14 Minimal Residual Disease Assessment in Myeloma

Jasmita Dass and Jyoti Kotwal

Multiple myeloma is a neoplastic proliferation of plasma cells that manifests as bone lesions, renal impairment, anaemia and hypercalcaemia [1]. Myeloma constitutes ~1% of all cancers diagnosed and ~13% of all haematological malignancies [2, 3]. Every year, ~86,000 new cases of myeloma are diagnosed [4]. The diagnostic criteria for myeloma were modified in 2017 by the International Myeloma Working Group (IMWG) to include asymptomatic patients with a high risk of progression to symptomatic myeloma within 2 years. These biomarkers were serum free light chain ratio abnormality with involved to uninvolved light chain ratio of >100, clonal plasma cells (PCs) ≥60% and presence of ≥1 lytic lesions on magnetic resonance imaging (MRI) of ≥5 mm in size [1]. The presence of these biomarkers was added to the classical 'CRAB' criteria that include hypercalcaemia, renal impairment, anaemia and bone lesions to form myeloma-defining events [1].

Nearly all myelomas come from a preceding monoclonal gammopathy of undetermined significance (MGUS) [5]. MGUS may be followed by an asymptomatic smouldering stage in which the clonal PC percentage is ≥30% and/or the monoclonal M band is ≥3 g/dL [1].

J. Dass (✉) · J. Kotwal
Department of Hematology, Sir Ganga Ram Hospital, New Delhi, India

14.1 Why Assess Minimal Residual Disease (MRD) in Myeloma?

There has been a consistent improvement in survival in myelomas from <50% in 1980–1989 to ~75% in 2000–2009 [6]. The median overall survival (OS) has improved considerably in the last 15 years. In a series of >1000 myeloma patients diagnosed from 2001 to 2010, it was seen that median OS in patients treated from 2001 to 2005 was ~4.6 years while the median OS in patients treated from 2006 to 2010 was ~6.1 years [7]. The survival pre-2001 era was ~2.5 years [8]. This has been due to a marked improvement in the drug development and discovery with the advent of immunomodulatory agents like thalidomide [9], lenalidomide [10, 11] and bortezomib [3]. Further improvements in myeloma OS are expected with the flurry of new agents approved for treatment like pomalidomide [12], carfilzomib [13], daratumumab [14], elotuzumab [15] and ixazomib [16].

The improvements in OS also stem from higher rates of complete response (CR) and very good partial response (VGPR) [17, 18]. With the current anti-myeloma therapy, ~100% patients achieve an overall response with ≥VGPR seen in 80% patients [19–22]. This is in contrast to the

R. Saxena, H. P. Pati (eds.), *Hematopathology*, https://doi.org/10.1007/978-981-13-7713-6_14

earlier anti-myeloma agents which even when combined with autologous stem cell transplantation (ASCT) were able to achieve a ≥VGPR in <50% patients [23, 24]. These consistent improvements pave the need for better response assessment in myeloma to identify patients who do extremely well post ASCT and who will not.

14.2 Response Assessment in Myeloma

IMWG lays down the response assessment criteria for myeloma patients. The IMWG 2006 criteria were centred on the assessments of M bands in serum and urine by the use of serum and 24 h urine protein electrophoreses (SPE and UPE), serum and urine immunofixation electrophoresis (SIFE and UIFE) and bone marrow PC percentage. They divided patients into CR, VGPR, partial response (PR), stable disease (SD) and progressive disease. Patients in CR had a complete absence of M bands in SPE, UPE, SIFE and UIFE together with <5% PC in bone marrow. Patients in VGPR should have had ≥90% reduction in the amount of M band in all the serum and urine studies [25].

This definition will clearly be inadequate when assessing patients treated with immunomodulatory agents and proteasome inhibitors due to high numbers of patients in CR. To address this issue, the IMWG in 2011 gave a new set of response criteria to identify patients within the category of CR who would do better than the rest. The IMWG added serum free light chain ratio and bone marrow immunophenotyping using 2–4 colour flow cytometry to the response evaluation investigations. In addition, they included immunohistochemistry on bone marrow biopsy with an intent to identify clonal PCs. The category of stringent CR (sCR) was added, and this included absence of an abnormal free light chain ratio and ≤5% clonal PCs by either immunohisctochemistry on bone marrow biopsy or 2–4 colour flow cytometry in the bone marrow [26]. It has however been identified that immunohistochemistry may be relatively unreliable as the bone marrow post therapy generally shows regeneration of normal PCs, and this may lead to false-negative assessment for clonal PCs [27, 28]. In addition, the IMWG 2011 criteria added the categories of immunophenotypic CR and molecular CR for those patients who are negative by 2–4 colour flow cytometry and using allele-specific oligonucleotide-polymerase chain reaction (ASO-PCR), respectively [26].

However, even these definitions were relatively inadequate for the continuing improvements in the OS rates and high rates of sCR achieved with modern anti-myeloma therapy. Minimal residual disease (MRD) in the myeloma patients may exist in the intramedullary location or the extramedullary locations. The last 8–10 years have witnessed a major surge in publications, addressing assessment of intramedullary minimal residual disease (MRD) in myeloma [29–40]. Most of these have used multiparameter flow cytometry (MFC) for MRD detection [29–36] while others have used molecular methods [37–40]. For the detection of extramedullary MRD in myeloma, imaging techniques like functional Magnetic resonance imaging (MRI) [41, 42] and 18-fluorodeoxyglucose positron emission tomography-computed tomography (PET/CT) scan [41, 43, 44] have been used.

14.3 Intramedullary MRD in Myeloma

MFC has been extensively applied to detect MRD in myeloma [29–36, 45–53]. Panels of monoclonal antibodies used by various studies on MRD are given in Table 14.1. As the years advance, the improvement in MFC techniques with the addition of more colours and better software leading to acquisition of more events and better analysis capabilities become visible with high sensitivities achievable with current methods of analysis.

14.3.1 Clinical Utility

Bruno Paiva's group at PETHEMA/GEM (Programa para el Estudio de la Terapéutica en Hemopatías Malignas/Grupo Español de Mieloma) [30, 33, 36, 47] and Andy Rawstron's

Table 14.1 Various panels used for MRD assessment in myeloma

Study (year)	Panel used	Sensitivity (%)
Rawstron et al. (2002) [29]	CD45-FITC/CD38-CECy5/CD138-PE CD45-FITC/CD38-CECy5/CD19-PE CD45-FITC/CD38-CECy5/CD56-PE	0.01
Sarasquete et al. (2005) [39]	CD38-FITC/CD56-PE/CD19-PerCP Cy5.5/CD45-APC CD138-FITC/CD28-PE/CD33-PerCP Cy5.5/CD38-APC CD20-FITC/CD117-PE/CD138-PerCP Cy5.5/CD38-APC	0.01
de Tute et al. (2007) [45]	CyIgλ-FITC/CD19-PE/CyIgκ-PE Cy5/CD38-PE Cy7/CD138-APC/CD45-APC Cy7	0.01
Paiva et al. (2008) [30]	CD38-FITC/CD56-PE/CD19-PerCP Cy5.5/CD45-APC (only tube applied in 90% patients) CD38-FITC/CD27-PE/CD45-PerCP Cy5.5/CD28-APC β2 micro-FITC/CD81-PE/CD38-PerCP Cy5.5/CD117-APC	0.01
Gupta et al. (2009) [46]	CD19-FITC/CD56-PE/CD38-PerCP Cy5.5/CD138-APC CD45-FITC/CD52-PE/CD38-PerCP Cy5.5/CD138-APC CD20-FITC/CD117-PE/CD38-PerCP Cy5.5/CD138-APC	0.01
Paiva et al. (2011) [33], (2012) [35], (2014) [47]	CD38-FITC/CD56-PE/CD19-PerCP Cy5.5/CD45-APC CD38-FITC/CD27-PE/CD45-PerCP Cy5.5/CD28-APC β2 micro-FITC/CD81-PE/CD38-PerCP Cy5.5/CD117-APC	0.01–0.001
Puig et al. (2014) [40]	CD38-FITC/CD56-PE/CD19-PerCP Cy5.5/CD45-APC CD38-FITC/CD27-PE/CD45-PerCP Cy5.5/CD28-APC β2 micro-FITC/CD81-PE/CD38-PerCP Cy5.5/CD117-APC	0.01–0.001
Rawstron et al. (2013) [34]	CD27-FITC/CD56-PE/CD19-PerCPCy5.5/CD38-PE Cy7/CD138-APC/CD45-APC Cy7 CD81-FITC/CD117-PE CD52-FITC/CD200-PE	0.01
Robillard et al. (2013) [48]; Rousel et al. (2014) [49]	CD38-HV450/CyIgλ-FITC/CD56+CD28-PE/CD138-PE Cy5/CD19-PE Cy7/CyIgκ-APC/CD45-APC H7	0.001
Euroflow (2017) [50]	CD138-BV421/CD27-BV510/CD38-FITC/CD56-PE/CD45-PerCP Cy5.5/CD19-PE Cy7/CD117-APC/CD81-APC C750 CD138-BV421/CD27-BV510/CD38-FITC/CD56-PE/CD45-PerCP Cy5.5/CD19-PE Cy7/CyIgκ-APC/CyIgλ-APC C750	0.001
Euroflow (2010) [51]	CD138-BV421/CD27-BV510/CD117-BV605/CD38-FITC/CD56-PE/CD45-PerCP Cy5.5/CD19-PE Cy7/CyIgλ-APC/CyIgκ-APC A700/CD81-APC C750	NA
Roshal et al. (2017) [52]; Royston et al. (2017) [53]	CD81 PacBlue/CD38BV510/CD27 BV605/CD117 PerCPCy5.5/CD19 PECy7/CD138 APC/CD56 APC-R700/CD45 APC-H7	0.001

group at Leeds [29, 34] have generated the most clinical data on MRD in myeloma. Only data from key papers is being presented here.

In the PETHEMA/GEM analysis of trials GEM2000 and GEM2005 >65 years, Paiva et al. showed that in 241 patients in CR at D100 post ASCT, the patients who did not sustain CR at 1 year post ASCT had a poor prognosis. The only two variables that could predict this unsustained CR were high-risk cytogenetic profile and presence of MRD-positive status at D100 post ASCT. Their data gave proof that patients who remain MRD positive at D100 post ASCT do poorly and should be enrolled in trials to intensify therapy or treat MRD-positive status [35]. In another analysis of GEM05 >65 years confined to elderly patients, 102/260 patients achieved at least a PR and were analysed using a serum free light chain assay, immunofixation, and MFC. After six cycles of induction therapy, CR was seen in 43%, sCR in 30% and MRD negative by MFC in 30% of the 102 patients. Patients in sCR when compared with patients in CR did not show any survival advantage while patients who were MRD negative by MFC had a longer progression-free survival (PFS) and time to pro-

gression. Seven patients were MRD negative by MFC but were positive on IFE studies, and all of them attained an IFE-negative status on follow-up. In contrast, patients who were MRD positive by MFC but were IFE negative showed early relapses on repeat IFE examinations [33]. In a subsequent series on relapsed myeloma patients, the prognostic impact on time to progression of attaining a MRD-negative status after salvage chemotherapy with or without ASCT could also be demonstrated. Importantly, in this series, MRD status was not useful for patients who underwent allogeneic stem cell transplantation as this was uniformly associated with a poor time to progression [47].

Rawstron in 2013 published the results from Medical Research Council Myeloma IX Study on >600 patients of myeloma who underwent MRD assessment by a six-colour MFC panel at the end of induction therapy with cyclophosphamide, vincristine, doxorubicin and dexamethasone (CVAD) or cyclophosphamide, thalidomide and dexamethasone (CTD) for intensive therapy followed by ASCT or attenuated CTD or melphalan and prednisolone (MP) for non-intensive treatment. The patients underwent bone marrow examination at diagnosis, end of induction and at day 100 (D100) of ASCT procedure, and MFC analysis was performed at all time points. For each tube, minimum 500,000 events were acquired and ≥50 cell cluster with an aberrant immunophenotype was considered abnormal. Of the 397 patients who received ASCT following intensive therapy (62.2%), an MRD-negative status at D100 post ASCT had a significantly longer PFS and OS. In the sub-analysis, patients who were MRD negative at the end of induction before undergoing ASCT had the best PFS, but this benefit was not translated to OS. The favourable impact of MRD negativity on PFS and OS was independent of cytogenetic risk groups with the benefit seen in both favourable and adverse risk groups. When they looked at patients achieving CR, an MRD-negative status was associated with a better OS. There were patients who were MRD negative but not in CR, and these patients were similar to MRD-positive cases. The MRD negative but not in CR can be possibly explained by the longer half-life of M bands and possibly by patchy distribution of the disease. In a further analysis of patients randomized to receive thalidomide maintenance, it was seen that patients on maintenance thalidomide had higher chances of becoming MRD negative [34]. In a subsequent series from the same patient group, Rawstron et al. demonstrated that a reduction in MRD by each log predicted a better OS. Median OS achieved in ≥10% MRD group was 1 year while the corresponding figures for 1 to <10%, 0.1 to <1% and 0.01 to <0.1% groups were 5.9, 6.8 and >7.5 years, respectively. They concluded that 1-year OS benefit is achieved per log reduction of MRD [54].

Other studies have used molecular methods to assess MRD. Most published evidence is for ASO-PCR [37, 39, 40]. In the Spanish trials of myeloma, patients underwent both ASO-PCR and MFC to assess MRD. Of the 170 patients who achieved at least a PR, ASO-PCR could be successfully applied in only 42% patients as in many patients either clonality could not be demonstrated or sequencing analysis was unsuccessful or ASO performance was suboptimal. In patients who were assessable by both techniques, MRD could be demonstrated using MFC in 46% while ASO-PCR was positive in 52% patients. Overall, a good correlation was observed between both techniques. A better PFS and OS were reported for patients who were ASO-PCR negative versus those who were ASO-PCR positive [40]. Sarasquete et al. could identify an assessable ASO-PCR rearrangement in 24/32 patients of myeloma who achieved CR post ASCT and could predict improved PFS in MRD-negative group [39]. In an earlier analysis of patients who underwent either ASCT or allogeneic stem cell transplantation, MRD-negative patients by ASO-PCR were found to have a lower relapse rate and long-term relapse free survival than MRD-positive patients [38].

The benefit of attaining an MRD-negative status in myeloma has been the subject of two meta-analyses [55, 56]. In the first analysis by

Munshi et al., data from 21 eligible studies from January 1990 to January 2016 was pooled and impact on PFS and OS was examined. The meta-analysis showed that attaining an MRD-negative status was associated with a better PFS and OS. The median PFS was 54 months for MRD-negative patients while it was 26 months for MRD-positive patients. Interestingly, the benefit of MRD-negative status was seen in patients in CR also with patients who were in CR and MRD negative, having a better PFS and OS than patients who were MRD positive but in CR. They concluded that attaining MRD-negative status can be used as an end point in myeloma clinical trials [55]. This would mean that MRD-negative status could be used as a surrogate end point for studies on drug approval [57].

The second meta-analysis though started with 20 full texts, finally only included four studies—three using MFC and one using ASO-PCR. They also showed that MRD negativity was associated with a better PFS and OS. They also concluded that MRD can become a possible end point for regulatory drug approval in myeloma [56].

However, in all these papers, it was clear that MRD-negative status did not translate to cure. This clearly means that MRD-negative patients also possibly have a significant disease burden that is still present in the body. This could either be due to the fact that MRD techniques being used in pre-2015 era were possibly less sensitive than what is desirable or many patients have a patchy disease, and it might be that the site sampled for bone marrow may not contain MRD while other sites may have residual disease. A third possibility is that the relapses may originate from extramedullary sites.

14.3.2 Development of Next Generation Flow Cytometry for MRD Detection in Myeloma

To address the first issue, International Myeloma Foundation (IMF) initiated the Black Swan Research initiative who in concert with Euroflow Consortium tried to develop better methods and standardization of MFC technique. This was achieved with a two-tube eight-colour MFC approach with validated antibodies and bulk-lysis procedure to obtain ≥10 million events. This tube design was a result of five cycles of *design–evaluation–redesign*. The panel consists of two tubes: Tube 1 CD138 BV421/CD27 BV510/CD38 (multi-epitope) FITC/CD56 PE/CD45 PerCPCy5.5/CD19 PE-Cy7/CD117 APC/CD81 APC C750 and Tube 2 CD138 BV421/CD27 PE/CD38 (multi-epitope) FITC/CD56 PE/CD45 PerCPCy5.5/CD19 PE-Cy7/CyIgκ APC/CyIgλ APC C750. They also are validating a single-tube 10-colour panel version and are using the Infinicyt software, and automated identification of abnormal PCs is being explored. This method has been termed next generation flow cytometry (NGF) as it is capable of attaining sensitivity as high as next generation sequencing (NGS). NGF had a higher sensitivity of MRD detection than conventional MFC at 47% vs. 34%. This effectively meant that a quarter of patients who were MRD negative by conventional MFC were actually MRD positive by NGF, and this translated to an improvement in 75% PFS which was not reached in NGF-negative group compared to 75% PFS of 7 months in patients who were NGF positive. Interestingly this was regardless of the conventional response status of these patients. Interestingly, patients who were NGF positive but MRD negative by conventional MFC did worst. A small subset of patients also underwent NGS for the comparison of NGS and NGF, and they showed that NGF approach had higher applicability than NGS and higher sensitivity of MRD detection than NGS [50]. The validated panel is also being marketed through Cytognos (http://www.cytognos.com/index.php/euroflow/minimal-residual-disease-panels/1440-multiple-myeloma-mm-mrd-kits) [58]. There has been an attempt by other centres for the evaluation of a 10-colour panel that can give comparable results to the NGF using a two-tube eight-colour approach [52, 53].

14.3.3 Utility of Next Generation Sequencing for MRD Detection

Next generation sequencing (NGS) in the context of MRD in myeloma employs multiplex sequencing of the immunoglobulin heavy-chain regions (IgH) of plasma cells. It requires the diagnostic sample to identify the IgH rearrangements in the initial clone of neoplastic PCs, and in case of myeloma, multiple clones may be present at the time of diagnosis. All subsequent samples are also assessed using the same platform, and this technique can achieve a sensitivity of 10^{-6} or 0.0001%. Two studies have explored the use of a LymphoSIGHT™ (Sequenta, Inc., San Francisco, CA) high-throughput sequencing platform for immunoglobulin heavy-chain locus (IGH) complete (IGH-VDJH), IGH incomplete (IGH-DJH) and immunoglobulin κ locus (IGK) to assess MRD in myeloma [59, 60]. The first series from GEM trials included 133 patients whose diagnostic DNA was subjected to NGS followed by the analysis for MRD. Of 133 patients, an assessable clonotype was available in 121, and of these, 110 underwent MRD detection. Hence, NGS was applicable in 91% patients. MRD-positive status was present in 83% patients and MRD-negative patients by NGS had a higher time to tumour progression and OS. It was seen that patients who were in CR and MRD negative by NGS had the highest time to tumour progression. All patients had been assessed by MFC and ASO-PCR earlier. When MRD data by NGS was compared with MFC, 83% samples were concordant and for ASO-PCR, the corresponding figure was 85% [59].

The second study was a part of the IFM/DFCI 2009 Trial. Patients were assessed at pre-maintenance and post-maintenance time points. Patients who were MRD negative by NGS had a higher PFS than MRD-positive patients at both time points of assessments. The benefit of MRD negativity was also seen in patients in CR and was also seen in patients with t(4;14) cytogenetic abnormality [60].

Table 14.2 IMWG 2016 criteria pertaining to MRD [61]

Response category	Definition
Sustained MRD negative	MRD negativity in bone marrow by NGS, NGF or both and by imaging confirmed at least 1 year apart. There is a provision to identify further duration of MRD negativity
Flow MRD negative	Absence of aberrant clonal PCs by NGF on bone marrow samples using the standard operating procedure of the Euroflow consortium or a validated equivalent method; assay sensitivity of 0.001% or higher
Sequencing MRD negative	Absence of clonal PCs on bone marrow aspirate using the LymphoSIGHT platform or equivalent method at an assay sensitivity of 0.001% or higher
Imaging plus MRD negative	MRD negative as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or a matching uptake as mediastinal blood pool or less than surrounding normal tissue
Relapse from MRD negative	Loss of MRD-negative state (evidence of clonal plasma cells on NGF or NGS, or positive imaging study for recurrence of myeloma); or reappearance of M band or development of CRAB

With the exciting data from NGF and NGS, the two techniques were formally incorporated in the IMWG 2016 response and MRD assessment criteria for myeloma. The response criteria are mentioned in Table 14.2 [61].

14.4 Imaging to Detect Extramedullay MRD in Myeloma

In a series of 134 myeloma patients, an abnormal PET/CT was seen in 91% patients, and in ~32% of these patients, a normalization of PET/CT after three cycles of bortezomib/lenalidomide/dexamethasone therapy led to an improvement in progression free survival. RVD therapy was followed by ASCT. PET/CT normalization pre-maintenance was seen in 62% patients, and this

was also associated with better PFS and OS. MRI in contrast was abnormal in 95% patients, but its normalization following three cycles of RVD therapy was not associated with an improved PFS [41].

In another abstract presented at American Society of Hematology (ASH) annual meeting at Atlanta in 2017, retrospective data of 87 patients treated from 2008 to 2017 and put on lenalidomide therapy was presented. Patients who were flow MRD negative and PET negative after lenalidomide maintenance therapy had a significantly higher PFS and a trend to better OS than all other groups [43]. In an earlier published study, it was seen that in 282 patients treated up front, PET-CT was positive in 70% at diagnosis, and after last cycle of first-line therapy, PET-CT was positive in 30% patients. A PET-negative status was associated with a better PFS and OS. Patients in CR with a PET-positive status did worse and PET-CT was an independent prognostic variable in patients with conventional CR [44].

In another abstract at ASH 2017, the authors specifically looked at 46 patients (35 newly diagnosed and 11 post relapse) who were in MRD-negative CR using eight-colour MFC from a total of 294 patients of myeloma treated with novel agents and ASCT but relapsed subsequently. The limit of detection (LOD) of the MFC assay was between 0.01% and 0.001%. The patients also underwent PET/CT and diffusion-weighted magnetic resonance imaging with background suppression (DWIBS). DWIBS could detect focal lesions in 12/46 patients while PET/CT could detect focal lesions in 3/46 patients. It was also seen that 9/14 patients presented with lesions in the contralateral side to the bone marrow assessment by MFC. This could explain the MRD-negative status using MFC [42].

The IMWG2016 criteria have incorporated MRD assessment by PET/CT in the response and MRD assessment in myeloma, Table 14.2 [61].

A comparison of the techniques used to detect myeloma MRD has been provided in Table 14.3.

Table 14.3 Comparison of the techniques used for MRD detection in myeloma [61, 62]

Criteria	MFC	NGF	ASO-PCR	NGS	PET/CT
Applicability	~100%	~100%	60–70%	90%	~100% for extramedullary disease
Sensitivity	10^{-4}	10^{-5} to 10^{-6}	10^{-5} to 10^{-6}	10^{-6}	High, even 4 mm lesions
Requirement of diagnostic sample	Preferable; not mandatory	Preferable; not mandatory	Mandatory	Mandatory	Preferable; not mandatory
Turnaround time	~2–3 h	~2–3 h	~1 week; ~4 weeks for first identification	≥1 week	2 h
Availability	Most labs worldwide	Most labs worldwide can apply	Intermediate	Low	Intermediate
Requirement of fresh sample	Yes; ≤36 h	Yes; ≤36 h	None	None	Not applicable
Impact from patchy disease	Yes	Yes	Yes	Yes	No
Assessment of sample quality	Possible	Easy	No	No	No
Cost	<350 USD	~350 USD	~500 USD (follow-up); ~1500 (diagnostic)	~700 USD	~2000 USD ~INR 12,000–14,000
Standardization	Poor	Ongoing	EuroMRD since 15 years	No	Ongoing

14.5 Nitty-Gritty of MFC to Assess MRD Using Conventional and NGF Approaches

The use of MFC has evolved from three- or four-colour panels [29, 30, 33–35, 39, 40, 46, 47] to 6–8 colours [45, 48, 49] and then the development of the NGF [50] and 10-colour panels [51–53]. The first guidelines for the determination of MRD by MFC were given in 2008 by European Myeloma Network [63]. These were followed after 7 years by a full issue on myeloma MRD published in *Cytometry B: Clinical Cytometry* [17, 64–73].

14.5.1 Specimen Requirement

The sample recommendations for MRD assessment are limited to bone marrow aspiration. The acceptable anticoagulants have been EDTA and sodium heparin, but since CD138 is a heparin sulphate, there might be some decrease in its intensity following heparin anticoagulation. Hence, some centres prefer EDTA as an anticoagulant [74]. Bone marrow can be transported at room temperature but should ideally be processed within 48 h of sample collection. It is however better if the sample is processed within 24 h of collection [66].

14.5.2 Sample Requirement

If MFC is being applied without NGF approach, 1–2 mL bone marrow sample is adequate, but if NGF approach is to be used, ~5 mL bone marrow sample should be taken. Ideally, the first pull sample should be taken for MFC/NGF unlike the usual scenario where first pull samples are used to prepare marrow films. To acquire 5 million events as recommended by NGF, it is required to start with 10–20 million events as ~50% cells may be lost during processing [50, 65]. However, this is only when the aim is to reach a sensitivity of 0.001%. A lower sensitivity threshold of 0.01% does not require these many cells to begin with and ~55% patients may have MRD higher than this threshold and would therefore not require NGF for detection. NGF processing can be applied to the rest 45% patients, and this approach has been tried at Leeds as they also send sample for molecular analysis as the first priority [65].

14.5.3 Processing Technique

The MFC sample should be processed using a lyse-wash-stain-wash approach. A pre-lysis step for NGF is a bulk-lysis approach with the addition of ~0.5% bovine serum albumin, and a FACS-lysing-fixation step is recommended (Protocol A1) [50]. If utilizing this approach, titration protocol should also follow the same method of processing. Ficoll-hypaque processing is not recommended as it may lead to PC loss. People have tried using higher amounts of sample volume with an appropriate increase in antibody cocktail, but this leads to increased cost and reduced limit of detection (LOD) as fewer cells are acquired when compared to bulk-lysis approach [66].

14.5.4 Panel Requirements

Gating markers: To identify PCs, the single most useful marker is CD38 as it is expressed at a high intensity on both normal and neoplastic PCs. However, it is also expressed by hematogones at an intensity intermediate between PCs and other cells, and this might create a problem when assessing neoplastic PCs which may show a slight downregulation of CD38 [67, 75]. Since CD38 is very brightly expressed, it is best tagged with a weak fluorochrome like FITC. CD138/syndecan is a specific marker for PCs in the context of bone marrow with bright expression seen only in PCs [76]. However, the NGF data has shown that this marker should be tagged with a bright fluorochrome to identify all neoplastic PCs in the context of MRD assessment [75]. The dye recommended by Euroflow in its diagnostic panel is V450, but in the myeloma panel, BV-421 is the recommended dye due to its exceptional

brightness [50]. Other dyes like APC and PE can also be used [75].

In addition, CD45 should always be used as a gating marker to refine the PC gate and take care of contaminants. Forward and side scatter (FSC and SSC) are available with every acquisition and should be used to exclude debris and doublets that may contaminate the final gates [75].

With the advent of daratumumab therapy [14], which is an anti-CD38 monoclonal antibody, reductions in CD38 binding of some antibody clones may occur [50]. In addition, daratumumab may complicate IFE assessments as it comigrates with IgGκ bands and also complicates blood transfusion by interfering with serological cross-matches [77]. To circumvent its impact on MRD assessment, Euroflow has optimized a multi-epitope CD38 molecule that is capable of detecting MRD in patients treated with daratumumab [50, 58, 75].

There has also been an attempt to identify other potential gating markers for plasma cells. For this purpose, CD54, CD229 and CD319 were evaluated by the Euroflow group on 46 myeloma patients, 5 healthy controls, 3 extraosseous plasmacytomas and 2 uninvolved non-Hodgkin lymphoma (NHL) marrows. It was seen that all markers when combined with CD38 performed well, but if combined with CD138, only CD229 had a potential to identify all PCs [73]. However, in the final published manuscript and with detailed evaluation of the MRD panel, the group dropped CD229 also as it missed MRD in 8% myeloma cases and was also found at high levels in plasmacytoid dendritic cells and some lymphocytes [50].

Hence, the best approach is the use of a combination of CD138 tagged with a strong dye, CD38 tagged with a weak dye, and CD45 with FSC and SSC for plasma cell gating [63, 67]. In patients treated with daratumumab, a multi-epitope CD38 molecule should be used to identify neoplastic PCs.

Identification of neoplastic PCs: Identification of the abnormal always happens when we know what is normal. Normal PCs in the bone marrow express CD38 and CD138 but do not express the most mature B-cell markers like CD20, CD22 and surface immunoglobins. They however show cytoplasmic expression of light chains that can be used to assess clonality or lack thereof. Traditionally, it was thought that normal PCs show a dim expression of CD45, homogeneous expression of CD19 and lack CD56 [63, 78–80]. However, with modern MFC with acquisition of large number of events, small populations of PCs that could be considered abnormal or neoplastic could be identified in normal patients like CD19−, CD45−, CD56+, CD20+ and may complicate MRD analysis [81–84]. The immunophenotype of PCs shows a significant overlap between normal, neoplastic and reactive PC populations. After acquisition of 1 million events, populations like CD19–CD56/CD28+, CD19–CD56/CD28−, CD19+CD56/CD28+ could be discovered at less numbers [82]. CD81 is expressed at high intensity in normal plasma cells and hematogones and an underexpression is reported in neoplastic PCs [85], and this has not been observed in the normal PC compartment [81]. In contrast normal PCs do not express CD200, CD221 and CD117 [83].

Neoplastic PCs tend to be CD19 negative, and this is consistently seen in >90% myelomas [63, 67, 78–80]. Approximately 60–75% cases of myeloma are CD56 positive [63, 70, 78–80]. An incidence of aberrant patterns and the number of myelomas that show those patterns are given in Table 14.4. Some markers have been shown to have prognostic significance like CD28+CD117− myelomas were shown to have a high risk of

Table 14.4 Differently expressed markers between normal and neoplastic PCs

Marker	Incidence	Normal expression	Aberrancy
CD19	>90%	Positive	Negative
CD56	65–70%	Negative	Bright positive
CD81	55%	Bright positive	Negative or dimmer
CD117	30%	Negative	Positive
CD28	15–45%	Negative	Positive
CD27	40–68%	Positive	Negative or dim
CD45	73%	Dim positive	Negative
CD200	70%	Negative	Bright positive
CD38	80%	Bright	Dim
CD54	60–80%	Bright	Dim

progression [80]. Clonality assessment was not considered mandatory in the earlier published MRD literature [29, 30, 33–35, 39, 40, 46, 47], but the addition of cytoplasmic anti-κ and λ antibodies always adds utility for MRD assessment when a large number of events are acquired to establish clonality of small suspect populations and is included in most ≥8-colour combinations [48–53].

An abnormality in a few markers (one or two) should not be considered as evidence of neoplastic PCs. Multiple assessed markers must be considered when differentiating normal from neoplastic PCs [62, 70]. Addition of clonality assessment improves this distinction of minor populations of PCs. Increasing the number of markers assessed also adds utility to the analysis. Currently, the minimum recommended markers for MRD assessment in myeloma that can differentiate between normal and myelomatous PCs are CD38, CD19, CD45, CD56, CD27, CD81 and CD117. CD138 is required for gating PCs, and in addition, cytoplasmic light chain assessment adds utility when small suspicious PC subsets are present [70].

Acquisition of events: The number of acquired events determines the sensitivity of the assay. The EMN guidelines recommended a total of 1 million cells to be acquired with ~500,000 events per tube as a minimum guide [63]. However, as MFC and software capability have advanced, an acquisition of higher event numbers is achievable easily and is desirable to perform.

Most clinical data generated by MRD has used a threshold of 0.01% as a cut-off to differentiate MRD positive from negative [29, 30, 33, 35, 47]. However, relapses were seen in the MRD-negative group of patients also, and to circumvent that, NGF was developed [50]. This approach calls for an acquisition of a minimum of 3–5 million events to achieve a good limit of detection and lower limit of quantification (LOD and LLOQ, respectively).

Gating strategy: The gating strategy for neoplastic PCs should begin with refinement of data to exclude abnormal flow with CD38-time plot, doublet exclusion using FSC-area vs. FSC-height or SSC-area vs. SSC-height, exclusion of debris by FSC vs. SSC plot [67]. For the first identification of PCs from this refined data, CD38 vs. CD138 plot is used to generously gate all possible PC events. This can be further assessed on the CD38 vs. CD45 plot [67]. Further identification of neoplastic PCs and differentiating them from normal PCs uses a combination of multiple markers assessed in the panel [63, 67, 70]. Normal PCs most often are CD19+/CD45 dim/CD38 bright/CD138 bright/CD27+/CD81+/CD56−/CD117−/CD200− with a polyclonal light chain expression. In contrast most neoplastic PCs exhibit a combination of abnormalities including CD19−/CD45−/CD38 dim/CD138 bright/CD27− or dim/CD81− or dim/CD56+/CD117+/CD200+ [63, 67, 70]. An example of a case is shown in Fig. 14.1.

LOD and LLOQ: LOD and LLOQ are functions of total acquired events, the size of the cluster considered as neoplastic, number of events required to attain reproducibility of detection and quantification. LOD is estimated as having a cluster of at least 30 cells as a percentage from total cells while LLOQ is calculated using a cluster of 50 cells as a percentage of the total cells. This automatically means that in an analysis of 100,000 cells, LOD is 0.03% while LLOQ is ~0.05%. This increases to 0.003% and 0.005% when 1 million cells are analysed and increases further to 0.001% and 0.0017%, respectively, when 3 million cells are interrogated. If 5 million events are analysed, the LOD of 0.0006% and LLOQ of 0.001% are achievable.

How to report MRD: As described earlier, MRD was initially reported at a 0.01% cut-off at EMN recommendations [63]. Some studies reported MRD as aberrant to total PC number ratio [46] and some as neoplastic PC percentage from all leucocytes [34] while others from all events acquired [35, 40]. The current recommendations state that neoplastic PCs be determined as a percentage of total assessed PCs but MRD to be reported as percentage of all nucleated cells [67, 70]. This makes sense as MRD reported by molecular methods also utilizes the entire genomic DNA with no selection of populations [67].

Assessment of sample quality: Concerns pertaining to a representative marrow sample have

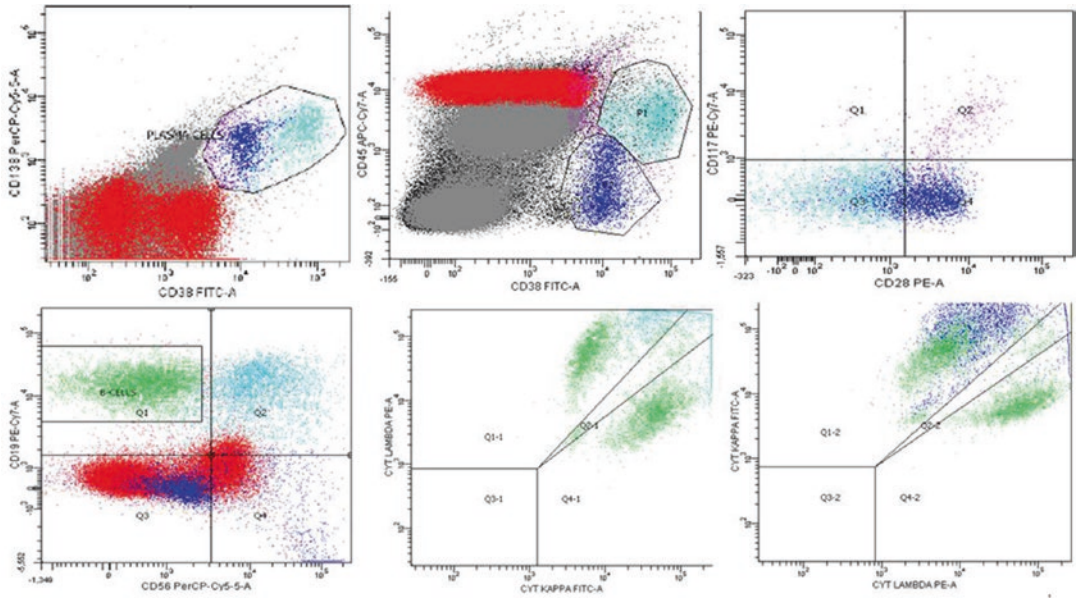

Fig. 14.1 Detection of MRD in myeloma: a gating approach. Plasma cells after exclusion of doublets and debris were gated on the CD38 vs. CD138 plot. From these, two populations were separated on the CD45 vs. CD38 plot into CD45–CD38 dim dark blue PCs and aqua CD38+ bright CD45+ PCs. These two populations exhibited a different immunophenotype. The dark blue is the neoplastic PC population that constituted ~0.1% of all events and was CD38 dim/CD45−/CD2+/CD117−/CD19−/CD56− and exhibited clonality for kappa. In contrast, the aqua population was CD38 bright/CD45+/CD28−/CD117−/CD19+/CD56+ and was polyclonal. Clearly the dark blue population is neoplastic while aqua population is normal PCs. This case highlights a variable immunophenotype within the normal PCs with enrichment of the CD19+/CD56+ subpopulation and shows that a single marker should never be relied upon while assessing for MRD

always been raised for MRD in myeloma. The EMN guidelines in 2008 stated that if polyclonal PCs are present within an MRD sample, it should be considered as representative. If they were undetectable, a recommendation was made to look for erythroid or normal myeloid blasts and hematogones. In a situation where MRD was present but sample lacked all these, a comment stating that sample is positive but unsuitable for MRD quantification was recommended [63]. There have been attempts to identify normal ranges for multiple normal populations that can be assessed using markers used for MRD assessment. In the paper on NGF [50], the authors have published ranges for mast cells, erythroid cells, %CD27+ B-cell precursors, %CD27− B-cell precursors, % mature B cells, % myeloid precursors and % endothelial and mesenchymal cells which can serve as a potential guide to validation of sample quality. In their analysis, they could demonstrate that the two patients who progressed despite being NGF MRD negative could be explained by a suboptimal sample submitted for NGF. However, this finding will require confirmation in other large series of patients to be considered as the only reason for progression while being NGF MRD negative. Other investigators have used normal PCs, mononuclear cells, erythroid cells, granulocytes, CD117+CD27− myeloid progenitors, CD27+CD117− lymphoid cells, B cells, NK cells and hematogones [69].

Final report of MRD by flow cytometry: In addition to the sample time point and patient demographic data, it is recommended that results be reported as event number of neoplastic PCs along with total events analysed as well as the LOD and LLOQ values. If the results are between LOD and LLOQ values, then MRD should not be reported as a percentage but as a range between LOD and LLOQ. Total events in this context

represents the denominator after doublets and debris have been excluded for refining the data acquired [67]. A comment on sample adequacy by reporting on normal cell types assessed in the sample should also be a part of the final report.

14.6 MRD in Myeloma: Indian Perspective

Myeloma MRD has lagged behind in India with published data only from AIIMS Gupta et al. [46]. They had reported their data in 2009 using a four-colour panel (given in Table 14.1) and targeted a threshold of 0.01% neoplastic plasma cells. They managed to detect aberrancies in at least two antigens in 90.7% cases and observed a change in immunophenotype at the time of MRD assessment with that from the diagnostic time point in 78% cases. Their initial work highlighted that neoplastic PCs may upregulate or downregulate antigens after therapy and stressed the need to evaluate multiple antigens. In their assessment, they did not assess clonality of PCs due to a limited four-colour panel, but this has been subsequently assayed and is now a part of the myeloma MRD detection.

We have used a six-colour and subsequently an eight-colour panel to detect MRD in myeloma. Using a six-colour approach also, it is feasible to detect MRD and determine clonality of neoplastic PCs.

Hopefully with the incorporation of MRD assessment in IMWG response criteria [61], there would be an increase in the centres evaluating it in India.

Take Home Message

MRD in myeloma has undergone a drastic progress in the past 10 years and has kept pace with the novel agent discovery that led to improved survivals in myeloma. The most utilized approach is multiparameter flow cytometry and is capable of detecting MRD at ≥0.01% threshold. This can be further improved by utilizing next generation flow cytometry and standardized approach to MRD, and increased sensitivity of 0.001% is achievable. Most molecular methods used like ASO-PCR and NGS require a diagnostic sample and still have limited availability and are expensive. MRD assessments in the extramedullary compartment require PET/CT, and the data on both intramedullary and extramedullary MRD in myeloma has been incorporated in the IMWG 2016 response criteria.

References

1. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15:e538–48.
2. Moreau P, Attal M, Facon T. Frontline therapy of multiple myeloma. Blood. 2015;125:3076–84.
3. Rajkumar SV. Multiple myeloma: 2014 update on diagnosis, risk-stratification, and management. Am J Hematol. 2014;89:998–1009.
4. Howlader N, Noone A, Krapcho M, et al. Seer Cancer Statistics Review, 1975–2009 (Vintage 2009 populations). Bethesda: National Cancer Institute; 2012.
5. Landgren O, Weiss BM. Patterns of monoclonal gammopathy of undetermined significance and multiple myeloma in various ethnic/racial groups: support for genetic factors in pathogenesis. Leukemia. 2009;23:1691–7.
6. Kristinsson SY, Anderson WF, Landgren O. Improved long-term survival in multiple myeloma up to the age of 80 years. Leukemia. 2014;28:1346–8.
7. Kumar SK, Dispenzieri A, Lacy MQ, et al. Continued improvement in survival in multiple myeloma: changes in early mortality and outcomes in older patients. Leukemia. 2014;28:1122–8.
8. Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008;111:2516–20.
9. Singhal S, Mehta J, Desikan R, et al. Antitumor activity of thalidomide in refractory multiple myeloma [see comments]. N Engl J Med. 1999;341:1565–71.
10. Richardson PG, Blood E, Mitsiades CS, et al. A randomized phase 2 study of lenalidomide therapy for patients with relapsed or relapsed and refractory multiple myeloma. Blood. 2006;108:3458–64.
11. Rajkumar SV, Hayman SR, Lacy MQ, et al. Combination therapy with lenalidomide plus dexamethasone (Rev/Dex) for newly diagnosed myeloma. Blood. 2005;106:4050–3.
12. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005;352:2487–98.
13. Siegel DS, Martin T, Wang M, et al. A phase 2 study of single-agent carfilzomib (PX-171-003-A1) in patients with relapsed and refractory multiple myeloma. Blood. 2012;120(14):2817–25. https://doi.org/10.1182/blood-2012-05-425934.

14. Lonial S, Brendan M, Usmani SZ, et al. Single-agent daratumumab in heavily pretreated patients with multiple myeloma (Sirius): an open-label, international, multicentre phase 2 trial. Lancet. 2016;387:1551–60.
15. Lonial S, Dimopoulos M, Palumbo A, et al. elotuzumab therapy for relapsed or refractory multiple myeloma. N Engl J Med. 2015;373:621–31.
16. Moreau P, Masszi T, Grzasko N, et al. Ixazomib, an investigational oral proteasome inhibitor (PI), in combination with lenalidomide and dexamethasone (IRd), significantly extends progression-free survival (PFS) for patients (Pts) with relapsed and/or refractory multiple myeloma (RRMM): the phase 3 tourmaline-MM1 study (NCT01564537). Blood. 2015;126. Abstract 727.
17. Landgren O, Owen RG. Better therapy requires better response evaluation: paving the way for minimal residual disease testing for every myeloma patient. Cytometry B Clin Cytom. 2016;90:14–20.
18. Mailankody S, Korde N, Lesokhin AM, et al. Minimal residual disease in multiple myeloma: bringing the bench to the bedside. Nat Rev ClinOncol. 2015;12:286–95.
19. Attal M, Lauwers-Cances V, Marit G, et al. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366:1782–91.
20. McCarthy PL, Owzar K, Hofmeister CC, et al. Lenalidomide after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366:1770–81.
21. Jakubowiak AJ, Dytfeld D, Griffith KA, et al. A phase 1/2 study of carfilzomib in combination with lenalidomide and low-dose dexamethasone as a frontline treatment for multiple myeloma. Blood. 2012;120:1801–9.
22. Kumar S, Flinn I, Richardson PG, et al. Randomized, multicenter, phase 2 study (EVOLUTION) of combinations of bortezomib, dexamethasone, cyclophosphamide, and lenalidomide in previously untreated multiple myeloma. Blood. 2012;119:4375–82.
23. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. N Engl J Med. 1996;335:91–7.
24. Child JA, Morgan GJ, Davies FE, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J Med. 2003;348:1875–83.
25. Durie BG, Harousseau JL, Miguel JS, et al. International uniform response criteria for multiple myeloma. Leukemia. 2006;20:1467–73.
26. Rajkumar SV, Harousseau JL, Durie B, et al. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel 1. Blood. 2011;117:4691–5.
27. Tatsas AD, Jagasia MH, Chen H, McCurley TL. Monitoring residual myeloma: high-resolution serum/urine electrophoresis or marrow biopsy with immunohistochemical analysis? Am J ClinPathol. 2010;134:139–44.
28. Chee CE, Kumar S, Larson DR, et al. The importance of bone marrow examination in determining complete response to therapy in patients with multiple myeloma. Blood. 2009;114:2617–8.
29. Rawstron AC, Davies FE, DasGupta R, et al. Flow cytometric disease monitoring in multiple myeloma: the relationship between normal and neoplastic plasma cells predicts outcome after transplantation. Blood. 2002;100:3095–100.
30. Paiva B, Vidriales MB, Cervero J, et al. Multiparameter flow cytometric remission is the most relevant prognostic factor for multiple myeloma patients who undergo autologous stem cell transplantation. Blood. 2008;112:4017–23.
31. Korde N, Roschewski M, Zingone A, et al. Treatment with carfilzomib-lenalidomide-dexamethasone with lenalidomide extension in patients with smoldering or newly diagnosed multiple myeloma. JAMA Oncol. 2015;1:746–54.
32. Mateos MV, Oriol A, Martinez-Lopez J, et al. GEM2005 trial update comparing VMP/VTP as induction in elderly multiple myeloma patients: do we still need alkylators? Blood. 2014;124:1887–93.
33. Paiva B, Martinez-Lopez J, Vidriales MB, et al. Comparison of immunofixation, serum free light chain, and immunophenotyping for response evaluation and prognostication in multiple myeloma. J ClinOncol. 2011;29:1627–33.
34. Rawstron AC, Child JA, de Tute RM, et al. Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: impact on outcome in the Medical Research Council Myeloma IX Study. J ClinOncol. 2013;31:2540–7.
35. Paiva B, Gutiérrez NC, Rosiñol L, et al. High-risk cytogenetics and persistent minimal residual disease by multiparameter flow cytometry predict unsustained complete response after autologous stem cell transplantation in multiple myeloma. Blood. 2012;119:687–91.
36. Mathis S, Chapuis N, Borgeot J, et al. Comparison of cross-platform flow cytometry minima residual disease evaluation in multiple myeloma using a common antibody combination and analysis strategy. Cytometry B Clin Cytom. 2015;88:101–9.
37. Silvennoinen R, Kairisto V, Pelliniemi TT, et al. Assessment of molecular remission rate after bortezomib plus dexamethasone induction treatment and autologous stem cell transplantation in newly diagnosed multiple myeloma patients. Br J Haematol. 2013;160:561–4.
38. Martinelli G, Terragna C, Zamagni E, et al. Molecular remission after allogeneic or autologous transplantation of hematopoietic stem cells for multiple myeloma. J Clin Oncol. 2000;18:2273–81.
39. Sarasquete ME, Garcıa-Sanz R, Gonzalez D, et al. Minimal residual disease monitoring in multiple myeloma: a comparison between allelic-specific oligonucleotide real-time quantitative polymerase

chain reaction and flow cytometry. Haematologica. 2005;90:1365–72.
40. Puig N, Sarasquete ME, Balanzategui A, et al. Critical evaluation of ASORQ-PCR for minimal residual disease evaluation in multiple myeloma. A comparative analysis with flow cytometry. Leukemia. 2014;28:391–7.
41. Moreau P, Attal M, Caillot D, et al. Prospective evaluation of magnetic resonance imaging and [18F] fluorodeoxyglucose positron emission tomography-computed tomography at diagnosis and before maintenance therapy in symptomatic patients with multiple myeloma included in the IFM/DFCI 2009 trial: results of the IMAJEM study. J Clin Oncol. 2017;35:2911–8.
42. Rasche L, Schinke C, Alapat D, et al. Functional imaging detects residual disease in MRD-negative multiple myeloma patients who subsequently relapse. Blood. 2017;130:4510.
43. Fernandez RA, Cedena MT, Rios R, et al. Maintenance treatment with lenalidomide for multiple myeloma increases the proportion of MRD negative (flow-/PET-CT-) patients. Blood. 2017;130:3098.
44. Zamagni E, Nanni C, Mancuso K, et al. PET/CT improves the definition of complete response and allows to detect otherwise unidentifiable skeletal progression in multiple myeloma. Clin Cancer Res. 2015;21(19):4384–90.
45. de Tute RM, Jack AS, Child J, et al. A single-tube six-colour flow cytometry screening assay for the detection of minimal residual disease in myeloma. Leukemia. 2007;21:2046–9.
46. Gupta R, Bhaskar A, Kumar L, Sharma A, Jain P. Flow cytometric immunophenotyping and minimal residual disease analysis in multiple myeloma. Am J Clin Pathol. 2009;132:728–32.
47. Paiva B, Chandia M, Puig N, et al. The prognostic value of multiparameter flow cytometry minimal residual disease assessment in relapse multiple myeloma. Haematologica. 2015;100:e53–5.
48. Robillard N, Bene MC, Moreau P, Wuilleme S. A single-tube multi-parameter seven-colour flow cytometry strategy for the detection of malignant plasma cells in multiple myeloma. Blood Cancer J. 2013;3:e134.
49. Roussel M, Lauwers-Cances V, Robillard N, et al. Front-line transplantation program with lenalidomide, bortezomib, and dexamethasone combination as induction and consolidation followed by lenalidomide maintenance in patients with multiple myeloma: a phase II study by the Intergroupe Francophone du Myelome. J Clin Oncol. 2014;32:2712–7.
50. Flores-Montero J, Sanoja-Flores L, Paiva B, et al. Next generation flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. Leukemia. 2017;31:2094–103.
51. Domingo E, Moreno C, Sanchez-Ibarrola A, et al. Enhanced sensitivity of flow cytometry for routine assessment of minimal residual disease. Haematologica. 2010;95:691–2.
52. Roshal R, Flores-Montero JA, Gao Q, et al. MRD detection in multiple myeloma: comparison between MSKCC 10-color single-tube and EuroFlow 8-color 2-tube methods. Blood Adv. 2017;1(12):728–32.
53. Royston DY, Gao Q, Nguyen N, et al. Single-tube 10-fluorochrome analysis for efficient flow cytometric evaluation of minimal residual disease in plasma cell myeloma. Am J Clin Pathol. 2016;146:41–9.
54. Rawstron A, Gregory WM, de Tute RM, et al. Minimal residual disease in myeloma by flow cytometry: independent prediction of survival benefit per log reduction. Blood. 2015;125:1932–5.
55. Munshi NC, Avet-Loiseau H, Rawstron A, et al. Association of minimal residual disease with superior survival outcomes in patients with multiple myeloma a meta-analysis. JAMA Oncol. 2017;3:28–35.
56. Landgren O, Devlin S, Boulad M, Mailankody S. Role of MRD status in relation to clinical outcomes in newly diagnosed multiple myeloma patients: a meta-analysis. Bone Marrow Transplant. 2016;51:1565–8.
57. Gromley NJ, Farrell AT, Pazdur R. Minimal residual disease as a potential surrogate end point—lingering questions. JAMA Oncol. 2017;3:18–20.
58. http://www.cytognos.com/index.php/euroflow/minimal-residual-disease-panels/1440-multiple-myeloma-mm-mrd-kits. Accessed 26 Dec 2017.
59. Martinez-Lopez J, Lahuerta JJ, Pepin F, et al. Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. Blood. 2014;123:3073–9.
60. Avet-Loiseau H. Minimal residual disease by next-generation sequencing: pros and cons. Am Soc Clin Oncol Educ Book. 2016;35:e425–30.
61. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol. 2016;17:e328–46.
62. Paiva B, van Dongen JJ, Orfao A. New criteria for response assessment role of minimal residual disease in multiple myeloma. Blood. 2015;125:3059–68.
63. Rawstron AC, Orfao A, Beksac M, et al. Report of the European Myeloma Network on multiparametric flow-cytometry in multiple myeloma and related disorders. Haematologica. 2008;93:431–8.
64. Wood BL. Principles of minimal residual disease detection for hematopoietic neoplasms by flow cytometry. Cytometry B Clin Cytom. 2016;90B:47–53.
65. Rawstron AC, Pavia B, Stetler-Stevenson M. Assessment of minimal residual disease in myeloma and the need for a consensus approach. Cytometry B Clin Cytom. 2016;90B:21–5.
66. Stetler-Stevenson M, Paiva B, Stoolman L, et al. Consensus guidelines for myeloma minimal residual disease sample staining and data acquisition. Cytometry B Clin Cytom. 2016;90B:26–30.
67. Arroz M, Came N, Lin P, et al. Consensus guidelines on plasma cell myeloma minimal residual disease analysis and reporting. Cytometry B Clin Cytom. 2016;90B:31–9.

68. Oldaker TA, Wallace PK, Barnett D. Flow cytometry quality requirements for monitoring of minimal disease in plasma cell myeloma. Cytometry B Clin Cytom. 2016;90B:40–6.
69. Rawstron AC, de Tute RM, Haughton J, Owen RG. Measuring disease levels in myeloma using flow cytometry in combination with other laboratory techniques: lessons from the past 20 years at the Leeds Haematological Malignancy Diagnostic Service. Cytometry B Clin Cytom. 2016;90B:54–60.
70. Flores-Montero J, de Tute R, Paiva B, et al. Immunophenotype of normal vs. myeloma plasma cells: toward antibody panel specifications for MRD detection in multiple myeloma. Cytometry B Clin Cytom. 2016;90B:61–72.
71. Gormley NJ, Turley DM, Dickey JS, et al. Regulatory perspective on minimal residual disease flow cytometry testing in multiple myeloma. Cytometry B Clin Cytom. 2016;90B:73–80.
72. Vittorio Emanuele M, Elona S, Milena G, et al. Multiple myeloma: new surface antigens for the characterization of plasma cells in the era of novel agents. Cytometry B Clin Cytom. 2016;90B:81–90.
73. Pojero F, Flores-Montero J, Sanoja L, et al. Utility of CD54, CD229, and CD319 for the identification of plasma cells in patients with clonal plasma cell diseases. Cytometry B Clin Cytom. 2016;90B:91–100.
74. Barnett DSI, Wilson GA, Granger V, Reilly JT. Determination of leucocyte antibody binding capacity (ABC): the need for standardization. Clin Lab Haematol. 1998;20:155–64.
75. Terstappen LW, Johnsen S, Segers-Nolten IM, Loken MR. Identification and characterization of plasma cells in normal human bone marrow by high-resolution flow cytometry. Blood. 1990;76:1739–47.
76. Wijdenes J, Vooijs WC, Clement C, et al. A plasmocyte selective monoclonal antibody (B-B4) recognizes syndecan-1. Br J Haematol. 1996;94:318–23.
77. Darzalex (daratumumab) injection [prescribing information]. Horsham: Janssen Biotech, Inc; 2015.
78. Lin P, Owens R, Tricot G, Wilson CS. Flow cytometric immunophenotypic analysis of 306 cases of multiple myeloma. Am J Clin Pathol. 2004;121:482–8.
79. Almeida J, Orfao A, Ocqueteau M, et al. High-sensitive immunophenotyping and DNA ploidy studies for the investigation of minimal residual disease in multiple myeloma. Br J Haematol. 1999;107: 121–31.
80. Mateo G, Montalban MA, Vidriales M, et al. Prognostic value of immunophenotyping in multiple myeloma: a study by the PETHEMA/GEM cooperative study groups on patients uniformly treated with high-dose therapy. J Clin Oncol. 2008;26:2737–44.
81. Tembhare P, Yuan CM, Venzon D, et al. Flow cytometric differentiation of abnormal and normal plasma cells in the bone marrow in patients with multiple myeloma and its precursor diseases. Leuk Res. 2014;38:371–6.
82. Robillard N, Wuilleme S, Moreau P, Bene MC. Immunophenotype o fnormal and myelomatous plasma-cell subsets. Front Immunol. 2014;5:137.
83. Cannizzo E, Bellio E, Sohani AR, et al. Multiparameter immuno-phenotyping by flow cytometry in multiple myeloma: the diagnostic utility of defining ranges of normal antigenic expression in comparison to histology. Cytometry B Clin Cytom. 2010;78:231–8.
84. vanDongen JJM, Lhermitte L, Bottcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia. 2012;26:1908–75.
85. Paiva B, Gutierrez NC, Chen X, et al. Clinical significance of CD81 expression by clonal plasma cells in high-risk smoldering and symptomatic multiple myeloma patients. Leukemia. 2012;26: 1862–9.