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

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Abstract

The third annual Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Myeloma Intergroup Workshop on Minimal Residual Disease and Immune Profiling was held on November 29, 2018 at the American Society of Hematology (ASH) annual meeting. This workshop featured the latest research focused on minimal residual disease assessment (MRD) and immune profiling (IP) in myeloma as well as discussion of the statistical and regulatory issues intrinsic to the development of MRD as a surrogate endpoint. In this report, we provide a summary of the workshop and focus on the integration of MRD and IP assessment into trial design and clinical practice.

Keywords

Minimal residual disease; immune profiling; multiple myeloma; endpoint; CAR T cell

Introduction:

Minimal residual disease (MRD) assessment increasingly is reported along with traditional response rates as part of the outcomes of clinical trials as well as being incorporated into clinical trial design. Consensus criteria for MRD assessment and MRD response have been established by the International Myeloma Working Group (IMWG).[1] Currently there are two sanctioned methodologies: multiparametric flow cytometry (MFC) and next generation sequencing (NGS).[1] More specifically, MFC is to be performed using the established Euro-Flow procedure which involves an eight-color, two-tube method and has a minimum sensitivity of 1×10^{-5}.[2] The clonoSEQ assay (Adaptive Biotechnologies) is a NGS assay that was approved by the United States Food and Drug Administration (FDA) for the detection of MRD from the bone marrow of myeloma patients in 2018. This assay has a sensitivity of 1×10^{-6}. As both of these methods rely on detection of disease in the bone marrow, and as myeloma can involve extramedullary sites, the IMWG has also defined an “Imaging plus MRD-negative” status as being MRD negative by MFC/NGS plus PET/CT negativity.

Achievement of MRD negativity has been consistently associated with improved survival outcomes in both the newly diagnosed and relapsed/refractory settings[3] and thus is currently considered to be a prognostic biomarker. However, there is significant interest in establishing MRD as a surrogate endpoint as this would enable clinical trials to be designed with much more rapid read-outs than the traditional endpoints of progression free survival (PFS) or overall survival (OS).[4] There is also significant interest in determining whether MRD status can be used to guide treatment decisions such as escalation or de-escalation of therapy. Furthermore, there are emerging data that suggest that particular immune profiles (IP) are associated with MRD status and survival outcomes.[5, 6] It is evident from several recent publications that the IP post-autologous stem cell transplant (ASCT) is particularly complex and that certain phenotypes may correlate with outcomes, however there continues to be a lack of consensus with respect to how the IP is assessed.[5–7]

Given the significant interest in the development of MRD and IP assessment, the BMT CTN Myeloma Intergroup has been holding annual workshops on MRD and IP since 2016 in order to discuss emerging technologies and data and to develop strategies that will enable routine incorporation of these assessments into clinical trial design.[8, 9] In the present report we provide a summary of the third annual workshop.

Pre-workshop survey:

Prior to the inaugural workshop held in 2016, a survey focused on utilization of MRD and IP was sent to 163 individuals representing 71 centers from around the world.[8] At that time, 70% (28/40) of respondents reported that their center measured MRD, with 57% utilizing flow cytometry, 18% utilizing NGS, 18% using both flow cytometry and NGS and 7% utilizing an alternative technique such as CD138-selected FISH or PET/CT.[8] Thirty-five percent (14/40) responded that their center measures immune reconstitution/IP before and/or after ASCT. A follow-up survey was distributed prior to the 2018 workshop to 205 individuals from 103 centers and 10 companies. Twenty-three individuals responded from 19 centers and 3 companies. Approximately two-thirds of respondents reported that their center measures MRD, with the majority utilizing flow cytometry and/or NGS. For those respondents who reported that their center does not measure MRD, 56% noted barriers in terms of access to proper technology, 89% noted issues related to reimbursements, 11% noted practice guidelines and 33% noted other, not specified, reasons for not measuring MRD. Similar to 2016, 63% of respondents reported that they do not measure MRD in all patients, with 60% reporting that it is measured only in patients in a complete response (CR). Time points at which MRD is analyzed include following induction (44%), following stem cell collection (13%), after ASCT (75%), at one-year post-ASCT (19%) and/or at other unspecified time points (63%). Overall, this pattern is very similar to the results of the survey from 2016.[8] New to the 2018 survey was a question regarding whether the MRD results are incorporated into clinical practice. Six percent reported that the results triggered a change in surveillance, 38% reported that the results triggered a change in treatment and 79% reported that the results did not change practice.

Similar to the 2016 survey results, 69% of respondents reported that their center does not measure immune reconstitution before and/or after ASCT. For those centers that do measure immune reconstitution, 29% utilize flow cytometry, 86% measure immunoglobulin levels, 14% perform Hevylite testing, and 14% check vaccine titers. New to the 2018 survey was a question regarding whether the immune reconstitution results are incorporated into practice. Forty-three percent of respondents reported that these results triggered a change in treatment. The majority of respondents reported that they do not utilize HevyLite testing (88%) or measure vaccine titers (84%). For those who do measure vaccine titers, 50% evaluate them post-ASCT and the other 50% evaluate them at one year post-ASCT. Finally, 56% of respondents reported that they are not billing commercial insurance for any of the MRD/IP tests. Overall, there continues to be heterogeneity with respect to the utilization of MRD/IP testing.

MRD assessment from recent clinical trials:

RV-MM-EMN-441 and RV-MM-COOP-0556 (EMN02/HO95 MM):

Stefania Oliva presented a pooled analysis of MRD testing in two phase III studies (RV-MM-EMN-441 and RV-MM-COOP-0556 (EMN02/HO95) that included lenalidomide maintenance following consolidation with ASCT or chemotherapy. MRD analysis was performed in patients who achieved at least a very good partial response (VGPR) after consolidation and then every six months during lenalidomide maintenance until time of disease progression.[10] ASO-RQ-PCR analysis was performed using patient-specific primers designed to evaluate IGH rearrangement in tumor cells. Euro-MRD guidelines were utilized for RQ-PCR data interpretation.[11] Molecular-CR (mCR) was defined as MRD-negativity with a minimal sensitivity of 10^{-5}. MRD analysis was also performed using MFC. For the RV-MM-EMN-441 study, two tubes with six colors (tube 1: CD138, CD56, CD20, CD117, CD45, CD38; tube 2: cytoplasmic kappa, cytoplasmic lambda, CD19, CD56, CD45, CD38) were used with a minimum of 1×10^6 events acquired. For the RV-MM-COOP-0556 study, two-tube eight-color MFC was performed (tube 1: CD81, CD27, CD138, CD19, CD20, CD38, CD45; tube 2: cytoplasmic kappa, cytoplasmic lambda, CD138, CD19, CD56, CD117, CD38, CD45) and a minimum of 2×10^6 events were acquired. The threshold for MRD negativity using MFC (flow CR) was defined as when <20 clonal plasma cells were detected among >200,000 leukocytes (10^{-4} to 10^{-5}).

A total of 105 patients were enrolled in this MRD sub-study, but only 73 (70%) had successful sequencing which allowed for the identification of a molecular marker. Of these 73 patients, 29 (40%) were in a VGPR post-consolidation and 44 (60%) achieved a CR. Approximately half of the patients underwent ASCT (48%), 59% had standard risk disease based on FISH and 33% had high-risk disease. MRD testing at the post-consolidation time point revealed that 46% of patients (56% of ASCT patients vs 37% of non-ASCT patients) achieved a mCR and 63% of patients (67% of ASCT patients vs 59% of non-ASCT patients) achieved a flowCR. The median PFS for patients who achieved a mCR was not reached compared to 37.1 months for patients who did not achieve a mCR. Similarly, the median PFS for patients who achieved a flow CR was not reached compared to 26 months for those who did not achieve MRD negativity by flow. Evaluation of MRD during maintenance revealed that 31% of patients who were initially MRD-positive converted to MRD-negative by ASO-RQ-PCR compared to 23% by MFC. Conversion to MRD negativity during ASCT was associated with improved PFS as well ($p<0.001$). Subgroup analysis revealed that achievement of MRD negativity was associated with improved PFS regardless of cytogenetic risk. Achievement of MRD negativity was associated with superior OS: the 4-yr OS rates were 84% and 80% for MRD-negative patients compared with 60% and 61% for MRD-positive patients (ASO-RQ-PCR and MFC, respectively). Finally, an analysis of MRD kinetics during maintenance was presented. Those patients who had persistently negative MRD tests over time had the lowest rates of relapse (~20%) compared to those who achieved transient or minimal MRD responses (~70%).

FORTE:

Stefania Oliva also presented an overview of data from the FORTE study, which was formally presented by Francesca Gay at ASH 2018.[12] In this study, patients are randomized to three treatment groups: 1) carfilzomib/cyclophosphamide/dexamethasone (KCD) induction followed by ASCT and KCD consolidation, 2) carfilzomib/lenalidomide/dexamethasone (KRD) induction followed by ASCT and KRD consolidation, or 3) KRD induction/consolidation without ASCT (KRD12). A second randomization occurs following completion of consolidation with randomization to lenalidomide vs lenalidomide + carfilzomib maintenance. The primary endpoint of this study is VGPR rate with key secondary endpoints including MRD and safety. MRD status is determined after induction, prior to maintenance and every six months thereafter until PD. MRD is being assessed using two-tube 8-color MFC with at least 3.5 million events acquired and MRD positivity being defined as >20 monoclonal plasma cell events. Thus far, rates of MRD-negativity are superior in the KRD arms compared to KCD, correlating with superior rates of ≥VGPR and sCR/CR.[12]

Subsequently, Francesca Gay presented updated data from the FORTE trial during the 2019 American Society of Clinical Oncology national meeting.[13] Of particular interest were the premaintenance and one-year persistent MRD negativity rates broken down by revised international staging system (R-ISS). With respect to the premaintenance MRD status, 69% of R-ISS I patients in the KRD-ASCT group were MRD-negative compared to 62% in the KRD12 group while 51% of R-ISS II/III patients in the KRD-ASCT group were negative compared to 49% in the KRD12 group. In the KRd-ASCT group, the one-year MRD-negativity persistent rate was 90% (all patients), 90% (R-ISS I) and 90% (R-ISS II/III) while in the KRD12 group, the rates were 78% (all patients), 85% (R-ISS I) and 72% (R-ISS II/III). The risk of early relapse, defined as relapse within 18 months after first randomization, was higher in patients with R-ISS II/III vs I (OR 3.78, p=0.001), lower in patients in the KRD-ASCT group compared to the KRD12 group (OR 0.41, p=0.022) and lower in MRD-negative patients (OR 0.21, p<0.001). These data highlight the importance of considering myeloma disease biology characteristics such as high-risk cytogenetics when attempting to determine the clinical significance of MRD-negative results and risk of relapse.[14–17]

BMT CTN 0702, 1302, 1401:

Theresa Hahn provided an overview of the MRD testing that has been conducted with several BMT CTN studies, including 0702, 1302 and 1401. BMT CTN 0702 is a phase III study which evaluated three different consolidation approaches: single ASCT, double ASCT, or single ASCT followed by 4 cycles of bortezomib/lenalidomide/dexamethasone (VRD).[18] No differences in PFS or OS have been observed amongst the three arms.[18] BMT CTN 1302 is a placebo-controlled randomized phase II study evaluating ixazomib maintenance following allogeneic stem cell transplantation in patients with high-risk myeloma. This study closed to enrollment in September 2018 and results have not yet been reported. BMT CTN 1401 is a randomized phase II study evaluating the addition of dendritic cell/myeloma fusion vaccine to lenalidomide maintenance post-ASCT. This study finished accruing in October 2018 and results have not yet been reported.

The MRD analysis for these studies was performed at Roswell Park Comprehensive Cancer Center utilizing MFC. The MFC panels and sensitivity of the MRD testing have evolved over time. When the MRD panel for BMT CTN 0702 was designed in 2009–2010, it consisted of 3 tubes, six colors each with a sensitivity of 0.004–0.001% (acquiring a minimum of 2.5×10^5 cells). In contrast, the panel utilized for the BMT CTN 1302 and 1401 studies consists of 2 tubes, 8 colors each with a sensitivity of 0.001–0.004% (acquiring a minimum of 2×10^6 cells). Samples for MRD for the 0702 study were obtained pre-ASCT (n=293), post-ASCT (n=311; timing dependent on the arm of the study) and approximately one year post-ASCT (n=279). For the 1302 study, optional MRD assessment for patients in CR was performed if samples were collected pre-ASCT (n=13), 60–80 days post-ASCT (n=17) and at one year post-ASCT. For the 1401 study, MRD assessment was performed 60–80 days post-ASCT (n=68) and at one year post-ASCT (n=13). The MRD data for the 0702 study have subsequently been presented at the Transplantation and Cellular Therapy Meetings.[19]

BMT CTN 1302 also incorporates assessment of peripheral blood IP with samples collected prior to initiation of maintenance, at start of cycle 5 of maintenance and within 4 weeks after the end of maintenance. The comprehensive panel includes six 8-color tubes: tube 1, inflammatory monocytes and dendritic cells; tube 2, recent thymic emigrant, CD4 and CD8 naïve and memory cells; tube 3, CD4 T regulatory cells; tube 4, natural killer cells; tube 5, γδ T cells; and tube 6, B cells.

Myeloma chimeric antigen receptor (CAR) T cell studies:

Edward Stadtmauer presented an overview of MRD assessment in CAR T cell studies. He noted that the candidate antigen targets that have been evaluated or are being considered include CD138, CD38, CD56, kappa light chain, CD19, Lewis Y, CD44v6/CD229, MAGE A3/NY-ESO-1, CS1/SLAMF7, BCMA and Integrin beta 7. To date the majority of the studies have evaluated BCMA, however these studies do vary with respect to conditioning regimen, cell dose, and timing of myeloma response assessment. The University of Pennsylvania's BCMA CAR T study utilized cyclophosphamide conditioning and evaluated MRD status at day 28 and day 90.[20] MRD was assessed using the EuroFlow protocol with 10^{-5} sensitivity. Four patients who were measurable by flow at baseline achieved MRD negativity post-treatment at least one time point. Of these four patients, two progressed within a few months while two had long term CR. The bb2121 BCMA CAR T cell study involved a fludarabine/cyclophosphamide conditioning regimen and bone marrow biopsies were obtained at week 2 and week 4.[21] MRD was assessed using NGS at a level of 10^{-4} to 10^{-6}. All responding patients that were evaluated for MRD were found to be MRD negative at one or more time points, but despite this high rate of MRD negativity, the median PFS was 17.7 months. A study of CD19 CAR T cell therapy following salvage ASCT demonstrated the feasibility of post-ASCT CAR T therapy.[22] While only one of twelve patients achieved sCR/MRD negativity, this patient did progress after 15 months. Interestingly, at time of progression only rare CD19-negative myeloma cells were found in the bone marrow but multiple extramedullary plasmacytomas were observed. Finally, results from a phase 1/2 study of NY-ESO1 TCR-engineered T cells were discussed.[23, 24] The T cells were administered two days after ASCT and response assessments performed on days

42, 100, 180, 270 and 360. MRD testing by PCR was performed at day 100 in 23 out of 25 patients and was successful in 18 patients. Five of those 18 post-infusion samples were found to be MRD negative. Interestingly, although there were three long-term survivors, defined as more than 3 years post-infusion, only 1 was MRD negative. The remaining two were not MRD negative but did have long-term persistence of the T cells.

In aggregate, the available data suggest that MRD assessment may not be the best predictor of outcome following CAR T cell therapy. Currently there are minimal IP data available in the post-CAR T setting. Work by Paiva et al., utilized MFC to evaluate 13 bone marrow immune cell populations in transplant ineligible patients who were also having MRD assessed.[25] Three different clusters were identified based on erythroblasts, B-cell precursors, mature naïve and memory B cells that had different survival outcomes that were independent of MRD status. Whether there are similar immune phenotypes in the post-CAR T setting that predict outcome remains to be determined, but it is evident that the future of myeloma therapy will involve CAR T-cells.[26]

Incorporating MRD and IP assessment into current and future clinical trials:

MASTER Trial:

Luciano Costa presented the MASTER (Monoclonal antibody-based sequential therapy for deep remission in multiple myeloma) trial. This is a single arm, multi-center phase 2 study utilizing MRD response-adapted therapy during induction (n=82 planned). The primary endpoint is to determine the frequency of MRD-negativity (at least 10^{-5}). Secondary endpoints include determining the toxicity profile of Dara-KRD, the frequency of imaging plus MRD-negative remissions, the feasibility of MRD-guided treatment discontinuation and to determine the risk and timing of resurgence of MRD-positivity after discontinuation of therapy in MRD-negative patients. MRD assessment will be performed using NGS (Adaptive Biotechnologies). Initial therapy consists of 4 cycles of Dara-KRD (Induction) followed by ASCT (consolidation 1) and up two blocks with 4 more cycles of Dara-KRD (Consolidations 2 and 3) until achievement of MRD negativity ($<10^{-5}$) in two consecutive assessments. MRD is assessed following completion of induction and after consolidation step. Patients who are MRD-negative at two consecutive time points are then observed but undergo repeat MRD assessment at 6 and 18 months following discontinuation of therapy. The remainder of patients then proceed to lenalidomide maintenance. For patients in observation, if conversion to MRD-positivity occurs, lenalidomide maintenance will be initiated. It is estimated that this study will finish accruing in late 2019. As of Dec 2018, 19/21 patients had trackable MRD testing and the ≥ VGPR rate after induction was 100%.

SWOG S1803/BMT CTN 1706:

Parameswaran Hari presented an update of the SWOG S1803/BMT CTN 1706 Study (). This is a phase III study comparing lenalidomide to lenalidomide + daratumumab (subcutaneous formulation) maintenance post-ASCT. After two years of maintenance therapy, MRD will be assessed. Those patients who are MRD-positive will continue their assigned maintenance treatment. Patients who are MRD-negative (defined at a sensitivity of 10^{-6} by NGS) will be randomized to stopping maintenance vs continuing maintenance. OS

is the primary endpoint with key secondary endpoints including ORR, PFS and MRD-negativity rates. Long-term follow-up of CALGB 100104 revealed a median OS of over 10 years in patients receiving lenalidomide maintenance.[27] A total of 950 patients accrued to maintenance over 6 years are required to detect an increase in the median OS from 10 to 16.7 years in the combination arm (HR 0.6). It is estimated that MRD-negativity rates following 2 years of maintenance will be 60% for the lenalidomide arm and 80% to the lenalidomide + daratumumab arm. MRD analysis will be performed at maintenance initiation and then at 12, 24, 36, and 48 months after the second randomization. Quality of life assessments will also be included to not only address the potential adverse effect of adding a second agent to the maintenance setting but also to address whether patients who are MRD-positive have more distress than those who are MRD-negative. This study opened for enrollment in August 2019.

Ongoing developments in MRD and IP:

Enhancing immune reconstitution post-ASCT:

Zaid Al-Kadhimi discussed issues related to immune suppression in the post-ASCT setting and his group's ongoing efforts to enhance immune reconstitution via graft manipulation. It was noted that immune suppression, characterized by low CD4/CD8 ratio,[28] slow CD4 reconstitution[29] and global functional suppression of T cell proliferative activity,[30] have been well-documented in the post-ASCT period. This immune suppression is associated with an increased risk of infections such as varicella zoster[31] but may be linked to myeloma relapse as well. It was noted that a higher lymphocyte count, as well as a lower monocyte count, early in the post-ASCT period has been associated with improved survival outcomes.[32, 33] Factors contributing to this post-ASCT immunosuppression likely include thymic dysfunction, poor CD4 homeostatic dysfunction as well as G-CSF-induced mobilization of T-regs and immature immune suppressive monocytes to the peripheral blood.[34, 35] He noted that in unpublished work, depletion of T-regs and myeloid derived suppressor cells (MDSCs) from peripheral blood stem cell (PBSC) grafts improves allogeneic mixed lymphocyte reactions in vitro as well as an increased homeostastic expansion in response to IL7 and IL15. He therefore hypothesized that it is the abundance of immune suppressor cells in the G-CSF-mobilized graft which is the main cause for post-transplant immune suppression. Thus, it would be predicted that depletion of these immune suppressor cells will result in faster immune recovery post-ASCT, improved vaccine response and less relapse. The three components of the graft include stem cells, immune competent cells and immune suppressive cells. As the latter are thought to be induced by G-CSF, one approach to producing a graft that is depleted of immune suppressive cells would be to collect peripheral blood leukocytes via apheresis prior to G-CSF, mobilize with G-CSF, collect stem cells and select for CD34 cells, and then combine the two populations as an indirect way of eliminating the immune suppressor cells. This approach was studied in a pilot study conducted at Emory University. The primary objectives of this study included 1) to compare the cellular and humoral vaccine responses post-ASCT and 2) to determine the feasibility and safety of this approach. Secondary objectives included comparison of post-ASCT recovery of innate and adaptive immune cells, T cell phenotype, post-transplant recovery of T-regs and MDSCs, two-year PFS and MRD status at three months. In this study, patients

received a series of pre-transplant vaccines consisting of a peptide myeloma vaccine, tetanus and influenza vaccines, and then underwent pheresis to obtain peripheral blood leukocytes. G-CSF treatment was then initiated and stem cell pheresis and CD34 selection were performed. High dose melphalan was administered at day −2 and the CD34+ cells infused on day 0. At day +2, peripheral blood leukocytes were administered followed by vaccines at various time points between day +15 and day +60. Only two patients were enrolled. The preliminary data obtained from these two patients demonstrated the safety and feasibility of indirectly depleting immune suppressor cells from the autologous graft. While both patients did engraft, neutrophil engraftment was delayed (day + 20–22). The vaccine response data, as evaluated by intracellular cytokine release assay and tetramer for the myeloma peptide vaccine, were reportedly uninterpretable. Dr. Al-Kadhimi is now initiating a similar trial at the University of Nebraska Medical Center. In this study, only the tetanus vaccine will be administered and the leukocyte infusion will be moved to day +7. A total of 30 patients will be enrolled: 15 in the experimental arm and 15 in the control arm in which a standard graft is used.

Allison Jacob, an employee of Adaptive Biotechnologies, discussed the use of next generation sequencing technology to evaluate MRD as well as other applications. She noted that immunosequencing, which involves the use of PCR and next-generation sequencing to identify DNA sequences of B- and T-cell receptors, has both diagnostic applications as well as research applications. The NGS-MRD assay identifies the dominant sequence or sequences associated with malignant lymphocytes. As has previously been discussed, multiple groups have reported that achievement of MRD negativity is associated with improved outcomes, regardless of time point and treatment regimen.[3, 36–39] She also discussed exploratory studies which utilized NGS to evaluate the T cell repertoire. In a single institution retrospective study involving 34 cord blood transplant patients, a reduced diversity of the TCR repertoire as early as 56 days post-transplant was correlated with nonrelapse mortality. In a follow-up prospective study including 79 patients, a significant difference in NRM-free survival was noted between patients with low TCR diversity and those with high TCR diversity (NRM-free survival of 55% vs 90%, respectively) at 56 days post-transplant.[40] In an analysis of peripheral blood TCR diversity following allogeneic stem cell transplant in myeloma, low TCR diversity at day 90 post-transplant was associated with disease relapse.[41] Interestingly, however, there was not a significant association between day 180 TCR diversity and risk of subsequent relapse/progression.[41]

Molecular testing in myeloma:

Nikhil Munshi provided an overview of the evolution of molecular testing in myeloma. He noted that FISH testing remains a useful tool to evaluate clonal heterogeneity. While FISH can identify common abnormalities, it can also be useful to estimate the degree of subclonality present. For example, multiple studies have shown that the level of del(17p) correlates with outcome.[42–44] More recently, alternative methods have been employed to evaluate genetics, including the evaluation of copy number changes using SNP arrays. Recent work by Samur et al., evaluated 336 newly diagnosed samples from patients enrolled in the IFM/DFCI 2009 study as well as 164 monoclonal gammopathy of undetermined significance (MGUS) samples from the IFM2008/02 study.[45] Samples were processed for

FISH, SNP array analysis and clonality analysis. The copy number analysis revealed two distinct types of myeloma characterized by different evolution patterns of copy number alterations: hyperdiploid myeloma and non-hyperdiploid myeloma.[45] The proportion of hyperdiploid myeloma cases was noted to increase with age. Discussion was also held regarding the use of other methods to evaluate myeloma genetics, including expression profiling via array-based or RNA-sequencing methods or DNA sequencing via whole exome, whole genome or targeted sequencing approaches. In work presented at ASH 2018, deep whole genome sequencing was performed on 260 myeloma samples in characterize recurrent somatic alterations in non-coding regions.[46] A median of almost 12,000 mutations and indels per sample were detected with more than 3.9 million total somatic mutations. The number of structural variants correlated with overall survival. Dr. Munshi also discussed his group's targeted sequencing approach that includes 246 genes known to be myeloma drivers or pan-cancer oncogenes, 2538 SNPs, and the IGH locus. In a study involving 426 diagnostic samples, this approach provided copy number and IGH translocations accuracy comparable to FISH. In addition, these studies demonstrated that copy number and karyotype serve as the major players determining prognosis. When the variables with prognostic value were broken down by class, gene copy number represented 50%, cytogenetics 38% and sequencing 12%. Thus while TP53 mutations predict poor overall survival, other genes had little significance in univariate analysis. It was proposed that at diagnosis, the evaluation would include mutation analysis, copy number alterations, chromosome rearrangements and gene signature in order to identify high risk patients and select treatment accordingly. In the pre-maintenance setting, evaluation would include analysis of the residual clone including IGH sequencing and mutations. For patients who are MRD positive, duration of maintenance should be longer or ideally, be modified to adapt to the residual clone characteristics. At relapse, evaluation would include analysis of mutations, clonal evolution and gene signature in order to detect potentially druggable targets (e.g., B-RAF).

Radiographic assessment of MRD:

Jens Hillengass discussed radiographic imaging in the context of MRD. As noted previously, [9] different bone marrow infiltration patterns can be observed using MRI: minimal, diffuse, focal or mixed. Focal lesions can be detected via PET/CT, MRI T1, MRI T2 or DW (diffusion weighted)-MRI. As there can be heterogeneous involvement of the bone marrow by myeloma, disparate MRD results can be obtained based on the location of the aspirate. He noted that an analysis of patients enrolled in the IFM 2009 study has shown a subgroup of patients for whom PET/CT remains positive after treatment but MRD testing is negative, which is likely a consequence of not sampling an area of residual disease. However, PET/CT false negatives can also be an issue and work by Rasche et al., have shown a PET/CT false negativity rate of 11% when FDG-PET is compared to DWI.[47] Of note, the expression of the gene encoding hexokinase-2 was significantly lower in PET false-negative patients, providing a possible mechanism for the observed false negativity.[47]

Comparison of MRI and PET/CT from the IFM 2009 study has shown that there is not a prognostic significance of residual lesions present on MRI prior to initiation of maintenance but there is with PET/CT.[48] Multiple studies have demonstrated a low rate (~10–20%) of

MRI normalization following treatment.[48–50] Patients whose lesions underwent cystic transformation on MRI (characterized by T2 hyperintense signal transformation) following transplant had higher CR/nCR rates but shorter PFS compared to patients without cystic transformation.[50] In addition, patients with a cystic transformation pattern also more frequently had del13q and a medium/high proliferation index per gene expression profiling.[50] In a study involving 404 transplant-eligible, newly diagnosed patients, the prognostic value of the size of focal lesions found on DW-MRI was evaluated.[51] The presence of at least three focal lesions with a product of perpendicular diameters >5 cm^2 was associated with poorer PFS and OS.[51]

Establishing MRD as a surrogate endpoint:

Qian Shi provided a statistical perspective of the issues involved in establishing MRD as a surrogate endpoint in myeloma. The goals of being able to replace a true endpoint with a surrogate endpoint include having an endpoint that can be measured sooner, more frequently, at less cost and/or less invasively. In turn, this would lead to reduced trial duration and cost, exposing fewer patients to potentially toxic treatment and lead to more rapid drug approvals. The definition of a prognostic biomarker is one that can be used to identify the likelihood of a clinical event, disease recurrence or progression in patients who have the disease of interest. An endpoint is defined as a precisely defined variable intended to reflect an outcome of interest that is statistically analyzed to address a particular research question. This precise definition typically includes the type of assessments to be made, the timing of those assessments and the assessment tools, along with other details if applicable such as how multiple assessments within an individual will be combined. A surrogate endpoint is an endpoint that is used in clinical trials as a surrogate for a direct measure of how a patient feels, functions or survives. The surrogate endpoint does not directly measure the clinical benefit of primary interest but rather is expected to predict the clinical benefit or harm based on epidemiologic, therapeutic, pathophysiologic or other scientific evidence. Dr. Shi noted that there are multiple factors that must be considered when determining the relationship between the disease, the surrogate endpoint and the true clinical outcome. In specific, the following must be demonstrated: 1) that the surrogate is not in the disease causal pathway; 2) that if there are several causal pathways that an intervention does not only affect the pathway mediated by the surrogate; 3) that the surrogate is neither in the intervention's pathway nor is it insensitive to the intervention's effect; and 4) that the intervention has mechanisms of action independent of the disease process.[52]

A discussion was held regarding the different types of data required to validate a prognostic marker vs a surrogate endpoint. For the former, the data points are generated by individual patients (e.g., Kaplan Meier curves showing a difference in survival outcomes between MRD-negative patients and MRD-positive patients). In this setting, correlations can be made between MRD status and PFS for individual patients but does not involve treatment comparisons. Multiple studies provide assessment of consistency and robustness.[3] The prognostic biomarker can be used to assist individual patient care with respect to treatment choice or monitoring of disease. In contrast, when validating a surrogate endpoint, data points are generated by trial. In this setting, correlations can be made between odds ratio of MRD endpoint and hazard ratio on PFS. In this way, multiple studies provide the building

blocks that allow for treatment comparisons from each trial.[53] The surrogate endpoint will be used as a primary endpoint in a clinical trial to inform the efficacy of a new regimen. Notably, the prognostic value (at the individual level) and the trial level surrogacy can be different.[54] Therefore, two levels of surrogacy estimation should be considered for meta-analysis: the individual level (R^2_{indiv}) which captures the treatment-adjusted association between endpoints at a patient level and the trial level R^2_{trial}) which captures the prediction ability of the treatment effect on the true endpoint based on the observed treatment effect on the surrogate endpoint.[55, 56] When evaluating the individual patient data, consistent definition of endpoints and time scales should be used. In addition, subgroup and sensitivity analyses, as well as examination of unpublished endpoints can be performed. While both levels of surrogacy are important, the trial-level surrogacy is the key factor that allows a surrogate endpoint to be used in future trials as a replacement of the true endpoint.[57, 58] There are multiple factors that affect the estimation performance of the trial-level surrogacy, including number of trials and range of treatment effects, and it is critical to include all possible trials. Examples of this type of meta-analytic surrogacy evaluation previously have been performed for other surrogate endpoints such as disease-free survival, PFS and 30-month complete response rate in malignancies such as colorectal cancer and lymphoma.[53, 59–61]

From Dr. Shi's perspective, the MRD status should reflect all major features associated with a patient's clinical course, including performance status and tolerability to receive the pre-planned treatment, the biology of the tumor clone and its sensitive/resistant phenotype to the proposed treatment and the actual response to the treatment was administered and likelihood of relapse based on extent of tumor depletion. Currently many cohort studies demonstrate the strong prognostic value of MRD-negativity. However, formal validation at the trial-level is needed.

The i^{2}TEAMM (International Independent Team for Endpoint Approval of Myeloma MRD) Initiative represents a collaboration between academia and industry and is composed of the Mayo Clinic SHARE team (an independent statistics and data coordinating center), academic groups (BMT CTN, EMN/HOVON, PETHEMA/GEM, GMMG, MRC IFM) and industry (Amgen, Abbvie, BMS, Celgene, Janssen, Genentech/Roche, Sanofi, Takeda). The primary objective of the i^{2}TEAMM initiative is to evaluate and validate MRD as a surrogate endpoint for PFS through prospectively planned meta-analytic surrogacy analysis based on patient-level data. The inclusion criteria for the trials to be analyzed are: 1) randomized, multicenter studies which involve ≥ 2 treatment groups, 2) newly diagnosed/transplant eligible or newly diagnosed/transplant ineligible or relapsed/refractory myeloma patients, 3) more than 50 patients per trial, 4) study published after 2006 and 5) MRD testing performed. Exclusion criteria include issues related to definition of MRD endpoint, analysis population, surrogacy qualification strategy, data availability. In addition, consensus between i^{2}TEAMM and the FDA/EMA on the inclusion/exclusion of studies is needed. The current list of studies includes 8 newly diagnosed/transplant eligible studies, 5 newly diagnosed/transplant ineligible studies and 4 relapsed/refractory studies. The challenges intrinsic to this analysis include having three different patient populations, differing MRD testing sensitivity (these studies vary between 10^{-4} to 10^{-6}) and differing timing of MRD analysis. Furthermore, the MRD assessment is commonly dependent on the conventional response status.

Summary of ASH2018 abstracts on IP in myeloma:

There continues to be significant effort devoted to characterizing the IP in myeloma. As shown in Table 1, ASH 2018 included multiple abstracts exploring the IP in myeloma and other plasma cell dyscrasias. Similar to the previous year's set of abstracts,[9] there is heterogeneity with respect to the compartment (e.g., peripheral blood or bone marrow) or immune subset being evaluated, the analytic methodology utilized (e.g., MFC, CyTOF (cytometry by time of flight), single cell RNA sequencing), and the patient population of interest (e.g., MGUS, smoldering myeloma, newly diagnosed myeloma, relapsed/refractory myeloma, AL amyloidosis).

Milestones and Deliverables:

Ongoing discussions amongst regulatory agencies, clinical researchers and industry sponsors will be critical if the goal of establishing MRD as a surrogate endpoint is to be achieved. The data from previously completed studies may not be sufficient and it may be necessary to await the results of ongoing studies. The established guidelines for MRD assessment using MFC or NGS, as well as the recently published guidelines for imaging in myeloma[62] should facilitate consistent assessment of MRD in planned studies. Further understanding of the MRD "evolution patterns" which encompass time to MRD-negativity and sustainment of MRD negativity is needed.[63] At this time, there are no consensus guidelines for assessment of IP thus hindering interpretation of existing studies. It will be important to develop a standardized IP methodology, such as a MFC panel, that could be incorporated into larger clinical trials as an exploratory endpoint. In addition, it will be important that the myeloma research community continue to conduct smaller studies utilizing other methodologies such as molecular-based tools in order to explore the immune microenvironment and identify novel immune signatures that may be associated with response or outcomes. Recent data from studies exploring the gastrointestinal microbiome, suggest that we need to expand our scope beyond peripheral blood and bone marrow studies when evaluating the IP.[64, 65]

Conclusion:

Evidence continues to accumulate for the prognostic impact of achieving MRD negativity in both the newly diagnosed and relapsed/refractory settings. Whether this holds true in the post-CAR-T cell setting, the allogeneic transplant setting[66–69] and for patients with high-risk cytogenetics remains to be determined however. While there are substantial existing patient-level data, it is likely that further trial-level data are needed before MRD can become established as a surrogate endpoint. In addition, the use of MRD as a decision tool for treatment has not yet been established through prospective clinical trials. Therefore, at this time the use of MRD to make treatment decisions outside of the context of a clinical trial is not recommended. The emerging data that are establishing new links between the IP and plasma cell dyscrasia evolution, MRD status and survival outcomes are highly intriguing and merit further evaluation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Comprehensive summary from the 3rd annual BMT CTN Myeloma Intergroup MRD/IP workshop
- MRD is not yet established as a surrogate endpoint but is a prognostic biomarker
- Incorporation of IP as an exploratory endpoint in trial design is recommended

Table 1.

Summary of ASH 2018 abstracts evaluating immune profiling (IP) in plasma cell disorders

Study	IP methodology	Patient Population
Bhutani et al.[70]	MFC of PB and BM; TCR clonotyping of PB and BM	MGUS, smoldering MM and MM
Croft et al.[71]	MFC of BM	R/R receiving PD vs CyPD
Danzinger et al.[72]	GEP of MM and BM	ND MM
Descalzi-Montoya et al.[73]	MFC of PB	ND and R/R MM
Kourelis et al.[74]	CyTOF of BM	MGUS, smoldering MM, MM, AL amyloidosis
Lutz et al.[75]	MFC of PB and BM; scRNA-seq of BM	MM with long-term remission
Neri et al.[76]	scRNA-seq of BM	R/R receiving daratumumab or daratumumab/ pomalidomide
Neri et al.[77]	Single cell TCR sequencing	R/R receiving daratumumab, DRd or DPd
Perez Ruiz et al.[78]	MFC of BM, RNAseq	HC and MM
Pierceall et al.[79]	MFC of PB	R/R receiving daratumumab/PD
Pietz et al.[80]	MFC of PB; mRNA-Seq of BM	ND and R/R
Pontes et al.[81]	MFC of BM	ND, post-induction, post-ASCT, maintenance
Seymour et al.[82]	CyTOF of BM; MFC of PB; RNAseq of BM	R/R receiving daratumumab/durvalumab
Seymour et al.[83]	CyTOF of BM	HC and ND MM
Yoo et al.[84]	WGS of MM and saliva; WTS of MM cells and BM	MM
Young et al.[85]	CyTOF of BM	MGUS, smoldering MM and MM
Zavidij et al.	scRNA-seq of MM cells and BM	HC, MGUS, smoldering MM, MM

Abbreviations: ASCT, autologous stem cell transplant; BM, bone marrow; CyPD, cyclophosphamide/pomalidomide/dexamethasone; CyTOF, cytometry by time of flight; DPd, daratumumab/pomalidomide/dexamethasone; DRd, daratumumab/lenalidomide/dexamethasone; GEP, gene expression profiling; HC, healthy control; MFC, multiparametric flow cytometry; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; ND, newly diagnosed; PB, peripheral blood; PD, pomalidomide/dexamethasone; R/R, relapsed/refractory; scRNA-seq, single cell RNA-seq; WGS, whole genome sequencing; WTS, whole transcriptome sequencing