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Minimal Residual Disease in Indolent Lymphomas: A Critical Assessment

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Opinion statement

Indolent non-Hodgkin lymphomas (iNHL) are a heterogeneous group of pathologies characterized by a prolonged natural history and good response to treatment. They also have a tendency to relapse, in some cases with a worse prognosis. One of the main objectives in the newest clinical trials is to identify patients at high risk of relapse. This cannot be accomplished using only clinical prognostic scores. Detection of minimal residual disease (MRD) is effective in evaluating long-term disease response, with a strong and independent predictive value that was demonstrated in large cohorts of patients. Analysis of MRD allows patient stratification based on the risk for relapse; therefore, different therapeutic programs can be designed based on the response characteristics. This tailored therapy is already happening in current clinical trials. Limits imposed by traditional PCR-based tools are being overcome due to new molecular biology techniques like droplet digital PCR and next generation sequencing. Although these techniques are not yet standardized, they will likely increase the reliability and ensure broad applicability of MRD detection in future years.

Introduction

Indolent non-Hodgkin lymphomas (iNHL) are a heterogeneous group of pathologies that include follicular lymphoma (FL), marginal zone lymphoma (MZL), and lymphoplasmacytic lymphoma (LPL). Clinically, these disorders are characterized by an indolent course and good response to treatment. Nevertheless, relapses still occur frequently and a proportion of patients die from their disease. Therefore, the identification of patients at high risk of relapse is a major goal of clinical and translational research in the iNHL field. Currently, clinical scores such as FLIPI and FLIPI-2 [1, 2] and clinical-related parameters such as POD 24 [3] can provide important insight into the identification of high-risk patient populations. Also, positron emission tomography (PET) scanning (in terms of tumor metabolic volume or metabolic response) is very important for patient prognostication [4–6]. However, there is considerable evidence that biological parameters might provide critical prognostic information. In particular, biomarkers assessed at baseline (such as mutations in the M7 FLIPI or gene expression profiling) [7–9] and molecular monitoring of minimal residual disease (MRD) after treatment are reliable tools to help classify patients based on their prognosis. In MRD, a small population of lymphoma cells remain after very effective treatment and are not detectable with traditional laboratory or imaging techniques. They can be detected with more sensitive molecular biology techniques that can identify the neoplastic clone. This review will focus on the technical features and clinical relevance of MRD monitoring in iNHL. Since the vast majority of results have been generated in FL, the bulk of the review will focus on this neoplasm, followed by a section on the findings for other iNHL subtypes. The final section will concentrate on the application MRD as a decision tool in current and future clinical trials.

Prognostic value of MRD analysis: positive studies

Approximately 85% of FLs feature the translocation t(14, 18) that juxtaposes the Bcl2 gene, encoding the protein involved in the anti-apoptotic pathway, to the immunoglobulin heavy chain (IGH) gene locus. The Bcl2/IGH juxtaposition leads to malignant B cells with overexpression of proteins that escape the physiologic apoptotic process that generally occurs in the germinal center, giving clear advantages to the proliferation and survival of the clonal cells. The Bcl2/IGH rearrangement was used for decades as a disease marker for MRD using qualitative PCR techniques, and then quantitative ones [10, 11]. The first studies focusing on the role of MRD were published in the early 1990s. Gribben et al. monitored MRD to evaluate the efficacy of "purging" techniques or cell selection using the first monoclonal antibodies on leukapheresis samples for FL patients treated with autologous transplantation. Moreover, these studies showed that traditional induction chemotherapy did not achieve molecular remission (MR) [12, 13]. After a clinical follow-up of 12 years, the same cohort of patients was reviewed by Brown et al., who observed that most patients who had completed the therapeutic program were still alive to assess the efficacy of this treatment regimen and the indolent course of this pathology. Moreover, the statistical analysis showed that the bone marrow MRD status after purging was an independent prognostic factor with significant impact on progression-free survival (PFS) [14].

At the beginning of the year 2000, several studies showed the efficacy of rituximab-containing therapy, both in consolidation and induction regimes, for obtaining higher MR rates than traditional chemotherapy schemes [15, 16]. Despite these results, FL remained particularly sensitive to high-dose

chemotherapy (HDC) compared with other lymphoproliferative diseases [17]. In the prospective multicenter clinical trial of the Italian Group of Bone Marrow Transplantation (GITMO), HDC was given followed by an autologous stem cell transplant (ASCT) as induction therapy for 92 patients with newly diagnosed FL. The therapy was effective and 90% of patients achieved complete remission (CR) and 65% showed MR [18]. An update of these data, after 4 years follow-up, showed the long-term impact of HDC in terms of survival: 80% of patients were alive at 5.5 years and about 50% remained in CR after HDC [19].

Subsequently, the role of HDC was studied in a randomized prospective phase 3 trial by the GITMO and Italian Lymphoma Intergroup (IIL). This study was the first to prospectively compare R-CHOP chemo-immunotherapy with HDC in a large cohort of patients with a new diagnosis of high-risk FL. In this population, high-dose therapy proved to be more effective in terms of the CR rate (85% vs. 62%) and MR rate (80% vs 44%), and was associated with better control of long-term disease in terms of PFS and event-free survival (EFS) at 4 years. In addition, statistical analyses showed that the positive or negative MRD status after treatment was the main predictive factor for clinical outcome in terms of PFS, regardless of the treatment received. The best results, in terms of MR, were observed in the patients receiving high-dose therapy who had an increased median PFS, while the 4 year overall survival (OS) was the same for both cohorts. However, the higher efficacy of high-dose therapy was associated with longer bone marrow toxicity. In fact, in the population analyzed, the onset of secondary neoplasms (myelodysplastic syndrome and acute myeloid leukemia) was greater in patients receiving high-dose therapy, with a 4-year cumulative incidence of 6.6% vs. 1.7% for patients who received R-CHOP. Moreover, patients who relapsed early post R-CHOP were successfully treated with second-line therapy with HDC and obtained response rates very similar to patients who received HDC as a first-line therapy, without a significant increase in toxicity. Although a number of early recurrences occurred in the R-CHOP arm, about one third of the patients had a positive long-term course when treated with low-toxicity therapy such as R-CHOP, suggesting that HDC may represent an excessive first-line therapy [20]. These findings supported the role of HDC as a highly effective therapy, applicable for patients with relapsed/refractory FL; while, MR emerged as the best independent parameter for long-term control of the disease.

To increase the MR rate and improve long-term control of disease in the study by Goff et al., MRD was used to evaluate the impact of therapy that combined radio-immunotherapy with 90Y-ibritumomab, an anti-CD20 monoclonal antibody conjugated to radioisotope Ittrium90, in patients with a new diagnosis of FL. In the study, MRD was detected less in the combined treatment arm than in the standard observational arm. In addition, the median PFS was 3 years for the 90Y-ibritumomab arm and 13 months for the observational arm. Radioimmunoassay consolidation therapy seemed to play an important role in achieving MR and, therefore, in long-term control of the disease [21].

In the last few years, several European groups have integrated MRD studies in phase 3 multicenter randomized clinical trials and samples were analyzed using molecular techniques. These samples were from different laboratories belonging to the Euro-MRD group, which previously used international standardization of MRD study techniques. In 2013, data were published on the use

of rituximab as maintenance therapy in elderly FL patients enrolled in the Italian Lymphoma Foundation (FIL) ML17638 study. Briefly, in this trial, FL patients received an induction chemo-immunotherapy and then a consolidation therapy with four infusions of weekly rituximab. Then, they were randomized to observation or maintenance with additional cycles of rituximab (one every 2 months for four total administrations). In this population, after induction therapy, the bone marrow (BM) of 80% of patients was negative for MRD, demonstrating the efficacy of an "intensive" program with rituximab. After randomization, the maintenance arm obtained better results in terms of MR, associating the persistence of negative MRD in the various contiguous time points with a progressive improvement in clinical outcome, in terms of PFS [22•].

Until 2015, few reports described MRD detection in peripheral blood (PB). In the clinical Protocol NHL1-2003, a randomized and prospective phase 3 clinical trial, the Bcl2/IGH translocation was researched and quantified using PB. It emerged that patients who had BM invasion, splenomegaly, or an advanced stage had higher marker levels than the others, suggesting that the marker level in PB could be used as an index for tumor burden. However, the remarkable variability in the marker level among patients suggested diverse behavior and spread of the disease. In addition, the study showed the prognostic impact on outcome of Bcl2/IGH levels; in particular, high levels of translocation were associated with low PFS, when compared with patients with intermediate or low levels of Bcl2/IGH (high vs. intermediate: HR 4.28; IC 95%, 1.7–10.77; P. 002; High vs. Low HR 3.02, IC 95%, 1.55–5.86, p. 001). Moreover, multivariate analysis showed that the marker level at diagnosis better stratified patients at medium and high risk according to FLIPI. Concerning the primary objective of the study, there was no significant difference in efficacy between the two chemotherapy regimens (R-CHOP and R-bendamustine), and post-therapy MRD values were predictive in both induction regimes. The lack of a MR was associated with a worse median PFS (8.7 months vs. not reached) and marker reappearance was observed 9.5 months (range 0.6–20.3) before clinical recurrence [23].

The prognostic value of MRD in the diagnosis and post-treatment was subsequently detailed in the study by Galimberti et al. in the context of the FOLL05. In this FIL prospective phase 3 trial, three induction chemotherapy regimens, R-CHOP, R-FC, and R-CVP, were compared. The first scheme was the best in terms of efficacy and toxicity. The molecular marker level was associated with the quality of response and the risk of long-term recurrence. In fact, patients with low tumor burden achieved CR and good outcomes with a 3-year PFS of 80% vs. 59% for patients with a high tumor burden ($P = .015$). In addition, multivariate analysis confirmed that MRD was the most powerful and independent predictor of outcome. In fact, PCR-negative patients with partial remission (PR) had a better outcome, in terms of PFS, than patients who reached CR, but remained PCR positive (3-year PFS was 32% for CR/PCR-positive patients vs. 62% for PR/PCR negative patients [24].

Bendamustine is another drug investigated as induction therapy for FL. In the study published by Rummel et al., the induction schedule including bendamustine seemed to give a higher advantage in terms of PFS in patients with indolent lymphomas [25]. Recently, an Italian retrospective study on a small cohort of patients also described the high efficacy of induction therapy

with R-bendamustine, obtaining a MR rate of 93% that was associated with at least a 4-logarithm reduction in MRD levels. Nevertheless, in contrast to previously mentioned studies, the levels of the molecular target Bcl2/IGH evaluated at diagnosis did not show a prognostic impact, suggesting the high efficacy of bendamustine in MRD clearance [26]. The role of bendamustine was investigated by two other prospective phase 3 trials: GADOLIN (NCT01059630) and GALLIUM (NCT01332968), in which the efficacy of bendamustine was tested in association with obinutuzumab (GA101), a new monoclonal antibody [27, 28•].

In the GALLIUM study, MRD was assessed during the induction program (MI) and at the end of induction (EOI). In 2016, the first data analysis described a superiority of the scheme containing obinutuzumab compared to the scheme containing rituximab, in terms of MR rates and reduction of the Bcl2/IGH levels at both monitored time-points. In the rituximab arm, treatment with bendamustine and rituximab was characterized by a higher MR rate (96% after R-bendamustine, 93% after R-CHOP, 79% after R-CVP). The same advantage was not observed in the obinutuzumab arm, suggesting that the antibody activity cancels out the difference in efficacy between the different chemotherapy regimens.

In conclusion, MRD monitoring has been used widely to evaluate the depth of response to treatments and to study different therapeutic strategies. Its prognostic impact has been validated in large multicenter prospective phase 3 trials, and it emerged as the most important and independent predictor of outcome.

Prognostic value of MRD analysis: negative studies

There are also works that negatively describe the predictive role of the MRD in the context of the FL [29, 30]. Generally in these studies, the failure of using MRD in prognosis prediction is due to the small number of patients included and the heterogeneity of the tissues analyzed. In the work of Van Oers [31], the benefits of rituximab in association with high-dose chemotherapy and as maintenance were evaluated in patients with FL who were enrolled in the 20981 clinical trial of the European Organisation for Research and Treatment of Cancer (EORCT). In this work, the authors confirmed the predictive value of the tumor burden for the diagnosis and that the predictive value of MRD was statistically significant only in the maintenance phase. Contrary to the previous mentioned analyses, no clinical impact was observed during the chemo-immunotherapy. This negative result can be easily explained by some specific characteristics of the project and some technical aspects of the analysis. First, all patients defined as PCR-negative after treatment were included in the assay, independent of their Bcl2/IGH (positive or negative) status at diagnosis. Moreover, the patient series studied was poorly characterized in terms of the tissue analyzed (for example, the number of BM samples evaluated after treatment is not reported) and the analysis of MRD monitoring on PB is less reliable than for BM, especially after intense exposure to rituximab. The time of collection of the tissues is not detailed, suggesting that the lack of prognostic prediction of the MRD could be due to a sampling very close to rituximab

administration. Finally, the manuscript does not provide a technical definition of MR and does not discuss the results obtained from a methodological point of view. However, despite these limitations, this paper indicates that MRD analysis requires a very robust interpretation setting to provide clinically useful results.

Limits and new technical opportunities for MRD detection

PCR represents the gold standard technique for MRD monitoring in FL patients. PCR techniques are able to identify well-described breakpoints involved in the FL translocation. In fact, the juxtaposition between chromosome 18 and chromosome 14 combines four Bcl2 genomic loci to JH regions, identifying four different (in terms of frequency) rearranged regions. The first described breakpoint, identified in at least 50% of FL patients, was the Major Breakpoint Region (MBR); this was followed by the Minor Cluster Region (mcr) and other two rearrangements defined as "minor Bcl2", located at the 3′ end of the MBR (3′ MBR) and 5′ end of the mcr locus (5′ mcr), had a frequency of < 10% [32].

A qualitative approach was developed to detect MBR and mcr. The MBR-mcr nested PCR assays, using two sets of primers annealing to chromosome 18 and 14 in a two-step PCR, are able to detect one clonal cell harboring the translocations out of 500,000 analyzed cells (i.e., 1×10^{-5}), reaching high sensitivity levels [33]. Although Bcl2/IGH nested PCR was applied in several studies assessing the role of MRD in patient stratification, a quantitative PCR approach (q-PCR) was recently developed to better monitor the disease kinetics. Technically, the qPCR approach uses two primers complementary to the rearranged loci and a specific Bcl2 probe and it features the same high sensitivity levels of nested PCR [34].

Qualitative and quantitative PCR approaches and Sanger sequencing determined that the Bcl2/IGH rearrangements unrelated to the original tumor clone can lead to false positivity during the follow-up of FL patients [14]. Later, the finding that non-lymphoma-associated Bcl2/IGH rearrangements are frequently amplified in the blood of lymphoma-free subjects highlighted a long-term clonal population that rarely changes its phenotype in malignant indolent lymphoma [35–37]. In the last 10 years, standardization of the Bcl2/IGH qPCR has been one of the main goals of the EuroMRD International group, developed within the European Scientific Foundation for Laboratory Hemato Oncology (ESLHO) Consortium. The group, including almost 20 international laboratories, organizes periodic quality control checks to obtain high levels of standardization in the interpretation of qPCR data. This has led to international methodological harmonization of MRD monitoring and the introduction of qPCR in several clinical trials.

Nevertheless, qPCR has some pitfalls often due to the standard curve required for the target quantification. This is a laborious process that requires a sufficient amount of material to carry out the different measurements. To perform the reaction, it is necessary to have specific patient reagents that increase the cost of the procedures, and in some cases the reaction does not lead to the identification of any marker. Often this happens because of the low tumor infiltration, which makes it impossible

to construct the standard curve. This can also be due to the high mutation rate of the IGH making primer pairing difficult or the quantification of the disease is not possible due to the limits of sensitivity of the assay (not exceeding 1×10^{-5}). The MRD assay with ASO RQ-PCR is still difficult for some tumors, such those that undergo clonal evolution, necessitating the identification of a new specific clone primer. Some cases of FL may remain localized in the tissue and region of origin and not spread to the PB or BM, making it impossible to identify the disease in these samples. Due to these technical limitations, it is not possible to identify a molecular marker in 35–40% of patients, and these patients are not eligible for MRD detection.

The newly introduced droplet digital PCR (ddPCR) assay, currently developed to only detect the MBR rearrangement, could overcome these disadvantages. As recently shown [38], this technology uses the same qPCR primer and probe set but the DNA molecules are partitioned into oil droplets and amplified; therefore, ddPCR does not need a standard curve for Bcl2/IGH quantification. This could solve the problems of low tumor burden or qPCR positive, but not quantifiable, samples that are amplified in the range between the qPCR quantitative and sensitivity thresholds. As far as we know, ddPCR sensitivity levels are perfectly comparable to qPCR (1×10^{-5}) [38]. The approach was described as a more accurate and promising method to quantify the disease at diagnosis and to monitor MRD during the clinical follow-up, especially in those FL patients with localized disease and low BM infiltration [39, 40].

Nevertheless, qPCR and ddPCR did not increase the number of patients eligible for MRD studies because both are strictly dependent on qualitative nested PCR as the Bcl2/IGH marker screening tool. Recently, the introduction of next generation sequencing (NGS) has expanded the techniques available for monitoring FL. The clonal IGH rearrangements, which had no optimal marker (by classical PCR) in FL patients due to the high rate of hyper-somatic mutations in VH regions, are a new target for NGS. In fact, preliminary data demonstrated the standardized IGH-NGS approach of the EuroClonality-NGS Consortium robustly identified IGH clonotypes in FL patients and may become a complement to current MRD methods, allowing reliable MRD assessment in the majority of FL patients [41, 42•]. The new Targeted Locus Amplification (TLA) technology was used for FL patients who were positive for Bcl2/IGH by FISH, but negative by classic PCR marker screening. The TLA identified new breakpoints, mapping different genomic positions from those previously mentioned in 40% of analyzed patients, highlighting new biological features of t(14;18) [43]. Although NGS data are relevant and promising, these high-throughput approaches are strictly dependent on gold standard PCR methods to validate their results.

MRD in LPL and MZL

Unlike FL, there are few reports about the clinical impact of MRD in LPL and MZL. In 2012, whole genome sequencing (WGS) identified a recurrent somatic mutation in LPL cells: MYD88 L265P [44]. The mutation was confirmed by Sanger sequencing in 49 of 54 patients with Waldenstrom macroglobulinemia (WM) and in three patients with non-IgM LPL. The intracellular protein MYD88

mediates the intracellular signal from the cell surface through the Toll Like Receptor (TLR) and the Interleukin 1 Receptor (IL1-R), activating the IRAK signaling pathway converging on the nuclear factor NF-KB. Several other studies demonstrated the MYD88 mutation was present, using Sanger sequencing and PCR, in more than 90% of patients with WM and non-IgM LPL [45–47]. The MYD88 L265P mutation seemed to be a good molecular marker because it is not present in multiple myeloma cells, including those secreting IgM; thus, it defines a distinct biologic entity. Some studies reported a low incidence of MYD88 L265P in MZL and other lymphoproliferative disorders such as non GC (germinal center) diffuse large B cell lymphoma and chronic lymphocytic leukemia (CLL) [48, 49].

In addition to its potential diagnostic role in WM and potential prognostic role in monoclonal gammopathy of unknown significance [50, 51], the MYD88 L265P mutation could be used as an MRD marker. Although MR is less common in this pathology, the MYD88 mutation was reduced after effective therapy with carfilzomib, rituximab, and dexamethasone (CaRD) [52]. Based on these preliminary data, MYD88 L265P could be used as a marker of disease to monitor the response to therapeutic programs and to assess the long-term impact of treatment on outcome. Initially, MYD88 detection was performed by Allele Specific PCR (AS-PCR), based on a relative ratio between the amplification of mutated and wild type alleles, but due to its low sensitivity, reported as the detection of one mutation in 1000 analyzed cells, this approach is not optimal for MRD monitoring in WM [45]. Recently, Drandi et al. developed a new technique based on ddPCR to detect MYD88 L265P, especially in WM. They assessed the feasibility of the approach and the limit of detection and sensitivity, which were increased about 1.5-logaritihms compared with that of AS-PCR. In addition, this is the first study that detected MYD88 L265P in three different tissues: BM, peripheral blood (PB), and plasma through free circulating DNA (cfDNA), to verify the correlation between different tissues and identify the most patient-friendly and suitable method for MRD detection. Data showed that, as expected, BM is the most sensitive tissue. Moreover, there was good correlation between BM and cfDNA, highlighting the important role of this newly investigated tissue, which has a less invasive sampling procedure than a BM biopsy. Finally, the PB analysis seemed less reliable with increasing false negatives and reduced sensitivity, especially in patients who had previously received treatments based on rituximab [40].

In MZL, the detection of the clonal rearrangement of the immunoglobulin heavy chain (IGHV) was an important tool to understand the cell of origin, probably due to clonal B progenitor selection after super-antigen recognition in the spleen. In this disorder, WGS identified some recurrent mutations in the genes encoding NOTCH2 and KLF2, which are involved in cell differentiation and splenic homing [53]. The MRD monitoring for this type of lymphoma is still preliminary due to the low incidence of the disease and because it often does not require treatment; thus, the enlistment in clinical trials is poor. The FIL-IELG36 protocol (EudraCT Number 2011-000880-28) is a phase 2 prospective clinical trial that assessed the efficacy of rituximab and bendamustine as a frontline therapy for symptomatic MZL patients. The MRD study detected IGHV rearrangements at diagnosis and during follow-up. This clinical trial is not MRD-driven, therefore MRD monitoring was for observational purposes only and did not affect the clinical decisions.

Conclusions and future perspectives

In recent years, molecular biology has played an important role in the biological characterization of hematologic diseases, as well as in the evaluation of the therapeutic response. In fact, in some diseases such as WM, the identification of single somatic mutations by DNA sequencing contributed to the differential diagnosis and even found targets for new drugs. In MZL patients, studying the IGHV rearrangement improved the understanding of the probable origin of the lymphoma cell. By evaluating the treatment response by MRD it was possible to identify the most effective therapeutic strategy in different patient settings, and the standard for the first line treatment for FL. The impact of MRD on outcome has been extensively verified and validated by large prospective clinical trials evaluating different treatment regimens for FL. From these studies, the MRD status emerged as the main independent factor for outcome prediction in the post-induction phase, strengthening its impact during follow-up. Its potential role as a surrogate of tumor burden during the diagnosis was also evaluated.

Previous studies extensively reported on using rituximab along with standard chemotherapies to achieve high clinical response rates and MR in the majority of patients, and obtained a median PFS of about 6 years and a 5-year survival of 90% [54•]. Despite these successes, Casulo et al. identified with POD24 that about 20% of the patients have an unfavorable course regardless of the risk category assessed at diagnosis [3]. Although this high-risk group may need different and probably more intensive therapeutic approaches, other studies showed that the same induction chemo-immunotherapy was able to control the disease for a long time in about 30% of patients, with rituximab maintenance having a non-significant advantage on overall survival. Thus, some patients may be eligible for a less intensive treatment regimen while benefiting from reduced toxicities compared with prolonged pharmacological treatments. In view of these different responses to therapy, it may be necessary to design individual treatment strategies and use MRD monitoring and PET scanning as fundamental tools for patient stratification.

Two current FIL "MRD driven" clinical trials, the FOLL12 (EudraCT number 2012-003170-60) and the MIRO (EudraCT number 2012-001676-11) are ongoing and require MRD monitoring at different time points to make therapeutic decisions. The FOLL12 evaluates, in low-risk patients, the impact of de-intensification therapy (observation) vs. maintenance therapy with rituximab and, in high-risk patients, the benefit of intensification with radio-immuno consolidation therapy. It also assesses the impact of preemptive therapy to induce MR and its impact on long-term clinical follow-up. The possibility to more accurately identify patients according to the risk of relapse can help define personalized therapeutic programs, optimizing benefits and reducing toxicity for a better overall management of the patient.

Moreover, the development of increasingly reliable and sensitive techniques, such as ddPCR and NGS, has made it possible to exceed the limits imposed by standard PCR. Other tissues, such as PB and urine, have less invasive sampling techniques than the traditional BM biopsy and are being investigated to see if they can be used for MRD detection. Promising studies on WM are already moving in this direction. All these new developments have made it possible to

imagine that MRD detection will become part of the routine management of patients with lymphoma, leading to the more and more personalized medicine, based on the biological characteristics of each patient's disease.

Compliance with Ethical Standards

Conflict of Interest

Daniele Grimaldi declares that he has no conflict of interest.
Elisa Genuardi declares that she has no conflict of interest.
Martina Ferrante declares that she has no conflict of interest.
Simone Ferrero has received compensation from Janssen for service as a consultant; compensation from Janssen and Pfizer for participation on advisory boards; and compensation from Janssen, Pfizer, and Gilead for service on speakers' bureaus.
Marco Ladetto declares, in the past 5 years, the following relationships in terms of consultancy, participation on advisory boards, invitations to scientific meetings, institutional research support, and contracts: AbbVie, Acerta, Amgen, Archigen, Celgene, ADC Therapeutics, Gilead, Novartis, Johnson & Johnson, Roche, Roche Diagnostics, Sandoz, and Takeda.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- Of importance

1. Solal-Celigny P. Follicular lymphoma international prognostic index. Blood. 2004;104:1258–65.
2. Massimo F, Bellei M, Marcheselli L, Luminari S, Lopez-Guillermo A, Vitolo U, 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.
3. Casulo C, Byrtek M, Dawson KL, Zhou X, Farber CM, Flowers CR, 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;33:2516–22.
4. Nagham NO, Abolela MS, Abdelgawad MI, Ibrahim A, Mourad AF, Rezk K. Diagnostic Utility of 18F-FDG PECT/CT in Assessment of Post-therapy Remission or Relapse of Lymphoma. Cancer Res Treat. 2016;4(5):88–95.
5. Trotman J, Luminari S, Boussetta S, Versari A, Dupuis J, Tychyj C, et al. Prognostic value of PET-CT after first-line therapy in patients with follicular lymphoma: a pooled analysis of central scan review in three multicentre studies. Lancet Haematol. 2014;1:e17–27.
6. Dupuis J, Berriolo-Riedinger A, Julian A, Brice P, Tychyj-Pinel C, Tilly H. et al. Impact of [18F] 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.
7. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger A, Szczepanowski M, 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;16:1111–22.
8. Huet S, Szafer- Glusman E, Xerri L, Bolen C, Punnoose C, Tonon L, et al. Evaluation of clinicogenetic risk models for outcome of follicular lymphoma patients in the PRIMA trial. Hematol Oncol. 2017;35:96–7.
9. Huet S, Tesson B, Jais JP, Feldman AL, Magnano L, Thomas E, et al. A gene-expression profiling score for

prediction of outcome in patients with follicular lymphoma: a retrospective training and validation analysis in three international cohorts. Lancet Oncol. 2018;19:549–61.

10. Voena C, Ladetto M, Astolfi M, Provan D, Gribben JD, Boccadoro M, et al. A novel nested-PCR strategy for the detection of rearranged immunoglobulin heavy-chain genes in B cell tumors. Leukemia. 1997;11:1793–8.
11. van der Velden VHJ, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, van Dongen JJM. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. Leukemia. 2003;17:1013–34.
12. Gribben JG, Freedman AS, Neuberg D, Roy DC, Blake KW, Woo SD, et al. Immunologic purging of marrow assessed by PCR before autologous bone marrow transplantation for B-cell lymphoma. N Engl J Med. 1991;325:1525–33.
13. Gribben JG, Freedman AS, Woo SD, Blake K, Shu RS, 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;78:3275–80.
14. Brown JR, Yang F, Gribben JG, Nueberg D, Fisher DC, Mauch P, et al. Long-term survival after autologous bone marrow transplantation for follicular lymphoma in first remission. Biol Blood Marrow Transplant. 2007;13:1057–65.
15. Rambaldi A. Monitoring of minimal residual disease after CHOP and rituximab in previously untreated patients with follicular lymphoma. Blood. 2002;99:856–62.
16. Hirt C, Schüler F, Kiefer T, Schwenke C, Haas A, Niederwieser D, 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;141:631–40.
17. Corradini P, Ladetto M, Zallio F, Astolfi M, Rizzo E, Sametti S, et al. Long-term follow-up of indolent lymphoma patients treated with high-dose sequential chemotherapy and autografting: evidence that durable molecular and clinical remission frequently can be attained only in follicular subtypes. J Clin Oncol. 2004;22:1460–8.
18. Ladetto M. 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;100:1559–65.
19. Ladetto M, Vallet S, Benedetti F, Vitolo U, Martelli M, Callea V, et al. Prolonged survival and low incidence of late toxic sequelae in advanced follicular lymphoma treated with a TBI-free autografting program: updated results of the multicenter consecutive GITMO trial. Leukemia. 2006;20:1840–7.
20. Ladetto M, De Marco F, Benedetti F, Vitolo U, Patti C, Rambaldi A, 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;111:4004–13.
21. Goff L, Summers K, Iqbal S, Kuhlmann J, Kunz M, Louton T, 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;27:6094–100.
22.• Ladetto M, Lobetti-Bodoni C, Mantoan B, Ceccarelli M, Boccomini C, Genuardi E, et al. Persistence of minimal residual disease in bone marrow predicts outcome in follicular lymphomas treated with a rituximab-intensive program. Blood. 2013;122:3759–6.

MRD was the most powerful and independent predictor of outcome in a phase III prospective trial.. Accumulation of MRD negative values increased the predictive value.

23. Zohren F, Bruns I, Pechtel S, Schroeder T, Fenk R, Czibere A, et al. Prognostic value of circulating Bcl-2/IgH levels in patients with follicular lymphoma receiving first-line immunochemotherapy. Blood. 2015;126:1407–14.
24. Galimberti S, Luminari S, Ciabatti E, Grassi S, Guerrini F, Dondi A, 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;20:6398–405.
25. Rummel MJ, Niederle N, Maschmeyer G, Banat GA, von Grünhagen U, Losem C, et al. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. Lancet. 2013;381:1203–10.
26. Galimberti S, Ciabatti E, Ercolano G, Grassi S, Guerrini F, Cecconi N, et al. The combination of rituximab and bendamustine as first-line treatment is highly effective in the eradicating minimal residual disease in follicular lymphoma: an Italian retrospective study. Front Pharmacol. 2017;8.
27. Pott C, Belada D, Danesi N, Fingerle-Rowson G, Gribben J, Harbron C, 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.
28.• Pott C, Hoster E, Kehden B, Unterhalt M, Herold M, van der Jagt RH, et al. Minimal residual disease in patients with follicular lymphoma treated with obinutuzumab or rituximab as first-line induction immunochemotherapy and maintenance in the phase 3 GALLIUM study. Blood. 2016;128:613

Preliminary data about a prospective phase III trial suggest that G-based regiment induces rapid and more effective tumor cell

clearance than R-containing therapy as first line induction immunochemotherapy in FL.

29. Mandigers C, Meijerink J, Mensink E, Tönnissen E, Hebeda K, Bogman M, et al. Lack of correlation between numbers of circulating t(14;18)- positive cells and response to first-line treatment in follicular lymphoma. Blood. 2001;98:940–4.
30. Schmitt C, Grundt A, Buchholtz C, Scheuer L, Benner A, Hensel M, et al. One single dose of rituximab added to a standard regimen of CHOP in primary treatment of follicular lymphoma appears to result in a high clearance rate from circulating bcl-2/ IgH positive cells: is the end of molecular monitoring near? Leuk Res. 2006;30:1563–8.
31. van Oers M, Tönnissen E, Van Glabbeke M, Giurgea L, Jansen J, Klasa R, 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;28:2246–52.
32. Pott C, Brüggemann M, Ritgen M, van der Velden VH, van Dongen JJ, Kneba M. MRD detection in B-cell non-Hodgkin lymphomas using Ig gene rearrangements and chromosomal translocations as targets for real-time quantitative PCR. Methods Mol Biol. 2013;971:175–200.
33. Gribben JG, Neuberg D, Freedman AS, Gimmi CD, Pesek KW, et al. Detection by polymerase chain reaction of residual cells with the Bcl-2 translocation is associated with increased risk of relapse after autologous bone marrow transplant for B-cell lymphoma, n.d., 1993 by The American Society of Hematology edition.
34. Ladetto M, Sametti S, Donovan JW, Ferrero D, Astolfi M, Mitterer M, 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;29:183–93.
35. Ladetto M, Mantoan B, De Marco F, Drandi D, Aguzzi C, Astolfi M, et al. Cells carrying nonlymphoma-associated bcl-2/IgH rearrangements (NLABR) are phenotypically related to follicular lymphoma and can establish as long-term persisting clonal populations. Exp Hematol. 34(12):1680–6.
36. Huet S, Sujobert P, Salles G. From genetics to the clinic: a translational perspective on follicular lymphoma. Nat Rev Cancer. 2018;18:224–39.
37. Roulland S, Kelly RS, Morgado E, Sungalee S, Solal-Celigny P, Colombat P, et al. t(14;18) Translocation: a predictive blood biomarker for follicular lymphoma. J Clin Oncol. 2014;32(13):1347–55.
38. Drandi D, Kubiczkova-Besse L, Ferrero S, Dani N, Passera R, Mantoan B, et al. Minimal residual disease detection by droplet digital PCR in multiple myeloma, mantle cell lymphoma, and follicular lymphoma. J Mol Diagn. 2015;17:652–60.
39. Cavalli M, De Novi LA, Della Starza I, Cappelli LV, Nunes V, Pulsoni A, et al. Comparative analysis between RQ-PCR and digital droplet PCR of BCL2/IGH gene rearrangement in the peripheral blood and bone marrow of early stage follicular lymphoma. Br J Haematol. 2017;177(4):588–96.
40. Drandi D, Genuardi E, Dogliotti I, Ferrante M, Jiménez C, Guerrini F, et al. Highly sensitive MYD88 L265P mutation detection by droplet digital polymerase chain reaction in Waldenström Macroglobulinemia. Haematologica. 2018;103:1029–37.
41. Pott C, Knecht H, Herzog A, Genuardi E, Unterhalt M, Mantoan B, et al. Standardized IGH-based next-generation sequencing for MRD detection in follicular lymphoma. ASH annual meeting abstracts. Blood. 2017;130:1491.
42.• Ladetto M, Bruggemann M, Ferrero S, Pepin F, Drandi D, Monitillo L, et al. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. Leukemia 2014. 2012a;28(6):1299–307 A straightforward comparison between PCR-based and NGS based methodologies.
43. Genuardi E, Klous P, Drandi D, Mantoan B, Monitillo L, Daniela B, et al. Targeted locus amplification (TLA): a novel next generation sequencing (NGS) technology to detect new molecular markers and monitoring minimal residual disease (MRD) in mantle cell and follicular lymphoma. Blood. 2017;130:2742.
44. Steven P, Treon XL, Yang G, Zhou Y, Cao Y, Sheehy P, et al. MYD88 L265P somatic mutation in Waldenström's Macroglobulinemia. N Engl J Med. 2012;367(9):826–33.
45. Xu L, Hunter ZR, Yang G, Zhou Y, Cao Y, Liu X, et al. MYD88 L265P in Waldenstrom Macroglobulinemia, immunoglobulin M monoclonal gammopathy, and other B-cell lymphoproliferative disorders using conventional and quantitative allele-specific polymerase chain reaction. Blood. 2013;121(11):2051–8.
46. Ansell SM, Hodge LS, Secreto FJ, Manske M, Braggio E, Price-Troska T, et al. Activation of TAK1 by MYD88 L265P drives malignant B-cell growth in non-Hodgkin lymphoma. Blood Cancer J. 2014;4:e183.
47. Varettoni M, Arcaini L, Zibellini S, Boveri E, Rattotti S, Riboni R, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's Macroglobulinemia and related lymphoid neoplasms. Blood. 2013;121:2522–8.
48. Jiménez C, Sebastián E, Chillón MC, Giraldo P, Mariano Hernández J, Escalante F, et al. MYD88 L265P is a marker highly characteristic of, but not restricted to, Waldenström's Macroglobulinemia. Leukemia. 2013;27:1722–8.
49. Ngo VN, Young RM, Schmitz R, Jhavar S, Xiao W, Lim K, et al. Oncogenically active MYD88 mutations in human lymphoma. Nature. 2011;470:115–9.
50. Landgren O, Staudt L. MYD88 L265P somatic mutation in IgM MGUS. N Engl J Med. 2012;367:2255–6 author reply 2256-2257.

51. Varettoni M, Zibellini S, Defrancesco I, Ferretti VV, Rizzo E, Malcovati L, et al. Pattern of somatic mutations in patients with Waldenström Macroglobulinemia or IgM monoclonal gammopathy of undetermined significance. Haematologica. 2017;102:2077–85.
52. Treon SP, Tripsas CK, Meid K, Kanan S, Sheehy P, Chuma S, et al. Carfilzomib, rituximab, and dexamethasone (CaRD) treatment offers a neuropathy-sparing approach for treating Waldenstrom's macroglobulinemia. Blood. 2014;124:503–10.
53. Arcaini L, Rossi D, Paulli M. Splenic marginal zone lymphoma: from genetics to management. Blood. 2016;127:2072–81.
54.• Luminari S, Ferrari A, Manni M, Dondi A, Chiarenza A, Merli F, 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 With the aim to maximize treatment activity and increase the chance of durable disease control, R-CHOP should be the preferred option among the other options R-CVP and R-FM.