The combination of rituximab, bendamustine, and cytarabine for heavily pretreated relapsed/refractory cytogenetically high-risk patients with chronic lymphocytic leukemia

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Treatment of patients with B-cell chronic lymphocytic leukemia (CLL) relapsed/refractory (R/R) to conventional treatments is particularly challenging. The combination of bendamustine and cytarabine has demonstrated distinct and synergistic mechanisms of action in preclinical studies on cell lines and primary tumor cells of several B-cell lymphomas, including 17p deleted or TP53 mutated CLL. The efficacy of rituximab (375 mg/m², Day 1), plus bendamustine (70 mg/m², days 1–2), and cytarabine (800 mg/m², Day 1–3; R-BAC), every 28 days for up to four courses, was evaluated in a pilot trial enrolling 13 patients with very selected high-risk R/R CLL. All patients (median age 60 years, range 53–74) had symptomatic Binet stage B or C active disease requiring treatment, were characterized by adverse cytogenetics (17p deletion, 11q deletion, or both), unmutated immunoglobulin heavy-chain variable region, and were heavily pretreated (1–5, median three previous lines). Overall, R-BAC was well tolerated with limited non-hematological toxicity. Major toxicities were transient Grade 3/4 neutropenia and thrombocytopenia in 84% and 85% of patients, respectively. Overall response rate (OR) was 84%, including complete and partial response in 38% and 46% of patients, respectively. Patients with 17p deletion had an OR of 78%. After a median follow-up of 17 months, median progression-free survival was 16 months while median overall survival (OS) was not reached (1-year OS: 75 ± 13%). R-BAC is an active regimen in R/R heavily pretreated high-risk patients with CLL, representing an option for the treatment of patients that are usually refractory to standard therapy. Am. J. Hematol. 88:289–293, 2013. © 2013 Wiley Periodicals, Inc.

Introduction

The prognosis of patients with chronic lymphocytic leukemia (CLL) relapsing after first line treatment with alkylating agents and/or fludarabine is poor, with a need for improved treatment strategies [1,2]. Molecular abnormalities have been identified at relapse, including chromosome deletions (i.e., 17p-, 11q-) or single gene mutations (i.e., p53, Notch1) that confer to these patients additional risk, being associated to resistance to conventional therapeutic agents and propensity to disease transformation [3,4]. 17p and 11q deletions are found in around 5% and 20% of cases at diagnosis (de novo deletions), respectively, but can also be acquired during the evolution of the disease. Indeed, the incidence of 17p- in patients with relapsed or refractory CLL can be up to 30% [5].

In particular, for 17p- patients, alternative more toxic therapeutic approaches are generally recommended, such as immunotherapy, high-dose methylprednisolone (HDM), or allogeneic hematopoietic cell transplantation (alloHCT) [6,7]. The overall response rate (OR) to the anti-CD52 antibody alemtuzumab in the relapsed setting was 34%, with OR of 39% in patients with 17p-, 30% in patients with 11q-, and a median progression-free survival (PFS) of 7.7 months [5]. The majority of responses are only partial, particularly in patients with bulky lymphadenopathy, with a high burden of opportunistic infections. The humanized anti-CD20 antibody ofatumumab was recently approved for the treatment of fludarabine- and alemtuzumab-refractory patients, resulting in 58% OR and a median PFS of 5.7 months. However, this antibody showed little benefit in fludarabine-refractory patients harboring the 17p deletion and bulky lymphadenopathy (OR 14%) [8]. Therapy with HDMP, with or without anti-CD20 antibody rituximab, was shown to be effective in refractory CLL [9–11], including cases with the TP53 mutation, with reported OR of 69% and median PFS of 12 months [12].

Bendamustine is an alkylating agent that has purine analogue properties and exhibits considerable activity in indolent lymphomas and CLL [13,14]. It has been reported that bendamustine exerts a cytotoxic effect in CLL cell lines, involving both p53-dependent and p53-independent mechanisms [15]. However, when combined with rituximab in-vivo it resulted in 90% OR in previously untreated patients with del(11q), 94.7% with trisomy 12, and 89.4% with unmutated immunoglobulin heavy-chain variable (IGHV) gene mutational status, while response in 17p- patients was unsatisfactory (37.5%) [16].

The combination of bendamustine and cytarabine has demonstrated distinct and synergistic mechanisms of action in preclinical studies on cell lines and primary tumor cells of several B-cell lymphomas [17,18]. Our group recently

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Conflict of interest: Nothing to report

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Contract grant sponsors: Associazione Vicentina per la Leucemie, i Linfomi e il Mieloma/Associazione Italiana Leucemie (AVILL’AL), Vicenza, Italy; Hematology Project Foundation (HPF), Fondazione Progetto Ematologia, Vicenza, Italy; Mundipharma Pharmaceuticals srl granted the HPF to partially cover the management costs of this study.

Received for publication 30 December 2012; Accepted 8 January 2013


Published online 24 January 2013 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/ajh.23391

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reported that bendamustine significantly potentiated the cytoxic effect of cytarabine in primary tumor cells from patients with CLL, also when 17p- or p53 mutated CLL cells were analyzed [18]. Furthermore, the combination of rituximab, bendamustine, and cytarabine (R-BAC regimen) has shown remarkable activity with acceptable toxicity in older patients with mantle cell lymphoma [19].

With this pilot trial we explored the efficacy and tolerability of R-BAC in a cohort of relapsed or refractory patients with CLL, all showing a clinical, biological and cytogenetic high-risk profile.

Patients, Materials, and Methods

Patients

Patients with previously treated B-CLL were enrolled in this study between February 2010 and September 2012. All patients met the CLL diagnostic criteria of National Cancer Institute Working Group (NCI WG) [20]. To be included patients should have (i) symptomatic Binet stage B or C active disease requiring treatment, as defined by the NCI WG; (ii) previous treatment with at least one immunchemotherapy regimen; (iii) high cytogenetic risk, defined as the presence of 17p-, 11q-, or both. Other eligibility criteria included Eastern Cooperative Oncology Group performance status score 0–2, no major organ dysfunction, age ≥ 18 years (no upper age limit). All patients gave written informed consent. The study was approved by the central ethics review board of the participating institutions as ancillary clinical study of the “CLL Veneto” project, which is a prospective clinical and biological ongoing registry of incident patients diagnosed with CLL recruiting in all major hematology Institution of our geographical region.

Study design, end points, and statistical analysis

This four-center, single arm, open-label, prospective study was conducted to investigate the activity of the R-BAC regimen in a population of heavily pretreated high-risk symptomatic patients with CLL. The primary end-point was the response rate. Secondary end-points included PFS, and overall survival (OS), which were calculated according to the Kaplan and Meier method. Time to progression and OS were measured from the start of R-BAC treatment until disease progression or death, respectively.

Molecular and cytogenetic assessment

To assess IGHV gene mutational status, RNA was obtained from peripheral blood or bone marrow specimens. Sequences were aligned to IMGT and analyzed using IMGT/ VQUEST software. Sequences differing <2% from the corresponding germ-line gene were considered unmutated. Cytogenetic abnormalities involving deletions at chromosomes 11q23, 13q14, 17p13, and trisomy 12 were evaluated by fluorescence in situ hybridization (FISH), either at the time of CLL diagnosis or at the time of study entry.

Treatment regimen and supportive care

The R-BAC regimen [19] consisted of rituximab (375 mg/m² Day 1 of first cycle, then 500 mg/m² for subsequent cycles), bendamustine (70 mg/m², Days 1–2, given as a 1-hr infusion) and cytarabine (800 mg/m², Day 1–3, given as a 2-hr infusion starting 2 hr after bendamustine). Cycles were repeated on Day 29 if the patient had sufficient hematopoietic recovery, or otherwise postponed for at least two more weeks. A maximum of four cycles was planned. Therapy was stopped after two cycles if no partial or complete response was obtained. A cytarabine reduction to 500 mg/m² was planned for patients older than 70 years, for those with inadequate cell count recovery on Day 29 and for Patient 10 who had relapsed after alloHCT.

Primary prophylaxis with granulocyte colony-stimulating factor was routinely used starting from Day 5 after chemotherapy completion, and lasting for 3–6 days or until neutrophil count recovery. The use of erythropoietin was allowed. Rituximab premedication included antihistamines and paracetamol/acetaminophen. Allopurinol was used as prevention of tumor lysis syndrome for at least the first week of treatment. Corticosteroids were not routinely used to premedicate rituximab, but were used in case of infusion-related reactions. The use of systemic steroids or steroid-based collyrum was allowed during treatment with cytarabine, but was restricted to the days of active treatment. Cytarabine was preceded by Dexamethasone 4 mg IV to avoid treatment related systemic side effects.

Criteria for response and toxicity

Responses were graded according to NCI WG criteria [20]. After completion of therapy, patients were followed at 3-month intervals until documented relapse or death to assess duration of response. Response assessment after two and four cycles included physical examination, complete blood count, peripheral blood, and bone marrow examination inclusive of flow cytometry evaluation. Computed tomography or ultra sound was performed in all patients with evidence of tumor mass before treatment.

Hematological toxicity was graded according to NCI WG guidelines [20]. Non-hematological toxicity was assessed according to the NCI Common Toxicity Criteria version 3.0 [http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf].

Results

Patients

Thirteen patients from four participating centers were enrolled and treated between February 2010 and September 2012. Pretreatment characteristics are shown in Table I. The median age was 60 years (range 53–74). Median number of previous lines of immunchemotherapy was 3 (range 1–5). All patients had been pretreated with rituximab, eight (62%) had upfront fludarabine, cyclophosphamide, rituximab (FCR), while in five patients (38%) the first line treatment consisted of rituximab plus chlorambucil or cyclophosphamide, vincristine, prednisone (R-CHVP). These five patients had been considered too old or unfit to receive first-line FCR by the treating physicians. Subsequent lines of treatment included alemtuzumab in five patients, bendamustine in three, while one patient had relapsed after alloHCT (number 10, see Table I). Five patients (38%) were refractory to previous chemoimmunotherapy, including four fludarabine-refractory patients according to the NCI definition (31% of the whole cohort, 50% of patients previously treated with FCR).

At study entry, median time since CLL first diagnosis was 34 months (range 7–117), median absolute lymphocyte count was 46,000/mmc (range 4,100–382,000), Binet stage was B or C and all tested patients had elevated b2-microglobulin. In line with the aggressive clinical behavior of included patients, all showed unmutated IGHV status. FISH analysis revealed the presence of 17p- in six (46%), 11q- in four (31%), or both deletions in three (23%). These abnormalities were accompanied by at least one additional chromosomal gain or deletion in four (Table I).

Administered cycles and toxicity

Nine patients (69%) completed the planned four treatment cycles. Four patients discontinued after <4 R-BAC cycles due to adverse events (n = 1), progressive disease (PD; n = 2), or physician’s decision (n = 1). The adverse event consisted of a fatal pneumonia after the first cycle in a patient who had otherwise achieved partial remission (PR). The decision to stop treatment after three cycles in patient number 10 was taken because this patient had relapsed after alloHCT, had a brilliant response to R-BAC, and was
candidate to donor lymphocyte infusions. Of the 44 treatment cycles administered, four (9%) were delayed. However, each delay lasted < 14 days.

Overall, R-BAC was well tolerated (Table II). The primary toxicity was reversible myelosuppression. Severe neutropenia (median duration 2 days, range 0–5) and thrombocytopenia (median duration 3 days, range 0–6) occurred in 84% and 85% of patients, respectively. Febrile neutropenia occurred in one patient (8%). There was no clear trend towards an increase in cytopenia severity or transfusion requirements with subsequent cycles.

Most non-hematologic adverse events attributed to R-BAC were Grade 1–2 (Table II). Three patients had infections, one of whom had pneumonia which was fatal. Isolated gamma-glutamyl transferase (gamma-GT) elevation (46%) was the second Grade 3–4 non-hematologic event (Table II). One of whom had pneumonia which was fatal. Isolated gamma-glutamyl transferase (gamma-GT) elevation (46%) was the second Grade 3–4 non-hematologic event.

Of the three deaths reported during the study, two were attributed to disease progression and one was attributed to pneumonia.

**Response and survival**

According to NCI-criteria, OR was 84%, with 38% of patients achieving complete remission (CR), 46% who had PR and 16% who had PD. Response according to number and type of prior regimens and clinico-biological characteristics of the disease are shown in Table I. Of note, patients with 17p- had an OR of 78% (CR 33%). After a median follow-up of 17 months (range 2–34), eight patients (62%) were alive and disease-free. Median PFS was 16 months while median OS was not reached (1-year OS: 75 ± 13%, CI: 5%, Fig. 1).

The PFS rates were similar in patients with Binet stage B or C (P = 0.56), in patients with relapsed or refractory disease (P = 0.27), in those who had previous bendamustine or not (P = 0.83), or in patients who had previous FCR or not (P = 0.79). The presence of 17p- was not associated with impaired PFS or OS (P = 0.80).

**Discussion**

The prognosis of CLL patients relapsing or refractory to initial treatments is poor and no clear standard treatment is defined. In patients with these characteristics that are still in good shape and not exceedingly old, which is the case of our study cohort, difficult decisions have to be made, including what treatment, if any, should the patient receive, or whether a donor search should be initiated or not. Based on the results we obtained in a small cohort of very unfavorable patients with CLL, R-BAC appeared well tolerated and very active, being able to induce objective response in 84% of patients, with a remarkable fraction of CR (38%). Moreover, responses appeared somewhat durable, with an encouraging median PFS of 16 months and none of the five patients achieving CR experiencing progression. Median OS was not yet reached (Fig. 1).

The prognosis of patients with 17p- or 11q- CLL has been shown to be secondary to the clinical presentation and for this reason not always dismal. Patients with de novo
deletions, Stage A disease and low percentage of cells harboring the gene deletion should be reassured as they have a favorable outcome [21,22]. In contrast, patients requiring therapy have a significantly worse outcome and, when possible, should be referred for alloHCT due to the absence of alternative curative approaches [23]. Beside the documentation of cytogenetic high-risk features and unmutated IGHV status in all enrolled patients, the clinical behavior was unequivocally aggressive, with refractoriness to standard treatments (including 31% fludarabine refractory) and/or responses to previous therapies lasting <24 months.

Bendamustine, in combination with rituximab, is a treatment of choice in older patients with CLL [13], but its efficacy in patients with high-risk disease (i.e., 17p deletion) was quite low [16]. In a recent trial in the relapsed/refractory setting, rituximab and bendamustine (RB, at a dose of 70 mg/m² combined with rituximab at standard doses) achieved an OR of 59% in a cohort of 72 patients. Responses were 45.5% in fludarabine-refractory patients and 60.5% in fludarabine-sensitive patients, 92.3% in patients with 11q-, 7.1% in patients with 17p-, and 58.7% with unmutated IGHV status [24]. This study included 28% fludarabine-refractory patients and 16% with 17p-. After a median follow-up time of 24 months, the median PFS was 15.2 months (6.8 months for patients with 17p-). Compared with RB, the R-BAC regimen appeared more active in patients with 17p- (OR 77.7% vs. 7.1%), while apparently similar responses were achieved among other risk groups, although our population was more heavily pretreated (median three lines) and had more unfavorable clinical (Stage B or C in all cases) and biologic (100% IGHV unmutated and high-risk FISH) than that of the reported study. Although the number of treated patients in our study is low to drive any definitive conclusion, our preliminary observations confirm that cytarabine is likely to potentiate the anti-tumor effect of bendamustine in CLL patients, as already observed in tumor cell lines of patients with or without 17p- or in patients with other B-cell malignancies including mantle cell lymphoma [18,19].

In CLL patients carrying 17p deletions no therapeutic approach has proved satisfactory, with the exception of alemtuzumab or HDMP [6,9–12]. For this reason, such patients should be recruited, whenever possible, in clinical trials aiming at investigating not only new cytotoxic agents but also the possible role of cellular therapy and immunomodulatory drugs. Alemtuzumab is effective in relapsed or refractory CLL and in patients with high-risk disease, but unfortunately, life-threatening complications and lack of activity in patients with bulky disease limit the use of this monoclonal antibody [25]. Of note, the European Medicines Agency has recently withdrawn the marketing authorization for alemtuzumab, unless for compassionate use. A recent study reported results of treatment with HDMP and rituximab in a cohort of 29 high-risk relapsed patients that had clinical (34% were fludarabine-refractory) and biological features (44% had TP53 abnormalities, 39% had the 11q deletion) similar to our patients. Steroids are known to induce apoptosis in lymphocytes through a number of TP53-independent mechanisms, including suppression of transcription factors, c-myc, and nuclear factor κB, down-regulation of cyclin D3, activation of caspase and multicatalytic proteasome, inhibition of cytokine production, and altered signaling cross-talk [26]. The OR for all patients was 62%. This rate was comparable with that achieved in pretreated patients within the REACH trial with the FCR regimen and with FC plus alemtuzumab (OR: 69.9% and 67%, respectively) [27,28], and higher than that reported for alemtuzumab monotherapy (OR: 34%) [5]. It is noteworthy that the REACH trial did not include fludarabine-refractory patients, and the 17p deletion was present in only 7% of the patients. Importantly, patients with the 17p deletion treated with HDMP and rituximab achieved an OR of 69%, which compares favorably with the 39% reported for alemtuzumab treatment [29]. The OR obtained with R-BAC in this difficult-to-treat group (84%) compares favorably with response rates achieved after HDMP or alemtuzumab treatment [12,21]. In line with this, our median PFS (16 months) was longer than what reported in comparable subgroups of patients treated with HDMP (12 months) or alemtuzumab (8 months). Of note, two of our patients (Patients 7 and 9) underwent alloHCT after four cycles of R-BAC and are both in ongoing remission after 10 and 8 months, similarly to Patient 10 who achieved a CR with R-BAC that was followed by donor lymphocyte infusion from a previous alloHCT.

The toxicity of salvage therapy regimens is always a major concern. Patients treated with R-BAC experienced profound but transient thrombocytopenia and neutropenia in 85% and 64% of cases, respectively (Table II), despite the use of granulocyte colony-stimulating factor. Nevertheless, neutropenic fever was rare (8%). The majority of patients treated with the FCR regimen [27] for relapsed CLL experienced grade III–IV neutropenia (89%), which was complicated by neutropenic fever in 12% of the patients. Because of the presence of adverse events, approximately 50% of refractory patients treated with fludarabine combination regimens need to have dose reductions, skip courses of treatment, or have delays in administration of therapy because of myelosuppression [30,31], while our patients completed the planned 4 R-BAC cycles in 69% of cases with only 9% of cycles that were delayed. The RB regimen was associated with severe infections in 12.8% of patients, in front of a
lower rate of Grade 3 or 4 neutropenia, thrombocytopenia, and anemia (23.1%, 28.2%, and 16.6% of patients, respectively) [24]. Also the HDMP, which are considered low myelosuppressive agents, have been complicated by infections. Despite a low frequency of reported grade III–IV neutropenia (14% of patients) [10], 29% of patients developed infectious complications before completing one month of therapy in one series [11], while Grade 3 infections including two cases of pneumonia and two cases (7%) of febrile neutropenia were reported by Pileckyté et al [12]. In the latter study, one patient died of sepsis.

Other less toxic investigational agents are being actively investigated for the treatment of CLL. Forodesine is a transition-state inhibitor of the purine nucleoside phosphorylase with anti-leukemic activity, bypassing TP53-mediated cell death. In vitro studies showed encouraging activity of single-agent forodesine and its combination with rituximab, regardless of ATM and TP53 status [32]. Lenalidomide has recently shown activity in relapsed patients with CLL with 11q or 17p deletions, with an ORR of 38%, CR of 19%, and median PFS of 12.1 months [33]. A Phase I and a subsequent Phase II study with the serine/threonine kinase inhibitor flaviporid showed promising activity in pretreated patients with CLL; the ORRs for patients classified as 17p-, 11q-, or without these abnormalities were 48%, 57%, and 34%, respectively, and PFS was not significantly different among the cytogenetic groups (around 10 months) [34]. More recently, the Btk inhibitor Ibrutinib (PCI-32765) in combination with Rituximab exhibited impressive early response rates in a patient population that was very similar to our one: of 20 patients evaluable for early response assessment at 3 months, the OR was 85% [35].

In conclusion, our preliminary results demonstrate that the R-BAC combination is an effective and well-tolerated treatment option for patients with relapsed CLL with adverse biologic and genetic features, including TP53 dysfunction. Should these findings be confirmed in larger series of patients with CLL, R-BAC might be proposed as a treatment option in high-risk patients with CLL, also as bridge to allogeneic bone marrow transplant.

References