# Minimal residual disease status predicts outcomes in patients with follicular lymphoma treated with chemo-immunotherapy on the SWOG S0016 trial

Follicular lymphoma (FL) is characterized by variable clinical behavior. Current prognostic tools, such as the Follicular Lymphoma International Prognostic Index (FLIPI), are unable to identify high-risk patients, including those who experience early relapse or progression within 24 months (POD24), even with incorporation of molecular biomarkers.[1-3] Minimal residual disease (MRD) assessment has emerged as a transformative tool in hematologic malignancies, yet in FL it is challenged by spatial and clonal heterogeneity, the potential for transformation, and the very long natural history that mandates many years of follow-up.[4,5] The ClonoSeq assay has become a gold standard for quantifying tumor-specific clonal immunoglobulin gene rearrangements and has been cleared by the Food and Drug Administration for use in several hematologic malignancies.[6] Here we show that MRD accurately predicts outcomes in patients with advanced-stage FL patients treated with frontline chemo-immunotherapy regimens on SWOG S0016 (*clinicaltrials gov. Identifier: NCT00006721*), a randomized phase III trial with very mature follow-up.[5,7]

Between 2001 and 2008, 554 patients with previously untreated advanced-stage FL were randomly assigned to receive either six cycles of R-CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone, rituximab) or CHOP-RIT (radiommunotherapy, $I^{133}$-tositumomab) on SWOG S0016 phase III study.[5,8] All participating sites obtained institutional review board approval and the study was conducted in accordance with the Declaration of Helsinki. All eligible patients enrolled on S0016 were eligible for this analysis, including patients who were randomized to receive CHOP only.

Archival tumor formalin-fixed paraffin-embedded (FFPE) biopsy specimens were obtained prior to the start of treatment. Five paraffin curls taken from the face of a block containing a standard FFPE lymph node needle biopsy slice was used to prepare a library of *IGH* rearrangements. DNA was extracted using QIAamp DNA FFPE tissue kit (Qiagen). Bone marrow biopsy specimens were collected at a 1-year mark and mononuclear cells were isolated using standard Ficoll technique. DNA was extracted from 5,000,000 mononuclear cells using a DNEasy Blood and Tissue Kit (Qiagen).

MRD analysis was performed using the ClonoSeq research assay from Adaptive Biotechnologies.[6] We used high-throughput sequencing (HTS) of the *IGH* and *IGK/L* loci from tumor biopsy specimens taken prior to treatment to define complementarity-determining region 3 (CDR3) rearrangements that tag the tumor. Biopsy libraries with less than 50 estimated number of genomes (ENG) were classified as failures. Successful libraries were then screened for the presence of a dominant clone, which was inferred to represent a tumor-tagging CDR3 rearrangement. Large pre-libraries (>1,000 ENG) with an obvious dominant tumor-tagging clone accounting for greater than 20% of the library were used to estimate how much MRD was present within the tumors. Following analysis of baseline tumor biopsies, MRD status was determined on a 1-year bone marrow biopsy using starting material equivalent to 100,000 nucleated cells. MRD-positive state (MRDp) was defined as a minimum Hamming distance (HD) ≤6 in a patient's post-library (relative to any clone detected in a pre-library that met our criteria for tagging the tumor), as well as conserved pre-existing somatic hypermutation (SHM; if HD>0). Undetectable MRD (MRDu, at $<10^{-4}$) was defined as zero detected clones at CDR3 HD>6 in a post-library with a depth >10,000 ENG.

Five-year progression-free survival (PFS) was the primary endpoint of the study, defined as the time from randomization to the first observation of progression or death due to any cause. Secondary endpoints included 5-year overall survival (OS), and 10-year PFS and OS. OS was defined as the time from randomization to date of death due to any cause or last follow-up. Landmark analysis, defined at 1 year after randomization at the time of MRD determination, was used for assessing associations with PFS and OS. PFS and OS estimates and 95% confidence interval were calculated using the Kaplan-Meier and Greenwood methods. Comparisons use the two-sided log-rank test. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated using the Cox regression model. Nominal *P* values were reported without correction for multiplicity for all association tests.

Tumor biopsy tissue was available for 189 patients (Figure 1). Of these, 24 patients were not evaluable: four patients had an early event (disease progression prior to 1-year mark); 16 patients did not consent for tissue banking, and six patients were ineligible. Thus, 163 patient samples were analyzed. Thirty-six baseline tumor biopsies did not yield a trackable clone. An additional 11 1-year bone marrow specimens did not yield libraries large enough to confidently detect MRD. Thus, MRD status could not be ascertained in 47 patients due to the absence of a clear trackable clone in either pre- or post-library, con-

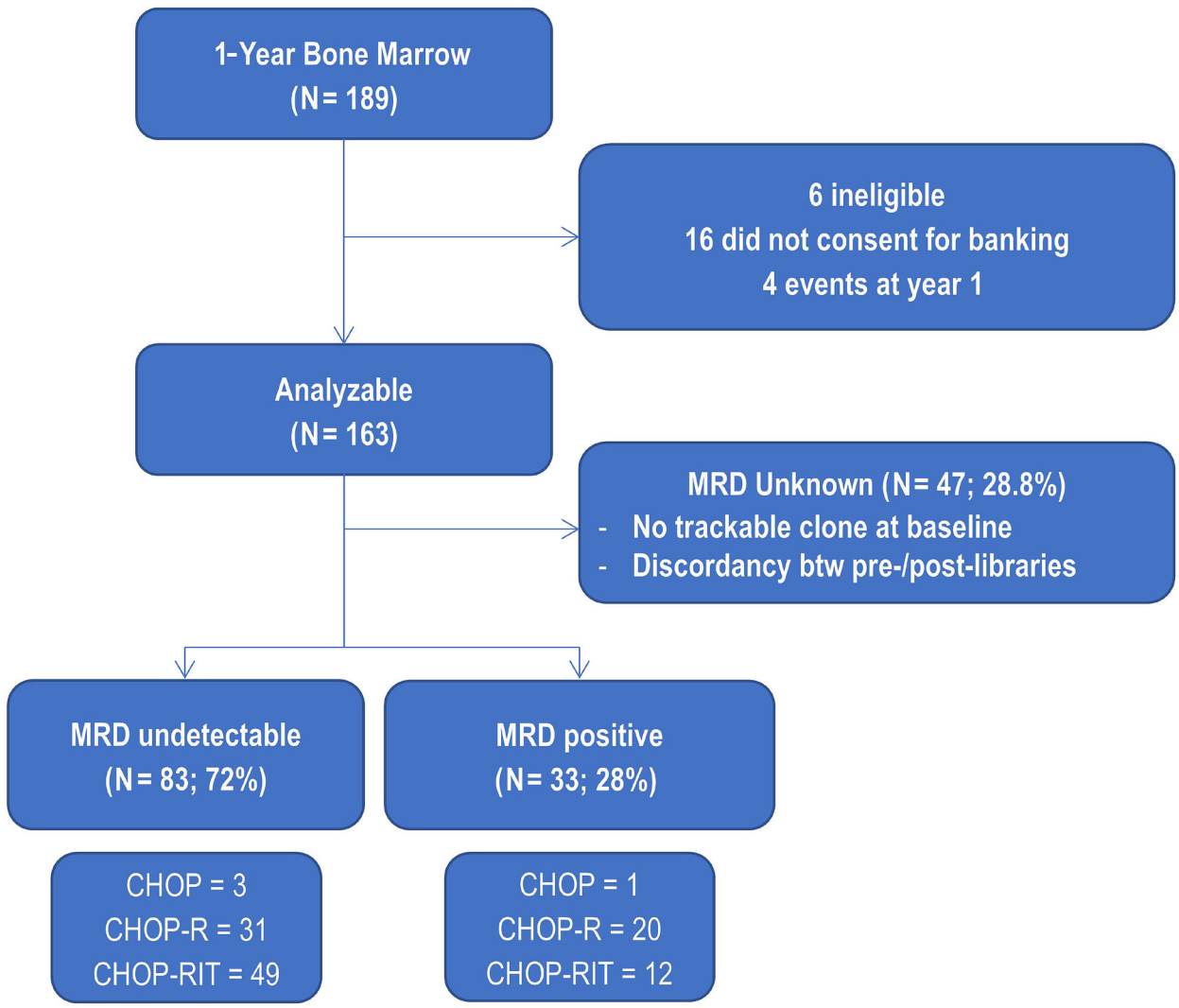


**Figure 1. Study consort diagram.** MRD: minimal residual disease. CHOP: cyclophosphamide, doxorubicin, vincristine; CHOP-R: CHOP plus rituximab; CHOP-RIT: CHOP-R plus iodine$^{-133}$tositumoab radioimmunotherapy.

**Table 1.** Patient characteristics.

| Characteristics | MRDp*, N=33 | MRDu*, N=83 | Two-sided *P* |
|---|---|---|---|
| Age, years, median (range) | 49.8 (27.6-66.4) | 56.4 (23.4-75.3) | 0.06 |
| Sex, male, N (%) | 20 (61) | 43 (52) | 0.42 |
| Race, White, N (%) | 28 (85) | 73 (88) | 0.76 |
| B symptoms, N (%) | 10 (30) | 19 (23) | 0.48 |
| Bone marrow involvement, N (%) | 27 (82) | 46 (55) | 0.01 |
| Histologic grade, N (%)<br>Grade 1-2<br>Grade 3 | <br>31 (94)<br>2 (6) | <br>74 (89)<br>9 (11) | 0.73 |
| Stage, N (%)<br>II<br>III<br>IV | <br>0 (0)<br>6 (18)<br>27 (82) | <br>1 (1)<br>32 (39)<br>50 (60) | 0.056 |
| Bulky disease >10 cm, N (%) | 9 (27) | 15 (18) | 0.31 |
| β2-microglobulin >ULN, N (%) | 22 (67) | 45 (54) | 0.30 |
| FLIPI risk, N (%)<br>Low<br>Intermediate<br>High | <br>8 (24)<br>17 (52)<br>8 (24) | <br>20 (24)<br>41 (49)<br>22 (27) | 1.00 |

*MRDp: minimal residual disease positive; MRDu: minimal residual disease undetectable; FLIPI: Follicular lymphoma International Prognostic Index; ULN: upper limit of normal reference range.

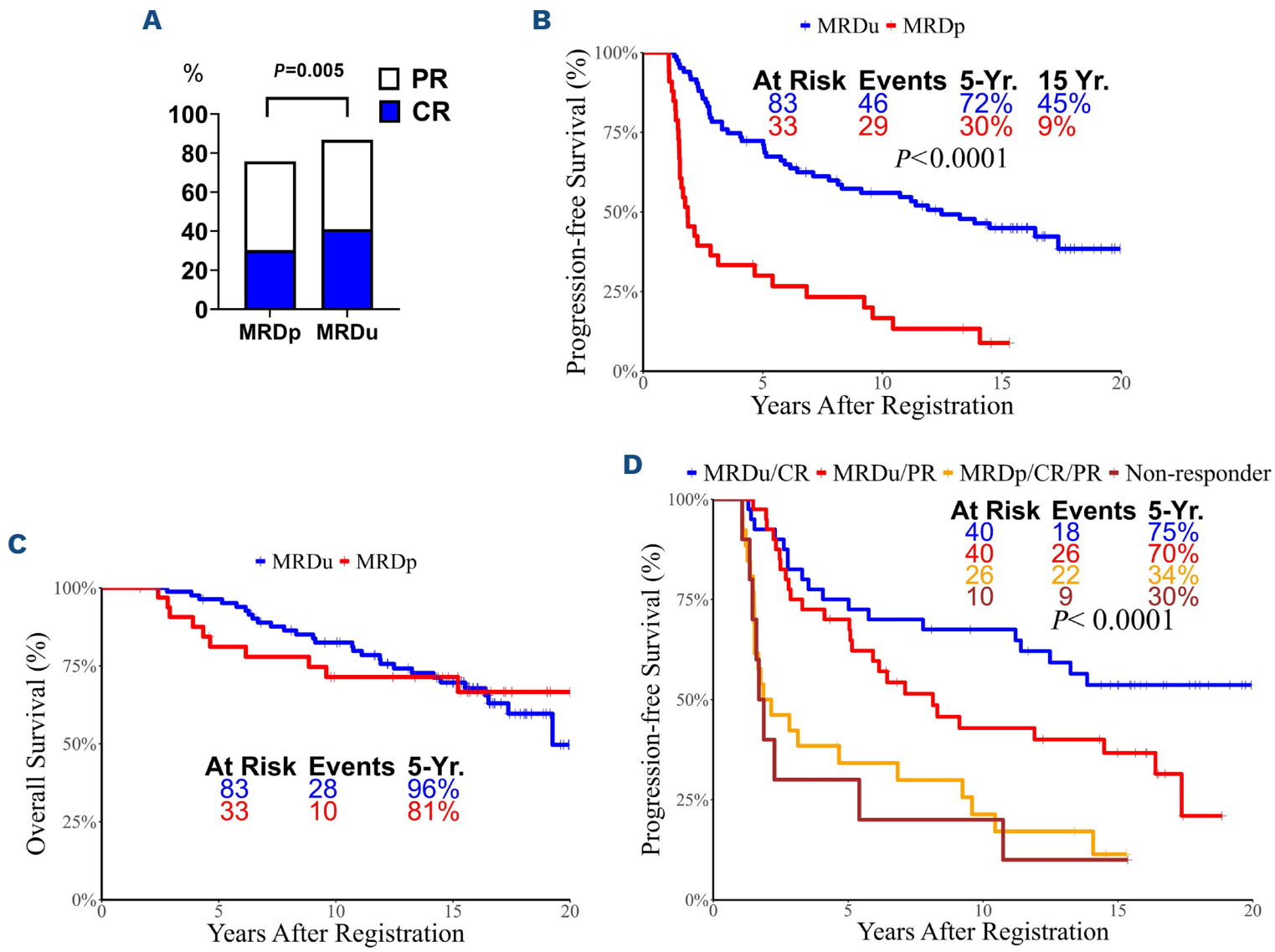


**Figure 2. Minimal residual disease status is associated with response and progression-free survival but not overall survival in patients with follicular lymphoma.** (A) Response rates in patients with FL depending on minimal residual disease (MRD) status; (B) 5-year and 15-year progression-free survival (PFS) of patients with follicular lymphoma (FL) depending on MRD status; (C) 5-year overall survival (OS) of patients with FL depending on MRD status; (D) PFS depending on response and MRD status. CR: complete response; PR: partial response; Yr: year. MRDu: MRD undetectable; MRDp: MRD positive.

tributing to a 28.8% failure rate.

Of 116 patients which yielded a trackable clone in the baseline library and a follow-up library of adequate size to be evaluated for MRD, 83 (72%) were MRDu and 33 (28%) were MRDp (Figure 1). Baseline patient characteristics (including age, sex, race, presence of B symptoms, histologic grade, disease stage and presence of bulky lymphadenopathy and FLIPI risk) were similar between MRDp and MRDu patients (Table 1). Of note, MRDp patients demonstrated higher frequency of bone marrow involvement by FL at the baseline compared with patients who reached MRDu (82% *vs*. 55%; *P*=0.01).

We next evaluated the prognostic significance of MRD state in FL. Overall response rate (ORR; computed tomography [CT]-assessed) was higher in patients who achieved MRDu compared with those who did not (96.4% *vs*. 78.8%; *P*=0.005; Figure 2A). Similarly, these patients had numerically higher rate of complete response (CR) or unconfirmed CR (48.2% *vs*. 30.3%; *P*=0.098), while the frequency of partial response (PR) was similar (48.2% *vs*. 48.5%).

The estimated 5-year PFS (primary endpoint) was significantly prolonged among patients who achieved MRDu (72%, 95% CI: 61-81%) compared with MRDp patients (30%, 95% CI: 16-46%) (*P*<0.0001; Figure 2B) with a median follow-up time of 15.8 years. Similarly, 10-year and 15-year PFS remained superior among patients with MRDu (56%, 95% CI: 45-66% and 45% *vs*. 17%, 95% CI: 6-31% and 9% in MRDp, respectively; *P*<0.0001).

MRDp state was associated with early progression of FL, as patients who failed to achieve MRDu status had a significantly increased risk of experiencing POD24 (RR=6.5, 95% CI: 3.0-14.0; *P*<0.0001).

Meanwhile, 1-year bone marrow MRD status was not associated with OS. Five-year and 10-year OS were not different between the two groups (96% and 82% in MRDu *vs*. 81% and 71% in MRDp, correspondingly; *P*=0.91; Figure 2C).

In patients who achieved MRDu, 5-year PFS was not shown to be associated with the depth of response (75% for CR and 70% for PR). Meanwhile, in patients who remained MRDp, 5-year PFS was numerically similar to that of non-responders (34% and 30%, respectively; Figure 2D; *Online Supplementary Table S1*). There was no statistically significant difference in 5- or 10-year OS rates

among the subgroups, although non-responders had a numerically shorter 10-year OS (*Online Supplementary Figure S1A; Online Supplementary Table S1*). The OS was not statistically different between the two treatment arms regardless of MRD status (*Online Supplementary Figure S1B; Online Supplementary Table S2*). Almost none of the MRDp patients who received CHOP-R were progression-free at 10 years, although this subgroup contained few patients.

Our study underscores the prognostic value of MRD status as assessed by the ClonoSeq assay. Our results are consistent with prior studies indicating that MRD negativity correlates with favorable outcomes in FL.[9,10] In a GALLIUM study which randomized patients with previously untreated FL to CHOP in combination with rituximab or obinutuzumab, achievement of MRD was associated with improved PFS.[9] In this study, 10.1% of patients remained MRDp at the end of induction. Interestingly, positron emission tomography (PET)-positivity rate was ~12%.[11] By contrast, we observed 28% MRDp rate in our study, suggesting that testing next-generation sequencing-MRD may have superior sensitivity over PET and polymerase chain reaction (PCR)-based MRD assessments. Still, the role of MRD as a biomarker to guide therapy in FL remains controversial. FOLL12 study used PCR-based MRD assessment to guide post-induction management of high-tumor burden FL but failed to demonstrate superiority of response-adapted treatment paradigm over standard rituximab maintenance, suggesting that further validation is needed before MRD is incorporated as an integral biomarker in future trials of FL.[12]

The 29% failure rate for MRD detection in our study highlights a critical limitation of current ClonoSeq assay technologies. Factors such as poor DNA quality, insufficient sequencing depth, and high rates of somatic mutations accompanied by clonal heterogeneity may contribute to assay failures. Future efforts should focus on optimizing DNA extraction protocols and exploring alternative sample types, such as circulating tumor DNA.[6,13]

Furthermore, whereas harmonization efforts will be essential for adoption of MRD as a clinical tool, incorporation of MRD testing as an early endpoint in future clinical trials, similar to other hematologic malignancies including chronic lymphocytic leukemia and multiple myeloma, is poised to accelerate evaluation of efficacy of emerging therapies.[14,15] MRD assessment in FL may fulfil promise of precision medicine, where patients with early MRDu may minimize treatment and associated toxic effects, while MRDp patients may receive pre-emptive therapy. Overall, this study illustrates the potential of MRD by molecular analysis as a powerful prognostic biomarker in FL. Its strong association with PFS highlights its potential in guiding therapeutic decisions. While challenges remain, the integration of MRD into treatment algorithms has the potential to transform FL management.

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**Contributions**

AVD, CC, SS and JWF designed the study. AVD, HL, MS, LR, AZ, ML and JYS conducted analysis. AVD and JYS wrote the manuscript. All authors edited and approved the manuscript.

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**Data-sharing statement**

Data from the clinical trial (*clinicaltrials gov. Identifier: NCT00006721*) and correlative analysis are available upon request. Please contact the corresponding author.

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