Biol Blood Marrow Transplant 25 (2019) e89–e97

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Report

Summary of the Second Annual BMT CTN Myeloma Intergroup Workshop on Minimal Residual Disease and Immune Profiling

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Article history:
Received 11 September 2018
Accepted 1 November 2018

Key Words:
Minimal residual disease
Immune profiling
Multiple myeloma
Autologous stem cell transplantation
Endpoint

A B S T R A C T

The second annual Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Myeloma Intergroup Workshop on Minimal Residual Disease and Immune Profiling was convened on December 7, 2017, at the American Society of Hematology (ASH) meeting. During this workshop, investigators from around the world presented their latest research involving assessment of minimal residual disease (MRD) and immune profiling (IP) in myeloma. This document summarizes the workshop presentations as well as relevant ASH abstracts and focuses on the regulatory issues involved in the integration of MRD and IP assessment in clinical trial design and practice.

INTRODUCTION

The incorporation of such therapies as immunomodulatory drugs, proteasome inhibitors, and monoclonal antibodies into the newly diagnosed and relapsed/refractory setting have led to higher response rates and improved survival for patients with multiple myeloma (MM). In particular, achievement of minimal residual disease (MRD)-negativity has been associated with improved survival outcomes [1]. Previously reported studies evaluating MRD have been heterogeneous with respect to the sensitivity of the MRD assay and the technique used, leading to international efforts to standardize MRD assessment [2–4]. Although MRD status is now incorporated into the International Myeloma Working Group (IMWG) response criteria [5], and it is evident that MRD negativity can serve as a prognostic biomarker, whether MRD can be used as a surrogate endpoint or to guide treatment decisions is not clear. In addition, although several reports have highlighted varying immune profiles that appear to correlate with survival outcomes [6–8], the clinical utility of immune profiling (IP) remains to be determined.

In 2016, the first annual Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Myeloma Intergroup Workshop on MRD and IP was convened to discuss emerging data and technologies for MRD and IP assessment and to develop strategies to incorporate MRD IP into clinical trial design [9]. The second annual workshop was convened at the American Society of Hematology (ASH) meeting on December 7, 2017. Here we summarize the presentations from the workshop, review the

Financial disclosure: See Acknowledgments on page e95.

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https://doi.org/10.1016/j.bbmt.2018.11.001
1083-8791/

relevant myeloma MRD/IP abstracts from ASH 2017, and discuss future considerations for the clinical and/or regulatory uses of MRD and IP.

Defining MRD

In 2016, the IMWG published their consensus criteria for MRD assessment and incorporation of MRD status into the response criteria [5]. They clarified that a first-pull bone marrow aspirate of 2 to 5 mL should be used for MRD analysis. The criteria distinguish between flow MRD-negativity and sequencing MRD-negativity. If multiparametric flow cytometry (MFC) analysis, also known as next- generation flow (NGF), is used, then a validated 8-color, 2-tube method should be used, in accordance with the established Euro-Flow procedure [10]. Five million cells are to be assessed, and the MFC method should have a sensitivity of at least 1 in 10^5 plasma cells. If NGS is to be used, then the IMWG specifies the use of a validated assay, such as Lympho-SIGHT (Sequenta, San Francisco, CA) with a minimum sensitivity of 1×10^{-5}. In September 2018, the US Food and Drug Administration (FDA) granted de novo designation for the clonoSEQ assay (Adaptive Biotechnologies, Seattle, WA) for the detection and monitoring of MRD in patients with myeloma or B cell acute lymphoblastic leukemia (https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm622004.htm). The clonoSEQ assay has a sensitivity of 1×10^{-6}. The IMWG also defined "imaging plus MRD-negative" status as MRD negativity by NGF or NGS in addition to positron emission tomography (PET)/computed tomography (CT)-negativity. Finally, the concept of "sustained MRD-negativity" was introduced as achieving imaging plus MRD-negativity in assessments that are a minimum of 1 year apart. Although these definitions will be incorporated into ongoing and future studies, there continues to be significant heterogeneity in the sensitivity thresholds used in recently presented abstracts, as detailed below.

MRD and IP Analysis from Completed Clinical Trials

Myeloma MRD from UK studies

Roger Owen provided an overview of the MRD testing performed in the Myeloma XI trial, as well as several other recent UK studies. The Myeloma XI trial enrolled 1971 patients with newly diagnosed myeloma, including 1248 transplantation-eligible (TE) and 723 transplantation-ineligible (TNE) patients. Following completion of a variety of induction regimens, consolidation based on response to induction, and then autologous stem cell transplantation (ASCT) for patients in the TE group, all patients underwent randomization to lenalidomide maintenance, no maintenance, or lenalidomide plus vorinostat maintenance. Data regarding the lenalidomide plus vorinostat subgroup were not presented. MRD assessment was performed via MFC at 10^{-4} using International Clinical Cytometry Society/European Society for Clinical Cell Analysis consensus performed at a single central laboratory. DNA was also stored for future NGS testing.

An analysis was performed on a subset of patients (n = 389) who had evaluable samples at 6 and 12 months after maintenance randomization [11]. MRD negativity at 6 months was observed in 206 (56%) of these patients. MRD status at 6 months after initiation of maintenance therapy had a significant impact on progression-free survival (PFS). The median PFS was not reached for the MRD-negative subgroup, whereas it was 24 months for the MRD-positive subgroup (hazard ratio [HR], .22; 95% confidence interval [CI], .14 to .34; $P < .0001$). In addition, superior overall survival (OS) was observed in the MRD-negative patients (HR, .42; 95% CI, .21 to .87; $P = .0153$). Of note, maintenance therapy improved PFS regardless of MRD status, and 30% of the patients receiving maintenance therapy converted from MRD-positive to MRD-negative, compared with 4% in the observation group ($P = .0045$).

The Myeloma X study treated patients with salvage therapy comprising bortezomib/doxorubicin/dexamethasone following relapse after ASCT. Patients were subsequently randomized to salvage ASCT or cyclophosphamide (400 mg/m^2 orally weekly $\times$ 12 weeks) [12,13]. In this study, 174 patients were randomized, and day 100 MRD testing was available in 95 patients. Both PFS (HR, .39; 95% CI, .24 to .61; $P < .0001$) and OS (HR, .52; 95% CI, .27 to .99; $P = .0434$) were prolonged in MRD-negative patients compared with MRD-positive patients. The PADIMAC trial (Phase II study of bortezomib, Adriamycin, and dexamethasone (PAD) therapy for previously untreated patients with multiple myeloma: Impact of Minimal Residual Disease in Patients with Deferred ASCT) treated patients with PAD induction followed by stem cell harvest [14]. Patients with partial response (PR) underwent ASCT, whereas those with very good partial response (VGPR) or complete response (CR) were observed and then offered ASCT at the time of relapse. MRD assessment was performed following stem cell harvest and at day 100 post-ASCT (n = 77). MRD negativity at the time of stem cell harvest was associated with superior PFS. In addition, patients who achieved a PR, underwent ASCT, and were MRD-negative at day 100 had similar PFS as those who were MRD-negative and in VGPR/CR after induction. Finally, the MUK *five* trial randomized patients in first relapse or with primary refractory disease to cyclophosphamide/bortezomib/dexamethasone (8 cycles of 21 days) versus carfilzomib/cyclophosphamide/dexamethasone (6 cycles of 28 days). Among the 292 evaluable patients, 182 (62%) had available MRD samples. MRD was assessed at the end of induction, and 18% of patients were MRD-negative (~10% MRD-negative in the intention-to-treat population).

BMT CTN PRIMeR

The PRIMeR (Prognostic Immunophenotyping for Multiple Myeloma Response) study is an ancillary MRD study associated with the BMT CTN 0702 STaMINA (Stem Cell Transplantation for Multiple Myeloma Incorporating Novel Agents) trial. The STaMINA study involved 750 patients who were randomized to 1 of 3 arms: (1) single ASCT followed by lenalidomide maintenance, (2) single ASCT followed by consolidation with 4 cycles of VRD (bortezomib, lenalidomide, dexamethasone) and then lenalidomide maintenance, and (3) tandem ASCT followed by lenalidomide maintenance [15]. To date, no differences in PFS or OS have been observed among the 3 arms. Bone marrow and peripheral blood samples were collected at randomization, before initiation of maintenance, and at 1 year postrandomization. Marcelo Pasquini provided information regarding the design and results thus far from the PRIMeR study. The primary endpoint was to evaluate MRD status across treatment arms at the 1-year time point. The number of bone marrow samples available for MRD was 302 at baseline, 314 before maintenance, and 294 at year 1. MRD was assessed centrally using 4- and 6-color MFC with 10^{-5} sensitivity. MRD negativity rates were 43% before transplantation, 78% before maintenance and 84% at 1 year. MRD status is currently being analyzed to determine whether this is more prognostic for PFS than traditional disease response.

EMN 02/HO95

The RV-MM-COOP-0556 (EMN02/HO95) study enrolled 1499 newly diagnosed patients [16,17]. Patients received VCD

(bortezomib, cyclophosphamide, dexamethasone) induction, followed by stem cell collection and randomization to ASCT (single or double) versus 4 cycles of bortezomib, melphalan, and prednisone. Patients then underwent a second randomization (R2) to consolidation with 2 cycles of VRD versus nothing, and then all patients received lenalidomide maintenance. Stefania Oliva discussed the MRD testing that was performed as part of this trial [18]. MRD was assessed in patients with suspected CR before randomization (R2), before maintenance, and then every 6 months during maintenance therapy until clinical relapse occurred. MRD assessment was performed using the EuroFlow protocol [3], with a maximum sensitivity of 10^{-5}, centralized in 3 European laboratories. The cutoff for MRD positivity was defined as ≥20 clonal plasma cells out of at least 1×10^4 acquired plasma cells or at least 2 million leukocytes. Quality checks were done to compare sensitivity and demonstrate correlation of protocols among the 3 laboratories. Before maintenance therapy, 76% of the patients were MRD-negative. Among Of the 24% who were MRD-positive before maintenance and underwent subsequent MRD analysis after at least 1 year of maintenance, 44% and 48% became MRD-negative after 1 year and 2 years of maintenance, respectively. Among the 316 patients assessed for MRD, the median PFS was not reached for those who achieved MRD-negativity, whereas it was 38 months for those who were MRD-positive (HR, .33; 95% CI, .20 to .53; $P < .001$). A landmark analysis after 1 year of maintenance therapy showed a statistically significant difference for the 2-year PFS rate: 92% for MRD-negative versus 65% for MRD-positive ($P < .001$). In subgroup analysis, patients with high-risk cytogenetics and ISS stage III were at greatest risk for MRD-positivity. Despite this, PFS was superior in patients with high-risk cytogenetics or ISS stage III who achieved MRD-negativity compared with those with MRD-positivity.

Incorporating MRD and IP Assessment into Current and Future Clinical Trials

GMMG-CONCEPT

Katja Weisel presented the GMMG-CONCEPT study (A clinical Phase II, multicenter, open-label study evaluating induction, consolidation, and maintenance treatment with isatuximab (SAR650984), carfilzomib, lenalidomide, and dexamethasone (I-KRd) in primary diagnosed high-risk multiple myeloma patients). This study will involve 117 TE patients and 36 TNE patients, all with high-risk disease as defined by del(17p), t(4;14) or gain(1q21) and ISS II/III. In the TE arm, patients will receive 6 cycles of I-KRd induction, followed by single or double ASCT, consolidation with 4 cycles of I-KRd and then I-KR maintenance until progression. In the TNE group, patients receive a total of 12 cycles of I-KRd, followed by I-KR maintenance until PD.

The study's primary objective is MRD-negativity after consolidation using MFC at 10^{-5} sensitivity, with experimental MRD assessment evaluated with allele-specific oligonucleotide-PCR, NGS, and diffusion-weighted magnetic resonance imaging (DW-MRI). All patients in VGPR/CR will undergo MRD assessment, and all MRD-negative patients will undergo MRD assessment every 6 months. The secondary objective of the study is PFS, and tertiary objectives include overall response rate, duration of MRD-negativity, and OS. Exploratory objectives include determining the best method for defining the MRD-negative state; evaluating genetic polymorphisms, such as HLA, KIR, and FcGR variations, at baseline; and evaluating immunologic reconstitution during maintenance. The latter will be evaluated in both the bone marrow and peripheral blood using IP via MFC and will assess CD4 T cells, CD8 T cells, regulatory T cells, natural killer (NK) cells, invariant NK T cells, monocytes, neutrophils, myeloid-derived suppressor cells, and dendritic cells. At the time of the workshop, 7 patients had been enrolled.

SWOG S1803 (DRAMMATIC Study)

Amrita Krishnan presented the concept for the DRAMMATIC Study: Phase III study of daratumumab + lenalidomide (LD) or lenalidomide (L) as post-autologous stem cell transplant maintenance therapy in patients with multiple myeloma (MM) using minimal residual disease to direct therapy duration. Lenalidomide maintenance following ASCT has been shown to significantly prolong both PFS and OS [19]. Furthermore, as noted above, the data from the Myeloma XI trial show improved PFS in patients who achieve MRD-negativity post-ASCT [11]. In the relapsed/refractory setting, the addition of daratumumab to lenalidomide/dexamethasone significantly increased the rate of MRD-negativity [20]. The DRAMMATIC study plans to address whether lenalidomide + daratumumab maintenance is superior to single-agent lenalidomide maintenance and whether MRD status can be used to guide the duration of maintenance therapy. Patients will be randomized to single-agent lenalidomide versus lenalidomide/daratumumab maintenance post-ASCT. After 2 years of maintenance, MRD will be assessed by NGS (sensitivity, 10^{-6}). Patients who are MRD-positive will continue maintenance therapy, and those who are MRD-negative will be randomized to either continuation or discontinuation of maintenance therapy. The primary objective is to compare the OS between the 2 maintenance arms. Secondary objectives for the first randomization include PFS, overall response rate, MRD-negativity rate, and toxicity, and those for the second randomization include comparing PFS between MRD-negative patients randomized to indefinite maintenance therapy versus those randomized to discontinued maintenance therapy. A total cohort of 950 patients enrolled over 6 years is needed to detect an increase in the median OS from 10 to 16.7 years in the combination arm (HR, .6). At the time of the workshop, the plan was to assess MRD status via NGS over multiple time points.

GEM2014MAIN

Noemi Puig discussed the Spanish Myeloma Group's (GEM) past and current efforts to assess MRD and IP. As has been reported previously, second-generation 8-color MFC was used to evaluate MRD in 162 transplantation-ineligible patients enrolled in the PETHEMA/GEM2010MAS65 study [8]. Thirty-four percent of patients achieved MRD-negativity, and this was associated with improved time to progression and better OS. In addition, IP was assessed by evaluating 15 bone marrow cell subsets (erythroid and myeloid hematopoietic progenitors; erythroblasts; mast cells; eosinophils; basophils; monocytes; neutrophils; B lymphocytes and their respective precursor, naïve, and memory subsets; natural killer T cells; and natural killer cells and remaining T lymphocytes) using a single 8-color combination (CD45, CD138, CD38, CD56, CD27, CD19, CD117, and CD81). Based on the IP phenotype, patients were grouped into 3 clusters with different times to progression and OS outcomes. The authors identified a subset of MRD-positive patients with a favorable IP. Currently this group is performing immune monitoring in the GEM2014MAIN study, which is evaluating lenalidomide/dexamethasone versus lenalidomide/ixazomib/dexamethasone maintenance therapy post-ASCT. Following completion of 2 years of maintenance therapy, patients who are MRD-negative will be observed, whereas those who are MRD-positive will continue lenalidomide/dexamethasone maintenance for an additional 3 years. A single

8-color (9-marker) flow strategy is being used to identify 18 different B cell subsets, including precursors, naïve, and memory with heavy chain and light chain distribution. In addition, 2 more 8-color tubes are being used, including 1 tube to identify more than 10 T cell subsets and the 1 tube to characterize NK subpopulations. An example was shown demonstrating the IP of 4 patients in the GEM2014MAIN study after 1 year of maintenance therapy with different patterns observed when evaluating NK cells, CD4 and CD8 T cells, B cells, and plasma cells. The overall goal of this work is to integrate patient factors with the tumor landscape, MRD assessment, and IP to identify predictors of outcome.

Ongoing Developments in MRD and IP

Peripheral blood IP and MRD assessment

Manisha Bhutani presented work done by the Levine Cancer Institute investigating whether the peripheral IP is distinct in MRD-positive versus MRD-negative patients following ASCT [21]. Thirty-six patients who underwent ASCT had bone marrow and blood specimens obtained between days 60 and 90 post-ASCT. The bone marrow was analyzed for MRD using next-generation flow cytometry, and blood was analyzed for activation, polarization, and functionality using flow cytometry. The MRD assay was based on EuroFlow, with the threshold for MRD positivity defined as >15 abnormal plasma cells in 1 million nucleated cells (1.5×10^{-5}). Using this assay, 6 of 36 patients (17%) were MRD-negative. Mature NK, NKT-like, and T cells were identified based on the distributions of CD3 and CD56 and were then subsequently evaluated for major histocompatibility complex I cytotoxicity, CD1d cytotoxicity, activation and anergy via surface expression of NK "inhibitory" Ig-like receptors (KiR2DS4 and KiR3DL1), NK group 2 proteins (NKG2A and NKG2D), NK p46 protein (NKp46), programmed death receptor 1 (PD1), and T cell inhibitory receptor (Tim3). Differences in the peripheral IP of MRD-positive and -negative patients were found, including lower NK/$\gamma\delta$ T cell numbers and a higher NK/NK T activation phenotype in MRD-positive patients. In addition, they monitored the IP of patients undergoing immunomodulatory drug-based maintenance therapy and found that the differences in NK/$\gamma\delta$ T cell numbers between MRD-positive and -negative patients normalized in response to maintenance therapy. Future directions for this work include blood and marrow IP using NGF (62 variables) and NGS ($\alpha\beta$/$\gamma\delta$ T cell receptor sequencing) in a variety of contexts, including monoclonal gammopathy of undetermined significance, smoldering and active myeloma [22] longitudinal monitoring during lenalidomide maintenance [21] and as correlative endpoints in ongoing and planned clinical trials.

Evaluation of clonal heterogeneity and MRD

Niels Weinhold presented data from studies in which bone marrow aspirates and focal lesions were molecularly evaluated and compared. In these studies, both a standard iliac crest aspirate and a CT-guided aspirate of focal lesions were obtained. CD138 cells were subsequently selected from the specimens, and whole-exome sequencing and copy number arrays were performed. As recently reported by Rasche et al. [23], these studies revealed the spatial heterogeneity of myeloma. In particular, evidence was provided for the regional evolution of myeloma as well as the limited exchange between sites, likely as a consequence of spatial constraints within the bone marrow. In addition, focal lesions appeared to contain advanced clones. Differential responses of these clones to therapy were shown in a patient who underwent tissue biopsies at multiple sites across multiple time points. At baseline, 3 different biopsy sites yielded the same dominant clone; however, at 2 months after chemotherapy, 1 residual focal lesion now had a different genomic profile. Seven months later, the patient had progressed, and biopsy of 2 different sites revealed that the second clone was now dominant in both sites.

In another example, a patient had 4 different clones identified from 4 different biopsy sites at baseline. After 4 months of treatment, including a first ASCT, the patient was noted to be in a stringent CR with MRD-positivity in the bone marrow (.004%). Three months later, after the second ASCT, the marrow was now MRD-negative (<.001%); however, focal lesions were still observed in the marrow on imaging. The patient received consolidative chemotherapy, but then relapsed 4 months after the second ASCT. At that time, biopsies of 2 sites showed the presence of the baseline clone, which had now acquired an additional 144 mutations, as well as a second dominant clone.

Finally, a third case was presented in which a patient with relapsed/refractory MM with known disease progression was assessed as MRD-negative in bilateral iliac crest aspirates. Taken together, these studies reveal the extraordinary complexity of MM with clonal evolution and spatial heterogeneity and underscore the importance of interpreting marrow MRD testing in the context of imaging.

Radiographic assessment of MRD

Jens Hillengass presented an overview of the different bone marrow infiltration patterns observed using MRI, including minimal, diffuse, focal, and mixed, which are estimated to be present in 20%, 30%, 30%, and 20% of patients, respectively. Focal lesions can be detected using PET/CT, T1-weighted MRI, T2-weighted MRI, or DW-MRI. The heterogeneous infiltration of the bone marrow by myeloma can result in disparate MRD results depending on where the aspirate is obtained. Although several studies have reported the prognostic significance of residual lesions on PET/CT at the time of or after ASCT, the prognostic significance of MRI has been less evident [24–27]. Advances in DW-MRI, which uses differences in the motion of water molecules between tissues, has led to the development of whole-body DW-MRI, which could be used to evaluate burden of disease and changes following therapy [28]. Finally, there are emerging imaging modalities that could be used to evaluate MRD and IP. The incorporation of zirconium-89-labeled monoclonal antibodies into PET imaging (immuno-PET) has been evaluated in a variety of malignancies [29]. This technique has the potential not only to provide visualization of the tumor, but also to quantify uptake of the radiolabeled monoclonal antibodies [30]. A recent report described the development of zirconium-89-labeled reconstituted high-density lipoproteins that have high uptake by tumor-associated macrophages, which may have potential for noninvasively monitoring tumor-associated macrophage immunology [30].

Memorial Sloan Kettering Cancer Center-National Cancer Institute Consortium

Ola Landgren provided updates on the Memorial Sloan Kettering Cancer Center (MSKCC)-National Cancer Institute MRD consortium, which is part of the MSKCC Biomarker Development Initiative. This initiative is focused on establishing MRD as a novel surrogate endpoint to accelerate novel drug development and encompasses evaluation of myeloma biology, MRD assay development, and development of assays that are independent of bone marrow sampling. As noted in other

Table 1
Summary of ASH 2017 Abstracts Evaluating MRD in Plasma Cell Disorders

Study	MRD Methodology (Sensitivity)	Patient Population
ALCYONE; Mateos et al. [37]	NGS (10^{-5})	TNE ND randomized to D-VMP versus VMP
IFM2009; Avet-Loiseau et al. [38]	NGS (10^{-6})	TE ND receiving RVD induction randomized to consolidation with ASCT/RVD versus RVD, followed by lenalidomide maintenance
Myeloma XI; de Tute et al. [11]	MFC (4×10^{-5})	TE and TNE ND
GEM2000 MM, GEM2010, GEM2012; Medina et al. [39]	NGS compared with MFC ($\geq 10^{-5}$)	TE and TNE ND
EMN02/HO95; Cavo et al. [16]	MFC (10^{-5})	TE ND
Chu et al. [40]	qPCR for patient-specific variable region sequence (10^{-5})	TE ND
Huang and Li [41]	MFC	TE ND
Pethema/GEM2012; Paiva et al. [42]	MFC (3×10^{-6})	TE ND
Terpos et al. [43]	MFC (2×10^{-6})	Sustained CR for $\geq$2 years after first-line therapy
Rasche et al. [44]	MFC (10^{-4} to 10^{-5}); functional imaging: PET/CT, DWIBS	TE ND or following salvage therapy
PADIMAC; Popat et al. [14]	MFC 10^{-4}	TE ND
MM5; Huhn et al. [45]	PCR of paired BM and PB samples	TE ND
Fernandez et al. [46]	MFC and PET/CT	TE and TNE ND
Pawarode et al. [47]	MFC (3×10^{-5} to 5×10^{-6})	TE ND, not achieving CR pre-ASCT
Solovev et al. [48]	MFC (10^{-5})	TE ND
MMY1001; Facon et al. [49]	NGS (10^{-4}, 10^{-5}, 10^{-6})	RR receiving DPd
Gay et al. [50]	MFC (10^{-5})	TE ND receiving KRD versus KCD induction
CASTOR; Spencer et al. [51]	NGS (10^{-4}, 10^{-5}, 10^{-6})	RR receiving Vd versus DVd
POLLUX; Dimopoulos et al. [52]	NGS (10^{-4}, 10^{-5}, 10^{-6})	RR receiving Rd versus DRd
Rosinol et al. [53]	MFC (3×10^{-6})	TE ND receiving RVD
Brudno et al. [54]	MFC	RR receiving BCMA CAR T-cells
Korde et al. [55]	MFC	ND receiving KRD induction
GEM-CESAR; Mateos et al. [56]	MFC	High-risk smoldering MM treated with KRD induction, ASCT, and KRD consolidation and maintenance
Jimenez-Zepeda et al. [57]	MFC	Newly diagnosed and relapsed AL patients
Foureau et al. [21]	MFC (1.5×10^{-5})	Post-ASCT receiving IMiD maintenance

TNE indicates transplantation-ineligible; ND, newly diagnosed; D-VMP, daratumumab/bortezomib/melphalan/ dexamethasone; VMP, bortezomib/melphalan/prednisone; RVD, lenalidomide/bortezomib/dexamethasone; TE, transplantation eligible; MFC, multiparametric flow cytometry; DWIBS, diffusion-weighted magnetic resonance imaging with background suppression; BM, bone marrow; PB, peripheral blood; RR, relapsed/refractory; DPd, daratumumab/pomalidomide/dexamethasone; KRD, carfilzomib/lenalidomide/ dexamethasone; KCD, carfilzomib/cyclophosphamide/dexamethasone; Vd, bortezomib/dexamethasone; DVd, daratumumab/bortezomib/dexamethasone; Rd, lenalidomide/dexamethasone; DRd, daratumumab/lenalidomide/dexamethasone; AL, light chain amyloid; IMiD, immunomodulatory drug.

presentations, the genetic landscape of myeloma is quite complex. This institution is developing a targeted exome sequence platform, "myTYPE," which uses fluorescein in situ hybridization sequencing to evaluate 120 myeloma-specific genes. This panel includes genes frequently mutated in myeloma, genes in the NFκB pathway, treatment targets such as proteasome subunit genes, immunotargets (eg, *BCMA, PD1, CD38*), and candidate genes that may be associated with the development of myeloma. Given a recent study showing that at the time of diagnosis, patients have multiple subclones [31], and that these subclones may have differing responses to therapy, the incorporation of an assay such as myTYPE at diagnosis may lead to improved decision making regarding the choice of therapy. With respect to MRD assessment, Roshal et al. [32] recently reported the development of a single-tube 10-color panel MFC panel, which has the advantage of decreased antibody costs and equivalent sensitivity to the EuroFlow 2-tube 8-color method. With respect to an assay that is bone marrow-independent, MSKCC has been investigating the use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis of monoclonal immunoglobulins. In this assay, either 20 μL of serum or 1 mL of urine is purified in 5 separate reactions using nanobody immunoenrichment to identify the different immunoglobulin isotypes [33]. As has been reported previously, this assay is more analytically sensitive than immunofixation electrophoresis and can simultaneously measure free light chain ratios for IgG, IgA, and IgM [33]. Finally, preliminary studies in mice have demonstrated the feasibility of using ^{89}Zr-labeled daratumumab as CD38 immuno-PET to visualize myeloma involvement, with initial human studies planned in 2018.

Summary of ASH2017 Abstracts on MRD and IP in Myeloma

The number of studies incorporating MRD and IP as exploratory endpoints is rapidly increasing. As shown in Table 1 (MRD) and Table 2 (IP), multiple studies were presented at the 2017 ASH annual meeting that reported included analysis of MRD and IP in patients with MM. Despite established guidelines for MRD assessment [5], there continues to be heterogeneity with respect to how MRD is assessed; although the majority of studies used MFC or NGS analysis, these analyses were performed with varying sensitivity thresholds. Although historically, assessment of MRD focused on response to treatment in the newly diagnosed population, an increasing number of studies are now incorporating this analysis in the relapsed/refractory setting. In aggregate, these studies add to the body of existing literature demonstrating improved survival outcomes associated with achievement of MRD-negativity, regardless of the methodology used to assess MRD status. However, none of these studies directly address the question of whether MRD status can be used to tailor therapy. Once these abstracts are published in full, it will be important to undertake a formal analysis of the data so that evidence-based recommendations can be generated. Finally, as shown in Table 2, it is difficult to draw any conclusions regarding the studies assessing IP, because these studies evaluated different patient populations with different assays at different time points, highlighting the need for consensus guidelines in this area.

Table 2
Summary of ASH 2017 Abstracts Evaluating IP in Plasma Cell Disorders

Study	IP Methodology	Patient Population
Terpos et al. [43]	MFC BM	Sustained CR for ≥2 yr after first-line therapy
Bhutani et al. [22]	MFC PB	MGUS, smoldering MM, and MM
Foureau et al. [21]	MFC PB and BM	Post-ASCT receiving IMiD maintenance
Manasanch et al. [58]	MFC, gene expression profiling, and exome sequencing of PB and BM	High-risk smoldering MM treated with pembrolizumab
Ocio et al. [59]	MFC PB and BM	Patients receiving pembrolizumab for persistent residual disease after 1-2 previous lines of therapy
POLLUX; Van de Donk et al. [60]	CyTOF PB	RR receiving Rd versus DRd
Neri et al. [61]	scRNA-seq BM	RR receiving daratumumab + IMiD
Danziger et al. [62]	RNA microarray from paired whole BM core biopsies and CD138-enriched BM aspirates	ND
MM-014; Qian et al. [63]	MFC PB	RR receiving DPd

MGUS indicates monoclonal gammopathy of undetermined significance; CyTOF, cytometry by time of flight; scRNA-seq, single-cell RNA-seq.

Establishing MRD as a Surrogate Endpoint

The i²TEAMM Initiative

Nikhil Munshi presented the ongoing efforts of the i²TEAMM (International Independent Team for Endpoint Approval of Myeloma MRD) Initiative. As has been noted above and in a recent meta-analysis [1], achievement of MRD-negativity in newly diagnosed patients is associated with superior PFS and OS outcomes. The i²TEAMM initiative is composed of myeloma research groups from the US and Europe (GEM, IFM/DFCI, MRC, EMN/HOVON, GMMG, and BMT-CTN), a pharmaceutical industry group, and an independent statistical/analytical group. The primary objective of this initiative is to evaluate and validate MRD as a surrogate endpoint for PFS in myeloma clinical trials through prospectively planned meta-analytic surrogacy analysis based on patient-level data. Fourteen clinical trials will be evaluated, including 13 randomized trials, 2 trials with multiple cohorts, 2 trials with more than 2 treatment arms, and 5 trials with more than 1 randomization. Among the 11 randomized trials involving newly diagnosed patients, 7 trials used first-generation flow cytometry (10^{-4}), 2 used second-generation flow cytometry (10^{-5}), and 2 used next generation flow cytometry (10^{-6}). All of these 11 trials assessed MRD before maintenance, and 4 assessed MRD after or during maintenance. The primary assessment of surrogacy will be trial level, to measure how precisely the treatment effect on the true endpoint can be predicted based on the observed treatment effect on the surrogate endpoint. It was noted that individual patient-level correlation is insufficient to establish surrogacy of an endpoint, because it does not directly follow that a treatment that alters an intermediate event will alter the long-term event in a predictable manner. The inclusion and exclusion criteria for study selection are under evaluation; however, currently proposed inclusion criteria included randomized multicenter studies involving at least 200 patients per trial. Surrogacy will need to be determined for each of the different myeloma patient populations (eg, newly diagnosed versus relapsed/refractory) separately. The data elements that will be collected include MRD status, cycle-by-cycle conventional response, PFS, and OS data.

Regulatory perspectives of MRD and IP testing

Nicole Gormley from the FDA discussed some of the regulatory issues that must be considered when proposing the use of MRD or IP. She noted that MRD has distinct potential uses as a clinical tool to monitor for relapse and guide therapeutic decisions and as a regulatory tool to determine patient stratification, patient selection, or risk-based treatment assignment or as a surrogate endpoint. Similarly, the potential uses for IP include as a prognostic biomarker, a predictive biomarker, a clinical tool (eg, to identify patients at risk for relapse, toxicity, or response, or to guide therapeutic decisions), and a regulatory tool for determining patient stratification, patient selection, and risk-based treatment assignment.

When considering the use of MRD assessment in trial design as an enrichment or stratification factor, various trial designs may be considered, including enrichment design, biomarker-stratified design, or biomarker strategy design [34]. In the enrichment design, all patients are assessed for eligibility, but only those who are biomarker-positive are randomized onto the trial. This approach does not provide any information about the biomarker-negative population, and the indications for use of the biomarker assay would be limited to the selected population. In the biomarker-stratified design, all patients are assessed for eligibility, and the biomarker assay is used to stratify patients into separate arms. This approach has the advantage of providing information about both the biomarker-negative and the biomarker-positive populations and can evaluate the predictive and prognostic attributes of the biomarker. Finally, the biomarker strategy design allows for evaluation of the adequacy of the biomarker to guide therapy. In this design, all patients regardless of biomarker status are randomized to a traditional therapeutic approach or to a biomarker-guided treatment approach in which patients who are biomarker-negative receive standard therapy and those who are biomarker-positive receive additional or investigational treatment.

A number of factors are considered during the regulatory assessment of a biomarker [35]. First is the degree of risk introduced by using the biomarker. If the biomarker is being used in the selection/stratification process, there is less risk, but if it is being used as a surrogate endpoint for regulatory approval, the risk is greater if the biomarker does not perform as expected. A second factor is the underlying biological rationale for the biomarker and understanding of its position in the disease pathway. Factors associated with the assay must be considered, including the analytical validation of performance characteristics (ie, reliability, reproducibility, sensitivity, and specificity). Another factor is the types of data available to assess the strength of the association of the biomarker with its proposed clinical outcome (eg, PFS, OS). Finally, reproducibility of the data must be demonstrated in test and confirmatory datasets.

From a regulatory perspective, MRD is currently considered a prognostic biomarker. Although the use of MRD testing in patient selection and risk-based treatment assignment may be reasonable, it must be recognized that issues related to assay performance could introduce more risk. Consultation with the Centers for Devices and Radiological Health may be needed

depending on the risk posed by use of the assay and the assignment of patients based on the assay. At this time, although MRD is more commonly included as a secondary or exploratory endpoint, it is not yet being used as a surrogate endpoint. For MRD to be used as a surrogate endpoint, a number of issues must be resolved. More information is needed to determine the threshold that best correlates with clinical benefit and the optimal time points to assess MRD. The role of the disease setting must be taken into account, given that whether what we know about MRD in the newly diagnosed setting translates to other settings (eg, relapsed/refractory, smoldering) remains unclear. In addition, as noted above, it is evident that other important clinical factors, such as cytogenetics, extramedullary disease, and residual disease on advanced imaging, impact survival outcomes. How to incorporate these factors with MRD assessment remains to be determined. The degree of MRD improvement that is clinically meaningful also has yet to be determined; thus, designing a trial with MRD as the primary endpoint is difficult from a statistical perspective. Finally, it remains to be determined how to handle the problem of missing data associated with MRD assessments performed in previous studies.

A review of the FDA's internal databases for 2014 to 2016 evaluated 34 original or supplemental applications submitted to the Division of Hematology Products [36]. Of those, 13 (38%) included MRD data for chronic myelogenous leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, acute lymphoblastic leukemia, and MM. On the FDA's review of these 13 applications, 3 were not proposed for inclusion in the United States Prescribing Information (USPI), 6 were deemed adequate for inclusion of MRD in the USPI, and 4 were deemed inadequate. Reasons for excluding MRD data from the USPI included missing data, inconsistent testing across samples sources (ie, blood versus bone marrow), high test failure rates due to inability to detect a clonal rearrangement, lack of test validation in the disease setting, incomplete test characteristics data (ie, limit of detection), and incomplete planned statistical analysis. Thus, it is critical that data collection and assay performance characteristics be of sufficient rigor and completeness to allow for a comprehensive review by the FDA. It is recommended that plans for MRD assessment be discussed with the FDA in advance.

Milestones and Deliverables

Future annual meetings are planned to discuss the development and implementation of MRD and IP testing in myeloma. The ongoing efforts of the i^{2}TEAMM will provide key data regarding the validity of MRD as a prognostic biomarker, but might not be sufficient to establish MRD as a surrogate endpoint. Continued discussions with regulatory agencies are critical as clinical trials using MRD as a decision tool are planned. Currently, the field is hindered by a lack of standardization with respect to IP assessment, and consensus is needed to determine the source (bone marrow versus blood), markers/cell populations, and time points to be assessed. The development of a standardized NGF panel for IP would allow routine incorporation of IP as an exploratory endpoint in ongoing/future clinical trials. It is also recognized that molecular-based methodologies that evaluate the immune microenvironment are promising and should be pursued as well. The myriad issues related to regulatory approval of MRD and/or IP as endpoints will require considerable efforts by the myeloma research community to overcome.

CONCLUSION

Significant progress has been made in the standardization of MRD analysis. Correlations between achievement of MRD negativity and survival outcomes have been documented across many different patient populations, including newly diagnosed TE patients, newly diagnosed TNE patients, and relapsed/refractory patients; however, MRD status has not yet been established as a surrogate endpoint. Furthermore, there is insufficient evidence to support the use of MRD status to guide treatment decisions outside of the context of a clinical trial. Further efforts are needed to develop consensus guidelines for standardizing IP and to more routinely incorporate these studies into prospective clinical trials.

ACKNOWLEDGMENTS

Financial disclosure: Support for the Blood and Marrow Transplant Clinical Trials Network was provided by Grant U10 HL069294 from the National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute. Support for the PRIMeR ancillary study was provided by NHLBI Grant R01 HL107213. Support for the IP studies performed at Levine Cancer Institute was provided by Conquer Cancer Foundation ASCO Young Investigator Award, the Carolinas Myeloma Research Fund, and the Leukemia Lymphoma Society.

Conflict of interest statement: S.A.H. has served on advisory boards for Celgene, Amgen, Adaptive Biotechnology, and Takeda and has served as a consultant for Celgene. M.B. has received institutional clinical trial funding from MedImmune, Millennium, Prothena, and Janssen and has received speaking fees from Amgen, Bristol Myers Squibb and Takeda Oncology. T. H. has received research funding from Celgene. J.H. has received honoraria and travel support from Amgen, Bristol-Myers Squibb, Celgene, Janssen, Novartis, and Takeda and research funding from Celgene and Sanofi. A.K. has served as a consultant for Celgene and Janssen; has served on speakers bureaus for Celgene, Takeda, Onyx, and Janssen; has received research funding from Janssen; and has stock in Celgene. C.O.L. has received institutional research funding from Amgen, Celgene, Janssen, and Takeda. N.C.M. has served as a consultant for Biotest, Celgene, Janssen, Pfizer, Merck, Oncopep, and Takeda and is a part owner and shareholder of Oncopep. S.O. has received honoraria from Amgen, Celgene, Janssen, and Takeda. R.G.O. has received honoraria from Celgene, Janssen, and Takeda; has served on advisory boards for Celgene and Janssen; and has received travel and meeting support from Janssen. M.P. has received honoraria from Amgen and Celgene. N.P. has received honoraria and/or research funding from Amgen, Celgene, Janssen, and Takeda. K.W. has received institutional research funding from Amgen, Celgene, and Sanofi. P.L.M. has received honoraria from Bristol-Myers Squibb, Celgene, Sanofi-Aventis, Takeda, and Binding Site; has received research funding from Celgene; and has served on advisory committees/review panels/boards for Bristol-Myers Squibb, Celgene, Sanofi-Aventis, Takeda, Binding Site, and Karyopharm. The other authors have no conflicts of interest to report.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found https://doi.org/10.1016/j.bbmt.2018.11.001.

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