Rituximab induction immunotherapy for first-line low-tumor-burden follicular lymphoma: survival analyses with 7-year follow-up


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Background: The purpose of this study was to report long-term results of rituximab induction monotherapy in patients with low-tumor-burden follicular lymphoma (LTBFL).

Patients and methods: Of 49 first-line LTBFL patients who received weekly doses of rituximab (375 mg/m²), 46 have been followed with a long-term analysis of clinical and molecular responses.

Results: Best clinical response (at any staging within a year following treatment) was 80%, 24 (52%) patients had complete or unconfirmed complete response, 13 (28%) had partial response and 9 (20%) had stable or progressive disease. Of 31 patients having a positive bcl2-JH rearrangement, 15 (48%) became negative following treatment. After 83.9 months of follow-up (95% confidence interval 6.4–92.8 months), the median progression-free survival is 23.5 months and overall survival (OS) is 91.7%. Five patients died (one progression, one myelodysplasia, one diffuse large B-cell lymphoma and two solid tumors). Seven patients (15%) are progression-free including five who are bcl2 informative. No unexpected long-term adverse event has been observed.

Conclusion: A significant proportion of patients remain progression-free 7 years after a single 4-dose rituximab treatment in first-line LTBFL. The 7-year overall survival (OS) is very high in this selected population of patients.

Key words: follicular lymphoma, induction monotherapy, low-burden, rituximab

introduction

Although a number of standard chemotherapeutic and immunotherapeutic agents are active in the treatment of patients with follicular lymphoma (FL), these treatments do not cure the patient. In patients with low-tumor-burden and without adverse prognostic factors, three randomized studies failed to show any survival benefit of chemotherapy compared with the observation [1–3]. Hence, watchful waiting is currently considered as the standard strategy, until clinical signs or symptoms warrant intervention [4, 5].

Rituximab (MabThera) is a chimeric murine/human anti-CD20 monoclonal antibody capable of lysing CD20+ lymphoma cells through multiple mechanisms of action,
including complement-mediated cytotoxicity, antibody-dependent cellular toxicity and direct induction of apoptosis [5–7]. In high-tumor-burden FL, rituximab added to chemotherapy is now widely used, because it significantly improves response rates, progression-free survival (PFS) and most importantly, overall survival (OS), compared with the same chemotherapy regimen given alone [8–11].

In 2001, we reported the effectiveness of rituximab in terms of clinical and molecular response, when given at a dose of 375 mg/m²weekly for 4 weeks in 49 FL patients with low tumor burden [12]. The clinical response rate was 73% and 16 of the 26 patients (62%) who responded and were informative for BCL2 rearrangement were polymerase chain reaction (PCR) negative in peripheral blood at month 12. Three other studies have addressed the efficacy of rituximab monotherapy in previously untreated advanced (high tumor burden) indolent FL with a response rate between 47% and 72% [13–15].

However, the long-term results of rituximab monotherapy have not yet been published in terms of PFS and OS and minimal residual disease negativity. This report summarizes the long-term results of the patients included in our study.

patients and methods

patients

As previously published, the patients were eligible if they were adults, 18–75 years of age with a histologically confirmed diagnosis of FL according to the Revised European American Lymphoma classification [16], stage II–IV of the Ann Arbor classification, with at least one measurable site and low tumor burden according to the GELF criteria as detailed in our previous report [12].

The study protocol was approved by the institutional ethics committee of Angers (France), and informed written consent was obtained from each patient before therapy in accordance with the Helsinki protocol. The trial was registered at http://www.roche-trials.com/patient/trials/trial167.html. A 3-year follow-up period was initially planned, but because of the prolonged remissions and survival observed in the study population, an extension was warranted to better assess long-term efficacy of rituximab. A second written informed consent for both clinical and biological monitoring had, therefore, to be obtained before inclusion in the extended follow-up study.

treatment

Rituximab was given on an outpatient basis at a weekly dose of 375 mg/m² by intravenous infusion, for a total of 4 doses (days 1, 8, 15 and 22).

clinical monitoring

To assess all possible sites of disease involvement, baseline evaluation (radiography, CT scan, bone marrow biopsy and laboratory testing) has been described in a previous publication [12]. Monitoring included hematology and serum chemistry assessments before each treatment cycle and full tumor restaging 28 days after the end of therapy, 1 month later, every 3 months for 1 year and then every 6 months for the following 6 years.

pathological review

For this study, biopsy specimens at initial diagnosis were centrally reviewed to confirm the initial diagnosis and to analyze the intratumoral macrophage count.

The available biopsy specimens at relapse were also collected for centralized review and compared with the initial biopsy specimens. Morphologic features of transformation were assessed and CD20 expression was performed using a standard immunohistochemical technique.

molecular monitoring of BCL2—IgH gene rearrangements

All collected samples were centralized in a single laboratory (GS, Lyon-Sud University Hospital, Pierre-Bénite, France). DNA extraction and amplification have been described in a previous publication [12]. The nested PCR analysis was performed using MBR (CAGCCCTGAAACATTGATGG) or mcr (CGTGGCTGTCACCTCTCCTG) with JH (ACCTGAGGAGACGGTGACC) specific primers for the first round (30 cycles for MBR, 25 cycles for mcr), then a reamplification of 4% of the reaction product with internal MBR (CTATGTGTTGTGACCTTTAGAG) or mcr (GGACCTTCCTTTGTTGTGTTG) and JH (ACCAAGGTCCCTTGGCCCCACG) oligonucleotides (30 cycles each). The sensitivity of this assay was routinely greater than or equal to 10⁻⁴.

Molecular evaluation was performed on both blood and marrow samples before treatment and, for informative patients, 1 month after the end of study treatment, at months 12, 24 and 36. The bcl-2 monitoring was then performed on blood only every 6 months for a total of 7 years.

response assessment and statistical analysis

The protocol was designed before the first report of response assessment on lymphoma was published by Cheson et al. in 1999 [17] and was thus based on criteria used in solid tumors. The primary efficacy end point was clinical response 1 month after the end of rituximab treatment (day 50) with confirmation of the response 4 weeks later (day 78), at 6 months (day 180), then every 6 months for 7 years. After the publication of Cheson et al., all clinical and radiological data for response assessment were reviewed by an independent committee (panel of radiologists and clinicians), which also determined the ‘best response’ status (i.e. the most complete clinical response) from any evaluation during the follow-up, according to the Cheson criteria [17].

The secondary end points were PFS, OS and bcl-2 status. PFS was measured from the start of treatment until progression/relapse or death and OS was defined as time from the start of treatment until death. Response duration curves were constructed from the response assessment on day 78 until relapse. Survival curves were calculated according to the method of Kaplan and Meier and compared using the log-rank test.

results

Overall, 50 patients were enrolled and 49 patients were analyzed in the initial study from October 1997 to August 1998 [12]. Subsequently, three further patients were excluded as the initial diagnosis of FL was not confirmed by centralized pathology review using additional immunohistochemistry analysis, which was not available at the time of the initial study.

Therefore, a total of four patients were excluded (transformed FL, MALT, small lymphocytic non-FL and diffuse large B-cell lymphoma). Efficacy results are presented for the population of 46 patients with FL. Two patients chose not to participate in the long-term part of the study and one patient died from squamous lung cancer before month 24. Four further patients died before month 84 (one patient from urothelial cancer, one from tumor progression, one from myelodysplasia and one
from diffuse large B-cell lymphoma) (see Figure 1 for CONSORT flow diagram).

The characteristics of the patient population are described in Table 1. There were 24 males and 22 females. About 70% of patients had stage IV disease, with 56% presenting with an intermediate or high FLIPI score upon inclusion.

Initial clinical and molecular response to rituximab (evaluation up to month 12)

The clinical response rate at day 78 was 74% with 13 complete responses (CR)/unconfirmed complete responses (CRu) and 23 partial responses (PR). As frequently observed in advanced indolent non-Hodgkin’s lymphoma (NHL), a high proportion of patients reached maximal response to rituximab monotherapy beyond the study primary criteria assessment deadline (day 78): 12 of the 23 patients initially assessed as having PR converted to CR/CRu at month 12, and 3 of the SD patients converted to PR at month 12, with one of these eventually converting to CR at month 36. When best response was taken into consideration, a total of 24 (52%) patients reached maximal response to rituximab monotherapy beyond the study primary criteria assessment deadline (day 78): 12 of the 23 patients initially assessed as having PR converted to CR/CRu at month 12, and 3 of the SD patients converted to PR at month 12, with one of these eventually converting to CR at month 36. The best clinical response rate at any staging during the first year of follow-up after rituximab treatment was 80%.

Thirty-two of the 46 (70%) patients were deemed bcl-2 informative at inclusion, defined by positivity at baseline of the bcl-2/IgH specific PCR, in blood or bone marrow. Of the 32 bcl-2 informative patients, 31 patients were assessable for molecular response; 10 patients (32%) became bcl-2 negative at day 50 and 15 (47%) patients became bcl-2 negative at month 12. No patients were withdrawn due to their bcl-2 status. Details regarding assays results are presented in supplementary Table S1, available at *Annals of Oncology* online.

**Long-term results**

The median follow-up time was 83.9 months [6.4–92.8]. The median PFS for the entire group was 23.5 months [95% confidence interval (CI) 13.6–36.7] (Figure 3), and the median response duration for the responders after 3 months (n = 36) was 28.7 months (95% CI 14.9–54.4).

The prognostic value of clinical and molecular response at 3 months on PFS was analyzed. Clinical response (Cheson criteria) was predictive of progression, with median PFS of 51.0 months for CR/CRu, 23.0 months for PR and 9.5 months for SD/PD patients (P = 0.057 log rank) (Figure 4). While, initial bcl-2 clearance in blood was not correlated with subsequent clinical response, molecular response at day 50 was correlated with clinical response at 12 months (15 responders/17 bcl-2 negative patients versus 4 responders/13 bcl-2 positive patients, P = 0.002). Also, the median PFS for patients still bcl-2 positive in blood at day 50 (12.5 months) was significantly shorter than the median PFS of 36.7 months for patients who responded and became bcl-2 negative after rituximab treatment with (P = 0.019) (Figure 5).

Table 1. Patients characteristics (n = 46)

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<th>Patients characteristics</th>
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<tr>
<td>Follicular histology</td>
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<td>Age (years)</td>
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<td>Median</td>
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A significant difference was also found when the same analysis was done considering the results of bcl-2 in the blood and/or bone marrow at 12 months ($P < 0.001$). For patients bcl-2 negative in the blood and/or bone marrow at 12 months, the median PFS from the date of start of treatment was 66.8 months and 10/15 patients (66.7%) were in progression. For bcl-2 positive patients at 12 months, the median PFS was only 12.5 months and all 11 patients (100%) were in progression. Importantly, more than 7 years after receiving 4-weekly doses of rituximab, seven patients (15% of the study population) are still progression-free. Five of these patients were bcl-2-informative and all were serum PCR negative at their latest follow-up visit. Long-term response does not seem to be correlated to FLIPI score, because 3 and 4 of the long-term responders had, respectively, low and intermediate FLIPI scores. Moreover, two patients also had bone marrow involvement at diagnosis.

pathological review at relapse

Of the 36 patients who relapsed during the study, 26 underwent a new tumor biopsy that was available for central pathological review. Over the 7 years of follow-up, progression to an aggressive type of lymphoma was infrequent, with only one case of diffuse large B-cell lymphoma transformation. A follicular-type relapse was confirmed in the 24 remaining patients. One patient developed a Hodgkin lymphoma 9 months after entering the study. Her initial biopsies have been reviewed and the initial diagnosis of FL was confirmed. CD20 expression was confirmed by immunostaining for each patient.

safety results

No additional safety signals have been identified since our first report (mainly grade 1 and 2 fever, headache, laryngitis and rhinitis), despite continuous monitoring for infectious and cancer events.

deaths

Five patients died during the 7-year study period from disease progression, myelodysplasia, squamous lung cancer, urothelial cancer and aggressive diffuse large B-cell lymphoma.

discussion

Watchful waiting is currently considered as the standard therapeutic approach for FL with low tumor burden or low FLIPI score. Indeed, none of the three randomized trials have shown an OS benefit of chemotherapy or immunotherapy when compared with the observation [1–3]. Ideally, new therapeutic approaches for this group of patients should aim at
improving survival, but demonstrating an OS benefit would require a prohibitively large and lengthy trial, considering the median survival of this specific population (estimated at 8–10 years [1]). More realistically for this group of patients where the medical need is nevertheless high, new therapies should at least demonstrate both high response rates and prolonged progression-free survival, with minimal impact on quality of life and a good safety profile.

Even if watchful waiting of newly diagnosed but asymptomatic advanced-stage FL patients is nowadays more frequently questioned, the optimal therapeutic approach for these patients remains to be defined. Rituximab appears to be a good therapeutic candidate in this setting due to its low toxicity profile and its proven efficacy in symptomatic indolent NHL. One trial was published on asymptomatic low-tumor-burden patients since our first report in 2001 [12]. Witzig et al. reported phase II trial results involving of 37 patients with asymptomatic newly diagnosed grade 1 FL, treated with rituximab monotherapy using the same dose and schedule as in our trial. An overall response rate of 72% was observed, with 36% CR and a median time to progression of 2.2 years. The initial median follow-up was short (2.6 years) and no long-term follow-up has since been reported [15]. Recently, Arshedna et al. has presented the interim analysis results of a randomized trial in this setting. The patients were randomly assigned to a wait and watch strategy (n = 186) or rituximab 375 mg/m² weekly for 4 weeks (n = 84) or rituximab 375 mg/m² weekly for 4 weeks followed by rituximab 375 mg/m² maintenance every 2 months for 2 years (n = 192). The primary end point, time to initiation of new therapy (TTINT), was clearly in favor of rituximab arms (Rituximab versus wait and watch), HR = 0.37, 95% CI 0.25–0.56, P < 0.001. The median TTINT was not reached in rituximab arms and was 33 months in the W + W arm. No benefit on OS was observed so far [18]. The benefit of rituximab in early treatment of FL patients with low tumor burden requires a longer follow-up to assess a long-term benefit.

The long-term results of our study, now with a 7-year follow-up, confirm the high efficacy of single-agent rituximab given as 4-weekly doses in 46 untreated asymptomatic patients with FL. Based on the delayed clinical and molecular responses seen in many patients, the Reviewing Committee of the study decided to also assess response rates to rituximab in terms of best response, in addition to early clinical evaluation at day 50 and 3 months. In 2001, we reported the response rates evaluated at 3 months (73% ORR, 27% CR/CRu), but the best response rate was 80% with 41% of patients being in CR/CRu. At 12 months, 15 patients became bcl-2 negative (10 patients were bcl-2 negative at day 50). These results indicate that even in low-tumor-burden patients, maximum clinical and molecular response may be delayed for up to 12 months after rituximab monotherapy [12].

With nearly 2 years of median PFS after 4-weekly doses of rituximab (and a median relapse-free survival of 28.7 months for responders), these results are in line with those obtained by Witzig et al. on a smaller cohort [15]. Other studies exploring the efficacy of rituximab as monotherapy for upfront treatment of FL, have been performed in symptomatic patients and included a rituximab maintenance phase, which limits any results comparison with our trial [13, 14]. Martinelli et al. reported the long-term results (follow-up of 9.5 years) of a randomized trial, in 202 patients with FL (first line n = 64, relapse n = 138), assessing the benefit of induction therapy with 4-weekly doses single-agent rituximab followed or not by four additional doses of rituximab every 2 months in non-progressive disease. The median event-free survival for the whole study population was 13 months versus 24 months (P < 0.001) for the induction arm compared with the prolonged exposure arm. Maintenance therapy was effective regardless of the line of treatment. Efficacy results were consistent with our results in first line patients [14].

In contrast with the observed delay in clinical response, the molecular follow-up of the bcl-2 informative patients showed that early bcl-2 clearance in blood (at day 50) seemed to be correlated with better clinical response at 12 months (88% of responders in negative patients versus only 31% if bcl-2 were still positive), prolonged PFS and response duration. But the key issue would be to know whether or not long-term PCR negative disease-free survivors are cured. We observed no relapse beyond the sixth year, and a significant number of patients (n = 7) were still disease-free 7 years after 4 weekly doses of rituximab. Persistent PCR negativity could suggest cure for these patients, but an even longer follow-up is warranted to confirm this assumption.

While these results are encouraging, the optimal administration schedule of rituximab in these patients remains unknown, and 4-weekly rituximab infusions may not be sufficient. Indeed, the fact that we observed delayed responses indicates that although rapid-onset ADCC and induction of apoptosis are important mechanisms of action of rituximab, the achievement of full clinical efficacy is a lengthy process in FL. Therapeutic efficacy is probably not only linked to the magnitude of serum rituximab concentrations, but also to the duration of rituximab exposure, as suggested by the results of several rituximab maintenance therapy trials in FL patients with high tumor burden [6, 7, 19]. The intrinsic efficacy of rituximab might also potentially be improved with adjuvant agents such as granulocyte–macrophage colony-stimulating factor, Interleukin or vaccine, but clinical results of these agents are still preliminary [20–22].

Additional important information from this trial was obtained by the systematic central pathology review of all biopsies that could be obtained for patients at relapse. CD20 expression on tumor cells was confirmed by immunohistochemistry staining and consistently observed on every follicular relapse observed in this population. This finding does not confirm, the few published reports showing a reduction or loss of CD20 expression by tumor cells of patients relapsing after rituximab therapy [23–25].

Furthermore, no additional safety signals have been identified since our first report. Only five patients died during the 7-year study period. The observed incidence of transformation was remarkably low in this study, with only one patient evolving to an aggressive diffuse large B-cell lymphoma.

The results of this study evaluating long-term results after rituximab given as a 4-weekly infusion in first-line FL patients with low tumor burden indicate that rituximab could be a...
promising and safe alternative to watchful waiting strategy. As reported in high-tumor-burden FL, recent data support the use of induction plus maintenance in FL with low tumor burden. However, further follow-up is required to prove the benefit of early treatment and the induction plus maintenance schedule in this setting.

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