

PERSONALIZED MEDICINE IN LYMPHOMA: TAILORING TREATMENT ACCORDING TO MINIMAL RESIDUAL DISEASE

Authors:

Irene Dogliotti and Simone Ferrero

Division of Hematology, Department of Molecular Biotechnologies and Health Sciences,
University of Torino and AOU “Città della Salute e della Scienza di Torino”, Torino, Italy

Corresponding Author

Simone Ferrero, MD

Division of Hematology

Department of Molecular Biotechnologies and Health Sciences - University of Torino

AOU “Città della Salute e della Scienza di Torino”

Via Genova 3, 10126 Torino, Italy

T: +390116334220-6884

F: +390116963737

E-mail: simone.ferrero@unito.it

Research Support

SF was supported by: Progetto di Rilevante Interesse Nazionale (PRIN2009) from Ministero Italiano dell'Università e della Ricerca (MIUR), Roma, Italy [7.07.02.60 AE01]; Progetto di Ricerca Sanitaria Finalizzata 2009 [RF-2009-1469205] and 2010 [RF-2010-2307262 to S.C.], A.O. S. Maurizio, Bolzano/Bozen, Italy; Fondi di Ricerca Locale, Università degli Studi di Torino, Italy; Fondazione Neoplasie Del Sangue (Fo.Ne.Sa), Torino, Italy; Fondazione Cassa di Risparmio di Torino (CRT, project codes: 2015.1044 and 2016.0677), Torino, Italy; Associazione DaRosa, Torino, Italy.

Abstract

Mature B-cell disorders have recently become highly curable diseases thanks to the introduction of new treatment strategies; nonetheless, despite evidence of complete remission, many patients affected by lymphoma or myeloma eventually relapse and need salvage therapy. Minimal residual disease (MRD) detection and quantification is a powerful tool to evaluate treatment efficacy, to stratify patients, and to predict long-term outcome. In fact, in the current landscape of novel, highly efficacious but toxic and often costly targeted therapies, early identification of factors predictive of treatment response or refractoriness is the key to avoid overtreatment of patients and thus also to reduce costs for health care system.

In this article, we reviewed the prognostic role of MRD in follicular lymphoma, mantle cell lymphoma and multiple myeloma. We analyzed published clinical studies and available methodologies, and we described the major ongoing studies of MRD-driven tailored treatment in these diseases. Finally, we discussed novel applications and techniques for MRD identification in hematologic malignancies and future directions of MRD-oriented research. In particular, we hypothesized some possible, next-generation, precision medicine trials in lymphoproliferative diseases based on the most promising currently available biomarkers.

Keywords: lymphoma, MRD, PCR, clinical trial, personalized medicine

Acknowledgements

The Authors would like to thank the Fondazione Italiana Linfomi (FIL), Marco Ladetto, Massimo Federico, Alessandro Pulsoni, Sergio Cortelazzo, Christiane Pott, Christian Andersen, Steven Le Gouill and Brad Kahl for sharing the information on MRD-driven clinical trials; Luigia Monitillo and Daniela Drandi for their scientific support; the Authors are grateful to Giorgio Schirripa for language assistance.

1. Background and rationale: is personalized therapy needed in lymphoproliferative diseases?

Lymphoproliferative diseases (LPDs) account for around 5% of all human malignant neoplastic diseases; in 2014, 70890 new cases of non-Hodgkin lymphomas (NHL) were reported in the US, which constituted 4% of all new cancers in both male and female patients (1). In 2015, the Fondazione Italiana Linfomi (FIL) registered 3002 new cases of lymphoma in Italy, and 15011 cases from 2010 to 2015 (<http://www.filinf.it/registro-fil>). The incidence rises steadily with age, especially after age 40, but lymphomas are also among the most common malignancies in patients between the ages of 20 and 40 years. Moreover, the incidence of NHL nearly doubled between 1970 and 1995, and it has slowly continued to rise since then by 1.5% - 2% each year. Despite the great improvements in the past decades, the outcome of LPDs is still quite poor, leading to a mortality of 4% per year in 2010; NHL ranks as the ninth most common cause of cancer-related death in men and the eighth in women in the US (2, 3). Despite evidence of transient complete remission, many patients with lymphoma eventually relapse and need a salvage therapy; nonetheless, several steps forward have been made in the last years. We have recently gained a better insight into the complex biological and pathogenetical characterization of these neoplasms, which has also led to an improvement in the diagnostic accuracy. For instance, new entities of lymphomas with potentially different prognosis and treatment response have been identified (4); moreover, traditional imaging assays and laboratory analyses (such as multiparameter flow cytometry, MFC) have been considerably improved, and we are now starting to determine the biological mechanisms of resistance to conventional therapies. In

parallel, powerful pre-treatment prognostic tools, mainly based on clinical scores, have been defined. Some examples are the International Scoring System (ISS) in multiple myeloma (MM), Follicular Lymphoma International Prognostic Index (FLIPI) and FLIPI2 in follicular lymphoma (FL) and Mantle Cell Lymphoma International Prognostic Index (MIPI) in mantle cell lymphoma (MCL) (5-8). These scores are able to divide patients into different risk categories, enabling clinicians to better foresee the prognosis of each patient. Moreover, biological and genetic features, analyzed through highly sensitive and innovative laboratory techniques, are being integrated into new prognostic indexes and appear to impact on patients' final outcome (R-ISS in MM (9), m7-FLIPI in FL (10) and MIPI-c in MCL (11)).

Other advancements in research have been made in the evaluation of individual response to treatments based on the morphological and metabolic results of imaging studies - such as computed tomography (CT) scans and fluorodeoxyglucose-positron emission tomography (FDG-PET), performed both after the first few courses of chemotherapy ("interim-PET") and at the end of induction therapy (12-14); but also based on the identification of minimal residual disease (MRD) through highly sensitive methods. MRD can be defined as the smallest number of malignant cells beyond the sensitivity level of routine laboratory and imaging techniques that potentially remain during or after appropriate therapy, even when the patient has no clinical sign of disease. MRD detection and quantification are used to evaluate treatment efficacy and to identify patients at risk of relapse. Therefore, MRD can be a valid tool to stratify patients and to predict long-term outcome (15).

Thanks to the growing information on molecular cell biology in LPDs, new targeted treatments have been developed in the last few years. First, immunotherapy was introduced in clinical practice, particularly rituximab and its derivatives, chimeric anti-CD20 monoclonal antibodies (MoAb). These agents showed to have a great impact on disease outcome in all kinds of B-NHL and in chronic lymphocytic leukemia (CLL), as compared with standard chemotherapy alone (16, 17); oral immunomodulatory agents, such as lenalidomide, were first approved for MM but were later found to be highly effective as single-agent therapy also in relapsed/refractory aggressive and indolent B-cell NHL, including MCL, diffuse large B-cell lymphoma (DLBCL), and FL, because they have direct and indirect effects on malignant cells (18).

The approval of oral, non-chemotherapeutic agents represents the latest innovation in molecular targeted treatment; for instance, ibrutinib is a Bruton's tyrosine kinase (BTK) inhibitor active on the B-cell receptor (BCR) signalling pathway, a critical mechanism in the survival of these malignancies. This agent has shown promising activity in certain subtypes of DLBCL, in CLL, in relapsed or refractory MCL and in Waldenström's Macroglobulinemia (WM), for which it has recently received FDA approval (19-23). Idelalisib, an oral inhibitor of PI3K δ , employs a similar strategy to target the BCR signalling pathway and thus the growth and survival of malignant B-cells. It has been shown to be highly active in relapsed and extensively pre-treated indolent B-NHL, particularly in a subset of FL patients (24). Combined with rituximab, idelalisib significantly improved progression-free survival (PFS) and overall survival (OS) of relapsed CLL patients with significant coexisting medical conditions (25). Finally, venetoclax, a powerful inhibitor of the anti-

apoptotic protein Bcl-2, is able to restore the natural mechanism of apoptosis in CLL and lymphoma cells and to overcome chemotherapy resistance (26).

The landscape of LPD treatment has therefore changed dramatically and several different strategies are currently available. In the future, in selected patients, LPDs may likely become chronic diseases requiring continuous oral, well-tolerated, non-chemotherapy agents as maintenance or consolidation treatment, while upfront high-dose conventional chemotherapy may remain an option for younger patients with highly aggressive diseases. Of note, safety concerns can arise also with targeted therapies: toxicities can still occur, especially with continuous therapy and in patients receiving multiple drugs for pre-existing comorbidities. The costs of these new agents is also a matter of debate: due to the limited resources of many health care systems, selection of patients who will benefit more from high-cost medications will be critical from both ethical and economical points of view. Therefore, early prediction of treatment response or refractoriness is the key to avoid overtreatment of patients and unnecessary toxicities, while reducing the costs for health care systems. A more personalized approach including newest compounds and aiming at achieving long-term benefits is therefore needed in lymphoma.

Among all the available biomarkers potentially impacting on clinical outcome, post-treatment MRD analysis is one of the few predictive tools validated in large prospective trials (27-29); therefore, MRD is currently the most mature biomarker that can be used to tailor treatment, and it is already under evaluation in precision medicine trials in LPDs (30).

In this article, we reviewed the prognostic role of MRD in mature LPDs. We

analyzed the methodologies and results from published clinical trials, and major ongoing clinical trials of MRD-driven tailored treatment in LPDs. Finally, we addressed novel applications and techniques for MRD identification in hematologic malignancies, and we discussed future directions of MRD-oriented research. In particular, we hypothesized some possible, next-generation, precision medicine trials in LPDs based on the most promising currently available biomarkers.

2. Current molecular methods for minimal residual disease (MRD) detection

Real-time quantitative polymerase chain reaction (RQ-PCR) is currently the most employed technique for MRD detection in LPDs (with the exception of MFC in MM) (31) and it has been fully standardized in the multinational context of the EuroMRD group (32). PCR assays are based on the amplification of a tumor-specific molecular marker; primers and probes are designed from a chosen DNA sequence, which in mature B-cell disorders essentially belongs to two categories, i.e. chromosomal translocations and antigen-receptor rearrangements (15). Tumor translocation markers, being the hallmark of lymphoma, remain stable during the natural history of the disease and are therefore a reliable MRD target; however, often only a portion of patients displays a detectable translocation marker. BCL2/IGH rearrangement relative to t(14;18) is currently detectable by primers targeting the Major Breakpoint Region (MBR) and minor breakpoint region (mcr) in no more than 50-55% of FL (28, 33), and a number of "minor" breakpoints might account for another small percentage of cases (34, 35). Similarly, in MCL only 30-40% of patients can be currently monitored through BCL1/IGH rearrangement ("Major

Translocation Cluster" breakpoint, MTC) relative to t(11;14) due to the widely disperse breakpoint region on BCL1 locus (36-38).

On the other hand, antigen-receptor rearrangements of the immunoglobulin heavy chain (IGH) gene are a widely applicable clonality and MRD marker in B-cell disorders. The allele-specific oligonucleotide PCR (ASO-PCR) used in this kind of assay is in fact based on the generation of the specific DNA sequence of the V(D)J junctional region, encoding the variable domains of immunoglobulin molecules, and this is characteristic of each B-lymphocyte. This occurs during the physiological recombination processes, first in pre-B lymphocytes, and later during the germinal center somatic hypermutation events. The random insertion and deletion of nucleotides during this process determine the "fingerprint-like" sequences of the junctional regions of IGH genes, which differ in each lymphocyte and, as a consequence, in each lymphoid malignancy (35). Therefore, junctional regions of malignant lymphoma cells can be used as tumor-specific targets for PCR-based MRD analysis, particularly in MCL and MM. Indeed, in MCL and MM, neoplastic cells derive from mature B-cells that do not undergo a continuous somatic hypermutation process, in contrast to what happens in FL. However, the rate of successful IGH sequencing is not uniform across different histologies - ranging from 65-85% in MCL (27, 37, 38) to 50% in MM (39-41) - and, although IGH-based ASO-PCR has a very good sensitivity, it requires the development of patient-specific reagents. Therefore, it is a complex, time consuming and expensive assay (15).

Despite the described current limitations, the standardized RQ-PCR is the most employed tool for MRD monitoring, at least in MCL and FL. It relies on

translocation-derived markers and on IGH rearrangements, which have slightly different performances and can be done both on bone marrow (BM) and on peripheral blood (PB) samples (15, 32). Nevertheless, many groups continue to perform both RQ-PCR and nested, qualitative, PCR for MRD monitoring (29, 30, 37). This latter approach is simpler, faster and slightly more sensitive; however, it is less standardized, more prone

to contaminations and still highly dependent on ASO-primers (15, 28, 38). Finally, new technologies for MRD monitoring have been introduced in the last few years, and they are likely to overcome some limits of the ASO-qPCR approach.

Figure 1 describes the main nested and RQ-PCR approaches for MRD in mature LPDs.

Standard MRD molecular techniques

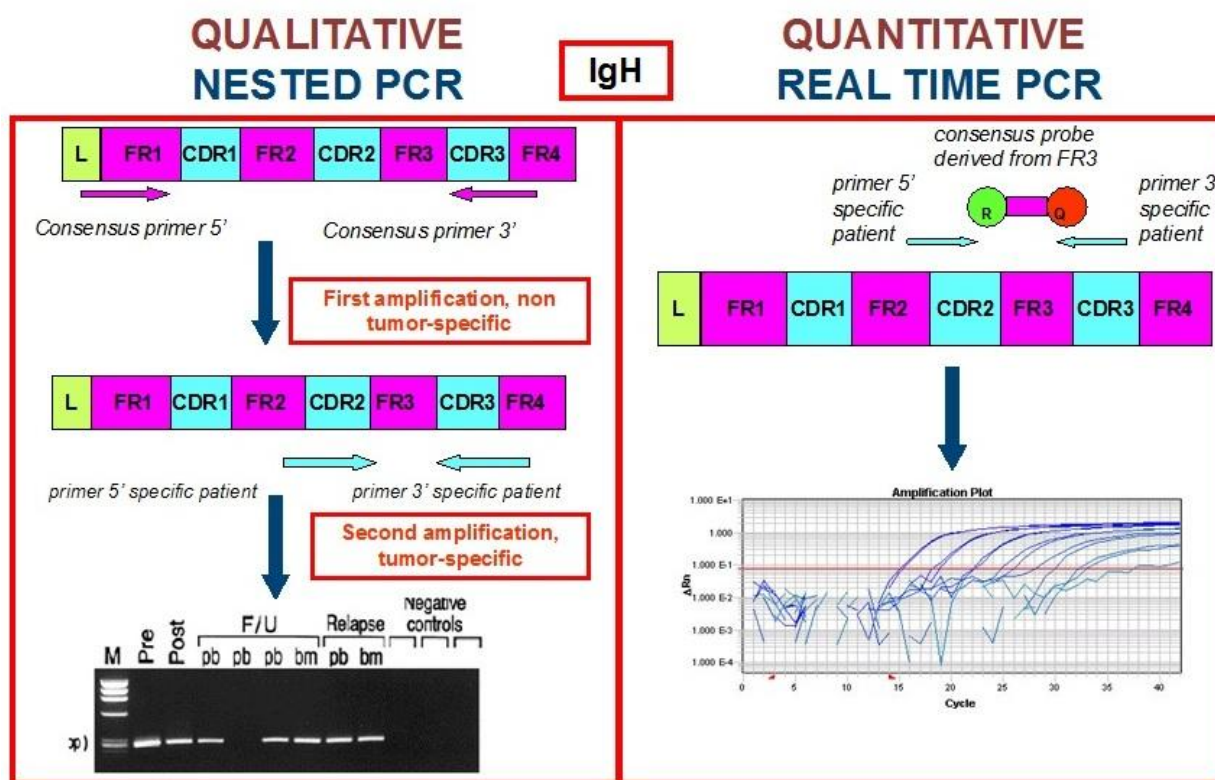


Figure 1. Standard molecular MRD techniques. Comparison between nested PCR and quantitative real-time PCR applied on IgH rearrangements. Abbreviations. PCR, polymerase chain reaction; IgH, immunoglobulin heavy chain.

3. Clinical impact and predictive role of minimal residual disease (MRD) in mature B-cell lymphoproliferative disorders

3.1. Follicular lymphoma

In the mid-1980s, FL was the first lymphoma to be studied for MRD due to the

availability of the MBR and mcr BCL2/IGH rearrangements for nested-PCR analysis in more than half of patients. The seminal study by Gribben et al. was the first that demonstrated that anti B-cell monoclonal antibodies could induce immunologic ex vivo purging of BM by residual lymphoma cells before autologous stem cell

transplantation (ASCT) (33). Moreover, the absence of detectable MRD in BM after purging was strongly associated with an advantage in long-term disease-free survival (DFS) after ASCT (33). An update of this study with 12 years of follow-up further confirmed that MRD positivity was an independent adverse predictor of progression-free survival (PFS), Hazard Ratio (HR) 4.18, 95% CI 1.99-8.8 ($p = 0.0002$) (42).

Since then, many studies have been published on the role of MRD in FL and various prospective clinical trials have been designed including MRD analysis as secondary endpoint. The first studies reported on the ability of novel treatment strategies (such as ASCT and new MoAbs) to induce MRD clearance in comparison to standard chemotherapy. Standard chemotherapy alone was not able to eradicate disease from the PB or BM in the majority of patients (33, 43), whereas rituximab as single agent or rituximab-chemotherapy led to a clearance of BCL2/IGH-positive cells in most patients (44, 45). Rambaldi et. al evaluated the BCL2/IGH status in BM and PB of 86 FL patients treated with either CHOP or a sequential R-CHOP scheme: with a median follow-up of 56 months, the freedom from recurrence (FFR) of MRD negative patients was 64% as compared to 32% for MRD positive patients ($P < 0.006$) (43, 46). In another study including patients treated with mitoxantrone, chlorambucil and prednisone (MCP) \pm rituximab, the addition of rituximab led to a rapid eradication of circulating lymphoma cells, with improved clinical response and event-free survival (EFS) for patients with a reduction ≥ 2 log (44). Finally, radio-immunotherapy consolidation of complete or partial response with $(90)\text{Y}$ -ibritumomab tiuxetan also induced high rates of conversion to MRD

negativity and subsequently prolonged PFS (38.4 v 8.2 months, $P < 0.01$) (47).

Many studies showed that MRD evaluation before and after ASCT may predict disease outcome in FL patients. In the pre-rituximab era, among indolent lymphoma patients treated with an intensified high-dose chemotherapy program followed by ASCT, the achievement of post-ASCT molecular remission (MR) was common in patients with FL subtype (70%); the incidence of relapse was markedly lower in patients with durable MRD negativity compared with patients who had never attained MR (8% vs 88%, $P < 0.005$) (48). Of note, the predictive value of the achievement of MR was repeatedly confirmed, independently of treatment. A randomized multicenter study of 136 patients compared 6 courses of R-CHOP with rituximab-supplemented, high-dose, sequential chemotherapy with autografting (R-HDS) as first-line therapy in high-risk FL patients. MR was achieved in 44% of R-CHOP and 80% of R-HDS patients ($P < 0.001$) and was predictor of PFS: the outcome of patients achieving MR was similar regardless of treatment (29). Similar results were reported in the prospective, phase III FOLL05 trial (NCT00774826), where untreated FL patients were randomized to receive either R-CVP, R-CHOP or R-FM conventional chemo-immunotherapy. PFS correlated significantly with PCR status, with a 3-year PFS of 66% for MRD negative cases versus 41% at 12 months for MRD positive cases, respectively ($P=0.015$), and 84% versus 50% at 24 months ($P=0.014$). The prognostic value of MRD at these time points was confirmed also in multivariate analysis, regardless of treatment (49). Even among elderly patients receiving a short chemo-immunotherapy course plus rituximab consolidation, both the prognostic role of MRD and the clearing activity of rituximab were confirmed.

Ladetto et. al investigated the role of rituximab maintenance after first-line chemo-immunotherapy with rituximab, fludarabine, mitoxantrone, dexamethasone in elderly patients with advanced FL. BCL2/IGH MRD was extensively determined on BM cells in a centralized Euro-MRD laboratory and conversion to PCR negativity predicted PFS at all post-treatment time points (3-year PFS, 72% vs 39%; $P < .007$) (28).

More recently, the first prospective MRD data on the widely used Bendamustine-rituximab (BR) regimen have been published by the StiL group. Zohren et. al prospectively studied PB BCL2/IGH levels in 173 first-line FL patients enrolled in the multicenter phase III clinical trial NHL1-2003 comparing BR with R-CHOP (50). Although only 92 patients had a follow-up sample analyzed (53% of the entire series, 48 in the BR arm and 44 in the R-CHOP arm), conversion to post treatment MRD negativity was observed in 78 of 92 patients (85%). Irrespective of the study arm, MRD positivity was associated with shorter PFS (HR, 3.15; $P=0.002$) (51). Similar impressive data on the potential of BR-like regimens in MRD clearance have been recently presented both in prospective and retrospective series (52, 53). Finally, the high activity of bendamustine in MRD clearance has been recently reported in the large, prospective, patients series of the GADOLIN (NCT01059630) and GALLIUM (NCT01332968) phase III trials, along with the first clues of a deeper MRD clearing potential of the new anti-CD20 MoAb GA-101 in comparison to rituximab (54, 55).

Based on the dismal prognostic role of MRD persistence or reappearance and on the demonstrated potential MRD-reverting activity of rituximab in FL and MCL (30, 56-58), MRD-driven pre-emptive strategies have been designed to be prospectively

tested in large, first-line clinical trials for FL patients. These trials will be extensively described in the next chapter of the review.

3.2. Mantle cell lymphoma

In the pre-rituximab era, molecular remissions could not be obtained in MCL patients treated with chemotherapy alone; this was consistent with the extremely poor prognosis of these patients, especially when compared to the better scenario observed in FL (48, 59). The prognosis of MCL patients changed substantially thanks to the introduction of sequential high dose chemotherapy in to clinical practice, in particular with cytarabine and rituximab – which for the first time proved to be able to induce a MR (60, 61). A subsequent RQ-PCR study on a prospective series was performed in 29 MCL patients treated with high dose chemotherapy + total body irradiation (TBI), followed by ASCT. MR after ASCT was a strong predictor of improved outcome, with a median PFS of 92 months in the MRD negative group versus 21 months in the MRD positive group ($P < .001$). These data were also confirmed in multivariate analysis (62).

In the larger phase II Nordic MCL2 trial, 160 young untreated patients received a dose-intensified induction therapy with R-maxi-CHOP alternating with R-high-dose cytarabine, followed by ASCT conditioned with BEAM or BEAC scheme (carmustine, etoposide, cytarabine, and melphalan/cyclophosphamide), with in vivo purging of peripheral blood stem cells (PBSC). This approach produced long-term PFS and a significantly higher proportion of MR and PCR-negative stem cell products as compared with the MCL-1 trial (high dose CHOP followed by ASCT) (37).

High rates of MR have been observed in all the subsequent clinical trials containing

rituximab and cytarabine, and an advantage in time to treatment failure (TTF) was also observed in the randomized, phase III European MCL Network (EuMCLNet) “Younger” trial (63-65). In particular, prospective quantitative MRD monitoring was a secondary endpoint of the two EuMCLNet phase III trials, namely the “Younger” and the “Elderly”. The pooled MRD data showed that after rituximab-based induction treatment, 106 of 190 evaluable patients (56%) achieved MR on PB or BM samples; MR resulted in a significantly improved response duration (RD), and emerged to be an independent prognostic factor for RD (hazard ratio 0.4, P .028), independently of clinical response, both before ASCT (in MCL Younger) and during maintenance (in MCL Elderly) (27). MRD thus proved to be a powerful predictor of outcome in large prospective studies, both in young and in elderly patients, and in the context of ASCT, both pre-transplant and during post-transplant follow-up. MRD persistence or reappearance, on the other hand, was associated to a shorter median time to relapse (18 months) (66).

On the basis of the prognostic relevance of MR, pre-emptive strategies were therefore tested in small retrospective trials (56, 57) and in one phase II study to obtain MR re-induction, and indirectly to improve PFS (30, 67); in a study by Ladetto et al., patients experiencing a molecular relapse (M-rel) after R-HDS induction therapy, defined as PCR positivity in 2 consecutive samples in the absence of clinical relapse, were treated with 4 courses of rituximab plus 2 additional infusions if PCR remained positive, and monitored by nested qualitative PCR and RQ-PCR. After 4-6 courses of rituximab, all 4 patients achieved again a MR, and no clinical relapses were recorded at 3, 6, 18, and 62 months from rituximab re-treatment (56). This study was later extended to another 18

patients (9 MCL and 9 FL); 23 cases of M-rel or MRD persistence were reported and were treated with rituximab, which re-induced MR in 17/23 cases. PFS after pre-emptive treatment was 64% at a median follow-up of 6 years (57). A prospective analysis of the efficacy of pre-emptive treatment was conducted in the Nordic MCL-2 trial, in which a significant (5-fold) increase in the RQ-PCR detectable MRD level in two consecutive BM or PB samples defined M-rel. In case of M-rel with increasing MRD levels, patients were offered pre-emptive treatment with rituximab 375 mg/m² weekly for 4 weeks. Twenty-six patients underwent pre-emptive treatment, and MR was again achieved in 92%. Median molecular and clinical relapse-free survival after pre-emptive treatment were 1.5 and 3.7 years, respectively (30, 67).

Both PB and BM are adequate tissues for MRD detection in MCL, but PB showed to have a faster disease clearance. Therefore, M-rel in PB is highly predictive of upcoming clinical relapse (11, 66).

Finally, recent trials in MCL employ MRD to describe the depth of response to novel treatment schemes and agents, such as lenalidomide, bendamustine and bortezomib (68-71).

3.3. Multiple myeloma

The role of MRD monitoring in MM is more recent than in lymphoma; until the introduction of “new drugs”, the achievement of CR was not common in MM patients and the MRD-negativity status was often confined only to the small group of patients undergoing allogeneic transplantation (40, 72, 73). Moreover, because of the elevated somatic hypermutation rate of the IGHV gene in MM, it was difficult to get a reliable molecular marker for MRD studies in a large

subset of patients (41, 74, 75). Therefore, multicolor flow cytometry (MFC) on BM samples was the most widely used method to detect MRD in MM, and despite a slightly inferior sensitivity, it was more widely applicable than PCR-based technologies (31, 74, 76).

After the introduction of the novel, non-chemotherapeutic agents (namely thalidomide, bortezomib and lenalidomide) in the 2000s, CR rates have dramatically increased, even among transplant-ineligible patients (77). Consequently, MRD studies acquired a new importance, both to describe depth of response achievable with the new drugs and to predict outcome in these patients. Ladetto et al. firstly described in a clinical trial the occurrence of durable MRs in a subset of MM patients achieving at least very good partial remission after ASCT and receiving a bortezomib, thalidomide, and dexamethasone (VTD) consolidation (39). MR rates in BM by nested PCR increased from 3% after ASCT to 18% after VTD, and consolidation was able to induce a reduction in tumor burden of more than 4 logs by RQ-PCR; notably, patients with a MRD value lower than the median had significantly improved outcomes (PFS 100% vs 57%; $P < .001$). Mature results of this study after 93 months of median follow-up reported a superior OS for patients with MR (72% vs 48% at 8 years, $P = 0.041$) and showed that MRD relapse predicts clinical relapse, with a median lag between MRD reappearance and salvage treatment of 9 months (78). Similar results were reported by the Spanish group on 170 patients enrolled in three consecutive clinical trials. The first wide comparison between PCR and MFC was performed in this study, with a good correlation between the two techniques ($r=0.881$, $P<0.001$). Among patients in CR ($n=62$), PCR was able to identify two risk groups with different PFS (49 vs 26 months, $P=0.001$) and OS (not reached vs 60 months, $P=0.008$) (74).

Finally, MRD has recently been used to evaluate the effect of maintenance therapies, such as lenalidomide (41).

Differently from FL and MCL, much less data are available about MRD monitoring on PB samples in MM, as only small numbers of clonal plasma cells can be detected in PB by sensitive approaches. In the largest retrospective study, MRD by RQ-PCR was evaluated in 42 MM patients undergoing high-dose therapy followed by ASCT as first-line therapy. The MRD level of PB samples was in median 40-fold lower than in paired BM samples. Nonetheless, patients with MRD negativity at early time points after ASCT (3 months) had a prolonged median EFS and OS (52 vs 17 months; $p=0.03$). Importantly, sequential monitoring of MRD levels in PB was able to predict disease progression in 19 of 29 patients (66%) (79).

Recently, thanks to the development of next-generation sequencing (NGS) techniques, the feasibility of molecular MRD monitoring has increased and it can be applied to the majority of MM patients (80, 81). Indeed, many ongoing clinical trials are currently investigating the anti-MM activity of many new targeted compounds (such as carfilzomib and daratumumab) by measuring the MRD shrinkage induced by these drugs (e.g. in the “FORTE study”, NCT02203643, combining carfilzomib with lenalidomide or cyclophosphamide in newly diagnosed MM patients eligible for ASCT). In a small phase II trial in newly diagnosed MM patients treated with carfilzomib-lenalidomide-dexamethasone, 12-month PFS for MRD-negative vs MRD-positive status by MFC and NGS was 100% vs 79% ($p<0.001$) and 100% vs 95% ($P = 0.02$), respectively (82). Finally, to determine the ability of the new anti-CD38 MoAb daratumumab to further clear the neoplastic clone beyond CR, MRD was assessed on BM by ClonoSEQ™ NGS-

based assay in two large phase III studies, CASTOR (daratumumab-bortezomib-dexamethasone versus bortezomib-dexamethasone) and POLLUX (daratumumab-lenalidomide-dexamethasone versus lenalidomide-dexamethasone): daratumumab in combination with standard of care significantly improved MRD-negative rates at all sensitivity thresholds, leading

to a lower risk of progression in MRD-negative patients, even in high-risk subjects (83).

Table 1 reports the most important published clinical trials assessing the prognostic role of MRD by classical molecular methods in mature B-cell lymphoproliferative disorders.

Study	Disease	Marker, method	Evaluable patients	Sample analyzed	Study Treatment	MRD impact on outcome
Gribben, NEJM 1991 [34]	FL	BCL2, N-PCR	114	Harvest	TBI+ASCT+ex vivo purging	Relapse incidence at 24 m FU: 39% vs 5%; p <.00001
Ghielmini, Blood 2004 [46]	FL	BCL2, N-PCR, qPCR	70	PB, BM	RTX x 4 ± RTX q2m x4	CR/PR patients: 84% PCR neg vs SD/PD 11% PCR neg (P <.0001)
Rambaldi, Blood 2005 [47]	FL	BCL2, N-PCR	86	PB, BM	R-CHOP	FFR 64 vs 32% , p <.006
Corradini, JCO 2004 [49]	FL, SLL, MCL	BCL2, IGH, N-PCR	35	PB, BM, harvest	R-HDS + ASCT	Relapse incidence 88% vs 8% at 75 m FU (p <.005)
Ladetto, Blood 2008 [30]	FL	BCL2, N-PCR	60	BM	R-HDS + ASCT vs R-CHOP	PFS 78% vs 25% at 4y FU (p <.001)
Hirt, BJH 2008 [45]	FL	BCL2, IGH, qPCR	43	PB	R-MCP vs MCP	PFS NR vs 27 m (p <.02)
Ladetto, Blood 2013 [29]	FL	BCL2, IgH, N-PCR, qPCR	51	BM	R-FND + R consolidation or maintenance	PFS 72% vs 39% at 3y; P <.007
Galimberti, CCR 2014 [50]	FL	BCL2, IgH, N-PCR, qPCR	415	PB, BM	R-CHOP vs R-FM vs R-CVP	PFS at 24 months 84% vs 50% (P=.014)
Zohren, Blood 2015 [52]	FL	BCL2, qPCR	173	PB	R-CHOP vs B-R	PFS NR vs 8.7 months (p = .002)
Pott, Blood 2006 [63]	MCL	IgH, qPCR	29	BM, PB, harvest	R-HDS +TBI + ASCT	PFS 92 vs 21 m (p <.001)
Geisler, Blood 2008 [38]	MCL	BCL1, IgH, N-PCR	79	PB, BM	R-maxiCHOP/R-HD-AraC + ASCT + in vivo purging	PFS NR vs 18 m (p <.001)
Andersen, JCO 2009 [31]	MCL	BCL1, IgH, N-PCR, qPCR	78	PB, BM	R-maxiCHOP/R-HD-AraC + ASCT ± R preemptive	RFS 3.7y after pre-emptive R
Pott, Blood 2010 [28]	MCL	BCL1, IgH, qPCR	190	BM, PB, harvest	R-CHOP +TBI + ASCT vs R-CHOP/R-DHAP + R-HD-AraC +TBI + ASCT (younger); R-FC vs R-CHOP (elderly)	PFS 77% vs 34% at 2y FU (p=.021)
Ladetto, JCO 2010 [40]	MM	IgH, N-PCR, qPCR	37	BM	ASCT + VTD consolidation	PFS 100% vs 57% (P <.001)
Ferrero, Leuk 2015 [79] (update of Ladetto, 2010)	MM	IgH, N-PCR, qPCR	37	BM	ASCT + VTD consolidation	OS 72% vs 48% at 8 years (P = 0.041); RD NR vs 38 m (p=.001)
Khortals, BBMT 2013 [80]	MM	IgH, qPCR	42	PB	HD-CHT + ASCT	OS 52m vs 17 m (p=.03)
Puig, Leukemia 2014 [75]	MM	IgH, ASO-qPCR	71	BM	Bortezomib-containing regimens ± ASCT	PFS NR vs 31 months (P=.002)
Ladetto, Leuk 2015 [41]	MM	IgH, N-PCR, qPCR	26	BM	ASCT + RIC-allograft	OS NR vs 40 m (p = .027)

Table 1. Clinical studies (> 20 patients) investigating the impact of MRD on patients' outcome. *Abbreviations:* FL, follicular lymphoma; MCL, mantle cell lymphoma; SLL, small lymphocytic lymphoma; MM, multiple myeloma;R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; R-FND, rituximab, fludarabine, mitoxantrone, dexamethasone; TBI, total body irradiation;ASCT, autologous stem cell transplant; B-R, bendamustine, rituximab; R-DHAP, rituximab, cytarabine, dexamethasone, cisplatin; HD-CTX, high dose cyclophosphamide, HD-Ara-C, high dose cytarabine; RIC, reduced intensity conditioning; HD-CHT, high dose chemotherapy; VTD, bortezomib, thalidomide, dexamethasone; MCP, mitoxantrone, chlorambucil, prednisolone; NR, not reached; FU, follow up.

4. Ongoing MRD-tailored clinical trials in mature LPD

4.1. Phase III, randomized, FOLL12 clinical trial

FOLL12 (EudraCT number: 2012-003170-60) is a multicenter, phase III, randomized study sponsored by FIL; its aim

is to evaluate whether, after conventional chemo-immunotherapy, a FDG-PET and MRD response-based maintenance therapy with rituximab is not less effective in terms of PFS than a standard maintenance in patients with untreated, advanced FL; primary objective is to evaluate efficacy of a response-adapted strategy in defining

maintenance therapy (“personalized medicine”).

This trial started recruitment in 2013 and, as of January, 2017, it randomized 595 of the 810 planned patients. Patients with naïve, previously untreated FL, stage II-IV, Follicular Lymphoma International Prognostic Index 2 (FLIPI2) >0 requiring a therapeutic intervention (according to the GELF criteria) (84, 85) are randomly assigned in a 1:1 ratio to either standard or experimental arm. At baseline, patients are assessed for molecular BCL2/IGH status (both major and minor rearrangements) on PB and BM (33, 35) and staged by CT scan and FDG-PET scan. (86) Both arms receive induction therapy with either 6 cycles of R-CHOP followed by 2 additional doses of rituximab or 6 cycles of BR 90 mg/m² plus 2 rituximab courses (at physician discretion). At the end of chemo-immunotherapy (“end of induction”), all patients are assessed for disease response by common clinical and laboratory examinations, CT scan, FDG-PET (centrally revised) and BCL2/IGH MRD both on PB and BM, both by qualitative nested PCR and by quantitative RQ-PCR, according to Euro-MRD guidelines (only for patients with a molecular marker at diagnosis) (32, 35).

In both arms, patients with stable or progressive disease (PET positive and less than PR on CT scan) can be assigned to salvage treatment, at physician’s discretion. In the standard arm, patients responding to induction therapy receive standard maintenance with rituximab 375 mg/m² every 2 months for 2 years. In the experimental arm, patients with a positive FDG-PET scan (Deauville score 4-5) (87-89), regardless of MRD status, are defined as high-risk group and therefore receive intensified consolidation with radio-immunotherapy: a single dose of ⁹⁰Y ibritumomab tiuxetan 0.4 mCi/kg (preceded

by the two standard doses of rituximab 250 mg/m²) (90), followed by rituximab maintenance (375 mg/m² every 2 months) for the remaining 11 infusions. On the contrary, experimental arm FDG-PET negative (Deauville score 1-3) patients, defined as low-risk, are further divided into two subgroups according to their MRD status, based on nested-PCR results: MRD negative patients do not receive maintenance therapy and are followed up with MRD monitoring every 6 months for two years (both PB and BM), whereas MRD positive patients undergo a cycle of pre-emptive rituximab therapy with four weekly doses of rituximab (375 mg/m²) (57). Pre-emptive treatment should also be repeated in case of persistent MRD positivity after pre-emptive rituximab or subsequent MRD reappearance in MRD negative patients, for a maximum of three courses in total. Patients without a molecular marker who are FDG-PET negative do not receive further treatment and are followed up every 6 months without MRD assessments (see Figure 2).

4.2. Phase II, multicenter, MIRO’ clinical trial

The MIRO’ (Molecularly Oriented Immuno-Radio-therapy) study (EudraCT number: 2012-001676) is a FIL-sponsored phase II prospective multicenter study for stage I/IIA FL. It aims to evaluate whether a pre-emptive therapy with the new anti-CD20 monoclonal antibody ofatumumab is able to reduce or eliminate MRD after conventional treatment with local radiotherapy of the involved site(s), which is the current standard of care in patients with localized FL (91). This trial started the recruitment in 2015 and, as of January 2017, it enrolled 52 of the 110 planned patients.

In this trial, all the enrolled patients, grade 1-3a, stage IA/IIA FL without bulky disease, are assessed for BCL2/IGH status

on PB and BM samples at baseline; they are all treated with involved-field radiotherapy (IFRT) at doses of 24 Gy in 12 fractions; after radiotherapy, patients lacking a molecular marker on baseline BM or PB do not receive further treatment, but are followed every 3 months without further MRD detection. BCL2/IGH positive patients at baseline are re-staged after radiotherapy with physical examination, laboratory analysis and MRD evaluation on PB and BM. MRD-negative patients stop study treatment, while MRD-positive patients receive ofatumumab for 8 weekly doses of 1000 mg total dose: after ofatumumab therapy, patients who are still MRD-positive do not repeat treatment. Patients who become MRD-negative are followed with BCL2/IGH detection every 6 months for 3 years: in case of subsequent MRD conversion from negative to positive, treatment with ofatumumab is permitted maximum twice, with the same schedule (see Figure 2). The primary objective of the study is the MRD negativity after ofatumumab: the effectiveness of anti-CD20 monoclonal antibody treatment will be determined by the proportion of negativization of residual BCL2/IGH positive cells after radiotherapy, evaluated by qualitative and quantitative PCR MRD detection. Secondary endpoints include clinical response rate, overall, partial and complete response rate, PFS and relapse free survival.

4.3. Phase III, randomized, MCL0208 clinical trial

MCL0208 (NCT02354313) is a multicenter, phase III, randomized study, sponsored by FIL; its aim is to compare lenalidomide maintenance vs observation after an intensified induction regimen containing rituximab followed by high-dose chemotherapy and ASCT as first line

treatment in adult patients with advanced MCL.

An induction phase with 3 cycles of R-CHOP21 and high-dose (HD) R-cyclophosphamide was followed by a consolidation phase with 2 cycles of R-HD-Ara-C and a BEAM/FEAM-conditioned ASCT. Patients achieving at least partial response were randomized to maintenance with lenalidomide (15 mg once daily on days 1-21, every 28 days, for two years) or observation. IGH-based or BCL1/IGH MRD was examined both on PB and BM by ASO qualitative nested PCR and by quantitative RQ-PCR at different time points: at diagnosis, after R-HD-cyclophosphamide, on CD34+ cell harvests, before and after ASCT, during maintenance/observation and during follow-up every six months, according to Euro-MRD guidelines (only for patients with a molecular marker at diagnosis) (32, 35).

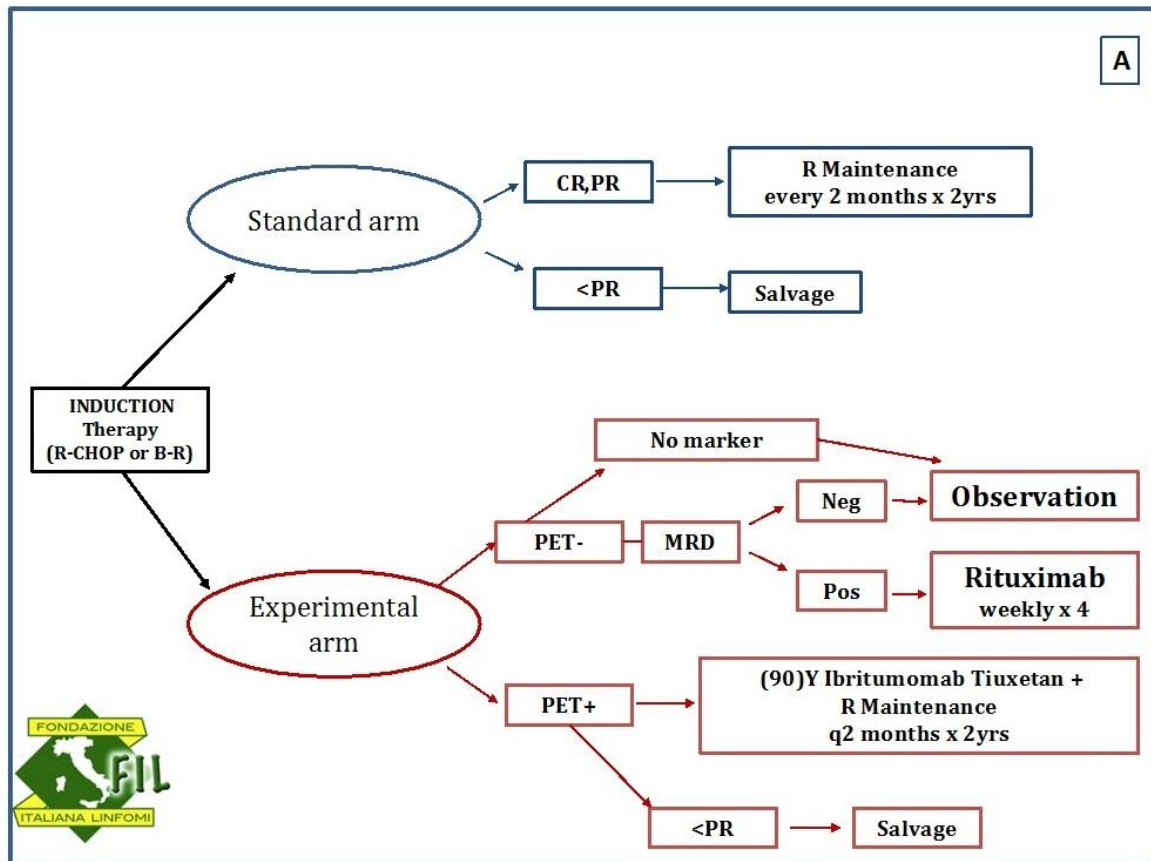
According to the well-known activity of cytarabine in MCL (60, 61), a CD34+ cell harvest is performed after the first course of R-HD-Ara-C and tested for MRD; a second harvest is performed after the second R-HD-Ara-C course, only if the first harvest is still MRD positive (or if the harvest is not adequate or the patient has no molecular marker for MRD detection) (see Figure 2).

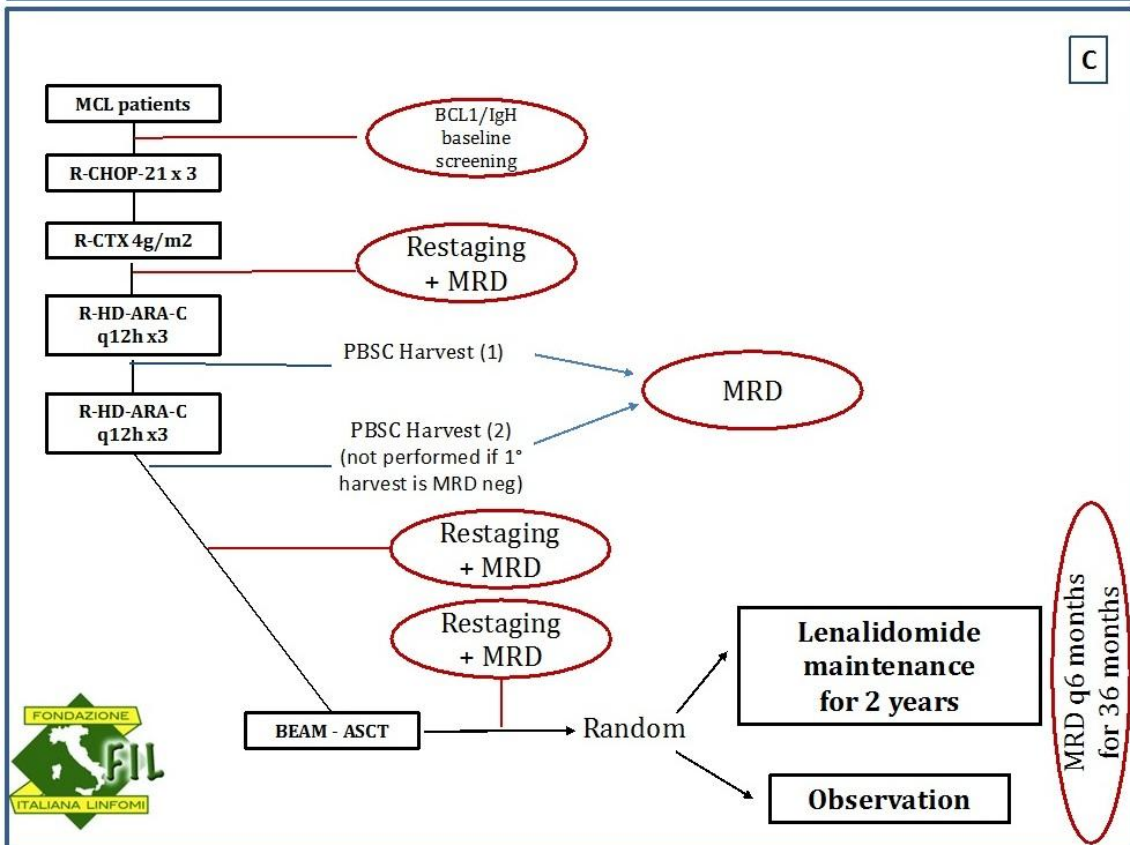
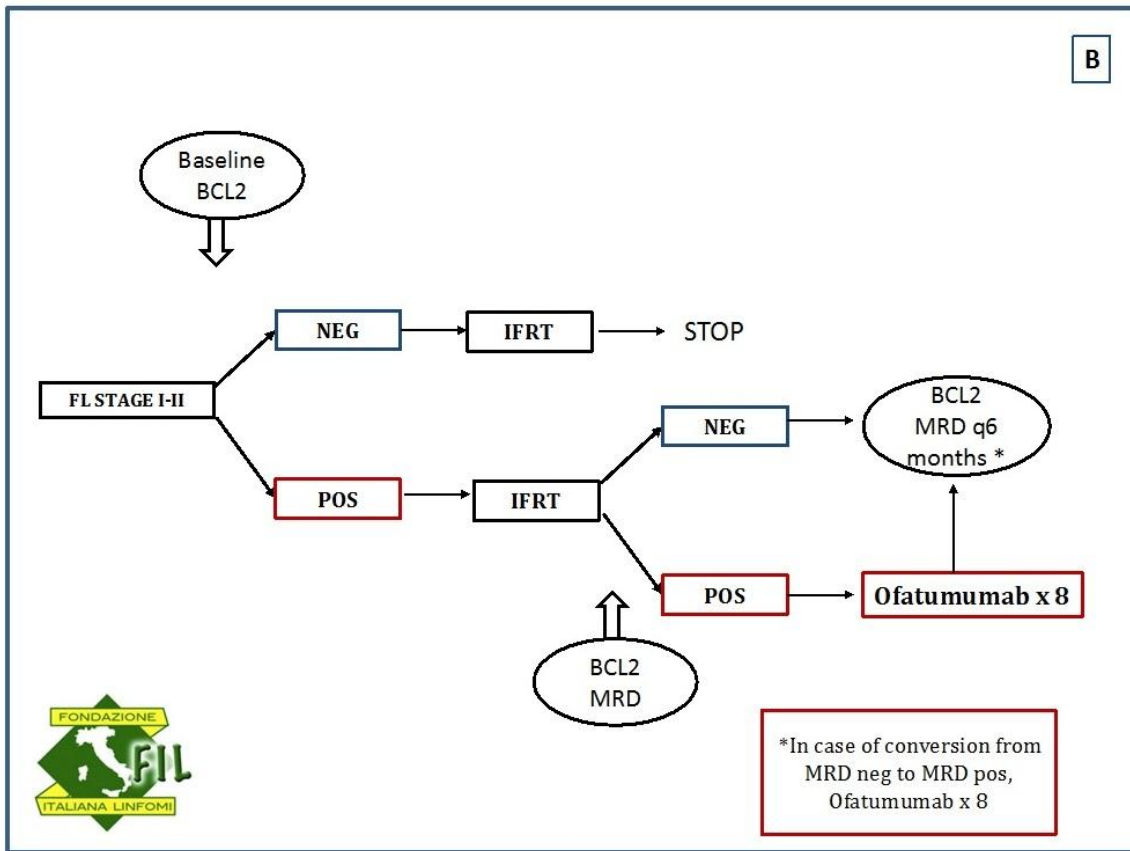
The recruitment of the planned 300 patients was completed in August, 2015 and the second interim analysis of the clinical results is currently ongoing (as of January, 2017). According to the results of the first interim analysis on 260 enrolled patients, a molecular MRD marker was found in 87% of cases: molecular responses after ASCT were 79% and 50% by nested PCR and 86% and 73% by RQ-PCR, on PB and BM, respectively (65). Importantly, MRD determination on the first CD34+ cell harvest allowed to spare a second

leukapheresis in 147 out of 182 patients (81%), further confirming that the use of rituximab in combination with high-dose cytarabine represents a very effective in vivo purging method in MCL patients. However,

the clinical impact of such MRD-driven strategy on patients' outcome will be better clarified only when more mature data from this trial is available.

Figure 2. Panel A: FIL-FOLL12 (EudraCT 2012-003170-60) phase III trial schedule. 810 first line FL patients to be enrolled (595 randomized so far). *Abbreviations*. MRD, minimal residual disease; FL, follicular lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; B-R, bendamustine, rituximab; CR, complete remission; PR, partial remission. **Panel B: FIL-MIRO' (EudraCT 2012-001676) phase II trial schedule.** 110 first line, stage I/II FL patients to be enrolled (52 enrolled so far). *Abbreviations*. IFRT, involved field radiotherapy. **Panel C: FIL-MCL0208 (NCT02354313) phase III trial schedule.** 300 first line MCL patients enrolled. *Abbreviations*. MCL, mantle cell lymphoma; R-CTX, rituximab-high dose cyclophosphamide; HD-Ara-C, high dose cytarabine; PBSC, peripheral blood stem cells.





4.4. Other current and planned MRD-driven clinical trials in mature LPDs.

The ALTERNATIVE study (NCT02689869) is a prospective, single-arm multicenter phase 2 study, sponsored by the German Low Grade-lymphoma Study Group (GLSG) in up to 98 subjects with advanced stage, previously untreated FL and with high tumor burden requiring treatment. The primary objective is to evaluate the efficacy of the chemotherapy-free combination of ibrutinib and obinutuzumab (GA 101) in this subset of patients. Primary endpoint is the rate of PFS at 12 months. This trial started recruiting patients in April 2016 and aims to enroll 98 patients. The hypothesis of this study is that ibrutinib in combination with obinutuzumab will achieve PFS, response rates and rates of MRD negativity comparable to currently used chemotherapy-containing regimens, such as R-CHOP or BR. According to the trial design, patients receive 6 initial cycles of Ibrutinib 560 mg once daily every day until start of maintenance for a total of 24 weeks, in association to obinutuzumab 1000 mg I.V. on days 1, 8, 15 of cycle 1 and on day 1 of cycles 2-6. In patients in clinical remission after the last induction cycle, maintenance consists of additional 24 months of ibrutinib plus GA101: Ibrutinib 560 mg/day, and GA101 1000 mg I.V. every 2 months for a total of 24 months. The total duration of ibrutinib plus obinutuzumab therapy is therefore 30 months.

According to protocol, MRD central molecular monitoring should be regularly performed on PB samples collected before the start of therapy and at 3, 6, 9, 12, 18, 24 and 30 months. Subsequently, MRD analyses are performed every 6 months until clinical progression of the disease or for a maximum of 4 years (until the end of the study). In patients remaining MRD positive

at 30 months without clinical progression, single agent ibrutinib therapy is continued for another 12 months. In MRD-negative patients at 30 months no further treatment is given, while MRD monitoring should be continued.

The ACVDL trial (EudraCT number: 2011-002751-34) is an open-label phase II study sponsored by the Vejle Hospital, in Denmark. The study is conducted in newly diagnosed MM patients and investigates the efficacy and safety of the combination of doxorubicin, cyclophosphamide, bortezomib, dexamethasone and lenalidomide, followed by consolidation therapy with bortezomib for subjects who are not in molecular CR (mCR). (92)

This trial started in November 2011, and it has now completed its recruitment, with 35 enrolled patients. All patients were assigned to receive at least four cycles of ACVDL in the following combination: doxorubicin 50 mg/m² IV day 1, cyclophosphamide 750 mg/m² IV day 1, bortezomib 1.3 mg/m² IV day 2 and 9, dexamethasone 20 mg PO day 2, 3, 9 and 10 and lenalidomide 15 mg PO day 1-14 in a 21-day cycle. Transplant-eligible patients proceeded with standard ASCT after four cycles. Patients not eligible for ASCT received four additional cycles of ACVDL, for a total of eight cycles of ACVDL. Therefore, 18 patients received four induction cycles of ACVDL followed by ASCT and 11 patients received eight induction cycles of ACVDL. In case of MRD positivity detected by ASO-PCR on BM after induction therapy (both ASCT and non-ASCT arms) patients who did not attain the mCR were offered five cycles of consolidation therapy with subcutaneous bortezomib 1.6mg/m² day 1, 8, 15 and 22 in a 35-day cycle. Out of 23 candidates, 19 patients followed this option, and response improved in 4 of them (1 SD → CR and 3

PR → VGPR). In 12 patients treatment response was unchanged, and 3 patients experienced disease progression. There was no difference in PFS among patients who received consolidation therapy and those who did not, and the median PFS was 785 and 581 days, in the two groups respectively [HR 0.96 (95% CI: 0.32;2.91); log-rank $p=0.94$ (Christian Andersen, personal communication).

The LyMa101 trial (NCT02896582) is a multicentric, single arm phase II trial, sponsored by LYSARC (The Lymphoma Academic Research Organization), aiming at evaluating the efficacy of upfront obinutuzumab (GA101) plus a cytarabine-containing regimen, ASCT and a subsequent MRD-driven GA101 maintenance in younger MCL patients. Treatment consists of 4 cycles of GA101-DHAP every 21 days, followed by ASCT conditioned with GA101-BEAM; GA101 maintenance is administered every 2 months for 3 years, then when requested according to MRD persistent positivity or reappearance. The primary objective of this study is the rate of MRD negativity on BM after induction therapy. The LyMa101 trial started in November 2016 with an estimated enrollment of 83 MCL patients.

The EA4151 trial by the ECOG (Eastern Cooperative Oncology Group) intergroup in MCL is a randomized phase III trial comparing ASCT consolidation followed by rituximab maintenance vs. rituximab maintenance alone for patients with MCL in MRD-negative first CR. Tumor tissue from initial diagnostic biopsy will be tested in a central laboratory with the highly sensitive ClonoSEQTM assay (Adaptive Biotechnologies, Seattle, WA, USA) (83); “no marker” patients will receive high-dose chemotherapy and ASCT followed by 2 years of rituximab maintenance; all patients with a molecular marker will receive induction therapy; at post-induction restaging, MRD-positive patients in CR or PR will undergo ASCT + rituximab maintenance, while MRD-negative patients in CR will be stratified using the MIPI-c score and randomized between ASCT plus rituximab maintenance or rituximab maintenance alone. Patient enrollment is expected to begin in April 2017; estimated sample size will be 689 patients enrolled in the US and Canada. (Brad Kahl, personal communication)

A summary of the ongoing MRD-driven trials in mature LPD is described in Table 2.

Study code	Short title	Disease	Study features	Estimated enrollment (n° of patients)	Study Treatment	Primary Outcome
EudraCT 2012-003170-60	FIL_FOLL12	Follicular lymphoma	Phase III, randomized	810	R-CHOP or B-R + either R maintenance or PET/MRD adapted consolidation	PFS
EudraCT 2012-001676	FIL_MIRO'	Follicular lymphoma (stage IA/IIA)	Phase II	110	IFRT (24 Gy) ± ofatumumab	Bcl-2 negativization after ofatumumab
NCT 02689869	ALTERNATIVE	Follicular lymphoma	Phase II, single-arm	98	ibrutinib + obinutuzumab + maintenance with ibrutinib + obinutuzumab	PFS
NCT 02354313 [84]	FIL_MCL0208	Mantle cell lymphoma	Phase III, randomized	300	R-CHOP + HD-CTX + HD-Ara-C + BEAM and ASCT ± lenalidomide maintenance	PFS
NCT02896582	LyMa 101	Mantle cell lymphoma	Phase II, single-arm	83	GA-DHAP + GA-BEAM + ASCT + GA maintenance	MRD negativity at end of induction
Not available yet	EA4151 - ECOG group	Mantle cell lymphoma	Phase III, randomized	689	induction therapy ± ASCT + R maintenance	OS
EudraCT 2011-002751-34	ACVDL	Multiple Myeloma	Phase II	35	ACVDL ± ASCT ± bortezomib consolidation	PFS

Table 2. Ongoing clinical MRD-driven studies. *Abbreviations:* MRD, minimal residual disease; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; ASCT, autologous stem cell transplant; B-R, bendamustine, rituximab; PFS, progression free survival; OS, overall survival; GA-BEAM, obinutuzumab, carmustine, etoposide, cytarabine, melphalan; GA-DHAP, obinutuzumab, cytarabine, dexamethasone, cisplatin; HD-CTX, high dose cyclophosphamide, HD-Ara-C, high dose cytarabine; ACVDL, doxorubicin, cyclophosphamide, bortezomib, dexamethasone, lenalidomide.

5. Future directions of MRD studies: novel tools, tissues, and entities

Despite the described robust prognostic value in MCL (27) and FL (28), overall MRD analysis in NHL is not yet used in clinical practice for several reasons. Firstly, NHL involves multiple sites and not exclusively PB and BM; secondly, available MRD studies have focused only on some NHL subtypes (mainly FL and MCL); finally, current MRD tools are complex and not applicable to all patients. Actually, MRD detection has substantial advantages over alternative disease monitoring tools (including novel imaging) in terms of safety, sensitivity, length of event anticipation and costs. However, to become a strategic tool for treatment personalization in NHL, substantial implementation is needed, particularly in terms of: a) developing simpler and more applicable tools and

increasing the number of molecular targets; b) validating the impact of alternative tissue sources, other than BM and PB; c) demonstrating MRD predictive value in NHL, besides FL and MCL.

In this scenario, recent technical developments offer major opportunities. In particular, "next generation" MRD assays with broader applicability, increased reproducibility, reduced labor-intensiveness and high standardization potential, are under development. These include droplet-digital PCR (ddPCR), NGS-based assays and use of alternative molecular targets. Indeed, ddPCR will be rapidly implemented in the routine MRD diagnostics of NHL. Based on recently published results, this tool - relying on absolute rather than relative quantification of tumor invasion - correlates very well with the standardized RQ-PCR and is able to overcome some of its intrinsic limitations,

allowing more patients to be studied for MRD, particularly those with low-infiltrated baseline tumor samples or with no available MFC data (93).

Moreover, NGS does not rely on patient-specific reagents, and it might provide more standardized approaches in routine diagnostics. NGS can find an immunoglobulin-based MRD marker in the majority of patients, it can reach higher sensitivity levels and is a valid strategy to describe tumor clonal heterogeneity and evolution (80, 81, 94, 95). In addition, other NGS-based techniques, such as targeted-locus amplification (TLA), can provide a newly identified, translocation-based MRD marker in patients without an IGH-derived molecular marker, and may help to perform MRD analysis on subsequent follow-up samples (96, 97).

Finally, alternative DNA sources, such as plasma, urine and cerebrospinal fluid, can allow a polidistrictual MRD analysis, targeting the circulating tumor DNA (ctDNA), which will widen the MRD application in most NHL subtypes. CtDNA is already employed in molecular oncology to investigate the most common driving mutations of solid tumors, in order to target specific treatments (98). In hematology, the feasibility of ctDNA detection and monitoring has been already retrospectively proven in aggressive, non-leukemic tumors, such as DLBCL and HL (99-101). Therefore, ctDNA analysis might overcome some limitations of the current MRD approach in NHL. First, it can avoid false-negative MRD results: as ctDNA gives a better representation of tumor masses (lymph nodes) rather than leukemic disease, ctDNA might help in localized relapses, not detectable either in PB or in BM; in addition, it may extend the application of MRD to non- or less leukemic disorders, such as DLBCL (102, 103), primary central nervous

system lymphoma (PCNSL) (104), stage I FL or WM (105); finally, it is likely to decrease or avoid invasive diagnostic procedures (BM biopsy or lumbar puncture) to monitor MRD.

All these advances in MRD methodology will play a central role in the development of future, no-profit clinical trials aiming at investigating tailored treatment options in multiple NHL subtypes.

6. Conclusions

MRD monitoring in lymphoma is essential to offer patients tailored therapies, based on their specific risk of relapse. Many national research groups are already carrying out clinical trials to optimize treatment, mainly in FL and MCL, and, in such studies, consolidation and maintenance therapies are modulated on the basis of the patient's MRD profile (Table 2).

Such strategies not only represent an opportunity for patients, as they can substantially improve quality of life, but they can also reduce costs for the national healthcare system (105, 106). The need to best use novel agents has certainly led to a growing interest in tailored treatment. In fact, by selecting the best drug and appropriate combinations for each patient, physicians can be able to reduce toxicities and costs. In addition, imaging studies and several biomarkers are being investigated as possible prognostic factors, and they may considerably improve stratification of lymphoma patients at diagnosis.

Ideally, future clinical trials should include a two-step approach for risk stratification, in order to build up a real personalized medicine for lymphoma (Figure 3). The first step of such stratification may consist in a thorough baseline risk evaluation, which takes into

account conventional prognostic scores, novel imaging data (e.g. the PET-derived Total Metabolic Tumor Volume, TMTV) (106) and novel biomarkers that can predict disease aggressiveness and response to targeted treatments. Therefore, novel interventional trials could include different treatments for different risk groups: intensified treatments, including molecularly targeted compounds, could be used for high-risk patients, while less intense strategies could be adopted for low-risk cases. The second step of risk stratification may consist in a re-evaluation of the relapse risk after induction treatment: disease restaging will become more and more MRD-centered, thanks to the increase in feasibility and sensitivity of laboratory techniques. Moreover, the role of metabolic response by FDG-PET and other new imaging tools will

be extended thanks to their progressive standardization and growing applications. Both MRD and FDG-PET will become fundamental in the treatment decision-making process at the end of induction therapy: based on these tools, clinicians may choose subsequent treatments, ranging from simple observation to consolidation and maintenance strategies with emerging drugs.

In conclusion, personalized medicine represents the next step in the treatment of mature B-cell diseases. A deeper understanding of the molecular mechanisms in each individual tumor can be achieved thanks to a “total prognostic assessment” approach. In the near future, such approach will be crucial to offer tailored and effective therapies to patients with highly heterogeneous diseases like lymphoma.

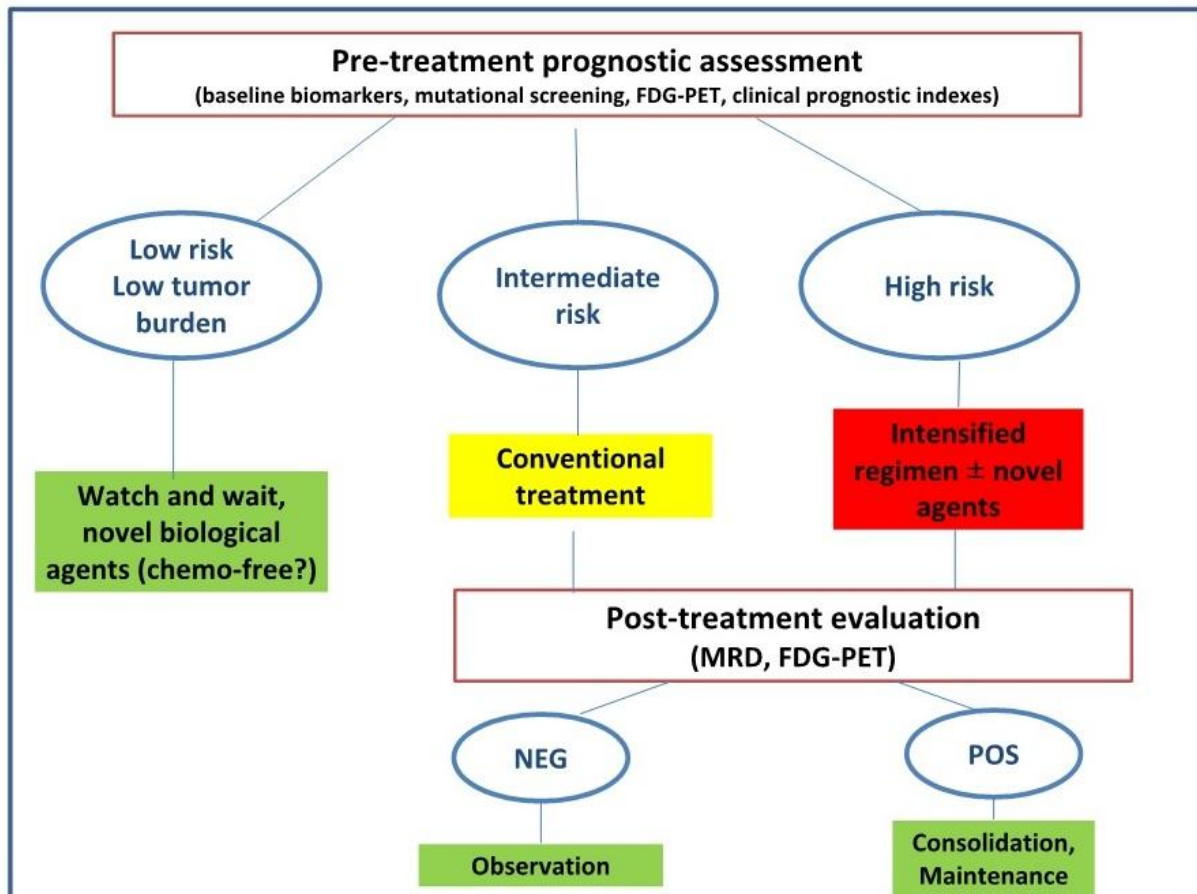


Figure 3. Total prognostic assessment approach. *Abbreviations.* MRD, minimal residual disease; NEG, negative; POS, positive.

References:

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA: a cancer journal for clinicians*. 2014;64(1):9-29.
2. DL Longo AF, DL Kasper, SL Hauser, J Jameson, J Loscalzo. *Harrison's Principles of Internal Medicine*. 18th ed. New York, NY: McGraw-Hill; 2012.
3. DeVita VT, T S Lawrence, S A Rosenberg. DeVita, Hellman, and Rosenberg's *Cancer: Principles and Practice of Oncology*. 10th ed 2014.
4. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375-90.
5. Greipp PR, San Miguel J, Durie BG, Crowley JJ, Barlogie B, Blade J, et al. International staging system for multiple myeloma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2005;23(15):3412-20.
6. Solal-Celigny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, et al. Follicular lymphoma international prognostic index. *Blood*. 2004;104(5):1258-65.
7. Federico M, 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. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2009;27(27):4555-62.
8. Hoster E, Dreyling M, Klapper W, Gisselbrecht C, van Hoof A, Kluijn-Nelemans HC, et al. A new prognostic index (MIPI) for patients with advanced-stage mantle cell lymphoma. *Blood*. 2008;111(2):558-65.
9. Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, et al. Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2015;33(26):2863-9.
10. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, 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. *The Lancet Oncology*. 2015;16(9):1111-22.
11. Hoster E. Minimal Residual Disease in Mantle Cell Lymphoma: Insights Into Biology and Impact on Treatment. *ASH annual meeting abstracts*. 2016.
12. Gallamini A, Hutchings M, Rigacci L, Specht L, Merli F, Hansen M, et al. Early interim 2-[18F]fluoro-2-deoxy-D-glucose positron emission tomography is prognostically superior to international prognostic score in advanced-stage Hodgkin's lymphoma: a report from a joint Italian-Danish study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2007;25(24):3746-52.
13. Pregno P, Chiappella A, Bello M, Botto B, Ferrero S, Franceschetti S, et al. Interim 18-FDG-PET/CT failed to predict the outcome in diffuse large B-cell

- lymphoma patients treated at the diagnosis with rituximab-CHOP. *Blood*. 2012;119(9):2066-73.
14. Adams HJ, Nievelstein RA, Kwee TC. Prognostic value of interim and end-of-treatment FDG-PET in follicular lymphoma: a systematic review. *Annals of hematology*. 2016;95(1):11-8.
15. Ferrero S, Drandi D, Mantoan B, Ghione P, Omede P, Ladetto M. Minimal residual disease detection in lymphoma and multiple myeloma: impact on therapeutic paradigms. *Hematological oncology*. 2011;29(4):167-76.
16. Feugier P. A review of rituximab, the first anti-CD20 monoclonal antibody used in the treatment of B non-Hodgkin's lymphomas. *Future oncology*. 2015;11(9):1327-42.
17. Goede V, Fischer K, Busch R, Engelke A, Eichhorst B, Wendtner CM, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. *The New England journal of medicine*. 2014;370(12):1101-10.
18. Witzig TE, Nowakowski GS, Habermann TM, Goy A, Hernandez-Ilizaliturri FJ, Chiappella A, et al. A comprehensive review of lenalidomide therapy for B-cell non-Hodgkin lymphoma. *Annals of oncology : official journal of the European Society for Medical Oncology*. 2015;26(8):1667-77.
19. Wang ML, Rule S, Martin P, Goy A, Auer R, Kahl BS, et al. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. *The New England journal of medicine*. 2013;369(6):507-16.
20. Treon SP, Xu L, Hunter Z. MYD88 Mutations and Response to Ibrutinib in Waldenstrom's Macroglobulinemia. *The New England journal of medicine*. 2015;373(6):584-6.
21. Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *The New England journal of medicine*. 2013;369(1):32-42.
22. Treon SP, Tripsas CK, Meid K, Warren D, Varma G, Green R, et al. Ibrutinib in previously treated Waldenstrom's macroglobulinemia. *The New England journal of medicine*. 2015;372(15):1430-40.
23. Wilson WH, Young RM, Schmitz R, Yang Y, Pittaluga S, Wright G, et al. Targeting B-cell receptor signaling with ibrutinib in diffuse large B-cell lymphoma. *Nat Med*. 2015;21(8):922-6.
24. Gopal AK, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ, et al. PI3Kdelta inhibition by idelalisib in patients with relapsed indolent lymphoma. *The New England journal of medicine*. 2014;370(11):1008-18.
25. Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *The New England journal of medicine*. 2014;370(11):997-1007.
26. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *The New England journal of medicine*. 2016;374(4):311-22.
27. Pott C, Hoster E, Delfau-Larue MH, Beldjord K, Bottcher S, Asnafi V, et al. Molecular remission is an independent

predictor of clinical outcome in patients with mantle cell lymphoma after combined immunochemotherapy: a European MCL intergroup study. *Blood*. 2010;115(16):3215-23.

28. 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(23):3759-66.

29. Ladetto M DMF, Benedetti F, Vitolo U, Patti C, Rambaldi A., Pulsoni MM, Liberati A. M, Olivieri A, Gallamini A, Pogliani E., Rota Scalabrini D CV, Di Raimondo F, Pavone F, Tucci A., Cortelazzo S LA, Boccadoro M, Majolino I, Pileri A, M. Gianni A., Passera R CP, and Tarella C. 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(8):4004-13.

30. Andersen NS, Pedersen LB, Laurell A, Elonen E, Kolstad A, Boesen AM, et al. Pre-emptive treatment with rituximab of molecular relapse after autologous stem cell transplantation in mantle cell lymphoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2009;27(26):4365-70.

31. Paiva B, Gutierrez NC, Rosinol L, Vidriales MB, Montalban MA, Martinez-Lopez J, et al. High-risk cytogenetics and persistent minimal residual disease by multiparameter flow cytometry predict unsustained complete response after autologous stem cell transplantation in

multiple myeloma. *Blood*. 2012;119(3):687-91.

32. van der Velden VH, Cazzaniga G, Schrauder A, Hancock J, Bader P, Panzer-Grumayer ER, et al. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia*. 2007;21(4):604-11.

33. Gribben JG FA, 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(22):1525-33.

34. 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. *The Journal of molecular diagnostics : JMD*. 2007;9(4):530-7.

35. Pott C, Bruggemann 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 in molecular biology*. 2013;971:175-200.

36. Pott C, Tiemann M, Linke B, Ott MM, von Hofen M, Bolz I, et al. Structure of Bcl-1 and IgH-CDR3 rearrangements as clonal markers in mantle cell lymphomas. *Leukemia*. 1998;12(10):1630-7.

37. Geisler CH, Kolstad A, Laurell A, Andersen NS, Pedersen LB, Jerkeman M, et al. Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo-purged stem cell rescue: a nonrandomized phase 2 multicenter study by the Nordic

- Lymphoma Group. Blood. 2008;112(7):2687-93.
38. Ghione P, Monitillo, L., Barbero, D., Verardo, G., Drandi, D., Grimaldi, D., Genuardi, E., Mantoan, B., Cavallo, F., Vasta, M., Zaja, F., Visco, C., Cortelazzo, S., Ladetto, M., Ferrero, S. A Technical Comparison Between Igh And Bcl1/Igh Molecular Markers For Minimal Residual Disease Analysis In Mantle Cell Lymphoma. SIES meeting abstract. 2016.
39. Ladetto M, Pagliano G, Ferrero S, Cavallo F, Drandi D, Santo L, et al. Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010;28(12):2077-84.
40. Ladetto M, Ferrero S, Drandi D, Festuccia M, Patriarca F, Mordini N, et al. Prospective molecular monitoring of minimal residual disease after non-myeloablative allografting in newly diagnosed multiple myeloma. *Leukemia*. 2015.
41. Oliva S, Gambella M, Gilestro M, Muccio VE, Gay F, Drandi D, et al. Minimal residual disease after transplantation or lenalidomide-based consolidation in myeloma patients: a prospective analysis. *Oncotarget*. 2016.
42. Brown JR, Feng Y, Gribben JG, Neuberg D, Fisher DC, Mauch P, et al. Long-term survival after autologous bone marrow transplantation for follicular lymphoma in first remission. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2007;13(9):1057-65.
43. Rambaldi A, Lazzari M, Manzoni C, Carlotti E, Arcaini L, Baccarani M, et al. Monitoring of minimal residual disease after CHOP and rituximab in previously untreated patients with follicular lymphoma. *Blood*. 2002;99(3):856-62.
44. Hirt C, Schuler 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(5):631-40.
45. Ghielmini M, Schmitz SF, Cogliatti SB, Pichert G, Hummerjohann J, Waltzer U, et al. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly x 4 schedule. *Blood*. 2004;103(12):4416-23.
46. Rambaldi A, Carlotti E, Oldani E, Della Starza I, Baccarani M, Cortelazzo S, 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(9):3428-33.
47. 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. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2009;27(36):6094-100.
48. 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. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2004;22(8):1460-8.

49. 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. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2014;20(24):6398-405.

50. Rummel MJ, Niederle N, Maschmeyer G, Banat GA, von Grunhagen 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(9873):1203-10.

51. 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(12):1407-14.

52. Boccomini C, Ladetto M, Rigacci L, Rattotti S, Evangelista A, Ferrero S, Volpetti S, Mantoan B, Meli E, Freilone R, Corradini P, Franceschetti S, Rusconi C, Stelitano C, Dutto F, Puccini B, Bolis S, Salvi F, Fabbri A, Billio A, Tucci A, Balzarotti M, Vitolo U. Sicurezza Ed Efficacia Di Una Breve Chemioimmunoterapia Di Prima Linea Con Rituximab, Bendamustina E Mitoxantrone (R-Bm) Seguita Da Consolidamento Con Rituximab Nei Pazienti Anziani Affetti Da Linfoma Follicolare In Stadio Avanzato: Risultati Finali Di Uno Studio Prospettico Di

Fase Ii Della Fondazione Italiana Linfomi (FIL). SIE meeting abstracts. 2015.

53. Galimberti S EG, Ciabatti E, Grassi S, Metelli MR, Guerrini F et al. Galimberti S, Ercolano G, Ciabatti E, Grassi S, Metelli MR, Guerrini F et al. Rituximab-bendamustin: an effective treatment for minimal residual disease eradication in patients affected by follicular lymphoma. SIES meeting abstracts. 2016;Abstract C060.

54. Pott C, Belada D, Danesi N, Fingerle-Rowson G, Gribben J, Harbron C, Hoster E, Kahl B S, Mundt K, Sebban C, Sehn L H, Cheson B D. 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.

55. Pott C, Hoster E, Kehden B, Unterhalt M, Herold M, van der Jagt R H, Janssens A, Kneba M, Mayer J, Pocock C, Danesi N, Fingerle-Rowson G, Harbron C, Mundt K, Marcus R E, Hiddemann W. 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.

56. Ladetto M, Magni M, Pagliano G, De Marco F, Drandi D, Ricca I, et al. Rituximab induces effective clearance of minimal residual disease in molecular relapses of mantle cell lymphoma. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2006;12(12):1270-6.

57. Ferrero S, Monitillo L, Mantoan B, Barbero D, Genuardi E, Barbiero S, et al.

Rituximab-based pre-emptive treatment of molecular relapse in follicular and mantle cell lymphoma. *Annals of hematology*. 2013;92(11):1503-11.

58. Morschhauser F, Recher C, Milpied N, Gressin R, Salles G, Brice P, 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. *Annals of oncology : official journal of the European Society for Medical Oncology*. 2012;23(10):2687-95.

59. Andersen NS, Donovan JW, Borus JS, Poor CM, Neuberg D, Aster JC, et al. Failure of immunologic purging in mantle cell lymphoma assessed by polymerase chain reaction detection of minimal residual disease. *Blood*. 1997;90(10):4212-21.

60. Gianni AM, Magni M, Martelli M, Di Nicola M, Carlo-Stella C, Pilotti S, et al. Long-term remission in mantle cell lymphoma following high-dose sequential chemotherapy and in vivo rituximab-purged stem cell autografting (R-HDS regimen). *Blood*. 2003;102(2):749-55.

61. Magni M, Di Nicola M, Devizzi L, Matteucci P, Lombardi F, Gandola L, et al. Successful in vivo purging of CD34-containing peripheral blood harvests in mantle cell and indolent lymphoma: evidence for a role of both chemotherapy and rituximab infusion. *Blood*. 2000;96(3):864-9.

62. Pott C, Schrader C, Gesk S, Harder L, Tiemann M, Raff T, et al. Quantitative assessment of molecular remission after high-dose therapy with autologous stem cell transplantation predicts long-term remission

in mantle cell lymphoma. *Blood*. 2006;107(6):2271-8.

63. Hermine O, Hoster E, Walewski J, Bosly A, Stilgenbauer S, Thieblemont C, et al. Addition of high-dose cytarabine to immunochemotherapy before autologous stem-cell transplantation in patients aged 65 years or younger with mantle cell lymphoma (MCL Younger): a randomised, open-label, phase 3 trial of the European Mantle Cell Lymphoma Network. *Lancet*. 2016;388(10044):565-75.

64. Callanan MB, Delfau M, Macintyre E, Thieblemont C, Oberic L, Gyan E, Bouabdallah K, Gressin R, Damaj G, Casasnovas O, Ribrag V, Gimenez E, Hermine O, & Le Gouill S. Predictive Power of Early, Sequential MRD Monitoring in Peripheral Blood and Bone Marrow in Patients with Mantle Cell Lymphoma Following Autologous Stem Cell Transplantation with or without Rituximab Maintenance; Interim Results from the LyMa-MRD Project, Conducted on Behalf of the Lysa Group. *Blood*. 2015;126(23):338.

65. Cortelazzo S, MM, Ladetto M, Ferrero S, Ciccone G, Evangelista A, Mian M, Di Rocco A, Chiappella A, Rossi G, Re A, Zinzani P. L., Balzarotti M, Cavallo F, Rusconi C, Gotti M, Arcaini L, Gobbi M, Gomes M, Molinari A, Liberati A.M., Michieli M, Latte G, Cabras M. G., Novero D, Paulli M, Zamò A, Chilosi M, Federico M, Vitolo U. High Dose Sequential Chemotherapy With Rituximab And ASCT As First Line Therapy In Adult MCL Patients: Clinical And Molecular Response Of The MCL0208 Trial, A FIL Study. EHA Meeting abstracts. 2015.

66. Pott C, Macintyre E, Delfau-Larue M-H, Ribrag V, Unterhalt M, Kneba M, et al. MRD Eradication Should be the Therapeutic

Goal in Mantle Cell Lymphoma and May Enable Tailored Treatment Approaches: Results of the Intergroup Trials of the European MCL Network. *Blood*. 2014;124(21):147-.

67. Kolstad A, Pedersen L. B., Eskelund, C. W., Husby, S., Grønbaek K., Jerkeman M., Laurell A., Rätty, R., Elonen, E., Andersen, N.S., de Nully Brown, P., Geisler, C.H. Molecular Monitoring and Tailored Strategy with Pre-Emptive Rituximab Treatment for Molecular Relapse; Results from the Nordic Mantle Cell Lymphoma Studies (MCL2 and MCL3) with Median Follow-up of 8.5 Years. ASH annual meeting abstracts. 2016;abstract n° 146.

68. Zaja F ea. Rituximab, lenalidomide, bendamustine second line therapy in mantle cell lymphoma: a phase II study of the Fondazione Italiana Linfomi (FIL). *ICML Abstracts* 2015:014. *ICML Abstracts* 2015;014.

69. Visco C, Chiappella A, Nassi L, Patti C, Ferrero S, Barbero D, et al. Rituximab, bendamustine, and low-dose cytarabine as induction therapy in elderly patients with mantle cell lymphoma: a multicentre, phase 2 trial from Fondazione Italiana Linfomi. *Lancet Haematol*. 2016.

70. Albertsson-Lindblad A, Kolstad A, Laurell A, Rätty R, Gronbaek K, Sundberg J, et al. Lenalidomide-bendamustine-rituximab in untreated mantle cell lymphoma > 65 years, the Nordic Lymphoma Group phase I+II trial NLG-MCL4. *Blood*. 2016.

71. Kaplan LD, Jung, S. H. , Bartlett, N. C. , Johnson, J. , Byrd, J. . Bortezomib maintenance (BM) vs consolidation (BC) following aggressive immunochemotherapy and autologous stem cell transplant (ASCT) for untreated mantle cell (MCL): CALGB

50403 (Alliance). *ASH - Hematol Oncol.*, 2013.

72. Corradini P, Voena C, Tarella C, Astolfi M, Ladetto M, Palumbo A, et al. Molecular and clinical remissions in multiple myeloma: role of autologous and allogeneic transplantation of hematopoietic cells. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 1999;17(1):208-15.

73. Martinelli G, Terragna C, Zamagni E, Ronconi S, Tosi P, Lemoli RM, et al. Molecular remission after allogeneic or autologous transplantation of hematopoietic stem cells for multiple myeloma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2000;18(11):2273-81.

74. Puig N, Sarasquete ME, Balanzategui A, Martinez J, Paiva B, Garcia H, et al. Critical evaluation of ASO RQ-PCR for minimal residual disease evaluation in multiple myeloma. A comparative analysis with flow cytometry. *Leukemia*. 2014;28(2):391-7.

75. Ferrero S, Capello D, Svaldi M, Boi M, Gatti D, Drandi D, et al. Multiple myeloma shows no intra-disease clustering of immunoglobulin heavy chain genes. *Haematologica*. 2012;97(6):849-53.

76. Paiva B, Vidriales MB, Cervero J, Mateo G, Perez JJ, Montalban MA, et al. Multiparameter flow cytometric remission is the most relevant prognostic factor for multiple myeloma patients who undergo autologous stem cell transplantation. *Blood*. 2008;112(10):4017-23.

77. Palumbo A, Hajek R, Delforge M, Kropff M, Petrucci MT, Catalano J, et al. Continuous lenalidomide treatment for newly diagnosed multiple myeloma. *The*

New England journal of medicine. 2012;366(19):1759-69.

78. Ferrero S, Ladetto M, Drandi D, Cavallo F, Genuardi E, Urbano M, et al. Long-term results of the GIMEMA VEL-03-096 trial in MM patients receiving VTD consolidation after ASCT: MRD kinetics' impact on survival. *Leukemia*. 2015;29(3):689-95.

79. Korthals M, Sehnke N, Kronenwett R, Schroeder T, Strapatsas T, Kobbe G, et al. Molecular monitoring of minimal residual disease in the peripheral blood of patients with multiple myeloma. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2013;19(7):1109-15.

80. Ladetto M, Bruggemann M, Monitillo L, Ferrero S, Pepin F, Drandi D, et al. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. *Leukemia*. 2014;28(6):1299-307.

81. Martinez-Lopez J, Lahuerta JJ, Pepin F, Gonzalez M, Barrio S, Ayala R, et al. Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. *Blood*. 2014;123(20):3073-9.

82. Korde N, Roschewski M, Zingone A, Kwok M, Manasanch EE, Bhutani M, et al. Treatment With Carfilzomib-Lenalidomide-Dexamethasone With Lenalidomide Extension in Patients With Smoldering or Newly Diagnosed Multiple Myeloma. *JAMA oncology*. 2015;1(6):746-54.

83. Avet-Loiseau H, Casneuf, T., Chiu, C., Laubach, J., , Lee J, Moreau, P., Plesner, T., Nahi, H.,, Khokhar NZ, Qi, M., Schechter, J., Carlton, V., Qin, X.,, Liu K, Wu, K., Zhuang, S. H., Ahmadi, T., Sasser, A. K., J.

S-M. Evaluation of Minimal Residual Disease (MRD) in Relapsed/Refractory Multiple Myeloma (RRMM) Patients Treated With Daratumumab in Combination With Lenalidomide Plus Dexamethasone or Bortezomib Plus Dexamethasone. *ASH annual meeting abstracts*. 2016.

84. Ardeshtna KM, Smith P, Norton A, Hancock BW, Hoskin PJ, MacLennan KA, 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(9383):516-22.

85. Brice P, Bastion Y, Lepage E, Brousse N, Haioun C, Moreau P, 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. Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 1997;15(3):1110-7.

86. Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2014;32(27):3059-68.

87. Barrington SF, Qian W, Somer EJ, Franceschetto A, Bagni B, Brun E, et al. Concordance between four European centres of PET reporting criteria designed for use in multicentre trials in Hodgkin lymphoma. *European journal of nuclear medicine and molecular imaging*. 2010;37(10):1824-33.

88. Gallamini A, O'Doherty M. Report of satellite workshop on interim-PET in Hodgkin lymphoma: 8th International Symposium on Hodgkin Lymphoma, Cologne, 23 October 2010. *Leukemia & lymphoma*. 2011;52(4):583-6.
89. Meignan M, Gallamini A, Meignan M, Gallamini A, Haioun C. Report on the First International Workshop on Interim-PET-Scan in Lymphoma. *Leukemia & lymphoma*. 2009;50(8):1257-60.
90. Witzig TE, Molina A, Gordon LI, Emmanouilides C, Schilder RJ, Flinn IW, et al. Long-term responses in patients with recurring or refractory B-cell non-Hodgkin lymphoma treated with yttrium 90 ibritumomab tiuxetan. *Cancer*. 2007;109(9):1804-10.
91. Ladetto M, Buske C, Hutchings M, Dreyling M, Gaidano G, Le Gouill S, et al. ESMO consensus conference on malignant lymphoma: general perspectives and recommendations for prognostic tools in mature B-cell lymphomas and chronic lymphocytic leukaemia. *Annals of oncology : official journal of the European Society for Medical Oncology*. 2016;27(12):2149-60.
92. Rajkumar SV, Harousseau JL, Durie B, Anderson KC, Dimopoulos M, Kyle R, et al. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel 1. *Blood*. 2011;117(18):4691-5.
93. Drandi D, Kubiczkoa-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: A Comparison with Real-Time PCR. *The Journal of molecular diagnostics : JMD*. 2015;17(6):652-60.
94. Faham M, Zheng J, Moorhead M, Carlton VE, Stow P, Coustan-Smith E, et al. Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. *Blood*. 2012;120(26):5173-80.
95. Brüggemann M KH, Bartram J, et al. International multi-laboratory next-generation sequencing for MRD analysis in ALL. A pilot study by the Euroclonality-NGS Consortium. *Haematologica*. 2015;100(s1):P164:31.
96. de Vree PJ, de Wit E, Yilmaz M, van de Heijning M, Klous P, Versteegen MJ, et al. Targeted sequencing by proximity ligation for comprehensive variant detection and local haplotyping. *Nat Biotechnol*. 2014;32(10):1019-25.
97. Hottentot QP, van Min M, Splinter E, White SJ. Targeted Locus Amplification and Next-Generation Sequencing. *Methods in molecular biology*. 2017;1492:185-96.
98. Diaz LA, Jr., Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2014;32(6):579-86.
99. Oki Y, Neelapu SS, Fanale M, Kwak LW, Fayad L, Rodriguez MA, et al. Detection of classical Hodgkin lymphoma specific sequence in peripheral blood using a next-generation sequencing approach. *Br J Haematol*. 2015;169(5):689-93.
100. Rossi D, Diop F, Spaccarotella E, Monti S, Zanni M, Rasi S, et al. Diffuse large B-cell lymphoma genotyping on the liquid biopsy. *Blood*. 2017.
101. Scherer F, Kurtz DM, Newman AM, Stehr H, Craig AF, Esfahani MS, et al. Distinct biological subtypes and patterns of

genome evolution in lymphoma revealed by circulating tumor DNA. *Sci Transl Med.* 2016;8(364):364ra155.

102. Kurtz DM, Green MR, Bratman SV, Scherer F, Liu CL, Kunder CA, et al. Noninvasive monitoring of diffuse large B-cell lymphoma by immunoglobulin high-throughput sequencing. *Blood.* 2015;125(24):3679-87.

103. Roschewski M, Dunleavy K, Pittaluga S, Moorhead M, Pepin F, Kong K, et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *The Lancet Oncology.* 2015;16(5):541-9.

104. Fontanilles M. MF, Bohers E., Viailly P., Bertrand, P., Dubois S., Mareschal S., Camus V., Veresezan E., Ruminy P., Tilly H., Laquerriere A., Picquenot J., Jardin F. Somatic Mutations Detected in Plasma Cell-Free DNA By Targeted Sequencing:

Assessment of Liquid Biopsy in Primary Central Nervous System Lymphoma. *ASH annual meeting abstracts.* 2015.

105. Drandi D GE, Dogliotti I, Sciascia I, Guerrini F, Mantoan B., Gilestro M MV, Ghione P, Omedè P, Galimberti S, Orsucci L, Cavallo F., Boccadoro M LM, Ferrero S. A Novel Digital PCR Assay For MYD88L265P Mutation Detection In Waldenström Macroglobulinemia: Minimal Residual Disease Monitoring And Characterization On Circulating Free Dna. *EHA Meeting abstracts.* 2016.

106. Meignan M, Cottreau AS, Versari A, Chartier L, Dupuis J, Boussetta S, et al. Baseline Metabolic Tumor Volume Predicts Outcome in High-Tumor-Burden Follicular Lymphoma: A Pooled Analysis of Three Multicenter Studies. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2016.