International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma

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Treatment of multiple myeloma has substantially changed over the past decade with the introduction of several classes of new effective drugs that have greatly improved the rates and depth of response. Response criteria in multiple myeloma were developed to use serum and urine assessment of monoclonal proteins and bone marrow assessment (which is relatively insensitive). Given the high rates of complete response seen in patients with multiple myeloma with new treatment approaches, new response categories need to be defined that can identify responses that are deeper than those conventionally defined as complete response. Recent attempts have focused on the identification of residual tumour cells in the bone marrow using flow cytometry or gene sequencing. Furthermore, sensitive imaging techniques can be used to detect the presence of residual disease outside of the bone marrow. Combining these new methods, the International Myeloma Working Group has defined new response categories of minimal residual disease negativity, with or without imaging-based absence of extramedullary disease, to allow uniform reporting within and outside clinical trials. In this Review, we clarify several aspects of disease response assessment, along with endpoints for clinical trials, and highlight future directions for disease response assessments.

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Introduction

The treatment landscape for multiple myeloma has been radically transformed during the past decade by the introduction of several new drugs with different mechanisms of action, which has led to improved survival for patients with multiple myeloma.[1,2] Progress has been made in other areas, including an improved understanding of disease biology, enhanced diagnostic criteria, availability of sensitive and specific tools for disease prognostication, increasingly effective treatment strategies, and enhanced supportive care.[3–10] The most recent iteration of the response criteria was developed in 2006[11] by the International Myeloma Working Group (appendix). Response evaluation in multiple myeloma has traditionally been based on the assessment of serum and urine monoclonal protein concentrations via protein electrophoresis or immunofixation, or both, as a surrogate for tumour burden, allowing for the detection of trace amounts of paraprotein.[11] The response criteria for multiple myeloma have evolved considerably since then with the substitution of monoclonal protein concentrations for synthetic rates and the use of different cutoffs for monoclonal protein concentrations, as well as inclusion of serum free light chain (sFLC) values for the assessment of oligo-secretory myeloma. Traditional quantitation of bone marrow plasma cells was performed on trephine biopsies (with a combination of haemotoxylin and eosin stains and immunohistochemistry) or bone marrow aspirates (with or without clot section). The importance of bone marrow plasma-cell quantitation for accurate response assessment (even in patients with negative serum and urine immunofixation) has been confirmed.[12] The original definition of a complete response only required bone marrow with less than 5% plasma cells, irrespective of their clonal nature.[11] The definition was further refined to stringent complete response, by the addition of the sFLC assay plus immunohistochemical clonal assessment on the trephine biopsy.[11,13] Additional clarifications, especially with respect to the use of sFLC, were introduced during the International Myeloma Workshop in 2011.[14]

The consensus criteria were uniformly incorporated into clinical trials, allowing improved comparison of different drugs, drug combinations, and treatment strategies, and the revisions over the years have allowed them to remain applicable despite advances in treatment. With older therapies, including autologous stem-cell transplantation (ASCT), less than half of patients achieve a complete response.[15,16] With the introduction of more effective multidrug combinations in the past 15 years, especially when used with ASCT, post-transplant consolidation, and prolonged maintenance therapy, nearly all patients achieve a treatment response, with more than 50% of these patients reaching a complete response in some studies.[17–21] Frustratingly, most patients relapse despite achieving such deep responses, reflecting a persistent disease that cannot be detected with the recommended disease evaluation techniques. Consequently, new methods are urgently required to detect and quantify the level of minimal residual disease beyond the detection of the present clinical response criteria, and the definition of

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See **Online** for appendix

disease response needs to be revised for it to evolve with the changing treatment framework. In this Review, we report the new International Myeloma Working Group consensus criteria for redefining disease response with a particular emphasis on the definitions and methods to assess minimal residual disease.

Depth of response and long-term outcome

The association between depth of response and long-term outcomes is a hotly debated topic in multiple myeloma. This debate has been particularly contentious for complete response, which has been generally considered as the deepest response level and a surrogate for improved outcome after any given treatment.[22–25] The relationship between complete response and progression-free survival, or time-to-progression, has been more consistent than the relationship between complete response and overall survival. This association is frequently seen in cancer therapy and is probably caused by multiple factors, including interactions between disease biology, different treatment strategies after reaching complete response, and the true depth of response beyond the conventional (and low-sensitive) approaches defining complete response after different therapies.[26] Nevertheless, a meta-analysis reported a significant correlation between the achievement of a complete response and improved overall survival in eight out of ten studies.[27] Several studies using sensitive new techniques have been able to demonstrate the presence of minimal residual disease that is not detected by current complete response (and stringent complete response) evaluation methods in a large proportion of patients.[28–30] The level of minimal residual disease, undetected by conventional methods, is probably one of the most important features contributing to the link between the depth of response and long-term outcomes. Independent of the method used to define minimal residual disease (cell-based, molecular-based, or imaging-based), previous studies consistently show that among patients who achieve a complete response, minimal residual disease-positive cases consistently have an inferior progression-free survival than minimal residual disease-negative patients.[28–35] Given the substantial proportion of patients achieving a complete response with current therapies, response criteria need to be expanded to define minimal residual disease accurately for patients with multiple myeloma.[35]

Detection of minimal residual disease in bone marrow

Bone marrow examination has been the cornerstone of disease assessment in the absence of a measurable monoclonal protein in the serum or urine, whether this represents non-secretory disease or complete response to therapy (ie, complete response or stringent complete response).[11,12] Increasingly, sensitive assays have been adopted for the evaluation of bone marrow aspirates, including multiparametric flow cytometry (MFC), allele-specific oligonucleotide (ASO)-qPCR and next-generation sequencing of VDJ sequences, in an effort to increase the sensitivity of the detection of multiple myeloma cells.[28,32,33,36–40] Such methods allow the quick examination of several hundreds of thousands to millions of bone marrow cells (or the corresponding amount of DNA) per assay and can provide a quantitative assessment of any residual tumour cells in the bone marrow.

MFC methods for minimal residual disease detection

First-generation methods

MFC is now a key tool in the management of haematological malignancies, and improvements in technology have increased the number of fluorochromes that can be used simultaneously and the number of cells that can be interrogated. This advance allows a large number of cell types, or different characteristics of the same cell type, to be studied concurrently in a fast and efficient way.[41,42] Although MFC-based assessment of bone marrow has been done in multiple myeloma for a number of years, the technology has only recently gained wide acceptance in the past decade for routine testing of patients with multiple myeloma.[32,36,43–45] MFC is now an integral part of laboratory investigations and the management of plasma-cell disorders, and can play an important part in the diagnosis, prognostic stratification, and monitoring of response to therapy via minimal residual disease detection,[28–30,32–34,46,47] the understanding of the biology of disease progression,[26,48–50] the study of the role of the tumour microenvironment in plasma cell disorders,[51] and the identification of potential therapeutic targets on the malignant plasma cell.[43,52] Many surface markers have been described for the identification of plasma cells and for distinguishing multiple myeloma plasma cells from normal plasma cells. The most commonly used surface markers used for discriminating and categorizing normal and multiple myeloma plasma cells include CD138, CD38, CD45, CD56, CD19, and cytoplasmic κ and λ immunoglobulin light chains. Additional markers, many of which are aberrantly expressed on multiple myeloma plasma cells, are also of value and include CD20, CD27, CD28, CD81, CD117, and CD200.[53] Other markers that are being studied include CD54, CD229, CD319, and VS38c, some of which could help with plasma cell recognition in patients undergoing therapy with monoclonal antibodies against CD38 or CD138. However, in view of the heterogeneity of expression of these markers and differences in both the number of events studied and in the analytical strategies used, substantial confusion and inconsistent clinical interpretation of results from different studies has occurred.[54] Attempts have been made to develop consensus guidelines to standardise the MFC-based assessment of disease in multiple myeloma and other plasma cell-related disorders.[36,41,55]

Several studies have demonstrated the use of MFC in the detection of minimal residual disease in the bone marrow (table 1). In a study of flow-based minimal residual disease assessment in multiple myeloma, Rawstron and colleagues[46] used a sensitive MFC assay that quantified normal and neoplastic plasma cells in the bone marrow of 45 patients who received ASCT. Monoclonal plasma cells were detectable 3 months after ASCT in 19 (42%) of 45 patients, in whom the median progression-free survival was 20 months compared with 35 months for those with undetectable multiple myeloma plasma cells. The sensitivity of the flow assay was highlighted by the presence of detectable plasma cells in nearly a third of the patients with negative immunofixation results and patients who were minimal residual disease-positive had a worse outcome. San Miguel and colleagues[45] reported almost identical results. Subsequently, larger prospective studies have reproduced these initial observations. The Spanish Myeloma Group (PETHEMA/GEM) used four-colour flow cytometry to study minimal residual disease in 295 patients newly diagnosed with multiple myeloma receiving uniform treatment including ASCT, and showed that minimal residual disease was one of the most important predictors of outcome.[28] Minimal residual disease negativity at day 100 after ASCT correlated with improved progression-free survival and overall survival and, furthermore, the effect of minimal residual disease negativity was equally relevant among patients that had achieved a conventional complete response. Similarly, Rawstron and colleagues[32] evaluated the role of six-colour MFC in the assessment of minimal residual disease at various stages of therapy in patients with newly diagnosed multiple myeloma enrolled on the MRC IX clinical trial. Among patients undergoing an ASCT, absence of minimal residual disease at day 100 was associated with statistically significantly improved progression-free survival, irrespective of cytogenetics or achievement of a complete response. Paiva and colleagues[30] studied a series of 241 patients enrolled in the Spanish GEM2000 and GEM2005MENOS65 studies. They identified the best independent predictors of early relapse after achieving a complete response were persistent minimal residual disease, using four-colour flow cytometry at day 100 after ASCT, presence of baseline high-risk cytogenetics, by use of fluorescence in-situ hybridisation.[30] Early relapse after achieving a complete response was associated with very poor survival in this group of patients, as was previously reported by Barlogie and colleagues.[56] These results again highlight the close association between disease biology and depth of response after therapy in determining long-term outcomes, but also highlight the immediate identification of patients with imminent relapse and poor survival (≤2 years). Of note, in all these studies, three six-colour MFC approaches with a sensitivity of one in 10^4 myeloma cells were used. The Intergroupe Francophone du Myelome reported on

	Disease status and treatment	N	MRD-negative patients	Outcomes
Paiva et al[28]	Patients with newly diagnosed multiple myeloma from GEM2000*. MRD status by MFC was determined at day 100 after ASCT	295	125 (42%)	PFS (median 71 months *vs* 37 months; p<0·001) and OS (median not reached *vs* 89 months; p=0·002) were longer in patients who were MRD-negative at day 100 after ASCT
Rawstron et al[32]	MRC IX trial of newly diagnosed multiple myeloma: intensive pathway with CTD *vs* CVAD followed by ASCT	397	246 (62%)	Median PFS for MRD-positive patients of 15·5 months *vs* 28·6 months for MRD-negative patients (p<0·001). Median OS of 59·0 months in MRD-positive patients *vs* 80·6 months in MRD-negative patients (p=0·02)
	MRC IX trial of newly diagnosed multiple myeloma: non-intensive pathway (melphalan and prednisone *vs* CTD)	245	37 (15%)	MRD-positive at end of induction associated with non-significantly inferior PFS (median 7·4 months *vs* 10·5 months, p=0·1)
Puig et al[33]	GEM2000*and GEM2005MENOS65† trials	102	52 (51%)	MRD-negative patients had longer PFS, both in intensively treated patients (median 45 months *vs* 27 months, p=0·02) and in non-intensively treated patients (not reached *vs* 27 months; p=0·002)
Sarasquete et al[39]	Patients with multiple myeloma who had achieved a complete response after transplantation	24	13 (53%)	Improved PFS for MRD-negative patients (median 27 months *vs* 10 months; p=0·05)
Paiva et al[29]	Transplant-ineligible patients with multiple myeloma who had achieved >75% reduction in the myeloma component after induction	102	31 (30%)	Achieving MRD-negativity translated into superior PFS and TTP compared with conventional complete response or stringent complete response (without clonality assessment on trephine biopsy)
Paiva et al[30]	Newly diagnosed patients with multiple myeloma from GEM2000* and GEM2005MENOS65† who achieved a complete response at day 100 after ASCT	241	154 (64%)	Presence of baseline high-risk cytogenetics and persistent MRD at day 100 after ASCT were the only independent factors that predicted unsustained complete response
Roussel et al[56]	Phase 2 study with three induction cycles followed by ASCT, consolidation, and 1-year lenalidomide maintenance	31	21 (68%)	Estimated 100% relapse-free survival at 3 years for MRD-negative patients

N=total number of patients. MRD=minimal residual disease. MFC=multiparametric flow cytometry. ASCT=autologous stem-cell transplantation. PFS=progression-free survival. OS=overall survival. CTD=cyclophosphamide, thalidomide, dexamethasone. CVAD=cyclophosphamide, vincristine, doxorubicin, dexamethasone. TTP=time to progression. *Vincristine, carmustine, melphalan, cyclophosphamide, and prednisone (VBMCP)/vincristine, carmustine, doxorubicin, dexamethasone (VBAD) induction plus ASCT. †Transplant-eligible patients: VBMCP/VBAD plus bortezomib in the last two cycles, thalidomide/dexamethasone or bortezomib/thalidomide/dexamethasone immediately after diagnosis; elderly patients: six induction cycles with bortezomib, melphalan, prednisone or bortezomib, thalidomide, prednisone.

***Table 1:* Studies using conventional flow cytometry-based assays for minimal residual disease detection**

a phase 2 study of 31 patients treated with three induction cycles of lenalidomide, bortezomib, dexamethasone (RVD) followed by cyclophosphamide harvest, ASCT, and then two RVD consolidation cycles followed by 1 year of lenalidomide maintenance.[57] Overall, 18 (58%) of 31 patients achieved a complete response, with 21 (68%) being minimal residual disease-negative as measured by MFC. With a median follow-up of 39 months, the estimated 3-year progression-free survival for the whole patient series was 77% and overall survival was 100%. None of the patients who achieved minimal residual disease negativity relapsed afer a median of 39 months.

The advances in MFC technology that allow interrogation of several million cells have significantly improved the sensitivity of the assay, particularly when combined with the use of eight or more colours or markers for increased specificity. Current consensus indicates that such approaches are optimally suited for minimal residual disease testing of multiple myeloma.[53] In addition, MFC-based assessment of the post-therapy bone marrow provides important information regarding the immune-cell profile, which can provide additional prognostic information. A report[58] from the PETHEMA group showed that normal plasma-cell recovery and normal B-cell maturation was associated with improved survival outcomes irrespective of the minimal residual disease status.

Next-generation flow

Attempts to standardise and automate readouts for MFC make it a potentially attractive test for sensitive, routine detection of minimal residual disease in the bone marrow compartment.[59,60] However, to have a uniform MFC-based minimal residual disease response criteria, consensus in the way minimal residual disease is evaluated will be mandatory. Accordingly, a concerted effort has been made to standardise the flow-based approaches and remove subjectivity by defining reagent characteristics, defining the acquisition and plasma-cell identification variables, and by introducing novel common data analysis tools.[41,42]

The current EuroFlow next-generation flow method for minimal residual disease detection in multiple myeloma relies on two eight-colour combinations that combine surface antigens for the identification of phenotypically aberrant clonal plasma cells and cytoplasmic κ and λ light-chain expression to confirm their clonality.[30] The technique has been modified to include an initial bulk lysis step to consistently measure more than 5×10^6 leucocytes per tube.[59,60] The EuroFlow group has also developed software algorithms for automated identification of clonal plasma cells (ie, minimal residual disease) in multiple myeloma samples. This two-tube next-generation flow approach has now been extensively validated (ie, >1000 minimal residual disease samples). It is very robust and improves reliability, consistency, and sensitivity because of the acquisition of a greater number of cells. The eight-colour technology is widely available globally and the next-generation flow method has already been fully adopted by multiple flow laboratories.

The complete eight-colour method can be done using individual antibodies or made more efficient by using a lyophilised mixture of the required antibodies, which reduces errors, time, and costs. Ongoing quality-control assessment is required for all laboratories reporting minimal residual disease flow results. Use of the automated software package is ideal because it makes the method user independent, identifies and counts all bone marrow cell subsets in addition to plasma cells, and reinforces the adoption of standard operation procedures for accurate and automated readouts of patient samples. Although many advantages are lost when not using this software, the method can remain satisfactory when adequately validated with quality controls.

One of the most attractive features of the eight-colour method is its balance between effectiveness (ie, sensitivity plus specificity) and wide availability, because eight-colour instruments are commonly used in many hospitals. To improve efficiency and to reduce costs, alternate single-tube ten-colour and 14-colour methods have been suggested by some centres. The single-tube approach will undergo detailed cross-validation with reference to the next-generation flow method to allow for standardisation. Documentation of cross-validation with reference next-generation flow, ongoing quality-control assessment, routine assessment of more than 5 million mononuclear cells to estimate minimal residual disease, and a sensitivity of one in 10^5 cells or higher is needed to fulfil the criteria for the next-generation flow method.

Molecular methods for minimal residual disease detection

ASO-qPCR

Another method that has been studied extensively in the past is ASO-qPCR, and it has been compared head-to-head with MFC assays (table 2). Use of ASO-qPCR to identify clonal multiple myeloma plasma-cell-specific immunoglobulin heavy chain (*IGH*) gene rearrangements allows the detection of very low levels of multiple myeloma plasma cells with a sensitivity that can detect one in 10^5 cells. Therefore, unlike the early PCR methods that were qualitative and semi-quantitative, ASO-qPCR provides an accurate quantification of minimal residual disease. ASO-qPCR involves making primers complementary to the junctional region of the rearranged *IGH* genes, which are used to interrogate bone marrow samples at different times to determine the response depth. This step requires availability of the baseline diagnostic sample. Bakkus and colleagues[65] examined the usefulness of using an ASO-qPCR assay at 3–6 months post-ASCT to detect minimal residual disease in 67 patients. By using specific thresholds to define the quantitative PCR results, the authors identified patients

with minimal residual disease positivity and, subsequently, short time to relapse. Lipinski and co-workers[66] retrospectively analysed the tumour load in bone marrow samples from 13 patients at the time of remission after ASCT and at the time of progression using ASO-qPCR. Progression was detected earlier with this method than with serum monoclonal protein estimation, showing the higher sensitivity of the ASO-qPCR technique. Galimberti and colleagues[67] examined the prognostic value of PCR-based monitoring of minimal residual disease in 20 patients after ASCT versus non-myeloablative allogeneic transplantation. After ASCT, only three patients (15%) achieved PCR negativity, whereas 12 (60%) were negative after allogeneic transplantation. At 2 years, 15 (75%) of 20 minimal residual disease-negative patients were still alive compared with five (25%) of 20 minimal residual disease-positive cases. In another study, 130 newly diagnosed patients with multiple myeloma from the GEM2000/GEM2005 trials who achieved a very good partial response to induction therapy were studied using multiplex PCR for *IGH D-J*, *IGK V-J*, and κ-deleting element rearrangements, at baseline and after induction therapy.[68] 64 (48%) of 120 minimal residual disease-negative patients had an improved median progression-free survival compared with minimal residual disease-positive patients (61 months *vs* 36 months; p=0·001), and improved median overall survival (not reached *vs* 66 months; p=0·03). Puig and colleagues[33] compared minimal residual disease status using ASO-qPCR versus four-colour MFC in a large series of 170 patients from different clinical trials who achieved at least a partial response after treatment. The authors found a significant correlation in predicting minimal residual disease between the two techniques (r=0·881; p<0·001), with minimal residual disease-negativity, using either method, predicting better progression-free and overall survival. However, more than half the patients could not be evaluated by the molecular approach either due to the inability to detect a clone, unsuccessful sequencing, or suboptimal ASO-qPCR performance. These technical limitations are in part due to the presence of multiple somatic mutations in the immunoglobulin genes. In these cases, primers and probes that are adapted to each patient to match the somatic hypermutations are needed. This will be particularly important in bone marrow baseline samples with relatively low levels of plasma-cell infiltration. Production of specific primers and probes has not been done consistently in the reported studies, leading to suboptimal results in the identification and sensitivity of identified targets. In view of the substantial proportion of patients with unsuccessful PCR-based minimal residual disease estimation, the same investigators examined the capacity of CD138 selection to increase the proportion of informative patients by comparing CD138-positive selected samples with paired unselected bone marrow samples.[69] Within the CD138-positive selected group, *VDJH* rearrangements were detected in all 25 cases (100%), compared with the control samples in which *VDJH* rearrangements were detected in 19 (76%) of 25 cases. After sequencing, 24 (96%) of 25 cases within the CD138 selected group had a PCR target for minimal residual disease detection compared with only 15 (60%) of 25 cases in the control group. Despite minimal residual disease

	Disease status and treatment	N (total)*	MRD-negative patients	Outcomes
Puig et al[33]	GEM2000† and GEM05‡ trials	103 (170)	47%	MRD-negative patients had significantly longer PFS, both in the intensively treated patient group (median 54 months *vs* 27 months; p=0·001) and in the non-intensively treated group (median not reached *vs* 31 months; p=0·029)
Korthals et al[61]	Induction: 2–4 cycles of idarubicin and dexamethasone followed by ASCT	53 (70)	49%	Median EFS in the low-MRD group was significantly longer than in the high-MRD group (35 months *vs* 20 months; p=0·001). Overall survival was significantly longer for the low-MRD group (70 months *vs* 45 months; p=0·04)
Putkonen et al[62]	Patients with multiple myeloma who had achieved a complete response/near to complete response after ASCT or SCT	30 (37)	57%	Low/negative-MRD after ASCT or SCT was a significant predictive factor for the prolongation of PFS (median 70 *vs* 19 months; p=0·003)
Martinez-Sanchez et al[38]	Patients enrolled in the GEM2000* protocol	53 (88)	53%	PFS not reached in MRD-negative patients *vs* 31 months for MRD-positive patients (p=0·001)
Ladetto et al[63]	Four cycles of bortezomib, thalidomide, and dexamethasone consolidation after ASCT	39 (112)	18%	Improved PFS; 100% *vs* 77% at 6 months (grouped by median tumour load as detected by allele-specific oligonucleotide qPCR [p=0·02])
Sarasquete et al[39]	Patients with multiple myeloma who had achieved a complete response after transplantation	24 (32)	29%	Improved PFS for MRD-negative patients (median 34 months *vs* 15 months; p=0·04)
Martinelli et al[64]	Patients who achieved a complete response following ASCT or SCT	44 (50)	27%	MRD-negative patients had a significantly lower relapse rate (41% *vs* 16%; p<0·05) and longer relapse-free survival than MRD-positive patients (median 35 months *vs* 110 months; p<0·005)

MRD=minimal residual disease. PFS=progression-free survival. ASCT=autologous stem-cell transplantation. EFS=event-free survival. SCT=allogeneic stem-cell transplantation. *N reflects the number of patients in which allele-specific oligonucleotide qPCR was successfully done. †Vincristine, carmustine, melphalan, cyclophosphamide, and prednisone (VBMCP)/vincristine, carmustine, doxorubicin, dexamethasone (VBAD) induction plus ASCT. ‡Transplant-eligible patients: VBMCP/VBAD plus bortezomib in the last two cycles, thalidomide/dexamethasone or bortezomib/thalidomide/dexamethasone immediately after diagnosis; elderly patients: six induction cycles with bortezomib, melphalan, prednisone or bortezomib, thalidomide, prednisone.

Table 2: **Studies using allele-specific oligonucleotide qPCR-based assay for minimal residual disease detection**

evaluation by ASO-qPCR being a sensitive and specific approach, it is only applicable in a low proportion of patients with multiple myeloma and is more timeconsuming than MFC.[39]

This has been recently reiterated by Drandi and colleagues,[70] who compared qPCR and droplet-digital PCR for minimal residual disease assessment in multiple myeloma, acute lymphocytic leukaemia, and mantle-cell lymphoma. The investigators showed that droplet-digital PCR was less applicable and more labour intensive.

Next-generation sequencing

Next-generation sequencing is of considerable interest for the detection of multiple myeloma minimal residual disease in the bone marrow. Most published data have been generated with the LymphoSIGHT platform (Sequenta Inc, San Francisco, CA, USA), which uses sets of multiple primers for the amplification and sequencing of immunoglobulin gene segments. Specifically, genomic DNA is amplified using locus-specific primers designed for $IGH\text{-}VDJ_H$, $IGH\text{-}DJ_H$, or *IGK*. Once amplified, the immunoglobulin gene DNA is sequenced and the frequencies of the different clonotypes in the sample are determined. To avoid disproportional amplification of the *IGH* and *IGK* rearrangements, the extensive sets of primers need to be attuned and validated to guarantee equal (proportional) amplification of each target rearrangement between the many rearrangements derived from remaining normal B cells. Patients with detectable multiple myeloma clones (>5%) at baseline can then be studied at subsequent timepoints to determine the presence and quantity of that particular clone using sequencing approaches. Ladetto and colleagues[71] compared *IGH* gene-based minimal residual disease detection by ASO-qPCR and next-generation sequencing to assess whether next-generation sequencing could overcome some of the limitations of ASO-qPCR, and further increase its sensitivity and specificity. Clonotypes identified by next-generation sequencing and ASO-qPCR were either identical or more than 97% homologous in 41 (96%) of 43 cases. Both tools had a sensitivity of about one in 10^5 cells, but next-generation sequencing had the added advantage of not requiring patient-specific primers. Previous studies show that next-generation sequencing can achieve a sensitivity of one in 10^6 nucleated cells.[72] Next-generation sequencing, as with other DNA sequence-based approaches, needs a baseline sample to identify tumour-specific sequences.

Martinez-Lopez and colleagues[34] compared next-generation sequencing with first-generation four-colour MFC. Bone marrow samples at baseline and from the time of very good partial response or complete response were studied by next-generation sequencing to identify a tumour clonotype at baseline and then re-evaluated for the presence of the same clonotype in the subsequent sample. Transplantation-ineligible patients were studied at the end of induction therapy, whereas patients who were younger in age were studied at 3 months after ASCT. A dominant multiple myeloma clone could be identified at baseline in 121 (91%) of 133 patients, with $IGH\text{-}VDJ_H$ rearrangements in 84 (69%), $IGH\text{-}DJ_H$ in 66 (55%), and *IGK* in 58 (48%) of patients with a dominant clone. This observation suggests that some clones are missed by the next-generation sequencing approach, most probably because of somatic mutations. Of the 121 patients with an identifiable clonotype at baseline, 110 had follow-up samples taken. Sequencing showed that 80 (73%) remained positive for minimal residual disease, with at least one tumour cell in 10^6 cells. Among the 110 patients who achieved a very good partial response, those who had a minimal residual disease-negative status (more than one tumour cell in 10^5 cells) had a better progression-free survival and overall survival compared with those who were minimal residual disease-positive. Among the group of patients with a complete response, a higher proportion of cases had minimal residual disease-negativity that also associated with improved progression-free survival compared with patients who were minimal residual disease-positive; however, overall survival did not differ significantly. Information on minimal residual disease detection by MFC was available in 99 patients and 41 patients by ASO-qPCR analysis, respectively, and the agreement between sequencing and MFC and ASO-qPCR was 83% and 85%, respectively.[34] Among those with different results, 12 patients were negative by MFC but were positive by sequencing; the five remaining patients had the opposite pattern (MFC-positive/next-generation sequencing-negative). Korde and colleagues[73] also used next-generation sequencing in 43 patients with multiple myeloma treated with carfilzomib, lenalidomide, and dexamethasone, and observed a 12-month progression-free survival for minimal residual disease-negative patients of 100% versus 79% for minimal residual disease-positive patients ($p<0\cdot001$). The IFM2009 trial randomised 700 patients to receive either eight cycles of bortezomib, lenalidomide, and dexamethasone (VRD; arm A), or three VRD cycles plus ASCT followed by two consolidation VRD cycles (arm B).[72] All patients then received lenalidomide maintenance therapy for 12 months. 289 patients were evaluated by next-generation sequencing and 475 patients with MFC before maintenance and 178 by next-generation seqencing and 310 by MFC after completion of maintenance therapy. Minimal residual disease detection by next-generation sequencing was feasible in 266 (92%) of 289 patients with a sensitivity of one tumour cell in 10^6 cells. Among those patients who achieved a complete response, the 3-year progression-free survival was 87% for minimal residual disease-negative patients and 42% for minimal residual disease-positive patients, pre-maintenance therapy. The corresponding numbers were 83% and 30% when minimal residual disease was tested post-maintenance.

A formal comparison with MFC with next-generation sequencing cannot be done given the low sensitivity (one tumour cell in 10^5 cells) for the MFC method used in this study.

Comparison of techniques

As described previously, various techniques have been studied for the detection of minimal residual disease. Each of these techniques (based on the plasma-cell phenotype, or genotype, or both) have advantages and disadvantages that need to be taken into consideration (table 3). The ideal minimal residual disease test should fulfil several relevant characteristics: high applicability (useful in most patients), high sensitivity and specificity, excellent feasibility (result can be obtained in most patients), easily accessible, rapid turnaround, small sample size that can be transported with relative ease, reproducibility, proven clinical value, and cost-effectiveness. A notable disadvantage of the sequence-based approach is the requirement of a baseline sample to identify tumour-specific sequences.

While no currently available tests fully satisfy all these ideal criteria, next-generation sequencing and next-generation flow fulfil most of them and can be translated into an advanced platform that can be uniformly applied across institutions and countries.[34,41,42,71,73] Next-generation sequencing and next-generation flow have been reported to have variable levels of sensitivity. Both methods have the ability to detect one multiple myeloma cell in 10^5–10^6 cells. We strongly encourage the inclusion of both methods in prospective trials, if possible, to find out the advantages and disadvantages of the individual approaches, as well as the sensitivity of detection required in various clinical settings. The purpose of this Review is not to judge the relative merits of the two approaches, or to imply that minimal residual disease assessment is a proven therapeutic goal in multiple myeloma, but to provide clear criteria that can be uniformly applied to and validated in future clinical trials and studies.

Defining a bone marrow minimal residual disease-negative response category

The current proposal builds on the existing International Myeloma Working Group response criteria by adding additional assessment for the detection of minimal residual disease in the bone marrow (table 4). A comprehensive approach to detect very small amounts of disease both inside and outside of the marrow space will require a panel of tests assessing different tumour compartments and probably use different technologies. However, these additional evaluation methods will require more data to show they complement existing methods and their clinical usefulness, to support their inclusion in future iterations of International Myeloma Working Group response criteria. Furthermore, the added criteria should allow researchers to define a response state that reflects a higher degree of tumour eradication than is possible with the current definition of complete response or stringent complete response. At this time, we recommend the use of next-generation sequencing or next-generation flow for the detection of minimal residual disease in the bone marrow based on the availability of the two techniques at each centre and the feasibility for individual clinical trials.

For more on **EuroMRD** see http://www.EuroMRD.org

	Allele-specific oligonucleotide qPCR	MFC	*VDJ* sequencing
Applicability	60–70%	Nearly 100%	≥90%
Need for baseline sample	Yes, requires production of patient-specific probes	Not required; abnormal plasma cells can be identified in any sample by their distinct immunophenotypic pattern *vs* normal plasma cells	Baseline samples required for identification of the dominant clonotype; alternatively, a stored sample from a time point with detectable disease can be used to define baseline status
Sample requirements	<1 million cells	>5 million cells	<1 million cells; higher numbers improve sensitivity
Sample processing	Can be delayed; can use both fresh and stored samples	Needs assessment within 24–48 h; requires a fresh sample	Can be delayed; can use both fresh and stored samples
Sample quality control	Not possible. Additional studies required	Immediate with global bone marrow cell analysis	Not possible. Additional studies required
Sensitivity	≥1 in 10^5	≥1 in10^5	≥1 in 10^5
Information regarding sample composition	No further information available	Detailed information available on leucocyte subsets and their relative distribution	Information about immunoglobulin gene repertoire of B cells in the studied patient samples
Turnaround and complexity	Labour intensive; requires the development of patient-specific primers/probes; can take several days	Can be done in a few hours; automated software available	Can take several days for turnaround; requires intense bioinformatics support. Use of local laboratories could speed up this limitation
Standardisation	Has been done for other diseases (EuroMRD), can be done for myeloma as well	Standardised by the EuroFlow consortium	In process
Availability	Wide*	Most hospitals with four-colour flow cytometry. Eight or more-colour flow cytometry requires more experienced centres/laboratories. Many laboratories have adopted the EuroFlow laboratory protocols and use the EuroFlow MRD tubes	So far limited to one company/platform

*Globally, about 60 MRD laboratories are EuroMRD members and participate twice per year in the external quality assurance rounds. MFC=multiparametric flow cytometry. MRD=minimal residual disease.

Table 3: **Comparison of different bone marrow minimal residual disease assessment techniques**

	Response criteria*
IMWG MRD criteria (requires a complete response as defined below)	
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 (eg, 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-positive 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¶
Standard IMWG response criteria‖	
Stringent complete response	Complete response as defined below plus normal FLC ratio** and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells)††
Complete response	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h
Partial response	≥50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≥90% or to <200 mg per 24 h; If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria; If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50–89%. In addition to the above listed criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required
Stable disease	Not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates. Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease
Progressive disease ¶¶,‖‖	Any one or more of the following criteria: Increase of 25% from lowest confirmed response value in one or more of the following criteria: Serum M-protein (absolute increase must be ≥0·5 g/dL); Serum M-protein increase ≥1 g/dL, if the lowest M component was ≥5 g/dL; Urine M-protein (absolute increase must be ≥200 mg/24 h); In patients without measurable serum and urine M-protein levels, the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg/dL); In patients without measurable serum and urine M-protein levels and without measurable involved FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥10%); Appearance of a new lesion(s), ≥50% increase from nadir in SPD§§ of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis; ≥50% increase in circulating plasma cells (minimum of 200 cells per μL) if this is the only measure of disease

(Table 4 and footnotes continue on the next page)

Accordingly, when minimal residual disease results are reported, the assessment should be qualified by the method(s) used (flow minimal residual disease-negative or sequencing minimal residual disease-negative), and the level of sensitivity (eg, one in 10^5 or one in 10^6 cells). Several ongoing studies are simultaneously testing both methods, which will allow researchers to identify whether both techniques perform equally or whether one approach is better than the other. Alternatively, both methods might be required given the evolving clonal diversity of plasma cells. Further work should be done to establish whether potentially emerging alternative cytometric and sequencing techniques can be standardised and directly compared with the next-generation flow EuroFlow and next-generation sequencing LymphoSIGHT methods.

Detection of extramedullary disease

Present approaches for the detection and measurement of tumour burden after therapy rely on bone marrow assessment. However, bone marrow involvement in multiple myeloma can be heterogeneous, thus increasing the likelihood of a false-negative assessment. Furthermore, such involvement does not allow detection of the disease outside the bone marrow. Extramedullary disease is increasingly seen in the clinic as a result of sensitive imaging studies and extended survival of patients with multiple myeloma. The estimated incidence of clinically detected extramedullary disease among a cohort of patients seen over a 10-year time period was 9%, with high-risk patients having a high risk of extramedullary disease later in the disease course.[74] In the future, these rates might increase as increasingly sensitive imaging technologies and novel biomarkers are used to detect minimal residual disease, and as overall survival continues to increase. This factor is of great relevance when response and disease progression are redefined, and particularly relevant when eradication of minimal residual disease is redefined in the context of new therapies. To ensure complete eradication of the tumour, assessment of the extramedullary compartment will be important as part of the disease assessment in multiple myeloma, particularly for defining high-quality complete response.

(Continued from previous page)

Clinical relapse	Clinical relapse requires one or more of the following criteria: Direct indicators of increasing disease and/or end organ dysfunction (CRAB features) related to the underlying clonal plasma-cell proliferative disorder. It is not used in calculation of time to progression or progression-free survival but is listed as something that can be reported optionally or for use in clinical practice; Development of new soft tissue plasmacytomas or bone lesions (osteoporotic fractures do not constitute progression); Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and ≥1 cm) increase as measured serially by the SPD§§ of the measurable lesion; Hypercalcaemia (>11 mg/dL); Decrease in haemoglobin of ≥2 g/dL not related to therapy or other non-myeloma-related conditions; Rise in serum creatinine by 2 mg/dL or more from the start of the therapy and attributable to myeloma; Hyperviscosity related to serum paraprotein
Relapse from complete response (to be used only if the end point is disease-free survival)	Any one or more of the following criteria: Reappearance of serum or urine M-protein by immunofixation or electrophoresis; Development of ≥5% plasma cells in the bone marrow; Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcaemia see above)
Relapse from MRD negative (to be used only if the end point is disease-free survival)	Any one or more of the following criteria: Loss of MRD negative state (evidence of clonal plasma cells on NGF or NGS, or positive imaging study for recurrence of myeloma); Reappearance of serum or urine M-protein by immunofixation or electrophoresis; Development of ≥5% clonal plasma cells in the bone marrow; Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcaemia)

For MRD assessment, the first bone marrow aspirate should be sent to MRD (not for morphology) and this sample should be taken in one draw with a volume of minimally 2 mL (to obtain sufficient cells), but maximally 4–5 mL to avoid haemodilution. IMWG=International Myeloma Working Group. MRD=minimal residual disease. NGF=next-generation flow. NGS=next-generation sequencing. FLC=free light chain. M-protein=myeloma protein. SPD=sum of the products of the maximal perpendicular diameters of measured lesions. CRAB features=calcium elevation, renal failure, anaemia, lytic bone lesions. FCM=flow cytometry. SUV_{max}=maximum standardised uptake value. MFC=multiparameter flow cytometry. ^{18}F-FDG PET=^{18}F-fluorodeoxyglucose PET. ASCT=autologous stem cell transplantation. *All response categories require two consecutive assessments made any time before starting any new therapy; for MRD there is no need for two consecutive assessments, but information on MRD after each treatment stage is recommended (eg, after induction, high-dose therapy/ASCT, consolidation, maintenance). MRD tests should be initiated only at the time of suspected complete response. All categories of response and MRD require no known evidence of progressive or new bone lesions if radiographic studies were performed. However, radiographic studies are not required to satisfy these response requirements except for the requirement of FDG PET if imaging MRD-negative status is reported. †Sustained MRD negativity when reported should also annotate the method used (eg, sustained flow MRD-negative, sustained sequencing MRD-negative). ‡Bone marrow MFC should follow NGF guidelines.[30] The reference NGF method is an eight-colour two-tube approach, which has been extensively validated. The two-tube approach improves reliability, consistency, and sensitivity because of the acquisition of a greater number of cells. The eight-colour technology is widely available globally and the NGF method has already been adopted in many flow laboratories worldwide. The complete eight-colour method is most efficient using a lyophilised mixture of antibodies which reduces errors, time, and costs. 5 million cells should be assessed. The FCM method employed should have a sensitivity of detection of at least 1 in 10^5 plasma cells. §DNA sequencing assay on bone marrow aspirate should use a validated assay such as LymphoSIGHT (Sequenta). ¶Criteria used by Zamagni and colleagues,[85] and expert panel (IMPetUs; Italian Myeloma criteria for PET Use).[81,97] Baseline positive lesions were identified by presence of focal areas of increased uptake within bones, with or without any underlying lesion identified by CT and present on at least two consecutive slices. Alternatively, an SUV_{max}=2·5 within osteolytic CT areas >1 cm in size, or SUV_{max}=1·5 within osteolytic CT areas ≤1 cm in size were considered positive. Imaging should be performed once MRD negativity is determined by MFC or NGS. ||Derived from international uniform response criteria for multiple myeloma.[11] Minor response definition and clarifications derived from Rajkumar and colleagues.[14] When the only method to measure disease is by serum FLC levels: complete response can be defined as a normal FLC ratio of 0·26 to 1·65 in addition to the complete response criteria listed previously. Very good partial response in such patients requires a ≥90% decrease in the difference between involved and uninvolved FLC levels. All response categories require two consecutive assessments made at any time before the institution of any new therapy; all categories also require no known evidence of progressive or new bone lesions or extramedullary plasmacytomas if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements. Bone marrow assessments do not need to be confirmed. Each category, except for stable disease, will be considered unconfirmed until the confirmatory test is performed. The date of the initial test is considered as the date of response for evaluation of time dependent outcomes such as duration of response. **All recommendations regarding clinical uses relating to serum FLC levels or FLC ratio are based on results obtained with the validated Freelite test (Binding Site, Birmingham, UK). ††Presence/absence of clonal cells on immunohistochemistry is based upon the κ/λ/L ratio. An abnormal κ/λ ratio by immunohistochemistry requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is κ/λ of >4:1 or <1:2. ‡‡Special attention should be given to the emergence of a different monoclonal protein following treatment, especially in the setting of patients having achieved a conventional complete response, often related to oligoclonal reconstitution of the immune system. These bands typically disappear over time and in some studies have been associated with a better outcome. Also, appearance of monoclonal IgG κ in patients receiving monoclonal antibodies should be differentiated from the therapeutic antibody. §§Plasmacytoma measurements should be taken from the CT portion of the PET/CT, or MRI scans, or dedicated CT scans where applicable. For patients with only skin involvement, skin lesions should be measured with a ruler. Measurement of tumour size will be determined by the SPD. ¶¶Positive immunofixation alone in a patient previously classified as achieving a complete response will not be considered progression. For purposes of calculating time to progression and progression-free survival, patients who have achieved a complete response and are MRD-negative should be evaluated using criteria listed for progressive disease. Criteria for relapse from a complete response or relapse from MRD should be used only when calculating disease-free survival. ||||In the case where a value is felt to be a spurious result per physician discretion (eg, a possible laboratory error), that value will not be considered when determining the lowest value.

Table 4: **IMWG criteria for response assessment including criteria for minimal residual disease**

PET/CT scans

Improved imaging techniques have shown that multiple myeloma can be heterogeneous in its distribution pattern. For example, the pattern of bone marrow infiltration by malignant plasma cells can vary between patients and within the same patient. In addition, studies suggest that up to 10% of patients (probably higher with more sensitive technologies) have extramedullary disease with the involvement of soft tissue or major organs at the time of diagnosis and suggest that a high proportion of patients have these findings at the time of disease relapse.[75–80]

^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET is a powerful tool to assess tumour metabolic activity and the effect of therapy on tumour-cell metabolism. Multiple studies support the notion that the detection of PET-positive lesions has prognostic value in patients with multiple myeloma at diagnosis and at time of relapse.[31,81–84] In addition to metabolic assessment, the low-dose CT that is typically done for localisation along with ^{18}F-FDG PET is a sensitive screen for multiple myeloma-associated bone disease. In an initial study,[78] complete ^{18}F-FDG suppression in the focal lesions before first transplantation was associated with better survival outcomes. Another

study[81] showed that persistent ^{18}F-FDG avidity 7 days after the initiation of therapy was associated with worse survival outcomes and was independent of other prognostic factors. In an Italian study,[31] PET/CT was performed at diagnosis, after thalidomide-dexamethasone induction therapy and after double ASCT, in 192 patients newly diagnosed with multiple myeloma. Persistence of maximum standardised uptake values (SUV_{max}) greater than 4·2 after induction therapy predicted an early relapse, and 4-year progression-free survival and overall survival was better for those patients with negative PET/CT at day 100 post-ASCT. PET/CT was negative in 125 (65%) of 192 patients 3 months post-ASCT. 4-year progression-free survival was 47% and overall survival was 79% for PET/CT-negative patients, compared with 32% (p=0·02) progression-free survival and 66% (p=0·02) overall survival, for PET/CT-positive patients. The Italian group presented updated results from their study,[85] including 282 patients with newly diagnosed multiple myeloma who had PET imaging at baseline. After treatment, PET negativity was achieved in 132 (70%) of 189 patients, whereas conventionally defined complete response was achieved in 104 (55%) patients. Among the proportion of patients who achieved a complete response, 30 (29%) had positive PET scans and lower progression-free survival (median 44 months *vs* 84 months, p=0·0009) and overall survival (5-year estimate of 70% *vs* 90%, p=0·0032) compared with those with a positive PET/CT. In this study, persistence of SUV_{max} higher than 4·2 was the only factor independently associated with skeletal progression in the absence of conventional measures of disease progression. The IFM2009 trial[86] showed a clear value for PET imaging in response assessment in myeloma. In this trial, 134 patients had a PET/CT scan and MRI (spine and pelvis) at study entry, at 3 months, and before maintenance therapy. MRI of the spine and pelvis and whole-body PET/CT were equally effective in the detection of bone involvement in symptomatic patients at diagnosis. The median number of focal lesions detected by PET/CT was three (range 0 to more than ten lesions), with a median SUV_{max} of 4·1 (range 1·5–28·4). Normalisation of the PET/CT was noted in 43 (32%) of 134 patients after three cycles of induction, and this group had improved progression-free survival compared with those with positive PET/CT; however, overall survival did not significantly differ. Normalisation of PET was seen in 83 (62%) of 134 patients before maintenance and progression-free survival and overall survival were improved. The results of this study show the value of PET scanning in assessing treatment response during therapy in patients with multiple myeloma.

MRI

MRI examination is a sensitive method to detect bone marrow infiltration by multiple myeloma cells before bone destruction is present and detectable by conventional radiographs.[9,87] The role of MRI—both limited to the spine and whole-body approaches—has been studied extensively in the setting of symptomatic and asymptomatic patients with multiple myeloma. Walker and colleagues[88] studied 611 patients given different total therapy protocols, 452 (74%) of whom had focal lesions detected by baseline MRI that correlate with known prognostic factors in multiple myeloma. Hillengass and colleagues[89] compared conventional treatment response in 100 patients with multiple myeloma with whole-body MRI before and after ASCT. Good concordance was noted between serological response and changes in imaging. In this study, the number of focal lesions present on post-therapy MRI was informative for survival outcomes. Data from the IFM2009 trial[86] demonstrated equivalent efficacy for MRI and PET in the detection of bone lesions at diagnosis. MRI normalisation was noted in a small number of patients (four [3%] after three cycles of induction and 15 [11%] before maintenance), and did not translate into any improvement in progression-free survival or overall survival in this study. The usefulness of MRI for the assessment of residual disease after therapy remains unclear at this time due to the lack of sufficient data.[90]

Defining an imaging response category

Improvement of the limits of disease detection with available technologies will also require evaluation of disease outside the bone marrow. Present data favour the use of ^{18}F-FDG PET. One study examined the diagnostic efficacy of whole-body MRI versus ^{18}F-FDG PET in 31 patients after stem-cell transplantation.[87] In this study, 104 lesions were detected in 21 patients: PET/CT had a lower sensitivity than MRI (50·0% *vs* 80·0%), a higher specificity (85·7% *vs* 38·1%), a higher positive predictive value (62·5% *vs* 38·1%), a lower negative predictive value (78·3% *vs* 80·0%), and was more accurate overall for the determination of remission status (74·2% *vs* 51·6%). While some studies suggest that MRI is more sensitive in picking up lesions at the time of initial evaluation, ^{18}F-FDG PET has distinct advantages for follow-up evaluation. Metabolic changes on ^{18}F-FDG PET can detect early responses, but MRI responses are usually delayed as marrow signal abnormalities can take a long time to resolve depending on the size of the lesion.[84,91,92] MRI also has a low specificity in the differentiation of viable disease from bone remodelling compared with ^{18}F-FDG PET.[93,94] However, for minimal residual disease monitoring (in which ^{18}F-FDG uptake is important rather than lytic bone lesion detection), both false-negative and false-positive results (in case of other coexisting infectious or inflammatory processes) may be seen. Data from the IFM2009 trial[86] have shed some light on the additive value of imaging-based and marrow-based assessments of minimal residual disease. Among the 134 patients assessed by PET at various stages of therapy,

results of minimal residual disease by flow cytometry were available in 86 patients. Progression-free survival was improved for the 41 patients who had negative bone marrow PET results compared with those patients who had positive results using either or both methods.

The data available from these studies show an inferior outcome for patients with positive PET scans even in those who achieved deep responses, highlighting the relevance of this assessment method in patients with myeloma. PET/CT has become standard for response assessment in lymphomas, where baseline scans, interval scans during treatment, and end of treatment scans are integrated into the response criteria. A specific five-point scoring system has been developed to standardise the scoring of images to define response on serial scans (Deauville criteria).[95] In the present criteria, we have defined the imaging response stringently as the disappearance of every area of increased tracer uptake found at baseline, or a preceding PET/CT; or a decrease to less than the mediastinal blood pool SUV; or a decrease to less than that of surrounding normal tissue. These criteria are analogous to what has been used in lymphoma in which a complete metabolic response has been defined as a score of one or two on the five-point scale. Response assessments should be conservative, because myeloma remains incurable and use of these criteria in prospective clinical trials should not lead to the undertreatment of patients. Future prospective trials will allow fine tuning of the cutoffs used for defining absence of disease on PET imaging.

Many questions remain incompletely answered—eg, how many flow minimal residual disease-negative or molecular minimal residual disease-negative patients are imaging positive? In which patients should clinicians be particularly aware of the potential for extramedullary disease? Do investigators need the same imaging technique at baseline and after treatment to evaluate metabolic response? Should treatment (consolidation/maintenance) be tailored on imaging-defined minimal residual disease? For example, extramedullary relapses are likely even among minimal residual disease-negative patients after ASCT suggesting that, at least for this particular therapeutic strategy, response assessment might benefit from combined medullary and extramedullary (PET/CT) measure of minimal residual disease.[96] In turn, standardised interpretation of imaging techniques remains a challenge. Several attempts to standardise criteria for PET/CT imaging definitions and the use of semi-quantitative SUV evaluations are now ongoing to consolidate the use of this technique as a prognostic tool.[97] New imaging technologies such as PET/MRI have been introduced. PET in combination with MRI is a novel and promising method, in which the PET detects active focal lesions, while the MRI shows the location of the lesions and provides information on myeloma-cell infiltration of the bone marrow.[98] By substitution of the CT component in PET/CT, MRI not only provides the anatomical localisation, but also brings two active modalities into a single study, with relatively short acquisition time without compromising on the imaging quality and avoiding the radiation exposure associated with CT. The results of a study[99] that compared PET/CT and functional MRI—namely, diffusion-weighted imaging—as a whole-body protocol in a small group of patients with multiple myeloma, showed that diffusion-weighted imaging is superior in detecting focal and diffuse infiltration of the bone marrow. Further studies should investigate which imaging technique or which combination brings the most final benefit for patients with multiple myeloma in initial investigations and response assessment.

Special considerations based on therapy

Monoclonal antibodies are a promising area for the treatment of multiple myeloma, and several will be available in the clinic in the future. Use of monoclonal antibodies can present unique challenges for clinical response assessment techniques. These challenges include interference with the monoclonal protein assessment on serum protein electrophoresis, or immunofixation, and with MFC-based assessment of monoclonal plasma cells in the bone marrow aspirates.

The monoclonal antibodies that have been approved, as well as those in clinical development, can be detected on the immunofixation assays currently used in the clinic for the detection of small amounts of monoclonal protein. This factor is important because complete response is defined as the complete disappearance of the monoclonal protein on serum and urine immunofixation. When the infused monoclonal antibody shares the same isotype as the monoclonal multiple myeloma protein, low levels of the therapeutic antibody can lead to a false-positive immunofixation result, potentially under-reporting the drug's depth of response.[100,101] Anti-idiotype antibodies that bind the offending drug and alter its migration out of the range of the endogenous M-protein, allow confirmation of interference on serum immunofixation and protein electrophoresis, and assays based on this strategy are being developed for mitigation of this problem.[102] To help with this issue, mass spectrometry-based techniques that enable the discrimination of different proteins based on their masses are being developed.[103,104] Although confirmation of serological complete response might not alter treatment decisions in day-to-day practice, these endpoints are key in the clinical trial setting; therefore, reflex testing to distinguish between the monoclonal protein and the therapeutic antibody in patients who are immunofixation positive only should be mandatory in clinical trial settings.

The therapeutic approach taken can also have an effect on minimal residual disease testing by MFC. CD38 is a critical surface marker that is extensively used for the

identification of plasma cells by flow cytometry, and the use of anti-CD38 antibodies can potentially interfere with the flow cytometry-based assay. To this end, specific CD38 antibody clones or reagents, together with the most sensitive CD138-fluorochrome conjugates, such as those validated and incorporated in the current EuroFlow 2 tube eight-colour panel (eg, the CD38 multiclone and CD138-BV421 reagents), will allow for a treatment-independent minimal residual disease assay with the greatest sensitivity and specificity. By contrast, next-generation sequencing is not affected by monoclonal antibody-based treatments. Other promising therapies that are currently going through clinical trials include chimeric antigen receptor T cells, which can influence the immune-cell types and may require additional strategies that are yet to be defined.

Updated consensus response criteria

The present iteration of the International Myeloma Working Group consensus response criteria has been crucial in light of the progress witnessed over the past decade in the development of new drugs and treatment approaches, including high-dose therapy, consolidation, and maintenance approaches. Ambiguities and nuances have become apparent in these criteria as they are used in multicentre clinical trials performed across different geographical regions, with highly effective treatment regimens, including drugs with new methods of action. Uniform response criteria should not only be used across all clinical trials, but they should also be uniformly interpreted and applied. To provide a clear approach to the application of the response criteria, we have incorporated many practical clarifications in the current consensus criteria (table 4). We hope that this will serve as a practical guide for investigators and pharmaceutical companies involved in clinical trials for multiple myeloma.

Baseline measurements and required testing during follow-up

In addition to tumour burden-based response assessment, other laboratory measurements have been incorporated into the current response criteria to define a category of clinical progression. This categorisation is particularly important as oncologists increasingly encounter oligo-secretory disease or non-secretory disease in patients who had measurable levels of monoclonal protein at the time of diagnosis. While we believe that this situation reflects clonal evolution of the multiple myeloma cells, the precise mechanisms remain poorly understood. Thus, guidelines that reflect functional consequences of disease progression such as haemoglobin, renal function, and serum calcium need to be followed closely. Table 5 defines the required baseline and ongoing testing in patients with multiple myeloma that are key for appropriate application of the consensus criteria. The panel provides guidance on commonly observed situations in patients enrolled in clinical trials. Definitions for time-to-event endpoints can be found in a previous publication.[14] We propose to redefine disease-free progression using minimal residual disease rather than complete response: duration from the start of minimal residual disease

	Every response assessment timepoint (every cycle)	If electrophoresis shows no measurable protein	At suspected CR	At suspected progression (clinical or biochemical)
SPEP (serum M-spike ≥1 g/dL[1])*	X	..	X	X
Serum immunofixation (any)	..	X	X	X
UPEP (urine M-spike ≥200 mg/24 h)	X	..	X	X
Urine immunofixation (any)	..	X	X	..
Serum FLC				
Serum M-spike <1 g/dL, urine M-spike <200 mg/24 h, but involved immunoglobulin FLC is ≥10 mg/dL	X	..	X	X
Any	..	..	X	X
Bone marrow aspirate/ biopsy				
Serum M-spike, urine M-spike, or involved immunoglobulin FLC not meeting above criteria but bone marrow plasma cell percentage ≥30%	X (to be done every three or four cycles till a plateau or complete response, or as clinically indicated and then at suspected progression)	..	X	..
Any	..	..	X	..
Plasmacytoma (PET imaging)				
Serum M-spike, urine M-spike, involved Ig FLC or bone marrow not meeting above criteria, but at least one lesion that has a single diameter of ≥2 cm	X (to be done every three or four cycles till a plateau or complete response, or as clinically indicated, and then at suspected progression)	..	X	..
Any	..	..	X	..
Haemoglobin, serum calcium, creatinine (any)	X	..	..	X

SPEP=serum protein electrophoresis. UPEP=urine protein electrophoresis. FLC=free light chain. IMWG=International Myeloma Working Group. X=test performed. ··=test not performed. *A baseline M-spike of ≥0·5 g/dL is acceptable if very good partial response or higher is the response endpoint to be measured and in situations where progression-free survival or time to progression are the endpoints of interest.

Table 5: **Required baseline and follow-up tests for response assessment using IMWG consensus criteria**

Panel: Practical considerations for application of IMWG consensus criteria

- If partial or minimal response rate is an endpoint, patients must have measurable disease at baseline, within the window defined by the study protocol; if multiple measurements are available, the measurement closest to cycle 1, day 1 will be used as baseline
- If patients do not have measurable disease at baseline they can only be assessed for at least a complete response or progressive disease
- Measurable disease is defined as
  - Serum M-protein ≥1 g/dL
  - Urine M-protein ≥200 mg/24 h
  - Serum FLC assay: involved FLC level ≥10 mg/dL provided serum FLC ratio is abnormal
- Missing serum and/or urine electrophoresis during disease follow-up remains a significant problem. In general, the following considerations will allow a more uniform assessment:
  - In the context of a clinical trial, missing serum or urine electrophoresis, or both, can only be accepted at the discretion of an independent review committee
  - If the immunofixation of the serum or urine is negative at baseline, any lack of follow-up testing of the serum or urine can be accepted at the discretion of the independent review committee
  - Parameters that are considered measurable at baseline (serum and urine, FLC if both serum and urine are not measurable) should be performed at each assessment
  - Urine M-protein is not needed to document partial response or minor response if baseline urine M-protein was not measurable; however, it is still required for complete response and very good partial response
- A plasmacytoma that has been radiated is not suitable for response assessment; however, it must be monitored to assess for progressive disease
- A baseline bone marrow examination must always be attempted; if the patient declines or if the sampling is unsuccessful this must be documented; when bone marrow plasma-cell infiltration is assessed by both bone marrow aspirate and by bone marrow biopsy, the highest value of bone marrow plasma-cell infiltration should be used
- For patients achieving very good partial response by other criteria, a soft tissue plasmacytoma must decrease by more than 90% in the sum of the maximal perpendicular diameter (SPD) compared with baseline
- Single discrepant results can be ignored at the discretion of an independent review committee
- For IgA and IgD myelomas, quantitative immunoglobulin measurements are preferred for disease assessments; the same percentage changes applies as for serum M-spike
- Serum FLC levels should only be used for response assessment when both the serum and urine M-component levels are deemed not measurable
- Documentation of response requires two consecutive readings of the applicable disease parameter (serum M-protein, urine M-protein, or serum FLC), performed at any time (no minimum interval is required, it can be done the same day); however, to confirm response or progressive disease, two discrete samples are required; testing cannot be based upon the splitting of a single sample
- Whenever more than one parameter is used to assess response, the overall assigned level of response is determined by the lower or lowest level of response
- Patients should be categorised as stable disease until they meet criteria for any response category or have progressive disease
- Patients will continue in the last confirmed response category until there is confirmation of progression or improvement to a higher response status; patients cannot move to a lower response category
- If alternate therapy is started before confirming progressive disease any additional testing during subsequent therapy can be used to confirm progressive disease
- The lowest confirmed value before suspected progression will be used as baseline for calculation of progression; if a serum and/or urine spike is considered too low to quantitate, this value can be assigned as zero as a baseline for documentation of subsequent progressive disease
- Any soft tissue plasmacytoma documented at baseline must undergo serial monitoring; otherwise, the patient is classified as inevaluable
- Patients will be considered to have progressive disease if they meet the criteria for progression by a variable that was not considered measurable at baseline; however, for patients who had a measurable serum or urine M-spike at baseline, progression cannot be defined by increases in serum FLC alone
- In patients with two monoclonal protein bands at the start of therapy, the sum of the two spikes should be used for monitoring of disease
- Careful attention should be given to new positive immunofixation results appearing in patients who have achieved a complete response, when the isotype is different, it probably represents oligoclonal immune reconstitution and should not be confused with relapse; these bands typically disappear over time

FLC=free light chain. IMWG=International Myeloma Working Group.

negativity to the time of reappearance of minimal residual disease. In this definition, disease-free survival only applies to patients who are minimal residual disease-negative.

Future directions

The development of an accurate framework for the assessment of minimal residual disease is a work in progress and this report is the first and probably the

most important step in that direction. Ongoing work will continue to define what level of minimal residual disease is clinically relevant and when it should be evaluated. Specific aspects of disease biology will also need to be incorporated into future definitions of the minimal residual disease state (eg, identification of minimal residual disease-positive patients who will nevertheless experience long-term survival).

Detection of minimal residual disease in blood

Clonal plasma cells in multiple myeloma are typically restricted to the bone marrow, although small numbers can be detected by sensitive approaches in the peripheral blood of most patients with newly diagnosed or relapsed multiple myeloma. In both newly diagnosed and relapsed disease, the presence of circulating tumour cells has been associated with shorter progression-free survival and inferior overall survival.[105–107] In a study[106] of 647 consecutive patients with previously treated multiple myeloma who had their peripheral blood evaluated for multiple myeloma plasma cells by MFC, none of the patients who achieved a complete response had circulating plasma cells at the time of initial evaluation at the study site compared with 62 (9·6%) of 647 patients with relapsed disease. Demonstration of absence of multiple myeloma cells in circulation may be important for all patients with multiple myeloma, particularly for those with large numbers of circulating cells at initial evaluation. DNA-sequencing methods have also been applied to detect small numbers of circulating tumour cells in the peripheral blood. In one study, minimal residual disease was assessed in 42 patients undergoing ASCT using circulating DNA in the peripheral blood that was analysed by ASO-qPCR to identify rearranged *IGH* genes.[108] Even though the minimal residual disease level in peripheral blood samples was significantly lower than in bone marrow samples, patients with negative ASO-qPCR results 3 months after ASCT had a longer event-free survival (median 15 months *vs* 4 months; p=0·004) and longer overall survival (median 52 months *vs* 17 months; p=0·03). Importantly, sequential monitoring of clonotypic cells in peripheral blood allowed the early identification of disease relapse. Another study used a sequencing-based method to identify multiple myeloma cells in peripheral blood samples, and was able to detect clones at less than one in a million leucocytes (0·0001%).[109] The authors detected multiple myeloma cells in the peripheral blood in 44 (96%) of 46 patients. Although there was a correlation between multiple myeloma clone levels in paired bone marrow and peripheral blood samples, almost all patients investigated in these studies did not achieve a complete response. Prospective studies should examine the true prognostic value of the detection of multiple myeloma cells in the circulation of patients who achieve a complete response and compare these results to those obtained in paired bone marrow samples before these methods can be adopted.

Ongoing studies[110–113] are examining the assessment of circulating tumour DNA as a sensitive measure of small amounts of residual cells. In addition to quantification, assessment of circulating tumour DNA levels could allow investigators to track individual tumour clones. The sensitivity of blood for the evaluation of minimal residual disease remains unknown and the development of peripheral blood-based monitoring should be the ultimate goal as it would allow for serial sampling without the trauma of repeated bone marrow aspirations.

Hevylite assay

In conjunction with the International Myeloma Working Group response criteria, the ability to quantitate free immunoglobulin light chains greatly enhanced oncologists' ability to detect deeper responses and to define stringent complete response. The development of antibodies against conjunction epitopes between the light and heavy chains enables the quantitation of specific pairs of heavy/light chains (IgGκ/IgGλ, IgAκ/IgAλ, and IgMκ/IgMλ) in the serum and is the basis of the Hevylite assay (Binding Site, Birmingham, UK). The Hevylite assay provides information on both the involved immunoglobulin (eg, IgGκ in an IgGκ patient) and the polyclonal non-involved pair (eg, IgGλ in an IgGκ patient). The Hevylite assay is useful in patients with oligo-secretory disease and can overcome limitations associated with monitoring β-migrating monoclonal IgA by electrophoresis. Studies have also indicated a role of the Hevylite assay in minimal residual disease assessment. Increased IgAκ/IgAλ and IgMκ/IgMλ ratios of the uninvolved isotype were associated with longer progression-free survival compared with normal ratios.[114] This probably reflects the degree of immune recovery post-ASCT, which could enhance the capacity to immunologically control the disease for longer. Unlike the other tests described so far, heavy/light chain ratios could reflect a functional consequence of minimal residual disease negativity on the recovery of normal B cells and plasma cells, in addition to the quantitative estimate of residual disease. In most cases, responses assigned by the Hevylite assay have shown to be equivalent to those assigned by conventional methods. In some cases, however, heavy/light chain ratios provided additional sensitivity. Ludwig and colleagues[115] studied sequential sera of 156 patients with IgG or IgA multiple myeloma comparing the heavy/light chain measurements with conventional assays such as serum protein electrophoresis, immunofixation, nephelometry, and sFLC tests. When both heavy/light chain and sFLC testing were applied for response assessment, clonal excess was noted in 14 (45%) of 31 patients who achieved a complete response. The heavy/light chain ratio indicated the presence of disease in eight (26%) of 31 patients who achieved a complete response and, in sequential studies, indicated evolving relapse in

three patients before immunofixation became positive. It is probable that the test not only allows for the detection of persistent secretory clones of plasma cells, but it is also an indicator of the normalisation of the immune system, suggesting a deeper eradication of the tumour clone and a lack of negative effect on the immune status. However, more data must be collected, particularly among patients who achieve a complete response, to allow conclusions to be drawn for the use of the Hevylite assay.

Timing and frequency of disease assessment

Disease biology plays a key role in the determination of the degree and the duration of disease control after therapy. For example, a rapid and deep response is commonly seen in patients with multiple myeloma with features of high-risk disease, which is often—though not always—poorly sustained and followed by a rapid relapse. In a study by van Rhee and colleagues,[116] sFLC levels were measured at baseline, within 7 days of starting the first cycle, and before both the second induction cycle and the first ASCT. Patients within the top tercile for sFLC reductions from baseline until cycle 2 or before transplantation (reflecting either a more rapid response or a higher tumour burden at presentation) had an inferior event-free survival and overall survival compared with the other two terciles. Barlogie and colleagues[56] examined the effect of complete response on survival among patients undergoing total therapy protocols. The authors observed that patients who had achieved a complete response and then relapsed had inferior survival compared with those who never achieved a complete response. These patients were more likely to have gene expression profile-defined high-risk multiple myeloma and more likely to present with other poor prognostic factors. It has become clear that these patients not only have high-risk features at baseline but also have persistent minimal residual disease in the context of achieving complete response. We propose that the assessment of minimal residual disease kinetics over the disease course, rather than at a single timepoint when complete response is first documented, could provide a more robust evaluation of disease control in patients with multiple myeloma after achieving a complete response or stringent complete response. Conversely, one group[117] proposed that a small proportion of patients have a monoclonal gammopathy of unknown significance (MGUS)-like gene expression profile signature, and they experience significantly better outcomes compared with the vast majority of (non-MGUS-like) patients with multiple myeloma without necessarily increased complete response rates. More recently, a Spanish group[26] proposed that there are patients with multiple myeloma with an MGUS-like flow cytometry signature, and that they have better outcomes (estimated 60% time to progression and overall survival at 10 years) independently of their complete response status. Altogether, in addition to the amount of tumour burden that persists after therapy, the genetic and epigenetic make-up of chemotherapy-resistant minimal residual disease cells might dictate the duration of survival.

Conclusion

The proposed guidelines form a framework for future investigation into minimal residual disease in multiple myeloma. Prospective studies are being incorporated in newly designed clinical trials, and we encourage new studies to incorporate (whenever reasonable) minimal residual disease monitoring by next-generation flow or next-generation sequencing, or both.[59,60] In addition, existing archived samples from various clinical trials and different institutions are being evaluated for the validation of the clinical usefulness of minimal residual disease monitoring as a predictive variable. In view of the increasing incidence of extramedullary disease in patients with multiple myeloma, the presence of extramedullary disease should be ruled out as part of minimal residual disease assessment. Ongoing studies are evaluating the role of a PET scan at the time of minimal residual disease assessment along with the previously mentioned testing, especially when minimal residual disease negativity is achieved in the bone marrow.

Finally, the use of heavy/light chain ratios might have an important role in the definition of a minimal residual disease-negative state. The combination of a negative cell-based assay, negative PET scan, and a normal heavy/light chain ratio probably represents a composite endpoint reflecting the eradication of tumour cells from all compartments and recovery of the normal plasma-cell population to the currently available level of detection. This aspect needs further study in prospective clinical trials and large retrospective datasets. Development of a blood-based assay, either testing for rare circulating cells or circulating tumour DNA, would be ideal, and ongoing work should be focused on developing these approaches.

As more sensitive flow-based assays become more commonplace, we anticipate that the stringent complete response criteria will be used less frequently and may eventually be dropped. This factor is particularly relevant, as the contribution of sFLC normalisation as part of the stringent complete response criteria has been challenged by data from the Intergroupe Francophone du Myélome group. If indeed the usefulness of stringent complete response over complete response comes mostly from the lack of detectable plasma cells by less sensitive methods, use of minimal residual disease methods will make this criterion obsolete. Another area of active investigation has been the substitution of sFLC measurements for 24-h urine measurements. While this substitution would greatly reduce the burden for patients and physicians, no definitive data support this change at this time.[118–120] The most important question that this approach raises is

Search strategy and selection criteria

We searched PubMed for articles published in English between Jan 1, 1980, and June 30, 2014, that contained the term "myeloma" and any one of the following terms: "response" or "minimal residual disease" or "imaging" or "bone marrow" or "monoclonal protein". We also reviewed recent reviews on multiple myeloma. Members of the International Myeloma Working Group were then asked to identify any appropriate citation that was of interest but not detected by the search strategy.

the effect of the minimal residual disease results on decisions regarding treatment. Can treatment duration and need for alternative therapies be guided by the results of the minimal residual disease assessment? This question will have to be answered prospectively through well-designed response-adapted clinical trials.

Contributors

SK wrote the first draft of the manuscript after reviewing the scientific literature and discussing the proposed criteria with members of the International Myeloma Working Group. All authors reviewed the draft, provided detailed input and comments, and contributed to the final report.

Declaration of interests

SK reports personal fees for Skyline Diagnostics, Noxxon Pharma, and Kessios Pharma and serves on advisory boards for Takeda, Celgene, Janssen, Abbvie, Bristol-Myers Squibb, and Merck. KCA reports personal fees from Celgene, Millennium, Gilead, and Bristol-Myers Squibb. BP has been a consultant for Sanofi, serves on advisory boards and has received honoraria for Takeda, Celgene, Amgen, and Janssen, and reports grants from Celgene, Takeda Sanofi, and EngMab. PM reports personal fees from Celgene, Takeda, Janssen, Bristol-Myers Squibb, Novartis, and Amgen. NM has been a consultant for Celgene, Merck, Takeda, Pfizer, Amgen, and Janssen. M-VM reports personal fees from Janssen, Celgene, Bristol-Myers Squibb, and Amgen. MD has received personal fees from Celgene, Amgen, Janssen, and Novartis. EK has received honoraria from Janssen, Takeda, and Onyx. MB has received honoraria from Celgene, Onyx, Janssen-Cilag, Sanofi, and Amgen. RO reports grants from Bristol-Myers Squibb, Celgene, Takeda, Onyx, and Spectrum Pharma, and serves on advisory boards for Array BioPharma, Bristol-Myers Squibb, Celgene, FORMA Therapeutics, Janssen, Takeda, and Onyx. HG reports grants and personal fees from Celgene, Janssen, Chugai, Novartis, Onyx, Millennium, and reports grants from Bristol-Myers Squibb, Amgen, and Takeda. SJ serves on advisory boards for Celgene, Novartis, Bristol-Myers Squibb, and Merck. ET has received honoraria from Amgen, Celgene, Genesis, Takeda, Novartis, and Janssen-Cilag, serves on advisory boards for Amgen, Takeda, and Janssen-Cilag, and reports grants from Genesis, Amgen, and Janssen-Cilag. AR reports personal fees from Celgene. PS reports grants and personal fees from Celgene, Janssen, and Amgen, and reports grants from Karyopharm, Pharmamar, and Oncopeptide. PGR serves on advisory boards for Celgene, Takeda, and Johnson & Johnson. PM reports grants from Celgene and reports personal fees from Bristol-Myers Squibb, Celgene, Takeda, Karyopharm, Sanofi, Janssen, and The Binding Site. HL reports grants from Takeda and reports fees from BMS, Janssen-Cilag, Celgene, Onyx, and Boehringer Ingelheim. MC reports personal fees from Celgene, Janssen, Amgen, Takeda, and Bristol-Myers Squibb. SL serves on advisory boards for Celgene, Bristol-Myers Squibb, Novartis, and Celgene. JH reports grants from Novartis and Sanofi and reports personal fees from Amgen, Celgene, and Janssen. AP reports personal fees from Amgen, Novartis, Bristol-Myers Squibb, Genmab A/S, Celgene, Janssen-Cilag, Takeda, Sanofi, and Merck. JSM serves on advisory boards for Celgene, Novartis, Onyx, Janssen, Bristol-Myers Squibb, Takeda, and MSD. All other authors declare no competing interests.

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