluorescent fluorescein by cells with intact cell membranes as previously described [9]. In each well, 100 000 viable leukemic cells in 180 μl culture medium were seeded. The culture medium consisted of Sigma (St Louis, MO) cell culture RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 60 μg/ml penicillin and 50 μg/ml streptomycin. Cell viability was measured after a continuous 72-h exposure to the cytotoxic agents in 96-well microtiter plates. Six blank wells contained only culture medium and six control wells contained culture medium and cells. The cell survival was calculated as the ratio between the fluorescence in drug-treated wells and the fluorescence in control wells with blank values subtracted and was expressed as percentage [9]. Low numerical values thereby indicated a high cytotoxic effect. The proportion of leukemic cells was estimated on May–Grunwald–Giemsa-stained cyto-centrifuged preparations, using a light microscope. All drugs and combinations were tested in triplicates. Quality criteria for an approved assay included a mean coefficient of variation in control cultures less than 30%, a fluorescence signal in control cultures of more than 5 times blank values and a tumor cell fraction exceeding 70%.

Measurement of the BCR–ABL fusion transcript

Quantitative real-time RT-PCR (QR-PCR) was used to detect and quantify BCR/ABL mRNA as previously described using GAPDH as control gene [10,11].

Statistical analysis

Non-parametric statistical analysis was used. The mean cell survival at different concentrations of imatinib for BCR–ABL-positive and -negative samples was compared using the Mann–Whitney test for unpaired comparisons. Spearman rank correlations were used to investigate the correlation between the level of expression of BCR/ABL mRNA and mean cell survival. The level of significance was set at $p < 0.05$.

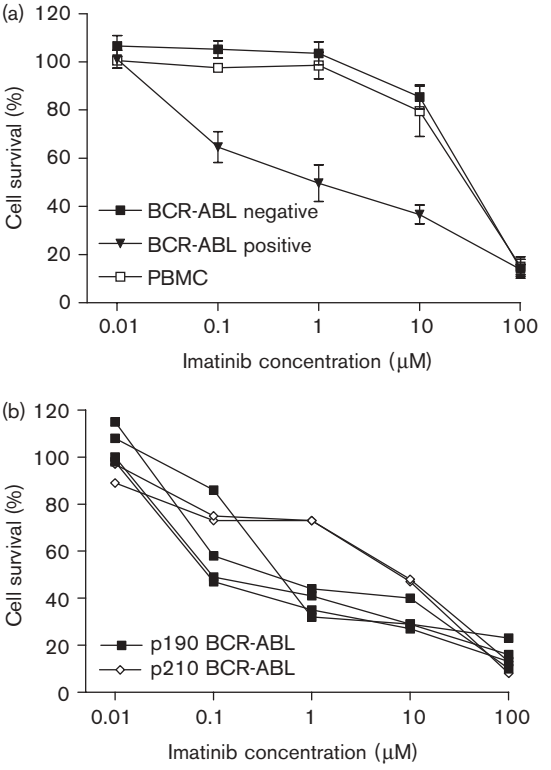
Results

A successful drug sensitivity analysis and QR-PCR was performed in 15 of the 25 tumor samples according to the quality criteria of the tests [9]. The most common cause of assay failure was fluorescence signal in control cultures of less than 5 times blank values (eight of 10 failed samples). Pre-B immunophenotype was dominating, including 14 patients; the remaining patient had T-ALL. The samples were taken at the time of diagnosis in nine patients and at relapse in the remaining six patients. None of the patients had received imatinib.

BCR–ABL fusion transcripts were found in six samples (two p210 and four p190) and were absent in nine samples. The BCR–ABL-positive samples were significantly ($p < 0.05$) more sensitive to imatinib than the BCR–ABL-negative at the concentrations 0.1, 1 and 10 μM (Fig. 1a). The BCR–ABL-negative samples and the normal lymphocytes responded to imatinib only at 100 μM, and to a slight extent at 10 μM. A similar sensitivity to high concentrations of imatinib has been observed in tumor cells from acute myelocytic leukemia and chronic lymphocytic leukemia (not shown).

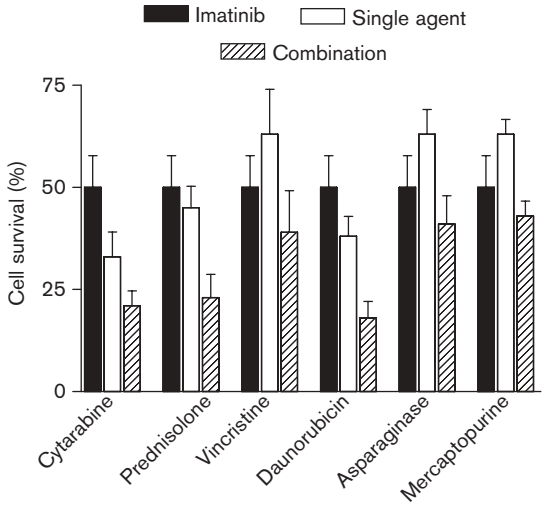
All BCR–ABL-positive samples had high levels of BCR–ABL mRNA ranging from 107 to 404 BCR–ABL molecules relative to 10^4 GAPDH molecules (data not shown). No correlation was found between the level of expression of BCR–ABL mRNA and imatinib sensitivity

Fig. 1



Concentration–effect curve of a cell survival for primary ALL cells exposed to five concentrations of imatinib. (a) Mean concentration–effect curve of BCR–ABL-positive and BCR–ABL-negative ALL samples and healthy lymphocytes (PBMC) exposed to imatinib. The error bars show SEM. (b) Concentration–effect curve of the four p190 and two p210 BCR–ABL-positive ALL samples exposed to imatinib

Fig. 2



Mean cell survival of primary ALL cells when exposed to imatinib, single agent and imatinib in combination with the single agent ± SEM.

at the concentration 1 μM (Rho = −0.4, NS, Spearman rank correlation). Cells expressing the p190 BCR–ABL fusion were slightly more sensitive than cells expressing the p210 fusion, particularly at 1 μM (Fig. 1b).

The mean cell survival for BCR–ABL-positive samples to imatinib, single agent and imatinib in combination with the single agent is shown in Figure 2. The drugs showing the largest benefit from combination with imatinib, defined as the highest mean difference in cell survival (%) of the combination compared to the most active single agent, were daunorubicin (51% higher effect of combination), prednisolone (41%) and cytarabine (26%). A less pronounced effect was found for vincristine (17% better effect of combination) and asparaginase (12%). When imatinib was combined with mercaptopurine, the effect of the combination was similar to the effect of the most active constituent (mean difference 8%) and a possible antagonistic interaction was observed in one sample. The mean cell survival rates for the BCR–ABL-negative cells were not significantly lower with any of the combinations of imatinib and cytotoxic agent than with the cytotoxic agent alone (not shown).

Discussion

The selective effect for Ph-positive leukemia is well described in previous *in vitro* tests and confirmed in clinical trials [6,12,13]. This study confirms that the drug sensitivity to imatinib was significantly higher for BCR–ABL-positive compared to the BCR–ABL-negative samples from ALL patients. At high concentrations of imatinib (especially at 100 μM) even the BCR–ABL-negative cells were sensitive, and no differences were found between samples from the ALL-patients and the samples from healthy donors. These results suggest that the activity at high concentration is less specific and independent of the Bcr–Abl tyrosine kinase.

p190 Bcr–Abl has been shown to have a higher tyrosine kinase activity compared to the p210 version [14]. This may correlate to our results that cells expressing the p190 BCR–ABL fusion were slightly more sensitive to imatinib than cells expressing the p210 fusion, even if the material is small. Beran *et al.* described similar activity of imatinib in p190 BCR–ABL lymphoblastic and p210 BCR–ABL myeloid cell lines [15], but this has not previously been examined in primary ALL cells and further investigations are required.

The short remissions achieved in Ph-positive ALL and the blast phase of CML [6,7] with imatinib are probably due to development of drug resistance by amplification or mutations in the Bcr–Abl tyrosine kinase [16,17]. In order to prevent development of resistance and to achieve an enhanced drug effect, combinations of imatinib and cytotoxic agents are interesting, and have been the subject of trials [18,19]. Synergistic or additive interactions between imatinib and, for example, cytarabine, daunorubicin, doxorubicin or vincristine have been found in *in vitro* tests using cell lines or samples from CML patients [20–24]. The observations from this study, concerning primary tumor cells from patients with BCR–ABL-positive ALL, are well in accordance with these findings and the *in vitro* results may be of guidance for future trials.

To conclude, the current study demonstrates a high concordance between *in vitro* sensitivity to imatinib and BCR–ABL expression, p210 being less sensitive than p190 BCR–ABL. The study also suggests that combinations of imatinib with cytotoxic drugs, such as daunorubicin, predisolone and cytarabine, are interesting for further evaluation in the treatment of Ph-positive ALL.

References

1 Gleissner B, Gokbuget N, Bartram CR, Janssen B, Rieder H, Janssen JW, *et al.* Leading prognostic relevance of the BCR–ABL translocation in adult acute B-lineage lymphoblastic leukemia: a prospective study of the German Multicenter Trial Group and confirmed polymerase chain reaction analysis. *Blood* 2002; **99**:1536–1543.

2 Westbrook CA, Hooberman AL, Spino C, Dodge RK, Larson RA, Davey F, *et al.* Clinical significance of the BCR–ABL fusion gene in adult acute lymphoblastic leukemia: a Cancer and Leukemia Group B Study (8762). *Blood* 1992; **80**:2983–2990.

3 Gambacorti-Passerini C, le Coutre P, Mologni L, Fanelli M, Bertazzoli C, Marchesi E, *et al.* Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL$^+$ leukemic cells and induces apoptosis. *Blood Cells Mol Dis* 1997; **23**:380–394.

4 Deininger MW, Goldman JM, Lydon N, Melo JV. The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR–ABL-positive cells. *Blood* 1997; **90**:3691–3698.

5 O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, *et al.* Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* 2003; **348**:994–1004.

6 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**:1965–1971.

7 Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, *et al.* 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. *N Engl J Med* 2001; **344**:1038–1042.

8 Larsson R, Kristensen J, Sandberg C, Nygren P. Laboratory determination of chemotherapeutic drug resistance in tumor cells from patients with leukemia, using a fluorometric microculture cytotoxicity assay (FMCA). *Int J Cancer* 1992; **50**:177–185.

9 Frost BM, Nygren P, Gustafsson G, Forestier E, Jonsson OG, Kanerva J, *et al.* Increased *in vitro* cellular drug resistance is related to poor outcome in high-risk childhood acute lymphoblastic leukaemia. *Br J Haematol* 2003; **122**:376–385.

10 Gabert J, Beillard E, Van Der Velden VH, Bi W, Grimwade D, Pallisgaard N, *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**:2318–2357.

11 Barbany G, Hagberg A, Olsson-Stromberg U, Simonsson B, Syvanen AC, Landegren U. Manifold-assisted reverse transcription-PCR with real-time detection for measurement of the BCR–ABL fusion transcript in chronic myeloid leukemia patients. *Clin Chem* 2000; **46**:913–920.

12 Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, *et al.* Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr–Abl positive cells. *Nat Med* 1996; **2**:561–566.

13 Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C, *et al.* Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med* 2002; **346**: 645–652.

14 Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of *bcr–abl* oncogene products. *Science* 1990; **247**:1079–1082.

15 Beran M, Cao X, Estrov Z, Jeha S, Jin G, O'Brien S, *et al.* Selective inhibition of cell proliferation and BCR–ABL phosphorylation in acute lymphoblastic leukemia cells expressing M_r 190,000 BCR–ABL protein by a tyrosine kinase inhibitor (CGP-57148). *Clin Cancer Res* 1998; **4**:1661–1672.

16 Mahon FX, Deininger MW, Schultheis B, Chabrol J, Reiffers J, Goldman JM, *et al.* Selection and characterization of BCR–ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. *Blood* 2000; **96**:1070–1079.

17 Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, *et al.* Clinical resistance to STI-571 cancer therapy caused by BCR–ABL gene mutation or amplification. *Science* 2001; **293**:876–880.

18 Thomas DA, Faderl S, Cortes J, O'Brien S, Giles FJ, Kornblau SM, *et al.* Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. *Blood* 2004; **103**:4396–4407.

19 Wassmann B, Scheuring U, Pfeifer H, Binckebanck A, Kabisch A, Lubbert M, *et al.* Efficacy and safety of imatinib mesylate (Glivec) in combination with interferon-alpha (IFN-alpha) in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL). *Leukemia* 2003; **17**:1919–1924.

20 Fang G, Kim CN, Perkins CL, Ramadevi N, Winton E, Wittmann S, *et al.* CGP57148B (STI-571) induces differentiation and apoptosis and sensitizes Bcr–Abl-positive human leukemia cells to apoptosis due to antileukemic drugs. *Blood* 2000; **96**:2246–2253.

21 Avramis IA, Laug WE, Sausville EA, Avramis VI. Determination of drug synergism between the tyrosine kinase inhibitors NSC 680410 (adaphostin) and/or STI571 (imatinib mesylate, Gleevec) with cytotoxic drugs against human leukemia cell lines. *Cancer Chemother Pharmacol* 2003; **52**:307–318.

22 Thiesing JT, Ohno-Jones S, Kolibaba KS, Druker BJ. Efficacy of STI571, an abl tyrosine kinase inhibitor, in conjunction with other antileukemic agents against bcr–abl-positive cells. *Blood* 2000; **96**:3195–3199.

23 Tipping AJ, Mahon FX, Zafirides G, Lagarde V, Goldman JM, Melo JV. Drug responses of imatinib mesylate-resistant cells: synergism of imatinib with other chemotherapeutic drugs. *Leukemia* 2002; **16**:2349–2357.

24 Kano Y, Akutsu M, Tsunoda S, Mano H, Sato Y, Honma Y, *et al. In vitro* cytotoxic effects of a tyrosine kinase inhibitor STI571 in combination with commonly used antileukemic agents. *Blood* 2001; **97**:1999–2007.