Pentostatin, Cyclophosphamide, and Rituximab Followed by Alemtuzumab for Relapsed or Refractory Chronic Lymphocytic Leukemia: A Phase 2 Trial of the ECOG-Acrin Cancer Research Group (E2903)

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Keywords

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Abstract

Patients with relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL) may benefit from salvage chemoimmunotherapy (CIT). To explore further the use of CIT in the pre-novel agent era, ECOG-ACRIN undertook a phase 2 trial (E2903) for R/R CLL utilizing pentostatin, cyclophosphamide, and rituximab (PCR) followed by a consolidation course of alemtuzumab. This trial enrolled 102 patients with a median age of 64 years. Treatment consisted of 6 cycles of PCR followed by alemtuzumab for either 4 or 18 weeks depending on the initial response to PCR. The overall response after PCR (complete remission, CR, nodular partial remission, nPR, and partial remission, PR) was 55%. Major responses (CR or nPR)

were achieved in 6%. The median overall survival (OS) and the median progression-free survival were 28 and 12 months, respectively. The most serious nonlethal adverse events were myelosuppression, febrile neutropenia, fatigue, nausea, and hyponatremia. PCR is an effective and well-tolerated nucleoside-based regimen for heavily pretreated CLL patients with R/R disease. The addition of alemtuzumab to CLL patients with a minor response (PR) or stable disease did not result in a significant number of higher responses (CR or nPR) nor an improvement in OS.

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Introduction

Prior to the development of B-cell receptor (BCR) signal inhibitors, treatment of relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL) consisted of purine nucleoside-based therapy in combination with cyclo-

phosphamide and an anti-CD20 monoclonal antibody (chemoimmunotherapy – CIT) [1–4]. The combination of fludarabine, cyclophosphamide, and rituximab can result in long-term survival of treatment-naïve, fit patients with low-risk genetics and mutated immunoglobulin heavy chain variable region (IGVH) [1]. The long-term administration of B-cell signal inhibitors alone [5], or in combination [6], in treatment-naïve patients with a good prognosis has resulted in long-term disease-free survival. Compared to CIT, continuous ibrutinib-based therapy induced superior progression-free survival (PFS) in previously untreated patients [7, 8]. However, only marrow ablative approaches [9, 10] and chimeric antigen receptor-modified T-cell administration [11] have resulted in molecular cure of the disease.

Pentostatin is active either alone or in combination therapy in CLL [12–19]. An earlier phase 2 trial tested a CIT regimen (pentostatin, cyclophosphamide, and rituximab – PCR) and demonstrated significant efficacy in previously untreated elderly patients with CLL [4]. Of particular importance was the ability to administer a full 6 cycles even in patients with diminished renal function [20]. Encouraged by these results, we designed and activated a phase 2 trial, E2903, for R/R CLL that incorporated 6 cycles of PCR followed by an anti-CD52 antibody, alemtuzumab, as consolidation. The use of alemtuzumab was based on its known effectiveness for the treatment of lymphoid neoplasms, including CLL [21–30], its activity in patients with 17p– and p53 mutations [25], and activity in fludarabine-resistant disease [26].

Patients and Methods

Clinical Sites, Patient Eligibility, and Trial Objectives

This was a phase 2 trial for R/R CLL conducted by the ECOG-ACRIN National Clinical Trials Network Group. Eligibility criteria included progressive disease or failure to achieve a meaningful response (less than a partial response [PR]) or relapse after prior therapy (including fludarabine, pentostatin, and rituximab). The definition of progressive disease was based upon the criteria of the National Cancer Institute (NCI) Working Group [31]. Patient requirements included an ECOG performance status of 0–3, serum creatinine <2 mg/dL, and a creatinine clearance >30 mL/min. Patients were ineligible if they had received prior alemtuzumab. Patients with infections were not eligible for study entry until resolution of the infection. All patients were tested for hepatitis B, and those found with evidence of viremia were ineligible. Patients with prior hepatitis B infection and antibodies were eligible. Patients with a history of malignancy, other than squamous/basal cell carcinoma of the skin or in situ carcinoma of the cervix, were not eligible unless the tumor was curatively treated at least 2 years prior to consideration for the protocol.

The primary clinical objectives of the trial were to determine the number of complete remissions (CRs), nodular PRs (nPRs), and PRs. Secondary objectives included estimation of overall survival (OS) and PFS, and the conversion rate to a higher response category after alemtuzumab. Hematologic toxicity was graded according to the NCI Working Group guidelines [31], and nonhematologic toxicity was assessed according to the NCI Common Toxicity Criteria (version 3.0). Clinical and laboratory correlates of outcome were analyzed, including the Rai staging classification [32], β_2 -microglobulin (β_2 -m) [33, 34], and IGVH mutation status. Elevated CD38 and ZAP-70 expression were both defined as >20% in the CLL population [35]. CLL FISH panel status [36] was categorized utilizing the following hierarchy: the high-risk group included 17p–, 11q–, 6q– defects, the intermediate-risk group trisomy 12, and the low-risk group 13q– or normal by FISH.

Study Design

Patients received pentostatin (4 mg/m² i.v.) and cyclophosphamide (600 mg/m² i.v.) on day 1. For the first cycle, rituximab was administered at 100 mg on day 1, followed by 375 mg/m² on days 3 and 5. For subsequent cycles (2–6), rituximab was given at a dose of 375 mg/m² on the same day as pentostatin and cyclophosphamide (PCR). This PCR regimen was given on a 21-day 6-cycle schedule. Bactrim DS (q.o.d.) and acyclovir (800 mg b.i.d.) were administered during the entire course of PCR and alemtuzumab administration. Surveillance for cytomegalovirus (CMV) was performed at each PCR cycle utilizing either pp65 antigenemia [37] or CMV DNA by real-time polymerase chain reaction [38]. Discovery of active disease resulted in cessation of therapy and treatment of CMV. The first cycle of PCR was to be administered at full dose regardless of preexisting cytopenias. During cycle 1, G-CSF (Neupogen) was administered subcutaneously daily, beginning 2 days after treatment for 10 consecutive days or until the neutrophil count was >1.0 × 10⁹/L for 2 consecutive days. Pegfilgrastim (6 mg/m² every 3 weeks) was an alternative option. After the first cycle, the decision to continue administration of growth factors was left up to the treating physician.

Patients achieving a CR or nPR after PCR were then observed during an 8-week treatment-free interval after which a bone marrow study was performed for pathological confirmation of response. Response was evaluated by sites for treatment decisions and reviewed centrally by data managers. After confirmation of response, alemtuzumab (30 mg s.c.) was administered 3 times a week for 4 weeks. The subcutaneous route was utilized because of ease of administration and efficacy [39, 40]. The 4-week course for CR or nPR patients as opposed to the 18-week course for a lesser response was based upon the concept that robust responding patients would need less intensive exposure to alemtuzumab and have less infectious complications [41]. Patients achieving a PR or stable disease after 6 cycles or progressive disease after at least 2 cycles of PCR received alemtuzumab (30 mg s.c. 3 times/week) for 18 weeks after a treatment-free period determined by the investigator.

Criteria for Response

CR and nPR, determined after completion of PCR and after the 8-week hiatus noted above, were defined by the 1996 CLL Working Group criteria [31]. Computerized imaging response criteria were not used to evaluate responses. Progressive disease was characterized by at least 1 of the following criteria: >50% increase in nodal

Fig. 1. CONSORT diagram showing the analytic population of E2903.

disease or appearance of new lymph nodes, increase in the size of liver or spleen, >50% increase in blood lymphocytes, or appearance of the Richter syndrome. Patients who did not achieve a CR, nPR, or PR but exhibited no findings consistent with progressive disease were considered to have stable disease.

Statistical Analyses

The primary endpoint was the response rate to PCR. Response rates and confidence intervals (CIs) were calculated using the method of Atkinson and Brown [42]. The distribution of pretreatment characteristics between responders and nonresponders was determined using Fisher's exact [43] and Wilcoxon rank sum tests [44]. A multivariate logistic model was performed on the response rate to examine the effects of potential risk factors. OS was defined as the time from registration until death from any cause or censored at the last date known to be alive. PFS was defined as the time from registration to disease progression or any cause of death in the absence of progression. OS and PFS were estimated using the Kaplan-Meier method [45]; log rank tests were used to examine the effects of risk factors on OS and PFS. Univariate and multivariate Cox models were performed on OS and PFS to examine the potential risk factors. Factors with $p < 0.15$ in the univariate model were included in the multivariate model as covariates. If >10% of patients had a missing value for a particular covariate, an indicator for whether or not the covariate was missing was indicated in the multivariate model. Landmark analysis at 6 months was done when patients had scheduled PCR treatment cycles completed and

was utilized to compare OS between responders and nonresponders. All p values reported are for two-sided significance tests with p values <0.05 considered significant.

Results

Patient Characteristics

A total of 102 patients were enrolled in E2903 beginning in December 2004 (Fig. 1). The study was closed to accrual in May 2013. Two patients did not begin the assigned therapy (declined to receive therapy or received alternative therapy), and 4 patients were ineligible for response because of violations of the inclusion criteria. Efficacy analysis included 96 patients, and safety analysis (toxicity) included all 100 patients who started treatment. The baseline clinical, laboratory, and prognostic factor characteristics are shown in Table 1. The median patient age was 64 years (range 38–81 years), and the majority of patients were male (79%). An ECOG performance status of 0–1 was present in 96% of patients, and 57% of patients were Rai stages III–IV. The number of patients having received ≥ 2 prior treatments was 56%. Among the 91 pa-

Table 1. Baseline characteristics

	N (%)
Age	
≤70 years	79 (82.3)
>70 years	17 (17.7)
Gender	
Male	76 (79.2)
Female	20 (20.8)
ECOG performance status	
0	53 (55.2)
1	39 (40.6)
2	4 (4.2)
Rai CLL stage	
Missing	2 (2.1)
0–I	29 (30.2)
II	10 (10.4)
III–IV	55 (57.3)
Clinical status at therapy	
First relapse	41 (42.7)
Second or later relapse	27 (28.1)
Refractory	6 (6.3)
Progressive	22 (22.9)
Prior regimens	
Missing	2 (2.1)
1–2	40 (41.7)
>2	54 (56.3)
β ₂ -Microglobulin	
Missing	5 (5.2)
≤3.5 µg/mL	35 (36.5)
>3.5 µg/mL	56 (58.3)
ZAP-70	
Missing	30 (31.3)
Negative	36 (37.5)
Positive	30 (31.3)
CD38	
Missing	30 (31.3)
Negative	32 (33.3)
Positive	34 (35.4)
IGHV	
Missing	45 (46.9)
Unmutated	35 (36.5)
Mutated	16 (16.7)
FISH risk category	
Missing	18 (18.8)
Low	29 (30.2)
Intermediate	12 (12.5)
High	37 (38.5)

tients with serum β₂-m information, 56 (58%) had a level ≤3.5 µg/mL. Of the 66 patients with ZAP-70 and CD38 studies, 45 and 51%, respectively, were positive based upon previous flow threshold determinations [33]. Unmutated IGHV status was found in 68% of the patient material available. Before therapy, FISH data were avail-

able in 78 patients and demonstrated high-risk defects in 47% (17p–, 11q–, 6q), intermediate risk in 15% (trisomy 12), and low risk in 32% (13q–, normal).

Responses to Therapy and Prognostic Factors

The median number of PCR cycles received was 5 (range: 1–6) with 46 completing all 6 cycles (47%). There were 11 patients not evaluable for response to PCR, all having received <2 cycles. The reasons included: death (4), thrombocytopenia (2), disease progression (1), myocardial infarction (1), adverse events (1), treatment with only 1 cycle (1), or no measurements of lymph node size (1). The OR was 55%, consisting of 3 CRs (3%), 3 nPRs (3%), and 47 PRs (49%). Three patients (3%) who were classified as having maintained a complete clinical remission (CCR) after PCR at 8 weeks off-therapy, but without bone marrow confirmation for CR status, received alemtuzumab consolidation. A significant number of patients did not receive consolidation (Fig. 1). The reasons for this included: adverse events/side effects (32), patient withdrawal/refusal (7), death on study (6), disease progression/relapse during active treatment (2), or alternative therapy/other (9). As a result, only 39 eligible patients subsequently received either alemtuzumab for 4 weeks (9 patients) or for 18 weeks (30 patients). Of the 9 patients on alemtuzumab for 4 weeks, all maintained their response status. Of the 30 patients on alemtuzumab for 18 weeks, 2 patients subsequently achieved a CR (7%). Of these 30 patients, 2 were not evaluable for response. One patient refused further therapy after day 1, and 1 patient was taken off treatment after week 3 during the first consolidation course.

Patients with a lower (0–2) Rai stage ($p = 0.04$), fewer prior regimens (<2) ($p = 0.02$), hemoglobin >10 g/dL ($p = 0.03$), β₂-m ≤3.5 mg/L ($p = 0.03$), and CD38 negativity ($p = 0.01$) were more likely to respond to PCR. In a multivariate logistic model, only CD38 <20% was significantly associated with a PCR response ($p = 0.01$). The response rates are 81 and 50% for patients with CD38-negative and -positive status, respectively. Although baseline IGHV status and FISH studies were largely unfavorable, neither was associated with response (IGHV mutation: $p = 0.36$; FISH risk: $p = 0.61$).

Overall Survival and Progression-Free Survival

The median OS was 27 months (90% CI: 20–42) (Fig. 2a) and the median PFS was 12 months (90% CI: 9–14) (Fig. 2b). There was no significant difference in OS between CR/nPR responders (21 months) and lesser responders (29.0 months) ($p = 0.74$).

Fig. 2. Kaplan-Meier estimation of overall survival (a) and progression-free survival of all patients (b). CNSR, censored.

Fig. 3. Kaplan-Meier curves of overall survival according to β_2 -microglobulin (a) and FISH risk category (b). CNSR, censored.

In a multivariate model of OS, both β_2 -m ≤ 3.5 mg/L ($p = 0.01$) and low FISH risk ($p = 0.02$) were significant factors for OS. If β_2 -m was ≤ 3.5 mg/L, the median survival was 56 versus 19 months if the β_2 -m was > 3.5 mg/L (Fig. 3a). For low-risk FISH, the median survival was 47.2 versus 15.9 months for high-risk FISH (Fig. 3b). In a multivariate model, β_2 -m (≤ 3.5 mg/L), IGHV mutation status, and lower FISH risk were not significantly associated with PFS ($p = 0.08$).

Treatment-Related Toxicity

Treatment-related toxicities during PCR and alemtuzumab were those expected in patients with R/R CLL receiving CIT (Table 2). Anemia, thrombocytopenia, and neutropenia were observed in 16, 34, and 60%, respectively, during PCR treatment. The worst degree of non-hematologic treatment-related toxicities during PCR treatment were grades 3–4 in 47 patients (47%) and grade 5 in 5 patients (5%). These included fatigue (9%), nausea

Table 2. Treatment-related toxicities (grades 3–5)

Step 1		Step 2			
PCR treatment, 6 weeks (arm A)	N (%)	alemtuzumab treatment, 4 weeks (arm B)	N (%)	alemtuzumab treatment, 18 weeks (arm C)	N (%)
Anemia	16 (16)	Lymphopenia	8 (89)	Anemia	7 (23)
Lymphopenia	60 (60)	Neutropenia	4 (44)	Lymphopenia	28 (90)
Neutropenia	38 (38)	Platelets	3 (33)	Neutropenia	17 (55)
Platelets	34 (34)			Platelets	13 (42)
Fatigue	9 (9)			Febrile neutropenia	5 (16)
Diarrhea	5 (5)			Fatigue	4 (13)
Nausea	8 (8)			Pneumonitis	2 (6)
Vomiting	7 (7)			Lung infection	5 (16) ^b
Febrile neutropenia	10 (10)			Multiorgan failure	1 (3) ^a
Hyponatremia	6 (6)				
Renal failure	6 (6)				
Tumor lysis syndrome	6 (6)				
Pneumonitis	3 (3) ^a				
Multiorgan failure	1 (1) ^a				
Respiratory disorder	2 (2) ^a				
Septicemia	2 (2) ^a				
ARDS	1 (1) ^a				

ARDS, acute respiratory distress syndrome. ^a One patient had grade 5. ^b Two patients had grade 5.

(8%), and vomiting (7%). Metabolic abnormalities were observed in 18 (18%) patients, but all were less than grade 4. The tumor lysis syndrome was documented in 6 patients, all grade 3. Febrile neutropenia occurred in 10 patients (10%). Most infections, either presumed or proven, were grades 1–3. These included respiratory tract, urinary tract, skin, and ocular site infections. Colitis, unrelated to CMV, occurred in 2 patients (*Clostridium difficile*, unknown). Septicemia was documented in 2 patients, 1 of whom died. Pneumonia, attributable to CMV, occurred in 2 patients. There were 5 treatment-related deaths during the PCR therapy, including multiorgan failure (1), acute respiratory distress syndrome, non-CMV (1), infection (1), and pneumonitis (2).

During the alemtuzumab treatment arms, pain at the injection site, fever, and myalgias were grades 1 or 2. One patient with a grade 4 local injection site reaction was excluded from the study after 2 doses of alemtuzumab. Myelosuppression was as common as during the PCR. One patient developed CMV-positive serology during the 4-week alemtuzumab treatment and 2 patients during the subsequent follow-up. Two patients developed CMV-positive serology during the 18-week alemtuzumab treatment and developed pneumonia.

The autoimmune disorders found in this study are those previously described in CLL patients [46]. Although autoimmune cytopenia occurs in CLL, occasionally related to purine nucleoside or alemtuzumab administration [47–49], there were no cases in the present study. Second malignancy in patients with CLL has been well documented [50–53]. The spectrum of second malignancies documented in this trial included non-Hodgkin lymphoma (5), including cases of Richter's syndrome (3), myelodysplastic syndrome (2), non-melanoma skin tumors (7), breast cancer (1), colorectal cancer (1), esophageal cancer (1), bladder cancer (1), and metastatic undifferentiated adenocarcinoma (1). No cases of melanoma were observed [54].

Discussion

In this study, 55% of R/R CLL patients achieved CR, nPR, or PR during PCR treatment. However, only 3% achieved a CR and 3% an nPR. CD38 <20% was the only prognostic factor associated with a major response. β 2-m was the only significant risk factor for OS, consistent with previous reports [33, 34]. No differences were found in OS and PFS between patients who achieved CR or nPR versus

others, which may be due to the subsequent salvage therapies that the non-major responders received off-study, such as high-dose chemotherapy, immunotherapy, and stem cell transplantation. These results were inferior to those of a previous PCR study without FISH data [55]. Our results of this salvage PCR trial are similar to the results of the fludarabine/cyclophosphamide/rituximab combination in a series of patients having failed multiple prior regimens [56]. In a recent analysis of pentostatin-based therapies for upfront therapy of CLL, an overall response rate across all trials of >90% with a 41% CR rate was reported [57].

The toxicity of PCR CIT appears tolerable, although, during PCR 40 patients (40%) developed grade 3 non-hematologic toxicities, and 12 (12%) patients had grade 4–5 nonhematologic treatment-related toxicities. The rationale for PCR CIT is based on ease of administration and its side effect profile particularly when administering to older patients with renal insufficiency [4].

Numerous publications have documented the use of post-CIT consolidation therapy with alemtuzumab in previously untreated patients, reporting successful eradication of minimal residual disease and clonal mutations, improved survival, and PFS [58–66]. The present study in previously treated patients demonstrated the inability of alemtuzumab to convert a significant number of lesser clinical responses (i.e., PR) to CRs after PCR induction. CMV viremia occurred when alemtuzumab was used alone in heavily pretreated patients [67] or combined with other agents in frontline therapy [68]. In the present study, 4 patients (4%) developed clinical CMV disease.

Therapeutic options for patients with CLL with a poor prognosis have been recently expanded to include ibrutinib [5], idelalisib with rituximab [6], and venetoclax [69]. Although highly active, they may be accompanied by significant side effects such as atrial fibrillation [70], bleeding [71, 72], infectious complications (ibrutinib) [73], immune-related events such as rash, pneumonitis, and/or colitis [74], and the tumor lysis syndrome

(venetoclax). Drug resistance and progression over time are observed with these agents [75]. Our PCR data support the use of CIT in CLL patients particularly if they choose to have short-term rather than long-term therapy [76] or in those in whom administration of ibrutinib may be contraindicated or difficult to administer (i.e., patients on anticoagulants or antiplatelet agents).

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Statement of Ethics

The protocol was reviewed and approved by the institutional review board at each participating institution. All patients were required to provide written informed consent prior to study entry, in accordance with the Declaration of Helsinki.

Disclosure Statement

The authors declare no competing financial interests.

Author Contributions

S.K., N.E.K., Z.S., R.P.K., and E.M.P. analyzed the data and wrote the paper. J.J.M., O.F., D.F.C., J.N.S., G.S., N.S.C., G.G. and M.S.T. provided clinical care, contributed clinical observations, and reviewed the paper.

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