vival considering disease-specific death in patients with chronic myeloid leukemia. Leukemia. 2016;30(1):48-56.
15. Hughes TP, Ross DM. Moving treatment-free remission into mainstream clinical practice in CML. Blood. 2016;128(1):17-23.
16. Saussele S, Richter J, Hochhaus A, Mahon F-X. The concept of treatment-free remission in chronic myeloid leukemia. Leukemia. 2016;30(8):1638-1647.
17. Baccarani M. Treatment-free remission in chronic myeloid leukemia: floating between expectation and evidence. Leukemia. 2017;31(4):1015-1016.
18. SUSTRENIM (Sustained treatment-free remission in BCR-ABL+ chronic myeloid leukemia: a prospective study comparing nilotinib versus imatinib with switch to nilotinib in absence of optimal response). ClinicalTrials.gov: NCT02602314.
19. Russo D, Martinelli G, Malagola M, et al. Effects and outcome of a policy of intermittent imatinib treatment in elderly patients with chronic myeloid leukemia. Blood. 2013;121(26):5138-5144.
20. OPTkIMA (Phase-III randomized study to optimize TKIs multiple approaches and quality of life in elderly patients with Ph+ chronic myeloid leukemia and MR 3.0 / MR 4.0 stable molecular response). ClinicalTrials.gov:NCT02326311.
21. Clark R, Polydoros F, Apperley JF, et al. Chronic myeloid leukaemia patients with stable molecular responses (at least MR3) may safely decrease the dose of their tyrosine kinase inhibitor: data from the British Destiny study. Blood. 2016;128(22):938.
22. Mahon F-X, Richter J, Guilhot J, et al. Cessation of tyrosine kinase inhibitors treatment in chronic myeloid leukemia patients with deep molecular response: results of the Euro-Ski trial. Blood. 2016;128 (22):787.
23. Etienne G, Guilhot J, Rea D, et al. Long-term follow-up of the French Stop Imatinib (STIM1) study in patients with chronic myeloid leukemia. J Clin Oncol. 2017;35(3):298-305.

Minimal residual disease in mantle cell lymphoma: are we ready for a personalized treatment approach?

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Mantle cell lymphoma (MCL) is nowadays recognized as a spectrum of diseases, characterized by significantly different treatment responses and outcomes. Some predictors of clinical and biological outcome have been established and validated over recent years, and these are either assessable at baseline (mainly MCL international prognostic indexes, Ki-67 proliferative index and genomic aberrations) or during treatment (functional imaging and minimal residual disease, MRD). MRD is defined as the minimal traceable persistence of lymphoma cells after a successful treatment. Many methods to monitor MRD have been published; however, the most sensitive and the most commonly used and best standardized approach in MCL is represented by the allele-specific oligonucleotide (ASO) quantitative polymerase chain reaction (qPCR) method.[1] The most relevant prospective trials investigating the impact of MRD on MCL patient outcome are listed in Table 1A.

The clinical role of MRD analysis in MCL is reflected according to four major aspects (Figure 1).

MRD provides early feedback on the efficacy of the clearance of different induction regimens. The dynamics and stability of tumor shrinkage after treatment can currently be precisely tracked by MRD kinetics; these data might be useful as an early *in vivo* predictor of the anti-lymphoma effect of a new compound.[6,9,10] Moreover, MRD can be used as a surrogate end point for progression-free survival (PFS) comparing the efficacy of different treatments in randomized trials, thus accelerating the development, and eventually the approval, of new drugs. For example, the superior outcome of the cytarabine-containing experimental arm of the "MCL Younger" phase III trial of the European MCL Network was heralded by a higher rate of MRD clearance many years before publication of the final results.[5,15]

MRD can provide an early prediction of disease recurrence. Even in the context of an incurable disease like MCL, the deepness of treatment response measured by MRD widely reflects patient outcome in large, prospective trials.[3-5] The predictive role of MRD analysis in MCL was confirmed in different patient subsets (both younger and elderly), treatment strategies (autologous transplantation and conventional immuno-chemotherapy), tissues (bone marrow and peripheral blood) and time points (end of induction and during maintenance treatment).[4]

MRD allows for risk stratification of patients after treatment. MRD describes the efficacy of therapy and presence of even minimal, resistant tumor clones; thus, this approach identifies patients at higher risk of recurrence after an apparently successful treatment. Actually, persistence of MRD positivity or recurrence after a transient MRD negativity precedes clinical relapse, with a median time lag of 18 months.[16]

MRD might drive pre-emptive treatment. As MRD positivity predicts upcoming clinical relapse, this approach can guide treatment tailoring, with the aim of preventing or delaying overt disease progression. In a number of prospective reports, a pre-emptive rituximab treatment of MRD positive patients was able to reconvert them to MRD negativity, with the possibility of also prolonging their PFS.[8,17]

Nevertheless, some limitations still hamper the widespread use of MRD analysis in clinical routine. The two major obstacles as far as methodology is concerned are the need for patient-specific primers and standardization issues. At present, the ASO-qPCR strategy relies upon either the clonal rearrangement of the IGH gene or the BCL-1/IGH rearrangement, derived from the t(11;14); both of these DNA sequences are unique for each B-cell clone, so individual primers are required for each patient to guarantee a reliable sensitivity. Thus a "one-fits-all" easy-to-use *in vitro* diagnostic medical device (IVD-kit) is not conceivable in MRD diagnostics so far, and access to an experienced and dedicated laboratory is mandatory. In addition, a rigorous standardization of the methods is essential in order to provide comparable results among different centers. Only selected laboratories across Europe

have so far been certified by the Euro MRD consortium, a standardization group regularly performing quality control rounds for MRD analysis in leukemia and lymphoma (*http://www.euromrd.org*).[18] Only by standardized analyses in this laboratory network can reliable MRD results be obtained in large, international clinical trials, such as the

Table 1A. Prospective clinical trials investigating the impact of minimal residual disease (MRD) on patients' outcome in mantle cell lymphoma (MCL) patients.

Study	Study population	Study features	Marker, method	Evaluable patients	Sample analyzed	Study treatment	MRD evaluation or MRD impact on outcome
Pott, 2006[2]	MCL, <70 y and GLSG	Local protocol	IgH, qPCR	29	BM, PB, harvest	R-HDS +TBI + ASCT	PFS 92 *vs.* 21 m (P<0.001)
Geisler, 2008[3]	MCL <66 y	Prospective trial phase II, non-randomized	BCL1, IgH, N-PCR	79	PB, BM	R-maxiCHOP/R-HD-AraC + ASCT + *in vivo* purging	PFS NR *vs.* 18 m (P<0.001)
Pott, 2010[4]	MCL, younger and elderly	Phase III, randomized	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 2 y FU (P=0.021)
Hermine, 2016[5]	MCL, <65 y	Phase III, randomized	Unspecified, qPCR	497	PB, BM	R-CHOP +TBI + HD CTX + ASCT *vs.* R-CHOP/R-DHAP + AraC +TBI + Mel + ASCT	EOI MRD neg: 47% *vs.* 79% (PB), 26% *vs.* 61% (BM)
Albertsson-Lindblad, 2017[6]	MCL, elderly	Phase I-II	BCL1, IgH, N-PCR	51	PB, BM	Len-BR x6 + Len maintenance	EOI MRD neg: 32%
Liu, 2012[7]	MCL, <69 y	Phase II, non-randomized	BCL1, IgH, qPCR	39	PB, BM	R-HD-MTX + maxi-CHOP + ASCT + R maintenance	TTP at 3 y 82% *vs.* 48% (MRD at EOI)
Kolstad, 2016[8]	MCL, <66 y	Phase II, non-randomized	BCL1, IgH, N-PCR	183	BM	R-maxiCHOP + ASCT ± RIT	PFS 20 m *vs.* 142 m (MRD post-ASCT); median OS NR *vs.* 35 m
Visco, 2016[9]	MCL, elderly	Phase II	BCL1, IgH, N-PCR	57	PB, BM	R-BAC500	No association between MRD status and PFS
Zaja, 2017[10]	MCL, >18 y	Phase II, non-randomized	BCL1, IgH, N-PCR, qPCR	42	PB, BM	R2B + R2 consolidation + Len maintenance	36% of MRD negativization, predictive of PFS
Armand, 2016[11]	MCL, transplant eligible (42-69 y)	Phase II, non-randomized	IgH, NGS	23	PB, plasma	BR + R-HD-ARA-C	93% MRD neg at EOT
Callanan, 2015[12]	MCL, <66 y	Phase III, randomized	IgH, qPCR	178	PB, BM	R-DHAP +R-BEAM-ASCT ± R maintenance	MRD status pre-ASCT in BM and PB predicts longer PFS (P=0.0451, P=0.0016)
Gressin, 2014[13]	MCL, elderly	Phase II, non-randomized	IgH, qPCR	76	PB, BM	RiBVD	MRD neg: 83% (PB) and 74% (BM) at 6 months
Kaplan, 2015[14]	MCL, <70 y	Phase II, randomized	BCL1, IgH, unspecified	151	Not available	augCHOP+MTX+EAR+ CBV-ASCT+ bortezomib maintenance *vs.* consolidation	5-y PFS: 93% if MRD-neg *vs.* 51% if MRD-pos following induction therapy

Table 1B. Current MRD-driven trials in MCL.

Clinicaltrials.gov identifier	Short title characteristics	Patients'	Study features (n. of patients)	Estimated enrollment	Study treatment	Primary outcome
02354313	FIL_MCL0208	MCL , <65 y	Phase III, randomized	300	R-CHOP + HD-CTX + HD-Ara-C + BEAM and ASCT ± lenalidomide maintenance	PFS
02896582	LyMa 101	MCL, <65 y	Phase II, single-arm	83	GA-DHAP + GA-BEAM + ASCT + GA maintenance	MRD negativity at end of induction
Not available	EA4151 - ECOG group	MCL, transplant eligible	Phase III, randomized	689	induction therapy ± ASCT + R maintenance	OS

MCL: mantle cell lymphoma; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; TBI: total body irradiation; ASCT: autologous stem cell transplant; Mel: melphalan; EOI: end of induction; B-R: bendamustine, rituximab; Len: lenalidomide; R-DHAP: rituximab, cytarabine, dexamethasone, cisplatin; R-BAC500: rituximab, bendamustine, cytarabine; EOT: end of treatment; GLSG: German Lymphoma Study Group; HD-CTX: high-dose cyclophosphamide; HD-Ara-C: high-dose cytarabine; RIC: reduced intensity conditioning; HD-CHT: high-dose chemotherapy; RIT: radio-immunotherapy; RiBVD: rituximab, bendamustine bortezomib and dexamethasone; y: years; m: months; BM: bone marrow; PB: peripheral blood; vs.: versus; neg.: negative; pos: positive; PFS: progression-free survival; OS: overall survival.

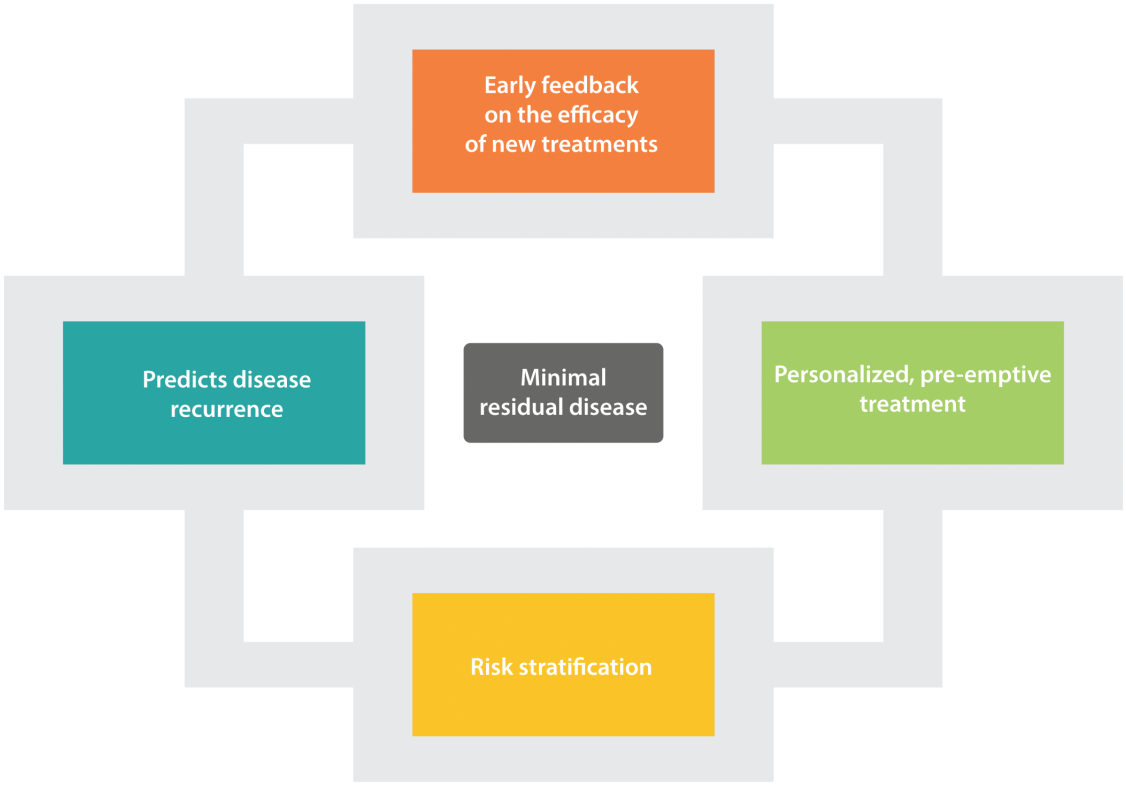

Figure 1. The clinical role of minimal residual disease analysis in mantle cell lymphoma.

recently launched TRIANGLE trial (EudraCT n. 2014-001363-12), sponsored by the European MCL Network. Moreover, another limitation of the current technique is that 10-15% of patients still lack a reliable molecular marker for MRD. For the moment, in MCL, an IGH-based marker is available in approximately 70% of cases and a BCL-1/IGH marker in approximately 35-40%, with some overlapping cases.[4] In particular, patients with low or absent bone marrow invasion often do not carry a marker and cannot, therefore, be analyzed for MRD. In addition, hypermutated IGH genes may hamper an optimal primer design. Finally, although the predictive role of MRD has been established in MCL,[3-5] evidence for the usefulness of subsequent treatment tailoring based on the MRD results is unfortunately still scarce due to the lack of MRD-driven phase III trials in MCL.[8,17] Moreover, no MRD data are available yet in the context of the new targeted treatments, such as the Bruton's tyrosine kinase inhibitor ibrutinib.

However, many technical innovations have recently been introduced in the MRD field, and these have the potential to overcome the issues of applicability and sensitivity described above. The droplet digital PCR (ddPCR), a 3rd-generation, end point, quantitative PCR has been shown to provide comparable results to ASO-qPCR for MRD monitoring in MCL with the advantage that it is less labor intensive. Moreover, since it does not require a standard curve for tumor quantification, ddPCR might provide reliable and sensitive MRD results also in cases in which the classical approach has failed.[19] Currently, a totally innovative approach is represented by the application of next-generation sequencing (NGS) techniques to the MRD field. The LymphoSIGHT™ approach was first published for MRD detection in acute lymphoblastic leukemia,[20] and was subsequently shown to be feasible also in MCL.[21] Its main advantages rely on the fact that it does not require patient-specific reagents (being thus suitable for an IVD-kit), it can provide additional MRD targets for patients lacking a "classical" molecular marker, it should easily reach high sensitivity levels, and might overcome some false-negative results (e.g. deciphering the clonal evolution issues). However, until now, this promising NGS technology has been available as a commercial tool only in US. Nevertheless, many laboratories are currently implementing alternative NGS-based approaches for MRD and an international development and standardization effort is ongoing within the EuroClonality-NGS laboratory consortium (*http://www.euroclonality.org/wp-content/uploads/2015/03/EuroClonality-NGS.pdf*).[22] Moreover, further promising NGS-based approaches for the identification of new molecular markers are being studied and might effectively provide an MRD target for each patient in the near future (Targeted Locus Amplification and Rapid Capture techniques).[23,24] Finally, MRD targeting on plasmatic, circulating tumor DNA is extremely promising as a means to track lymphoma clones residing outside the peripheral blood or bone marrow compartments.[25] Despite all of these encouraging data, a large-scale validation of each of these new technologies is required before their introduction into clinical practice.

Nowadays, MRD evaluation is mostly based on ASO-qPCR in the most important ongoing European clinical trials and, as explorative investigation, as part of clinical routine in a few selected centers. However, for the moment, the published diagnostic and treatment guidelines discourage any clinical decision-making based on MRD results,[26] mainly due to limitations of technical standardization and to the lack of prospective, randomized trials evaluating modified therapy according to MRD. Reliable and reproducible MRD data can currently be guaranteed only by the standardized methodological guidelines of the Euro MRD laboratory network. Therapeutic decisions based on MRD results obtained by different, as yet not validated techniques may even compromise the patient's long-term outcome. Moreover, convincing data on the real usefulness of such an MRD-based treatment modulation are not yet available in lymphoma, with only retrospective evidence or evidence derived from a single, phase II trial available so far.[8,17] Some MRD-driven phase II and III trials are ongoing in MCL and also in follicular lymphoma to assess the clinical impact of personalized treatment, with first results eagerly awaited in the near future (reviewed by Dogliotti and Ferrero[27] and summarized for MCL in Table 1B).

In conclusion, given that there is compelling evidence as to the predictive role of MRD in MCL, and ongoing standardization and methodological efforts are highly advanced, MRD analysis in MCL is already included in the majority of prospective trials. The next steps will consist of large trials investigating an MRD-driven tailored therapeutic approach (Table 1B). Thanks to the expected results and the development of rapidly evolving MRD techniques, we foresee that, in the near future, the huge research efforts over the last years will finally translate into personalized treatment strategies as part of clinical routine, leading to a further benefit for lymphoma patients in general.

References

1. Ferrero S, Drandi D, Mantoan B, Ghione P, Omedè P, Ladetto M. Minimal residual disease detection in lymphoma and multiple myeloma: impact on therapeutic paradigms. Hematol Oncol. 2011;29(4):167-176.
2. Pott C, Schrader C, Gesk S, 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):2271-2278.
3. Geisler CH, Kolstad A, Laurell A, 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):2687-2693.
4. Pott C, Hoster E, Delfau-Larue MH, 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):3215-3223.
5. Hermine O, Hoster E, Walewski J, 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;38 8(10044):565-575.
6. Albertsson-Lindblad A, Kolstad A, Laurell A, et al. Lenalidomide-bendamustine-rituximab in untreated mantle cell lymphoma > 65 years, the Nordic Lymphoma Group phase I+II trial NLG-MCL4. Blood. 2016 Jun 27 [Epub ahead of print]
7. Liu H, Johnson JL, Koval G, et al. Detection of minimal residual disease following induction immunochemotherapy predicts progression free survival in mantle cell lymphoma: final results of CALGB 59909. Haematologica. 2012;97(4):579-585.
8. Kolstad A, Pedersen LB, Eskelund CW, et al. Molecular Monitoring after Autologous Stem Cell Transplantation and Preemptive Rituximab Treatment of Molecular Relapse; Results from the Nordic Mantle Cell Lymphoma Studies (MCL2 and MCL3) with Median Follow-Up of 8.5 Years. Biol Blood Marrow Transplant. 2017;23(3):428-435.
9. Visco C, Chiappella A, Nassi L, 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. 2017;4(1):e15-e23.
10. Zaja F, Ferrero S, Stelitano C, et al. Second-line rituximab, lenalidomide, and bendamustine in mantle cell lymphoma: a phase II clinical trial of the Fondazione Italiana Linfomi. Haematologica. 2017;102(5):e203-e206.
11. Armand P, Redd R, Bsat J, et al. A phase 2 study of Rituximab-Bendamustine and Rituximab-Cytarabine for transplant-eligible patients with mantle cell lymphoma. Br J Haematol. 2016;173(1):89-95.
12. Callanan MB, Delfau M, Macintyre E, et al. 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.
13. Gressin R, Callanan M, Daguindau N, Tempescul A, Carras S, Moles MP, et al. Frontline Therapy with the Ribvd Regimen Elicits High Clinical and Molecular Response Rates and Long PFS in Elderly Patients Mantle Cell Lymphoma (MCL); Final Results of a Prospective Phase II Trial By the Lysa Group. Blood. 2014;124(21):148.
14. Kaplan LD, Jung SH, Stock W, Bartlett NL, Pitcher B, Byrd JC. Bortezomib Maintenance (BM) Versus Consolidation (BC) Following Aggressive Immunochemotherapy and Autologous Stem Cell Transplant (ASCT) for Untreated Mantle Cell Lymphoma (MCL): CALGB (Alliance) 50403. Blood. 2015;126(23):337.
15. Pott C, Hoster E, Beldjord K, et al. R-CHOP/R-DHAP Compared to R-CHOP Induction Followed by High Dose Therapy with Autologous Stem Cell Transplantation Induces Higher Rates of Molecular Remission In MCL: Results of the MCL Younger Intergroup Trial of the European MCL Network. Blood. 2010;116(21):965.
16. Pott C, Macintyre E, Delfau-Larue MH, 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.
17. Ferrero S, Monitillo L, Mantoan B, et al. Rituximab-based pre-emptive treatment of molecular relapse in follicular and mantle cell lymphoma. Ann Hematol. 2013;92(11):1503-1511.
18. van der Velden VH, Cazzaniga G, Schrauder A, et al. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. Leukemia. 2007;21(4):604-611.
19. Drandi D, Kubiczkova-Besse L, Ferrero S, et al. Minimal Residual Disease Detection by Droplet Digital PCR in Multiple Myeloma, Mantle Cell Lymphoma, and Follicular Lymphoma: A Comparison with Real-Time PCR. JMD. 2015;17(6):652-660.
20. Faham M, Zheng J, Moorhead M, et al. Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood. 2012;120(26):5173-5180.
21. Ladetto M, Brüggemann M, Monitillo L, et al. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. Leukemia. 2014;28(6):1299-1307.
22. Brüggemann M, Knecht H, 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.
23. Hottentot QP, van Min M, Splinter E, White SJ. Targeted Locus Amplification and Next-Generation Sequencing. Methods Mol Biol. 2017;1492:185-196.
24. Wren D, Walker BA, Brüggemann M, et al. Comprehensive translocation and clonality detection in lymphoproliferative disorders by next-generation sequencing. Haematologica. 2017;102(2):e57-e60.
25. Kurtz DM, Green MR, Bratman SV, et al. Noninvasive monitoring of diffuse large B-cell lymphoma by immunoglobulin high-throughput sequencing. Blood. 2015;125(24):3679-3687.
26. Dreyling M, Geisler C, Hermine O, et al. Newly diagnosed and relapsed mantle cell lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2014;25 (Suppl 3):iii83-92.
27. Dogliotti I, Ferrero S. Personalized medicine in lymphoma: tailoring treatment according to minimal residual disease. Medical Research Archives, Vol. 5, Issue 5, May 2017.