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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)

Sanford Kempin[1], **Zhuoxin Sun**[2], **Neil E. Kay**[3], **Elisabeth M. Paietta**[4], **Joseph J. Mazza**[5], **Rhett P. Ketterling**[6], **Olga Frankfurt**[7], **David F. Claxton**[8], **Joel N. Saltzman**[9], **Gordan Srkalovic**[10], **Natalie S. Callander**[11], **Gerald Gross**[12], **Martin S Tallman**[13]

[1]Beth Israel Comprehensive Cancer Center, New York, NY

[2]Dana Farber Cancer Institute-ECOG-ACRIN Biostatistics Center, Boston, MA

[3]Mayo Clinic, Rochester, MN

[4]Montefiore Medical Center, Bronx, NY

[5]Marshfield Clinic, Marshfield, WI

[6]Mayo Clinic, Rochester, MN

[7]Northwestern University, Chicago, IL

[8]Penn State Milton S. Hershey Medical Center, Hershey, PA

[9]University Hospitals of Cleveland, Cleveland, OH

[10]Sparrow Herbert-Herman Cancer Center, Lansing, MI

[11]University of Wisconsin Hospital and Clinics, Madison, WI

[12]Sanford Medical Center, Fargo, ND

[13]Memorial-Sloan-Kettering Cancer Center, New York, NY.

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 PCR for six cycles followed by alemtuzumab for either 4 or 12 weeks depending upon the initial response to PCR. The overall response after PCR (complete remission (CR), nodular partial remission (nPR),

Corresponding Author: Sanford Kempin, M.D., 11 Fifth Avenue-11-O, New York, NY 10003, 917 887 1615, skmd3@aol.com.

Authorship

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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 (PFS) were 28 months and 12 months respectively. The most serious non-lethal 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.

Keywords

Pentostatin/cyclophosphamide/rituximab; Chemoimmunotherapy; Chronic lymphocytic leukemia; Relapsed/refractory disease; Alemtuzumab

INTRODUCTION

Treatment for relapsed/refractory (R/R) CLL, prior to the development of BCR signal inhibitors, consisted of purine nucleoside based therapy in combination with cyclophosphamide and an anti-CD20 monoclonal antibody (chemoimmunotherapy-CIT).[1–4] The combination of fludarabine, cyclophosphamide and rituximab (FCR) can result in long-term survival for treatment naïve, fit patients with low risk genetics and mutated IGVH.[1] The long-term administration of B-cell signal inhibitors alone,[5] and in combination[6] in treatment naïve and good prognosis patients, has resulted in long-term disease -free survival. Ibrutinib-based therapy versus CIT, in previously untreated patients, resulted in a superior PFS.[7,8] However, only marrow ablative approaches [9,10] and CAR-T cell administration [11] have resulted in molecular cure of the disease.

Pentostatin is active alone and in combination in CLL.[12–19] An earlier phase 2 trial tested a CIT regimen (PCR) and demonstrated significant efficacy in previously untreated elderly patients with CLL.[4] Of particular importance was the ability to administer a full six 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 NCTN Group (ECOG-ACRIN). Eligibility criteria included progressive disease or failure to achieve a meaningful response (< 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 > 30ml/min. Patients were ineligible if they had received prior alemtuzumab. Patients with infections were not eligible for entry onto the study 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 two years prior to consideration for the protocol. 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 entry, in accordance with the Declaration of Helsinki.

The primary clinical objectives of the trial were to determine the number of complete remissions (CR), nodular partial remissions (nPR) and partial remissions (PR). Secondary objectives included estimation of the overall survival (OS) and progression-free survival (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 non-hematologic 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] β2-microglobulin (β2-m) [33,34] and immunoglobulin heavy chain variable region (IGVH) mutation status. Elevated CD 38, 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: high-risk group included 17p-, 11q-, 6q- defects, intermediate risk patients were trisomy 12 and low risk patients were 13q- or normal by FISH.

Study design

Patients received pentostatin (4mg/m^2 IV) and cyclophosphamide (600mg/m^2 IV) on day one. Rituximab was administered for the first cycle at 100 mg on day 1, followed by 375 mg/m^2on days 3 and 5. For subsequent cycles (2–6) rituximab was given at a dose of 375 mg/m^2 on the same day as the pentostatin and cyclophosphamide (PCR). This PCR regimen was given on a 21-day 6-cycle schedule. Bactrim DS (QOD) and acyclovir (800mg BID) were administered during the entire course of PCR, and alemtuzumab administration. Surveillance for cytomegalovirus (CMV) was performed at each cycle of PCR 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 the CMV. The first cycle of PCR was to be administered at full dose regardless of preexisting cytopenias. During cycle one G-CSF (Neupogen) was administered SQ daily, beginning two days after treatment for 10 consecutive days or until the neutrophil count was greater than 1.0×10^9/L for 2 consecutive days. Pegfilgrastim (6mg/ m^2 every three 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 pathologic confirmation of response. Response was evaluated by sites for treatment decisions and reviewed centrally by data managers. After confirmation of response, alemtuzumab (30mg SQ) was administered

three times a week (TIW) for four weeks. The subcutaneous route of administration was utilized because of ease of administration and efficacy.[39,40] The four 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 (SD) after six cycles or progressive disease (PD) after at least 2 cycles of PCR received alemtuzumab (30mg SQ TIW) for 18 weeks after a treatment-free period determined by the investigator.

Criteria for response

CR and nPR response criteria, determined after completion of PCR and after the 8 week hiatus noted above, was defined by the 1996 CLL Working Group criteria.[31] Computerized imaging response criteria were not used to evaluate the responses. PD was characterized by at least one of the following criteria: >50% increase in nodal disease or appearance of new lymph nodes, increase in the size of liver or spleen, >50% increase in blood lymphocytes or appearance of Richter's syndrome. Patients who did not achieve a CR, nPR or PR but exhibited no findings consistent with PD were considered to have SD.

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 non-responders was determined using the 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 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 mode. Landmark analysis at 6 months was done when patients had scheduled PCR treatment cycles completed and was utilized to compare the overall survival between responders and non-responders. All p-values reported are for two-sided significance tests with p-values under 0.05 considered significant.

RESULTS

Patient characteristics

A total of 102 patients were enrolled in E2903 beginning in December 2004 (Figure 1). The study was closed to accrual in May 2013. Two patients did not begin assigned therapy (declined to receive therapy; received alternative therapy) and four 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 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 patients with serum β2-m information, 56(58%) had a level ≤3.5 mgs/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 available patient material. Pre-therapy FISH data was available 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 factor analysis

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 < 2cycles. The reasons included: death(4),thrombocytopenia(2), disease progression(1), myocardial infarction(1), adverse events(1), received one cycle(1), or no measurements of lymph node size (1). The OR was 55%, consisting of 3 CR's (3%), 3 nPR's (3%) and 47 PR's (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 (Figure 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, two patients were not evaluable for response. One patient refused further therapy after day 1 and one 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 >10g/dl (p= 0.03), β2-m ≤ 3.5 (p=0.03), and CD 38 negativity (p=0.01) were more likely to respond to PCR. In a multivariate logistic model only CD 38 <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)(Figure 2A) and the median PFS was 12 months (90% CI: 9–14)(Figure 2B). There was no significant difference in OS between CR/nPR responders (21 months) and lesser responders (29.0 months)(p=0.74).

In a multivariate model of OS, both β2-m ≤3.5 mg/L (p=0.01) and low FISH risk (p=.02) were significant factors for OS. If β2-m was ≤ 3.5 mg the median survival was 56 months versus 19 months if the β2-m was > 3.5 mg/L (Figure 3A). For low-risk FISH the median

survival was 47.2 months versus 15.9 months for high-risk FISH (Figure3B). In a multivariate model, β2-m(≤ 3.5mg/L), IGHV mutation status, and lower FISH risk were not significantly associated with PFS (p=.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 (8%) and vomiting (7%). Metabolic abnormalities were observed in 18(18%) patients but all were less than grade 4. Tumor-lysis syndrome was documented in six 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 two patients (C. difficile, unknown). Septicemia was documented in 2 patients, one of whom died. Pneumonia, attributable to CMV occurred in 2 patients. There were 5 treatment-related deaths during the PCR therapy including multi-organ failure (1), ARDS, non-CMV (1), infection (1), pneumonitis (2).

During the alemtuzumab treatment arms, pain at the injection site, fever, and myalgias were grades I or 2. One patient with a grade 4 local injection site reaction was removed from 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 two patients in subsequent follow-up. Two patients developed CMV positive serology during the 18-week alemtuzumab treatment, two of whom 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 (NHL) (5), including three cases of Richter's syndrome, 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,. CD 38 <20% was the only prognostic factor associated with a major response. β2-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 trial of PCR are similar

to the results of FCR 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 over 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 related toxicities and 12 (12%) patients had 4–5 non-hematologic treatment-related toxicities. The rationale for PCR CIT therapy is based on ease of administration and 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 MRD and clonal mutations, improved survival and progression-free survival. [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 occured when alemtuzumab was used alone in heavily pre-treated patients[67] or combined with other agents in frontline therapy. [68] In the present study four 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 tumor lysis syndrome(venetoclax). Drug resistance and progression over time are observed with these agents. [75] Our data with PCR support the use of CIT in CLL patients particularly if they choose to have short-term rather than long-term therapy [76] or 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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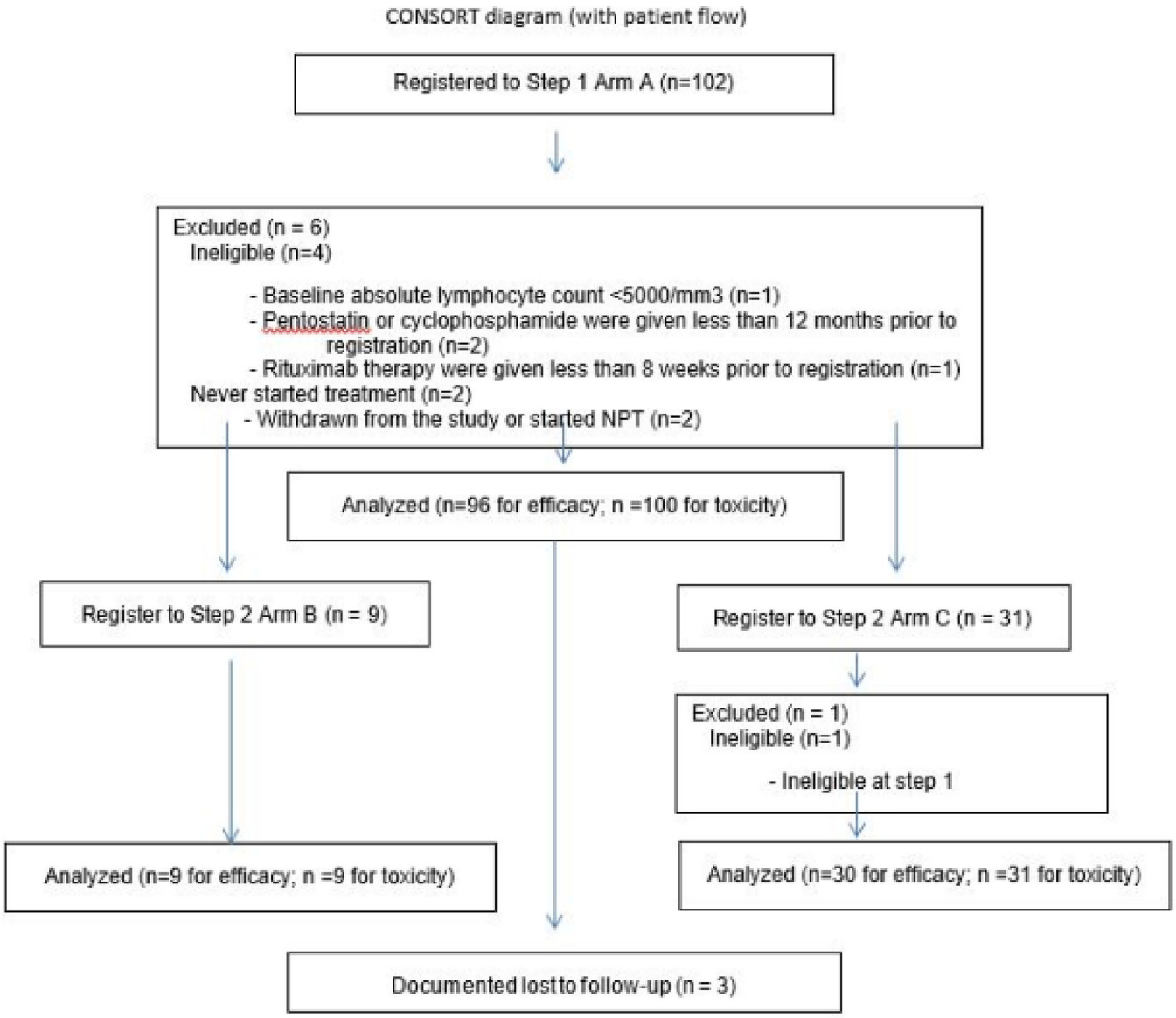
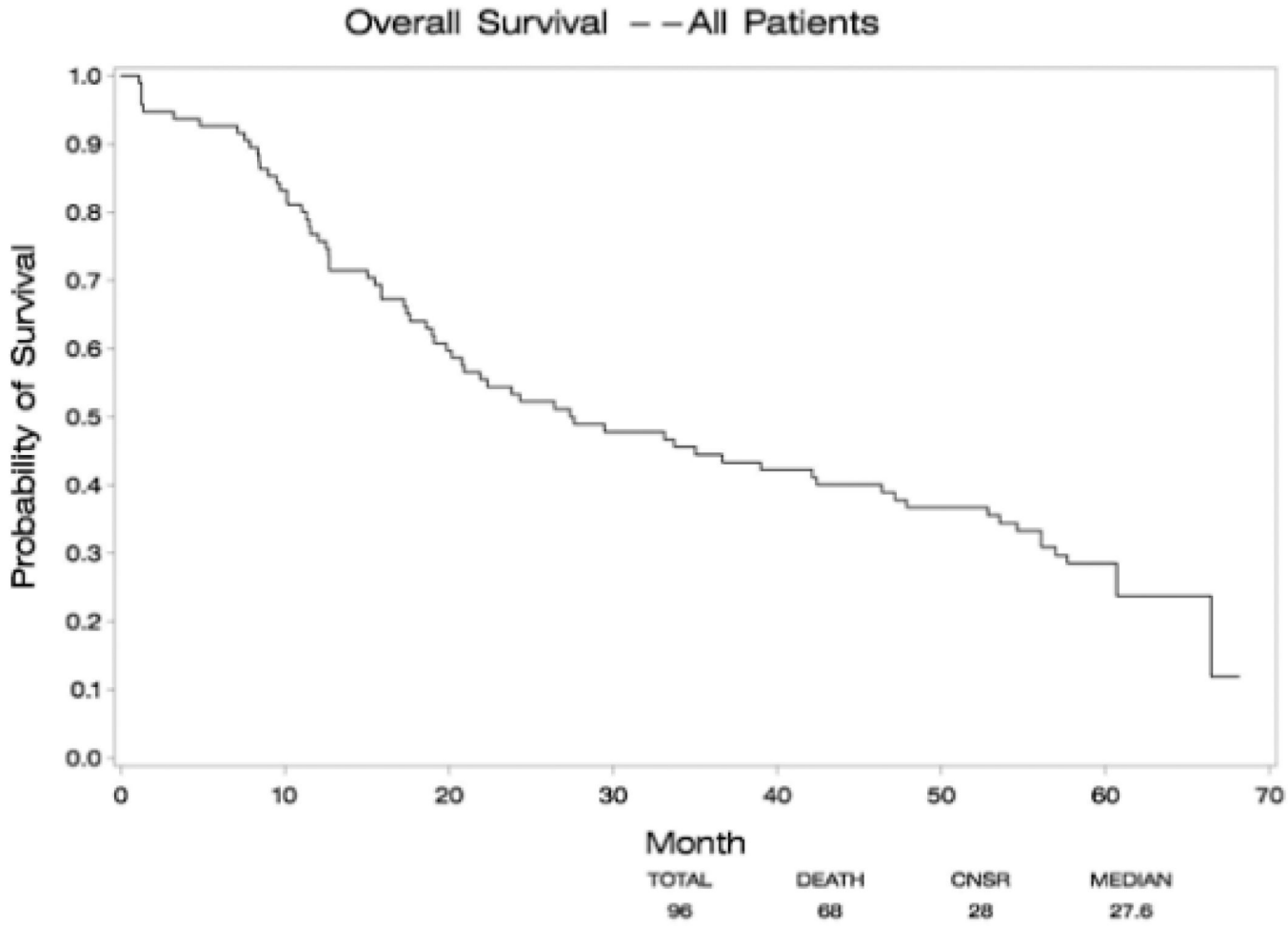


Fig 1.
CONSORT diagram showing the analytic population of E2903.



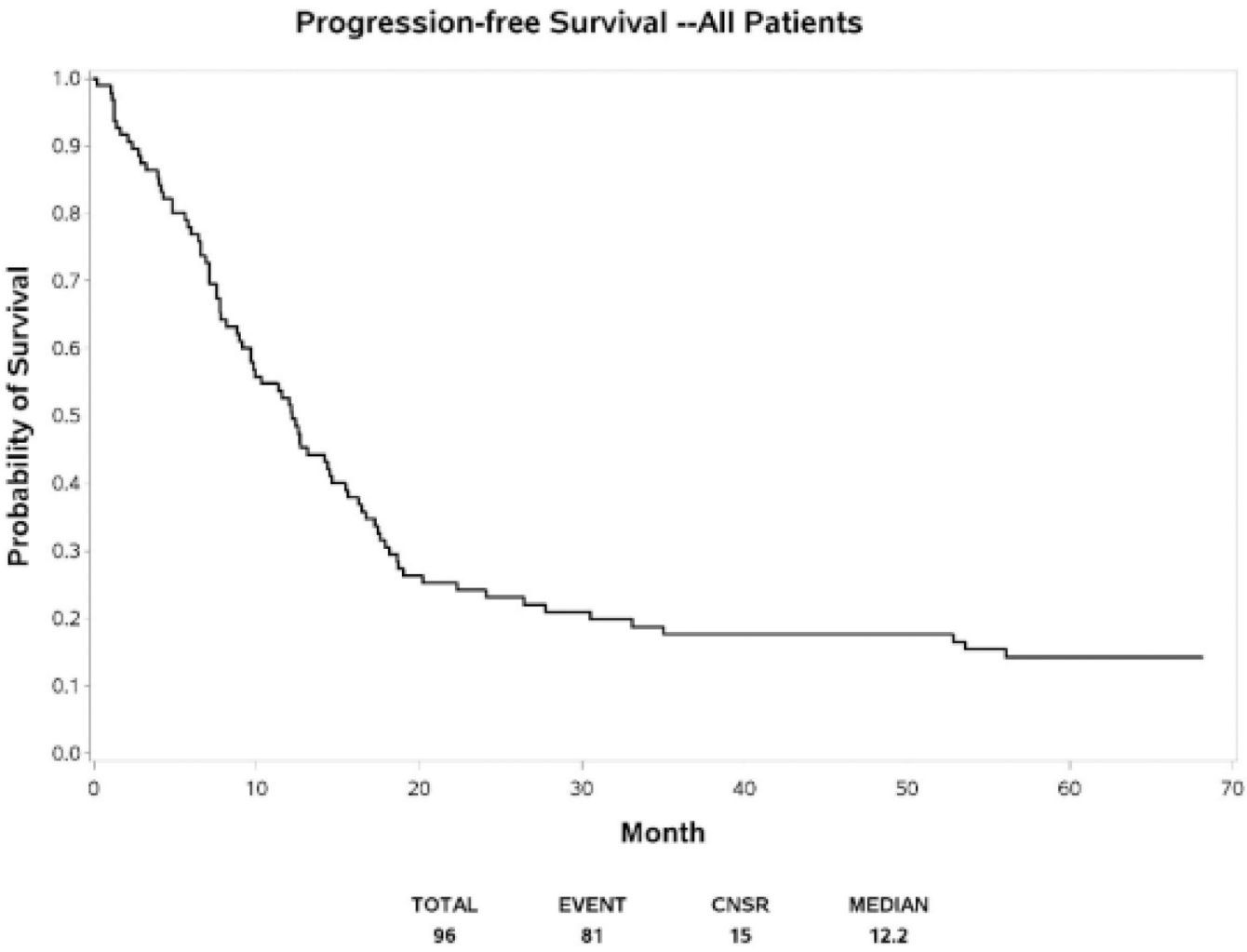
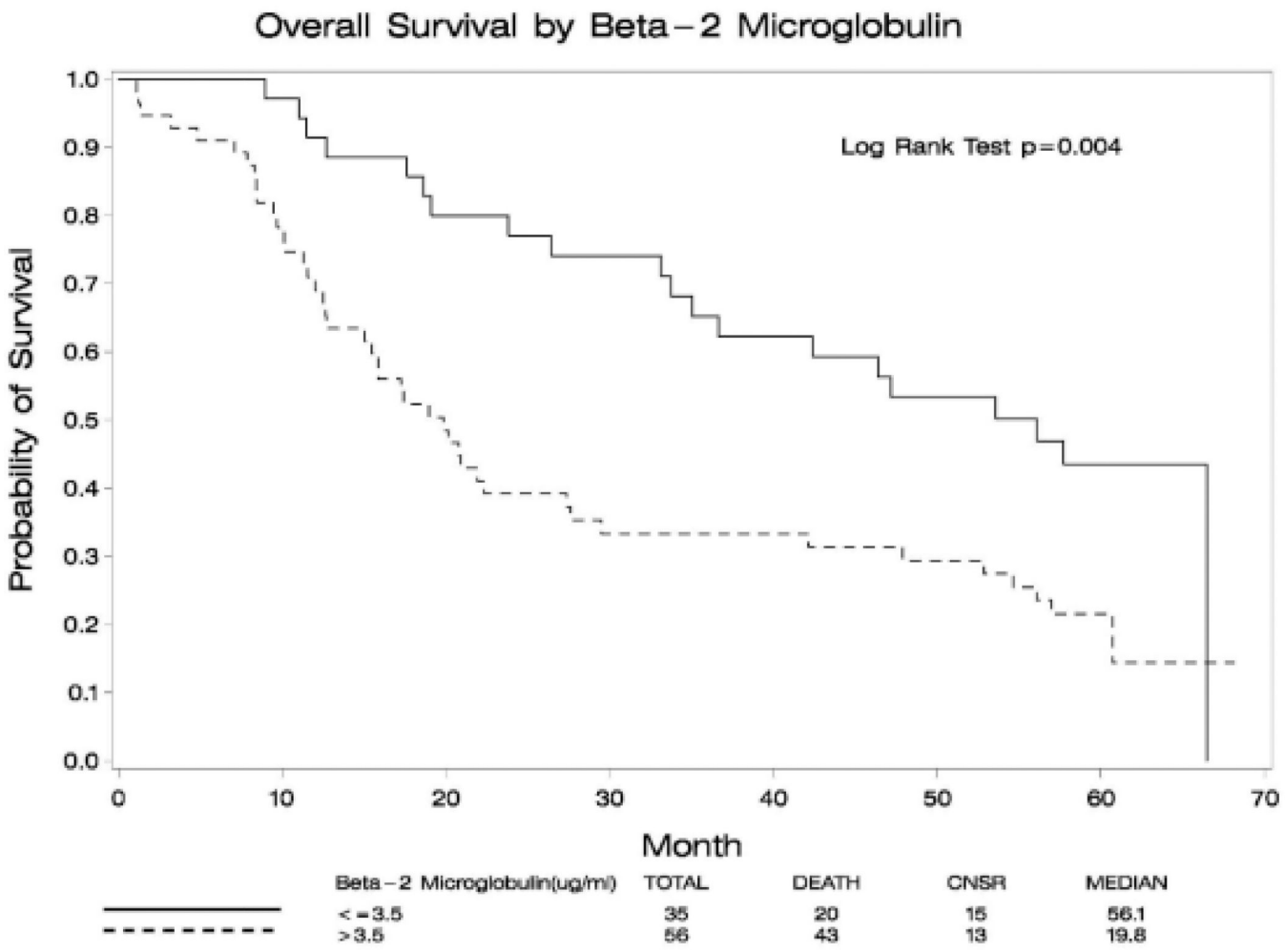


Fig 2.

Kaplan-Meier estimation of (A) overall survival (OS) of all patients, (B)progression-free survival (PFS) of all patients.



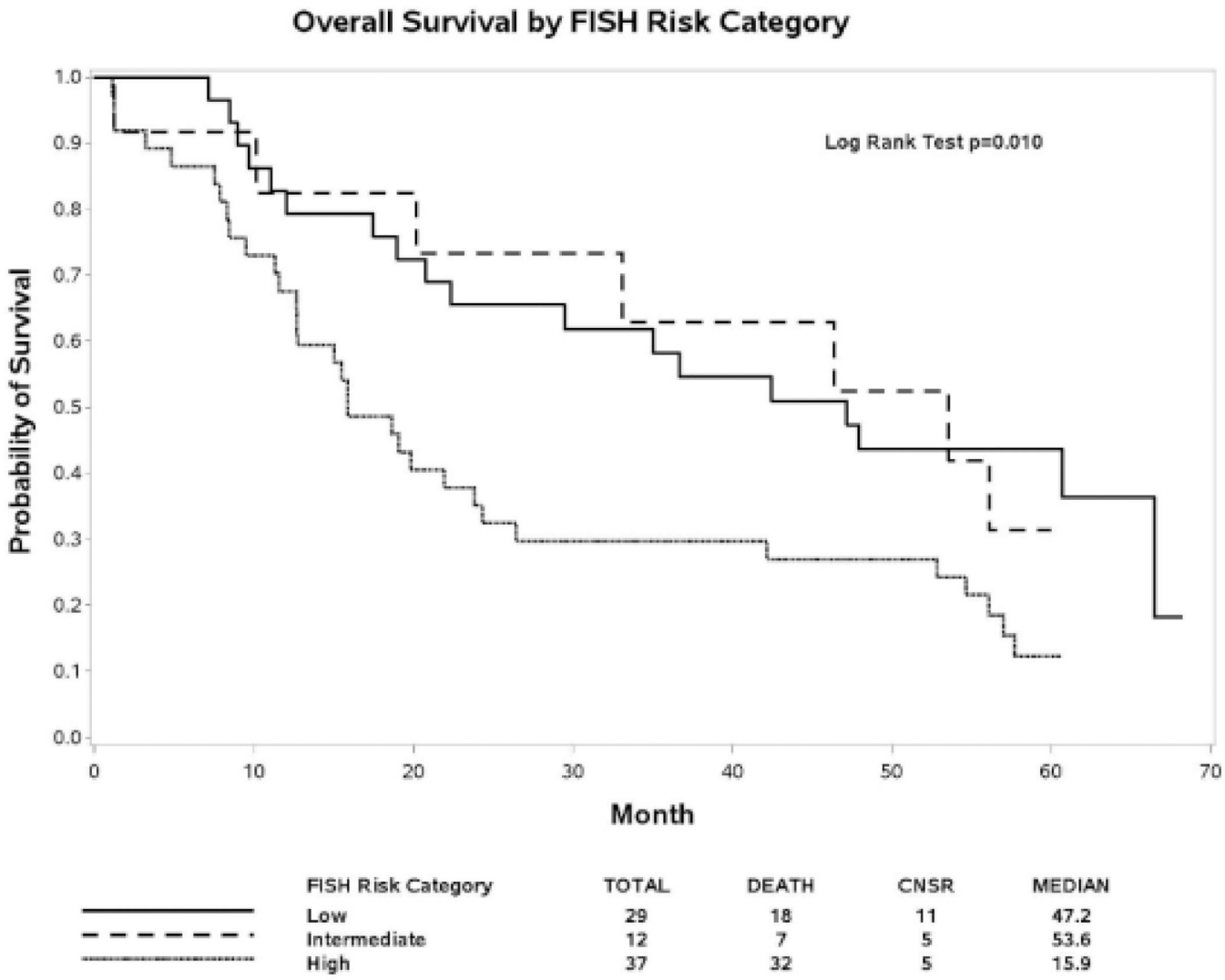


Fig 3.

Kaplan-Meier curves of OS according to (A) β−2 Microglobulin (B) FISH Risk Category.

Table 1:

Baseline characteristics

	N (%)
Age (yrs)	
<=70	79 (82.3)
>70	17 (17.7)
Gender	
Male	76 (79.2)
Female	20 (20.8)
ECOG PS	
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)
No. Prior Regimens	
Missing	2 (2.1)
1–2	40 (41.7)
>2	54 (56.3)
β-2 Microglobulin (μg/ml)	
Missing	5 (5.2)
<=3.5	35 (36.5)
>3.5	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	

	N (%)
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)

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- 18weeks (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)			F. Neutropenia	5 (16)
Diarrhea	5 (5)			Fatigue	4 (13)
Nausea	8 (8)			Pneumonitis	2 (6)
Vomiting	7 (7)			Lung infection	5 (16) **
F. Neutropenia	10 (10)			M-organ failure	1 (3) *
Hyponatremia	6 (6)				
Renal failure	6 (6)				
T. Lysis Syndrome	6 (6)				
Pneumonitis	3 (3) *				
M-organ failure	1 (1) *				
Respiratory disorder	2 (2) *				
Septicemia	2 (2) *				
ARDS	1(1) *				

* 1 of those patients had Grade 5

** 2 of those patients had Grade 5