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BMT CTN Myeloma Intergroup Workshop on Minimal Residual Disease and Immune Profiling: Summary and Recommendations from the Organizing Committee

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Abstract

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The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Myeloma Intergroup Workshop on Minimal Residual Disease and Immune Profiling was convened on December 1, 2016 at the American Society of Hematology (ASH) meeting to discuss the emerging data and technologies for minimal residual disease assessment and immune profiling in myeloma. Particular emphasis was placed on developing strategies to incorporate these techniques into clinical trial design. This document reviews the literature, summarizes the topics discussed in the workshop and provides recommendations for integration of these techniques into future clinical trial design.

Keywords

Minimal residual disease; immune profiling; multiple myeloma; autologous stem cell transplant

Introduction

The survival outcomes for patients with multiple myeloma have significantly improved over the past twenty years in large part because of the advent of novel therapeutic agents including immunomodulatory drugs (IMiDs), proteasome inhibitors (PIs), and monoclonal antibodies. There are substantial historical data showing that patients who achieve deeper responses (e.g., complete response (CR)) have prolonged survival compared to those who do not (reviewed in[1]). Induction regimens such as lenalidomide, bortezomib and dexamethasone (RVD) and carfilzomib, lenalidomide and dexamethasone (KRD) are associated with CR rates of approximately 25% and overall response rates nearly 100%.[2–5] However, not all patients who achieve CR have equivalent outcomes and this heterogeneity is in part due to the presence of minimal residual disease (MRD). Newer studies have demonstrated that achievement of MRD-negativity is a stronger predictor for survival than is traditional CR.[6] MRD has recently been incorporated into the IMWG response criteria.[7] However, there has been much heterogeneity with respect to how MRD is assessed and there are ongoing efforts to standardize MRD assessment.[8–10] There are emerging data which demonstrate that the immunophenotype of leukocytes before and/or after transplant (immune profiling (IP)) correlate with survival outcomes. Different studies have highlighted different immune cell populations.[11–13] Given the accumulating evidence for the associations between MRD status, immune profiling, and survival, a BMT CTN Myeloma Intergroup Workshop on Minimal Residual Disease and Immune Profiling was convened at the ASH meeting on December 1, 2016.

List of Speakers and Topics

Thursday, December 1	**Presented By**
Introduction	
Prognostic Markers Versus Endpoint Markers and Summary of Questionnaire regarding MRD and IP	Philip McCarthy
MRD Session	
Genetic Interrogation Of Circulating Multiple Myeloma Cells At Single-Cell Resolution	Jens Lohr

Thursday, December 1	Presented By
Utilizing Flow Cytometric Analysis	Bruno Paiva
Utilizing Molecular Analysis In Patients With MM	Hervé Avet Loiseau
Lessons Learned During The Implementation Of A Flow Cytometric MRD Assay For Multiple Myeloma	Joseph Tario
BMT CTN PRognostic Immunophenotyping in Myeloma Response (PRIMeR) from the BMT CTN 0702 And RPCI MRD data	Theresa Hahn
Molecular Analysis Of The Multiple Myeloma Patient	Nikhil Munshi
MRD: When To Measure And How To Incorporate Into Trial Design	Group Discussion
Immune Profiling Session	
Immune Profiling/Reconstitution Overview	Philip McCarthy
Immune Profiling To Predict Outcome	Bruno Paiva
Prospective Immunprofiling In A Multicenter Trial - The GMMG-CONCEPT Project	Katja Weisel
Immune Profiling As A Predictor Of MRD Negativity	Saad Usmani
Immune Profiling: When To Measure And How To Incorporate Into Trial Design	Group Discussion

Pre-workshop survey

Prior to the workshop, a survey was sent to 163 individuals representing 71 centers from around the world and 41 responses (38 complete, 3 partial) were received. The survey focused on the utilization of MRD and IP assessment. A listing of the institutions that participated in the survey is provided in the supplemental material for this manuscript. Seventy percent of respondents (28/40) reported that their center measures MRD, with 57% utilizing flow cytometry, 18% utilizing next generation sequencing (NGS), 18% using both flow cytometry and NGS and 7% utilizing an alternative technique such as CD138-selected FISH or PET/CT. Sixty-four percent (18/28) reported that they measured MRD in all patients while 78% (14/18) reported measuring MRD only in patients in CR. There was heterogeneity with respect to which time point(s) were assessed for MRD: 54% after induction, 21% after stem cell collection, 75% after autologous stem cell transplantation (ASCT), 32% at one year post-ASCT and 32% at other time points including at CR or sCR, at VGPR/nCR, during maintenance, in clinical trials, long-term CR, or after allogeneic transplant.

A summary of the responses related to measurement of immune reconstitution/IP is provided in Table 1. Thirty-five percent (14/40) responded that their center measures immune reconstitution/IP before and/or after ASCT. Of those, 64% utilize flow cytometry, 86% assess immunoglobulin levels, and 21% assess vaccine titers. For those respondents who use flow cytometry to measure immune reconstitution, 25% perform the assessment after stem cell collection, 88% after ASCT and 63% at one year. Fifty-six percent (22/39) reported that they bill commercial insurance for these tests (MRD, immune profiling, vaccination titers, other) and 18% (7/38) report that these tests are in part supported by research funding. Thus, there was heterogeneity as to how and when MRD is tested as well as how IP is conducted following primary therapy and after ASCT.

Of those who utilize flow cytometry for studying IP, 100% assess T cells, 75% assess B cells, 63% assess NK cells and 25% assess dendritic cells. For those respondents who measure immunoglobulin levels, 15% (6/40) use HevyLite testing. Forty-one percent (16/39) reported measuring immunoparesis primarily through the measurement of immunoglobulins. Only two respondents (5%) reported using other techniques such as cytokine secretion or mass cytometry (Cytometry by Time of Flight (CyToF)). Thirty-one percent (12/39) measure vaccine titers: 75% pneumococcal, 58% tetanus, 50% measles, 50% mumps, 50% rubella, 42% diphtheria, 42% pertussis, 42% varicella, 33% polio, 25% meningococcal, 25% influenza, and 17% other (hepatitis A, B, or haemophilus). The vaccine titers are assessed at induction (8%), pre-transplant (17%), post-transplant (50%), and at one year post-transplant (58%). Reasons given for not measuring vaccine titers included all patients being vaccinated post-transplant, the results not affecting management, or due to issues of cost or insurance. The following topics were presented at the workshop by the speakers listed above. Here we summarize the presentations, relevant literature, and discuss the future directions for these important issues in MM therapy.

Summary of the MRD literature

Dr. Bruno Paiva gave an overview of the role of MRD in predicting outcome in transplant eligible and transplant ineligible patients. Achievement of MRD-negativity following initial therapy for newly diagnosed myeloma patients has been associated with improved outcomes, regardless of the technique used to assess MRD. Paiva et al. assessed MRD by multiparametric flow cytometry (MFC) at day 100 post-transplant in 295 newly diagnosed patients treated on the GEM2000 protocol.[14] Both PFS and OS were significantly longer in patients who were MRD negative and MRD status was identified as the most important prognostic factor for both PFS and OS in multivariate analysis. In an analysis of GEM2000 and GEM2005 study patients, the presence of MRD positivity post-ASCT or high-risk cytogenetics at diagnosis predicted loss of CR status within one year.[15] Rawstron et al., assessed MRD by MFC in patients treated on the MRC Myeloma IX trial and reported that the presence of MRD post-transplant (day 100) was associated with inferior PFS and OS.[16] The use of thalidomide maintenance increased the PFS in the MRD-positive group but not the MRD-negative group.[16] MRD negativity was associated with improved PFS in patients treated on the IFM 2009 protocol, regardless of whether patients were randomized to the ASCT arm or the non-transplant arm.[3] de Tute et al., analyzed the MRD status of patients treated on the MRC Myeloma IX study. The conclusion from this report was that the benefit of achieving MRD negativity post-induction therapy is independent of the type of induction therapy used.[17] Chakraborty et al., reported on the outcomes of 185 patients at a single institution.[18] Those patients who achieved MRD negativity post-ASCT had improved PFS and OS compared to those who were MRD-positive. However, subgroups of patients with deletion 17p or more than two high-risk cytogenetic abnormalities achievement of MRD negativity did not confer improved survival. Paiva et al. assessed the prognostic impact of CR types in 102 elderly transplant ineligible patients after six cycles of induction therapy.[19] Patients in sCR and MRD negative by MFC had longer PFS than those in sCR alone, but no difference in OS was observed. Twenty percent of patients with negative IFE were MRD-positive, with 50% relapsing early. In a pooled analysis of three PETHEMA/GEM trials

which included both transplant eligible and transplant ineligible patients, achievement of MRD negativity was associated with prolonged PFS and OS.[20] A recent meta-analysis of clinical trials involving newly diagnosed myeloma patients reported that MRD negativity was associated with improved PFS and OS.[6, 21] Finally, achievement of MRD negativity has also been associated with improved outcomes in the relapsed/refractory setting.[22, 23] After this workshop, the EMN02/HO95 randomized study of ASCT after induction vs at relapse showed that MRD negativity at a sensitivity of 10^{-4} to 10^{-5} at initiation of maintenance was predictive of outcome in those patients achieving a VGPR.[24] Of the MRD positive patients, 44% became MRD negative by MFC after one year of maintenance. Of note, MRD positivity was most predictive of outcome followed by ISS II and high-risk cytogenetics.

Flow cytometric analysis of MRD

MFC involves the utilization of a panel of antibodies that can differentiate between normal and malignant plasma cells. Earlier generations of MFC assessed variable numbers of antigens and cell numbers and had a sensitivity of 10^{-4}. Improvements in flow cytometry technology have translated into increased acquisition time speeds and an ability to simultaneously analyze a larger number of fluorophores. In turn, this has allowed the field to develop panels with more antibodies and to acquire larger numbers of events, improving the sensitivity to as high as 10^{-6}. The goal of the International Myeloma Foundation's Black Swan initiative was to develop a consensus methodology and this work has led to the EuroFlow panel.[8, 9] This panel consists of two 8-color tubes (tube 1: CD138, CD27, CD38, CD56, CD45, CD19, CD117, CD81 ; tube 2: CD138, CD27, CD38, CD56, CD45, CD19, cIgK, cIgλ).

The advantages of utilizing MFC for MRD assessment include the availability of flow cytometers at the vast majority of centers, the standardized panels, feasibility, and lack of need for a diagnostic sample. However, in order to achieve the 10^{-5} –10^{-6} sensitivity, millions of cells need to be acquired and this can translate to lengthy acquisition times that interfere with the daily operating procedure of clinical flow cytometry labs. In addition, while there appears to be general agreement within the field regarding the identity of the epitopes to be analyzed, other variables such as the number of tubes, the commercial source of the antibodies and preparation of the sample continue to be assessed. Roshal et al., recently published an alternative method which utilizes the same epitopes but in a single ten-color tube.[25] During the workshop, Dr. Joseph Tario reported on the Roswell Park Cancer Institute experience with implementation of a flow cytometric MRD assay ("BuffaFlow").[26] This institution has performed a comparison of their methodology which utilized antibody incubation prior to red blood cell lysis to the EuroFlow methodology which utilizes a bulk pre-lysis protocol. They determined that while the bulk pre-lysis method is slightly less expensive it requires a dedicated technologist and it significantly decreases CD138 intensity. While CD45, CD56, CD19, CD81, CD27, and CD117 were found to be insensitive to pre-lysis, the intensity of CD138 was reduced by approximately 25-fold following the bulk lysis procedure. Finally, the quality of the bone marrow sample is a critical factor. A hemodilute specimen can lead to a false negative MRD result which becomes especially important if treatment decisions are being made on the basis of the MRD test result. Whether a standardized procedure for marrow collection for MFC MRD can be developed remains to

be determined. Certainly, it is essential that any MFC MRD report include an assessment of the quality of the marrow sample such that there can be confidence in the finding of MRD negativity.

Dr. Theresa Hahn gave an overview of the Prognostic Immunophenotyping in Myeloma Response (PRIMeR) Study, the ancillary study for BMT CTN 0702. Bone marrow was sampled after induction and prior to first ASCT for flow cytometric analysis for MRD. Bone marrow was further tested in each of 3 arms after primary therapy and at approximately 1 year post first ASCT. Aggregate results were presented without PFS and OS data as these were undergoing adjudication. There were 898 total samples available for analysis from 445 unique patients with 136 patients having samples at all three time points. We expect arm-specific and MRD correlation with PFS and OS results to be available in late 2017/first quarter 2018.

Dr. Nikhil Munshi gave an overview of the Molecular Analysis of the MM patient. His discussion included topics ranging from clonal heterogeneity and dispersed interstitial mutations in MM to the work of Bolli et al., describing mutational processes in MM.[27, 28] MM presents a challenge due to a wide mutational spectrum, variation in mutational load, clonal heterogeneity and evolution over time.

ASO-qPCR

Allele-specific oligonucleotides real-time quantitative PCR (ASO-qPCR) involves the use of patient-specific primers for immunoglobulin heavy chain gene rearrangements. The reported sensitivity of this methodology is 10^{-5}. One limitation of this methodology is the requirement for diagnostic samples. In addition, reported applicability rates have been noted to be in the 40–80% range due to factors such as lack of clonality detection and issues with sequencing.[29–31] A number of studies have compared ASO-qPCR to MFC which in general have demonstrated a higher sensitivity of the ASO qPCR technique, however these studies utilized different MFC protocols.[29, 32, 33]

Next Generation Sequencing

Next generation sequencing (NGS) utilizes locus-specific primers for *IGH-VDJ*^{H}, *IGH-DJ*^{H}, or *IGK*. This technique does not require the use of patient-specific primers, although baseline samples are still required in order to identify the dominant clonotype. The sensitivity of this technique can reach 10^{-6}.[34] Several studies have reported that the applicability of this technique is more than 90%.[34–36] In preliminary results from the IFM 2009 study, patients who achieved MRD negativity post-maintenance by NGS with less than 10^{-6} had a 3-yr PFS of 90% compared to 59% for those who with greater than 10^{-6}.[34] Dr. Herve Avet-Loiseau presented the results of the POLLUX (daratumumab + lenalidomide/dexamethasone (dex) (DRd) vs lenalidomide/dex (Rd)) and CASTOR (daratumumab + bortezomib/dex (DVd) vs bortezomib/dex (Vd)) trials, two randomized, phase 3 trials in patients with relapsed/refractory MM (RRMM).[37] MRD was assessed by NGS of the B cell receptor on marrow aspirate samples. In the POLLUX trial, MRD was tested at time of estimated CR, and at 3 and 6 months afterwards. In the CASTOR trial, MRD was tested at

time of estimated CR, and at 6 and 12 months afterwards. The addition of daratumumab induced deeper clinical responses manifested by MRD negativity leading to fewer PFS events compared to a MRD positive status. In both studies, attaining MRD negative status irrespective of study or control arm resulted in long-term disease control.

Emerging Methodologies

There is increasing evidence regarding the genetic complexity of the clonal evolution of myeloma cells and there is significant interest in characterizing this clonal evolution in order to understand the driving mutations for drug discovery purposes as well as for understanding drug resistance mechanisms.[28] Whether circulating plasma cells can provide similar information as bone marrow plasma cells is also an active area of investigation. Mishima et al., have reported on the use of whole exome sequencing on both circulating tumor cells and bone marrow samples which demonstrated that ≥ 99% concordance with respect to identification of clonal mutations.[38] Dr. Jens Lohr presented a methodology that allows for the isolation and characterization of myeloma cells at the single cell level.[39] This protocol can be performed on either peripheral blood or bone marrow samples. The isolated single cells can be used for DNA sequencing (DNA-seq) or RNA sequencing (RNA-seq), providing information regarding differences in the mutational profiles between circulating and marrow cells. While this technique has important implications for monitoring the emergence of resistant subclones following therapy, it may also serve as an adjunct in the measurement of MRD.

Incorporation of MRD status into clinical trial design

To date, studies that have assessed MRD status have included this as an exploratory endpoint. Moving forward, it is imperative to determine whether MRD status can serve as a surrogate endpoint for PFS and/or OS and whether MRD status can be used to make treatment decisions. With respect to the former, it is becoming increasingly difficult to design MM trials with OS as the primary endpoint as these studies require large numbers of patients and prolonged follow-up times given the ever-increasing OS rates. Thus, in addition to the feasibility of enrolling large numbers of patients and the cost of keeping a study open for 5–10 years, there is the issue that by the time the primary endpoint is reached, the clinical question may no longer be relevant. Even the use of PFS as a primary endpoint in the upfront setting is becoming more difficult now that novel induction regimens with transplant and maintenance are producing long-lasting remissions. The appeal of using MRD negativity (either at a single pre-specified time point or defined as persistent MRD negativity over a certain time period) as a primary endpoint is that this could allow for a much earlier read-out of studies. This would facilitate study designs with smaller numbers of patients and increase the likelihood that the study outcome would be clinically relevant in the face of rapid advances in the field. The possibility of response-adaptive therapy utilizing MRD status is also intriguing. For example, while there are now multiple phase III studies and a meta-analysis demonstrating that lenalidomide maintenance post-transplant prolongs survival outcomes,[40–45] the question remains whether all patients require maintenance therapy until disease progression or whether there are subsets of patients for whom maintenance is either not required or can be safely discontinued after a fixed duration of

time. Alternatively, MRD status may also be incorporated into study designs such that more intensive therapy is offered for patients who are MRD-positive. These studies would need to incorporate cytogenetic risk and higher clinical stage as these demographic features have been associated with outcome.

Recommendations:

1	Centers should follow IMWG consensus guidelines regarding the utilization of multiparameter flow cytometry and/or next generation sequencing to assess MRD.
2	MRD status is not yet a standard for making treatment decisions outside of the context of a clinical trial.
3	Clinical trials should be designed to determine whether MRD status can be used as the primary outcome.
4	Clinical trials should be designed to assess whether MRD status response-based approaches yield superior outcomes.

Immune profiling in multiple myeloma

There is a complex relationship between MM and the immune system. Thus, there is interest in determining whether specific patient immunophenotypes in blood and or bone marrow correlate with treatment outcome. Earlier studies assessed parameters such as CD4 count, absolute lymphocyte count, CD19 count, and NK cell count.[46–50] There is much heterogeneity in these studies as a consequence of differences in sample source (i.e., peripheral blood vs bone marrow), timing of analysis related to treatment, and the composition of the flow cytometric panel which has made it difficult to assess these studies in aggregate. More recently, advances in MFC has enabled the development of more comprehensive immunophenotyping/immune profiling studies. Drs. McCarthy, Paiva, Weisel and Usmani reviewed the current status of IP for predicting the effect of treatment and outcomes.

Immune profiling studies in the peri-ASCT period

Dr. Saad Usmani presented work from his group. Bhutani et al. developed a 9-color panel to assess NK and NK-T polarization as well as T and B cell activation.[51] This panel includes CD3 and CD56 to define NK (CD56+, CD3−), NKT (CD56+, CD3+) and T cell (CD56−, CD3+) subsets, programmed death receptor 1 (PD-1) and T-cell Ig and mucin receptor 3 (Tim3) to assess T cell activation state, and killer inhibitory Ig-like receptors (KiR2DS4, KiR3DL1), natural killer group 2 proteins (NKG2a, NKG2D) and natural killer p46 protein (NKp46) to assess NK and NK-T polarization in peripheral blood samples. Samples from 11 myeloma patients receiving lenalidomide maintenance post-transplant were analyzed. Significant heterogeneity of NK, NK-T, and T cell populations in the baseline (pre-lenalidomide) samples was noted. This work has been continued by Foureau et al., who utilized this 9-color panel, as well as multiplex protein assay to quantify inflammatory cytokines, chemokines and growth factors, to determine whether the immune profile of MRD+ patients was different from MRD negative patients sixty days post-ASCT.[52] MRD negative patients more frequently displayed an inflammatory/pro-angiogenic cytokine

profile and showed stronger TH1/17 immune polarization with γδ T cell activity. MRD positive patients had reduced expansion/killing potential of NK cells.

Dr. Philip McCarthy discussed work from his group on IP and MRD. Ho et al., reported on 101 myeloma patients who had comprehensive immune profiling performed prior to transplant and 100 days post-transplant.[12] The immune profiling panel consisted of 20 different T-cell subsets, 8 B-cell subsets, as well as NK and dendritic cell subsets. MRD by MFC was also performed in 80 of the patients at the post-transplant time-point. This study demonstrated associations between pre-transplant CD19+ B-cell counts and survival as well as post-transplant γδ T-cells and CD4+ central memory cells and survival outcomes. Interestingly, the associations noted with the γδ T-cells and CD4+ central memory cells were primarily in those patients who were MRD-negative or did not go on to receive maintenance therapy. MRD was also examined by MFC and we found a correlation between the number of events counted by MFC and a better correlation with PFS.[53] This study in conjunction with the study by Foureau et al., suggest an association between MRD status and the immunophenotype. Further research is needed to better understand whether the immunophenotype associated with MRD negativity is simply a marker of immunological health or whether the immunophenotype itself determines MRD status.

Immune profiling studies in smoldering myeloma

Immunophenotyping may be able to identify those patients with smoldering myeloma who are at higher risk for progression to active myeloma. In a study by Dosani et al., immunophenotyping was performed on peripheral blood samples from patients with MGUS, smoldering myeloma and myeloma. Dr. Bruno Paiva presented a study of patients with smoldering myeloma who eventually progressed to myeloma were found to have decreased proportions of CD57-CD56+ and CD57-CD16+ lymphocyte subsets.[54] In addition, in patients with high-risk smoldering myeloma treated on the QUIREDEX[55]: Revlimid (Lenalidomide) and Dexamethasone (ReDex) Treatment Versus Observation in Patients With Smoldering Multiple Myeloma With High Risk of Progression were analyzed by immune profiling. At baseline patients had decreased expression of markers of T cell activation (CD25/CD28/CD54), type 1 T helper (CD195/interferon-γ/tumor necrosis factor-α/interleukin-2) and proliferation compared to age-matched healthy controls.[56] Furthermore, following treatment with lenalidomide/dexamethasone, the levels of these markers were restored to normal and there was shift in the T-lymphocyte and NK-cell phenotype.[56]

Incorporation of immune profiling into clinical trial design

Several ongoing studies are prospectively collecting immunophenotyping data. Dr. Weisel presented the GMMG-CONCEPT (A Clinical Phase II, multicenter, open-label study evaluation induction, consolidation and maintenance treatment with isatuximab, carfilzomib, lenalidomide and dexamethasone (I-KRd) in primary diagnosed high-risk multiple myeloma patients; NCT03104842). The trial has a primary endpoint of MRD negativity (using MFC with a sensitivity of 10^{-5}) following consolidation. As an experimental objective, immune reconstitution during maintenance with I-KR will be assessed utilizing a 16-color flow cytometry panel. This panel will be used to analyze peripheral blood and bone marrow

samples for T cells (including effector, naïve, effector memory, central memory, transitional memory and regulatory), NK cells (including markers for differentiation and function), and myeloid cells. The MMY2004 study (Phase 2, randomized, open-label study comparing daratumumab, lenalidomide, bortezomib, and dexamethasone (D-RVd) versus lenalidomide, bortezomib, and dexamethasone (RVd) in subjects with newly diagnosed multiple myeloma eligible for high-dose chemotherapy and autologous stem cell transplantation; NCT02874742) has a primary endpoint of rate of stringent CR following consolidation. Secondary endpoints include MRD negativity as multiple time points throughout the trial and as an exploratory endpoint, this study is assessing immune profiling of NK, T, and B cells as well as T-cell receptor sequencing. The BMT CTN 1401 study (Phase II multicenter trial of single autologous hematopoietic transplant followed by lenalidomide maintenance for multiple myeloma with or without vaccination with dendritic cell/myeloma fusions; NCT02728102) has a number of secondary immunologic endpoints, including quantification of T cell subsets and NK cells.

Overall, we may speculate that immune profiling may have the potential to serve as a predictive biomarker in several settings. For example, it is possible that the immune phenotype at diagnosis could be used to identify the induction regimen predicted to have the best depth of response. In the maintenance setting, it is possible that the immune profile could be used to guide decisions regarding the choice of maintenance therapy as well as the duration of maintenance therapy. It is critical that immune phenotyping become incorporated into as many prospective studies as possible. In addition, emphasis needs to be placed on developing standardized panels by which to assess the immune phenotype such that the results from these studies can be more easily compared.

Recommendations:

1	Efforts are needed to standardize immunophenotyping/immune profiling studies.
2	Further prospective studies are needed to better understand the association between immunophenotype/immune profiling and MRD status.
3	Further studies are needed to determine whether immunophenotyping/immune profiling can be used to predict risk of progression to myeloma.
4	Further studies are needed to determine the effect of maintenance therapy on the immunophenotype/immune profile.

Milestones and Deliverables

This working group plans to continue to hold annual meetings to discuss the implementation of MRD and IP assessment. Goals for the future include updating the study of MRD as an endpoint for clinical trials and for clinical decision making. A MRD consortium: the International Independent Team for Endpoint Approval of Myeloma MRD (i^2 TEAMM) is developing a meta-analysis based on primary source data to be provided by investigators examining MRD in randomized Phase III trials. This meta-analysis will be submitted to the FDA for the designation of MRD as a surrogate endpoint for PFS/OS. Other initiatives will the presentation of new techniques for MRD and IP testing. In particular, the use of

peripheral blood for testing for MRD is an attractive alternative to bone marrow sampling. A major goal for IP is the development of standardized panels for study comparisons. Standardization of flow cytometric and molecular testing for MRD continues to advance so as to allow for study comparisons and meta analysis.

Conclusion

Treatment options for multiple myeloma are rapidly increasing, accompanied by the opportunity to achieve very deep responses, including MRD negativity. In aggregate, the available data regarding MRD status have demonstrated that achievement of MRD negativity is associated with improved survival outcomes. However, whether MRD negativity can be used as a surrogate endpoint or to determine treatment strategies remains unknown and it is imperative that clinical trials be designed to address these issues. In 2014, the FDA-NCI Roundtable Symposium on Flow Cytometry Detection of Minimal Residual Disease in Multiple Myeloma concluded that MRD should be considered for regulatory purposes, including drug approval, and therefore consensus guidelines needed to be developed.[10] There has been substantial effort devoted to the development of standardized assessment of MRD, including published consensus guidelines,[7] however currently we do not recommend that MRD status be used to determine treatment decisions outside of the context of clinical trials. Given the complex relationship between myeloma and the immune microenvironment, as well as the increasing number of drugs that modulate the immune system, it is not surprising that immune profiling studies have revealed associations between immune signatures and survival outcomes. Further research is needed to determine whether the immunophenotype could be used as a predictive biomarker. Routine incorporation of immune profiling into prospective clinical trials is therefore critical, as are efforts to standardize this assessment. The overall goal for studies utilizing MRD and immune profiling is to allow for personalized treatments for patients that results in optimal responses and long-term survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Review and recommendations from the BMT CTN Myeloma Intergroup MRD/IP workshop.
- Achievement of MRD negativity is associated with improved outcomes.
- Centers should follow IMWG consensus guidelines for MRD assessment.
- Efforts are needed to standardize immunophenotyping/immune profiling studies.

Table 1

Summary of survey responses regarding assessment of immune reconstitution and immunophenotyping (n=number of affirmative responses, N=total number of responses)

Methodology	n/N (%)
IP via flow cytometry	9/14 (63%)
T cells[a]	8/8 (100%)
B cells[b]	6/8 (75%)
NK cells	5/8 (63%)
Dendritic cells	2/8 (25%)
Immune paresis	12/14 (86%)
Quantitative immunoglobulins	16/39 (41%)
HevyLite	6/40 (15%)
Cytokine secretion/CyToF	2/39 (5%)
Vaccine titers	12/39 (31%)
Pneumococcal	9/12 (75%)
Tetanus	7/12 (58%)
Measles/mumps/rubella	6/12 (50%)
Diphtheria	5/12 (42%)
Pertussis	5/12 (42%)
Varicella	5/12 (42%)
Polio	4/12 (33%)
Meningococcal	3/12 (25%)
Influenza	3/12 (5%)
Other (hepatitis A, B or haemophilus)	2/12 (17%)

[a] T cell subsets assessed: CD4 (100%), CD8 (88%), CD4 subsets (e.g., naïve, central memory, effectors, T regs; 38%), and CD8 subsets (e.g., naïve, central memory, effectors; 25%)

[b] B cell subsets assessed: CD19 (100%), CD20 (67%), B cell subsets (e.g., naïve, memory, pre/post-switch; 33%)

Table 2

Summary of studies reporting outcomes associated with MRD status

Study and reference	Patient Population	MRD methodology	Outcome
GEM2000; Paiva et al.[14]	Day 100 post-ASCT	MFC	MRD negativity associated with improved PFS and OS
GEM2000/GEM2005; Paiva et al.[15]	Day 100 post-ASCT	MFC	MRD positivity associated with loss of CR status
MRC Myeloma IX; Rawstron et al.[16]	Day 100 post-ASCT	MFC	MRD negativity associated with improved PFS and OS
MRC Myeloma IX; de Tute et al.[17]	Post-induction	MFC	Impact of MRD negativity is independent of induction regimen
IFM 2009; Attal et al.[3]	Post-consolidation or post-maintenance	MFC	MRD negativity associated with improved PFS
IFM 2009; Avet-Loiseau et al.[34]	Post-maintenance	NGS	MRD negativity associated with improved 3-yr PFS
Chakraborty et al.[18]	Day 100 post-ASCT	MFC	MRD negativity associated with improved PFS and OS
GEM05>65y; Paiva et al.[19]	Post-induction, ASCT ineligible	MFC	MRD negativity associated with improved PFS
PETHEMA/GEM trials; Lahuerta et al.[20]	Nine months post-enrollment	MFC	MRD negativity associated with improved PFS and OS
Paiva et al.[22]	Relapsed/refractory	MFC	MRD negativity associated with improved TTP
POLLUX; Dimopoulos et al.[23]	Relapsed/refractory	NGS	MRD negativity associated with improved PFS
POLLUX/CASTOR; Avet-Loiseau et al.[37]	Relapsed/refractory	NGS	MRD negativity associated with fewer PFS events
EMN02/HO95; Oliva et al.[24]	Prior to maintenance	MFC	MRD negativity associated with improved 3-yr PFS