Mediterranean Journal of Hematology and Infectious Diseases

Review article

# Is There a Role for Minimal Residual Disease Monitoring in Follicular Lymphoma in the Chemo-Immunotherapy Era?

Giuseppe Gritti[1], Chiara Pavoni[1] and Alessandro Rambaldi[1,2]

[1] Hematology and Bone Marrow Transplant Unit, Ospedale Papa Giovanni XXIII, Bergamo, Italy
[2] Department of Oncology and Oncohematology, Università degli Studi di Milano, Milano, Italy

**Competing interests:** The authors have declared that no competing interests exist.

**Abstract. After 25 years, evaluation of minimal residual disease (MRD) in follicular lymphoma (FL) has become a standardized technique frequently integrated into clinical trials for its consistent and independent prognostic significance. Achievement of a sustained MRD negativity is a marker of treatment sensibility that has been associated with excellent clinical outcome in terms of clinical response and progression-free survival, independently from the employed therapy. However, no survival advantages have been reported for MRD negative patients and despite the compelling results of clinical trials, MRD evaluation has currently no role in clinical practice. Ongoing clinical trials will help in clarifying the potential setting in which MRD monitoring may have a routine clinical application i.e. allowing de-escalation of standard maintenance therapy in very low risk patients. In this review the clinical implications of MRD monitoring in Rituximab-era are discussed in light of the current treatment paradigms most aimed at reducing toxicities, and the response definition that now routinely integrates PET scan.**

**Keywords:** Follicular Lymphoma, Minimal Residual Disease, Molecular Remission, Outcome Prediction.

**Citation:** Gritti G., Pavoni C., Rambaldi A. Is there a role for minimal residual disease monitoring in follicular lymphoma in the chemo-immunotherapy era? Mediterr J Hematol Infect Dis 2017, 9(1): e2017010, DOI: http://dx.doi.org/10.4084/MJHID.2017.010

**Published: January 1, 2017** **Received: November 11, 2016** **Accepted: November 24, 2016**



Correspondence to: Prof. Alessandro Rambaldi, MD. Università degli Studi di Milano, Hematology and Bone Marrow Transplant Unit, Ospedale Papa Giovanni XXIII, Piazza OMS, 1, 24127 Bergamo, Italy. Tel.: +39-0352673684, Fax: +39-0352674968. E-mail: arambaldi@asst-pg23.it

**Introduction.** Follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma (NHL), accounting for 20-30% of all NHL in Western countries.[1] It is characterized by a chronic course, with a projected survival of more than 18 years in the modern chemo-immunotherapy era.[2] While some patients with limited stage disease may be cured, those presenting with advance stage or relapsing after local radiotherapy are generally considered not curable with standard treatments.[1] Early studies have shown that deferring treatment in asymptomatic patients with low tumor burden is not associated to a worse survival and, in many cases, the disease can remain stable for several years.[3,4] The usefulness of watchful waiting has been later on confirmed in the Rituximab era.[5] Thus only patients bearing a high tumor burden disease and/or symptomatic are currently treated with chemo-immunotherapy. Standard, first line treatment includes the use of Rituximab plus chemotherapy with an expected overall response rates of more than 90% and complete remissions in the range of 25–70% with median progression-free survival (PFS) exceeding 4 years.[6] A two years maintenance with Rituximab in responders results in significant prolongation of PFS, but not overall survival (OS).[7,8] Therefore, despite the excellent improvement gained by chemo-

immunotherapy, the majority of the patients eventually progress or relapse.

Several factors have been identified as of key importance in predicting PFS and OS, among those the quality of first line response have been shown to be remarkably associated to survival outcomes.[9] Traditionally, response evaluation in FL has been made with the use of contrast enhanced computed tomography (CT)-scan and bone marrow biopsy (BMB) along with standard laboratory tests and clinical parameters.[10,11] Immunohistochemical staining of BMB is the standard technique to assess lymphoma infiltration, but more sensitive assays have been developed to detect subclinical involvement. The presence of a hybrid BCL2/IGH gene in 80-90% of FL has spurred the interest in applying polymerase chain reaction (PCR) techniques to test the bone marrow (BM) and peripheral blood (PB) of patients before and after treatment.[12,13] In the current review we will discuss the methodological aspects of molecular monitoring and its clinical significance in the modern chemo-immunotherapy era.

**Technical Aspects.** The genetic hallmark of FL is the t(14;18)(q32;q21) translocation that leads to deregulated expression of the anti-apoptotic gene BCL2 in tumor cells, thus allowing for the acquisition of secondary chromosomal alterations in the germinal center environment, where the most non-neoplastic B cells undergo apoptosis.[14] The resulting hybrid gene BCL2/IGH is highly attractive for PCR based assays as it is a disease specific clonal sequence directly linked to FL pathogenesis and thus represents a highly stable marker. Five different clusters of BCL2/IGH rearrangements occur: the major breakpoint region (MBR), the minor clustering region (mcr), the intermediate cluster region (ICR), the 3′-BCL-2 region and the 5′-mcr region (**Figure 1**).[15] To date, the molecular detection of minimal residual disease (MRD) has been almost entirely based only on the study of MBR and mcr which account for about 50 and 10% of all BCL2 rearrangements, respectively.[15] Qualitative (nested) PCR (nPCR) has been widely used in molecular testing FL patients and proved as a highly reproducible method with an excellent sensitivity level able to detect 1 neoplastic cell in about a hundred thousand normal cells ($1x10^{-5}$).[16] The advent of TaqMan-based approaches allowed the introduction of quantitative PCR methods i.e. real-time quantitative PCR (RQ-PCR), a significant step forward from the mere presence or absence of a BCL2/IGH rearrangement.[17,18] This latter technique made possible the quantification of BCL2/IGH tumor burden at diagnosis and the dynamic of its reduction with treatment with lower risk of contamination and higher inter-laboratory reproducibility. Conversely, RQ-PCR has lower sensitivity than nPCR, probably as less amount of DNA is tested, and it is more expensive and technically complex needing the construction of standard reference curves.[19]

The occurrence of non-neoplastic BCL2/IGH rearrangements in the peripheral blood of healthy donors or patients without lymphoma was regarded as a possible confounder factor for MRD studies.[20,21] The low chimeric gene levels found in non-FL patients and its clearance after chemotherapy, however, confirmed the feasibility of MRD testing in this setting.[22] Another key point for the diffusion of MRD assessment is standardization of methodologies and definitions of common MRD terms. Standardization of RQ-PCR, including data interpretation and reporting, has been made by the efforts of the European network project EURO-MRD and has been applied in clinical trials.[19,23-26] New technical approaches could in the near future improve the frequency and the feasibility of BCL2/IGH rearrangements identification, as next generation sequencing (NGS) or droplet digital PCR.[27,28]

**Clinical Implication of Minimal Residual Disease Monitoring.** Twenty-five years have passed since the first observations that MRD negativity plays a role in predicting the outcome of patients with FL.[13] Early studies showed that standard chemotherapy programs can achieve a molecular remission (MR) in a minority of patients. First line anthracycline containing protocols could attain a MR in about 30-50% of the patients,[29,30] while intensification with autologous stem-cell transplant (SCT) can lead up to 60-70% of MRD negativity.[31] Conversely, the proportion was negligible in those with relapsed disease.[32] In all of these studies, patients achieving a MR were characterized by a significantly prolonged disease control. Notably, long term results of two trials aiming at reducing neoplastic cell contamination before ASCT with *ex vivo* purging, confirmed that persistence of residual

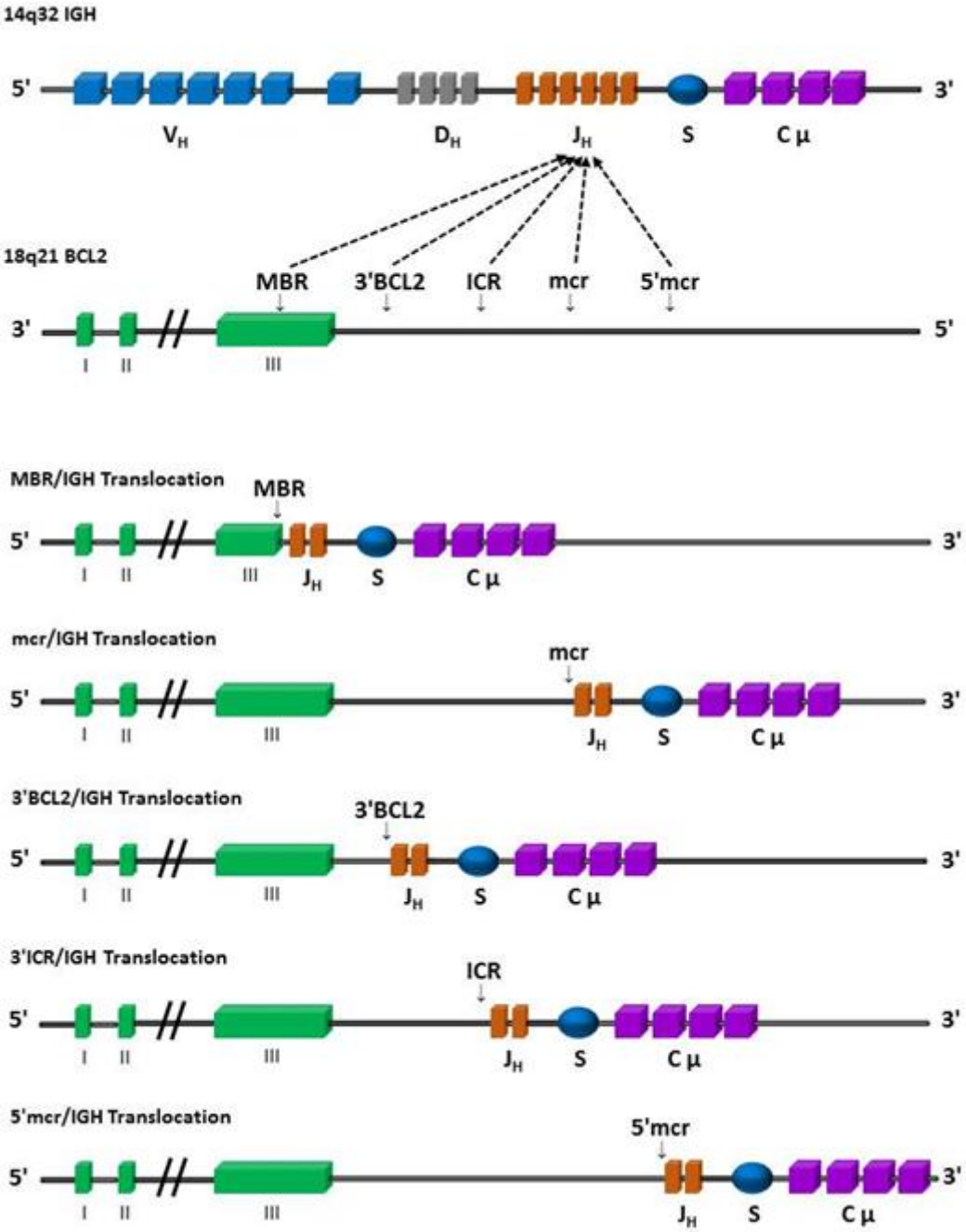


**Figure 1. Diagram of breakpoint sites of the IGH/BCL2 translocation.** In most cases the breakpoints of the IGH/BCL2 translocation are located downstream of the coding portion of the BCL2 gene and the IGH locus is mostly involved within the DJ recombination. In about 50% of cases the breaks occur in a 150-bp region in the 3′ noncoding portion of the third exon of the *BCL2* gene, named the major breakpoint region (MBR). The other less frequent breakpoints include the minor breakpoint region (mcr), the intermediate cluster region (icr), the 3′ BCL2 and 5′ mcr regions accounting for 5-10%, 5-10%, 6% and 1% of the cases, respectively.[15]

marrow involvement both at microscopic or molecular assessment were the only significant factors for long term remission, but not for survival.[33]

The association of Rituximab with chemotherapy dramatically improved response rates, PFS and, most notably, OS of advanced FL patients requiring treatment.[34,35] The ability of Rituximab to deplete FL neoplastic cells from peripheral blood and bone marrow, increased the rates of MR, accordingly. A clear demonstration of the Rituximab activity on MRD was shown in a study in which only responsive patients after CHOP therapy not achieving a MR were treated with 4 weekly infusion of Rituximab.[30] Overall, sequential administration of CHOP followed by Rituximab resulted in complete BM and PB molecular response in more than 70% of patients. Freedom from recurrence at 3 years was 52-57% for those patients obtaining a durable MR after CHOP or CHOP plus Rituximab, while it was significantly lower (20%) for those failing to

obtain or lost a MR. Residual disease kinetic showed that most of the patients in MR after CHOP were negative at the first interim evaluation after 3 cycles. Conversely, a delayed maximum effect was noted after Rituximab, with 59%, 74% and 63% of the patients in MR at week +12, +28 and +44 after treatment. This late effect of Rituximab was observed in other trials, as well as the more difficult clearance of MRD in the BM compared with the PB.[23,24] Rituximab have been used as consolidation therapy after autologous SCT in small series of patients, and proved to be safe and effective both in increasing the quality of clinical response i.e. converting PR into CR, as well as in achieving MR.[36,37]

The efficacy of front line Rituximab plus chemotherapy in inducing MR have been included as secondary end point in several large prospective trials (**Table 1**).

In two different controlled studies, R-CHOP resulted in MR of 39-44%.[23,25] Similar results were obtained with fludarabine and antracycline-based induction regimens (R-FM, R-FND) and with mitoxantrone, chlorambucil, and prednisolone (R-MCP).[23,24,38] Indirect evidence suggests that the less intensive regimen R-CVP could lead to inferior results both in term of clinical response and MR rates, explaining the shorter PFS observed compared to R-CHOP/R-FM.[23] Conversely, intensive regimens including upfront autologous SCT (R-HDS) increase the molecular response rates up to 80% of patients.[25] No data are currently available for the schema R-Bendamustine in the front line setting, but Bendamustine alone or combined with the novel anti-CD20 monoclonal antibody Obinutuzumab in relapsed/refractory patients can induce MR.[39] Induction therapy with $90^{Y}$-Ibritumomab-Tiuxetan was associated with achievement of a MR in nearly all of the CR patients.[40] Most importantly, all these trials confirmed the significant improvement in disease control in terms of PFS or relapse/event free survival in those patients achieving and maintaining the MR. The result was independent from other prognostic factors, most importantly the quality of response (CR vs PR), the chemotherapy induction regimen chosen and clinical risk factors as the FL prognostic index (FLIPI). However, the good results achieved in terms of disease control, did not translate in survival improvement. The importance of MR was shown also for a non-chemotherapy based consolidation. In a phase III trial $90^{Y}$-Ibritumomab-Tiuxetan was randomly given as consolidation therapy after standard first line therapy.[41] Interestingly, when compared to controls, consolidation with $90^{Y}$-Ibritumomab-Tiuxetan did not improve the PFS of patients who already were in MR while a significant prolongation of PFS was obtained in MRD positive patients (38.4 vs 8.2 months of the control group, $P<0.01$). In the relapsed/refractory setting, a randomized phase III study comparing CHOP vs R-CHOP therapy with subsequent Rituximab maintenance vs observation further confirmed the predictive value of MR, as almost all of the few patients who were still BCL2/IgH PCR positive at the end of the 2 years of maintenance treatment relapsed rapidly.[42]

Quantitative PCR methods have been used to measure the BCL2/IGH chimeric gene burden and early studies suggested that RQ-PCR evaluation before and after autologous transplantation may predict the clinical course of these patients.[43,44] In a clinical trial evaluating the sequential administration of CHOP and Rituximab in MRD positive patients, a high lymphoma cell burden at diagnosis was associated with lower probability to achieve a clinical and molecular CR.[45] The kinetic of BCL2/IGH positive cells during treatment showed that CHOP and Rituximab were both able to remove approximately 2 logs of tumor infiltration, thus explaining why patients with a limited lymphoma infiltration can achieve a molecular remission after CHOP chemotherapy alone, while the others necessitate the addition of Rituximab. Quantification of BCL2/IGH chimeric gene burden in the BM, but not in PB, was associated to a better event free survival. The result of the MRD analysis of a large phase III study confirmed the prognostic value of the molecular tumor burden both in term of likelihood to achieve a CR, and PFS.[23] Of note, high lymphoma cell burden at diagnosis was independent from FLIPI and clinical response in determining PFS. The importance of RQ-PCR was additionally shown in a study in which significant reduction ($\geq$2 logs) of circulating lymphoma cells rather than the mere MRD negativity was associated with a favorable clinical response and prolonged event-free survival.[38] However, not all the studies confirm these findings, probably due to the different induction regimen and Rituximab schedule.[24]

**Table 1.**

| | N evaluated (% of enrolled) | BCL2/IGH+ | Source | Treatment(s) | ORR (CR) | MRD negativity | EFS/PFS (MRD – vs +) | | Notes |
|---|---|---|---|---|---|---|---|---|---|
| **Rambaldi et al., 2002[30]** | 128 (100%) | 100% | BM and PB | CHOP plus R in MRD+ | 94% (57%) | 32% after CHOP, 74% after R | 3-year EFS: 52% (after CHOP) 57% (after CHOP+R) vs 20% | P<0.001 | |
| **Ladetto et al., 2008[25]** | 104 (78%) | 70% | BM | R-CHOP vs R-HDS | 70% (62%) vs 90% (85%) | 44% vs 80% | 3-y PFS: 77% vs 33% | P<0.001 | Age 18-60, high risk |
| **Hirt et al., 2008[38]** | 91 (45%) | 47% | PB | MCP vs R-MCP | 72% (28%) vs 100% (72%) | 0% vs 84% | Median EFS: not reached vs 27 months | P=0.02 | MRD- is considered a ≥2 log reduction of molecular burden |
| **Goff et al., 2009[41]** | 414 (100%) | 45% | PB | $90^{Y}$-IT consolidation vs observation | - | 90% vs 36% of previously MRD+ | Median PFS: MRD+ at randomization: 38 vs 8 months | P<0.01 | Consolidation of responding patients after chemotherapy +/- R |
| | | | | | | | MRD- at randomization: 37 vs 29 months | P=NS | |
| **Scholz et al., 2012[40]** | 59 (100%) | 49% | PB and/or BM | $90^{Y}$-IT | 87% (56%) | 93% | - | - | Age 50+, BM with <25% infiltration |
| **Ladetto et al., 2013[24]** | 227 (97%) | 51% | BM | R-FND plus R maintenance vs Observation | 86% (69%) | 84% | 34-months PFS: 72% vs 39% | P=0.007 | Age 60+ |
| **Galimberti et al., 2015[23]** | 415 (82%) | 52% | BM | R-CVP vs R-CHOP vs R-FM | 88% (67%) vs 93% (73%) vs 91% (72%) | 25% vs 39% vs 36% (P=NS) | 3-year PFS: 64% vs 53% | P=0.08 | |

**Legend**: ORR: overall response rate; MRD: minimal residual disease; CR: complete remission; EFS: event free survival; PFS: progression free survival; BM: bone marrow; PB: peripheral blood; R: Rituximab; CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisone; HDS: high-dose sequential; MCP: mitoxantrone, chlorambucil and prednisolone; $90^{Y}$-IT: 90-Yttrium Ibritumomab Tiuxetan; FM: fludarabine, mitoxantrone; NS: not significant.

**Current Issues and Future Perspective.** Compelling evidence indicates that MRD is a post treatment independent prognostic factor that can be consistently used to guide subsequent consolidation therapy in clinical trials. However a series of issues should be taken into consideration. Firstly, in large randomized prospective trials where molecular evaluation has been performed routinely, a molecular marker could be detected in only 50 to 60% of the patients.[23-25] In a large study including samples from 415 patients, a molecular marker was present in 53% of the cases; in particular, 67.5% of patients without BM infiltration were MRD positive, conversely 17.6% of patients with microscopic marrow involvement at BMB were lacking the molecular marker.[23] Several reasons could explain this finding, mainly the presence of uncommon rearrangements and the lack of significant marrow involvement in patients with nodal disease.[15] Despite the availability of primers and probes for detecting rare BCL2/IGH breakpoints will increase the cases with molecular marker, a significant proportion of patients are eventually excluded from this strategy. Persistence over time of MR is a major indication of sustained remission. As MRD detection is more informative on BM, especially in the Rituximab era, the need of multiple invasive procedures additionally limits the feasibility of MRD monitoring over time. Moreover, all the clinical trials reporting a prognostic implication of MR included the evaluation of response according to the 1999 or 2007 International Working Group (IWG) criteria with the use of the sole contrast enhanced CT-scan.[10,11] The introduction of FDG-PET scan improved the accuracy of staging and response assessment in FDG-avid lymphomas and is currently recommended for the definition of response in FL.[46] Several trials showed that concordance in CT-based and PET-based response designation is critical especially for patients in PR or CR unconfirmed, as PET scan is able to identify those patients with metabolically active disease and thus can improve the predictive value of response assessment.[47-49] To date, no data is available regarding the integration of PET based response and MRD evaluation. The only report in this setting is a retrospective evaluation of a very limited proportion of patient (8%) enrolled in a prospective trial.[50] This study suggests that PET and MRD are not strongly correlated with each other, and thus could be used as complementary techniques at the end of therapy to optimally explore the nodal and bone marrow compartments, but further studies are necessary to confirm the independent role of the two techniques.

The current clinical significance of MRD evaluation should also be evaluated when considering the evolving scenario of FL treatment. To date, given the satisfactory median results of chemo-immunotherapy and the lack of a survival impact of the chemotherapies available, the routine selection of induction treatment is guided more from the avoidance of unnecessary toxicity rather than the mere activity of the regimen.[1] However, while most patients achieve a prolonged disease control, a sizeable subset of cases remains substantially refractory to front line treatment with a poor prognosis.[9] Clinical scores currently available as FLIPI or FLIPI2 fail in identifying such cases, and a growing numbers of prognostic factors before or after treatment have been developed with this aim.[9,51-53] Thus, definition of high risk patient and, accordingly, end points for clinical trials are changing. Treatment results are satisfactory in low risk patients and integration of new molecules should be made with great caution in this group.[54] In this regard, achievement of sustained MR could allow the de-escalation of standard therapy in very low risk patients i.e. maintenance with Rituximab. Conversely, high risk patients are a group for which standard treatment need to be implemented and PFS should not represent *per se* the primary end point. Efforts to consistently characterize this latter group are ongoing and surrogate end points for survival as 2-year PFS have been proposed.[9,51-53]

**Conclusion.** Although not yet integrated in clinical practice as compared to other setting such acute lymphoblastic leukemia,[55] MRD evaluation is commonly integrated in clinical trials testing the efficacy of new treatment protocols in FL patients. In this setting MRD maintains its consistent and independent prognostic significance. Achievement of MR is a marker of treatment sensibility that has been associated with good clinical outcome in term of PFS, but not OS, independently from the specific therapy. Some technical limitations such as the limited coverage of the different breakpoints present in the BCL2/IgH rearrangements will be likely overcome in the near future by more appropriate molecular approaches.[27,28] These laboratory improvements, most likely in

combination with the new imaging technologies currently tested by an ongoing clinical trial (NCT02063685), will probably lead to a reappraisal of MRD evaluation in FL patients

## References:


1. Kahl BS, Yang DT. Follicular lymphoma: evolving therapeutic strategies. Blood. 2016;127:2055-2063. https://doi.org/10.1182/blood-2015-11-624288 PMid:26989204
2. Tan D, Horning SJ, Hoppe RT, et al. Improvements in observed and relative survival in follicular grade 1-2 lymphoma during 4 decades: the Stanford University experience. Blood. 2013;122:981-987. https://doi.org/10.1182/blood-2013-03-491514 PMid:23777769 PMCid:PMC3739040
3. Brice P, Bastion Y, Lepage E, et al. Comparison in low-tumor-burden follicular lymphomas between an initial no-treatment policy, prednimustine, or interferon alfa: a randomized study from the Groupe d'Etude des Lymphomes Folliculaires. Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol. 1997;15:1110-1117. PMid:9060552
4. Ardeshna KM, Smith P, Norton A, et al. Long-term effect of a watch and wait policy versus immediate systemic treatment for asymptomatic advanced-stage non-Hodgkin lymphoma: a randomised controlled trial. Lancet. 2003;362:516-522. https://doi.org/10.1016/S0140-6736(03)14110-4
5. Solal-Celigny P, Bellei M, Marcheselli L, et al. Watchful waiting in low-tumor burden follicular lymphoma in the rituximab era: results of an F2-study database. J Clin Oncol. 2012;30:3848-3853. https://doi.org/10.1200/JCO.2010.33.4474 PMid:23008294
6. Hiddemann W, Cheson BD. How we manage follicular lymphoma. Leukemia. 2014;28:1388-1395. https://doi.org/10.1038/leu.2014.91 PMid:24577532
7. Salles G, Seymour JF, Offner F, et al. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. Lancet. 2011;377:42-51. https://doi.org/10.1016/S0140-6736(10)62175-7
8. Barta SK, Li H, Hochster HS, et al. Randomized phase 3 study in low-grade lymphoma comparing maintenance anti-CD20 antibody with observation after induction therapy: A trial of the ECOG-ACRIN Cancer Research Group (E1496). Cancer. 2016;122:2996-3004. https://doi.org/10.1002/cncr.30137 PMid:27351685
9. Tarella C, Gueli A, Delaini F, et al. Rate of primary refractory disease in B and T-cell non-Hodgkin's lymphoma: correlation with long-term survival. PLoS One. 2014;9:e106745. https://doi.org/10.1371/journal.pone.0106745 PMid:25255081 PMCid:PMC4177839
10. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol. 1999;17:1244. PMid:10561185
11. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007;25:579-586. https://doi.org/10.1200/JCO.2006.09.2403 PMid:17242396
12. Vaandrager JW, Schuuring E, Raap T, Philippo K, Kleiverda K, Kluin P. Interphase FISH detection of BCL2 rearrangement in follicular lymphoma using breakpoint-flanking probes. Genes Chromosomes Cancer. 2000;27:85-94. https://doi.org/10.1002/(SICI)1098-2264(200001)27:1<85::AID-GCC11>3.0.CO;2-9
13. Gribben JG, Freedman AS, Neuberg D, et al. Immunologic purging of marrow assessed by PCR before autologous bone marrow transplantation for B-cell lymphoma. N Engl J Med. 1991;325:1525-1533. https://doi.org/10.1056/NEJM199111283252201 PMid:1944436
14. Basso K, Dalla-Favera R. Germinal centres and B cell lymphomagenesis. Nat Rev Immunol. 2015;15:172-184. https://doi.org/10.1038/nri3814 PMid:25712152
15. Weinberg OK, Ai WZ, Mariappan MR, Shum C, Levy R, Arber DA. "Minor" BCL2 breakpoints in follicular lymphoma: frequency and correlation with grade and disease presentation in 236 cases. J Mol Diagn. 2007;9:530-537. https://doi.org/10.2353/jmoldx.2007.070038 PMid:17652637 PMCid:PMC1975105
16. Weiss LM, Warnke RA, Sklar J, Cleary ML. Molecular analysis of the t(14;18) chromosomal translocation in malignant lymphomas. N Engl J Med. 1987;317:1185-1189. https://doi.org/10.1056/NEJM198711053171904 PMid:3657890
17. Holland PM, Abramson RD, Watson R, Gelfand DH. Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of Thermus aquaticus DNA polymerase. Proc Natl Acad Sci U S A. 1991;88:7276-7280. https://doi.org/10.1073/pnas.88.16.7276 PMid:1871133 PMCid:PMC52277
18. Donovan JW, Ladetto M, Zou G, et al. Immunoglobulin heavy-chain consensus probes for real-time PCR quantification of residual disease in acute lymphoblastic leukemia. Blood. 2000;95:2651-2658. PMid:10753847
19. Bruggemann M, Schrauder A, Raff T, et al. Standardized MRD quantification in European ALL trials: proceedings of the Second International Symposium on MRD assessment in Kiel, Germany, 18-20 September 2008. Leukemia. 2010;24:521-535. https://doi.org/10.1038/leu.2009.268 PMid:20033054
20. Summers KE, Goff LK, Wilson AG, Gupta RK, Lister TA, Fitzgibbon J. Frequency of the Bcl-2/IgH rearrangement in normal individuals: implications for the monitoring of disease in patients with follicular lymphoma. J Clin Oncol. 2001;19:420-424. PMid:11208834
21. Dolken G, Dolken L, Hirt C, Fusch C, Rabkin CS, Schuler F. Age-dependent prevalence and frequency of circulating t(14;18)-positive cells in the peripheral blood of healthy individuals. J Natl Cancer Inst Monogr. 2008:44-47. https://doi.org/10.1093/jncimonographs/lgn005 PMid:18648002
22. Ladetto M, Drandi D, Compagno M, et al. PCR-detectable nonneoplastic Bcl-2/IgH rearrangements are common in normal subjects and cancer patients at diagnosis but rare in subjects treated with chemotherapy. J Clin Oncol. 2003;21:1398-1403. https://doi.org/10.1200/JCO.2003.07.070 PMid:12663733
23. Galimberti S, Luminari S, Ciabatti E, et al. Minimal residual disease after conventional treatment significantly impacts on progression-free survival of patients with follicular lymphoma: the FIL FOLL05 trial. Clin Cancer Res. 2014;20:6398-6405. https://doi.org/10.1158/1078-0432.CCR-14-0407 PMid:25316810
24. Ladetto M, Lobetti-Bodoni C, Mantoan B, et al. Persistence of minimal residual disease in bone marrow predicts outcome in follicular lymphomas treated with a rituximab-intensive program. Blood. 2013;122:3759-3766. https://doi.org/10.1182/blood-2013-06-507319 PMid:24085766
25. Ladetto M, De Marco F, Benedetti F, et al. Prospective, multicenter randomized GITMO/IIL trial comparing intensive (R-HDS) versus conventional (CHOP-R) chemoimmunotherapy in high-risk follicular lymphoma at diagnosis: the superior disease control of R-HDS does not translate into an overall survival advantage. Blood. 2008;111:4004-4013. https://doi.org/10.1182/blood-2007-10-116749 PMid:18239086
26. van der Velden VH, Cazzaniga G, Schrauder A, et al. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. Leukemia. 2007;21:604-611. https://doi.org/10.1038/sj.leu.2404586
27. Drandi D, Kubiczkova-Besse L, Ferrero S, et al. Minimal Residual Disease Detection by Droplet Digital PCR in Multiple Myeloma, Mantle Cell Lymphoma, and Follicular Lymphoma: A Comparison with Real-Time PCR. J Mol Diagn. 2015;17:652-660. https://doi.org/10.1016/j.jmoldx.2015.05.007 PMid:26319783
28. Kotrova M, Muzikova K, Mejstrikova E, et al. The predictive strength of next-generation sequencing MRD detection for relapse compared with current methods in childhood ALL. Blood. 2015;126:1045-1047. https://doi.org/10.1182/blood-2015-07-655159 PMid:26294720 PMCid:PMC4551355
29. Lopez-Guillermo A, Cabanillas F, McLaughlin P, et al. The clinical significance of molecular response in indolent follicular lymphomas. Blood. 1998;91:2955-2960. PMid:9531606
30. Rambaldi A, Lazzari M, Manzoni C, et al. Monitoring of minimal residual disease after CHOP and rituximab in previously untreated patients with follicular lymphoma. Blood. 2002;99:856-862.

https://doi.org/10.1182/blood.V99.3.856 PMid:11806987
31. Ladetto M, Corradini P, Vallet S, et al. High rate of clinical and molecular remissions in follicular lymphoma patients receiving high-dose sequential chemotherapy and autografting at diagnosis: a multicenter, prospective study by the Gruppo Italiano Trapianto Midollo Osseo (GITMO). Blood. 2002;100:1559-1565. https://doi.org/10.1182/blood-2002-02-0621 PMid:12176870
32. Gribben JG, Freedman A, Woo SD, et al. All advanced stage non-Hodgkin's lymphomas with a polymerase chain reaction amplifiable breakpoint of bcl-2 have residual cells containing the bcl-2 rearrangement at evaluation and after treatment. Blood. 1991;78:3275-3280. PMid:1742487
33. Brown JR, Feng Y, Gribben JG, et al. Long-term survival after autologous bone marrow transplantation for follicular lymphoma in first remission. Biol Blood Marrow Transplant. 2007;13:1057-1065. https://doi.org/10.1016/j.bbmt.2007.05.012 PMid:17697968 PMCid:PMC4147857
34. Hiddemann W, Kneba M, Dreyling M, et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood. 2005;106:3725-3732. https://doi.org/10.1182/blood-2005-01-0016 PMid:16123223
35. Marcus R, Imrie K, Belch A, et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. Blood. 2005;105:1417-1423. https://doi.org/10.1182/blood-2004-08-3175 PMid:15494430
36. Brugger W, Hirsch J, Grunebach F, et al. Rituximab consolidation after high-dose chemotherapy and autologous blood stem cell transplantation in follicular and mantle cell lymphoma: a prospective, multicenter phase II study. Ann Oncol. 2004;15:1691-1698. https://doi.org/10.1093/annonc/mdh425 PMid:15520073
37. Morschhauser F, Recher C, Milpied N, et al. A 4-weekly course of rituximab is safe and improves tumor control for patients with minimal residual disease persisting 3 months after autologous hematopoietic stem-cell transplantation: results of a prospective multicenter phase II study in patients with follicular lymphoma. Ann Oncol. 2012;23:2687-2695. https://doi.org/10.1093/annonc/mds202 PMid:22767588
38. Hirt C, Schuler F, Kiefer T, et al. Rapid and sustained clearance of circulating lymphoma cells after chemotherapy plus rituximab: clinical significance of quantitative t(14;18) PCR monitoring in advanced stage follicular lymphoma patients. Br J Haematol. 2008;141:631-640. https://doi.org/10.1111/j.1365-2141.2008.07101.x PMid:18422779
39. Pott C, Belada D, Danesi N, et al. Analysis of Minimal Residual Disease in Follicular Lymphoma Patients in Gadolin, a Phase III Study of Obinutuzumab Plus Bendamustine Versus Bendamustine in Relapsed/Refractory Indolent Non-Hodgkin Lymphoma. Blood. 2015;126:2015-2012-2003 2016:2028:2015.
40. Scholz CW, Pinto A, Linkesch W, et al. (90)Yttrium-ibritumomab-tiuxetan as first-line treatment for follicular lymphoma: 30 months of follow-up data from an international multicenter phase II clinical trial. J Clin Oncol. 2012;31:308-313. https://doi.org/10.1200/JCO.2011.41.1553 PMid:23233718
41. Goff L, Summers K, Iqbal S, et al. Quantitative PCR analysis for Bcl-2/IgH in a phase III study of Yttrium-90 Ibritumomab Tiuxetan as consolidation of first remission in patients with follicular lymphoma. J Clin Oncol. 2009;27:6094-6100. https://doi.org/10.1200/JCO.2009.22.6258 PMid:19858392
42. van Oers MH, Tonnissen E, Van Glabbeke M, et al. BCL-2/IgH polymerase chain reaction status at the end of induction treatment is not predictive for progression-free survival in relapsed/resistant follicular lymphoma: results of a prospective randomized EORTC 20981 phase III intergroup study. J Clin Oncol. 2010;28:2246-2252. https://doi.org/10.1200/JCO.2009.25.0852 PMid:20368567
43. Ladetto M, Sametti S, Donovan JW, et al. A validated real-time quantitative PCR approach shows a correlation between tumor burden and successful ex vivo purging in follicular lymphoma patients. Exp Hematol. 2001;29:183-193. https://doi.org/10.1016/S0301-472X(00)00651-2
44. Galimberti S, Guerrini F, Morabito F, et al. Quantitative molecular evaluation in autotransplant programs for follicular lymphoma: efficacy of in vivo purging by Rituximab. Bone Marrow Transplant. 2003;32:57-63. https://doi.org/10.1038/sj.bmt.1704102 PMid:12815479
45. Rambaldi A, Carlotti E, Oldani E, et al. Quantitative PCR of bone marrow BCL2/IgH+ cells at diagnosis predicts treatment response and long-term outcome in follicular non-Hodgkin lymphoma. Blood. 2005;105:3428-3433. https://doi.org/10.1182/blood-2004-06-2490 PMid:15637137
46. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol. 2014;32:3059-3068. https://doi.org/10.1200/JCO.2013.54.8800 PMid:25113753 PMCid:PMC4979083
47. Le Dortz L, De Guibert S, Bayat S, et al. Diagnostic and prognostic impact of 18F-FDG PET/CT in follicular lymphoma. Eur J Nucl Med Mol Imaging. 2010;37:2307-2314. https://doi.org/10.1007/s00259-010-1539-5 PMid:20717826
48. Dupuis J, Berriolo-Riedinger A, Julian A, et al. Impact of [(18)F]fluorodeoxyglucose positron emission tomography response evaluation in patients with high-tumor burden follicular lymphoma treated with immunochemotherapy: a prospective study from the Groupe d'Etudes des Lymphomes de l'Adulte and GOELAMS. J Clin Oncol. 2012;30:4317-4322. https://doi.org/10.1200/JCO.2012.43.0934 PMid:23109699
49. Trotman J, Fournier M, Lamy T, et al. Positron emission tomography-computed tomography (PET-CT) after induction therapy is highly predictive of patient outcome in follicular lymphoma: analysis of PET-CT in a subset of PRIMA trial participants. J Clin Oncol. 2011;29:3194-3200. https://doi.org/10.1200/JCO.2011.35.0736 PMid:21747087
50. Luminari S, Galimberti S, Versari A, et al. Positron emission tomography response and minimal residual disease impact on progression-free survival in patients with follicular lymphoma. A subset analysis from the FOLL05 trial of the Fondazione Italiana Linfomi. Haematologica. 2016;101:e66-68. https://doi.org/10.3324/haematol.2015.132811 PMid:26471485 PMCid:PMC4938338
51. Casulo C, Byrtek M, Dawson KL, et al. Early Relapse of Follicular Lymphoma After Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone Defines Patients at High Risk for Death: An Analysis From the National LymphoCare Study. J Clin Oncol. 2015;33:2516-2522. https://doi.org/10.1200/JCO.2014.59.7534 PMid:26124482 PMCid:PMC4879714
52. Pastore A, Jurinovic V, Kridel R, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015;16:1111-1122. https://doi.org/10.1016/S1470-2045(15)00169-2
53. Jurinovic V, Kridel R, Staiger AM, et al. Clinicogenetic risk models predict early progression of follicular lymphoma after first-line immunochemotherapy. Blood. 2016;128:1112-1120. https://doi.org/10.1182/blood-2016-05-717355 PMid:27418643
54. Cheson BD. Speed bumps on the road to a chemotherapy-free world for lymphoma patients. Blood. 2016;128:325-330. https://doi.org/10.1182/blood-2016-04-709477 PMid:27222479
55. Spinelli O, Tosi M, Peruta B, et al. Prognostic significance and treatment implications of minimal residual disease studies in Philadelphia-negative adult acute lymphoblastic leukemia. Mediterr J Hematol Infect Dis. 2014;6:e2014062 https://doi.org/10.4084/mjhid.2014.062 PMid:25237475 PMCid:PMC4165493