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Minimal Residual Disease Status Predicts Outcome in Patients With Previously Untreated Follicular Lymphoma: A Prospective Analysis of the Phase III GALLIUM Study

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ABSTRACT

PURPOSE We report an analysis of minimal residual/detectable disease (MRD) as a predictor of outcome in previously untreated patients with follicular lymphoma (FL) from the randomized, multicenter GALLIUM (ClinicalTrials.gov identifier: NCT01332968) trial.

PATIENTS AND METHODS Patients received induction with obinutuzumab (G) or rituximab (R) plus bendamustine, or cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP) or cyclophosphamide, vincristine, prednisone (CVP) chemotherapy, followed by maintenance with the same antibody in responders. MRD status was assessed at predefined time points (mid-induction [MI], end of induction [EOI], and at 4-6 monthly intervals during maintenance and follow-up). Patients with evaluable biomarker data at diagnosis were included in the survival analysis.

RESULTS MRD positivity was associated with inferior progression-free survival (PFS) at MI (hazard ratio [HR], 3.03 [95% CI, 2.07 to 4.45]; $P < .0001$) and EOI (HR, 2.25 [95% CI, 1.53 to 3.32]; $P < .0001$). MRD response was higher after G- versus R-chemotherapy at MI (94.2% *v* 88.9%; $P = .013$) and at EOI (93.1% *v* 86.7%; $P = .0077$). Late responders (MI-positive/EOI-negative) had a significantly poorer PFS than early responders (MI-negative/EOI-negative; HR, 3.11 [95% CI, 1.75 to 5.52]; $P = .00011$). The smallest proportion of MRD positivity was observed in patients receiving bendamustine at MI (4.8% *v* 16.0% in those receiving CHOP; $P < .0001$). G appeared to compensate for less effective chemotherapy regimens, with similar MRD response rates observed across the G-chemo groups. During the maintenance period, more patients treated with R than with G were MRD-positive (R-CHOP, 20.7% *v* G-CHOP, 7.0%; R-CVP, 21.7% *v* G-CVP, 9.4%). Throughout maintenance, MRD positivity was associated with clinical relapse.

CONCLUSION MRD status can determine outcome after induction and during maintenance, and MRD negativity is a prerequisite for long-term disease control in FL. The higher MRD responses after G- versus R-based treatment confirm more effective tumor cell clearance.

ACCOMPANYING CONTENT

Data Supplement

Protocol

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INTRODUCTION

The widespread use of immunochemotherapy followed by maintenance with the anti-CD20 antibody rituximab (R) has improved outcomes for previously untreated patients with symptomatic follicular lymphoma (FL).[1-3] However, most patients will eventually relapse, with early relapse or non-response associated with particularly poor prognosis.[4] Obinutuzumab (GA101; G) is a glycoengineered type II anti-CD20 antibody with enhanced direct cell killing and antibody-dependent cellular phagocytosis,[5] and proven efficacy in patients with chronic lymphocytic leukemia (CLL)[6] and indolent non-Hodgkin lymphoma.[7-9]

The randomized GALLIUM trial (ClinicalTrials.gov identifier: NCT01332968) evaluated the efficacy and safety of G versus R in combination with chemotherapy as induction and maintenance in previously untreated patients with FL.[10] G plus

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CONTEXT

Key Objective

In the randomized GALLIUM trial, we explored the role of minimal residual/detectable disease (MRD) status during induction and maintenance to explain differences in outcome after obinutuzumab (G)- or rituximab-based treatment and evaluated the use of MRD as a marker for outcome and a dynamic parameter for treatment modification in patients with follicular lymphoma (FL).

Knowledge Generated

MRD positivity was associated with inferior progression-free survival during and after anti–CD20-based induction and was less frequently detected after G-based chemotherapy and maintenance. Outcome was favorable for early MRD responders during induction and throughout maintenance. MRD positivity or reappearance was prognostic for earlier clinical relapse.

Relevance *(J.W. Friedberg)*

This study leverages a large phase III trial to demonstrate the association of MRD status with outcomes in patients with FL. With these current data demonstrating feasibility, it is time to begin designing trials utilizing this as an integral biomarker.*

*Relevance section written by *JCO* Editor-in-Chief Jonathan W. Friedberg, MD.

chemotherapy (G-chemo) significantly improved investigator-assessed progression-free survival (PFS) compared with R plus chemotherapy (R-chemo; 3-year PFS, 80.0% *v* 73.3%; $P = .001$).[10,11]

FL still remains incurable, and the duration of remission in individual patients might be determined by quantitation of residual lymphoma cells after treatment (minimal residual/detectable disease [MRD]), as well as their proliferation kinetics during follow-up. There is increasing evidence that MRD status reflects depth of response and informs prognosis after first-line therapy and relapse in patients with FL.[12-15]

This study reports the results of preplanned MRD assessments during treatment periods in patients with FL enrolled in the GALLIUM trial. Study objectives were to evaluate the depth and kinetics of MRD response to first-line G-chemo or R-chemo, and explore the prognostic role of MRD-status in a prospective setting to evaluate the use of MRD as a dynamic parameter for treatment modification.

PATIENTS AND METHODS

Study Design and Treatments

Patients with untreated, histologically documented, CD20-positive FL were randomly assigned to G 1,000 mg versus R 375 mg/m^2 combined with chemotherapy (6 cycles of bendamustin on days 1 and 2 every 28 days, or 8 cycles of cyclophosphamide, doxorubicin, vincristine, prednisone [CHOP], or cyclophsphamide, vincristine, prednisolone once every 21 days).[10,11] Responding patients received G or R maintenance according to randomized treatment arm every 2 months for 2 years or until disease progression, patients with stable disease at end of induction (EOI) were observed. Positron emission tomography (PET) scans to assess metabolic response at EOI were retrospectively assessed according to the Lugano 2014 criteria.[16,17]

The study was conducted in accordance with the Declaration of Helsinki and the ICH guidelines for Good Clinical Practice. Approval was obtained from ethics committees of each participating center and written informed consent including MRD assessment was provided by patients.

MRD Assessment

MRD analysis was performed at the central reference laboratory in Kiel, Germany.

After screening diagnostic peripheral blood (PB) and bone marrow (BM) samples by polymerase chain reaction (PCR)[18] to detect a t(14;18) translocation and/or a clonal immunoglobulin (Ig) heavy or light chain rearrangement. Allele- or translocation-specific, real-time quantitative PCR (qPCR) assays were designed with a sensitivity of 10^{-5}.[19] Quantification by plasmid standards was conducted using a modified protocol.[20]

MRD results were evaluated according to European Study Group criteria for MRD detection/Euro-MRD.[21] To confirm qPCR results, qualitative nested PCR of two replicates (500 ng DNA input each) was performed using the ASO primer.[22]

MRD status was classified as positive if both qPCR and nested PCR were positive, and classified as negative if there was no specific PCR signal in a sample with at least 10^4 control gene copies.

MRD status was evaluated at mid-induction (MI; in PB), at EOI (pooled PB and BM), during maintenance (in PB), and during follow-up (in PB). MRD status at EOI was determined

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as positive if at least one sample (PB or BM) was positive, and the quantitatively higher MRD value was used for calculation.

The MI time point was defined as cycle 4 day 1 for patients treated with G-/R-bendamustine, or cycle 5 day 1 for those treated with G-/R-CHOP or G-/R-CVP (day 85 for all treatment groups). Assessments during maintenance were conducted every 4 months during the first year, every 6 months thereafter, and at the final or early termination/discontinuation visit. Assessments were conducted every 6 months during follow-up (Data Supplement, Fig S1, online only). Samples collected at or subsequent to documented clinical relapse were not included in statistical analysis.

Progressing patients during induction were excluded from the corresponding MRD analysis.

Statistical Analysis

MRD response at MI and EOI was tabulated against treatment arm and the association was tested using Fisher's exact test. The potential association between MRD status (MRD-negative or MRD-positive) and PFS and overall survival (OS) was assessed using Kaplan-Meier methodology and Cox proportional-hazard models. PFS was defined as the time from random assignment to first occurrence of progression, relapse, or death from any cause, and OS as the time from random assignment to death from any cause. Landmark analyses for PFS and OS included only patients in remission at the landmark time point, and patients were categorized according to MRD status at the corresponding landmark. Statistical analyses were conducted using R (The R Project for Statistical Computing,[23] version 4.0.3).

RESULTS

Patients

Among 1,202 patients in the FL cohort, 1,064 had a baseline sample and FL confirmed by reference pathology. A clonal marker was detected in 939 (88.3%) and a qPCR assay established in 815 (76.6%) patients (Fig 1; Data Supplement, Table S1). Patients with a clonal marker at diagnosis had more frequently advanced-stage and high-risk FL International Prognostic Index (FLIPI) than those without (Table 1). Availability of a clonal marker or a qPCR assay was not associated with PFS (P = .44; Data Supplement, Fig S2).

MRD Status at MI and EOI

MRD response was achieved early during treatment in both treatment arms. At MI, 57/680 (8.4%) patients were MRD-positive in PB: 37/333 (11.1%) after R-chemo versus 20/347 (5.8%; P = .013) after G-chemo (Data Supplement, Table S2). All 20 MRD-positive patients in the G-chemo arm had low-level MRD (positive below the quantitative range [BLQ]), while 24/37 MRD-positive patients (64.9%) treated with R-chemo showed quantifiable MRD.

At EOI, 70/693 (10.1%) were MRD-positive in PB or BM (Data Supplement, Table S2), with a higher number of patients MRD-positive after R-chemo (46/347 [13.3%]) than G-chemo (24/346 [6.9%]; P = .0077). When analyzed separately, the difference between the treatment arms was only significant in BM samples, where 34/209 (16.3%) R-chemo compared with 14/216 (6.5%) G-chemo patients were MRD-positive (P = .0019).

The chemotherapy backbone affected the quality of response. At MI, MRD positivity was more frequent in patients receiving CHOP (34/213, 16.0%) compared with bendamustine (20/415, 4.8%; P < .0001). At EOI, 31/225 (13.8%) patients treated with CHOP and 31/413 (7.5%) treated with bendamustine were MRD-positive (P = .019; Data Supplement, Table S2).

Antibody type also influenced MRD status in the context of the different chemotherapy backbones. At EOI, MRD positivity was more frequent after R-CHOP (23/111, 20.7%) and R-CVP (5/23, 21.7%) compared with G-CHOP (8/114, 7.0%) and G-CVP (3/32, 9.4%; Data Supplement, Table S3). In the bendamustine arm, the difference was less pronounced, with 18/213 (8.5%) patients showing MRD positivity after R-bendamustine and 13/200 (6.5%) after G-bendamustine (Fig 2; Data Supplement, Table S3). However, the difference was much more noticeable in BM after R-CHOP (19/77, 24.7%) compared with G-CHOP (5/81, 6.2%; Data Supplement, Fig S3). The Data Supplement contains further details on the association of MRD status with clinical response and the comparison of MRD in the PB and BM compartments.

Prognostic Value of MRD

After a median follow-up of 59 months, MRD positivity was strongly associated with poor PFS at both MI (hazard ratio [HR], 3.03 [95% CI, 2.07 to 4.45]; P < .0001) and EOI (HR, 2.25 [95% CI, 1.53 to 3.32]; P < .0001; Figs 3A and 3C). Although MRD status at MI was associated with PFS in both treatment arms (R: HR, 3.30 [95% CI, 2.06 to 5.29]; P < .0001; G: HR, 2.31 [95% CI, 1.16 to 4.62]; P = .018; Fig 3B), MRD positivity at EOI in the G arm was not strongly associated with adverse prognosis (HR, 0.72 [95% CI, 0.26 to 1.98]; P = .53; Fig 3D). Accordingly, prognostic effects of MRD status at EOI and antibody treatment revealed a significant interaction (interaction P = .0066). MRD status in clinical response groups was independently associated with prognosis (Data Supplement, Table S8 and Fig S4).

In a multivariate analysis including FLIPI and ECOG, MRD status at MI and EOI was an independent prognostic factor for PFS (MRD-positive at MI: HR, 3.08 [95% CI, 2.10 to 4.52]; P < .0001; MRD-positive at EOI: HR, 2.37 [95% CI, 1.59 to 3.51]; P < .0001).

Likewise, MRD status was associated with OS at both time points in univariable analysis (MI: HR, 2.58 [95% CI, 1.25 to 5.32]; P = .010; EOI: HR, 2.39 [95% CI, 1.15 to 4.97]; P = .019),

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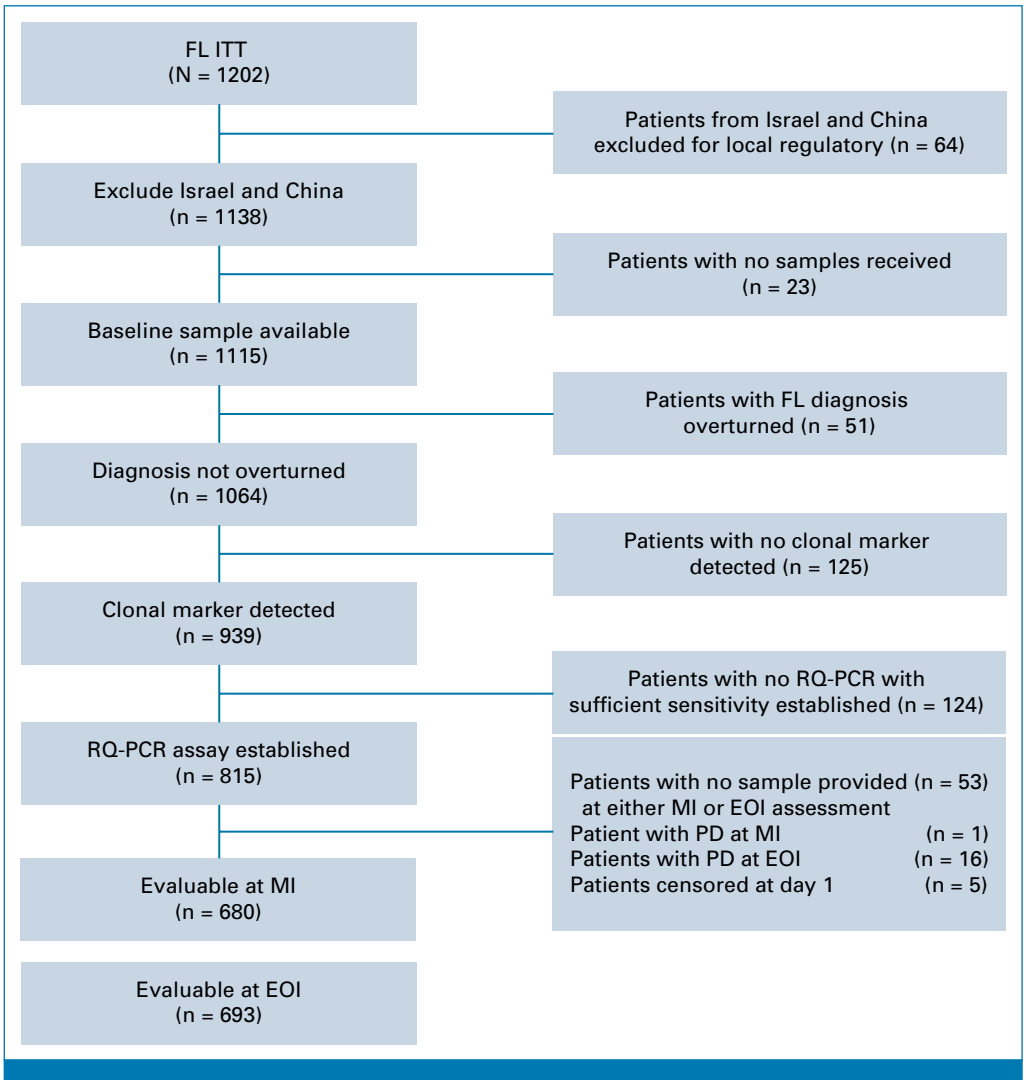

FIG 1. Flow diagram of the MRD-evaluable population of the GALLIUM trial. BM, bone marrow; EOI, end of induction; FL, follicular lymphoma; ITT, intention-to-treat; MI, mid-induction; MRD, minimal residual/detectable disease; PD, disease progression; RQ-PCR, real-time quantitative polymerase chain reaction.

and when adjusted for FLIPI and ECOG (MRD-positive at MI: HR, 2.45 [95% CI, 1.19 to 5.04]; P = .015; MRD-positive at EOI: HR, 2.34 [95% CI, 1.13 to 4.87]; P = .023). The association between MRD status and outcome was independent of the chemotherapy arm (Figs 3E and 4C); however, the difference in OS was more pronounced in bendamustine-treated patients (Fig 4C). Interestingly, late responders (MI-positive/EOI-negative) had a significantly poorer PFS than early responders (HR, 3.11 [95% CI, 1.75 to 5.52]; P = .00011; Fig 5).

MRD status at both MI and EOI in PB/BM was associated with progression within 24 months from the start of treatment (POD24; odds ratio [OR], 4.28; P < .0001 at MI; OR, 3.61; P = .00021 at EOI; Data Supplement, Fig S5). At MI/EOI, MRD status predicted 23%/25% of patients with POD24 and 93%/92% without POD24.

MRD Status and PET Response

Of 527 patients with FL with a PET result at EOI, 317 patients also had an MRD result at EOI. Complete metabolic response (CMR) was achieved by 246 patients (77.7%); of these, 229 (93%) were MRD-negative (Data Supplement, Table S4). Patients with both CMR and MRD-negative response had better PFS (3-year PFS after EOI, 81.1%; Data Supplement, Fig S6) than patients with CMR only (n = 17, 58.8%; HR, 2.53; P = .01) or MRD-negative response only (n = 65, 73.9%; HR, 1.50; P = .095). Both PET and MRD results were independently associated with poorer PFS in a Cox model including these two variables (MRD-positive: HR, 2.70 [95% CI, 1.53 to 4.79]; P = .00063; no CMR: HR, 1.57 [95% CI, 1.01 to 2.42]; P = .043). The results remained similar with adjustment for FLIPI and ECOG (Data Supplement, Table S5).

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TABLE 1. Disease Characteristics of Patients With and Without a Clonal Marker

Characteristic	Total Cohort (N = 1,064)	No Clone Detected (n = 125)	Clone Detected, No RQ-PCR Assay (n = 124)	Clone Detected, With RQ-PCR Assay (n = 815)	*P*
Age, years, median (range)	59 (23-88)	59 (33-88)	63 (33-81)	58 (23-85)	.092
Male, No. (%)	497 (46.7)	55 (44.0)	46 (37.1)	396 (48.6)	.047
ECOG PS, No. (%)					.37
0-1	1,027 (96.6)	122 (97.6)	122 (98.4)	783 (96.2)	
2	36 (3.4)	3 (2.4)	2 (1.6)	31 (3.8)	
Ann Arbor stage, No. (%)					<.0001
I	16 (1.5)	1 (0.8)	2 (1.6)	13 (1.6)	
II	78 (7.4)	22 (17.9)	15 (12.2)	41 (5.0)	
III	355 (33.6)	60 (48.8)	60 (48.8)	235 (28.9)	
IV	609 (57.6)	40 (32.5)	46 (37.4)	523 (64.4)	
FLIPI, No. (%)					<.0001
Low	223 (21.0)	46 (36.8)	31 (25.0)	146 (17.9)	
Intermediate	399 (37.5)	43 (34.4)	49 (39.5)	307 (37.7)	
High	442 (41.5)	36 (28.8)	44 (35.5)	362 (44.4)	
BM involvement, No. (%)					<.0001
Positive	562 (53.2)	16 (12.8)	37 (29.8)	509 (63.1)	
Negative	474 (44.9)	105 (84.0)	85 (68.5)	284 (35.2)	
Indeterminate	20 (1.9)	4 (3.2)	2 (1.6)	14 (1.7)	
Extranodal involvement, yes, No. (%)	705 (66.3)	50 (40.0)	61 (49.2)	594 (72.9)	<.0001
Antibody treatment, No. (%)					.80
Rituximab	528 (49.6)	61 (48.8)	65 (52.4)	402 (49.3)	
Obinutuzumab	536 (50.4)	64 (51.2)	59 (47.6)	413 (50.7)	

Abbreviations: BM, bone marrow; ECOG PS, Eastern Cooperative Oncology Group performance status; FLIPI, Follicular Lymphoma International Prognostic Index; RQ-PCR, real-time quantitative polymerase chain reaction.

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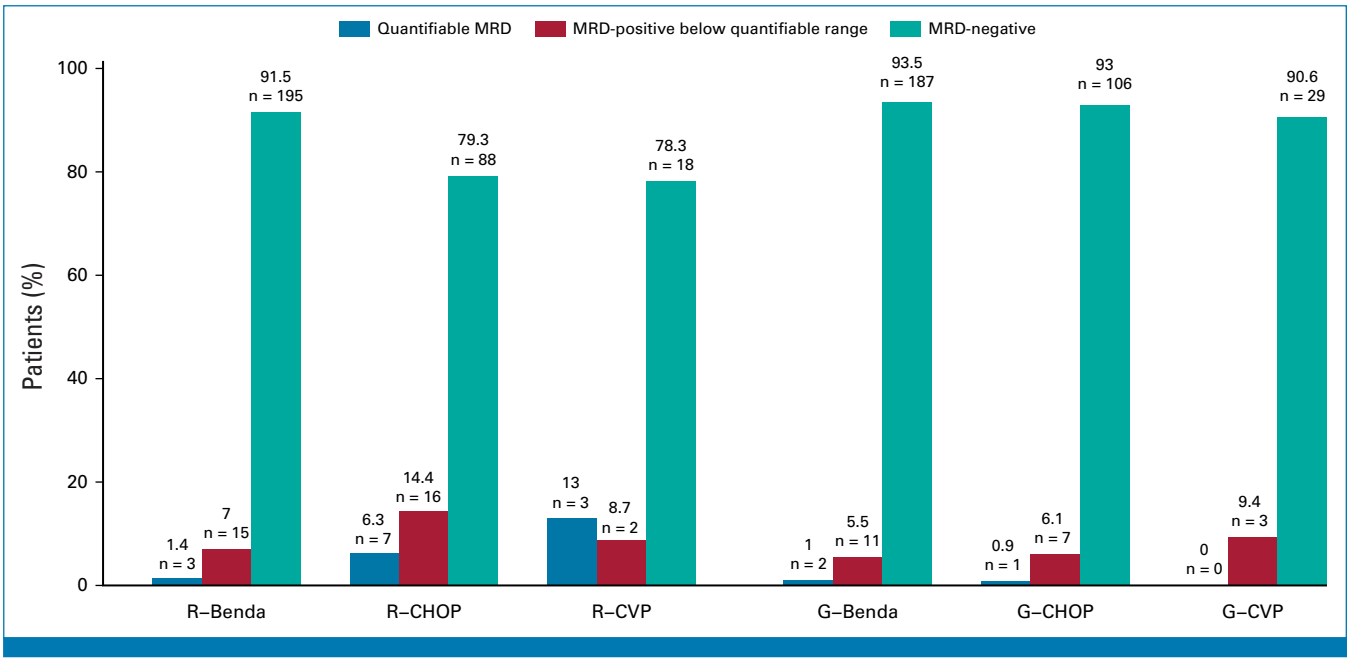

FIG 2. MRD response at EOI according to chemotherapy backbone and treatment arm (pooled PB/BM samples). Benda, bendamustine; BM, bone marrow; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CVP, cyclophosphamide, vincristine, prednisone; EOI, end of induction; G, obinutuzumab; MRD, minimal residual/detectable disease; PB, peripheral blood; R, rituximab.

MRD Status During Maintenance

The MRD status of patients in remission at EOI was analyzed at 4, 8, 12, and 18 months from the start of maintenance treatment and at the end of maintenance (Data Supplement, Fig S7). In the majority of patients, MRD status remained unchanged during maintenance; 575 patients remained negative throughout maintenance treatment and only six were continuously MRD-positive. Four of these patients showed progression (months 11, 12, 13, and 33), two of whom died within 2.5 years after progression.

Twenty-two patients changed from MRD-negative to MRD-positive and 12 from MRD-positive to MRD-negative, and 22 had a fluctuating MRD level during the course of maintenance treatment. Patients treated with R were more often consistently MRD-positive or switched more often from MRD-negative to MRD-positive, while patients treated with G were more frequently MRD-negative ($P = .16$; Data Supplement, Table S6).

Landmark analyses at 4, 8, 12, and 18 months from the start of maintenance demonstrated that throughout maintenance treatment, MRD positivity or MRD reappearance was highly associated with clinical relapse and had a strong impact on prognosis (Fig 6; Data Supplement, Table S7).

DISCUSSION

Sensitive MRD assessment is an important tool for predicting relapse and directing therapy in hematologic malignancies such as CLL, mantle cell lymphoma, and FL.[24,25] In FL, MRD analysis has demonstrated important prognostic value after immunochemotherapy[12-15] and after chemotherapy-free treatment, including R and lenalidomide.[26] However, one major limitation of published studies in FL is the restriction of MRD analysis to the patient population with a PCR-detectable t(14;18) translocation, thereby excluding around 40%-60% of patients. By additionally tracking the clonal IG rearrangement with a standardized allele-specific qPCR-based approach, we were able to (1) show the standardized applicability in the large prospective GALLIUM trial and (2) investigate a higher number of study patients (77% of patients with a baseline sample) for MRD.

The dropout rate of 23% was mainly because of low FL infiltration of the diagnostic PB and BM samples below the limits of detection and of quantification according to EuroMRD criteria, and was not improved by use of BM, known to contain a higher number of lymphoma cells than PB.[27] This is important information for prospective clinical trials using MRD by next-generation sequencing of Ig genes. Here, the critical threshold for identification of a trackable dominant Ig clonotype is >5% read abundancy in PB.[28,29] To further increase the proportion of evaluable patients for MRD-based treatment approaches, one should consider integrating diagnostic lymph node samples for establishment of the MRD assay. Of note, for MRD assessment after immunochemotherapy, using BM for MRD detection seems preferable with a higher MRD positivity rate, confirming data from other trials.[13,15]

MRD data in this trial were generated with a well-standardized qPCR assay targeting circulating lymphoma cells, which was the standard when this study was planned. New technologies

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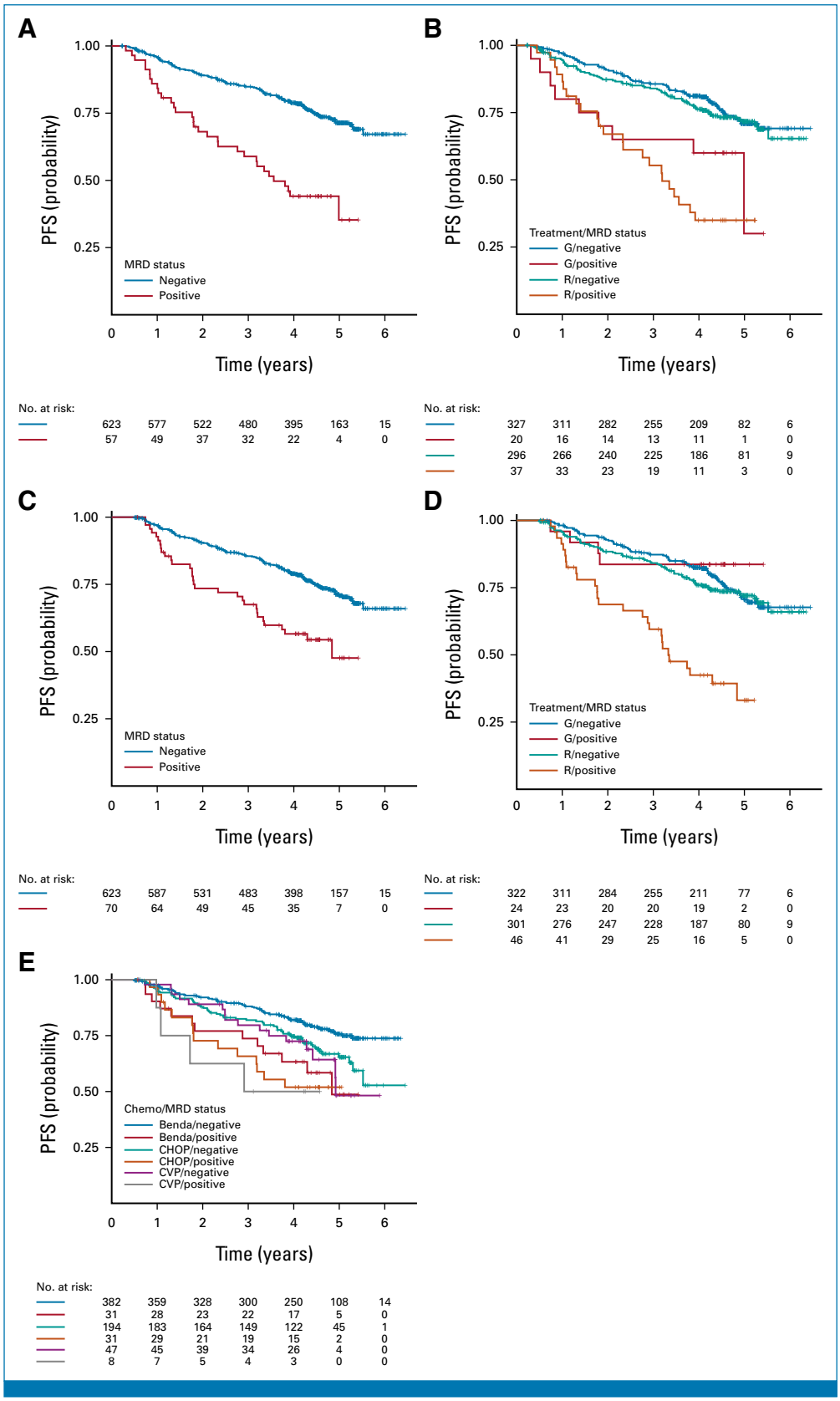

FIG 3. PFS according to MRD status (A) at MI, (B) at MI and according to treatment arm, (C) at EOI, (D) at EOI and according to treatment arm, and (E) at EOI according to chemotherapy arm. PFS calculated from the time of random assignment. Benda, bendamustine; chemo, chemotherapy; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CVP, cyclophosphamide, vincristine, prednisone; EOI, end of induction; G, obinutuzumab; MI, mid-induction; MRD, minimal residual/detectable disease; PFS, progression-free survival; R, rituximab.

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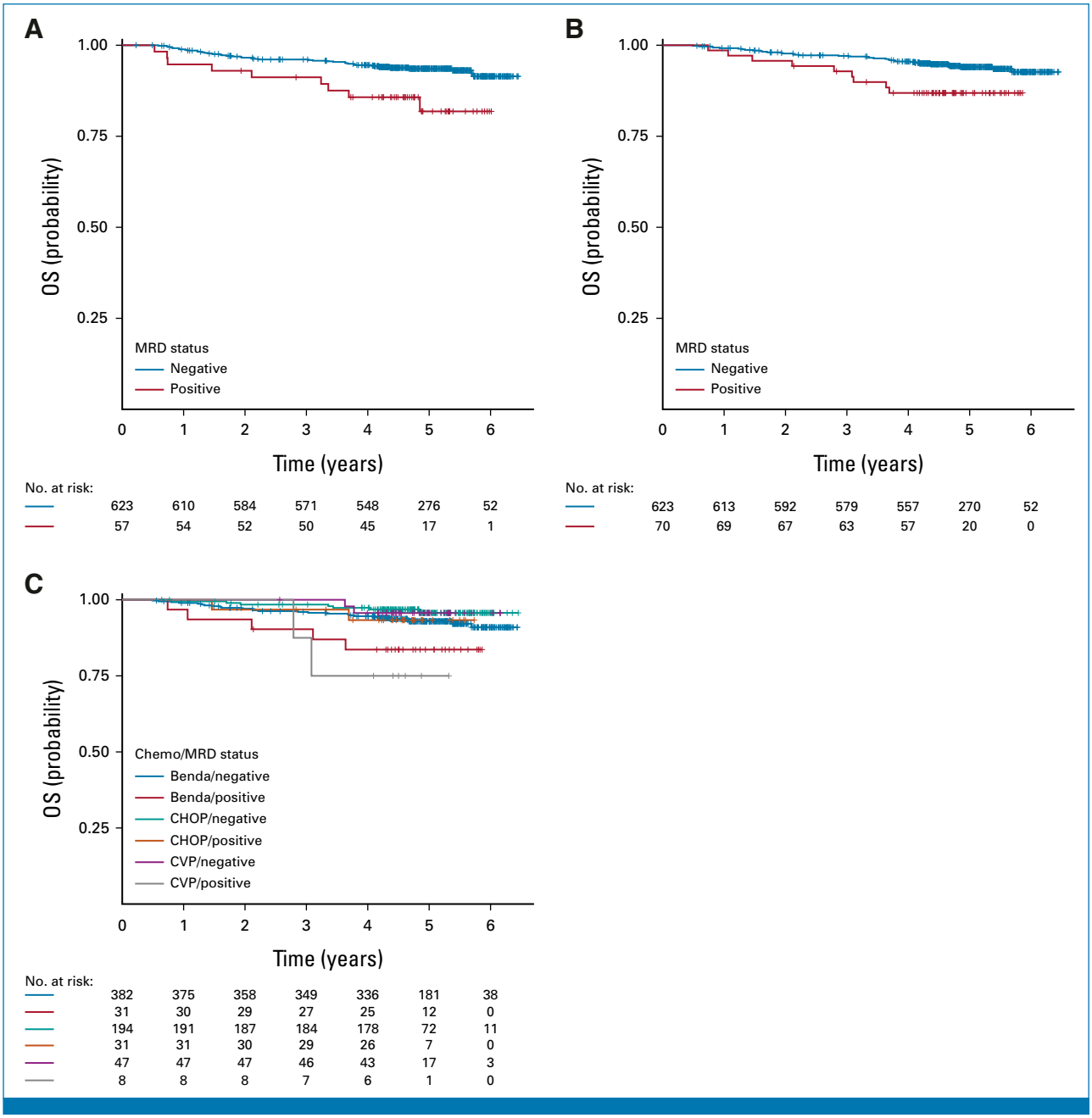

FIG 4. OS from random assignment according to MRD status (A) at MI, (B) at EOI, and (C) at EOI by chemotherapy arm. Benda, bendamustine; chemo, chemotherapy; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CVP, cyclophosphamide, vincristine, prednisone; EOI, end of induction; MI, mid-induction; MRD, minimal residual/detectable disease; OS, overall survival.

such as targeted high-throughput sequencing of cell-free circulating tumor DNA (ctDNA) currently used for MRD tracking and molecular profiling in aggressive lymphoma[30] might also be a suitable strategy for FL, not only overcoming disadvantages of qPCR-based MRD assessment such as the need for patient-specific assays and a limited sensitivity, but also providing additional information on mutational profile and potential clonal evolution. Whether dynamic ctDNA measurement is informative for MRD in a low proliferative disease such as FL with low ctDNA shedding should be explored in future clinical trials, including the investigation of earlier investigational time points for optimized risk stratification.

By measuring MRD, the better outcome after G in the GALLIUM trial[10] could be attributed to a more effective reduction of tumor cells during induction treatment, reflected by higher and deeper MRD response rates in the G-chemo arm across compartments (PB and/or BM) and chemotherapy partners. Residual disease was more frequently detected at MI

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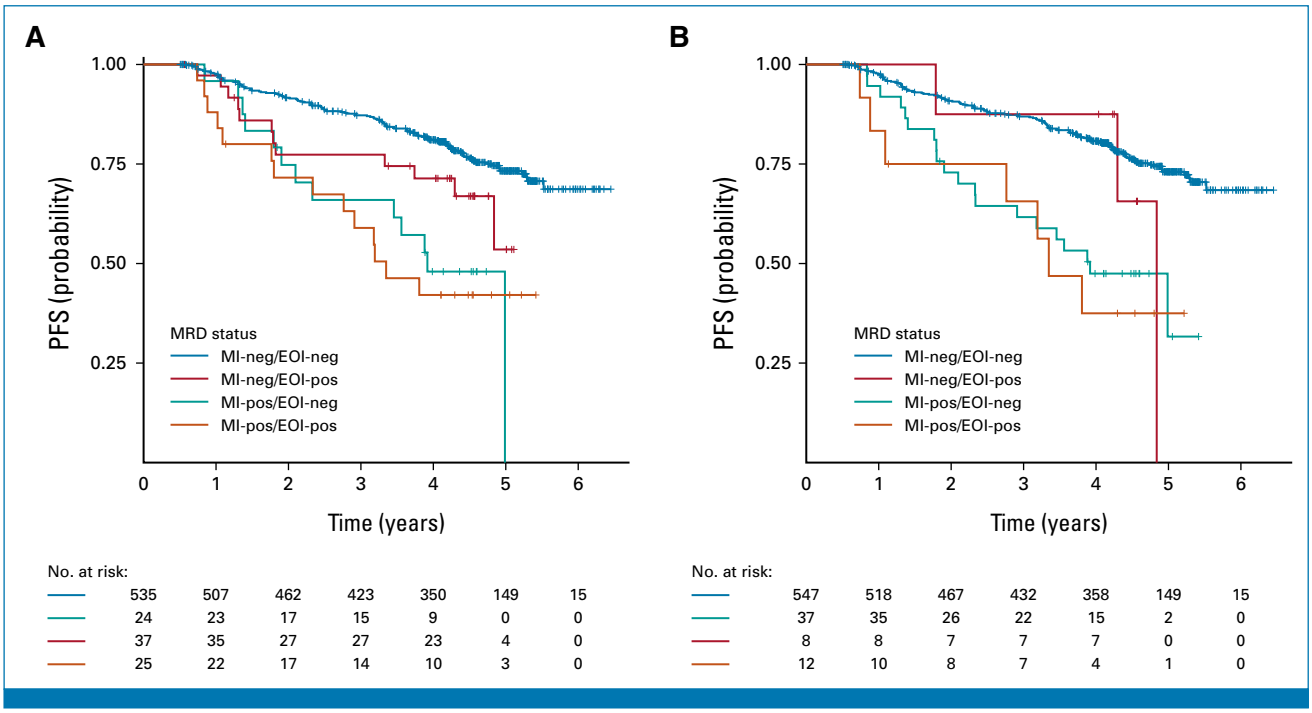

FIG 5. PFS from the time of random assignment by MRD status at EOI (A: in PB/BM, B: in PB only) and MI. (A) Late responders (MI-positive/EOI-negative) versus early responders (MI-negative/EOI-negative): HR, 3.11; $P = .00011$. (B) Late responders versus early responders: HR, 3.04; $P < .0001$. BM, bone marrow; EOI, end of induction; HR, hazard ratio; MI, mid-induction; MRD, minimal residual/detectable disease; neg, negative; PB, peripheral blood; PFS, progression-free survival; pos, positive.

in R-treated patients compared with the G-chemo arm; this difference was maintained at EOI and was demonstrated in particular in BM samples, where the number of MRD-positive samples was reduced by 50% in the G arm. To investigate future MRD-driven treatment strategies, the low MRD positivity rate already at MI should be overcome by looking at earlier time points or improving on MRD assay sensitivity.

The MRD response rate in the R-chemo arm of the GALLIUM trial (88.9%) was higher than previous reports of R-based immunochemotherapy induction in patients with previously untreated FL.[13-15] This can in part be attributed to the frequent use of bendamustine-based chemotherapy in GALLIUM (57%).[10] Although the trial was not powered to address differences by chemotherapy arms, 3-year PFS rates were highest in the bendamustine group and lowest in the CVP group, suggesting that CHOP/CVP were less effective partners.[31]

MRD data in this trial allowed a precise estimation of the contribution of each treatment component to the reduction of tumor burden over time. MRD response rates suggest that bendamustine is not only the most effective chemotherapy backbone in induction treatment of FL, but also that G can compensate for a less effective chemotherapy, resulting in comparable MRD response rates after CHOP-/CVP-based induction. Thus, MRD assessment is an attractive tool for measuring treatment efficacy of different therapies in a timely manner and is an important contributor to future study planning at the individual level.

Detectable MRD at both MI and EOI was highly associated with poor PFS, independent of chemotherapy backbone, clinical variables such as FLIPI and ECOG, and quality of clinical response. These findings are consistent with previous studies of first-line (immuno)chemotherapy in FL, which have suggested a prognostic role for MRD status at EOI.[13-15,32,33] However, our results suggest that G-chemo might reduce the prognostic effects of MRD status, for example, by achieving deeper MRD responses, a finding that requires further validation considering the reduced statistical power in light of the few PFS events observed so far with G-chemo.

Interestingly, in our study, early MRD response at MI was more predictive of outcome and could not be compensated for by later MRD response at EOI. The importance of early MRD response has been shown in other hematologic malignancies, such as ALL and CLL,[34,35] but data are scarce for FL. Our data emphasize the importance of early response assessment in effective induction treatment for an early readout of treatment efficacy and prognosis. Of note, MRD time points should be re-evaluated in the light of novel therapies with different response kinetics than immunochemotherapy.

By comparing MRD and PET response in a subgroup of patients, we confirmed the correlation between both measurements and show that in the setting of CMR, molecular assessment of response further improves the ability to predict patient outcomes. Therefore, in preplanned response-adapted trials, the

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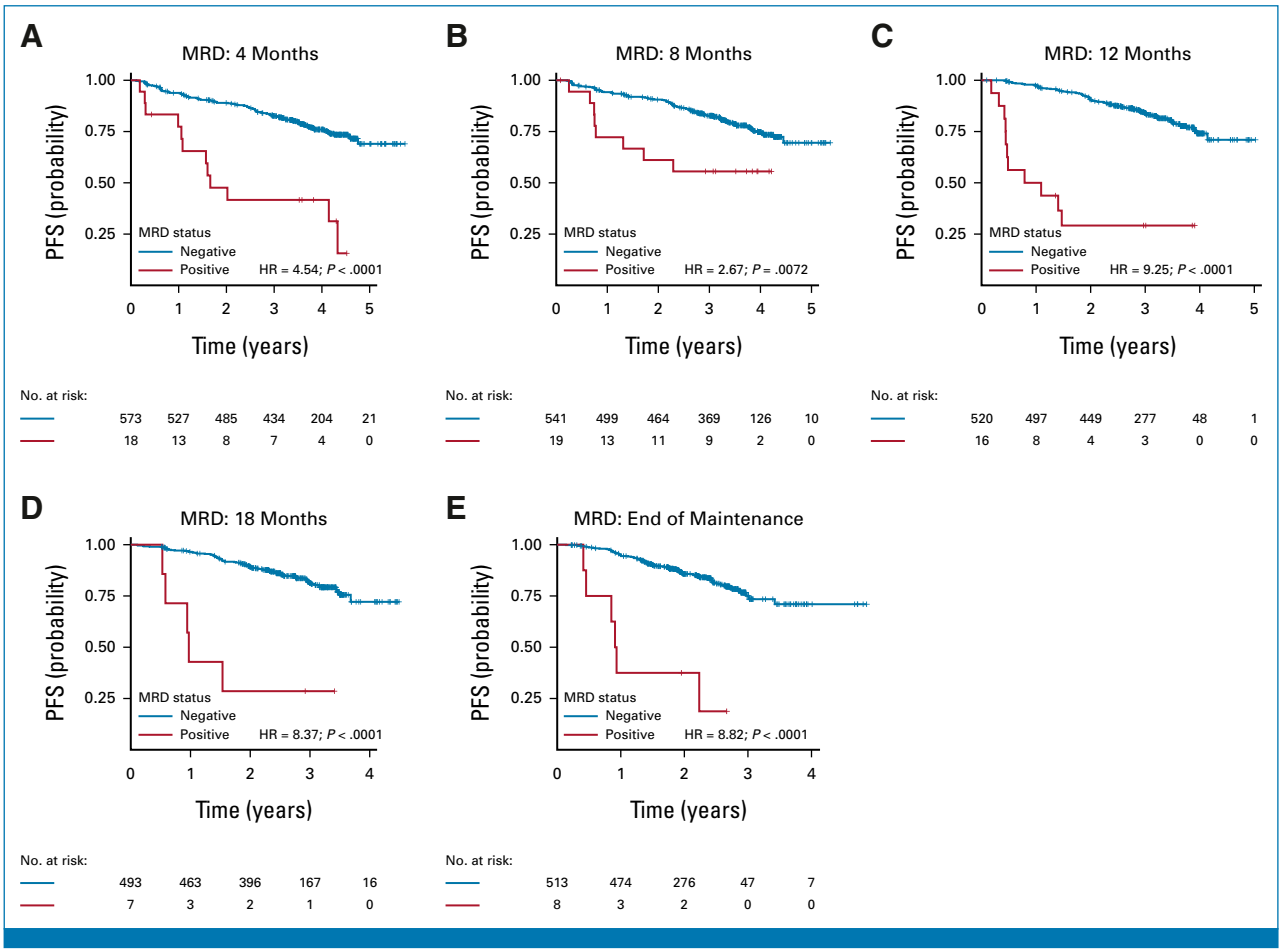

FIG 6. Landmark analysis of PFS and MRD status for patients in clinical remission at different landmark time points during maintenance: (A) 4 months, (B) 8 months, (C) 12 months, (D) 18 months, and (E) end of maintenance. The PFS is calculated from each landmark time point. Only patients who started maintenance treatment and had an MRD status in PB at EOI and had at least one MRD status during maintenance (n = 637) were evaluated. More than half of these (350/637, 54.9%) had evaluable data at all six follow-up time points (mean number of samples per patient, 5; range, 2-6). For landmark analyses, patients with a PFS event within 1 month from the respective MRD measurement were excluded. EOI, end of induction; HR, hazard ratio; MRD, minimal residual/detectable disease; PB, peripheral blood; PFS, progression-free survival.

combination of both methods should be performed for the most precise response assessment.

POD24 is associated with adverse survival outcomes in FL.[4] However, markers for early identification of these patients are currently lacking. In this trial, POD24 was significantly associated with MRD status during induction (both MI and EOI), but did not identify the majority of POD24 patients and was better suited to excluding than to predicting POD24. This reflects that early progression in FL is a multifactorial process, not only driven by the initial response to immunochemotherapy. However, as one quarter of POD24 patients can be identified early, strategies for treatment intensification according to MRD risk profiles during and after induction appear justified to improve prognosis of patients with FL at risk for relapse.

The importance of maintenance treatment for long-term disease control in FL has been shown by the PRIMA study.[36] There are few data published investigating the clinical relevance and prognostic role of MRD status during anti-CD20 maintenance treatment. A trial from the Fondazione Italiana Linfomi investigated the role of R-maintenance in elderly patients with FL, demonstrating a shorter PFS in MRD-positive patients during maintenance.[13] Similarly, the recently published FOLL12 phase III trial demonstrated that 2-year R maintenance prolonged PFS mainly in patients with CMR and MRD negativity, but was associated with inferior efficacy in CMR/MRD-positive patients even when administered in an intensified way.[32] MRD data in GALLIUM confirm the superiority of G for disease control in maintenance: patients in the R-arm were more often consistently MRD-positive or switched more often from MRD-negative to MRD-positive. In landmark analyses, we showed the strong prognostic impact of MRD status throughout maintenance treatment, with MRD positivity or MRD reappearance being highly associated with clinical relapse and adverse outcome. The strong association of clinical relapse with

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MRD positivity or reappearance raises the question whether an intensified maintenance including novel drugs could prevent clinical relapse and improve outcomes in MRD-positive patients, a question that should be addressed in future clinical trials. Our data confirm the importance of maintaining an MRD-negative status for long-term disease control of FL and discourage treatment reduction in MRD-negative patients.

In conclusion, this analysis demonstrates that early and continuous MRD response after immunochemotherapy in first-line treatment is the most important factor for long-term disease control and outcomes in FL and confirms the prognostic value of MRD status at EOI and throughout maintenance. It also provides valuable concepts for MRD-driven trials, including the timing of MRD analysis and potential intervention time points for MRD-positive patients.

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DATA SHARING STATEMENT

Qualified researchers may request access to individual patient-level data through the clinical study data request platform (https://vivli.org/). Further details on Roche's criteria for eligible studies are available here (https://vivli.org/members/ourmembers/). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here (https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm).

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Minimal Residual Disease Status Predicts Outcome in Patients With Previously Untreated Follicular Lymphoma: A Prospective Analysis of the Phase III GALLIUM Study

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