Efficacy and safety of front-line therapy with fludarabine-cyclophosphamide-rituximab regimen for chronic lymphocytic leukemia outside clinical trials: the Israeli CLL Study Group experience

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ABSTRACT

This study aimed to evaluate the efficacy and safety of the fludarabine-cyclophosphamide-rituximab regimen for young physically fit patients with chronic lymphocytic leukemia in the "real-life" setting. We specifically focused on the impact of dose reduction on patient outcomes. The patient cohort consisted of 128 patients with chronic lymphocytic leukemia (≤70 years) treated at 10 Israeli centers with front-line fludarabine-cyclophosphamide-rituximab. We defined reduced chemotherapy as two-thirds or less of the total indicated dose. Patients treated with rituximab were divided into two groups and compared: those who received full dosages of 375 mg/m^2 or 500 mg/m^2, and patients given less than six cycles with either dose. Overall and clinical complete response rates (92.8% and 70.4%), as well as toxicities and overall survival (median not reached at 6 years), were similar to other reported clinical trials, but progression-free survival was shorter (42.5 months). Almost 50% of patients had some dose reduction of chemotherapy, 21% receiving less than two-thirds of the indicated dose, while close to 30% did not complete six cycles of rituximab. Reduced doses of chemotherapy and rituximab were independently associated with shorter progression-free survival (hazard ratio 3.6, P<0.0001 for reduced chemotherapy; hazard ratio 2.5, P=0.003 for incomplete-treatment with rituximab). Achieving a complete response was associated with longer overall survival but was not linked to the given dose of chemoimmunotherapy. In younger physically fit patients, front-line fludarabine-cyclophosphamide-rituximab therapy in the "real-life" setting achieves long remissions (albeit shorter than in clinical trials) and prolonged overall survival. However, dose reductions are commonly administered and may impact outcome.

Introduction

The treatment of chronic lymphocytic leukemia (CLL) has evolved impressively over the years. For more than three decades, patients with CLL were treated mostly with alkylating agents as monotherapy, particularly chlorambucil.[1] In this regard, the first real advancement occurred during the 1980s, with the introduction of the purine analogs fludarabine,[2-4] cladribine,[5] and pentostatin[6] which were used effectively. Fludarabine (F) is the most extensively studied purine analog in patients with CLL. When used in combination with cyclophosphamide (C) in the FC regimen, F was clearly shown to be more effective than when given alone.[7] More recently, the addition of rituximab (R) to the FC combination (FCR) was also studied in CLL.[8-10] A single arm study in previously untreated patients with CLL conducted at the MD Anderson Cancer Center in Houston, showed that six cycles of rituximab (375 mg/m^2 cycle 1 and 500 mg/m^2 cycles 2-6) given together with fludarabine (25 mg/m^2, days 1-3) and cyclophosphamide (250 mg/m^2, days 1-3) achieved a high complete remission rate and prolonged duration of response.[8,9] Similarly, a large phase III study from the German CLL Study Group (CLL8) in previously untreated physically fit patients, demonstrated the superiority of FCR over FC with better complete response rates, progression-free survival (PFS) and overall survival (OS).[10] In the light of these data, FCR has become the mainstay of treatment for physically fit patients with CLL.

Despite its proven efficacy, FCR is associated with significant toxicities, in particular myelosuppression and a higher incidence of infections.[10] As a result, outside of formal clinical trials, patient adherence to the protocol is often compromised, and treating physicians are frequently tempted to decrease drug doses or reduce the number of treatment cycles.[11] Furthermore, the therapeutic concerns about toxicities become more problematic in older patients with comorbidities and renal dysfunction who have been under-represented in earlier clinical trials. Thus, there may be significant differences in outcome in patients treated with FCR within the framework of a clinical study when compared to patients treated in everyday life. In an attempt to reduce associated toxicity, some investigators used modified FCR-based regimens, employing protocols with reduced doses of chemotherapy and increased amounts of rituximab,[12] sequential F→C→R regimen,[13] and even substitution of fludarabine by pentostatin[14] or cladribine.[15,16]

The present study aimed to evaluate the efficacy and safety of the FCR regimen for physically fit CLL patients under 70

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years of age in the "real-life" setting. We specifically focused on the impact of dose reduction on patient survival and outcomes. In addition, we also examined treatment-related side-effects, including the rate of infections, degree of myelosuppression, frequency of autoimmune hemolytic anemia (AIHA) and the development of secondary malignancies.

Methods

Patients and study design

This was a retrospective cohort study of 128 young patients with CLL (≤70 years) from 10 Israeli medical centers who were treated with the intravenous FCR regimen as front-line therapy during the years 2002-2013. We extracted data from the patients' medical records including: complete blood count (CBC), creatinine clearance, Binet stage, available imaging results (abdominal ultrasound or CT scan of neck, chest and abdomen), analyses of genomic aberrations (fluorescent *in situ* hybridization, FISH), mutational status of immunoglobulin heavy-chain variable-region (IgHV) genes (using a cut off of 98% homology to the germ-line sequence), CD38 level (using a cut off of 30% $CD38^+$-CLL cells[17]), and ZAP70 expression (determined by flow cytometry as previously described by Crespo *et al.*[18] using a cut off of 20% $ZAP70^+$-CLL cells). We obtained data regarding: dose modifications of chemotherapy and rituximab, number of treatment cycles, severe adverse events documented according to the Common Toxicity Criteria for Adverse Events Classification (CTC-AE), use of antiviral and pneumocystis prophylaxis, the administration of granulocyte-colony stimulating factor (G-CSF), late-onset neutropenia (defined in the *Online Supplementary Appendix*),[19] and the occurrence of secondary malignancies. The study was approved by the institutional ethics committee of each participating center according to the principles of the Declaration of Helsinki.

Dose intensity

We defined reduced dose of chemotherapy as two-thirds or less of the total indicated dose. We also evaluated the subgroup of patients who received a total dose of less than 90% of the total indicated dose. Regarding rituximab, we divided the patient population into three subgroups: those who had completed a first cycle with rituximab 375 mg/m^2 followed by five subsequent cycles of rituximab 500 mg/m^2 (R-500); patients who had received six cycles of rituximab 375 mg/m^2 (R-375); and patients who did not receive the full six cycles of either regimen (R-incomplete).

Analysis of outcomes

Overall response rate was assessed as clinical complete response (CR), CR with incomplete bone marrow recovery (CRi), partial response (PR), or failure. Assessment of response followed the recommendations of the International Workshop on CLL 2008 (IWCLL2008) for general practice,[20] which includes physical examination, complete blood count (CBC), and a bone marrow biopsy and aspiration in patients with cytopenia of uncertain cause. Progression-free survival (PFS) was defined as the period from the time of commencing FCR therapy to the date of first documented disease progression, death from any cause or last follow up. Overall survival (OS) was calculated form the first date of FCR treatment to the date of death from any cause or last follow up.

Statistical analysis

We used the Fisher exact test to compare categorical variables, the Student *t*-test for normally distributed variables, the Mann-Whitney U test for non-normally distributed variables, and the Jonckheere-Terpstra test for non-normally distributed variables in ordered categories. Exploratory analyses for PFS and OS were performed using a univariate Cox proportional hazard models for continuous predictors and the Kaplan-Meier and log rank tests for categorical covariates. We then evaluated variables collectively using a multivariate Cox proportional hazard model. Two-sided statistical significance was set at P=0.05. All analyses were performed using SPSS (IBM, USA).

Table 1. Demographic data and base-line characteristics.

Parameter	N. patients (%) Total n=128
Median age, years (range)	57.9 (18-69)
Age >65 years	18 (14.1%)
Sex (male)	93 (72.7%)
Binet stage:	
A	11 (8.6%)
B	71 (55.5%)
C	46 (35.9%)
Splenomegaly (n=124)	102 (82.3%)
Bulky disease (lymph node≥5cm) (n=126)	29 (22.0%)
Positive CD38 (n=96)	43 (44.8%)
ZAP70- positivity (n=69)	33 (47.8%)
Unmutated IGHV (n=61)	42 (68.9%)
Chromosomal aberration by FISH: (n=96)	
del (13q)	22 (22.9%)
trisomy 12	14 (14.6%)
del (11q)	21 (21.9%)
del (17p)	7 (7.3%)
Absolute lymphocyte count (x10^9/L, mean±SD)	130±101
Hemoglobin (gr/dL, mean±SD)	11.7±2.2
Platelets (x10^9/L, mean±SD)	148±67
Creatinine clearance (mL/min, mean±SD)	82±22
Median time to treatment (months)	34.5

Results

Patients' characteristics

Patients' characteristics are shown in Table 1. A total of 128 CLL patients (≤70 years of age) from 10 medical centers treated with FCR as front-line therapy were reviewed. The majority of patients were males (72.7%, n=93) and under 65 years of age (85.9%, n=110). Eleven patients (8.6%) had Binet stage A disease, 71 (55.5%) were stage B, and 46 (35.9%) were stage C. Results of FISH analysis were available for 96 patients, with high-risk cytogenetics of del(11q) in 21 patients (21.9%) and del(17p) in 7 cases (7.3%). The majority of patients (55.9%, n=71) initially received treatment according to a protocol of 500 mg/m^2 rituximab, and the remainder according to a 375 mg/m^2 protocol. Of these, 31.5% (n=40) completed a 6-cycle treatment with 375 mg/m^2 (R-375), 38.6% (n=49) completed a full treatment with 500 mg/m^2 (R-500), and 29.9% (n=38) received less than six cycles of therapy (R-incomplete). Almost half of the cases (47.7%, n=61) had some dose reduction of chemotherapy (<90% of the indicated dosage), while 21.1% (n=27) had a considerable dose reduction of two-thirds or less of the indicated dosage. The median time from diagnosis to first treatment was

34.5 months. The median follow up for the entire cohort from the date of first treatment was 36.7 months (range 4-142 months).

Reduced total dose of chemotherapy

Twenty-nine patients (22.7%) were given a reduced dose of chemotherapy from the initiation of therapy. Reasons for dose reductions in this group were: treating physician's personal discretion in 75.9% (n=22), cytopenias in 13.8% (n=4), and low creatinine clearance in 10.3% (n=3). Twenty-seven patients (21.1%) had a secondary reduction in the chemotherapy doses, mainly because of cytopenias (74.1%, n=20), treating physician's discretion (18.5%, n=5), and infections (7.4%, n=2). Compared to patients receiving full dose (52.3%, n=67), patients who received lower doses of chemotherapy (less than 90%) had lower hemoglobin (Hb) level (11.2 *vs.* 12.2 g/L; *P*=0.009), lower platelet counts (136x10^9/L *vs.* 159x10^9/L; *P*=0.05) and accordingly higher Binet stages (48% *vs.* 25% Binet stage C; *P*=0.007). When evaluating the differences between patients receiving two-thirds of the total dose or less (21%, n=27) to the rest of the cohort, differences in Hb, platelet count and Binet stage C were more pronounced, and the difference in CCT values was statistically significant (70.4 *vs.* 85.0 mL/min; *P*=0.007). There was no difference in other patients' characteristics (e.g. age, sex, splenomegaly, bulky disease, CD38, ZAP70, chromosomal abnormalities).

Forty-two patients (32.8%) failed to complete six cycles of rituximab as a result of: cytopenias (45.2%, n=19), treating physician's discretion (33.3%, n=14), refractory disease (14.3%, n=6), infectious complications (4.8%, n=2), and associated second malignancy (2.4%, n=1). Patients with incomplete rituximab treatment had lower Hb compared to patients who received six courses of rituximab (10.9 g/dL for R-incomplete *vs.* 12.2 g/dL for R-375 and 11.9 g/dL for R-500; *P*=0.02). There were no other statistically significant differences between the rituximab treatment groups.

Response to treatment

The majority of patients achieved clinical CR [70.4%, n=88, including 12 (9.6%) cases of CRi], followed by PR in 22.4% (n=28); only 9 patients (7.2%) failed to respond. Lower response rates were associated with advanced Binet stage (rate of Binet stage C in the corresponding response groups: CR 29.5%, PR 46.4% and NR 66.7%; *P*=0.04) and presented with lower platelet counts (CR 160x10^9/L, PR 122x10^9/L, NR 112x10^9/L; *P*=0.007). Mutational status was significantly associated with clinical response (*P*=0.002). All NR patients (n=7) and 92.3% (12 of 13) patients with PR were IgHV unmutated compared to 55% (22 of 40) patients who achieved a CR. Patients with NR had higher rates of del(17p) compared to responders (25% in NR *vs.* 6% in CR+PR; *P*=0.05).

Non-response showed a trend towards a statistically significant association with lower doses of chemotherapy compared to PR and CR (two-thirds of the total indicated dose in 44.4% of NR, 25.0% of PR and 14.8% of CR; *P*=0.07). There was no difference in age, sex or other disease characteristics (Hb, absolute lymphocyte count, splenomegaly, bulky disease, CD38 and ZAP70) between the different response subgroups (*Online Supplementary Appendix*). Similarly, there was a higher rate of incomplete rituximab treatment in the non-responders as compared to

Table 2. Univariate analyses of prognostic factors for progression free survival.

Parameter	HR	95% CI	*P*
Age	1.00	0.97-1.06	0.696
Sex	1.17	0.68-2.04	0.574
Binet stage (C *vs.* A or B)	2.29	1.35-3.87	0.002
Splenomegaly	1.14	0.59-2.20	0.706
Bulky disease	0.61	0.29-1.30	0.200
Absolute lymphocyte count (per increase of 10x10^9 cells/L)	1.03	1.01-1.06	0.017
Hemoglobin (per decrease of 1gr/dL)	1.20	1.04- 1.35	0.009
Platelet count (per decrease of 10x10^9 platelets/L)	1.04	1.00-1.09	0.045
Del17p	3.91	1.63-9.36	0.002
Full FC dose (<90% *vs.* full dose)	1.72	1.03-2.88	0.038
FC dose (≤2/3 *vs.* full dose)	3.56	2.00-6.34	<0.0001
Rituximab full cycles (375 mg/m^2 *vs.* 500 mg/m^2)	0.57	0.31-1.04	0.065
Rituximab dose (375 mg/m^2 full cycles *vs.* incomplete dose)	0.39	0.21-9.73	0.003

HR: hazard ratio; CI: confidence interval.

PR and CR (R-incomplete in 77.8% of NR, 32.1% of PR, and 26.1% of CR; *P*=0.006). Treatment with six cycles of 500 mg/m^2 as opposed to 375 mg/m^2 of rituximab was not associated with a statistically significant difference in response rate (*P*=0.1).

Progression-free survival

During the follow-up period, 61 patients progressed or relapsed. The median progression-free survival for the entire cohort was 42.5 months (95%CI: 34.7-50.2) (Figure 1A). Binet stage C was associated with a reduced median PFS compared to stages B or A (25.0, 51.8 and 69.6 months, respectively; *P*=0.006). Chromosomal aberrations correlated with PFS (*P*=0.008), and in particular patients with del(17p) had a much shorter PFS compared to those without this abnormality (12.8 *vs.* 40.9 months; HR-3.9, *P*=0.002) (Figure 1B). Mutational status was significantly associated with PFS. Patients with unmutated IgVH had a median PFS of 36.46 months *versus* 63.8 months in the mutated group (HR=2.52, 95%CI: 1.05-6.04; *P*=0.03). Patients receiving a considerably reduced dose of chemotherapy (two-thirds or less) had almost a 4-fold increase in hazard for progression (HR 3.6, 95%CI: 2.0-6.3; *P*<0.0001) (Figure 1C), and an eventual decrease in estimated PFS from 46.9 months (for those receiving the full dose) to 18.7 months. Completing six cycles of 375 mg/m^2 of rituximab was associated with a longer PFS compared to an incomplete course (HR=0.39, 95%CI: 0.2-0.7; *P*=0.003) (Figure 1D). The full R-375 was also associated with a trend towards better PFS when compared to the R-500 (HR=0.57, 95%CI: 0.3-1.0; *P*=0.065). Additional factors associated with shorter PFS were higher ALC counts before treatment (HR=1.03 for every 10x10^9 increase in ALC counts; *P*=0.017), lower Hb (HR=1.2 for every 1 g/dL decrease in Hb; *P*=0.009), and lower platelet counts (HR=-1.04 for every 10x10^9 decrease in platelet count; *P*=0.045) (Table 2). There was no statistically significant difference in median PFS between the age groups of those patients under 65 years of age and those between 65 and 70 years (43.0 *vs.* 27.2 months; *P*=0.19) (*Online Supplementary Appendix*). In a multivariate analysis (including age, sex, Binet stage, CBC indices pre-treatment, and presence of bulky disease or splenomegaly) receiving a considerably reduced dose of chemotherapy (i.e. two-thirds), incomplete rituximab dose and del(17p) retained their statistical significance (HR=7.3, 95%CI: 2.6-20.5, *P*<0.0001 for del(17p); HR=3.3, 95%CI: 1.1-9.8, *P*=0.03 for reduced dose; HR=3.3, 95%CI: 1.3-7.7, *P*=0.01 for R-incomplete) (Table 3).

Overall survival

Overall there were 26 deaths during the study period. The median overall survival for the entire cohort was 99.5 months (95%CI: 76.4-122.5) (Figure 2A). The most common cause of death was related to infectious complications (46.1%, n=12) (mostly in cases with active CLL) followed by second malignancies (23.1%, n=6, including acute myeloid leukemia in 1 patient, Richter's transforma-

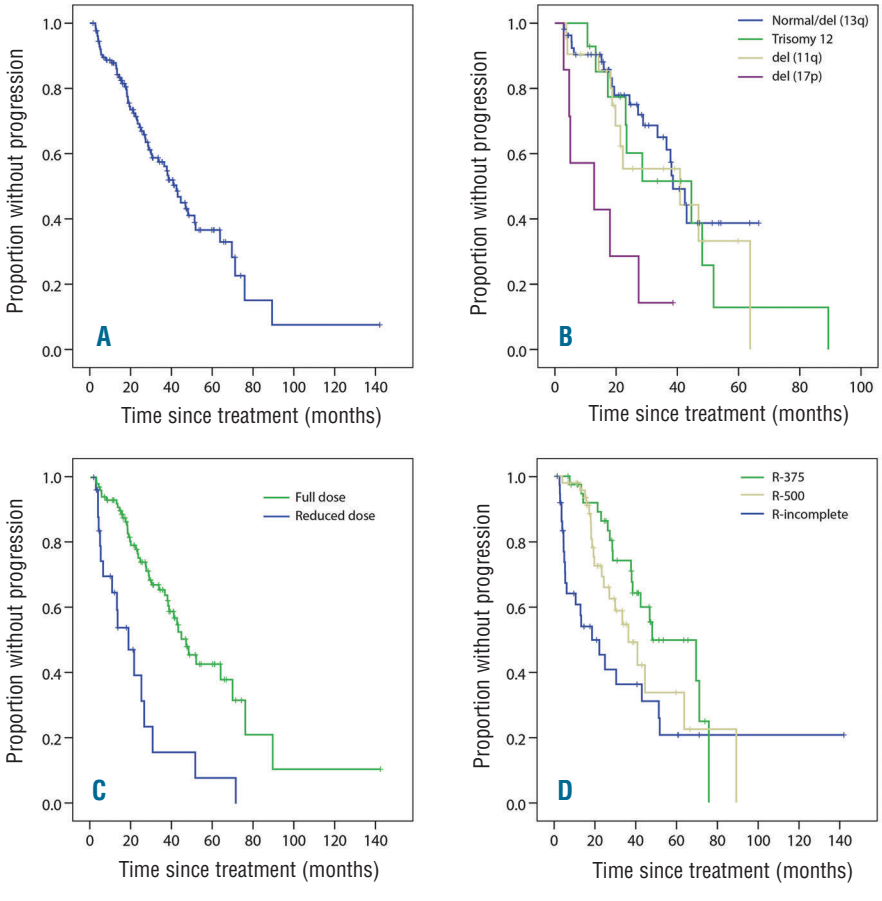

Figure 1. Progression-free survival in all patients (A), according to genetic subgroups (B), in patients given full and reduced (≤2/3) doses of chemotherapy (fludarabine and cyclophosphamide) (C), and in patients who had completed a first cycle with rituximab 375 mg/m^2 followed by five cycles of rituximab 500 mg/m^2 (R-500); patients who had received six cycles of rituximab 375 mg/m^2 (R-375); and patients who did not receive the full six cycles of either regimen (R-incomplete) (D).

tion in 2, melanoma in 1, metastatic carcinoma of unknown primary in 1, and carcinoma of colon in 1). There were no deaths during treatment. In univariate analyses, Binet stage, del(17p) and response were significantly associated with OS (Table 4). Binet stage C was associated with a reduced median survival compared to stages B or A (99.5, 124.5 months, and not reached, respectively; *P*=0.04). Chromosomal abnormalities by FISH also correlated with OS. In particular, del(17p) was linked to reduced median OS (21.6 *vs.* 94.0 months; *P*=0.03) (Figure 2B). Albeit the dismal prognosis of patients with del(17p), the OS curve of this subgroup showed a late plateau, representing patients who had a survival benefit following allogeneic stem cell transplantation. Patients with no response to treatment had a significantly worse overall survival (median survival months 23.3 for NR *vs.* 65.1 for PR and 124.5 for CR; *P*<0.0001) (Figure 2C). Patients with unmutated IgHV gene had a median OS of 94.0 months *versus* not-reached in mutated IgHV patients. However, this difference was not statistically significant (*P*=0.15). There was no statistically significant difference in median OS between the age groups of those patients under 65 years of age and those between 65 and 70 years (99.5 *vs.* 65.1 months; *P*=0.28) (*Online Supplementary Appendix*). In the multivariate analysis, only del(17p) and overall response rate retained statistical significance. Del(17p) was associated with a 7-fold increase in HR for all-cause mortality (HR=6.8, 95%CI: 1.4-33.5; *P*=0.019), and overall response with an over 10-fold decrease in HR (HR=0.08, 95%CI; 0.02-0.43; *P*=0.003) (Table 5). There was no statistically significant association between OS and chemotherapy dose, rituximab dose or any of the other demographic and clinical parameters (age, sex, disease stage, splenomegaly, bulky disease, blood count indices). Finally, when comparing patients who achieved CR to patients with PR or NR, CR was associated with a 4-fold decrease in HR (HR=0.25, 95%CI: 0.11-0.56; *P*=0.001). This association remained statistically significant in a multivariate analysis (*P*=0.04).

Table 3. Multivariable analyses of prognostic factors for progression-free survival.

Parameter	HR	95% CI	*P*
Age	0.9	0.9-1.1	0.88
Sex	1.3	0.6-2.9	0.52
Splenomegaly	1.1	0.4-2.9	0.84
Bulky disease	1.6	0.7-3.8	0.30
Absolute lymphocyte count	1.0	0.9-1.1	0.51
Hemoglobin	0.9	0.7-1.1	0.41
Platelet count	0.9	0.9-1.0	0.42
Binet stage	5.6	0.5-59.7	0.15
Del(17p)	7.3	2.6-20.5	<0.0001
≤ ⅔ FC dose	3.3	1.1-9.8	0.03
Rituximab-incomplete	3.3	1.3-7.7	0.01

HR: hazard ratio; CI: confidence interval.

Table 4. Univariate analyses of prognostic factors for overall survival.

Parameter	HR	95% CI	*P*
Age	1.03	0.97-1.09	0.358
Sex	1.02	0.44-2.37	0.971
Binet stage (C *vs.* A or B)	2.53	1.15-5.58	0.021
Splenomegaly	1.57	0.47-5.30	0.464
Bulky disease	0.88	0.33-2.36	0.799
Absolute lymphocyte count (per increase of $10x10^9$ cells/L)	1.01	0.98-1.05	0.533
Hemoglobin (per decrease of 1 gr/dL)	0.847	0.70- 1.03	0.093
Platelet count (per decrease of $10x10^9$ platelets/L)	0.95	0.88-1.02	0.145
Del17p	3.69	1.05-12.90	0.041
Full FC dose (<90% *vs.* full dose)	0.68	0.31-1.51	0.347
FC dose (≤2/3 *vs.* full dose)	0.66	0.24-1.80	0.418
Rituximab full cycles (375 mg/m² *vs.* 500 mg/m²)	0.60	0.69-1.96	0.601
Rituximab dose (375 mg/m² full cycles *vs.* incomplete dose)	0.69	0.24-1.99	0.493
Response (response *vs.* no-response)	0.08	0.03-0.22	<0.0001
Response (CR *vs.* PR or NR)	0.25	0.11-0.56	0.001

HR: hazard ratio; CI: confidence interval.

Adverse events

During treatment, the most commonly recorded severe adverse events (grade 3-4) included at least one episode of infection 25.0% (n=32) or neutropenia 23.4% (n=30). Treatment-related direct anti-globulin (DAT) positive autoimmune hemolysis occurred in 4.7% (n=6), and an additional 2 patients developed AIHA that was DAT negative (2 cases required ≥10 blood transfusions). Late onset neutropenia occurred in 6.5% of patients (n=7 out of 107 eligible for this evaluation), Richter transformation in 3.1% (n=4), and therapy-related myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML) developed in 3 patients (2.3%).

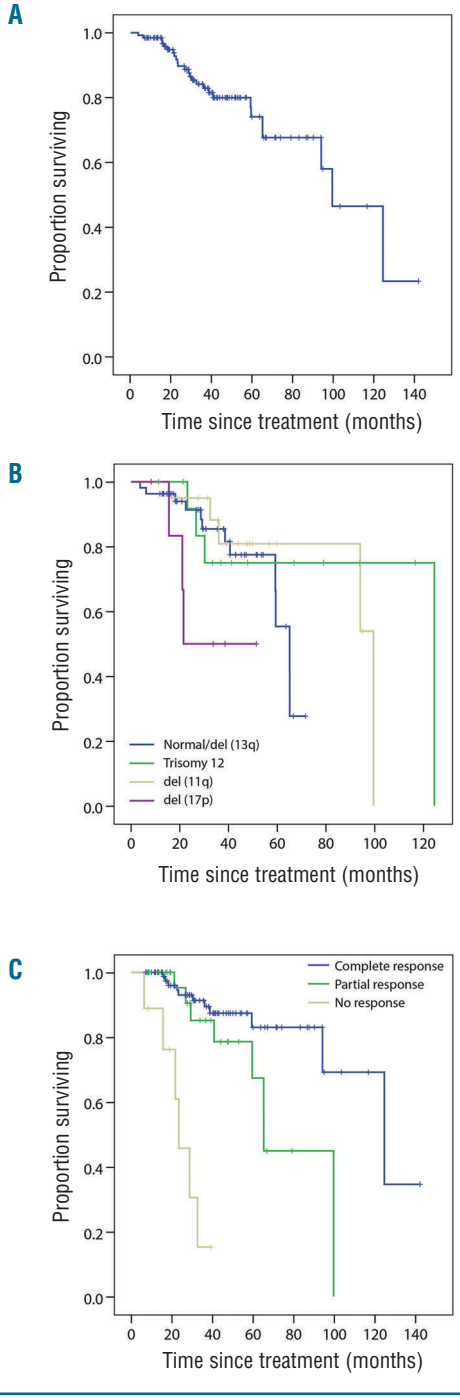

Figure 2. Overall survival in all patients (A), according to genetic subgroups (B) and response to therapy (C).

Infection prophylaxis

Prophylaxis with G-CSF was given to 71.5% of the patients: as primary prophylaxis in 51.2% of cases and as secondary in 20.3%. Prophylaxis for *Pneumocystis pneumonia* (PCP) was administered to most patients (93.8%, n=120) and for Herpes virus in only 36 patients (28.1%). During the period of treatment Varicella-Zoster reactivation was seen in only one patient who was not taking prophylaxis at the time. None of the patients received antibiotic prophylaxis.

Discussion

Fludarabine-cyclophosphamide-rituximab regimen was the first regimen shown to prolong survival in first-line treatment of physically fit CLL patients. However, despite its efficacy, this treatment is associated with significant toxicities. Subsequently, outside of clinical trials, when this regimen is used in a less selective patient population, dose modifications are common, and outcome may be compromised. In this study, we evaluated the outcome of FCR therapy in 128 young and physically fit patients treated in the "real-life" setting, focusing on the impact of dose reduction. Almost half of the cases had some dose reduction of chemotherapy, with 21% receiving a dose reduction of two-thirds or less of the indicated dose. Nearly one-third of the patients did not complete six cycles of rituximab. Reduced doses of chemotherapy and rituximab were independently associated with lower response rates and shorter PFS. Achieving a complete response was further associated with OS but not linked to the actual dose of chemoimmunotherapy.

In our study, clinical complete response was achieved in approximately 70% of the patients. This response rate is higher than that reported in previous clinical trials, probably due to a different definition of response.[10] In this regard, response in our patient population was evaluated outside of a clinical trial and was, therefore, assessed according to the IWCL2008 recommendations for general practice.[20] Based on these guidelines, response to therapy

Table 5. Multivariable analyses of prognostic factors for overall survival.

Parameter	HR	95% CI	*P*
Age	1.0	0.9-1.1	0.70
Sex	0.7	0.2-2.7	0.65
Splenomegaly	0.5	0.1-2.5	0.37
Bulky disease	1.6	0.5-5.8	0.46
Absolute lymphocyte count	1.0	0.9-1.0	0.79
Hemoglobin	0.9	0.6-1.3	0.62
Platelet count	1.0	0.9-1.0	0.69
Binet stage	1.5	0.4-6.4	0.55
Del(17p)	6.8	1.4-33.5	0.019
≤ ⅔ FC dose	0.5	0.1-4.0	0.49
Rituximab-incomplete	2.2	0.4-12.5	0.61
Overall response	0.08	0.02-0.4	0.003

HR: hazard ratio; CI: confidence interval.

was determined by physical examination and CBC, while a bone marrow examination was only required in cases with unexplained cytopenia. Accordingly, the CR rate of our cohort cannot be compared to that reported in clinical trials like CLL8, which assessed responses using a total body CT scan and bone marrow biopsy. Of note, previous studies of FCR employing the IWCL2008 response criteria for general practice showed similar response rates.[8,11]

Regarding PFS, the median of our population (42.5 months) was lower compared to the clinical trial experience of CLL8 (51.8 months) and MDACC (approx. 80 months). This discrepancy may be attributed to differences in the studied patient populations. For example, our cohort included patients with creatinine clearance of less than 70 mL/min (29%) and a greater proportion of patients with Binet stage C disease. In addition, in our study, the median chemotherapy cumulative dose was lower and the median follow up was shorter. Interestingly, CR rates and PFS were also considerably higher in the only other study evaluating FCR outside of a clinical trial (CR+CRi of 99% and median PFS of 56 months) performed by Bouvet *et al.*[11] These differences could be in part due to a patient population with milder disease [Binet stage C in only 18%, compared to 36% in our study and no patients with del(17p)] and exclusion of patients who did not complete six cycles of therapy.[11] Finally, in all the aforementioned studies, including ours, the median survival was not reached at six years, and the most common causes of death were infections and secondary malignancies. These observations reinforce the expected prolonged survival in CLL patients treated by FCR, but also suggest that outcomes may be poorer when administering FCR in the "real-life" setting as opposed to in a clinical trial.

Overall, the type and rate of severe complications during front-line FCR therapy seem to be quite similar both in the "real-life" setting and in clinical trials. As expected, the most common severe complications were infections. These occurred in over 25% of the patients but none were fatal. Conversely, grade 3-4 neutropenia was documented less frequently compared to other reports (23.4% *vs.* 33.7%),[10] probably since half of the patients received primary prophylaxis with G-CSF. In our patient cohort, the rate of MDS/AML was 2.3%, which is similar to that reported in other studies.[9] Interestingly, AIHA was encountered more frequently than in the German CLL8 trial (5.3% *vs.* <1%).[10] However, this rate was similar to that recorded in other studies where it was evident in cases with both pre-treatment positive and negative DAT,[8] supporting the concept that the DAT test does not necessarily predict subsequent development of AIHA.[21] In some reported cases, AIHA was DAT negative, but appeared to be autoimmune-mediated and responsive to corticosteroids.[12]

Similar to our results, Bouvet *et al*. recorded dose reduction in nearly one-third of CLL patients treated with FCR in the community (26% FC reduction of over 20% and 36% rituximab reduction). They too demonstrated a considerably reduced PFS in these patients.[11] Likewise, patients who received reduced doses generally had more severe disease and more comorbidities (as evident by a higher rate of Binet stage C and a reduced creatinine clearance). It is noteworthy that, in our study, reduced dose retained its statistical significance even after controlling for these covariates in a multivariate analysis. It is of interest that, in both studies, in nearly half of the cases dose reduction was made at the discretion of the treating physician and not necessarily because of disease status, toxicity or comorbidities. Thus, it is apparent that outside of clinical trials physicians readily lower the dose of chemotherapy irrespective of objective clinical and laboratory parameters.

The standard recommended dose of rituximab, namely one cycle of 375 mg/m followed by five cycles of 500 mg/m^2, was not associated with improved response rates, PFS or OS compared to six cycles of 375 mg/m^2. This is contrary to previous retrospective studies that demonstrated a PFS benefit for higher doses of the drug.[11] In this respect, the optimal dose of rituximab in combination with chemotherapy has not been prospectively tested in CLL. Rituximab at a dosage of 500 mg/m^2 was empirically chosen on the basis of early studies of monotherapy which demonstrated that dose escalation to greater than 375 mg/m^2 resulted in better overall responses.[22,23] Conversely, other studies demonstrated that rituximab at 375 mg/m^2 given concurrently with chemotherapy has good efficacy in CLL.[15,24-26] With the introduction of next generation anti-CD20 antibodies (e.g. obinutuzumab) and other novel agents into clinical practice, issues relating to the best dose of rituximab in CLL are unlikely to be addressed, and this question will probably remain unanswered.[27,28]

In conclusion, FCR given as front-line therapy to young and physically fit CLL patients in the "real-life" setting achieves long remissions (albeit shorter than in clinical trials) and prolonged overall survival with a similar toxicity profile to that reported in clinical trials. It is apparent that in "real-life", treating physicians readily reduce doses of chemotherapy based on their own personal judgment and not necessarily according to objective parameters. Reduced doses of fludarabine, cyclophosphamide and rituximab have an impact in CLL and appear to jeopardize PFS. Furthermore, and not unexpectedly, achieving response, and in particular CR, correlates well with prolonged overall survival irrespective of the drug doses administered. Recently, early discontinuation of treatment has been reported feasible in patients with CLL who achieved negative minimal residual disease following FCR treatment.[29] Our data further emphasize the fact that, although abbreviated therapy with FCR may be a reasonable approach in a subgroup of patients, in others it may considerably jeopardize clinical outcome.

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