Rituximab and the risk of transformation of follicular lymphoma: a retrospective pooled analysis

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Summary

Background Histological transformation of follicular lymphoma to aggressive lymphoma is a serious event with a substantial effect on patient outcome. The aim of the Aristotle study was to assess the effect of rituximab on the risk of histological transformation and its outcome.

Methods 11 cooperative groups or institutions across Europe contributed data to this study. Eligible patients (≥18 years) had histologically confirmed follicular lymphoma grade 1, 2, or 3a, diagnosed between Jan 2, 1997, and Dec 20, 2013. Histological transformation was defined as a biopsy-proven aggressive lymphoma that occurred as a first event after first-line therapy. The primary endpoints were the cumulative hazard of histological transformation and survival after transformation.

Findings Information was available for 10 001 patients with follicular lymphoma, 8116 of whom were eligible for analysis. 509 histological transformations were reported. After a median follow-up of 87 months (range 1–221; 2·5–97·5th percentile 5–160), the 10-year cumulative hazard of histological transformation was 7·7% (95% CI 6·9–8·5). The 10-year cumulative hazard of histological transformation was 5·2% (95% CI 4·5–6·2) in patients who received rituximab and 8·7% (7·2–10·6) in those who did not (hazard ratio [HR] 0·73, 95% CI 0·58–0·90; p=0·004). The 10-year cumulative hazard of histological transformation was 5·9% (95% CI 5·0–7·0) for patients who received induction rituximab only and 3·6% (95% CI 2·3–5·5) for those treated with induction and maintenance rituximab (HR 0·55, 95% CI 0·37–0·81; p=0·003). This finding was confirmed in a multivariate analysis (p=0·016). 287 deaths were recorded in 509 patients with histological transformation, resulting in a 10-year survival after transformation of 32% (95% CI 26–38). Survival after transformation did not differ between patients not exposed to rituximab and those who received rituximab in induction only (HR 0·94, 95% CI 0·69–1·28; p=0·70), and those who received rituximab in induction and maintenance (0·96, 0·58–1·61; p=0·88).

Interpretation The risk of histological transformation as a first event can be significantly reduced by the use of rituximab. These findings support the need to inform patients using rituximab nowadays that the risk of transformation is lower than it was before the introduction of rituxumab.

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Introduction

Follicular lymphoma is the second most frequent type of non-Hodgkin lymphoma and accounts for about 10–20% of all lymphomas in developed countries.[1,2] The clinical course of follicular lymphoma is heterogeneous, ranging from long-term responses to initial therapy to frequent recurrences, with a shorter treatment response duration after every relapse. The treatment priority for follicular lymphoma is to avoid relapses, transformation to more aggressive subtypes, and death. Transformation to an aggressive lymphoma, most commonly diffuse large B-cell lymphoma, is a serious clinical event with a marked effect on the course of the disease.[3–5] The reported incidence of histological transformation has varied over the past 35 years, ranging from 10% to 70%.[3,6–12] This large variability is believed to be related to several factors, including the absence of a unique definition of histological transformation, heterogeneity of the analysed populations, and differences in diagnostic methods, the initial therapeutic approach, and length of follow-up. The annual risk of histological transformation has been estimated to be 2–3% per year,[9–12] with a median post-transformation survival of less than 2 years with conventional chemotherapy.[9,10,13,14]

The addition of the anti-CD20 monoclonal antibody rituximab to chemotherapy regimens for follicular lymphoma has led to an impressive improvement in patient outcomes, although the effect of rituximab on

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Research in context

Evidence before this study

Histological transformation of follicular lymphoma to an aggressive lymphoma, most commonly a diffuse large B-cell lymphoma, is a serious clinical event with a substantial effect on the course of the disease. Before initiating this study, we searched PubMed on Oct 1–2, 2015, for English-language articles published since 1990 with the terms "follicular lymphoma", "rituximab", "histological transformation", and "outcome", either individually or as part of a combination. We found that most previous studies reported cumulative hazard of transformation, and not only histological transformation as a first event. Furthermore, several studies included cases in which transformation was diagnosed on the basis of clinical characteristics alone. Together, these issues make comparison of the absolute cumulative hazards of histological transformation in follicular lymphoma between studies difficult. The aim of the present study was to collect as large a series of cases of follicular lymphomas as possible, from archives of prospective clinical trials or population-based registries, to better explore the effect of rituximab on the risk of histological transformation as a first event after first-line therapy, and on the outcome after transformation.

Added value of this study

To the best of our knowledge, the number of patients with a biopsy-proven transformation as a first event after first-line therapy among the Aristotle cohort is the largest reported so far. Findings from the Aristotle study show that rituximab can have an important effect on histological transformation as a component of first-line therapy for follicular lymphoma. We found that patients initially treated with rituximab (alone or in combination with chemotherapy) had a lower cumulative hazard of histological transformation compared with patients who did not receive this treatment.

Implications of all the available evidence

Despite the potential limitations of our retrospective analyses, the Aristotle study showed that the cumulative hazard of histological transformation as a first event was significantly reduced by the introduction of rituximab. However, histological transformation still had an adverse effect on prognosis, albeit less catastrophic compared with the pre-rituximab era. Histological transformation remains a challenge for clinicians. A more comprehensive knowledge of the biological risk factors for follicular lymphoma transformation and its molecular pathways might help in making more accurate prognostic assessments and in predicting the putative usefulness of novel drugs. This, in turn, might contribute to both the prevention and potential cure of transformed follicular lymphomas.

the risk of transformation remains largely unknown. Studies from the past 5 years have proposed the notion of a reduced risk of histological transformation associated with rituximab-containing therapies.[15–19] Moreover, survival after histological transformation in patients treated with rituximab, although still poor, seems better than in patients who have not received rituximab.[12,15,17,19] In 2015, the European Lymphoma Institute (ELI) and the European Haematology Association Lymphoma Group together launched the Aristotle study, a pooled analysis of follicular lymphoma cases registered at the time of initial diagnosis in different clinical trials or lymphoma registries, and with long-term follow-up. In this study, we aimed to assess the effect of rituximab on the risk of histological transformation as a first event after first-line therapy, as well as the outcome after transformation.

Methods

Study design and patients

11 cooperative groups or institutions across Europe contributed to the Aristotle study, providing information on newly diagnosed follicular lymphoma cases retrieved from prospective clinical trials or population-based or hospital-based lymphoma registries. All included patients had histologically confirmed follicular lymphoma grade 1, 2, or 3a, as defined by the 2008 WHO classification system,[1] were diagnosed between Jan 2, 1997, and Dec 20, 2013, and were aged at least 18 years. Only biopsy-proven histological transformations occurring as a first event after first-line systemic therapy were included.

Patients initially assigned to a watch-and-wait period were included only if they had started systemic therapy owing to disease progression in the absence of transformation. Since Aristotle is a retrospective multicentre study, a prespecified interval between diagnosis and the start of treatment for establishing the watch-and-wait policy was not required. However, the minimum interval between diagnosis and first event was set to 3 months. The initial diagnosis and type of histological transformation as a first event was based on reporting by the local pathologists at the participating institutions; a central pathology review was not done.

Patients who presented at diagnosis with evidence of both follicular lymphoma and diffuse large B-cell lymphoma in the same tissue specimen were judged to be transformed at initial diagnosis and were excluded from the study, as were patients with follicular lymphoma grade 3b. Additionally, patients with a discordant histological diagnosis between nodal site and bone marrow were excluded. The initial therapy included different treatment categories (eg, single-agent or combined chemotherapy with or without rituximab, or

rituximab monotherapy). Maintenance with rituximab was prescribed according to local clinical practices or as per clinical trial design.

Study approval was obtained from the institutional review board at the coordinating centre (ELI, Lyon, France) and at each participating centre in accordance with institutional standards. For those patients enrolled in clinical trials, ethics approvals were already obtained; anonymised data from these trials were used in this study and therefore no further ethics approval was required.

Outcomes

The primary endpoints were the cumulative hazard of histological transformation and survival after transformation. The secondary endpoints were time to histological transformation, overall survival, and survival after relapse.

Relapse or recurrence was defined as the presence of follicular lymphoma disease after the achievement of a complete or partial response to first-line therapy lasting at least 3 months. Progression was defined as the presence of follicular lymphoma disease due insufficient response to first-line treatment. Transformation was defined as the presence, at the time of recurrence, relapse, or progression, of an aggressive lymphoma (from an initial diagnosis of indolent lymphoma). An event was defined as a relapse or recurrence of follicular lymphoma, a progression of follicular lymphoma, a biopsy-proven transformation, or death.

Statistical analysis

The cumulative hazard of histological transformation was calculated using the Nelson-Aalen estimator with a 95% CI.[20] The estimate was the absolute value multiplied by 100. Survival after transformation was measured as the time from histological transformation to either the date of death from any cause or last clinical contact. Survival after transformation was calculated by means of Kaplan-Meier estimates,[21] also with 95% CIs. Comparisons between categories were made using the log-rank test, and the effect of covariates was estimated with a Cox proportional hazard regression model[22] with 95% CIs, by either univariate or multivariable analysis. Proportionality of hazard was checked graphically by means of scaled Schoenfeld residuals.

We assessed the risk of transformation in patients not exposed to rituximab, those who received rituximab in induction only, and those treated with both induction and maintenance rituximab. We assessed the risk of histological transformation associated with rituximab in a multiple regression model, controlling for Follicular Lymphoma International Prognostic Index (FLIPI) score (0–1=low risk; 2=intermediate risk; 3–5=high risk), therapeutic approach (treatment upfront or previous watch and wait), histological grade at diagnosis, time to transformation (≤12 months or >12 months) and demographic variables (age, sex).

Time to histological transformation was calculated from the date of follicular lymphoma diagnosis to the date of histological transformation. The median time to transformation, overall survival, and corresponding 5-year and 10-year percentages were estimated using Kaplan-Meier methods. Patients alive at the last clinical contact or who died during follow-up were censored.

Survival after relapse was measured from the date of relapse to last follow-up or death from any cause. Time to relapse or progression was calculated from the date of follicular lymphoma diagnosis to the date of relapse or progression.

Age was the only continuous variable, and was summarised as the median with 2·5–97·5th percentiles and subsequently dichotomised according to the usual clinical thresholds reported in the literature (≤60 years *vs* >60 years).[23] We chose to use 2·5–97·5th percentiles rather than 95% CIs because they provide more detail about the dispersion of the data. Categorical variables were reported as absolute values and percentages. Fisher's exact tests or χ^2 tests were used to compare variables as appropriate. For this retrospective study, a specific sample size was not planned. All statistical tests were two sided and p values less than 0·05 were judged to be significant. All analyses were done in Stata 14.2.

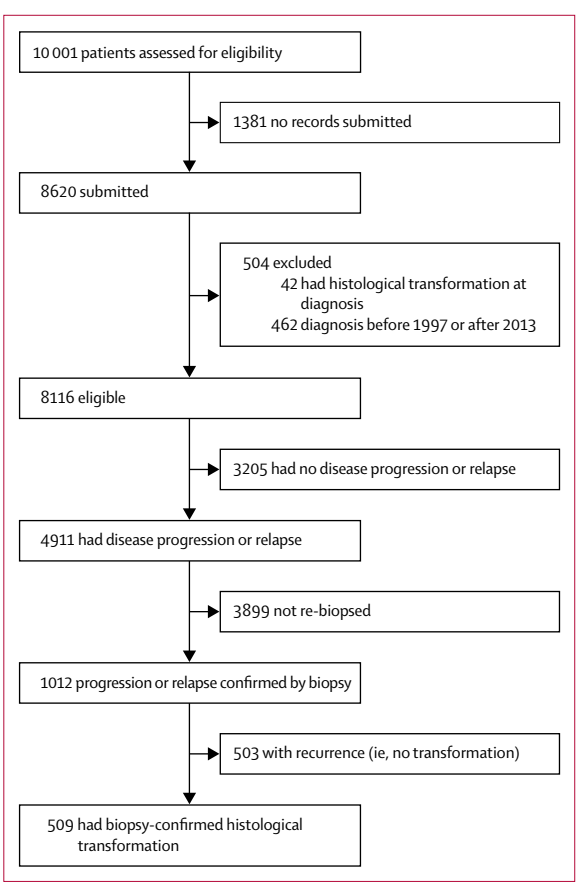

Figure 1: **Study profile**

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

See Online for appendix

	Lymphoma registries (n=2954)	Clinical trials (n=2360)	Population-based registries (n=2802)	Total (n=8116)
Age, years	58 (31–83)	56 (33–75)	61 (35–86)	59 (33–83)
Sex				
Women	1625 (55%)	1232 (52%)	1378 (49%)	4235 (52%)
Men	1329 (45%)	1128 (48%)	1424 (51%)	3881 (48%)
Histological grade				
1–2	2099 (71%)	1480 (63%)	1922 (69%)	5501 (68%)
3a	481 (16%)	232 (10%)	340 (12%)	1053 (13%)
Not assessed	374 (13%)	648 (27%)	540 (19%)	1562 (19%)
FLIPI risk group				
Low	767 (26%)	522 (22%)	870 (31%)	2159 (27%)
Intermediate	830 (28%)	828 (35%)	700 (25%)	2358 (29%)
High	896 (30%)	985 (42%)	704 (25%)	2585 (32%)
Data missing	461 (16%)	25 (1%)	528 (19%)	1014 (12%)
Ann Arbor stage				
I–II	450 (15%)	249 (11%)	859 (31%)	1558 (19%)
III–IV	2447 (83%)	2100 (89%)	1905 (68%)	6452 (79%)
Data missing	57 (2%)	11 (<1%)	38 (1%)	106 (1%)
Initial treatment				
Watch and wait	321 (11%)	54 (2%)	752 (27%)	1127 (14%)
Active treatment	2628 (89%)	2295 (97%)	2047 (73%)	6970 (86%)
Data missing	5 (<1%)	11 (<1%)	3 (<1%)	19 (<1%)
Treatment				
With rituximab				
CHOP or CHOP like	1236 (42%)	1062 (45%)	447 (16%)	2745 (34%)
CVP or chlorambucil	235 (8%)	591 (25%)	494 (18%)	1320 (16%)
Fludarabine combination	396 (13%)	216 (9%)	16 (1%)	628 (8%)
Rituximab monotherapy	19 (1%)	0	389 (14%)	408 (5%)
Other	230 (8%)	0	0	230 (3%)
High-dose treatment	0	0	0	0
Without rituximab				
CHOP or CHOP like	44 (1%)	0	73 (3%)	117 (1%)
CVP or chlorambucil	12 (<1%)	323 (14%)	53 (2%)	388 (5%)
Fludarabine combination	9 (<1%)	0	16 (1%)	25 (<1%)
Monotherapy	111 (4%)	0	368 (13%)	479 (6%)
Other	336 (11%)	0	187 (7%)	523 (6%)
High-dose treatment	0	103 (4%)	4 (<1%)	107 (1%)
Data missing	326 (11%)	65 (3%)	755 (27%)	1146 (14%)
Maintenance after rituximab*				
No	462/2116 (22%)	1203/1869 (64%)	1104/1346 (82%)	2769/5331 (52%)
Yes	231/2116 (11%)	666/1869 (36%)	131/1346 (10%)	1028/5331 (19%)
Not assessed	1423/2116 (67%)	0/1869	111/1346 (8%)	1534/5331 (29%)

(Table 1 continues on next page)

Results

The Aristotle database contained 10 001 follicular lymphoma cases received from the different sites between Feb 1, 2016, and July 24, 2017 (figure 1). We subsequently excluded 1885 (19%) cases: 1381 because no records were submitted (owing to the complexity in submitting the protocol to the ethics committees by some of participating sites), 42 owing to histological transformation at diagnosis, and 462 owing to diagnosis outside the allowed timeframe. 8116 patients (81%) were eligible for analysis. Data for 2954 (36%) patients were retrieved from lymphoma registries, 2360 (29%) from clinical trials (appendix p 4), and 2802 (35%) from population-based registries. The median age for the entire cohort was 59 years (2·5–97·5th percentile 33–83), and 5501 (68%) patients had a diagnosis of grade 1–2 follicular lymphoma (table 1). A FLIPI score was available in 7102 patients (88%), of whom 2159 (30%) were low risk, 2358 (33%) were intermediate risk, and 2585 (36%) were high risk.

Disease progression or relapse occurred in 4911 patients (61%), with confirmation by biopsy in 1012: 509 received a diagnosis of histological transformation (490 [96%] diffuse large B-cell lymphoma; 16 [3%] follicular lymphoma grade 3b; two [<1%] lymphoblastic lymphoma, and one [<1%] Burkitt lymphoma) and 503 were diagnosed with relapsed follicular lymphoma.

Details on treatment were available for 8097 patients. A watch-and-wait strategy before an active treatment for follicular lymphoma was reported in 1127 (14%) patients, whereas the remaining 6970 (86%) received an active treatment at time of initial diagnosis (table 1). 5331 (76%) of these 6970 patients were treated with rituximab: as a monotherapy in 408 and in combination with chemotherapy in 4923. With respect to chemotherapy, 2745 (51%) of 5331 were given CHOP (cyclophosphamide, doxo-rubicin, vincristine, and prednisone or prednisolone) or CHOP-like therapy, 1320 (25%) received CVP (cyclophosphamide, vincristine, and prednisone) or chorambucil, 628 (12%) received fludarabine-containing regimens, and 230 (4%) were treated with other regimens. 1028 (19%) of 5331 patients received maintenance treatment with rituximab after induction therapy.

The cumulative hazard of histological transformation was assessed in 483 cases (26 of the 509 patients with transformation had incomplete data and were excluded) and was 5·8% (95% CI 5·3–6·4) at 5 years and 7·7% (6·9–8·5) at 10 years (figure 2). Among patients with transformation, the median time from initial diagnosis to histological transformation was 19 months (2·5–97·5th percentile 2–110 months), whereas the median time to relapse or progression was 36 months (2·5–97·5th percentile 3–143 months).

After a median follow-up of 87 months (range 1–221; 2·5–97·5th percentile 5–160), 2065 deaths were recorded; overall survival for the whole cohort was 82% (95% CI 81–83) at 5 years and 68% (95% CI 67–70) at 10 years.

Transformation risk differed significantly between patients who received an active treatment upfront and those who were treated after an initial period of watch and wait. In the 6970 patients who received active treatment at diagnosis, 397 (6%) experienced transformation, whereas 84 (7%) of 1127 patients in the initial watch-and-wait group had histological transformation. Treatment information was not available for two cases with histological transformation. The overall cumulative hazard of histological transformation was 5·6% (95% CI 5·0–6·2) at 5 years and 7·0% (6·4–7·9) at 10 years for the initially treated group and 7·1% (5·6–9·1) at 5 years and 11·5% (9·0–14·7) at 10 years for the initial watch-and-wait group (hazard ratio [HR] 1·36, 95% CI 1·07–1·72).

A significant difference in the cumulative hazard of histological transformation was noted between patients treated with rituximab in induction or maintenance, or both, and those treated without rituximab: the cumulative hazard was 4·4% (95% CI 3·7–5·1) at 5 years and 5·2% (4·5–6·2) at 10 years in patients who received rituximab, and 6·9% (5·7–8·4) at 5 years and 8·7% (7·2–10·6) at 10 years in those who did not (HR 0·73, 95% CI 0·58–0·90; p=0·004; figure 3A). 54 patients without rituximab and 31 patients with rituximab were not evaluable for risk calculation.

Information on the use of rituximab for induction and maintenance was available for 5382 patients. In these patients, the cumulative hazard of transformation was 5·1% (95% CI 4·3–6·0) at 5 years and 5·9% (5·0–7·0) at 10 years for patients who received rituximab in induction only, and 2·6% (1·8–3·8) at 5 years and 3·6% (2·3–5·5) at 10 years for those who received it for both induction and maintenance (figure 3B). Compared with patients not exposed to rituximab, the HRs for histological transformation were 0·69 (95% CI 0·54–0·89; p=0·003) for rituximab in induction only and 0·38 (0·25–0·57; p<0·0001) for rituximab in induction and maintenance (table 2). Finally, the HR for transformation in the rituximab in induction and maintenance versus rituximab in induction only group was 0·55 (95% CI 0·37–0·81; p=0·003). In univariate and multivariate analyses, rituximab use in induction, maintenance, or both was associated with improved risk of transformation (table 2).

In addition to rituximab use, other factors that were significantly associated with the risk of transformation by univariate analysis were histological grade 3a follicular lymphoma, age older than 60 years at diagnosis, and FLIPI score of at least 2 (table 2). Conversely, in our dataset the type of chemotherapy did not affect the cumulative hazard of transformation (p=0·90; appendix p 4). A multivariable analysis using Cox regression was done after adjusting for FLIPI score 3–5 and follicular lymphoma grade 3a. In the 3866 patients with a complete dataset, the reduced risk of transformation for patients treated with rituximab-containing regimens was retained. Using patients not exposed to rituximab as reference, the HR for transformation was 0·59 (95% CI 0·42–0·85; p=0·004) for the induction rituximab only group and 0·33 (0·19–0·56; p<0·0001) for the induction and maintenance rituximab group (table 2). FLIPI score, but not follicular lymphoma grade, retained its prognostic value in multivariate analyses (table 2).

In the whole cohort of 8116 patients with follicular lymphoma, 2065 deaths were recorded. 287 deaths were recorded among the 509 patients with histological transformation, of which 155 (54%) were due to lymphoma progression, 15 (5%) infections, three (1%) second malignancies, and 18 (6%) other causes. In 96 (33%) of these histological transformation cases, the cause of death was unknown.

After a median follow-up of 59 months (range 1–167; 2·5–97·5th percentile 3–136) from histological transformation, survival after transformation was 43% (95% CI 38–48) at 5 years and 32% (26–38) at 10 years (figure 4A).

	Lymphoma registries (n=2954)	Clinical trials (n=2360)	Population-based registries (n=2802)	Total (n=8116)
(Continued from previous page)				
Proportion of responders to first-line therapy (95% CI)				
Overall response rate†	92% (90–93)	92% (91–93)	85% (83–86)	90% (89–91)
Complete remission	67% (66–69)	64% (62–66)	56% (54–59)	63% (62–64)
Overall survival (95% CI)				
5 years	82% (81–84)	87% (86–88)	78% (76–79)	82% (81–83)
10 years	69% (66–71)	68% (57–76)	64% (62–66)	68% (67–70)

Data are median (2·5–97·5th percentile), number (%), or n/N (%), unless otherwise specified. Some percentages do not add up to 100 because of rounding. FLIPI=Follicular Lymphoma International Prognostic Index CHOP=cyclophosphamide, doxorubicin, vincristine, and prednisone or prednisolone. CVP=cyclophosphamide, vincristine, and prednisone. *In those exposed to rituximab in induction. †Complete and partial remission.

Table 1: **Demographics and baseline characteristics**

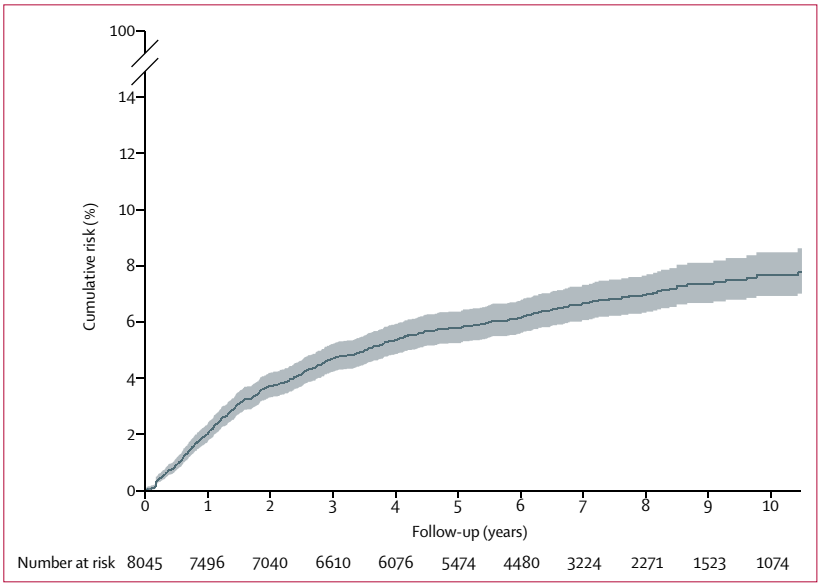

Figure 2: **Cumulative hazard of histological transformation as a first event in patients with follicular lymphoma**
The shaded area shows the 95% CI. 71 patients were excluded from this analysis because of they were not evaluable for risk calculation.

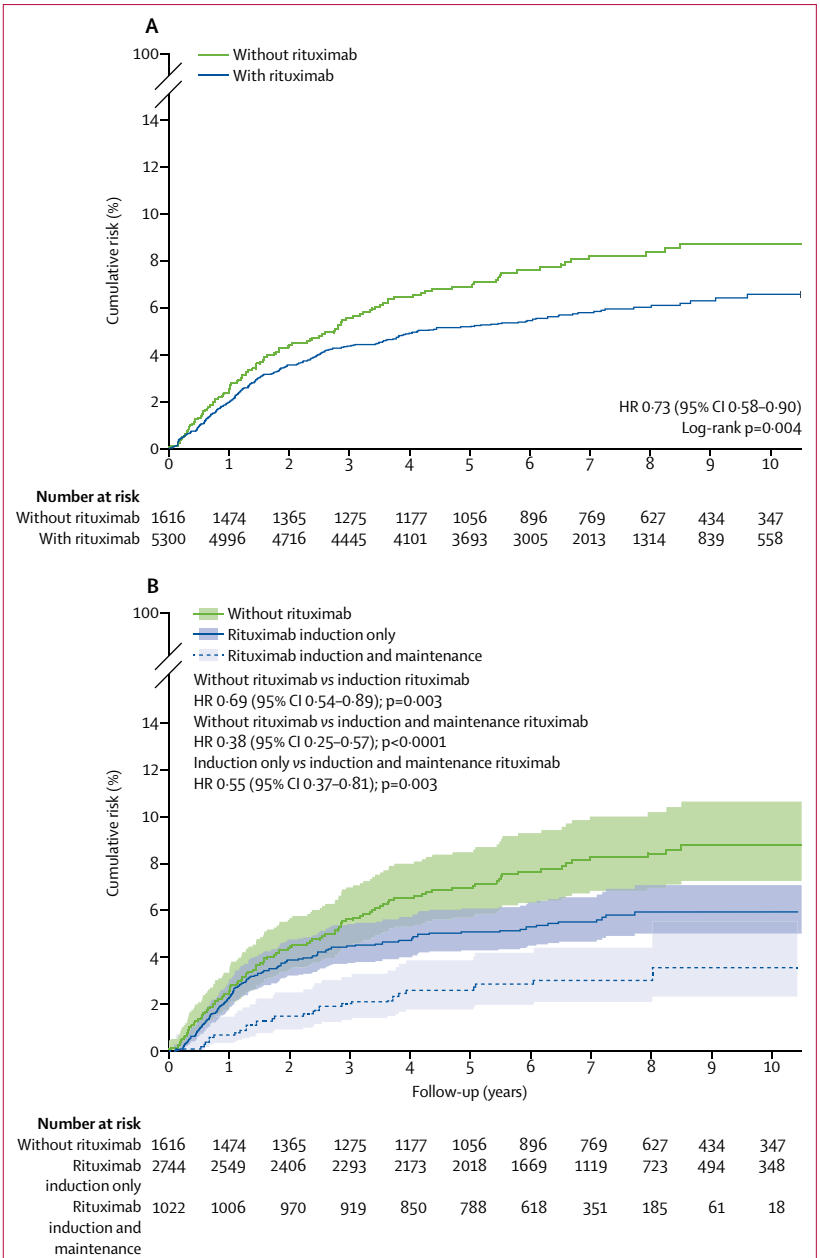

Figure 3: **Cumulative hazard of histological transformation**
Cumulative risk of histological transformation in patients with follicular lymphoma who did or did not receive rituximab (A) and in patients not exposed to rituximab, those who received induction rituximab, and those who received induction and maintenance rituximab (B). Shaded areas show the 95% CIs. HR=hazard ratio.

By contrast, 913 deaths (466 due to disease progression) were reported among the 4402 patients who experienced follicular lymphoma progression or relapse and either were not re-biopsied, or were re-biopsied and found to have recurrence rather than transformation. After a median follow-up of 64 months (2·5–97·5th percentile 2–142) from progression or relapse, survival after relapse for patients with progression or relapse (ie, recurrence) confirmed by biopsy was 71% (95% CI 70–73) at 5 years and 56% (53–59%) at 10 years (HR 2·82, 95% CI 2·47–3·22; p<0·0001; figure 4A). With regard to time to histological transformation, the 5-year survival after transformation was 34% (95% CI 26–41) for patients experiencing early (≤1 year) histological transformation and 48% (42–54) for those with late histological transformation (p<0·0001).

We also investigated the effect of previous rituximab exposure on outcome after transformation, and found no differences among patients who received induction rituximab (n=146), induction and maintenance rituximab (n=31), or who were never exposed to rituximab before transformation (n=126). Survival after transformation at 5 years was 38% (95% CI 28–46) for patients not exposed to rituximab, 42% (33–50) for those who received rituximab in induction only, and 43% (95% CI 24-60) for those who received rituximab in induction and maintenance, with no difference between the three groups (p=0·92; figure 4B).

Median time to histological transformation was 18 months (2·5–97·5th percentile 2–111), with a median of 19 months (2–142) for patients treated without and 17 months (1–101) for those treated with rituximab (p=0·16). Overall survival, progression-free survival, and event-free survival after transformation were not calculable owing to insufficient data.

Discussion

In our opinion, the most relevant information arising from the Aristotle study thus far is the protective role against histological transformation of rituximab as a component of a first-line therapy for follicular lymphoma. We found that patients initially treated with rituximab (alone or in combination with chemotherapy) had a lower cumulative hazard of histological transformation than patients who did not receive this treatment. To better understand the role of initial therapy as a risk factor for transformation, the present study focused on cases of histological transformation after first-line therapy as a first event only. Moreover, in an effort to reduce possible selection biases linked to the retrospective and multicentre nature of the study, only cases with biopsy-proven histological transformation were eligible for the analysis.

In addition to its retrospective nature, one possible weakness of our study was the absence of a centralised pathology review. However, we believe that this potential selection bias was overcome at least partly by the substantial experience of the haematopathologists of the participating centres, and the low level of disagreement after centralised revision of follicular lymphoma cases in a previous study.[24] Only 20% of patients with follicular lymphoma were re-biopsied at the time of the first event, whereas disease status was defined by clinical features in the remaining cases. This low proportion of re-biopsies was a result of the different practices of performing a re-biopsy at relapse or progression by the different

investigators at the participating institutes, and the fact that re-biopsy is not mandatory, although it is recommended.

The decision to include only patients with biopsy-confirmed histological transformation increased the homogeneity of the cohort, although patients without biopsies could also have had histological transformation. 50% of patients who underwent a biopsy had histological transformation. These patients probably presented with signs associated with suspected transformation, thus driving the treating physician to perform a biopsy.[11]

The variations in the risk of transformation reported for follicular lymphoma in the literature probably result from differences in diagnostic methods, definitions of transformation (according to clinical factors or histological confirmation), and follow-up durations, and variabilities in the initial treatment strategies, which have changed substantially over the past 20 years. Taking into account the restrictive selection criteria we used, the proportion of patients with transformation that we observed seemed to be lower than reported previously.[4,10–12] However, to the best of our knowledge, the number of patients with a biopsy-proven transformation as a first event after first-line therapy among the Aristotle cohort is the largest reported (appendix p 5). Most previous studies have reported not only histological transformation as first event, but also cumulative risk of transformation.[4,10–12,15,17–19,25] Furthermore, several studies included cases with transformation diagnosed on clinical characteristics only. Thus, these issues make comparison of the absolute cumulative risk of transformation between studies difficult, which is a limitation for the specialty.

The proportion of patients with transformation that we observed in our cohort confirm some of the findings reported by others.[12,15,17–19] Specifically, in a previous study done using the Molecular Epidemiology Resource of the University of Iowa/Mayo Clinic Lymphoma Specialized Program of Research Excellence,[12] the proportion of patients with transformation at 5 years was 3·2% in patients with follicular lymphoma who initially received rituximab monotherapy. In the PRIMA trial,[17] the cumulative hazard of documented histological transformation was 4·1% at 6 years after induction in patients with follicular lymphoma. In the FOLL05 trial,[19] histological transformation was documented in 11 patients with follicular lymphoma, with a cumulative hazard of transformation of 2·9% at 8 years from diagnosis. Finally, a retrospective multicentre study from the GELTAMO group in Spain reported the proportion of patients with transformation to be 7·2% at 10 years in patients with follicular lymphoma.[18] By contrast, the reported proportions of patients with transformation at 5 years and 8 years were 12·8% and 19·4%, respectively, in the National LymphoCare Study (NLCS)[15] in patients with follicular lymphoma, suggesting that the risk of transformation might not have been modified by the introduction of chemoimmunotherapy in recent years.

	Univariate analysis		Multivariate analysis (n=3866)*	
	HR (95% CI)	p value	HR (95% CI)	p value
Treatment with rituximab				
Not exposed	1·00	..	1·00	..
Induction only	0·69 (0·54–0·89)	0·003	0·59 (0·42–0·85)	0·004
Induction and maintenance	0·38 (0·25–0·57)	<0·0001	0·33 (0·19–0·56)	<0·0001
Induction and maintenance *vs* induction only	0·55 (0·37–0·81)	0·003	0·55 (0·34–0·89)	0·016
Aged >60 years at diagnosis	1·28 (1·05–1·56)	0·015	..	..
Female sex	0·92 (0·75–1·12)	0·40	..	..
FLIPI (n=6137)				
0–1	1·00	..	1·00	..
2	1·61 (1·15–2·25)	0·005	1·78 (1·20–2·66)	0·004
3–5	2·92 (2·15–3·95)	<0·0001	3·28 (2·15–5·01)	<0·0001
Watch and wait *vs* treated (n=8026)	1·36 (1·07–1·72)	0·011	..	..
Follicular lymphoma grade				
1–2	1·00	..	1·00	..
3a	1·43 (1·09–1·88)	0·011	1·22 (0·83–1·79)	0·31

HT=histological transformation. HR=hazard ratio. FLIPI=Follicular Lymphoma International Prognostic Index.
*174 patients had histological transformation.

Table 2: **Univariate and multivariate analyses of potential risk factors for HT in patients with follicular lymphoma**

However, in the NLCS, patients with clinically diagnosed transformation (ie, not confirmed by biopsy) were also eligible for inclusion, and, most importantly, the role of rituximab in reducing the risk of transformation could not be ruled out because in their cohort only 71 (3%) of 2652 patients were treated without rituximab.

In our study, a high FLIPI score, grade 3a follicular lymphoma, and a previous watch-and-wait approach were associated with increased cumulative hazards of histological transformation, whereas in the PRIMA study[17] the risk factors for histological transformation were an altered performance status, anaemia, high lactate dehydrogenase concentrations, systemic symptoms, and grade 3a follicular lymphoma. Additionally, our results on transformation risk between patients who received upfront treatment and those for whom an initial watch-and-wait strategy was chosen suggest that progression after watch and wait might increase the risk of subsequent transformation. However, our population of patients who were initially on watch and wait did not represent all cases, but only those who subsequently started systemic therapy owing to lymphoma progression in the absence of transformation.

We found that rituximab maintenance was associated with a statistically significant and clinically relevant reduction in transformation risk. In 2016, Sarkozy and colleagues[17] published an analysis on transformation risk in patients with follicular lymphoma enrolled in the PRIMA study, showing a reduced risk of biopsy-proven transformation (p=0·008) in patients in the rituximab maintenance group compared with those who did not

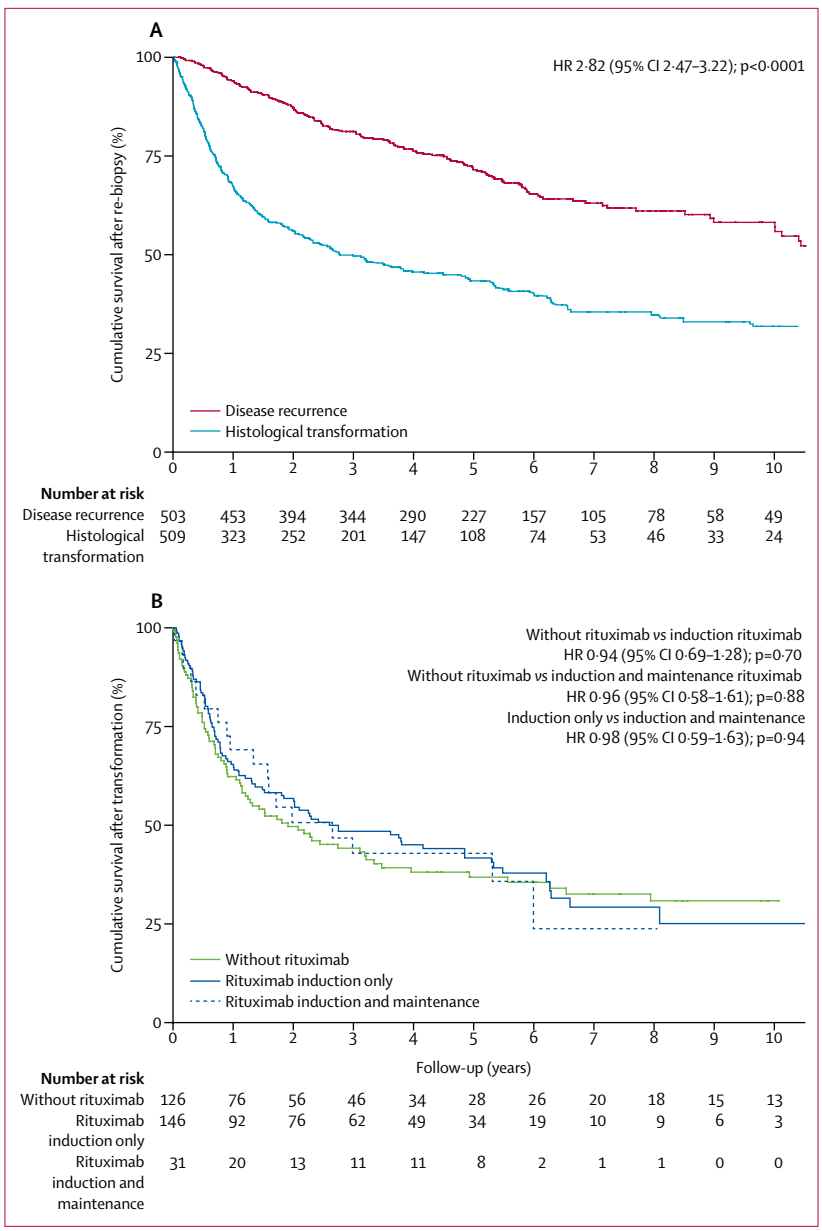

Figure 4: **Survival (A) after re-biopsy and (B) after transformation of patients with follicular lymphoma who received rituximab treatment**
HR=hazard ratio.

receive maintenance with rituximab. However, the investigators concluded that the small sample size (among the 40 patients with histological transformation, only 16 had received rituximab maintenance) represented a limitation that prevented definitive conclusions from being made. Nevertheless, in the Aristotle study, the large sample size has allowed us to definitively confirm the PRIMA findings.

Regarding the outcome after histological transformation, we observed overall survival to be 32% at 10 years, consistent with other published reports showing improved overall survival[12,15,17–19] compared with the pre-rituximab regimens.[10,11,25] However, survival after transformation remained significantly lower than that of patients with a biopsy-proven diagnosis of recurrent follicular lymphoma, confirming the adverse prognostic role of transformation. Finally, we observed a shorter median survival for patients with transformation within 1 year from diagnosis in our current investigation, confirming the negative effect of early transformation, as already reported by the NLCS.[15]

In summary, despite the intrinsic limitations related to the retrospective nature of our study, we confirmed that the cumulative hazard of histological transformation as a first event in follicular lymphoma can be reduced significantly by introducing rituximab to a backbone therapy. Moreover, our data also confirm that histological transformation still has an adverse effect on patient outcome, although it is less catastrophic than the pre-rituximab regimens. The challenge is to identify both clinical and biological risk factors associated with transformation, with the aim of establishing a diagnostic and therapeutic risk-adapted strategy. More comprehensive knowledge of the biological risk factors for follicular lymphoma transformation and the molecular pathways involved is likely to help clinicians make more accurate prognostic assessments and also inform the potential usefulness of novel drugs for the treatment of follicular lymphoma.

Contributors

MF, MDCB, and BC designed the study. LM did the statistical analyses. All authors provided study material, interpreted the data, and contributed to the drafting of manuscript. MF and MDCB approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

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References

1 Swerdlow SH, Campo E, Harris N, et al. WHO classification of tumors of haematopoietic and lymphoid tissues, 4th edn. Lyon, France: IARC Press, 2008.
2 Luminari S, Cesaretti M, Rashid I, et al. Incidence, clinical characteristics and survival of malignant lymphomas: a population-based study from a cancer registry in northern Italy. *Hematol Oncol* 2007; **25:** 189–97.
3 Casulo C, Burack WR, Friedberg JW. Transformed follicular non-Hodgkin lymphoma. *Blood* 2015; **125:** 40–47.
4 Conconi A, Ponzio C, Lobetti-Bodoni C, et al. Incidence, risk factors and outcome of histological transformation in follicular lymphoma. *Br J Haematol* 2012; **157:** 188–96.
5 Hubbard SM, Chabner BA, DeVita VT, et al. Histologic progression in non-Hodgkin's lymphoma. *Blood* 1982; **59:** 258–64.
6 Acker B, Hoppe RT, Colby TV, et al. Histologic conversion in the non-Hodgkin's lymphomas. *J Clin Oncol* 1983; **1:** 11–16.
7 Garvin AJ, Simon RM, Osborne CK, et al. An autopsy study of histologic progression in non-Hodgkin's lymphomas. 192 cases from the National Cancer Institute. *Cancer* 1983; **52:** 393–98.
8 Gallagher CJ, Gregory WM, Jones AE, et al. Follicular lymphoma: prognostic factors for response and survival. *J Clin Oncol* 1986; **4:** 1470–80.
9 Bastion Y, Sebban C, Berger F, et al. Incidence, predictive factors, and outcome of lymphoma transformation in follicular lymphoma patients. *J Clin Oncol* 1997; **15:** 1587–94.

10 Montoto S, Davies AJ, Matthews J, et al. Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. *J Clin Oncol* 2007; **25:** 2426–33.

11 Al-Tourah AJ, Gill KK, Chhanabhai M, et al. Population-based analysis of incidence and outcome of transformed non-Hodgkin's lymphoma. *J Clin Oncol* 2008; **26:** 5165–69.

12 Link BK, Maurer MJ, Nowakowski GS, et al. Rates and outcomes of follicular lymphoma transformation in the immunochemotherapy era: a report from the University of Iowa/MayoClinic Specialized Program of Research Excellence Molecular Epidemiology Resource. *J Clin Oncol* 2013; **31:** 3272–78.

13 Johnson PW, Rohatiner AZ, Whelan JS, et al. Patterns of survival in patients with recurrent follicular lymphoma: a 20-year study from a single center. *J Clin Oncol* 1995; **13:** 140–47.

14 Yuen AR, Kamel OW, Halpern J, Horning SJ. Long-term survival after histologic transformation of low-grade follicular lymphoma. *J Clin Oncol* 1995; **13:** 1726–33.

15 Wagner-Johnston ND, Link BK, Byrtek M, et al. Outcomes of transformed follicular lymphoma in the modern era: a report from the National LymphoCare Study (NLCS). *Blood* 2015; **126:** 851–57.

16 Bains P, Al Tourah A, Campbell BA, et al. Incidence of transformation to aggressive lymphoma in limited-stage follicular lymphoma treated with radiotherapy. *Ann Oncol* 2013; **24:** 428–32.

17 Sarkozy C, Trneny M, Xerri L, et al. Risk factors and outcomes for patients with follicular lymphoma who had histologic transformation after response to first-line immunochemotherapy in the PRIMA trial. *J Clin Oncol* 2016; **34:** 2575–82.

18 Alonso-Álvarez S, Magnano S, Alcoceba L, et al. Risk of, and survival following, histological transformation in follicular lymphoma in the rituximab era. A retrospective multicentre study by the Spanish GELTAMO group. *Br J Haematol* 2017; **178:** 699–708.

19 Luminari S, Ferrari A, Manni M, et al. Long-term results of the FOLL05 trial comparing R-CVP versus R-CHOP versus R-FM for the initial treatment of patients with advanced-stage symptomatic follicular lymphoma. *J Clin Oncol* 2018; **36:** 689–96.

20 Aalen O. Nonparametric Inference for a family of counting processes. *Ann Statist* 1978; **6:** 701–26.

21 Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53:** 457–81.

22 Cox DR. Regression models and life-tables. *J R Stat Soc Ser B* 1972; **34:** 187–220.

23 International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med* 1993; **329:** 987–94.

24 Federico M, Bellei M, Marcheselli L, et al. Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the International Follicular Lymphoma Prognostic Factor Project. *J Clin Oncol* 2009; **27:** 4555–62.

25 Giné E, Montoto S, Bosch F, et al. The Follicular Lymphoma International Prognostic Index (FLIPI) and the histological subtype are the most important factors to predict histological transformation in follicular lymphoma. *Ann Oncol* 2006; **17:** 1539–45.