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Minimal residual disease testing for multiple myeloma

Ocena minimalnej choroby resztkowej w szpiczaku plazmocytowym

Beatriz Sanchez-Vega[1, 2], Rosa Ayala[1, 2], Teresa Cedena[1, 2], Joaquin Martinez-Lopez[1, 2]

[1]Hematology Department, Hospital Universitario 12 de Octubre, Universidad Complutense, Madrid, Spain
[2]Hematological Malignancies Clinical Research Unit, Centro Nacional de Investigaciones Oncologicas, Madrid, Spain

Abstract

Minimal residual disease (MRD) in a patient with multiple myeloma (MM) is defined as the minimum levels of pathological plasma cells remaining after treatment when a patient is in complete response (CR). The ultimate aim of studying MRD is to identify patients with different prognosis and to tailor treatment for individual patients. MRD studies in MM should be recommended in young patients in CR after autologous hematopoietic stem cells transplantation and in older patients in CR after regimens including proteasome inhibitors. Bone marrow is the only recommend location to assess MRD in MM. The recommended methods of MRD testing include next generation sequencing of immunoglobulin genes or multiparametric flow cytometry (MFC), depending on the experience of each center and the possibility of study samples being available in the first 24 hours for MFC analysis. MRD should be considered as a therapeutic objective. However, there is not enough evidence for taking clinical decisions based on MRD status alone, and for this reason we encourage the design of new clinical studies to address these questions.

Key words: multiple myeloma, minimal residual disease, complete response, flow cytometry, next generation sequencing

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Streszczenie

Minimalną chorobę resztkową (MRD) u pacjenta z rozpoznaniem szpiczaka plazmocytowego definiuje się jako populację nowotworowych komórek plazmatycznych, która pozostała w organizmie chorego po osiągnięciu odpowiedzi całkowitej (CR). Ostatecznym celem badań MRD jest dążenie do identyfikacji chorych o odmiennym rokowaniu i indywidualizacji leczenia na tej podstawie. Ocenę MRD u chorych na szpiczaka plazmocytowego zaleca się u młodszych pacjentów, którzy osiągną CR po przeszczepieniu autologicznych krwiotwórczych komórek macierzystych oraz u chorych starszych osiągających CR po chemioterapii opartej na inhibitorach proteasomu. Powinno się oznaczać MRD wyłącznie w szpiku kostnym. Do rekomendowanych metod oceny MRD w szpiczaku plazmocytowym zalicza się sekwencjonowanie następnej generacji genów immunoglobulinowych oraz wieloparametryczną cytometrię przepływową, przy czym wybór jednej z tych metod powinien zależeć od doświadczenia danego ośrodka oraz możliwości wykonania badania cytometrycznego w czasie 24 godzin od pobrania próbki szpiku. Eradykację MRD powinna się obecnie uważać za

Address for correspondence: Joaquin Martinez-Lopez, Hematology Hematology Department, Hospital Universitario 12 de Octubre, Avd de Cordoba S/N, Madrid, 28041, Madrid, Spain, e-mail: jmarti01@ucm.es

istotny cel terapii szpiczaka plazmocytowego. Jednak, ze względu na brak wystarczających danych do podejmowania decyzji klinicznych wyłącznie na podstawie wyniku badania MRD, istnieje potrzeba dalszych, dobrze zaprojektowanych badań klinicznych w tym zakresie.

Słowa kluczowe: szpiczak plazmocytowy, minimalna choroba resztkowa, odpowiedź całkowita, cytometria przepływowa, sekwencjonowanie następnej generacji

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Complete response and minimal residual disease in multiple myeloma: a comprehensive vision

Minimal residual disease (MRD) is defined as the minimum levels of pathological plasma cells remaining after treatment when a patient is in complete response (CR). The ultimate goal of studying MRD is to identify patients with different prognosis and to adapt the treatment to individual patients; a means to precision medicine.

The importance of MRD was first addressed in acute lymphoblastic leukemia (ALL) in children and chronic myeloid leukemia [1–3]. It is very well documented that patients who achieve MRD negativity in ALL have longer survival; this leads to tailored treatment in the case of MRD status after induction, as Sant Jude or the German Acute Leukemia Group have shown [4, 5].

In multiple myeloma (MM), the last decade has seen an unprecedented increase in the survival of patients; due to impressive improvements in understanding the biology of the disease, and the treatments available. The remarkable increase of survival along with an increase of responses indicates that deepest responses are one of the best surrogate markers of longer survival in MM [6–10]. In this scenario, the proportion of patients who achieve CR has improved significantly in the last decade, from 30% to 70% in young patients with the newest combinations [11–13], and from < 5% to 30% in older patients [14, 15].

The first step in curing MM is achieving CR. This has increased to 50–70% with the newest therapeutic strategies [13, 16]. However, a majority of patients relapse, in part due to the persistence of MRD levels. It should be noted that although a patient achieves a CR, more than 10^8 pathologic plasma cells could sometimes persist (Figure 1) [17]. In addition, residual cells have a heterogeneous clonal architecture and undergo evolution, which means that the techniques employed for routine assessment of MRD cannot identify clonal evolution [18–20].

Nevertheless, MM hassingular characteristics confounding some aspects related to response as surrogate markers of survival: 1) some patients who fail to achieve CR have a good outcome, and return to a monoclonal gammopathy of undetermined significance (MGUS) phenotype after treatment [21]; 2) some patients in CR do not sustain CR showing reduced survival [9, 22–24]. In part, this could be due by the fact that the sensitivity of criteria used to define CR may be suboptimal (Figure 2). Despite this limitation, CR in MM is considered as a very good surrogate marker of survival in all clinical situations: both in younger and older patients, after new drug treatment (in several combination) and in relapsed patients [25, 26].

In classifying the response, several efforts have been made to incorporate new categories of deep response. First, stringent CR (sCR), immunophenotypic response (IR) and molecular response (MR) were defined (Table 1) [27, 28]. There is some controversy about the clinical impact of normalizing serum free light chains (sFLC) [29–32]. The first two analyses reflected the benefit in progression free survival of normalizing sFLC within the overall patient population, and not exclusively among patients in CR. The third study, from the Mayo Clinic, showed a significant prolongation of PFS and OS for those patients in CR with a simultaneous normal sFLC ratio, but there was not data about bone morrow (BM) clonality evaluation. However, the study of Martinez-Lopez et al. [32], demonstrated no clinical impact of normalizing the sFLC, contrary to the previous studies. In our opinion, this category of response (sCR) must be revised, and it should not be included in the categories of MRD in multiple myeloma. Also, the new serologic test HevyLite to study heavy light chain pairs, has no role as a technique for MRD assessment, although it could improve the definition of CR [33].

Several studies have demonstrated the use of multicolor flow cytometry (MFC) in detecting MRD in the bone marrow and showed that

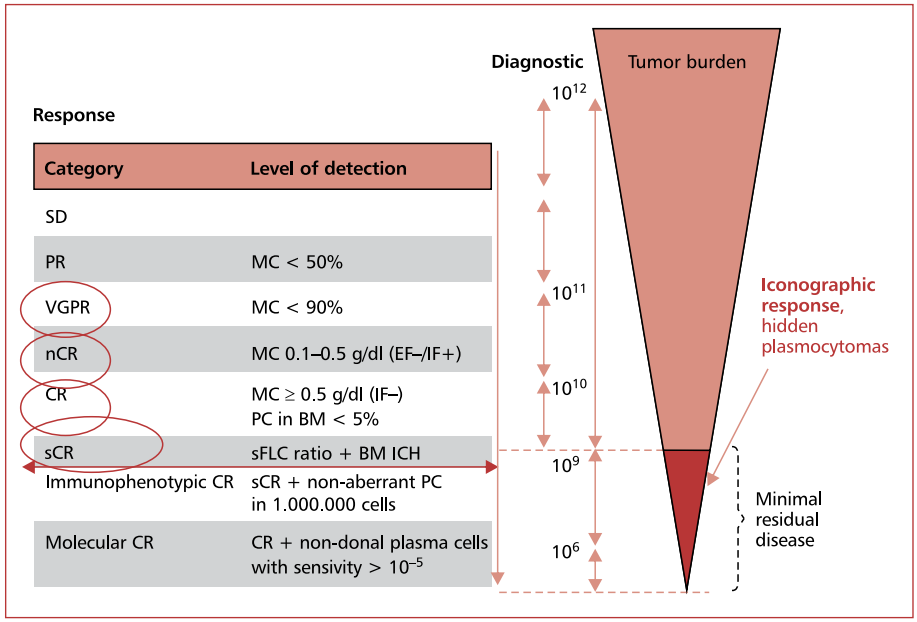

Figure 1. Response categories in multiple myeloma regarding tumor burden (based o n Rajkumar SV et al. *Blood* 2011, Durie B et al. *Leukemia 2006*, Blade J et al. *Br J Haematol.* 1998); SD — stable disease; PR — partial remission; MC — mast cell; VGPR — very good partial response; nCR — near complete remision; sFLC — serum free light chains; PC — plasma cells; BM — bone morrow; sCR — stringent complete remision; – — negative ; + — positive

Rycina 1. Zależność między uzyskaną kategorią odpowiedzi w szpiczaku plazmocytowym a liczbą pozostałych po leczeniu komórek nowotworowych (na podstawie Rajkumar S.V. i wsp. *Blood* 2011, Durie B. i wsp. *Leukemia 2006*, Blade J. i wsp. *Br. J. Haematol.* 1998); SD — stabilizacja choroby; PR — remisja częściowa; MC — komórki tuczne; VGPR — bardzo dobra odpowiedź częściowa; nCR — prawie całkowita remisja; sFLC — wolne łańcuchy lekkie w surowicy; PC — komórki plazmatyczne; BM — szpik kostny; sCR – przekonująca całkowita remisja; – — ujemne; + — dodatnie

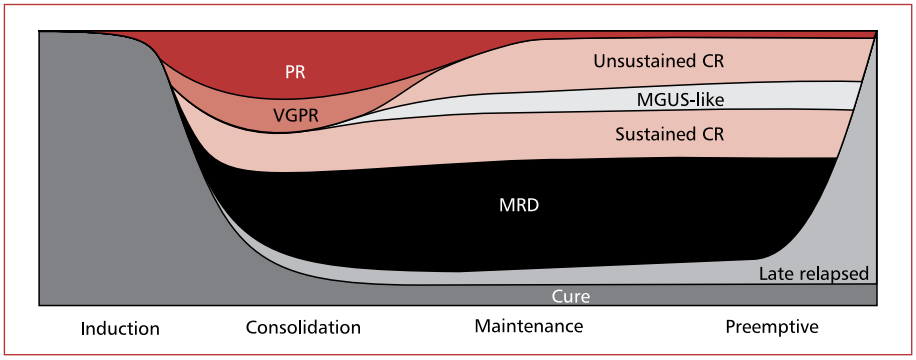

Figure 2. Dynamic evolution of the different patterns of response in multiple myeloma; PR — partial remission; CR — complete remision; VGPR — very good partial response; MGUS — monoclonal gammopathy of undetermined significance; MR — molecular response

Rycina 2. Ewolucja szpiczaka plazmocytowego w zależności od głębokości uzyskanej odpowiedzi na leczenie; PR — remisja częściowa; CR — całkowita remisja; VGPR — bardzo dobra odpowiedź częściowa; MGUS — gammapatia monoklonalna o nieokreślonym znaczeniu; MR — odpowiedź molekularna

MRD was one of the most important predictors of outcome [6, 8, 23, 34–37]. Of note, in all these studies, three to six-colour MFC approaches with a sensitivity of one in 10^4 myeloma cells were used. Moreover, although MRD evaluation by allele specific oligonucleotide – quantitative polymerase

Table 1. Response criteria for multiple myeloma are given according to the International Myeloma Working Group guidelines (source [28])

Tabela 1. Kryteria odpowiedzi na leczenie szpiczka plazmocytowego według wytycznych Międzynarodowej Grupy Roboczej ds. Szpiczaka (źródło [28])

CR	Stringent complete response (sCR)	Immunophenotypic CR	Molecular CR
Negative immunofixation of serum and urine, *and*	CR as defined, *plus*	Stringent CR *plus*	CR plus negative ASO-PCR, sensitivity 10^{-5}
Disappearance of any soft tissue plasmacytomas, *and*	Normal FLC ratio *and*	Absence of phenotypically aberrant PCs (clonal) in BM with a minimum of 1 mln total BM cells analyzed by multiparametric flow cytometry (with > 4 colors)	
< 5% PC in bone marrow	Absence of clonal PC by immunohistochemistry or 2- to 4-color flow cytometry		

CR — complete remission; sCR — stringent complete response; ASO-PCR— allele specific oligonucleotide polymerase chain reaction; PC — plasma cells; BM — bone marrow

Table 2. Categories of minimal residual disease (MRD) responses according to the International Myeloma Working Group (IMWG) guidelines (source [28])

Tabela 2. Kategorie odpowiedzi uwzględniające minimalną chorobę resztkową (MRD) według wytycznych Międzynarodowej Grupy Roboczej ds. Szpiczaka (IMWG) (źródło [28])

Sustained MRD-negative	MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (e.g. MRD-negative at 5 years)
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Imaging plus MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue

NGF — next generation flow; NGS — next generation sequencing; DNA — deoxyribonucleic acid; PET — positron emission tomography; CT — computed tomography

chain reaction (ASO-qPCR) is a very sensitive (one in 10^5 myeloma cells) and specific approach, it is only applicable in a low proportion of patients with MM due to technical limitations [27, 38]. Thus, it was important to define new response categories to identify deeper responses in MM patients as the ones proposed lately by the International Myeloma Working Group. (Table 2) [28]. The new criteria include a more sensitive molecular complete response to detect very low levels of pathologic plasma cells, supporting the use of several methodologies and techniques for MRD assessment in MM. In this way different high-sensitivity quantification methods are being developed and improved using flow cytometry, gene sequencing, and sensitive imaging approaches [37, 39–42].

In which patients and when should MRD be tested?

Most studies of MRD have been carried out in young patients after transplantation with or without novel agents [7, 8, 10, 43–50]. Recently, several studies in older patients treated with new drugs, particularly bortezomib after induction therapy have been published [6, 9, 39, 51].

There are only two studies analyzing the effect of maintaining MRD levels [8, 52]. The first employing thalidomide as maintenance, demonstrated that 28% of MRD-positive patients who received maintenance thalidomide became MRD negative, and only a 3% in the non-maintenance group. The second employing lenalidomide in a small

number of patients which improved response in eight patients (27%), and four (13%) became MRD negative [8, 52].

Although achieving CR after the first relapse is unlikely (less than 10%); there is only one study in this situation which found that those patients, who achieved MRD negativity after first relapse had longer progression free survival in a small series of patients [53]. So, in patients in relapse with CR, performing MRD could be indicated.

Which patients should be studied for MRD? It does not make sense to study patients who do not achieve CR, if there is already measurable disease. However, some studies found that a small fraction of patients in near CR (nCR) could achieve the status of MRD negative (5–7%) [6, 39]. If this small number of patients is analyzed, most of them achieve CR in a few months, this phenomenon could be due to the slow clearance of monoclonal immunoglobulins.

As an exception, there is a small group of patients who have an oligoclonal pattern in the immunofixation tests after therapy. In these patients, it is very difficult to determine CR status and they would therefore be candidates for the study and assessment of MRD [54].

Based on this evidence, we would recommend performing MRD studies in MM after transplantation in young patients in CR and in older patients in CR after regimens including proteasome inhibitors. At this moment, we would encourage studying the effect of maintenance and consolidation on MRD levels.

What is the optimal location to study MRD?

Although bone marrow BM infiltration in MM is patchy; BM aspiration has been the location classically used in MM to assess MRD. BM assessment has its pitfalls: 1) the pattern of BM infiltration in MM is not uniform, so the possibility of residual MM plasma cells in another region different from the one analyzed cannot be excluded (false negative results); 2) only BM is analyzed, thus extra-medullary relapses are not assessed. Nevertheless, MM is a bone marrow disease and pathological plasma cells are mostly in this niche.

In addition, to solve BM limitations, the use of imaging techniques to study hidden plasmocytomas has been postulated [55]. A study suggests that normalization of PET-CT receiving novel-agent based therapy can predict outcome in young MM patients [56, 57]. At this moment, bone marrow is the only recommended location to assess MRD in MM.

What is the recommended method of MRD assessment?

The two classical methods used for MRD assessment are multiparametric flow cytometry (MFC) and deep sequencing techniques. Other techniques such as serologic analysis of immunoglobulins: Hevylite or sFLC are not sensitive enough to be considered MRD techniques. What should be the ideal method for MRD assessment? 1) universally applicable; 2) easy to do; 3) minimally invasive; 4) cheap, and 5) fast results. However, at present such ideal technique does not exist.

Multiparametric flow cytometry

Detecting phenotypically aberrant clonal PCs through MFC can be performed in > 95% of MM patients, and with multidimensional staining (≥ 8--colors). It does not require information on the diagnostic samples, although this may be helpful. From a clinical point of view, achieving an immunophenotypic CR (CR plus no residual aberrant plasma cells in 10,000 normal cells; sensitivity of 10^{-4}) predicts extended survival both in young patients receiving intensive therapy and elderly patients treated with novel agents [6–8, 37, 58]. Patient risk-stratification can be further improved by combining cytogenetic baseline evaluation plus MRD monitoring in order to identify those cases at risk of unsustained CR [23]. Conventional flow cytometry has two particular disadvantages: limited sensitivity compared to molecular techniques, and lack of standardization [59]; however, novel multidimensional (digital ≥ 8--color) flow is already monitoring MRD levels in the same sensitivity range as ASO-PCR (10^{-5}), and current efforts by the EuroFlow consortium [60] and the Black Swan Research Initiative promoted by the International Myeloma Foundation are aiming to develop a fully automated MRD immunophenotypic method (Table 1). Recently, an ultrasensitive flow cytometry methodology has been published, termed next regeneration flow cytometry that reached sensitivity of 10^{-5}. This methodology is based on: automated analysis, eight color flow cytometers and studying more than 5 million cells of the same sample.

Gene sequencing

Since MM does not have a specific molecular marker, analyzing MRD relies on studying

immunoglobulin gene rearrangements. Using this strategy and following the recommendations of the BIOMED concert action, it is possible to identify a molecular marker in most of the 90% of MM patients. There are three major techniques to analyze immunoglobulin gene rearrangements: 1) fluorescent PCR (F-PCR) using family primers of immunoglobulin genes that, despite low sensitivity (10^{-3}), identifies patients in CR with longer survival [9]; 2) ASO-PCR has been the most employed molecular technique to define molecular response in MM, and most studies have shown that achieving complete molecular response improves progression free survival [38, 45, 46, 61], 3) high throughput sequencing or next generation sequencing (NGS). However, two first methods have important limitations such as low applicability (around 37–70%) [61] complexity, expertise needed, specific reagents and cost. These drawbacks prevent their use in the clinical setting. A promising novel methodology for analyzing immunoglobulin genes is high throughput sequencing; it has superior applicability compared to ASO-PCR (> 90% of patients) [62], it is highly sensitive — even 10^{-6} depending of the quality of the sample — and adequately stratifies patients with longer survival [39, 55, 63, 64]. However, it also has some pitfalls such as availability, lack of experience and cost (Table 1).

Based on the experience of Spanish Myeloma Group, ASO-qPCR or F-PCR of immunoglobulin genes should no longer be used. High-sensitive MFC and NGS of immunoglobulin genes will certainly be widely employed in the future. MFC or NGS will be used depending on the experience of each center and the possibility of study samples in the first 24 hours for MFC analysis. For clinical trials and studies for MRD assessment, we would recommend at this moment using both methods in parallel.

Summary

In conclusion MRD should be considered as a therapeutic objective. However, there is enough evidence to take clinical decisions depending on MRD status, and for this reason we would encourage the design of clinical studies to address such questions.

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References

1. O'Brien SG, Guilhot F, Larson RA, et al. IRIS Investigators. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003; 348(11): 994–1004, doi: 10.1056/NEJMoa022457, indexed in Pubmed: 12637609.
2. Leung W, Pui C, Coustan-Smith E, et al. Detectable minimal residual disease before hematopoietic cell transplantation is prognostic but does not preclude cure for children with very-high-risk leukemia. Blood. 2012; 120(2): 468–472, doi: 10.1182/blood-2012-02-409813, indexed in Pubmed: 22517895.
3. Bader P, Kreyenberg H, von Stackelberg A, et al. Monitoring of minimal residual disease after allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia allows for the identification of impending relapse: results of the ALL-BFM-SCT 2003 trial. J Clin Oncol. 2015; 33(11): 1275–1284, doi: 10.1200/JCO.2014.58.4631, indexed in Pubmed: 25605857.
4. Brüggemann M, Raff T, Kneba M. Has MRD monitoring superseded other prognostic factors in adult ALL? Blood. 2012; 120(23): 4470–4481, doi: 10.1182/blood-2012-06-379040, indexed in Pubmed: 23033265.
5. Campana D, Leung W. Clinical significance of minimal residual disease in patients with acute leukaemia undergoing haematopoietic stem cell transplantation. Br J Haematol. 2013; 162(2): 147–161, doi: 10.1111/bjh.12358, indexed in Pubmed: 23654352.
6. Paiva B, Martinez-Lopez J, Vidriales MB, et al. Comparison of immunofixation, serum free light chain, and immunophenotyping for response evaluation and prognostication in multiple myeloma. J Clin Oncol. 2011; 29(12): 1627–1633, doi: 10.1200/JCO.2010.33.1967, indexed in Pubmed: 21402611.
7. Paiva B, Vidriales MB, Cerveró J, et al. GEM (Grupo Español de MM)/PETHEMA (Programa para el Estudio de la Terapéutica en Hemopatías Malignas) Cooperative Study Groups. Multiparameter flow cytometric remission is the most relevant prognostic factor for multiple myeloma patients who undergo autologous stem cell transplantation. Blood. 2008; 112(10): 4017–4023, doi: 10.1182/blood-2008-05-159624, indexed in Pubmed: 18669875.
8. Rawstron AC, Child JA, de Tute RM, et al. Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: impact on outcome in the Medical Research Council Myeloma IX Study. J Clin Oncol. 2013; 31(20): 2540–2547, doi: 10.1200/JCO.2012.46.2119, indexed in Pubmed: 23733781.
9. Martinez-Lopez J, Fernández-Redondo E, García-Sánz R, et al. GEM (Grupo Español Multidisciplinar de Melanoma)/PETHEMA (Programa para el Estudio de la Terapéutica en Hemopatías Malignas) cooperative study group. Clinical applicability and prognostic significance of molecular response assessed by fluorescent-PCR of immunoglobulin genes in multiple myeloma. Results

from a GEM/PETHEMA study. Br J Haematol. 2013; 163(5): 581–589, doi: 10.1111/bjh.12576, indexed in Pubmed: 24117042.
10. Ladetto M, Pagliano G, Ferrero S, et al. Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma. J Clin Oncol. 2010; 28(12): 2077–2084, doi: 10.1200/JCO.2009.23.7172, indexed in Pubmed: 20308672.
11. Lahuerta JJ, Mateos MV, Martínez-López J, et al. Influence of pre- and post-transplantation responses on outcome of patients with multiple myeloma: sequential improvement of response and achievement of complete response are associated with longer survival. J Clin Oncol. 2008; 26(35): 5775–5782, doi: 10.1200/JCO.2008.17.9721, indexed in Pubmed: 19001321.
12. Jakubowiak AJ, Dytfeld D, Griffith KA, et al. A phase 1/2 study of carfilzomib in combination with lenalidomide and low-dose dexamethasone as a frontline treatment for multiple myeloma. Blood. 2012; 120(9): 1801–1809, doi: 10.1182/blood-2012-04-422683, indexed in Pubmed: 22665938.
13. Jakubowiak AJ, Dytfeld D, Griffith KA, et al. A phase 1/2 study of carfilzomib in combination with lenalidomide and low-dose dexamethasone as a frontline treatment for multiple myeloma. Blood. 2012; 120(9): 1801–1809, doi: 10.1182/blood-2012-04-422683, indexed in Pubmed: 22665938.
14. San Miguel JF, Schlag R, Khuageva NK, et al. VISTA Trial Investigators. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008; 359(9): 906–917, doi: 10.1056/NEJMoa0801479, indexed in Pubmed: 18753647.
15. Hernández JM, García-Sanz R, Golvano E, et al. Randomized comparison of dexamethasone combined with melphalan versus melphalan with prednisone in the treatment of elderly patients with multiple myeloma. Br J Haematol. 2004; 127(2): 159–164, doi: 10.1111/j.1365-2141.2004.05186.x, indexed in Pubmed: 15461621.
16. Rosiñol L, Oriol A, Teruel AI, et al. Programa para el Estudio y la Terapéutica de las Hemopatías Malignas/Grupo Español de Mieloma (PETHEMA/GEM) group. Superiority of bortezomib, thalidomide, and dexamethasone (VTD) as induction pretransplantation therapy in multiple myeloma: a randomized phase 3 PETHEMA/GEM study. Blood. 2012; 120(8): 1589–1596, doi: 10.1182/blood-2012-02-408922, indexed in Pubmed: 22791289.
17. Hauwel M, Matthes T. Minimal residual disease monitoring: the new standard for treatment evaluation of haematological malignancies? Swiss Med Wkly. 2014; 144: w13907, doi: 10.4414/smw.2014.13907, indexed in Pubmed: 24452390.
18. Melchor L, Brioli A, Wardell CP, et al. Single-cell genetic analysis reveals the composition of initiating clones and phylogenetic patterns of branching and parallel evolution in myeloma. Leukemia. 2014; 28(8): 1705–1715, doi: 10.1038/leu.2014.13, indexed in Pubmed: 24480973.
19. Walker BA, Wardell CP, Melchor L, et al. Intraclonal heterogeneity is a critical early event in the development of myeloma and precedes the development of clinical symptoms. Leukemia. 2014; 28(2): 384–390, doi: 10.1038/leu.2013.199, indexed in Pubmed: 23817176.
20. van Rhee F, Giralt S, Barlogie B. The future of autologous stem cell transplantation in myeloma. Blood. 2014; 124(3): 328–333, doi: 10.1182/blood-2014-03-561985, indexed in Pubmed: 24894774.
21. Paiva B, Vídriales MB, Rosiñol L, et al. Grupo Español de MM/Programa para el Estudio de la Terapéutica en Hemopatías Malignas Cooperative Study Group. A multiparameter flow cytometry immunophenotypic algorithm for the identification of newly diagnosed symptomatic myeloma with an MGUS-like signature and long-term disease control. Leukemia. 2013; 27(10): 2056–2061, doi: 10.1038/leu.2013.166, indexed in Pubmed: 23743858.
22. Kapoor P, Kumar SK, Dispenzieri A, et al. Importance of achieving stringent complete response after autologous stem-cell transplantation in multiple myeloma. J Clin Oncol. 2013; 31(36): 4529–4535, doi: 10.1200/JCO.2013.49.0086, indexed in Pubmed: 24248686.
23. Paiva B, Gutiérrez NC, Rosiñol L, et al. PETHEMA/GEM (Programa para el Estudio de la Terapéutica en Hemopatías Malignas/Grupo Español de Mieloma) Cooperative Study Groups. 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): 687–691, doi: 10.1182/blood-2011-07-370460, indexed in Pubmed: 22128143.
24. Barlogie B, Anaissie E, Haessler J, et al. Complete remission sustained 3 years from treatment initiation is a powerful surrogate for extended survival in multiple myeloma. Cancer. 2008; 113(2): 355–359, doi: 10.1002/cncr.23546, indexed in Pubmed: 18470907.
25. Martinez-Lopez J, Blade J, Mateos MV, et al. Grupo Español de MM, Programa para el Estudio de la Terapé utica en Hemopatía Maligna. Long-term prognostic significance of response in multiple myeloma after stem cell transplantation. Blood. 2011; 118(3): 529–534, doi: 10.1182/blood-2011-01-332320, indexed in Pubmed: 21482708.
26. San Miguel JF, Schlag R, Khuageva NK, et al. Persistent overall survival benefit and no increased risk of second malignancies with bortezomib-melphalan-prednisone versus melphalan-prednisone in patients with previously untreated multiple myeloma. J Clin Oncol. 2013; 31(4): 448–455, doi: 10.1200/JCO.2012.41.6180, indexed in Pubmed: 23233713.
27. Rajkumar SV, Harousseau JL, Durie B, et al. International Myeloma Workshop Consensus Panel 1. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel 1. Blood. 2011; 117(18): 4691–4695, doi: 10.1182/blood-2010-10-299487, indexed in Pubmed: 21292775.
28. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol. 2016; 17(8): e328–e346, doi: 10.1016/S1470-2045(16)30206-6, indexed in Pubmed: 27511158.
29. Giarin MM, Giaccone L, Sorasio R, et al. Serum free light chain ratio, total/ratio, and immunofixation results are not prognostic factors after stem cell transplantation for newly diagnosed multiple myeloma. Clin Chem. 2009; 55(8): 1510–1516, doi: 10.1373/clinchem.2009.124370, indexed in Pubmed: 19520760.
30. Hari P, Pasquini MC, Logan BR, et al. Immunoglobulin free light chain (FLC) and heavy chain/light chain (HLC) assays — comparison with electrophoretic responses in multiple myeloma (MM). Blood. 2011; 118(21): 2877.
31. Kapoor P, Gertz MA, Dispenzieri A, et al. importance of achieving sustained stringent complete response (sCR) following autologous stem cell transplantation in multiple myeloma. Blood. 2012; 120(21): 1988.
32. Martínez-López J, Paiva B, López-Anglada L, et al. Spanish Multiple Myeloma Group/Program for the Study of Malignant Blood Diseases Therapeutics (GEM/PETHEMA) Cooperative Study Group. Critical analysis of the stringent complete response in multiple myeloma: contribution of sFLC and bone marrow clonality. Blood. 2015; 126(7): 858–862, doi: 10.1182/blood-2015-04-638742, indexed in Pubmed: 26089396.

33. Ludwig H, Slavka G, Hubl W, et al. Usage of HLC-ratio, FLC--ratio, ife, PBMC infiltration and isotype suppression at best response reveals isotype suppression as most powerful parameter for identification of multiple myeloma patients with long survival. Blood. 2012; 120(21): 1817.
34. Kumar S, Flinn I, Richardson PG, et al. Randomized, multicenter, phase 2 study (EVOLUTION) of combinations of bortezomib, dexamethasone, cyclophosphamide, and lenalidomide in previously untreated multiple myeloma. Blood. 2012; 119(19): 4375–4382, doi: 10.1182/blood-2011-11-395749, indexed in Pubmed: 22422823.
35. Rawstron AC, Fazi C, Agathangelidis A, et al. A complementary role of multiparameter flow cytometry and high-throughput sequencing for minimal residual disease detection in chronic lymphocytic leukemia: an European Research Initiative on CLL study. Leukemia. 2016; 30(4): 929–936, doi: 10.1038/leu.2015.313, indexed in Pubmed: 26639181.
36. Paiva B, Almeida J, Pérez-Andrés M, et al. Utility of flow cytometry immunophenotyping in multiple myeloma and other clonal plasma cell-related disorders. Cytometry B Clin Cytom. 2010; 78(4): 239–252, doi: 10.1002/cyto.b.20512, indexed in Pubmed: 20155853.
37. Paiva B, Cedena MT, Puig N, et al. Grupo Español de Mieloma/Programa para el Estudio de la Terapéutica en Hemopatías Malignas (GEM/PETHEMA) Cooperative Study Groups. Minimal residual disease monitoring and immune profiling in multiple myeloma in elderly patients. Blood. 2016; 127(25): 3165–3174, doi: 10.1182/blood-2016-03-705319, indexed in Pubmed: 27118453.
38. Sarasquete ME, García-Sanz R, González D, et al. Minimal residual disease monitoring in multiple myeloma: a comparison between allelic-specific oligonucleotide real-time quantitative polymerase chain reaction and flow cytometry. Haematologica. 2005; 90(10): 1365–1372, indexed in Pubmed: 16219573.
39. Martinez-Lopez J, Lahuerta JJ, Pepin F, et al. Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. Blood. 2014; 123(20): 3073–3079, doi: 10.1182/blood-2014-01-550020, indexed in Pubmed: 24646471.
40. Fraioli F, Punwani S. Clinical and research applications of simultaneous positron emission tomography and MRI. Br J Radiol. 2014; 87(1033): 20130464, doi: 10.1259/bjr.20130464, indexed in Pubmed: 24234585.
41. Martinez-Lopez J, Sanchez-Vega B, Barrio S, et al. Analytical and clinical validation of a novel in-house deep-sequencing method for minimal residual disease monitoring in a phase II trial for multiple myeloma. Leukemia. 2017; 31(6): 1446–1449, doi: 10.1038/leu.2017.58, indexed in Pubmed: 28210002.
42. Wale A, Pawlyn C, Kaiser M, et al. Frequency, distribution and clinical management of incidental findings and extramedullary plasmacytomas in whole body diffusion weighted magnetic resonance imaging in patients with multiple myeloma. Haematologica. 2016; 101(4): e142–e144, doi: 10.3324/haematol.2015.139816, indexed in Pubmed: 26819048.
43. Corradini P, Voena C, Tarella C, et al. Molecular and clinical remissions in multiple myeloma: role of autologous and allogeneic transplantation of hematopoietic cells. J Clin Oncol. 1999; 17(1): 208–215, doi: 10.1200/JCO.1999.17.1.208, indexed in Pubmed: 10458235.
44. Davies FE, Forsyth PD, Rawstron AC, et al. The impact of attaining a minimal disease state after high-dose melphalan and autologous transplantation for multiple myeloma. Br J Haematol. 2001; 112(3): 814–819, doi: 10.1046/j.1365-2141.2001.02530.x, indexed in Pubmed: 11260088.
45. Galimberti S, Benedetti E, Morabito F, et al. Prognostic role of minimal residual disease in multiple myeloma patients after non-myeloablative allogeneic transplantation. Leuk Res. 2005; 29(8): 961–966, doi: 10.1016/j.leukres.2005.01.017, indexed in Pubmed: 15978948.
46. Korthals M, Sehnke N, Kronenwett R, et al. The level of minimal residual disease in the bone marrow of patients with multiple myeloma before high-dose therapy and autologous blood stem cell transplantation is an independent predictive parameter. Biol Blood Marrow Transplant. 2012; 18(3): 423–431.e3, doi: 10.1016/j.bbmt.2011.07.002, indexed in Pubmed: 21745451.
47. 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. 2013; 28(6): 1299–1307, doi: 10.1038/leu.2013.375, indexed in Pubmed: 24342950.
48. Martínez-Sánchez P, Montejano L, Sarasquete ME, et al. Evaluation of minimal residual disease in multiple myeloma patients by fluorescent-polymerase chain reaction: the prognostic impact of achieving molecular response. Br J Haematol. 2008; 142(5): 766–774, doi: 10.1111/j.1365-2141.2008.07263.x, indexed in Pubmed: 18637804.
49. Puig N, Sarasquete ME, Balanzategui A, 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): 391–397, doi: 10.1038/leu.2013.217, indexed in Pubmed: 23860448.
50. Putkonen M, Kairisto V, Juvonen V, et al. Depth of response assessed by quantitative ASO-PCR predicts the outcome after stem cell transplantation in multiple myeloma. Eur J Haematol. 2010; 85(5): 416–423, doi: 10.1111/j.1600-0609.2010.01510.x, indexed in Pubmed: 20722702.
51. Mateos M, et al. V., Martinez-Lopez J., Hernandez M.-T. Comparison of sequential vs alternating administration of bortezomib, melphalan, prednisone (VMP) and lenalidomide plus dexamethasone (Rd) in elderly pts with newly diagnosed multiple myeloma (MM) patients: GEM2010MAS65 Trial. Blood. 2014; 124(21): 178–178.
52. Roussel M, Lauwers-Cances V, Robillard N, et al. Front-line transplantation program with lenalidomide, bortezomib, and dexamethasone combination as induction and consolidation followed by lenalidomide maintenance in patients with multiple myeloma: a phase II study by the Intergroupe Francophone du Myélome. J Clin Oncol. 2014; 32(25): 2712–2717, doi: 10.1200/JCO.2013.54.8164, indexed in Pubmed: 25024076.
53. Paiva B, Chandia M, Puig N, et al. The prognostic value of multiparameter flow cytometry minimal residual disease assessment in relapsed multiple myeloma. Haematologica. 2014; 100(2): e53–e55, doi: 10.3324/haematol.2014.115162, indexed in Pubmed: 25381128.
54. Zent CS, Wilson CS, Tricot G, et al. Oligoclonal protein bands and Ig isotype switching in multiple myeloma treated with high--dose therapy and hematopoietic cell transplantation. Blood. 1998; 91(9): 3518–3523, indexed in Pubmed: 9558413.
55. Korde N, Mailankody S, Roschewski M, et al. Minimal residual disease (MRD) testing in newly diagnosed multiple myeloma (MM) patients: a prospective head-to-head assessment of cell--based, molecular, and molecular-imaging modalities. Blood. 2014; 124(21): 2105–2105.
56. Moreau P, Attal M, Caillot D, et al. Prospective evaluation of magnetic resonance imaging and [(18)F]fluorodeoxyglucose positron emission tomography-computed tomography at diagnosis and before maintenance therapy in symptomatic patients with multiple myeloma included in the IFM/DFCI 2009 trial: results

of the IMAJEM study. J Clin Oncol. 2017; 35(25): 2911–2918, doi: 10.1200/JCO.2017.72.2975, indexed in Pubmed: 28686535.
57. Zamagni E, Patriarca F, Nanni C, et al. Prognostic relevance of 18-F FDG PET/CT in newly diagnosed multiple myeloma patients treated with up-front autologous transplantation. Blood. 2011; 118(23): 5989–5995, doi: 10.1182/blood-2011-06-361386, indexed in Pubmed: 21900189.
58. Rawstron AC, Gregory WM, de Tute RM, et al. Minimal residual disease in myeloma by flow cytometry: independent prediction of survival benefit per log reduction. Blood. 2015; 125(12): 1932–1935, doi: 10.1182/blood-2014-07-590166, indexed in Pubmed: 25645353.
59. Flanders A, Stetler-Stevenson M, Landgren O. Minimal residual disease testing in multiple myeloma by flow cytometry: major heterogeneity. Blood. 2013; 122(6): 1088–1089, doi: 10.1182//blood-2013-05-506170, indexed in Pubmed: 23929839.
60. van Dongen JJM, Lhermitte L, Böttcher S, et al. EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708). EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia. 2012; 26(9): 1908–1975, doi: 10.1038/leu.2012.120, indexed in Pubmed: 22552007.
61. Puig N, Sarasquete ME, Balanzategui A, 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): 391–397, doi: 10.1038/leu.2013.217, indexed in Pubmed: 23860448.
62. Ladetto M, Bruggemann 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, doi: 10.1038/leu.2013.375, indexed in Pubmed: 24342950.
63. Takamatsu H, Murata R, Zheng J, et al. Prognostic value of sequencing-based minimal residual disease detection in multiple myeloma. Blood. 2014; 124(21): 2003–2003.
64. Jasielec J, Dytfeld D, Griffith KA, et al. Minimal residual disease status predicts progression-free survival in newly diagnosed multiple myeloma (NDMM) patients treated with carfilzomib, lenalidomide, and low-dose dexamethasone (KRd). Blood. 2014; 124(21): 2127–2127.