Brief Communication

Assessment of Minimal Residual Disease in Myeloma and the Need for a Consensus Approach

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Treatment options for myeloma continue to develop at a rapid pace, and it is becoming increasingly challenging to determine the optimal therapeutic approaches because demonstrating a clear survival benefit now requires many years of follow-up. The detection of minimal residual disease (MRD) is recognized as a sensitive and rapid approach to evaluate treatment efficacy that predicts progression-free and overall survival independent of categorical response assessment and patients' biology. The benefit of MRD analysis is reflected in the many different techniques (multiparameter flow cytometry, quantitative polymerase chain reaction, and high-throughput sequencing) and collaborative groups (including EMN, ESCCA, ICCS, EuroFlow, and EuroMRD) that have performed collaborative projects to harmonize quantitative MRD detection. The time has come to adopt a consensus approach, and this report reviews the benefits and disadvantages of different strategies for MRD detection in myeloma and highlights the requirements for a sensitive, reproducible, and clinically meaningful cellular analytical approach. © 2015 International Clinical Cytometry Society

Key terms: plasma cell myeloma; minimal residual disease; PCR; flow cytometry; high throughput sequencing; quantification; rare event detection

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Current therapeutic approaches for plasma cell myeloma (myeloma) offer an overall survival (OS) of more than 5 years for the majority of newly diagnosed patients. New and effective treatments are being developed at an unprecedented rate, but are becoming available to patients less rapidly because randomized Phase 3 trials now take several years to show benefit when measured by the most strict end-point, i.e., OS (1). Recognizing the increasing delay between drug development and approval, regulatory bodies are investigating whether biomarker evaluation of response such as minimal residual disease (MRD) assessment, can provide robust prediction of survival, thereby reducing the duration and cost of the drug approval process (2) and accelerating the safe translation of new therapies and their benefits to the patients.

MRD analysis in myeloma has been under evaluation as a more sensitive measure of response than conventional criteria (3) for more than two decades. More recently, several publications have demonstrated enhanced prediction of outcome using flow-MRD in comparison with categorical response in different clinical trials in different laboratories (4–9). In these studies, flow cytometry was demonstrated to be an independent predictor of PFS and OS in prospective studies, a critical feature for a surrogate trial endpoint. Although initially less sensitive than molecular assays, detection of MRD by flow cytometry (flow-MRD) became the preferred method by several cooperative groups to adopt in myeloma clinical trials for several

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reasons. First, flow-MRD is applicable to virtually every patient using a standard set of disease-associated markers, in contrast to sensitive molecular approaches that required, up until recently, the development of a specific assay for each patient (i.e., allele-specific oligonucleotide polymerase chain reaction, ASO-PCR). Moreover, flow-MRD assays incorporate a quality check of the whole sample cellularity that is critical for the identification of hemodilute aspirated bone marrow samples that can lead to false-negative results, and may allow for immediate communication between hospital and central laboratories for additional samples. In contrast, quantitative real-time PCR (qPCR) approaches, including ASO-PCR, require additional checks for sample quality. Finally, flow-MRD assays have become more sensitive (10^{-5}) and are directly quantitative with the same lower limits of detection (LOD) and quantification (LLOQ) in every case, in contrast to qPCR approaches that are calibrated to a standard curve with LOD/LLOQ that may vary according to the immunoglobulin heavy chain (IGH) variable region gene sequence.

The more recent development of high-throughput sequencing (HTS) provides a promising alternative to ASO-PCR. HTS is reported to offer an LOD of up to one myeloma cell in one million leukocytes (expressed as 10^{-6}, 1.0E-6, or 0.0001%), but unlike previous ASO-PCR molecular approaches, HTS strategies utilize the same set of primers for all patients, and such assays are now commercially available (10). However, HTS is an emerging technology requiring extensive prospective validation, as there are still multiple issues with quantification, including the calibration and correction approaches used to determine total leukocytes, B-lineage cell numbers, and the reproducible limit of quantification (11). Accurate quantification is also becoming more important because it has recently been demonstrated that the level of MRD is a more powerful predictor of PFS and OS than a categorical approach with MRD-negativity based on a threshold (12). Reproducible quantification is an absolute requirement for comparison of results across different trial centers and treatment strategies. In this regard, whereas initial reports for ASO-PCR suggested an LOD of 10^{-6}, more rigorous validation undertaken determined approximately one log less sensitivity (10^{-5}) (13,14). The potential impact of other conceptual issues, such as clonal heterogeneity, clonal evolution and selection under treatment, or concomitant indolent B-cell disorders (e.g., monoclonal B-cell lymphocytosis), of the outcome of both HTS and ASO-PCR techniques remain to be addressed. Similar to previous molecular approaches, HTS analysis requires additional checks to determine whether a negative result is due to sample quality (e.g., morphology and/or flow cytometry). Finally, the clinical value of the technology is yet to be proven prospectively in randomized trials. The major features of the available and evolving technologies for quantifying MRD in myeloma are compared and contrasted in Table 1.

Although further evaluation and valuation of new MRD methodologies in myeloma are underway, clinical trials that require a validated and sensitive assay with a proven track record of predicting outcome continue to rely on flow cytometry as the method of choice. Immunophenotypic complete response (i.e., undetectable MRD at the 10^{-4} level in the bone marrow) in myeloma has been shown to be one of the most relevant prognostic factors for patients undergoing autologous stem cell transplantation, as well as in nontransplant eligible patients treated with novel agents (6–9). In addition, baseline flow cytometric studies of bone marrow aspirates may also contribute to prediction of outcome of myeloma patients after standard chemotherapy and high-dose therapy followed by autologous stem cell transplantation (20–23). Furthermore, circulating phenotypically aberrant/clonal plasma cells can be detected in approximately 80% of myeloma patients at presentation, and the level of circulating neoplastic plasma cells in newly diagnosed myeloma patients is a predictor of PFS and OS (24–27). Quantification of circulating neoplastic plasma cells may become particularly useful to predict risk of transformation of monoclonal gammopathy of undetermined significance and smoldering myeloma cases (28,29). OS is significantly reduced in myeloma patients undergoing autologous stem cell transplantation, when flow cytometry detects neoplastic plasma cells in the stem cell grafts (25,30). Similarly, flow cytometric detection of circulating neoplastic plasma cells in the peripheral blood of myeloma patients 2 weeks prior to stem cell harvest is associated with inferior PFS and OS (31). Therefore, assessment of peripheral blood samples obtained at different time points during the course of the disease may also be relevant for prognostication and clinical management in the near future, though its complementary role with bone marrow MRD evaluation is yet to be demonstrated.

As treatment strategies for myeloma become more effective and progression-free survival becomes longer, assessing treatment efficacy according to MRD levels becomes increasingly important. Therefore, standardization of flow-MRD testing is vital to ensure superior uniform assessment of response and clinical prognostication. In line with this, and building on the earlier consensus of the European Myeloma Net (EMN) guidelines (15), the International Clinical Cytometry Society (ICCS) and European Society for Clinical Cell Analysis (ESCCA) recognized the need for a consensus flow cytometric approach that not only provides backward compatibility with established assays and is applicable in a significant number of central laboratories, but also offers sufficiently high sensitivity to remain relevant for the next decade as treatment strategies continue to evolve.

The following documents outline a recommended approach to the acquisition, analysis, and quality control steps of a consensus flow cytometry assay that would offer an LOD and quantification of comparable orders of magnitude achievable by ASO-PCR and potentially also HTS. Such an approach has the additional benefits of internal sample quality checks, no mandatory requirement for pretreatment samples, and a cost-effective proven track record of predicting outcome in prospective clinical trials.

Table 1
Comparison of Flow Cytometry and Molecular Techniques for MRD Analysis in Myeloma (6–9,14–19)

	Flow cytometry		Molecular techniques	
	2008 EMN consensus (4–6 color)	2014 ICCS/ESCCA consensus (≥8 color)	ASO-qPCR	High-throughput sequencing
Percentage of patients applicable	>95	>99	50–90	80–90
Lower limit of quantification (LLOQ)	0.01%, 1.0E-4, or 1 in 10,000	0.0025%, 2.5E-5, or 1 in 40,000	0.001%, 1.0E-5, or 1 in 100,000	0.001%, 1.0E-5, 1 in 100,000
Amount of DNA recommended for LLOQ/approximate number of cells	500,000 events per tube/ ≥1 million cells	2 million events per tube/≥3 million cells	500 ng DNA in triplicate/≥1 million cells	14 μg DNA/≥2 million cells
Lower limit of detection (sensitivity) (LOD)	0.004%, 4.0E-5, or 1 in 25,000	0.0004%, 4.0E-6, or 1 in 250,000	0.0001%, 1.0E-6, or 1 in 1,000,000	0.0001%, 1.0E-6, or 1 in 1,000,000
Is the assay the same for every applicable patient?	Yes—but additional markers may be required in up to 10% of cases	Yes	No—requires design and validation of patient-specific primers	Yes
Pretreatment evaluation	Required	Not mandatory	Required	Required
Does the assay require fresh material	Yes—samples must be <48 h old and should be processed immediately		Preferable—samples ideally should be <48 h old before DNA extraction, but analysis can be performed on archive material; extracted DNA may be stored indefinitely before processing	
Directly quantitative	Yes—neoplastic plasma cells are reported as a percentage of leukocytes		No—patient-specific IGH copy number calibrated to a standard curve generated from pretreatment DNA serially diluted into DNA extracted from pooled donor mononuclear cells	No—patient-specific IGH copy number calibrated to reference IGH sequence, reported as a proportion of total leucocytes calculated from total DNA content
Additional check for sample quality	Not required—identification of hematopoietic elements (progenitors and normal plasma cells) as well as other bone marrow associated cellular elements (NRBC) within the assay		Required—morphology or cytometry on the sample to assess quality and identify hemodilute specimens	
Harmonization	Yes (EMN consensus)	Yes (ICCS/ESCCA)	Yes	Ongoing (EuroMRD)
Independent prognostic factor for outcome in prospective clinical trial	Progression-free survival and overall survival		No—univariate analysis only	Under evaluation

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