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Dasatinib in combination with fludarabine in patients with refractory chronic lymphocytic leukemia: A multicenter phase 2 study

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ABSTRACT

Resistance to chemotherapy-induced apoptosis in CLL is associated with overexpression of antiapoptotic proteins induced by signals from the microenvironment. In vitro, dasatinib effectively inhibits expression of anti-apoptotic regulators and restores fludarabine sensitivity in activated CLL.

The aim of this study was to evaluate efficacy of one cycle of dasatinib monotherapy (100 mg/day, days 1–28) followed by combination of dasatinib with fludarabine (40 mg/m^2/day, days 1–3 every 28 day) for a total of 6 cycles in fludarabine-refractory CLL. The primary endpoint was overall response rate according to the IWCLL'08 criteria.

20 patients were enrolled: 18 completed at least one cycle of treatment of which 67% finished at least 2 cycles of combination treatment. 3 of these 18 patients reached a formal PR (16.7%). Majority of patients obtained some reduction in lymph node (LN) size. Most frequent toxicity was related to myelosuppression.

NF-κB RNA expression levels of circulating CLL cells decreased whereas the levels of pro-apoptotic NOXA increased during treatment.

In conclusion, dasatinib/fludarabine combination has modest clinical efficacy in fludarabine-refractory patients.

1. Introduction

In chronic lymphocytic leukemia (CLL), fludarabine refractoriness infers a poor prognosis [1] with a median survival of 10 months [2]. A variety of chemo-immunotherapeutic combinations have been studied in fludarabine-refractory CLL. Response rates vary between 15% and 39% (as reviewed by Tsimeridou and Keating) [3]. As such combinations induce significant side-effects, especially in these heavily pretreated patients, therapeutic regimens with less toxicity are highly needed for patients with refractory disease. An important mechanism of chemoresistance in CLL is the shift in the balance between pro- and anti-apoptotic proteins [4,5] which presumably occurs in CLL cells residing in secondary lymphoid tissues. By both in vitro and ex vivo studies we have indeed shown that stimuli from the microenvironment prevent apoptosis resulting in protection from the action of cytotoxic drugs [6]. It is postulated that clones harboring acquired cytogenetic changes affecting genes involved in the p53-response originate from these protected niches [7].

Dasatinib, a small-molecule kinase inhibitor used for the treatment of imatinib-resistant chronic myelogenous leukemia (CML), has activity against multiple kinases reported to be activated in CLL cells upon interaction with the microenvironment, including SRC, c-Abl and BTK [8–10]. We and others have recently shown in in vitro studies that dasatinib effectively inhibits the microenvironment-induced induction of anti-apoptotic proteins [11–13]. Specifically, it was found that dasatinib reversed overexpression of the anti-apoptotic Bcl-2 family members Bcl-xL, Bf1/A1, and Mcl-1 [12,14] and, as a consequence, restored fludarabine sensitivity [12]. In addition, dasatinib might have direct cytotoxic activity which may be

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independent of p53 function [14,15]. Moreover, very recent data indicated that dasatinib induced a reduction of cytoskeletal activity by its interaction in the LYN/HS1 signaling axis [16]. Together, these data indicate that both CLL cells with intrinsic apoptotic defects due to a dysfunctional p53 response as well as CLL cells within protective niches such as the lymph nodes could be sensitive to therapeutic strategies that include both dasatinib and purine-analogs. Findings of a recent study in relapsed and refractory CLL indicated that dasatinib has modest single agent activity with acceptable toxicity, although clinical outcome data were not reported [17]. Based on the observed in vitro synergy of dasatinib and fludarabine, we performed a phase 2 study combining fludarabine and dasatinib treatment in fludarabine-refractory CLL patients. To be able to assess the effect of dasatinib monotherapy in this patient group all patients started with one cycle of dasatinib monotherapy. When no response was observed fludarabine was added. In 5 patients correlative molecular studies were performed.

2. Patients and methods

2.1. Patients

CLL patients of at least 18 years old with fludarabine-refractory CLL according to IWCLL 2008 criteria (defined as either no response or relapse ≤6 months following fludarabine containing chemo(immuno)therapy) [18] and in need of treatment were eligible for the study provided they had a WHO performance status of ≤2. Three university hospitals in the Netherlands (Academic Medical Center, Amsterdam, University Medical Centre, Groningen, and Erasmus University Medical Center, Rotterdam) participated in the study. Patients were allowed to have been treated recently with alternative regimens as long as fludarabine refractoriness had been documented in the past. Furthermore, only patients were enrolled for whom reduced intensity allogeneic hematopoietic stem cell transplantation was not considered feasible at time of enrolment. The study was reviewed and approved by the institutional medical ethical review boards of all participating institutions. The study was registered at www.ClinicalTrials.gov as NCT01051115.

2.2. Treatment

Patients were treated with dasatinib (D) monotherapy 100 mg daily for 4 weeks. This dose was expected to be as effective as 140 mg as to inhibition of c-Abl and SRC-kinases but to result in less toxicity, notably in combination with fludarabine (F). After the first 4 weeks remission status was assessed. Patients with at least a partial remission (PR) according to IWCLL criteria continued on dasatinib monotherapy for up to another 24 weeks. In case of stable disease or progression, fludarabine was added to dasatinib (dasatinib fludarabine combination further described as DF) at a dose of 40 mg/m^2 orally. To avoid excess toxicity of the DF combination therapy, fludarabine was given for 3 days instead of 5 days. Whereas D was given daily, F was given at a 4 weeks interval. After two DF cycles, the remission status was re-evaluated. Patients with progressive disease went off study. The patients without PD received four additional cycles of fludarabine and continuous dasatinib.

2.3. Endpoints and response assessments

The primary endpoint in this study was overall response rate (ORR), defined as the percentage of patients with response classified as CR or PR, using the IWCLL 2008 criteria [18]. Secondary endpoints included toxicity, progression free survival (PFS) and overall survival (OS). LN-size changes were measured by CT-scan and calculated by comparing the sum of 6 target lesions according to the revised Cheson response criteria [19]. Patients were assessed for response after the first cycle of dasatinib monotherapy. Responding patients continued on D monotherapy and had another response assessment after the third cycle and at the end of protocol treatment (i.e. following 7 cycles of monotherapy). In addition, response evaluation was performed in case of clinically suspected progression prior to the end of the protocol. In case of DF combination treatment, response was assessed again after the second cycle of DF treatment and at the end of protocol treatment.

2.4. Toxicity assessments and dose adjustments

Toxicity was assessed according to the NCI Common Toxicity Criteria version 3.0, with the exception of hematologic toxicities which were scored according to the grading scale for hematologic toxicity in CLL studies [18].

In case of ≥grade 3 neutropenia and/or thrombocytopenia and/or non-hematological toxicity, dasatinib was withheld followed by dose reduction to 70 mg. In case toxicity recurred, treatment was discontinued.

2.5. Concomitant medication

Patients received allopurinol 300 mg daily day 0 until day 7 after the start of dasatinib.

All patients received sulphametoxazol/trimetoprim and valaciclovir from the start of fludarabine treatment throughout the study period until at least 3 months after the last treatment day.

2.6. Pharmacokinetics

Blood samples for pharmacokinetic (PK) assessment were collected pre-dose and at 1, 2, and 3 h after dosing at the beginning of the second cycle. Levels of dasatinib in plasma were determined using high-performance liquid chromatography and detection with tandem mass spectrometry (HPLC–MS/MS).

2.7. Western-blot analysis

From 3 patients that were included in this study protein, extracts were prepared from peripheral blood mononuclear cells by lysis in NP-40 lysis buffer (135 mM NaCl, 5 mM EDTA, 20 mM Tris–HCl pH 7.4) with protease and phosphatise inhibitors added (Roche Complete mini and Roche PhosStop, respectively). As a control, cells from baseline samples of the patients were treated for 1 h with 100 nM dasatinib as previously been described [16] before preparing protein extracts as described above. The lysates were then analyzed for phospho-Lyn (Y396) content by way of SDS/PAGE and subsequent Western-blot, using a Biorad Mini-Protean system (Bio-Rad Laboratories B.V., Veenendaal, The Netherlands). Proteins were blotted onto a PVDF membrane (Merck Millipore, Billerica, MA, USA), which, after blocking in fat-free milk, was incubated overnight with anti-phospho-Lyn (ab40660, Abcam, Cambridge, UK), anti-Lyn (610003, BD Transduction Laboratories, Breda, The Netherlands), and anti-Actin (sc-1616, Santa-Cruz Biotechnology, Dallas, TX, USA). The proteins were subsequently visualized with the Odyssey imaging system, using IRdye680 and IRDye800 secondary antibodies (LI-COR, Lincoln, NE, USA).

2.8. RNA isolation and RT-MLPA

From 5 patients with high leukocyte counts, blood was collected at baseline, following monotherapy of dasatinib and following two cycles of DF combination treatment. Total RNA was isolated using the GenElute mammalian total RNA miniprep kit (Sigma–Aldrich). A reverse transcription multiplex ligation-dependent probe amplification assay (RT-MLPA, kit R011-C1 Apoptosis, MRC-Holland, Amsterdam, the Netherlands) was performed as described previously to study changes in expression of apoptosis regulating genes [20].

For measuring changes in expression of NF-κB transcripts, RT-MLPA inflammation kit (kit P009; MRC-Holland, Amsterdam, The Netherlands) was used. 4 housekeeping genes (Diablo, Aif, Gusb and Parn) were selected, since their expression was not influenced by irradiation. Expression levels were normalized by the geometric mean of the expression of 4 housekeeping genes (Diablo, Aif, Gusb and Parn) within one sample. Subsequently, gene inductions were calculated by dividing the expression level in the treated sample by the expression in the sample obtained at base-line. As positive control for increased expression of NF-κB transcription, cells from a CLL patient were cultured for three days with 3T3 cells stably transfected with 3T40L or mock transfected as previously reported [21].

2.9. Statistical analyses

2.9.1. Sample size calculation

An overall response rate of at least 20% was considered to be clinically relevant in this poor prognosis patient group. According to the 95% confidence intervals table and nomogram [22], if no partial or complete responses (at 12 weeks) were obtained in the first 14 patients, this would imply a less than 5% chance that the overall response will be at least 20%. The study would then be prematurely terminated. To achieve a power of 80% to detect a RR of 20% (exact 95% CI of 8.4–36.9% (width = 28.5%)) we determined that 35 patients would need to be enrolled.

2.9.2. Statistical analyses

Statistical analyses were performed using Graphpad prism 5.0. Median time-to-event measures and graphs for time-to-event variables were generated using Kaplan–Meier analyses. PFS was measured from the day of first treatment until progression, relapse, or death due to any cause.

Differences in levels of expression of apoptosis genes (MLPA) were calculated by one-way ANOVA test. Data were considered significant in case both fold induction (FI) exceeded 2 (or 0.5) and a p value of less than 0.05 as compared to baseline was found as earlier described [20].