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ORIGINAL ARTICLE

Possible hampered effectiveness of second-line treatment with rituximab-containing chemotherapy without signs of rituximab resistance: a population-based study among patients with chronic lymphocytic leukemia

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Abstract

Rituximab-containing chemotherapy remains a viable frontline treatment option for patients with chronic lymphocytic leukemia (CLL) in the era of novel agents. However, its effectiveness in the second-line setting—in relation to previous rituximab exposure in first-line—has hardly been evaluated in a population-based setting. Therefore, in this comprehensive, population-based study, we assessed the impact of first-line treatment with rituximab-containing chemotherapy on the effectiveness of second-line treatment with rituximab-containing chemotherapy. We selected all 1735 patients diagnosed with CLL between 2004 and 2010 from the Dutch Population-based HAematological Registry for Observational Studies (PHAROS). The primary endpoint was treatment-free survival (TFS). First- and second-line treatment was instituted in 663 (38%) and 284 (43%) patients, respectively. In first line, the median TFS was 19.7 and 67.1 months for chemotherapy without ($n = 445$; 67%) and with rituximab ($n = 218$; 33%), respectively (adjusted hazard ratio [$HR_{adjusted}$], 0.83; $P = 0.031$). The median TFS among recipients of second-line chemotherapy without ($n = 165$; 57%) and with rituximab ($n = 121$; 42%) was 15.0 and 15.3 months, respectively ($HR_{adjusted}$, 0.93; $P = 0.614$). Of the 121 patients who received rituximab-containing chemotherapy in second-line, 89 (74%) and 32 (26%) received first-line chemotherapy without and with rituximab, respectively. Median TFS in these two treatment groups was 18.3 and 12.1 months, respectively ($HR_{adjusted}$, 1.71; $P = 0.060$). Collectively, in this population-based study, the effectiveness of first-line treatment with rituximab-containing chemotherapy was less pronounced in second-line treatment. The hampered effectiveness of rituximab-containing chemotherapy in second-line could not be explained by previous rituximab exposure.

Keywords Chronic lymphocytic leukemia · Rituximab · Chemotherapy · Cancer epidemiology · Population-based · Registry

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Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia diagnosed in adults, with an overall age-standardized incidence rate of 4 to 5 per 100,000 persons in Western countries [1, 2]. Over the past decades, significant advances have been made in the management of CLL, including the advent of purine analogs, chemoimmunotherapy, and, more recently, kinase inhibitors and anti-apoptotic agents [3–7]. Despite these advances, most patients with CLL will ultimately relapse after first-line therapy, highlighting the need for effective second-line therapy [8]. The management of CLL in the second-line setting is highly challenging since it has not been studied in all patient subsets in randomized clinical trials [9]. Patient- and disease-specific characteristics that guide the choice of second-line therapy include age, performance status, comorbidities, genetic aberrations (e.g., *TP53* mutation), and duration of response to first-line chemoimmunotherapy therapy [9–13].

Recently, two phase 3 trials reported the superiority of ibrutinib over chemoimmunotherapy in patients with untreated CLL, with respect to progression-free survival (PFS) but not concerning overall survival (OS) [14, 15]. However, the advantage of ibrutinib in first-line was not clear for mutated patients. Also, a phase 3 trial demonstrated profound PFS advantages with venetoclax-obinutuzumab over chlorambucil-obinutuzumab in previously untreated CLL patients [16]. Furthermore, in relapsed CLL patients, rituximab-venetoclax was shown to be more effective, as compared with chemoimmunotherapy [17]. Thus, it is reasonable to assume that these novel strategies will make their way into clinical practice across various lines of CLL treatment. In resource-limited countries, however, the application of novel agents in first and subsequent lines of treatment will be carefully weighed against alternative treatment options due to the substantial financial burden posed by these novel agents [18]. Against this background, rituximab-containing chemotherapy is a well-established first-line treatment for patients with CLL that still holds value, especially among the mutated patients and patients managed in resource-limited countries [7, 19, 20].

As for second-line treatment, one of the recommendations in current guidelines is to institute the same type of chemoimmunotherapy that was applied in first-line when the interval between the first remission and the need for second-line therapy exceeds 24 to 36 months [10, 12, 13]. That recommendation is largely based on findings from the REACH trial that demonstrated improved outcomes of fludarabine and cyclophosphamide (FC) with rituximab (FCR), as compared to FC alone, among previously treated patients with CLL. Of note, previous therapy with rituximab was an exclusion criterion in the REACH trial [21]. Therefore, the study population of that trial, which accrued patients between 2003 and 2007, may not entirely represent the current population of patients with CLL in need for second-line treatment, since these patients nowadays typically receive rituximab-containing chemotherapy in first-line [22, 23].

Recently, we and others have demonstrated the effectiveness of rituximab added to first-line chemotherapy, as compared to first-line chemotherapy without rituximab, in a population-based cohort of patients with CLL [22, 23]. In addition, we were the first to extend these observations by providing clues about the possible hampered effectiveness of rituximab added to subsequent lines of chemotherapy. We suggested that this finding could, in part, be attributed to the acquisition of rituximab resistance due to prior rituximab exposure. Indeed, several pre-clinical lymphoma studies have brought forward various mechanisms of acquired rituximab resistance. [24–26]. However, the small number of patients receiving subsequent treatment in our previous single-center study ($n = 58$) made it difficult to draw firm conclusions concerning this hypothesis in CLL [22].

Therefore, in this comprehensive population-based study, covering many hospitals within well-defined geographic regions in the Netherlands, we set out to assess the effectiveness of rituximab-containing chemotherapy, as compared to chemotherapy without rituximab, in first- and second-line CLL treatment. Moreover, emphasis was put to assess whether first-line treatment with rituximab affected the effectiveness of second-line treatment with rituximab-based regimens.

Methods

Registries and study population

Established in 1989, the nationwide population-based Netherlands Cancer Registry (NCR), which is managed by the Netherlands Comprehensive Cancer Organisation (IKNL), has an overall nationwide coverage of at least 95% of all malignancies in the Netherlands [27]. The NCR relies on comprehensive case notification of all newly diagnosed malignancies in the Netherlands via the Nationwide Network of Histopathology and Cytopathology, and the National Registry of Hospital Discharges (i.e., inpatient and outpatient discharges).

Trained registrars of the NCR routinely collect basic details on dates of birth and diagnosis, sex, hospital of diagnosis and treatment, disease topography and morphology, and primary treatment started within 12 months after diagnosis through retrospective medical records review. The date of last known vital status (i.e., alive, dead, or emigration) is retrieved by linking the NCR to the Nationwide Population Registries Network that holds vital statistics of all residents in the Netherlands.

Although the basic details recorded in the NCR are essential for national cancer surveillance activities, they

are insufficient to address more specific questions regarding the delivery of care to patients with CLL. Therefore, the Dutch Population-based HAematological Registry for Observational Studies (PHAROS) in CLL—the PHAROS CLL registry—was established to document additional details on various patient-, disease-, and treatment-related characteristics next to the basic details recorded in the NCR. The more detailed PHAROS CLL registry holds information about all patients diagnosed with CLL between January 1, 2004 and December 31, 2010 who were diagnosed in well-defined geographic regions that form the Western and Southern part of the Netherlands (~45% of the Dutch population). The PHAROS CLL registry is conceptually similar to the PHAROS registry in myelodysplastic syndromes [28]. Details about the NCR and the PHAROS registry, and their validity, logistics, and completeness were previously reported [1, 27–29]. Of note, both registries exclusively include CLL cases that were confirmed by the physician through bone marrow examination and/or immunophenotyping of the peripheral blood and/or bone marrow and classified according to the 2001 criteria of the World Health Organisation [30].

According to the Central Committee on Research involving Human Subjects (CCMO), this type of observational, non-interventional study does not require approval from an ethics committee in the Netherlands. The Privacy Review Board of the NCR approved the use of anonymous data for this study. Informed consent was obtained from all patients for being included in this study.

Treatment

Patients with CLL who received first- or second-line treatment were categorized into two groups, namely (i) patients who received rituximab-containing chemotherapy (+R) or (ii) chemotherapy without rituximab (NoR). Furthermore, patients who received second-line treatment with rituximab-containing regimens were categorized into those who received first-line chemotherapy with (+R/+R) or without rituximab (NoR/+R). Collectively, our study encompasses three treatment cohorts. Of note, treatment with rituximab monotherapy ($n = 11$) and the application of rituximab-containing therapy specifically for the treatment of auto-immune complications ($n = 13$), such as autoimmune cytopenia, were excluded from all analyses.

The specific chemotherapeutic backbone, with or without rituximab, was categorized into purine analogs (i.e.. fludarabine with or without cyclophosphamide), alkylating agents (i.e., chlorambucil monotherapy or cyclophosphamide with or without vincristine and prednisone), or other, less common regimens.

Endpoints

The primary endpoint was treatment-free survival (TFS), calculated from the start date of treatment until the institution of subsequent treatment or death [23, 31]. Patients who were alive in whom subsequent treatment was not started were censored at the date of last follow-up (i.e., December 31, 2014).

The secondary endpoints included time to next treatment (TTNT), best response, and OS. TTNT was calculated from the stop of first-line treatment until institution of second-line treatment or, in case of no subsequent therapy, the date of the last follow-up. The best response was determined by physicians' assessment following the guidelines that were valid at the time [13, 32]. The overall response rate (ORR) was calculated by adding the proportion of patients who achieved a complete response (CR) and partial response (PR). OS was calculated from the start date of treatment until death resulting from any cause. If death did not occur, patients were censored at the last of the last follow-up (i.e., December 31, 2014).

Of note, for the group of patients who underwent second-line treatment, TFS and OS were calculated from the start date of second-line treatment. Also, TTNT was calculated from the stop of second-line treatment until institution of third-line treatment, or in case of no third-line treatment, the date of the last follow-up.

Statistical analysis

Baseline characteristics were presented at the time of treatment according to the two treatment groups, stratified by the three treatment cohorts. The Fisher's exact test was used to compare categorical variables and the Kruskal-Wallis test for continuous variables.

The Kaplan-Meier method was used for time-to-event analyses and the log-rank test to compare survival distributions in a univariable fashion. Multivariable Cox proportional hazard models were constructed to assess TFS and OS, with adjustment for age at the time of treatment, sex, addition of rituximab to therapy, the chemotherapeutic backbone, receipt of rituximab in first-line (only application for analyses in second-line), type of therapeutic backbone in first-line (only applicable for analyses in second-line), and time to next treatment (TTNT; only applicable for analyses in second-line), unless stated differently. Results from the multivariable models produce hazard ratios (HRs) with 95% confidence intervals (CIs). The proportional hazard assumption was tested based on Schoenfeld residuals [33].

All statistical analyses were performed two-sided with a significance level of 5% (i.e., a P value of 0.05) using STATA Statistical Software Release 14.2 (College Station, TX, USA).

Results

First-line treatment

Patient characteristics

The PHAROS CLL registry includes 1735 adult (≥ 18 years) patients diagnosed with CLL between January 1, 2004 and December 31, 2010, with follow-up through December 31, 2014. For the current study, we selected 663 (38%) patients who initiated first-line treatment, of whom 445 (67%) and 218 (33%) without and with rituximab, respectively (Supplemental Fig. 1). Baseline characteristics of patients according to the three treatment cohorts are shown in Table 1. Overall, the median follow-up—calculated from the start of first-line treatment until death or last of follow-up, whichever occurred first—for the entire population was 28 months (range, 0–121 months). Cytogenetics was performed in a minority of the patient population at the time of diagnosis.

Patients in the +R group were significantly younger than those in the NoR group (median age, 66 versus 72 years; $P < 0.001$). Also, the chemotherapeutic backbone was different across the two treatment groups. Nevertheless, the majority of patients in both treatment groups were primarily treated with a backbone of alkylating agents (87% and 58% in the NoR and +R group, respectively), followed by purine analogs (10% and 26% in the NoR and +R group, respectively). The remaining patients received a variety of chemotherapeutic backbones, of whom in the +R group more often received such treatment modalities (16%) than those in the NoR group (3%). The specification of these other, less common chemotherapeutic backbones across the three treatment cohorts is listed in Supplemental Table 1. The distribution of chemotherapeutic backbones remained comparatively steady over the years studied (data not shown).

Effectiveness

Consistent with prior observations [22, 23], univariable and multivariable survival analyses demonstrated that first-line treatment with rituximab-containing chemotherapy resulted in significantly better TFS (Fig. 1a and Table 2) and OS

Table 1 Patient and treatment characteristics

	First-line treatment			Second-line treatment			Rituximab-containing therapy in second-line treatment		
	NoR ($n = 445$)	+R ($n = 218$)	*P*	NoR ($n = 165$)	+R ($n = 121$)	*P*	NoR/+R ($n = 89$)	+R/+R ($n = 32$)	*P*
Characteristics	No. (%)	No. (%)		No. (%)	No. (%)		No. (%)	No. (%)	
Male sex	288 (65)	142 (66)	0.794	110 (67)	80 (67)	1.00	58 (66)	22 (71)	0.662
Median age	72 (33–95)	66 (35–93)	*< 0.001*	72 (37–95)	68 (40–92)	*0.013*	70 (37–88)	67 (40–83)	0.187
18–64	145 (33)	109 (50)	*< 0.001*	51 (31)	56 (46)	*0.020*	39 (44)	17 (53)	0.260
65–74	154 (35)	79 (26)	0.171	66 (40)	45 (37)	0.504	34 (38)	11 (34)	0.770
≥ 75	146 (33)	30 (14)	*< 0.001*	48 (29)	20 (17)	0.064	16 (18)	4 (13)	0.081
FISH analysis									
Deletion 17p	3 (1)	3 (1)	0.370	1 (1)	2 (2)	0.737	1 (1)	1 (3)	0.446
Deletion 11q	5 (1)	6 (3)	0.123	1 (1)	2 (2)	0.737	1 (1)	1 (3)	0.446
Trisomy 12	14 (3)	10 (4)	0.351	5 (3)	7 (6)	0.251	5 (6)	2 (6)	0.896
Deletion 13q	25 (6)	19 (9)	0.132	7 (4)	9 (7)	0.245	5 (6)	4 (13)	0.203
Normal or none of above	30 (7)	20 (9)	0.676	20 (12)	18 (15)	0.498	15 (17)	3 (9)	0.308
Not performed	329 (74)	143 (66)	*0.026*	125 (76)	81 (67)	0.101	61 (69)	20 (63)	0.533
Unknown	37 (8)	17 (8)	0.804	6 (4)	2 (2)	0.129	1 (1)	1 (3)	0.094
Chemotherapeutic backbone in first-line									
Alkylating agents	389 (87)	127 (58)	*< 0.001*	150 (91)	101 (83)	0.058	77 (87)	24 (75)	0.133
Purine analogs	44 (10)	57 (26)	*< 0.001*	11 (7)	17 (14)	*0.038*	12 (13)	5 (16)	0.765
Other	12 (3)	34 (16)	*< 0.001*	4 (2)	3 (3)	0.976	0 (0)	3 (9)	*0.003*
Chemotherapeutic backbone in second-line									
Alkylating agents				118 (72)	66 (54)	*0.003*	56 (63)	10 (31)	*0.002*
Purine analogs				38 (23)	37 (31)	0.152	23 (25)	14 (44)	0.059
Other				9 (5)	18 (15)	*0.007*	10 (11)	8 (25)	0.061
Median TTNT[a]	10 (0–70)	11 (0–63)	0.200	10 (0–97)	10 (0–81)	0.461	12 (0–81)	8 (1–56)	0.260
Median follow-up time	32 (0–121)	25 (0–97)	*< 0.001*	21 (0–97)	36 (0–80)	*0.029*	19 (0–80)	8 (0–61)	*< 0.001*

NoR chemotherapy without rituximab, *+R* rituximab-containing chemotherapy, *TTNT* time to next treatment, *FISH* fluorescent in situ hybridization

[a] TTNT was calculated in months from the stop of first-line treatment until institution of second-line treatment. For patients in the second-line cohort, TTNT was calculated from stop of second until institution of third-line treatment

Italics denotes *P* values less than 0.05

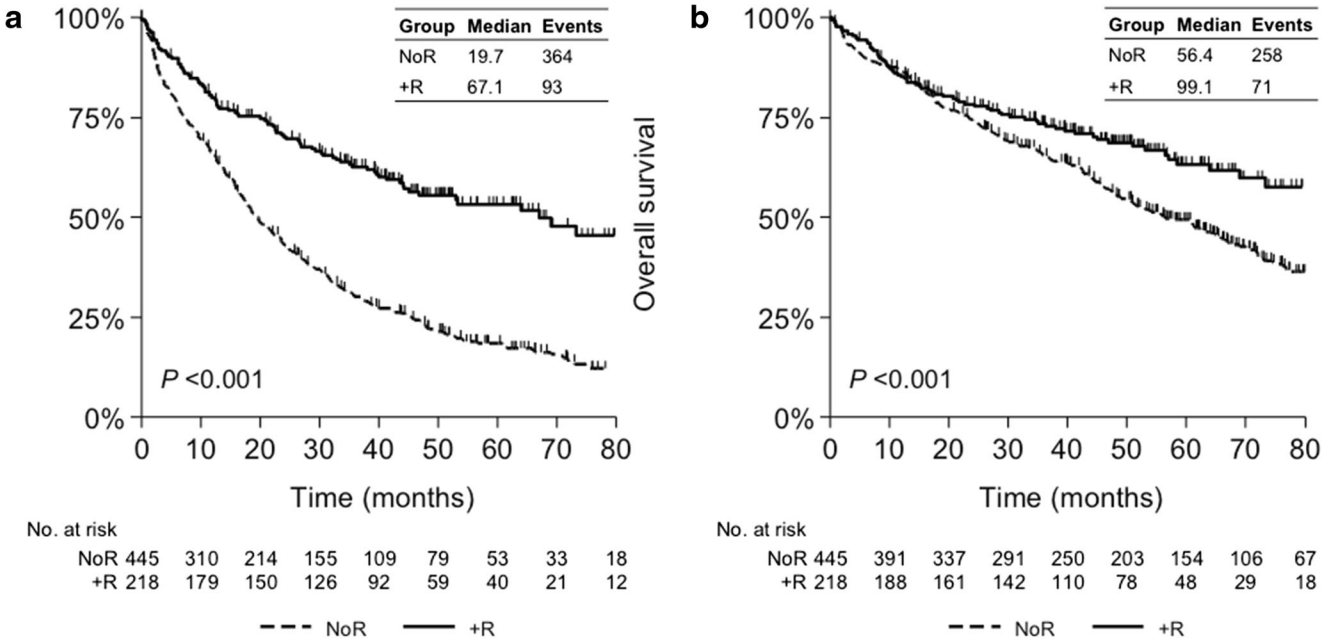

Fig. 1 Treatment-free survival (**a**) and overall survival (**b**) among patients with chronic lymphocytic leukemia who received first-line treatment with or without rituximab

(Fig. 1b and Table 2), as compared to chemotherapy without rituximab (*P* for all comparisons < 0.05). Variables associated with inferior TFS and OS were age per 1-year increase and male sex (for TFS and OS). In addition, treatment with a chemotherapeutic backbone including a purine analog or other, less common chemotherapeutic regimens was associated with inferior OS. The ORR was higher among recipients of first-line therapy with rituximab-containing chemotherapy, as compared to recipients of first-line chemotherapy without rituximab (79% versus 59%; *P* < .001).

Second-line treatment

Patient characteristics

Second-line treatment was initiated in 286 (43%) of 663 patients, of whom 165 (58%) without rituximab and 121 (42%) with rituximab (Table 1). Similar to the first-line cohort, patients in the +R group were significantly younger than those in the NoR group (median age, 68 versus 72 years; *P* = 0.013). In addition, most patients in the two treatment groups received

Table 2 Cox regression analyses for treatment-free survival and overall survival in first-line treatment

Covariate	Treatment-free survival					Overall survival				
	HR	95% CI			*P*	HR	95% CI			*P*
Age at treatment, years[a]	1.02	1.01	–	1.03	*< 0.001*	1.06	1.05	–	1.08	*< 0.001*
Sex										
Male	1	ref				1	ref			
Female	0.78	0.66	–	0.92	*0.004*	0.69	0.54	–	0.87	*0.002*
Receipt of rituximab										
No	1	ref				1	ref			
Yes	0.83	0.70	–	0.98	*0.031*	0.72	0.54	–	0.96	*0.023*
Chemotherapeutic backbone										
Alkylating agents	1					1	ref			
Purine analogues	0.87	0.69	–	1.09	0.231	1.44	1.00	–	2.06	*0.048*
Other	1.13	0.83	–	1.54	0.446	2.18	1.44	–	3.31	*< 0.001*

HR hazard ratio, *CI* confidence interval

Linear estimate per one-year increase

Italics denotes *P* values less than 0.05

second-line treatment with a backbone of alkylating agents (72% and 54% in the NoR and + R group, respectively). Interestingly, the vast majority of patients in the two treatment groups received first-line treatment with a backbone of alkylating agents (91% and 83% in the NoR and + R group, respectively).

Effectiveness

Univariable survival analysis showed similar TFS between second-line treatment with or without rituximab (median TFS, 15.3 versus 15.0 months; *P* for log-rank = 0.318; Fig. 2a). In multivariable analysis, the adjusted HR was not statistically significant different between the two treatment groups (HR, 0.93; 95% CI, 0.70–1.23; *P* = 0.614; Table 3). Predictors associated with poorer TFS included age per 1-year increase and first-line therapy with a backbone of purine analogs, as compared with a backbone of alkylating agents (Table 3). Conversely, patients who had a longer TTNT had better TFS (HR per 1-month increase, 0.98; 95% CI, 0.96–0.99; *P* < 0.001; Table 3).

Similar to TFS, the median OS (Fig. 2b) and the adjusted risk of death (Table 3) were not statistically significant different between the two treatment groups. Also, the multivariable analysis revealed associations similar to those for TFS (Table 3). In addition, patients who received second-line therapy with a therapeutic backbone other than the commonly applied backbones (i.e., alkylating agents and purine analogs) had a higher adjusted risk of death compared to patients who received second-line therapy with a backbone of alkylating agents (Table 3).

No differences with respect to the ORR were found between recipients of second-line therapy with or without rituximab (56% versus 52%; *P* = 0.746).

Rituximab-containing therapy in second-line

Patient characteristics

Next, we specifically focused on 121 patients who received rituximab-containing therapy in second-line. Of these patients, 32 (26%; +R/+R) and 89 (74%; NoR/+R) received first-line therapy with and without rituximab, respectively (Table 1). Of note, patients in the +R/+R group received purine analog-based chemotherapy more frequently, as compared to the NoR group (44% versus 25%; *P* < 0.001). Furthermore, 98 (34%) recipients of second-line therapy received the same chemotherapeutic backbone as was applied in first-line, namely alkylating agents (*n* = 96), purine analog (*n* = 1) and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP; *n* = 1).

Effectiveness

Univariable survival analysis showed similar TFS between patients in the NoR/+R and +R/+R group (median TFS, 18.3 months versus 12.1 months; *P* for log-rank = 0.243; Fig. 3a). In multivariable analysis, the adjusted HR was not statistically significant different between the two treatment groups (HR, 1.71; 95% CI, 0.98–2.96; *P* = 0.060; Table 4). First-line treatment with a backbone of purine analogs, as

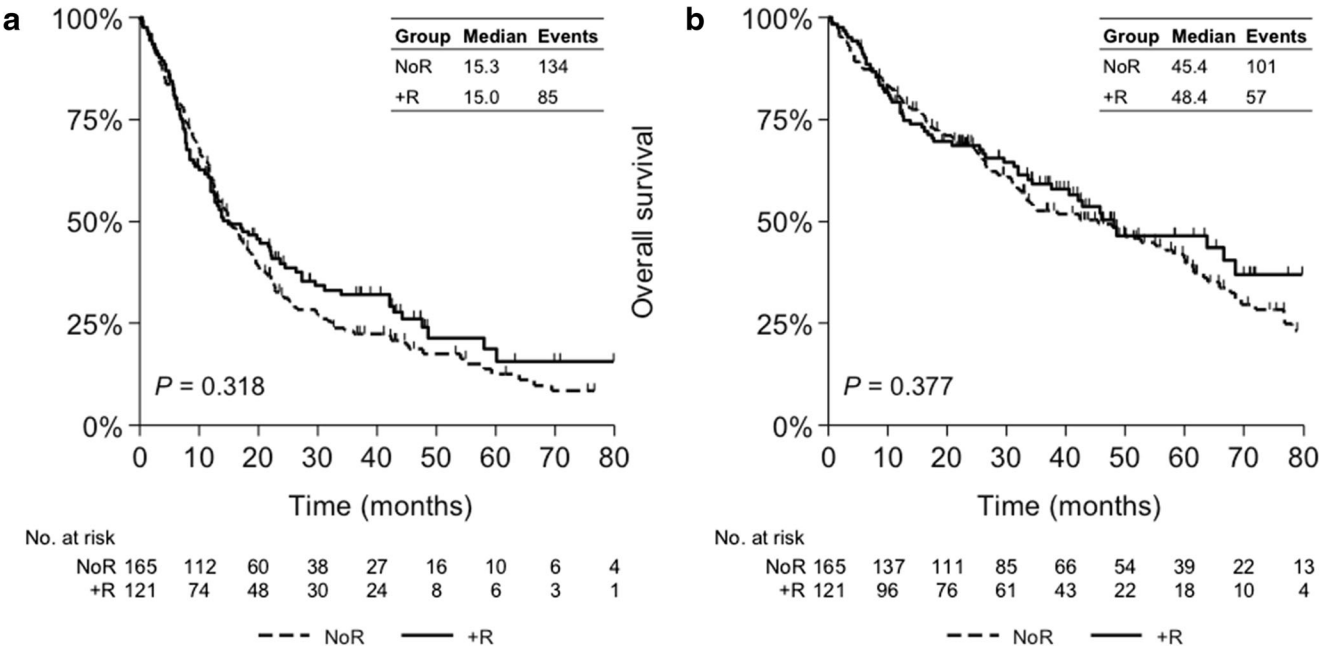

Fig. 2 Treatment-free survival (**a**) and overall survival (**b**) among patients with chronic lymphocytic leukemia who received second-line treatment with or without rituximab

Table 3 Cox regression analyses for treatment-free survival and overall survival in second-line treatment

Covariate	Treatment-free survival					Overall survival				
	HR	95% CI			*P*	HR	95% CI			*P*
Age at treatment, years[a]	1.02	1.00	–	1.04	*0.013*	1.05	1.04	–	1.07	*< 0.001*
Sex										
Male	1	ref				1	ref			
Female	0.98	0.74	–	1.31	0.880	0.74	0.53	–	1.05	0.096
Receipt of second-line rituximab										
No	1	ref				1	ref			
Yes	0.93	0.70	–	1.23	0.614	0.99	0.70	–	1.39	0.934
Chemotherapeutic backbone in first-line										
Alkylating agents	1	ref				1	ref			
Purine analogs	1.95	1.24	–	3.06	*0.004*	2.50	1.46	–	4.29	*0.001*
Other	0.95	0.38	–	2.34	0.906	1.41	0.51	–	3.91	0.505
Chemotherapeutic backbone in second-line										
Alkylating agents	1	ref				1	ref			
Purine analogs	0.84	0.61	–	1.15	0.269	1.12	0.77	–	1.63	0.550
Other	1.51	0.93	–	2.45	0.099	2.12	1.23	–	3.65	*0.007*
TTNT[b]	0.98	0.96	–	0.99	*< 0.001*	0.97	0.96	–	0.99	*0.001*

HR hazard ratio, *CI* confidence interval, *TTNT* time to next treatment

[a] Linear estimate per 1-year increase

[b] Linear estimate per 1-month increase

Italics denotes *P* values less than 0.05

compared with alkylating agents, was associated with a poorer TFS (HR, 2.62; 95% CI, 1.26–5.52, $P = 0.010$; Table 4), whereas patients with a longer TTNT had better TFS (HR per 1-month increase, 0.97; 95% CI 0.96–0.97; $P = 0.003$; Table 4).

Similar to TFS, the median OS (Fig. 3b) and the adjusted risk of death (Table 4) were not statistically significant different between the NoR/+R and +R/+R groups. In addition, in multivariable analysis, age per 1-year increase (HR 1.04; 95% CI, 1.01–1.07; $P = 0.009$) was associated with inferior OS, whereas a shorter TTNT (HR per 1-month increase, 0.98; 95% CI, 0.95–1.00; $P = 0.042$) was associated with better OS (Table 4).

No differences with respect to the ORR were found between recipients of second-line therapy with or without rituximab (50% versus 60%; $P = 1.00$).

Of note, exploratory analysis to assess TFS and OS in patients who were never treated with rituximab in first- and second-line (termed as the 'NoR/NoR group') showed no difference in outcome, as compared with the NoR/+R and +R/+R groups (Supplemental Table 2 and Supplemental Fig. 2).

Discussion

In this population-based study, we assessed the effectiveness of rituximab added to first- and second-line chemotherapy in CLL—with special emphasis whether the effectiveness of rituximab-containing therapy in second-line is affected by rituximab therapy in first-line. This emphasis was put on because chemoimmunotherapy is still regularly used in many countries across the globe in first- and second-line treatment. To our knowledge, this is the first population-based study that assessed the latter and more comprehensively assessed the former.

Congruent with findings from phase 3 trials [7, 19, 20], we demonstrated that the combination of rituximab to first-line chemotherapy—as compared to chemotherapy without rituximab—improved TFS, OS, and ORRs in patients with CLL within a population-based setting. Recently, two population-based studies demonstrated similar findings. However, one study only included patients managed within one region [23], whereas the other study only included patients managed within a single center [22]. Therefore, the present population-based study is the first that assessed the effectiveness of rituximab-containing therapy across several geographic regions encompassing multiple hospitals. Collectively, mainly owing to its relatively short-course of administration and cost-effective profile, first-line chemoimmunotherapy remains a viable treatment option in resource-limited countries and for the great majority of patients without adverse genetic factors such as *TP53* aberrations, especially in mutated patients.

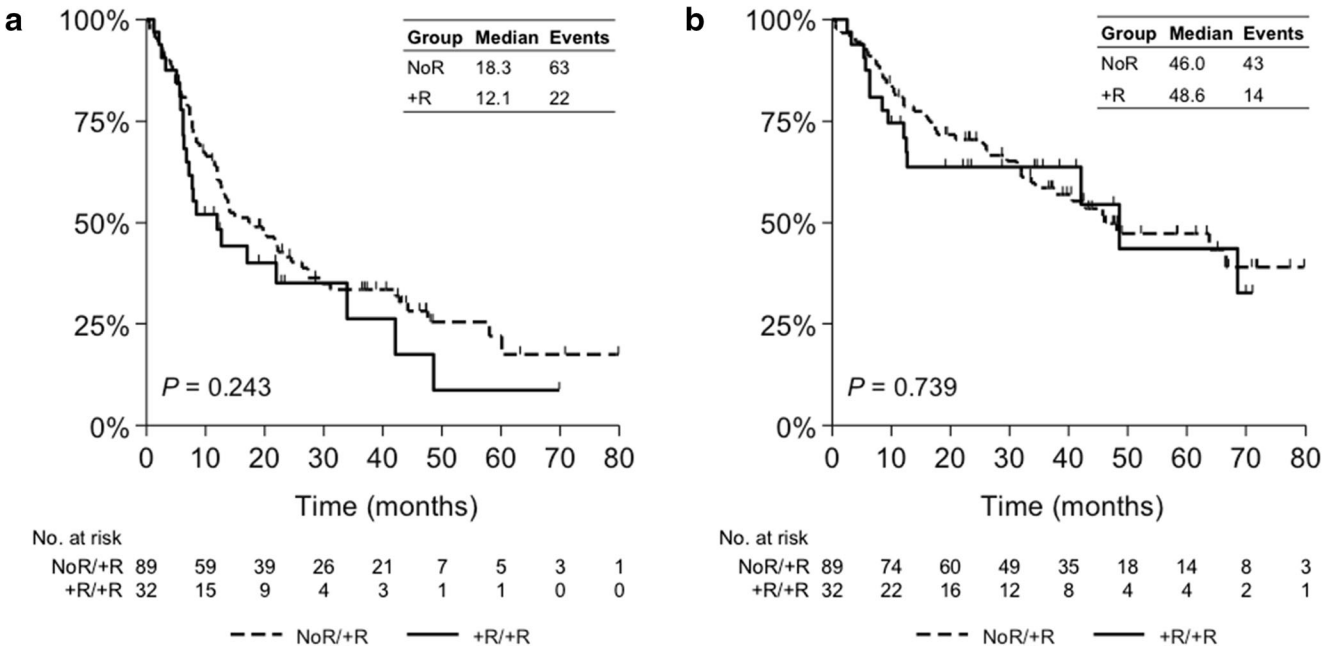

Fig. 3 Treatment-free survival (**a**) and overall survival (**b**) among patients with chronic lymphocytic leukemia who received rituximab-containing therapy in second-line according to the receipt of first-line therapy with or without rituximab

In contrast to the effectiveness of rituximab-containing therapy in first-line CLL treatment, an apparent lack of effectiveness of rituximab added to second-line chemotherapy was objectivated. Nevertheless, our finding contradicts that of the REACH trial [21] that demonstrated the efficacy of FCR, as compared to FC, among patients with CLL in the

Table 4 Cox regression analyses for treatment-free survival and overall survival in recipients of second-line treatment with rituximab-containing chemotherapy only

Covariate	Treatment-free survival					Overall survival				
	HR	95% CI			*P*	HR	95% CI			*P*
Age at treatment, years[a]	1.01	0.99	–	1.04	0.352	1.04	1.01	–	1.07	*0.009*
Sex										
Male	1	ref				1	ref			
Female	0.91	0.57	–	1.46	0.705	0.83	0.47	–	1.45	0.511
Receipt of first-line rituximab										
No	1	ref				1	ref			
Yes	1.71	0.98	–	2.96	0.060	1.09	0.54	–	2.20	0.818
Chemotherapeutic backbone in first-line										
Alkylating agents	1	ref				1	ref			
Purine analogs	2.62	1.26	–	5.42	*0.010*	2.34	0.96	–	5.68	0.061
Other	0.55	0.07	–	4.38	0.571	1.84	0.22	–	15.7	0.578
Chemotherapeutic backbone in second-line										
Alkylating agents	1	ref				1	ref			
Purine analogs	0.83	0.50	–	1.39	0.487	1.06	0.56	–	2.01	0.852
Other	1.33	0.66	–	2.67	0.420	1.80	0.78	–	4.12	0.166
TTNT[b]	0.97	0.95	–	0.99	*0.003*	0.98	0.95	–	1.00	*0.042*

HR hazard ratio, *CI* confidence interval, *TTNT* time to next treatment

[a] Linear estimate per 1-year increase

[b] Linear estimate per 1-month increase

Italics denotes *P* values less than 0.05

relapsed/refractory setting. Several arguments can be brought forward to explain the differences between our study and the REACH trial. As in most randomized controlled trials, the median age of patients is lower (e.g., 62 years in the REACH trial versus 72 years in this study) and patients generally have more favorable Eastern Cooperative Oncology Group (ECOG) performance scores and less serious comorbidities, as compared to patients managed in routine clinical practice. Furthermore, patients were not eligible for inclusion in the REACH trial when they were previously treated with rituximab. Thus, the exclusion of these patients results in selection bias. Although we demonstrated that second-line treatment with rituximab was comparable for patients with and without prior rituximab exposure, findings of the REACH trial cannot be extrapolated to a contemporary CLL population. Patients with CLL in the relapsed/refractory setting have often been treated with rituximab-based chemotherapy in first-line as part of standard care in contemporary clinical practices.

Very recently, the combination of venetoclax-rituximab was shown to be more effective in second-line treatment in terms of PFS [17]. However, considering the risk of tumor lysis syndrome, this combination is preferably not applied in patients with impaired kidney function. Furthermore, the duration of treatment with novel combinations, such as venetoclax-rituximab, is considerably longer, as compared to chemoimmunotherapy, which could lead to premature treatment discontinuation due to patient discouragement. Lastly, the market uptake of venetoclax (and ibrutinib) might be hampered in resource-limited countries [18]. As a result, the position of novel combinations in the treatment algorithm of CLL is continuously being debated. Taken collectively, chemoimmunotherapy is a viable option that still might be routinely applied in second-line treatment.

The hypothesis about the reduced effectiveness of rituximab in second-line treatment is deduced from the hypothesis of acquired rituximab resistance in non-Hodgkin lymphomas [34]. At present, only one study (that is, the prospective non-interventional PERLE study) specifically described the management of relapsed/refractory patients with CLL previously treated with rituximab in first-line and retreated with a rituximab-based regimen [35]. However, that study did not report on survival outcomes. We demonstrated that the effectiveness of second-line treatment with rituximab-containing regimens was not influenced by the application of rituximab in first-line. More specifically, these patients demonstrated TFS, OS, and ORR similar to those who received second-line therapy with rituximab-containing regimens without treatment with rituximab in first-line. Therefore, the hypothesis of acquired rituximab resistance could not be confirmed by the current study.

We certainly acknowledge that the comparatively low number of patients who received rituximab-containing therapy in both first- and second-line ($n = 31$) might have prevented to reveal a statistically significant difference between the NoR/+R and +R/+R groups. Furthermore, in a population-based setting, the choice of a particular treatment strategy is mainly influenced by the physician (i.e., confounding by indication). Therefore, the addition of rituximab to chemotherapy or the application of a more intensive chemotherapeutic backbone may reflect a heightened sense of urgency of response, thereby leading to variability regarding the initiation of a particular treatment. In addition, second-line treatment with rituximab in combination with alkylating agents appeared to be more often applied in the NoR/+R group (63% versus 31% in the +R/+R group). This might suggest that patients in the +R/+R group received a chemotherapeutic backbone in second-line with higher effectiveness (i.e., purine analogs and CHOP) that might counteract or conceal the resistance to rituximab. Therefore, it would be worthwhile to address the hypothesis regarding acquired rituximab resistance in a larger, broader population-based cohort of patients with CLL who received second-line treatment. Such an analysis will allow studying the effectiveness of specific types of rituximab-containing therapy in second-line while considering prior treatment with rituximab in first-line.

The strength of our study includes the use of a population-based cancer registry with comprehensive data available for individual patients. Furthermore, this is the first population-based study that provides comprehensive information on the effectiveness of second-line rituximab-based treatment with and without prior rituximab exposure. Limitations of our study mainly pertain to the lack of clinical information regarding the patient's fitness and response assessment (i.e., information on CT scans and bone marrow examination). Furthermore, since cytogenetic analysis was solely performed at diagnosis and not performed in a large number of patients, we were unable to include these well-known prognostic factors into the multivariable model.

In conclusion, in this population-based study, rituximab-containing therapy, as compared to therapy without rituximab, improves outcomes in patients with CLL in the front-line setting. However, the effectiveness of rituximab-containing therapy in second-line seems to be equal to that of second-line therapy without rituximab. Furthermore, its effectiveness seems not to be influenced by prior treatment with rituximab in first-line. Future population-based research is imperative to assess whether novel strategies, such as ibrutinib or rituximab with venetoclax, may improve outcomes among patients with CLL in the relapsed/refractory setting. In the meantime, while we await larger, broader population-based studies that address the effectiveness of rituximab-containing therapy in second-line CLL treatment, standard chemoimmunotherapy, ibrutinib, or rituximab and venetoclax remains the standard of care for second-line CLL treatment.

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Authors' contributions LvdS, AGD, and M-DL designed the study; LvdS analyzed the data; AGD provided statistical support; ECvdB collected the data; LvdS wrote the manuscript with contributions from all authors, who also interpreted the data, and read, commented on, and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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