Outcome of advanced chronic lymphocytic leukemia following different first-line and relapse therapies: a meta-analysis of five prospective trials by the German CLL Study Group (GCLLSG)

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ABSTRACT

To evaluate the effect of first-line and subsequent therapies, the outcome of 1,558 patients with chronic lymphocytic leukemia from five prospective phase II/III trials conducted between 1999 and 2010 was analyzed. The 3-year overall survival rate was higher after first-line treatment with chemoimmunotherapies such as fludarabine/cyclophosphamide/rituximab (87.9%) or bendamustine/rituximab (90.7%) compared to chemotherapies without an antibody (fludarabine/cyclophosphamide: 84.6%; fludarabine: 77.5%; chlorambucil: 77.4%). Furthermore, the median overall survival was longer in patients receiving at least one antibody-containing regimen in any treatment line (94.4 months) compared to the survival in patients who never received an antibody (84.3 months, P<0.0001). Univariate Cox regression analysis demonstrated that patients who did not receive antibody treatment had a 1.42-fold higher risk of death (hazard ratio, 1.42; 95% confidence interval: 1.185-1.694). Therapies administered at relapse were very heterogeneous. Only 55 of 368 patients (14.9%) who started second-line treatment >24 months after first-line therapy repeated the first-line regimen. Among 315 patients requiring treatment ≤24 months after first-line therapy, cyclophosphamide/doxorubicin/vincristine/prednisone with or without rituximab as well as alemtuzumab were the most commonly used therapies. In these early relapsing patients, the median overall survival was shorter following therapies containing an anthracycline and/or three or more cytotoxic agents (e.g. cyclophosphamide/doxorubicin/vincristine/prednisone or fludarabine/cyclophosphamide/mitoxantrone, 30.0 months) compared to single agent chemotherapy (e.g. fludarabine; 39.6 months) and standard chemoimmunotherapy (e.g. fludarabine/cyclophosphamide/rituximab: 61.6 months). In conclusion, the analysis confirms the superior efficacy of chemoimmunotherapies in patients with chronic lymphocytic leukemia. Moreover, the use of aggressive chemo(immuno)therapy combinations in patients with an early relapse does not offer any benefit when compared to less intensive therapies. *Trial identifier: NCT00281918, ISRCTN75653261, ISRCTN36294212, NCT00274989 and NCT00147901.*

Introduction

Chronic lymphocytic leukemia (CLL) has a highly variable clinical course: aggressive forms of CLL are as life-threatening as an acute leukemia whereas indolent forms can be managed with a watch-and-wait approach without treatment for many years.

The clinical management of CLL recently underwent considerable changes, which led to improved outcomes for patients with this disease.[1] After the use of monotherapies such as chlorambucil over several decades,[2-4] significant improvements in response rates and progression-free survival (PFS) were achieved with purine analog-based combination therapies [e.g. fludarabine and cyclophosphamide (FC)].[5-7] However, a prolongation of overall survival (OS) was first achieved by adding the monoclonal antibody rituximab to purine analog-based regimens.[8] As a consequence, chemoimmunotherapy with anti-CD20 antibodies became the standard first-line treatment for CLL both in physically fit patients[8-11] and in patients with relevant comorbidities.[12] The recently approved kinase inhibitors idelalisib and ibrutinib, and novel antibodies will further improve the outcomes of patients. Despite these advances CLL remains an incurable disease so far and most patients will eventually relapse.

Earlier versions of the European Society for Medical Oncology (ESMO) guidelines[13] recommended repeating first-line treatment in the case of a remission lasting 12–24 months after monotherapy or 24-36 months after chemoimmunotherapy. In the case of earlier progression and unfavorable prognosis, a change of treatment strategy was suggested, e.g. allogeneic stem cell transplantation in physically fit patients. As an allogeneic hematopoietic stem cell transplantation is not

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feasible in many cases because of the patient's age, fitness, burden of comorbidities or lack of a matching donor, intensive chemo(immuno)therapies, such as the combination of cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) with or without rituximab or FC plus mitoxantrone (FCM), as well as an alemtuzumab-based regimen have often been used, even though these regimens are relatively toxic.[14-18] Due to a lack of controlled clinical trials in patients with relapsed CLL, therapeutic decisions often rely on the experience of the treating physician and on preliminary evidence from phase II trials. The aim of this meta-analysis was, therefore, to evaluate the outcome of patients treated with different first-line and relapse therapies in five large prospective trials by the German CLL Study Group (GCLLSG). The patients included in this meta-analysis were heterogeneous, because these five trials were designed for different target populations and were performed consecutively between 1999 and 2010, which is a period of great changes in the treatment of CLL. Although the most innovative treatment options for CLL, such as the kinase inhibitors ibrutinib and idelalisib or the novel antibodies are not included in this analysis, the results of this meta-analysis are clinically relevant as these novel agents are not yet widely available in all countries.

Methods

This analysis includes 1,659 CLL cases treated in five phase II/III trials,[2,5,8,19-21] which were performed by the GCLLSG between 1999 and 2010. As 38 patients were identified who participated in two trials for different lines of treatment, the absolute number of patients is 1,621. The five trials evaluated the following treatment regimens: single agent chlorambucil (CLL5 trial) or fludarabine (CLL4 and CLL5 trials), FC (CLL4 and CLL8 trial), FC with rituximab (FCR) (CLL8 trial) or with alemtuzumab (FCA) (CLL2L trial) as well as bendamustine and rituximab (BR) (CLL2M-trial). CLL4, CLL5 and CLL8 were randomized phase III trials for first-line treatment while the CLL2L and CLL2M trials were phase II studies for both first-line and relapsed patients. Consequently, 91.6% (1,520) of all patients were included for first-line treatment and only 139 patients received a relapse therapy within one of the two phase II trials. With the exception of the 206 patients (12.4%) from the CLL5 trial with an advanced age >65 years, all other patients were required to be either younger, had a low burden of comorbidities and were physically fit for chemoimmunotherapy (for further details see *Online Supplementary Table S1* as well as the original publications of the trials).[2,5,8,19-21] As these five trials were run in an era of considerable changes in the treatment of CLL, the target populations, investigated regimens and therapeutic goals varied between the trials, resulting in a heterogeneous group of patients for this meta-analysis. The five studies were approved by the institutional review board or independent ethics committee at each participating institution and were conducted in accordance with the Declaration of Helsinki; all patients provided written informed consent.

Information on patients' subsequent therapies was collected prospectively as part of the follow-up documentation. In most cases the name of the therapeutic regimen or agents administered, as well as the treatment dates were documented, but information on dose intensity (dosage, number of cycles) and treatment outcomes, such as response to relapse treatment or duration of remission, were not provided. Hence, PFS could not be calculated; however, event-free survival (EFS: calculated from the start of the examined treatment until the start of the subsequent treatment or death), and OS (calculated from the start of the first-line treatment until death from any cause) according to Kaplan-Meier methodology were used as parameters for the efficacy of the relapse therapies. Statistical significance was defined as a P-value <0.05. No adjustments for multiple testing were performed. All data were medically reviewed and therapies unable to achieve long-term control of CLL, such as single-agent corticosteroids, intravenous immunoglobulins or immunosuppressive agents were excluded from the analyses.

As 63 of 1,621 patients had to be excluded from the analysis for different reasons, such as withdrawal of consent, application of treatment outside of a trial, and missing data because of early death or a short follow-up, the number of patients for this analysis was 1,558 (see *Online Supplementary Figure S1*). The median observation time for all patients was 51.5 months.

Results

Patients and treatment characteristics

The trials considered in this meta-analysis started consecutively during the pre-kinase-inhibitor era and examined various therapies in groups of patients with different inclusion/exclusion criteria, especially different limitations regarding age and comorbidities (see *Online Supplementary Table S1*). The median age at the time of starting first-line treatment for all 1,558 patients included in this analysis was 61 years (range, 30-81 years), whereas the median age at the start of second-line treatment for 704 patients receiving at least one relapse treatment was 64 years (range, 31-85 years). Almost three-quarters of the patients were male (72.4%). Prognostic factors were analyzed before the start of first-line treatment in 1,465 patients (94.0%): 7.1% (84/1184 patients) had a del(17p), 20.9% (248/1184) a del(11q), 12.7% (150/1184) a trisomy 12, 24.6% (291/1184) a normal diploid karyotype and 34.7% (411/1184) had a del(13q); 54.7% (621/1136 patients) had an unmutated *IGHV* status.

At the time of this analysis, 1,558 first-line and 1,437 relapse therapies had been documented. For 704 of the 1,558 patients (45.2%) at least one relapse treatment was documented. The median number of relapse therapies was two (range, 1-11). Among the 17 different first-line therapies, the most common were: FC (588 patients, 37.7%), FCR (402; 25.8%), single agent fludarabine (299; 19.2%), chlorambucil (118; 7.6%) and BR (116; 7.4%), which were mostly administered in the GCLLSG trials. In total, 60 different treatments were administered in second-line therapy (in 704 patients), 57 in third-line (in 392 patients), 43 in fourth-line (in 192 patients) and 32 in fifth-line therapy (in 87 patients) (Table 1 and *Online Supplementary Table S2*).

First-line therapies

The differences in the inclusion/exclusion criteria of the trials, which led to a heterogeneous group of patients in this analysis, also account for differences in patients´ baseline characteristics when comparing the five most prevalent first-line therapies (chlorambucil, fludarabine, FC, FCR, BR). Patients treated with chlorambucil were older (median age: 70 years) than those in all the other groups (59 years for patients treated with FC, 61 years for those treated with FCR, 62 years for fludarabine-treated patients, and 64 years for BR-treated patients). Moreover, due to the different dates of study activation, the median

Table 1. First-line and relapse therapies (for further details see *Online Supplementary Table S2*).

Line of treatment	1st line	2nd line	3rd line	4th line	5th line	6th line	7th line	8-12th line
1a) Heterogeneity of therapies								
N. of patients	**1558**	**704**	**392**	**192**	**87**	**37**	**16**	**4**
N. of different therapies	**17**	**60**	**57**	**43**	**32**	**22**	**15**	**9**
Missing information on therapies	0	0	2	0	0	0	0	0
1b) Type of therapies (absolute number and percentage of patients)								
Chemoimmunotherapy	526 (33.8%)	257 (36.5%)	134 (34.4%)	70 (36.5%)	26 (29.9%)	13 (35.1%)	3 (18.8%)	2 (50.0%)
Antibody-containing Rx	526 (33.8%)	318 (45.2%)	180 (46.2%)	105 (54.7%)	40 (46.0%)	18 (48.6%)	5 (31.3%)	3 (75.0%)
Rituximab-containing Rx	521 (33.4%)	239 (33.9%)	131 (33.6%)	74 (38.5%)	28 (32.2%)	14 (37.8%)	3 (18.8%)	3 (75.0%)
Alemtuzumab containing Rx	5 (0.3%)	81 (11.5%)	47 (12.1%)	30 (15.6%)	11 (12.6%)	3 (8.1%)	2 (12.5%)	–
Fludarabine containing Rx	1296 (83.2%)	276 (39.2%)	95 (24.4%)	34 (17.7%)	14 (16.1%)	7 (18.9%)	2 (12.5%)	–
Bendamustine containing Rx	118 (7.6%)	149 (21.2%)	113 (29.0%)	49 (25.5%)	14 (16.1%)	9 (24.3%)	1 (6.3 %)	1 (25.0%)
Anthracycline containing Rx	7 (0.4%)	132 (18.8%)	55 (14.1%)	30 (15.6%)	16 (18.4%)	3 (8.1%)	5 (31.3%)	1 (25.0%)
1c) Distribution of 10 most prevalent therapies (absolute number and percentage of patients, 3 most common regimen in bold):								
FCR	**402 (25.8%)**	54 (7.7%)	13 (3.3%)	7 (3.6%)	2 (2.3%)	–	–	–
BR	116 (7.4%)	**75 (10.7%)**	59 (15.1%)	34 (17.7%)	7 (8.0%)	**5 (13.5%)**	–	–
FCA	5 (0.3%)	32 (4.5%)	16 (4.1%)	4 (2.1%)	2 (2.3%)	–	1 (6.3%)	–
CHOP-R	1 (0.1%)	56 (8.0%)	20 (5.1%)	13 (6.8%)	**10 (11.5%)**	1 (2.7%)	**2 (12.5%)**	–
CHOP	3 (0.2%)	26 (3.7%)	21 (5.4%)	11 (5.7%)	5 (5.7%)	1 (2.7%)	1 (6.3%)	1 (11.1%)
FC	**588 (37.7%)**	**79 (11.2%)**	31 (7.9%)	7 (3.6%)	4 (4.6%)	2 (5.4%)	1 (6.3%)	–
Chlorambucil (± steroids)	134 (8.6%)	55 (7.8%)	17 (4.3%)	5 (2.6%)	**9 (10.3%)**	1 (2.7%)	**2 (12.5%)**	–
Fludarabine monotherapy	**299 (19.2%)**	**65 (9.2%)**	21 (5.4%)	5 (2.6%)	3 (3.4%)	1 (2.7%)	–	–
Benda mustine (± steroids)	1 (0.1%)	63 (9.0%)	**43 (11.0%)**	**14 (7.3%)**	7 (8.1%)	**4 (10.8%)**	1 (6.3%)	1 (11.1%)
Rituximab monotherapy	–	12 (1.7%)	21 (5.4%)	9 (4.7%)	5 (5.8%)	**3 (8.1%)**	1 (6.3%)	1 (11.1%)
Alemtuzumab monotherapy	–	43 (6.1%)	22 (5.6%)	**24 (12.5%)**	**8 (9.2%)**	1 (2.7%)	1 (6.3%)	–
1d) Use of stem cell transplantation								
Allogeneic HSCT	–	8 (1.1%)	20 (5.1%)	11 (5.7%)	8 (9.2%)	3 (8.1%)	1 (6.3%)	–
Autologous HSCT	–	3 (0.4%)	7 (1.8%)	3 (1.6%)	2 (2.3%)	–	–	–
Unknown type	–	3 (0.4%)	1 (0.2%)	3 (1.6%)	–	–	–	–
Donor lymphocyte infusions	–	–	2 (0.5%)	3 (1.6%)	2 (2.3%)	–	1 (6.3%)	–

BR: bendamustine/rituximab; CHOP: cyclophosphamide/doxorubicin/vincristine/prednisone; CHOP-R: cyclophosphamide/doxorubicin/vincristine/prednisone/rituximab; FC: fludarabine/cyclophosphamide; FCA: fludarabine/cyclophosphamide/alemtuzumab; FCR: fludarabine/cyclophosphamide/rituximab; HSCT: hematopoetic stem cell transplantation; N: number; Rx: therapies.

observation time varied between trials, being shortest in patients treated with BR (26.9 months) and longest for patients treated with fludarabine monotherapy (78.8 months).

The outcome of these five first-line therapies differed considerably; EFS and OS were shortest in patients treated with chlorambucil (median EFS: 14.8 months, median OS: 64.7 months) or fludarabine (32.8 and 78.0 months, respectively), followed by FC (50.3 and 93.2 months) and were longest in patients receiving chemoimmunotherapy with either BR or FCR (median EFS and OS: not reached, 3-year EFS rate: 72.3% and 77.1%, 3-year OS rate: 90.7% and 87.9%, respectively) (Table 2 and Figure 1A,B).

Use of antibodies in first-line and relapse therapies

Antibody-based regimens were administered to 58.3% of all patients, 909 patients received a total of 1,196 antibody-containing regimens. The majority of these patients received the antibody in first-line therapy (526 patients, 33.8% of all first-line therapies) or second-line regimens (239 patients, 15.3% of all second-line therapies); only 144 patients received the antibody later than second-line treatment. Most of these regimens (1,028 of 1,196 therapies, 86.0%) were chemoimmunotherapies and 655 of these antibody-containing therapies (54.8%) were administered within one of the trials included into this analysis. The vast majority of patients received rituximab (841 of 909 patients, 92.5%), followed by alemtuzumab (176 patients, 19.4%), ofatumumab (4 patients, 0.3%) and obinutuzumab (1 patient, 0.1%). The median age and observation time did not differ significantly between patients who were or were not treated with an antibody (ages: 61 and 64 years, respectively; P=0.08; observation time: 51.2 and 53.6 months, respectively; P=0.2). Adverse cytogenetic abnormalities, such as del(17p) and unmutated *IGHV* at baseline, were also balanced in patients who were or were not treated with an antibody [del(17p): 49/665 patients (7.4%) *versus* 35/519 (6.7%); unmutated *IGHV*: 373/669 patients (55.8%) *versus* 248/467 patients (53.1%)], whereas del(11q) was present at baseline more frequently in patients who received an antibody (161/665 patients, 24.2%) than in those who did not (87/519 patients, 16.8%).

The administration of antibody-containing therapies was associated with a statistically significant improvement of survival: the median OS was 94.4 months in patients treated with an antibody and 84.3 months in patients without antibody treatment (P<0.0001). Univariate Cox regression analysis demonstrated that patients who never received an antibody had a 1.42-fold increased risk of death (HR 1.42; 95% CI: 1.185-1.694). Patients who received an antibody in first-line treatment had a longer median OS (median OS not reached) than patients who received an antibody in second-line treatment (median OS 98.4 months) or even third-line or beyond (median OS 90.1 months). However, these differences of survival times in relation to the time point of administration of the antibody were not statistically significant (Figure 2). Similar results supporting the adminis-

tration of antibodies for first-line therapy, were obtained in an analysis including only anti-CD20 antibodies (i.e. predominantly rituximab) and excluding alemtuzumab (see *Online Supplementary Figure S2*).

Treatment with CHOP-like therapies

A total of 226 (R-)CHOP(-like) therapeutic regimens were administered to 202 patients (13.0% of all patients); 115 patients (7.4%) received a CHOP(-like) regimen combined with rituximab. Most of these regimens were administered as second-line therapy (106 patients; third- and fourth-line: 58 and 30 patients, respectively; see also *Online Supplementary Table S3*). (R-)CHOP(-like) therapies were administered in rather younger patients with a median age of 64 years and 35% being ≤60 years of age. While the median OS was significantly longer for patients never treated with (R-)CHOP(-like) regimens, there was no difference in OS between patients receiving CHOP(-like) regimens with and without rituximab (98.4 *versus* 67.1 and 67.7 months, respectively; $P<0.0001$) (Figure 3). The medi-

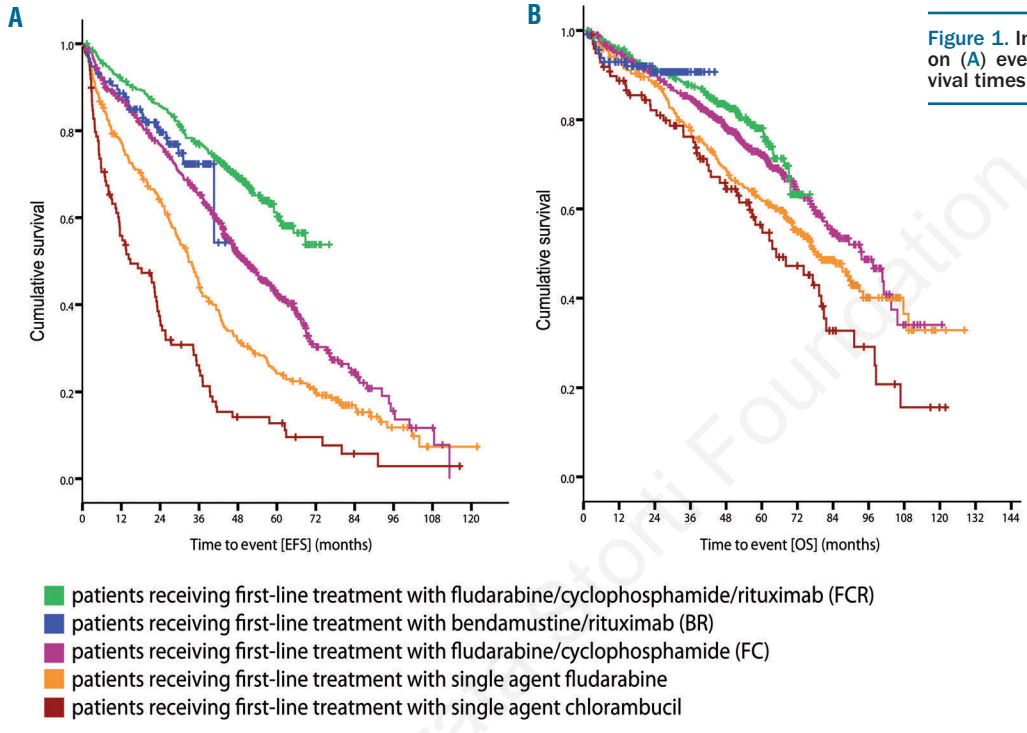

Figure 1. Impact of first-line treatment on (A) event-free and (B) overall survival times.

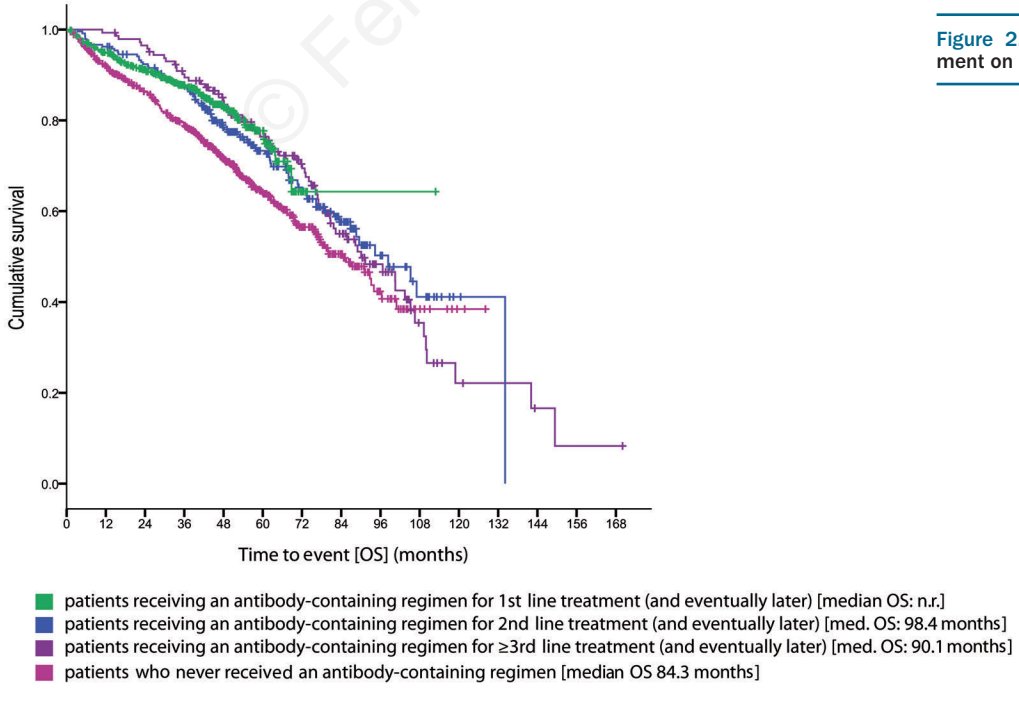

Figure 2. Impact of antibody-treatment on overall survival

an age of the patients and observation times did not differ between these three groups. Adverse cytogenetic abnormalities, such as del(17p) and del(11q), were more often present at baseline in patients receiving (R-)CHOP(-like) therapies than in those not receiving such therapies [del(17p): 19/154 patients (12.3%) *versus* 65/1030 (6.3%); del(11q): 40/154 (26.0%) *versus* 208/1030 (20.2%)]. The median time between the previous treatment and the start of the first (R-)CHOP(-like) therapy was only 7 months, reflecting the fact that high-risk patients were more likely to receive (R-)CHOP(-like) treatment. Interestingly, there was no difference in the percentage of patients with unmutated *IGHV* status between the two groups [78/152 patients (51.3%) who received (R-)CHOP(-like) therapies *versus* 543/984 patients (55.2%) who did not receive (R-)CHOP(-like) therapies].

Re-administration of treatment regimens

The same therapeutic regimen was repeated in a subsequent treatment line in 122 of 704 patients with documented relapse therapy (17.3%). Therapeutic regimens were repeated in all treatment lines and age groups, the median age at repetition of therapy was 66 years and 24.6% of patients were ≤60 years, 20.5% were 61 to 65 years old, 28.7% were 66 to 70 years old and 26.2% were >70 years of age. Seventy-one patients repeated their first-line regimen as second-line therapy: among them, 25 patients repeated FC, 18 repeated fludarabine, 16 repeated chlorambucil, 11 repeated FCR and one repeated BR (*Online Supplementary Table S4*). The EFS and OS of these 71 patients who received repeated first-line treatment were comparable to those of patients who received different treatment regimens for second-line therapy (median EFS: 17.0 and 16.0 months; median OS: 82.3 and 80.0 months).

Patients requiring second-line therapy within 24 months after initiation of first-line therapy received the same regimen less frequently; only 4.8% (15/315 patients) repeated the previous therapy in case of an early progression. Interestingly, eight of 15 patients were retreated with chlorambucil. Among patients who started second-line treatment within 25-36 months after first-line therapy, 12.2% (18/148 patients) received the same regimen

Table 2. Impact of first-line therapies event-free and overall survival.

Regimen	N. of patients	Age (mean, range)	Mean observation time	Median EFS	EFS rate [%] 2 years	3 years	4 years	Median overall survival	OS rate [%] 2 years	3 years	4 years
FCR	403	59.7, 30-80 years	52.5 months	not reached	85.7	77.1	69.4	not reached	91.7	87.9	83.2
BR	115	62.4, 34-78 years	26.1 months	not reached	80.8	72.3	54.3	not reached	90.7	90.7	90.7
FC	571	58.3, 36-81 years	56.1 months	50.3 months	76.8	65.7	51.6	93.6 months	89.9	84.6	78.2
Fludarabine	272	61.2, 33-80 years	74.8 months	32.8 months	64.2	44.7	31.6	78.2 months	88.1	78.0	68.0
Chlorambucil	99	70.6, 65-80 years	52.6 months	14.8 months	36.3	26.0	14.2	64.9 months	82.1	76.1	64.5

EFS: event-free survival; N.: number; OS: overall survival.

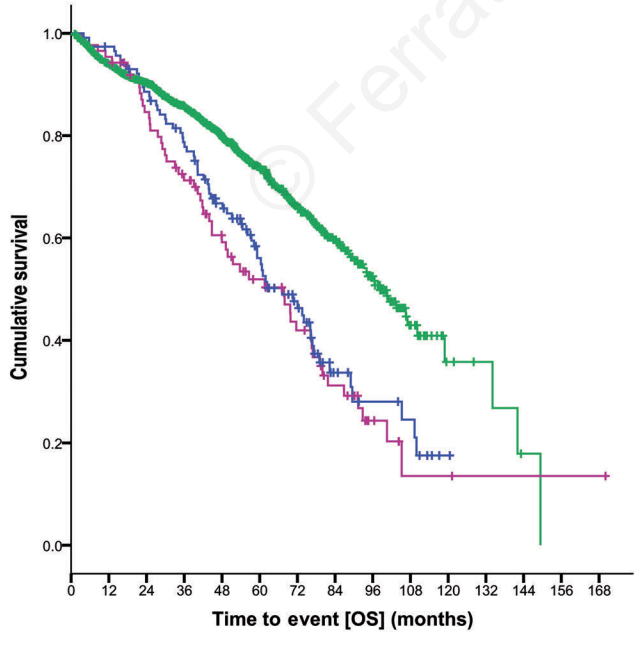

Figure 3. Impact of CHOP and related regimens with and without rituximab on overall survival.

patients never treated with (R-)CHOP(-like) regimen [median OS: 98.4 months]
patients treated with ≥ 1 R-CHOP(-like) regimen [median OS: 67.1 months]
patients treated with ≥ 1 CHOP(-like) regimen (without rituximab) [median OS: 67.7 months]

whereas among patients retreated >36 months after initial treatment this proportion was 16.8% (37/220 patients). The median EFS and OS were 14.7 and 109.3 months in patients retreated with the first-line regimen within 24 months, 14.8 and 69.7 months in patients with retreatment within 25-36 months and 21.4 and 82.3 months in patients retreated >36 months after first-line treatment (P=0.251 and P=0.571, respectively). For further evaluation of the efficacy of repeated treatment regimens, the 43 patients who received either fludarabine or FC for both first- and second-line therapy were pooled. The median EFS was 5.7 months in patients receiving the same purine analog chemotherapy within 24 months *versus* 18.2 months in patients repeating the treatment after 24 months (P=0.071). The median OS for these groups was 26.6 *versus* 82.3 months, respectively (P=0.6). Among patients who received relapse treatment within 36 months or after 36 months, the median EFS and OS were 14.9 *versus* 21.6 months (P=0.78) and 75.3 *versus* 89.0 months (P=0.157). Thus, a threshold of 24 months appeared to be more suitable for discriminating patients for whom a repetition of purine analog-based chemotherapy could be recommended. This calculation could not be performed for the repetition of chemoimmunotherapy because of the low number of patients.

Treatment after early relapse

In 315 of all patients (20.2%), the second-line treatment was initiated within 24 months after the start of first-line therapy. In this group, 100 (31.7%) relapses occured after FC, 85 (27.0%) after fludarabine, 70 (22.2%) after chlorambucil without/with steroids, 34 (10.8%) after FCR and 17 (5.4%) after BR. Forty-four different second-line therapies were used in these early relapsing patients (*Online Supplementary Figure S3*): the most frequently chosen regimens were CHOP with or without rituximab (32 and 24 patients, 10.2% and 7.6%, respectively), fludarabine (37 patients, 11.7%), alemtuzumab (27 patients, 8.6%) and FC (26 patients, 8.3%). Regarding the efficacy of these regimens, the median EFS and OS were longest with FC (30.8 and 88.4 months), followed by single agent alemtuzumab (18.6 and 49.6 months) and fludarabine (12.7 and 49.3 months) (*Online Supplementary Table S5*).

For further analyses, patients who relapsed early were grouped according to the intensity of their second-line treatment: 95 patients received an intensive chemo-immunotherapy, containing an anthracycline and/or three or more chemotherapeutic agents with or without an antibody [e.g. (R-)CHOP(-like) therapies or FCM]; 123 patients received either antibody monotherapy or standard chemo(immuno)therapy (e.g. FC, FCR, FCA, BR, alemtuzumab or rituximab) and 87 patients received mono-chemotherapy (e.g. single agent fludarabine, chlorambucil or bendamustine). Ten patients undergoing stem cell transplantation, irradiation, splenectomy or experimental therapies were excluded from this analysis. Patients who received a mono-chemotherapy for an early relapse were older (P<0.001) and had an ECOG performance status ≥1 in 61.6% of cases (P=0.002) as compared to the other two groups (see Table 3). However, there were no statistically significant differences with regards to median time between first- and second-line therapies, or in the distribution of adverse genetic factors. The EFS and OS of patients with an early relapse were shortest after intensive chemo(immuno)therapy containing an anthracycline and/or three or more chemotherapeutic agents, followed by mono-chemotherapy and was best after standard chemo(immuno)therapy or antibody monotherapy (P=0.009 and P=0.012, respectively) (Figure 4).

Discussion

This manuscript describes data from 1,558 CLL patients treated in five prospective phase II/III trials conducted by the GCLLSG. The results demonstrate that advances have been made with the introduction of chemoimmunothera-

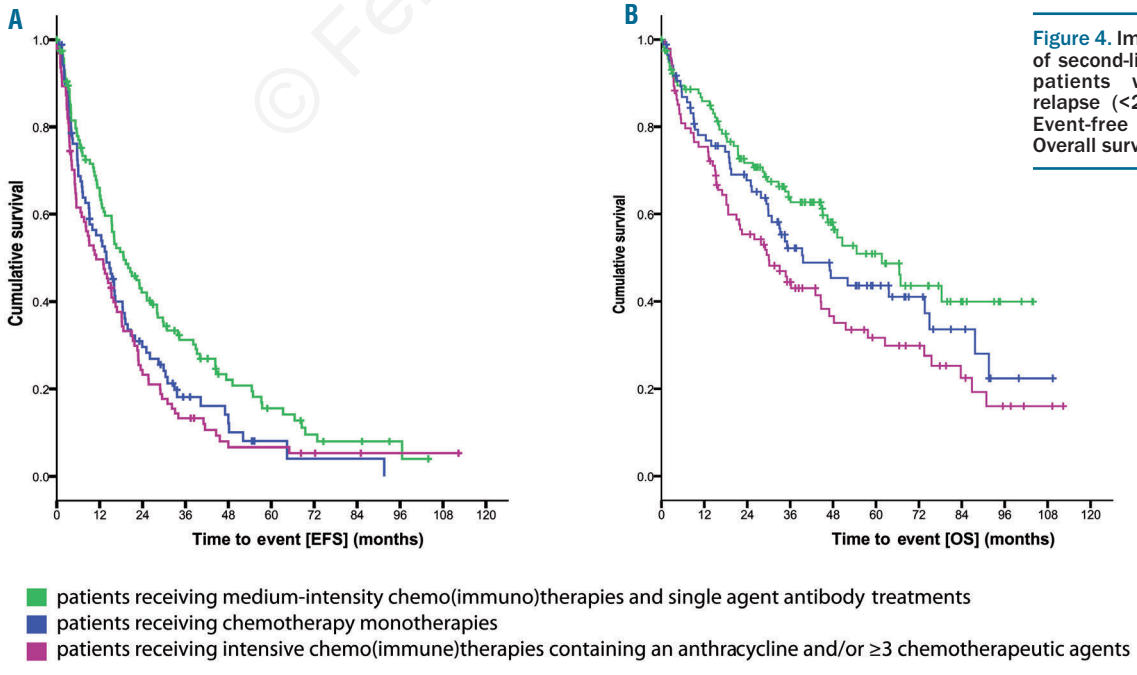

Figure 4. Impact of intensity of second-line treatment in patients with an early relapse (<24 months). (A) Event-free survival. (B) Overall survival.

py, confirm the recommendation of ESMO guidelines regarding repetition of treatment in cases of a relapse after 24-36 months and document that the use of aggressive chemo(immuno)therapy combinations is not beneficial in patients with an early relapse. However, this meta-analysis has several limitations. First, the trials evaluated different therapies in an era in which considerable changes occurred in the treatment of CLL and the inclusion/exclusion criteria of the trials varied, especially regarding age and comorbidities. Second, the documented information on therapies administered outside the trials (mainly relapse therapies) was not very detailed and, therefore, only EFS and OS were used as parameters for the efficacy of the therapies.

In this analysis, first-line therapy with FCR or BR led to significantly longer EFS and OS compared to other regimen. Besides two randomized phase III trials evaluating FCR,[8,10] several meta-analyses have confirmed the advantage of chemoimmunotherapies. Terasawa *et al.* performed a large meta-analysis of 25 randomized controlled trials, including 7,926 patients who received first-line treatment with chlorambucil or a CHOP(-like) regimen, but also fludarabine, bendamustine, cladribine, pentostatin, FC, FCR or alemtuzumab.[22] The PFS was better in patients treated with bendamustine- and fludarabine/rituximab-based chemoimmunotherapies than that in patients treated with chlorambucil, but no benefit in OS was detected. A meta-analysis by Cheng *et al.*[23] included 2,625 younger, physically fit patients who received first-line treatment in five randomized trials. The estimated median PFS was significantly longer with FCR than with FC, fludarabine, alemtuzumab or chlorambucil.

The choice of relapse therapy is often based on individual circumstances and the experience of the treating physician. In a recent analysis by the MD Anderson Cancer Center, 31 different second-line therapies were chosen in 136 patients relapsing after FCR.[24] In our analysis, 60 different second-line regimens were used in 704 patients and the treatment heterogeneity increased with every line of treatment. This reflects the fact that no therapeutic standard has been established so far for the relapse situation.

In the case of a relapse after frontline treatment with FCR, most patients at the MD Andersson Cancer Center received FCR-based therapies again; other rituximab-based and alemtzumab-based regimens were also often used.[24] In an analysis by the French intergroup group including 132 patients with first relapse after FCR, the most commen relapse therapies were BR and alemtuzumab-based regimens.[18] Both analyses demonstrated that patients with an early relapse <36 months after first-line therapy have a poor outcome, irrespective of the salvage therapy used.

As described above, the previous version of the ESMO guidelines[13] recommended repeating treatment in case of progression 12–24 months after monotherapy or 24-36 months after chemoimmunotherapy. In case of earlier progression, a change of the therapeutic approach is suggested. In our analysis, a cut-off of 24 months was found to be

Table 3. Previous therapies, patients' characteristics and outcomes of three groups treated for early relapse.

	Standard chemo(immuno)-therapy or antibody monotherapy e.g. alemtuzumab, FC, BR, FCR	Mono-chemotherapy e.g. fludarabine, chlorambucil ± steroids	Intensive chemo-(immuno) - therapy e.g. (R-)CHOP(like), FCM ± rituximab
Number of patients	123	87	95
Previous therapy (1st-line therapies)			
FCR	12x,	7x,	11x,
BR	11x	2x	4x
FC	45x	11x	40x
F	32x	21x	31x
C	-	-	1x
Clb/Clb-P	18x	44x	8x
(R-)CHOP(-like)	4x	2x	-
FCA	1x	-	-
Median time between 1st- and 2nd-line therapies	11.3 months	10.2 months	8.1 months
Median age			
at 1st-line therapy	61 years (35-79)	67 years (44-79)	60 years (37-77)
at 2nd-line therapy	62 years (36-79)	68 years (44-80)	61 years (37-78)
ECOG PS ≥1	34.4%	61.6%	53.4%
Risk factors at 1st-line therapy:			
IGHV unmutated	59.5%	54.4%	62.7%
del(17p)	16.9%	21.3%	13.1%
del(11q)	27.3%	20.0%	24.6%
Median EFS	18.7 months	13.9 months	11.0 months
Median OS	61.6 months	39.6 months	30.0 months

BR: bendamustine/rituximab; C: cyclophosphamide; (R-)CHOP(-like): cyclophosphamide/doxorubicin/vincristine/prednisone with/without rituximab and related regimen; Clb: chlorambucil; ClbP: chlorambucil/steroids; ECOG PS: Eastern Cooperative Oncology Group performance status; EFS: event-free survival; F: fludarabine; FC: fludarabine/cyclophosphamide; FCA: fludarabine/cyclophosphamide/alemtuzumab; FCM: fludarabine/cyclophosphamide/mitoxantrone; FCR: fludarabine/cyclophosphamide/rituximab; OS: overall survival.

more relevant than a cut-off of 36 months to identify patients in whom a repetition of first-line treatment with purine analogues proved effective. In the analysis by the French intergroup a cut-off of 36 months was found to differentiate best, because patients with a relapse <36 months after FCR had an equally poor outcome as patients with early relapses < 12 or 24 months. Yet, these results are both in line with the ESMO recommendation as patients from the French analysis had received chemoimmunotherapy and our analysis was performed with patients with repeated F- or FC-chemotherapy because of a low number of patients with a repetition of chemoimmunotherapy.

However, this recommendation by ESMO guidelines[13] was followed only in a minority of patients: only 55 of 368 patients (14.9%) with second-line therapy started ≥24 months after first-line therapy were re-exposed to the first-line regimen. This is probably explained by the introduction of newer agents and combination therapies (e.g. BR), which were expected to yield higher efficacy and/or better tolerability. In the analysis of patients initially treated with FCR at the MD Anderson Cancer Center,[24] FCR was repeated in 29 patients or slightly modified (e.g. combined with another antibody, fludarabine replaced by pentostatin) in 31 patients. The survival of patients with a late relapse ≥3 years was best when using FCR retreatment or a lenalidomide-based regimen. Patients with an earlier relapse seemed to benefit from allogeneic stem cell transplantation.

In our analysis, most patients with an early relapse within 24 months after first-line treatment received more aggressive therapies such as CHOP with/without rituximab or alemtuzumab. Patients treated with an intensive chemo(immuno)therapy (containing an anthracycline and/or ≥3 chemotherapeutic agents) had significantly shorter EFS and OS in comparison to patients treated with standard chemo(immuno)therapies, antibody monotherapy or single agent chemotherapy (figure 4). These findings could be biased by imbalances in the patients' characteristics, such as a higher age and more patients with a ECOG status >1 among patients receiving single agent chemotherapy, but no significant differences in the median time from first- to second-line therapy and the presence of adverse prognostic factors at start of first-line therapy were found (Table 3). Presumably, the worse outcome of patients treated with intensive chemo(immuno)therapies is influenced by several (prognostic) factors. However, the use of more intensive chemo(immuno)therapies appeared to be disadvantageous in this setting, which might be explained by a higher toxicity causing complications or dose-reductions and thereby leading to decreased efficacy. These results favor the use of standard chemoimmunotherapy or monotherapies in relapsed CLL patients. With the introduction of novel targeted agents, therapeutic alternatives become available, which will help to overcome the dismal prognosis of patients with an early relapse.

Comparisons of different therapies in patients with a late relapse or comparisons of therapeutic sequences are hampered by the heterogeneity of relapse therapies leading to small patient numbers and insufficient evidence to draw final conclusions. Nonetheless, the benefit of monoclonal antibodies, namely rituximab was confirmed, in line with the results of a Cochrane analysis of seven randomized controlled trials.[25] The Cochrane analysis included a total of 1763 patients receiving either chemotherapy alone or in combination with rituximab, ofatumumab or alemtuzumab for first-line or relapse therapy and demonstrated that the use of antibodies prolongs both PFS and OS.[25] Our analysis showed that patients who never received an antibody had a 1.42-fold increased risk of death (HR 1.42, 1.185-1.694 95% CI). Interestingly, the improvement of OS with antibody-based treatment was independent from the time point of administration of the antibody (Figure 2). However, in randomized trials an improvement of OS was shown only for the addition of an antibody in the first-line but not in the relapsed situation.[8,10,12,26]

Whereas most patients benefit from the use of monoclonal antibodies, the results of this metaanalysis also demonstrate that the outcome of patients with early progression remains unsatisfactory. A surprisingly long median OS of more than 9 years was documented for the small subgroup of 15 patients with a repetition of first-line treatment within 24 months. This finding might be explained by the fact that eight of these patients received chlorambucil repeatedly, unfavorable genetic risk factors were rare and the subgroup was rather small for an estimation of survival times. Furthermore, these analyses demonstrated that patients with an early relapse did not benefit from more intensive treatment with an anthracycline or ≥3 chemotherapeutic agents, such as CHOP or FCM (Figure 4). The treatment of these high-risk patients remains a challenge and further evaluation of novel treatments, such as kinase inhibitors or Bcl2 antagonists[27] is urgently needed and will help to treat these patients and might also prevent early relapses in patients with an adverse prognosis.

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