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Summary of the 2019 BMT CTN Myeloma Intergroup Workshop on Minimal Residual Disease and Immune Profiling

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Abstract

The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Myeloma Intergroup has organized an annual workshop focused on minimal residual disease (MRD) testing and immune profiling (IP) in multiple myeloma since 2016. In 2019, the workshop took place as an American Society of Hematology (ASH) Friday Scientific Workshop entitled “Immune Profiling and Minimal Residual Disease Testing in Multiple Myeloma”. This workshop focused on four main topics: the molecular and immunological evolution of plasma cell disorders, the development of new laboratory- and imaging-based MRD assessment approaches, chimeric antigen receptor T-cell therapy research, and the statistical and regulatory issues associated with novel clinical endpoints. In this report, we provide a summary of the workshop and discuss future directions.

Keywords

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

Introduction

In 2016, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Myeloma Intergroup began holding an annual workshop on minimal residual disease (MRD) and immune profiling (IP) in myeloma prior to the American Society of Hematology (ASH) annual meeting.[1–3] These workshops have focused on emerging methodologies to measure MRD and IP and discussed strategies to incorporate MRD and IP assessment into clinical trial design and clinical practice. In 2019, this meeting took place as an ASH Friday Scientific Workshop (entitled “Immune Profiling and Minimal Residual Disease Testing in Multiple Myeloma”) (Full agenda shown in Supplemental Material).

The International Myeloma Working Group (IMWG) published consensus guidelines in 2016 for the assessment of MRD in bone marrow samples and incorporation into response criteria.[4] The two methodologies discussed in those guidelines are multiparametric flow cytometry (MFC) and next generation sequencing (NGS).[4] In specific, if MFC is to be used for MRD assessment, it should be performed using the Euro-Flow procedure which has a minimum sensitivity of 1×10^{-5}.[5] For NGS, the FDA approved clonoSEQ assay (Adaptive Biotechnologies) has a sensitivity of up to 1×10^{-6}. At present, these established methodologies are used to assess MRD status from bone marrow aspirate specimens. However, as it is recognized that there can be extramedullary involvement, the IMWG also defined an “Imaging plus MRD-negative” status as being MRD negative by MFC/NGS plus PET/CT negativity. While these guidelines developed an initial framework for the use of

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MRD, the field is evolving rapidly due to advances in novel methodologies and ongoing efforts to establish the role of MRD assessment in routine clinical practice and as a potential surrogate endpoint for clinical trial and regulatory purposes. In addition, multiple groups are exploring the connection between MRD status, immunophenotype and survival outcomes.[6–9]

In the present report, we provide a summary of the 2019 workshop as well as discuss the results of the pre-workshop survey that was conducted. The workshop, which featured 20 speakers, focused on four main topics: the molecular and immunological evolution of plasma cell disorders, the development of new laboratory- and imaging-based MRD assessment approaches, chimeric antigen receptor T-cell therapy research, and the statistical and regulatory issues associated with novel clinical endpoints.

Pre-workshop survey

For the third time since 2016, we conducted a pre-workshop survey in order to better understand the MRD and IP practices of individuals and institutions. The 2016 survey indicated that 70% (28/40) of respondents measured MRD: 57% used flow cytometry, 18% used NGS, 18% used both flow cytometry and NGS and 7% used an alternative technique such as CD138-selected FISH or PET/CT.[1] Thirty-five percent (14/40) responded that their center measures immune reconstitution/IP before and/or after autologous stem cell transplant (ASCT). The 2018 survey was distributed to 205 individuals from 103 centers and 10 companies. Of the 23 respondents, approximately two-thirds reported that their center measures MRD, with the majority utilizing flow cytometry and/or NGS.[3] Sixty percent reported measuring MRD only in patients in a complete response (CR) and the majority (75%) assessed MRD post-ASCT. The 2018 survey asked whether the MRD results were incorporated into clinical practice and 79% reported that the results did not change practice. Similar to the 2016 survey results, 69% of the 2018 survey respondents reported that their center does not measure immune reconstitution before and/or after ASCT.

This year, 221 individuals from 112 centers and 7 companies were sent a survey. Of the 57 respondents, 45 (79%) reported that their center measures MRD. Sensitivity of the MRD testing used varies: 9% 10^{-4}, 39% 10^{-5}, 41% 10^{-6}, and 11% other. Fifty-eight percent only perform MRD testing in patients in very good partial response (VGPR) and CR while 29% only perform MRD testing in patients in CR. With respect to timing of MRD assessment, 47% perform it after induction, 9% after stem cell collection, 89% after ASCT, 44% at one-year post-ASCT and 40% at other time points. When asked how the MRD results are incorporated into practice, 42% responded that the results trigger change in surveillance, 38% reported that they trigger a change in treatment and 42% replied “other” which included answers such as no change, for prognostic purposes, for clinical trials, or data collection. Respondents could select more than on answer to the question about modality of MRD imaging: 67% use flow cytometry, 64% use NGS, 13% use MRI, and 38% use PET/CT. Seven of 12 (58%) reported that they use advanced imaging as part of the response assessment before/after transplant, with the majority using PET/CT. For those whose centers do not measure MRD, 42% cited barriers in terms of access to proper technology, 17% reported issues with reimbursement, 25% reported issues related to practice guidelines and

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50% gave other reasons such as the results being of unclear significance and lack of prospective data that support escalation of therapy based on MRD positivity.

Approximately half (53%) of respondents reported that their center measures immune reconstitution before and/or after ASCT. This is measured using peripheral blood flow cytometry (43%), bone marrow flow cytometry (30%), immunoglobulin levels (87%), vaccine titers (33%), or cytokine secretion (3%). When asked how these immune reconstitution results are incorporated into practice, 40% responded that they trigger a change in surveillance, 27% that they trigger change in treatment, and 43% responded "other" which included answers such as no practice change, immunizations, IVIG, or antibiotics. Of those who reported using flow cytometry to assess immune reconstitution, 92% evaluate T cells, 69% evaluate B cells, 46% evaluate NK cells and 15% evaluate dendritic cells. The majority (>92%) evaluate CD4 and CD8 T cells while 25% evaluate CD4 subsets and 8% evaluate CD8 subsets. For those who evaluate B cells, 100% evaluated CD19, 56% evaluate CD20 and 11% evaluate B cell subsets. Flow cytometry for immune reconstitution was assessed at varying time points including after induction (23%), after stem cell collection (15%), after ASCT (77%), at one-year post-ASCT (54%) and other time points (46%). The majority of respondents (89%) do not utilize HevyLite testing. Approximately half of respondents assess immunoparesis (55%), primarily via measurement of quantitative immunoglobulin levels. No respondents reported using methods to measure cytokine secretion or cytometry by time of flight (CyTOF). One third (34%) of respondents measure vaccine titers: 58% pneumococcal, 47% tetanus, 42% diphtheria, 32% pertussis, 26% polio, 53% measles, 47% mumps, 53% rubella, 37% varicella, 16% meningococcal, 5% influenza. These vaccine titers are measured at induction (5%), pre-ASCT (5%), post-ASCT (47%), and one-year post-ASCT (63%). For those who do not measure vaccine titers, reasons included lack of insurance coverage issues and uncertainty regarding clinical significance. Two-thirds of respondents reported that they bill MRD and/or immune reconstitution tests to commercial insurance and 80% of respondents reported that they are not supported by research funding to measure these tests.

Session 1: Molecular and Immunobiology of Plasma Cell Disorders (PCD) from Diagnosis to Therapy

This session focused on the recent developments in molecular assays to interrogate the molecular and immunological landscape of plasma cell disorders. While different research groups are utilizing different methodologies to explore the evolution of myeloma and the associated immune dysregulation, it is evident that these processes are extraordinarily complex and heterogeneous.

Mehmet Samur discussed research that evaluates the molecular landscape of myeloma via analysis of single nucleotide variants (SNVs), copy number alterations (CNA), structural variants, and gene expression profiling. This work has demonstrated that there are approximately 7000 mutations per 2.1 Mb and that different myeloma subtypes are characterized by different mutational loads and patterns, gene expression patterns and structural variant frequencies. In addition, analysis of paired samples (diagnosis and relapse) has revealed that driver mutations and translocations remain constant with clonality changes,

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while SNVs increase over time. While these detailed analyses provide insight into the mechanisms that underlie progression of a normal plasma cell to a malignant cell, as well events that occur during relapses, it is also evident from immune profiling studies that changes in the microenvironment play an important role in disease progression.

G. David Roodman discussed the bone and bone marrow microenvironment. He noted that tumor cells can engage with cells of the osteogenic lineage on the endosteal surface, which can induce long-term tumoral cell dormancy.[10] These dormant tumor cells can be released by osteoclast-mediated remodeling of the endosteal niche, enabling formation of micrometastases. In turn, the micrometastases can promote osteoclast formation and further modification of the microenvironment. Proliferating tumor cells may retain the potential to re-engage in an endosteal niche and become dormant. In myeloma bone disease, bone resorption is increased while bone formation is suppressed.[11] Stimulants of osteoclast activity include macrophage inflammatory protein (MIP1), interleukin-3, RANKL and interleukin-6. Suppression of osteoblast activity in myeloma bone disease is due to a number of factors including production of Dickkopf1 (DKK1) by myeloma cells and Gfi-1-induced epigenetic repression of the *Runx2* locus in pre-osteoblasts.[12, 13] The small molecule inhibitor XRK3F2 inhibits p62, which blocks the upregulation of Gfi-1. In preclinical studies, this agent abrogated myeloma-induced osteoblast suppression and thus might represent a novel therapeutic approach to myeloma bone disease.[14, 15]

Manisha Bhutani discussed the pervasive immune dysregulation associated with myeloma. She noted that dysfunctional T, NK and NKT cells are characterized by reduced proliferative capacity, decreased effector function and overexpression of multiple inhibitory receptors due to chronic antigen exposure and presence of various inhibitory and immune suppressive signals in the bone marrow microenvironment. To better understand the scope of the immune dysfunction in myeloma, the group at the Levine Cancer Institute has been performing immunophenotyping studies. They have established a next generation flow cytometry panel using two 10-color panels that evaluate T, NK, and NKT subsets as well as surface expression of inhibitory and activating receptors. In addition, the T-cell repertoire is evaluated by NGS. A pilot study in which patients with MGUS, smoldering (SMM) and active myeloma (MM) were immune profiled, revealed three immune clusters: cluster #1 (MGUS/SMM): precursor stage characterized by lack of innate inflammation, low Th/CTL mobilization, high polyclonal T response; cluster #2 (SMM/MM): immune ‘cold’ myeloma stage characterized by low inflammation but an oligoclonal T response (low Shannon index) and cluster #3 (MM primarily): immune ‘hot’ myeloma stage characterized by strong innate inflammation, Th terminal differentiation, CTL anergy (Tim3+), oligoclonal T response.[16] There is some evidence that therapies such as daratumumab may be able to alter the T cell repertoire.[17] Ongoing clinical trials in both the newly diagnosed and relapsed/refractory setting will evaluate the hypothesis that therapies such as daratumumab can renew the adaptive immune cell repertoire and rebuild a healthy immune system.

Zihai Li discussed immune dysregulation in solid tumors and commonalities with myeloma. One link may be the role that TGFβ plays in conferring resistance to PD-1/PD-L1 and T-cell therapies.[18, 19] Another potential link is GARP (Glycoprotein A Repetitions Predominant), which is a protein found on the cell surface of platelets, Tregs, and some tumor cells and can

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activate latent TGFβ.[20] In myeloma, TGFβ can be produced by the malignant plasma cells, resulting in inhibitory signals to $T_{effector}$ cells and osteoblasts as well as stimulation of stromal cells to produce pro-myeloma factors such as IL-6 and VEGF. There are multiple potential mechanisms by which to target the bone marrow microenvironment in myeloma, including targeting angiogenesis/hypoxia, mesenchymal stromal cells, osteoclasts/osteoblasts, cytokine signaling pathways, and immunostimulatory pathways. Notably, TGFβ is involved in all of these processes.[21] In addition, myeloma-associated thrombosis leads to increased levels of active TGFβ, likely via activation of platelets and thrombin-mediated cleavage of GARP. Intriguingly, Rachidi et al., reported that targeting platelets improves adoptive T cell therapy in preclinical studies.[22] Thus, targeting TGFβ, by blocking the thrombin-GARP-TGFβ pathway, is currently being evaluated for the treatment of solid tumors and should be considered in myeloma as well.

Madhav Dhodapkar also discussed the role that the immune microenvironment plays in myeloma progression. He noted the seeming paradox that many myeloma genomic alterations originate in the MGUS phase, yet many MGUS patients have long periods of clinical stability. Thus, it is evident that the microenvironment must change over time from a growth-restrictive environment to growth-permissive environment. Preclinical studies utilizing MGUS cells in humanized mice provided evidence for a dominant role of the bone marrow microenvironment in determining whether clonal plasma cells expand.[23] In addition, preclinical studies have revealed that alterations in components of the innate and adaptive immune systems, particularly the innate lymphoid cells, are early events in MGUS and affect the risk of progression to myeloma.[24–27] The prolonged premalignant phase of MGUS is associated with chronic antigen stimulation, raising the question as to how memory T-cells are maintained. There are data to suggest that as MGUS progresses to myeloma, there is a decline in stem cell-like CD8 T-cells expressing TCF-1 that have self-renewal potential and long-term survival and an increase in terminally differentiated CD8 effector T cells that express Blimp-1 and have a lower capacity for proliferation and cytokine production.[28–30] Therefore, a key question is what the mechanisms are that underlie the attrition of stem cell like T-cells in the myeloma microenvironment. One mechanism may be through DKK1 as studies have shown that there is an inverse relationship between bone marrow plasma DKK1 levels and attrition of TCF-1/7-expressing T cells.[28] In addition, the TCF-1/7+ T-cells may depend on the local bone marrow niche, including specific myeloid compartments.[28] Further understanding of the mechanisms by which the microenvironment provides immunological control of myeloma will provide insight into the development of new preventative and therapeutic immunomodulatory strategies.

Samir Parekh discussed immune profiling studies in patients receiving immunomodulatory therapy. Correlative studies performed during the course of a phase II study evaluating the combination of pomalidomide, cyclophosphamide and dexamethasone in relapsed/refractory myeloma, revealed shifts in T cell subsets. In specific, immune profiling studies performed on blood and marrow samples at baseline, on day 15 of cycle 3 and at progression showed a shift to effector (memory) phenotype at cycle 3, but that this pattern reverted to baseline at time of progression. In a phase I study evaluating iberdomide in relapsed/refractory myeloma patients, blood and marrow samples were analyzed using a comprehensive CyTOF immune profiling panel.[31] These studies demonstrated that iberdomide treatment increases

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marrow cytotoxic NK cells and CD8+ effector memory T-cells. In addition, single cell RNA-seq (scRNA) sequencing was performed which showed increased expression of genes associated with cytotoxic/cytolytic activity and interferon response in T cells at day 15 of cycle 2, as well as decreased expression of genes associated with a naïve state. Studies are also being performed in patients who have received CAR T-cell therapy. In these studies, peripheral blood samples are separated into CD138+ and CD138- fractions with the goal of being able to perform real-time cytokine profiling evaluating cytokine release syndrome and other clinical events. The CD138+ cells are analyzed using scRNA-seq and scCNV/genomics while the CD138- cells are analyzed using CyTOF and scRNA-seq. A CyTOF strategy is being used to identify the CAR T-cells. Preliminary data from a single patient reveals changes in the CAR T-cell phenotype over time, with expansion noted at day 8 and then a shift towards terminal differentiation/senescence and decreased activation by day 28. The technique of CITE-seq, which combines mass spectrometry with scRNA-sequencing allows for simultaneous cell surface protein and mRNA evaluation at a single cell level appears to be a powerful tool to enable detailed immune profiling studies.

Session 2: Adaptation of next generation sequencing, next generation flow cytometry, CyTOF and new approaches to bone and soft tissue imaging in PCD

This session focused on the use of novel techniques to assess MRD in myeloma, including marrow, peripheral blood and imaging-based modalities. Noemi Puig discussed the use of flow cytometry to assess MRD and immune profiling in myeloma. She noted that next generation flow cytometry (NGF) is a highly sensitive method to detect clonal plasma cells and that optimized reagents and methods have been developed in order to achieve standardization.[32] As of 2017, over 40 laboratories in 16 countries have implemented the EuroFlow method. This method is capable of a limit of detection of 2×10^{-6}.[33, 34] Recently reported data indicated that while achievement of MRD-negativity (as assessed by NGF) can overcome poor prognostic features at diagnosis, patients with R-ISS III disease and positive MRD have very poor survival outcomes.[35] However, there are cases of myeloma where patients have a stable small M-spike off-treatment without progression, raising the hypothesis that the immune system is controlling the disease. Simultaneous MRD and immune monitoring studies using flow cytometry have revealed that newly diagnosed patients undergoing induction, ASCT and consolidation who have higher CD27-/CD27+ T-cell ratios have prolonged PFS irrespective of MRD.[36] scRNA/TCRseq analysis of tumor infiltrating lymphocytes from a subset of patients revealed that CD27- T-cells were primarily CD8+ and included senescent, effector and exhausted clusters while CD27+ T-cells were mainly CD4+ and the remaining CD8+ T-cells had a predominant immune suppressive phenotype.[36] This work supports future studies integrating MRD and immune profiling.

Neha Korde discussed the use of MALDI (matrix associated laser desorption ionization) – TOF (time of flight) mass spectrometry to detect monoclonal immunoglobulins. Features of this methodology include ease of use and high-throughput data collection (<1 minute/sample). Immunoglobulins are purified from serum, separated into heavy and light chains, subjected to mass spectrometry and then analyzed based on molecular weight. She noted that MALDI is more sensitive than traditional immunofixation electrophoresis (IFE) with one study showing a four-fold lower limit of detection. She presented data from 29 patients

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receiving daratumumab that showed that MALDI was able to distinguish daratumumab from the M-protein in 86% of cases vs 48% with IFE. One key question is whether the blood-based MALDI testing could be used to assess MRD status. The potential advantages of a blood-based MRD assay include non-invasiveness, easy of performing serial testing, lower cost, and lower likelihood of false negatives due to the sampling issues associated with bone marrow aspirates. Her center evaluated 14 patients receiving carfilzomib/lenalidomide/dexamethasone (KRd) induction therapy and compared MALDI results with marrow MRD testing (NGF, 10^{-5} sensitivity). Nine out of ten patients who had MRD-negative marrows were positive by MALDI. This discrepancy may be a consequence of the half-lives of monoclonal proteins, and the inability to differentiate between newly produced monoclonal protein (from residual clonal plasma cells) vs previously produced monoclonal protein that has not yet been degraded in patients who are truly MRD-negative. A similar phenomenon has been described when evaluating MRD-negative patients who are IFE-positive vs negative.[37] Overall, the advantages of MALDI include cost-effectiveness, automation, improved sensitivity/specificity over SPEP/SIFE, the ability to identify monoclonal antibody drugs, and to more readily identify IgA monoclonal proteins that travel in the beta region on electrophoresis. At this time, the utility of this test to measure MRD status remains unclear. In addition, this methodology is currently not optimized for patients with IgD, IgE, or light chain-only myeloma.

Urvi Shah discussed the use of ^{89}Zr-labeled CD38 monoclonal antibody immuno-PET. The group at Memorial Sloan Kettering Cancer Center has developed a CD38-targeted PET tracer by conjugating the chelator deferoxamine (DFO) to daratumumab and then labeling the conjugate with ^{89}Zr. Preclinical studies involving a mouse model of myeloma demonstrated concordance with respect to localization of myeloma cells as determined via bioluminescent imaging and PET/CT imaging using [^{89}Zr]-DFOdaratumumab.[38] Results from the first-in-human, phase I study involving 10 patients (NCT 03665155) were discussed.[38] Importantly, an example was shown of a patient who was found to have multiple lesions using the ^{89}Zr-daratumumab immuno-PET method but was negative using traditional FDG PET/CT. A phase II study is planned.

Francesca Gay discussed data from the FORTE study in which MRD testing by MFC was compared to NGS. The first endpoint in this study is VGPR rate after 4 cycles of induction (either carfilzomib/cyclophosphamide/dexamethasone (KCd) or KRd) while the second endpoint occurs following completion of consolidation (ASCT followed by KRd x4; ASCT followed by KCd x 4; or KRd x 8 cycles), and includes assessment of the VGPR, stringent CR and MRD rates prior to starting maintenance therapy. MRD by MFC was conducted in all patients in VGPR while NGS assessment was conducted in a subgroup of CR patients (79%). Samples were obtained at the pre-maintenance time point and then every six months during maintenance. The MFC used in this study was the 8-color Euro-Flow method with sensitivity of 10^{-5} (at least 3.5 million events acquired with at least 20 events of monoclonal plasma cells needed to define MRD+). A sub-study of 79 patients was conducted using next generation flow (sensitivity 10^{-5} to 10^{-6}; two 8-color tubes, total of 12 markers). NGS was performed using the ClonoSEQ assay v2.0 (Adaptive Biotechnologies). Very similar rates of MRD-negativity were observed using either MFC or NGS in patients CR, with 86% agreement when comparing 10^{-6} NGS with 10^{-5} MFC and 78% agreement when comparing

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10^{-6} NGS with 10^{-6} MFC. The one year sustained MRD-negativity rate in patients with CR was 83% as assessed by either NGS or MFC (10^{-5}).

Andrew Spencer discussed the use of CyTOF as a correlative study performed during the ALLG MM14 study which evaluated pomalidomide/dexamethasone for relapsed/refractory myeloma (lenalidomide-refractory patients). Following four cycles of induction, patients were randomized to receive either pomalidomide alone or pomalidomide/dexamethasone continued until progression. Samples were obtained prior to initiation of treatment and then on day 1 of cycles 1, 3, 6, and 10 of maintenance. The CyTOF panel evaluated a broad panel of immune cell markers (35 in total) with a focus on NK and B cells. Cluster analysis revealed 131 immune cell populations at baseline. Key findings from the analyses conducted once patients were on treatment included enrichment of several neutrophil populations as well as activated NK cells during maintenance. In addition, patients receiving dexamethasone had decreased Treg levels.

Paola Neri discussed the use of single cell immune profiling in samples obtained from patients treated on a daratumumab/pomalidomide study (MM014). Serial bone marrow aspirates were obtained prior to initiation of treatment, on day 1 of cycle 3 and at time of relapse. CD138+ (tumor) and CD138- (immunome) cells were subjected to single-cell RNAseq, single-cell Assay for Transposase-Accessible Chromatin (ATAC)-seq and single-cell CNV analysis using the 10X GemCode system. TCR single-cell RNAseq was also performed on CD3+ cells. Preliminary results shared during this workshop included the finding that CD27+ effector memory T cells are expanded in treatment-sensitive patients while cytotoxic T cells are terminally exhausted in treatment-resistant patients. Other patterns were observed, such as lower expression of MHCII on CD1C+ dendritic cells in resistant patients. Overall, this work shows how single cell immune profiling in myeloma can be a useful approach to identify mechanisms of tumoral escape to anti-CD38 therapies and could facilitate the development of TCR engineered T cells targeting neo-epitopes, leading to the development of personalized CAR T-cells.

Session 3: Chimeric Antigen Receptor T-cell therapy

This session focused on novel cellular therapies for myeloma. David Avigan discussed vaccine and adoptive T-cell therapies. The personalized dendritic celltumor cell fusion vaccine approach has been evaluated in AML[39] and several studies are ongoing. This approach has also been utilized in myeloma, and the phase II study (BMT CTN 1401), which randomized post-ASCT patients to lenalidomide maintenance alone vs lenalidomide maintenance plus personalized dendritic cell/myeloma cell fusion vaccine, finished accruing (n=205) in less than two years. Numerous correlative studies are being performed on samples from this study, including in-depth characterization of the post-vaccine immune response. He also discussed a new approach to the development of cellular cancer vaccines involving the creation of an artificial lymph node using alginate to form a 3D scaffold. Co-stimulatory molecules are covalently bound to the scaffold and this scaffold can then serve as a supporting microenvironment for the co-culture of T cells and fusion vaccine. Preliminary results from both *in vitro* and *in vivo* studies demonstrate that the scaffold is more effective than the fusion vaccine at eliciting immune responses. Work is ongoing

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investigating the use of vaccine-educated T-cells as adoptive immunotherapy. Additional strategies utilizing vaccination include combination therapy with checkpoint inhibitors or CAR T-cells.

Adam Cohen discussed T-cell phenotypes associated with response to BCMA CAR T-cells. Multiple groups have investigated whether responses to CD19 CAR T-cells are associated with specific T-cell phenotypes and/or functions in patients with B-cell malignancies.[40–42] Work done by the Penn group with BCMA CAR T-cells showed statistically significant differences in CAR T-cell peak expansion and percentage of CD45RO-/CD27+ T cells (characteristic of T_{SCM} cells) in the apheresis product in patients who achieved partial response (PR) vs <PR.[43] Immunophenotyping studies of pre-manufacturing apheresed T-cells, the infusion product and post-infusion T-cells were performed in an effort to identify response-associated subpopulations.[44] This work revealed that responders are characterized by naïve or central memory CD8+ and CD4+ T-cells while non-responders are characterized by activated effector and effector memory CD8+ and CD4+ T-cells in the apheresis product. Differences were also observed at the time of peak expansion, with responders having increased numbers of non-cycling, central memory, CD8+ CAR T-cells while non-responders had increased numbers of granzyme B+, PD-1 high, CD8+ CAR T-cells. Ongoing and planned studies will evaluate whether these patterns are observed with other BCMA CAR T-cell products and whether there are differences between short-term vs long-term responders. The ultimate goal would be to perform phenotypic selection of favorable T-cell subsets prior to CAR T-cell manufacturing.

Sham Mailankody discussed next steps in the development of CAR T-cells. While multiple BCMA CAR T-cell studies have demonstrated high response rates, relapses are still common. There are a number of potential CAR targets, aside from BCMA, including NKGD2 ligand, CD19, SLAMF7, CD38, GPRC5D, CD138, kappa and CD56. With respect to GPRC5D, preclinical studies have demonstrated the effectiveness of targeting that antigen, regardless of BCMA expression.[45] Several groups are working on the development of dual-targeted CAR T-cells, including BCMA/GPRC5D and BCMA/CD38.[46, 47] Other approaches include optimizing the manufacturing process to enrich in particular T cell subsets, such as the bb21217 product (bb2121 cells grown in the presence of a PI3K inhibitor)[48] or the JCARH125 product (a defined cell product comprised of purified CD4 and CD8 CAR T-cells enriched for central memory T cells).[49] Finally, allogeneic CAR T-cells have potential advantages, including bulk manufacturing, the ability to provide repeat dosing with no need for bridging therapy and assurance about cell quality. However, issues with graft-vs-host and host-vs-graft remain. Potential solutions including knock-out of the TCR and CD52 blockade.

Damian Green discussed the role of antibody binding capacity in BCMA CAR T-cell therapy. Cell surface BCMA is cleaved by the gamma secretase complex. From a therapeutic perspective, this enzymatic activity can diminish efficacy of BCMA-directed CAR T-cell therapy by two mechanisms: by reducing the ligand density on the tumor cell for CAR T-cell recognition and by releasing a soluble BCMA fragment (sBCMA) which can inhibit CAR T-cell function. Previous preclinical work has demonstrated that gamma secretase inhibitors (GSI) can increase surface BCMA levels, decrease sBCMA levels and augment the efficacy

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of BCMA CAR T-cell therapy.[50] This has led to clinical trials utilizing an oral GSI. Preliminary data have revealed that pre-treatment BCMA antibody binding capacity (ABC) correlates with response and that GSI treatment increases the ABC.[51, 52] Intriguingly, no patients who received the GSI plus BCMA CAR T-cells had progressed. The optimal timing for administration of GSIs remains to be determined.

Session 4: Adapting Novel Techniques into Clinical Endpoints

This session focused on the statistical and regulatory issues associated with the use of novel endpoints. Nicole Gormley discussed the challenges facing myeloma drug approval and issues of drug safety. As an example, she noted that while checkpoint inhibitors such as nivolumab have been shown to have promising activity in lymphoma, no single agent activity was observed in myeloma patients.[53] Despite this, three randomized phase 3 studies were conducted evaluating the addition of checkpoint inhibitors to standard anti-myeloma therapy: KEYNOTE-183 (compared pembrolizumab/pomalidomide/dexamethasone to pomalidomide/dexamethasone in relapsed/refractory patients), KEYNOTE-185 (compared pembrolizumab/lenalidomide/dexamethasone to lenalidomide/dexamethasone in newly diagnosed transplant-ineligible patients) and CheckMate 602 (compared nivolumab/pomalidomide/dexamethasone to pomalidomide/dexamethasone in relapsed/refractory patients). Interim analyses of the two KEYNOTE studies revealed an increased risk of death for patients in the pembrolizumab arms relative to the control arms with OS hazard ratios (HR) of 1.61 (95% CI 0.912.85; KEYNOTE-183) and 2.06 (95% CI 0.93–4.55; KEYNOTE-185) (https://www.fda.gov/Drugs/DrugSafety/ucm574305.htm). No significant differences in response rates or time to progression were found. Enrollment in the CheckMate 602 study was halted after a futility analysis of PFS was performed and analysis of the OS data revealed a HR of 1.19 (nivolumab/PD vs PD; (95% CI 0.64–2.20). There were also higher rates of serious adverse events in the nivolumab-containing arms.[54] Another example highlighting the potential for unexpected findings from phase 3 studies, she discussed the BELLINI trial, which compared venetoclax/bortezomib/dexamethasone to bortezomib/dexamethasone in relapsed/refractory myeloma. While this study met its primary objective demonstrating the addition of venetoclax to bortezomib/dexamethasone improved PFS (HR 0.630, 95% CI 0.443–0.897), the venetoclax arm was associated with higher mortality (HR 2.027, 95% CI 1.0423.945).[55] A subgroup analysis found that patients with t(11;14)-positive disease had a much more robust PFS benefit and did not have an inferior OS, although the small numbers of this subpopulation limit its interpretation.[55] These examples highlight key points about the difficulties of designing trials. That studies can have divergent results between response rates, PFS and OS emphasizes the importance of including endpoints that can be assessed at both early and late time points, and the importance of including ones that provide definitive evidence of clinical benefit (i.e., OS). It is possible that randomized phase 2 studies could provide earlier comparative data in the context of backbone regimens. In addition, the heterogeneity of myeloma could be addressed through the use of enrichment strategies, stratification and biomarker-driven analyses. Finally, she noted that while the use of MRD as a potential surrogate endpoint holds promise, further standardization of MRD assessment in clinical trials is needed and MRD assays used in clinical trials should be analytically valid.[56] The FDA has issued a guidance

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regarding the regulatory considerations for use of MRD (https://www.fda.gov/media/117035/download).

Vera Suman discussed the challenges of using PFS and OS as endpoints in myeloma. She noted that the gold standards for endpoints for trials evaluating experimental therapies include overall survival and/or patient-reported outcomes and that there needs to be evidence that improvements in those metrics outweigh adverse effects. When OS is used as a primary endpoint, there are a number of factors that must be considered including the incidence and prevalence of the disease being studied, the percentage of eligible patients willing to enroll in a clinical trial, the median OS time for the standard of care treatment, and the minimum increase in OS that would be considered clinically meaningful. Depending on these factors, the trial design may entail a large number of patients, multiple years of enrollment and prolonged follow-up. In order for a surrogate endpoint to be valid, there has to be evidence that achievement of substantial effects on the surrogate endpoint reliably predicts achievement of clinically important effects on a clinically meaningful endpoint (i.e., OS). Reasons for failure of surrogate endpoint to predict clinically meaningful endpoints include: 1) the surrogate endpoint is not in the causal pathway, 2) the intervention affects only the pathway mediated through the surrogate and not the other causal pathways, 3) the surrogate is not in the pathway of the intervention's effect or is insensitive to its effect, or 4) the intervention has mechanisms of action independent of the disease process.[57] With respect to PFS as a surrogate endpoint, there are many possibilities for why treatments that increase PFS fail to demonstrate an improvement in OS, including that the agent may delay progression but induce a more aggressive disease biology that offsets earlier delays in progression or by having off-target effects that impact overall survival. In addition, there are several other factors, including that the date of disease progression is subject to measurement error, there can be attrition bias or evaluation-time bias, or there may be informative censoring in which patients initiate non-protocol treatment, develop a second primary malignancy or die due to other causes. Depending on the censoring rules used, the hazard ratio for PFS may be different from a hazard ratio obtained from an intention-to-treat analysis.[58] It is also noted that PFS can be a subjectively determined endpoint, which can lead to discrepancies between PFS estimates determined by an independent adjudicating committee versus the study investigators.

Qian Shi provided an update on the International Independent Team for Endpoint Approval of Myeloma MRD (i^{2}TEAMM) project. The i^{2}TEAMM initiative represents a collaboration between academia and industry, including oncologists and statisticians. The Mayo Clinic is serving as an independent statistical center for the project. The primary objective of the i^{2}TEAMM initiative is to establish MRDbased endpoint as a surrogate endpoint for PFS in myeloma in order to reduce the duration of clinical trials and expedite patient access to new therapies, through the formal FDA qualification process. The initiative will perform a prospectively designed meta-analytic surrogacy evaluation using individual patient data (IPD) from a collection of randomized clinical trials. The primary trial-level surrogacy (R^2_{WLS} and R^2_{Copula}) measures how precisely the treatment effect on PFS may be predicted based on the observed treatment effect on the MRD surrogate endpoint. She provided an overview of the 16 studies (encompassing almost 11,000 patients) that are being collected to date, noting the varying patient populations (e.g., newly diagnosed, relapsed/refractory),

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varying methodologies used to assess MRD (NGS vs MFC), varying degrees of sensitivity (ranging from 10^{-4} to 10^{-6}), and varying timing of MRD assessment (e.g., based on response status vs fixed time points). These heterogeneities raise significant challenges for this initiative.

Luciano Costa discussed the need to harmonize MRD assessment and outcomes reporting. He noted that currently MRD assessment in trials is sometimes evaluated using assays with unknown analytical performance, that there is a lack of consistency for when patients in clinical trials undergo MRD assessment (e.g., event-driven (achievement of CR) vs milestone driven (e.g., post-ASCT)) and that reporting of MRD is not consistent in the literature. He raised the following questions: 1) what kinds of myeloma trials should include MRD assessment, 2) which marrow sample should be used for MRD assessment, 3) what analytical parameters and validation should be required for MRD assays, 4) which MRD methods are acceptable, 5) should MRD assessment in trials be response-driven or milestonedriven, 6) should imaging (e.g., PET/CT) be paired with MRD assessment, and 7) how should MRD results be reported in the scientific literature. He proposed that consensus recommendations be developed to address these issues.

Future directions

Considerable efforts are ongoing to try to establish MRD as a surrogate endpoint. While there are guidelines for MRD assessment using MFC/NGS[4] as well as guidelines for imaging[59], it is recognized that these methodologies continue to evolve, whether it is through achieving higher levels of sensitivity (e.g., 10^{-6} or better) or through novel modalities (e.g., CD38-targeted immuno-PET[38]). In addition, the majority of the studies performed to date have evaluated MRD status at discrete time points, but it is clear that MRD "evolution patterns" [60] which account for the time it takes to achieve MRD-negativity as well as the duration of sustained MRD negativity also need to be considered when evaluating outcomes. There is a clear need for the development of sensitive peripheral blood-based MRD assays (e.g., mass spectrometry based) which could more readily be adapted into clinical practice. As is evident from the pre-workshop survey there is significant heterogeneity amongst physicians and institutions with respect to MRD testing outside of clinical trials. While this is in part due to lack of access or lack of reimbursement, the more significant factor is that the field currently does not have evidence supporting the use of MRD status to make treatment decisions. Thus until there are results from ongoing and planned clinical trials in which treatment decisions (e.g., switching therapies, intensifying therapy, de-escalating therapy, discontinuing therapy) based on MRD status are shown to improve long-term outcomes, we do not recommend using MRD in routine clinical practice given the lack of available evidence.

Understanding of the role of the immune microenvironment in both the development of myeloma and association with response to therapy continues to evolve. The identification of specific immunophenotypes to study has been a challenge with the utilization of disparate methodologies (e.g., flow cytometry vs scRNAseq vs CyTOF), patient populations (e.g., post-ASCT vs relapsed/refractory) and samples (e.g., peripheral blood vs bone marrow vs gastrointestinal microbiome[61, 62]). As a specific example from this workshop, it is noted that

Dr. Neri described that CD27+ effector T memory cells are expanded in lenalidomide-refractory patients who are sensitive to salvage daratumumab/pomalidomide therapy while Dr. Puig reported an association between a higher CD27-/CD27+ T-cell ratio and prolonged PFS in newly diagnosed patients treated with bortezomib/lenalidomide/dexamethasone (VRD) induction, ASCT and VRD consolidation. Aside from studying two very different patient populations, these two studies also used different techniques to interrogate the T-cell repertoire, thus highlighting the issues that complicate interpretation of immunophenotyping studies. Furthermore, as cellular therapies and other immune-modifying therapies such as bispecific T-cell engagers become increasingly incorporated into the myeloma armamentarium, it is likely that our understanding of the immune signatures associated with disease responsiveness will evolve. Allogeneic stem cell transplantation remains an option for some relapsed/refractory patients and the use of donor lymphocyte infusions and immunotherapy post-transplantation[63–65] represents another setting where comprehensive immune profiling studies should be incorporated.

It is evident that the research efforts represented by the speakers at this workshop, as well as those across the world, will ultimately lead to improved understanding of the pathophysiology of myeloma, which in turn will lead to improvements in treatments, clinical trial designs and evidencebased guidelines. Thus, continued work across the translational spectrum, in association with partnership with industry and regulatory agencies, is necessary to achieve this goal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of Interest:

SAH: has served on advisory boards for Adaptive Biotechnologies, Celgene, Genentech, Oncopeptides, Takeda; has served as a consultant for GlaxoSmithKline, Celgene, Sorrento.

AH: has nothing to disclose

DA: has served on advisory boards for Celgene, Juno, Partners Tx, Karyopharm, Bristol-Myers Squibb, Aviv MedTech Ltd, Takeda; has served as a consultant for Janssen, Parexel, Takeda, Kite Pharma; has received research support from Celgene, Kite Pharma, Pharmacyclics.

MB: has served on speaker bureaus for Amgen, Bristol-Myers Squibb, Takeda; has served as a consultant for Sanofi Genzyme; has received research funding from Janssen, MedImmune, Takeda, Prothena ADC: has served on advisory boards or as a consultant for Celgene/ Bristol-Myers Squibb, Takeda, Janssen, GlaxoSmithKline, Oncopeptides, Seattle Genetics, Kite Pharma; has received research support from Novartis; has intellectual property licensed by his institution on his behalf from Novartis.

LJC: has received research funding from Janssen and Amgen; has received consulting honorarium from Adaptive Biotechnologies, Celgene, Sanofi and Karyopharm.

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MVD: has served on advisory boards for Amgen, Janssen, Lava Therapeutics and Roche.

FG: has received honoraria from Janssen, Celgene, Bristol-Myers Squibb, Amgen, Takeda; has served on advisory boards for Janssen, Celgene, Bristol-Myers Squibb, Amgen, Takeda, Adaptive Biotechnologies, Roche, Abbvie and Oncopeptides.

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JH: has served on advisory boards for Adaptive Biotechnologies, Amgen, Bristol-Myers Squibb, Celgene, GlaxoSmithKline, Janssen, and Oncotracker.

NK:

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Highlights

- Comprehensive summary of the 2019 BMT CTN Myeloma Intergroup MRD/IP workshop
- MRD is a prognostic biomarker and should be incorporated into trial design
- IP provides insight into disease biology and response to treatment