Twenty Years of Advancing Discoveries and Treatment of Mantle Cell Lymphoma:

*Report of the 2023 Lymphoma Research Foundation's Mantle Cell Lymphoma Consortium Workshop*

A Lymphoma Research Foundation Publication

Published Fall 2023
Mantle Cell Lymphoma Consortium Executive Committee

Eduardo Sotomayor, MD  
Tampa General Hospital Cancer Institute  
(Chair)

Peter Martin, MD  
Weill Cornell Medicine

Leticia Quintanilla-Fend, MD  
Institute of Pathology, University Hospital/Tuebingen/Eberhard-Karls-University

Michael Wang, MD  
MD Anderson Cancer Center

Julie Vose, MD  
University of Nebraska Medical Center
Abstract

Mantle cell lymphoma (MCL) is an aggressive subtype of non-Hodgkin lymphoma (NHL) characterized by the t(11;14) chromosomal translocation, which leads to the dysregulation of the cell cycle through overexpression of cyclin D1. Though advances in treatment have improved outcomes, in particular the introduction of Bruton’s tyrosine kinase (BTK) inhibitors to the treatment armamentarium, and more recently CAR-T therapy, MCL often rapidly develops resistance and has a high rate of relapse. In addition, MCL is clinically heterogeneous, and response to treatment can vary, making it difficult to establish a standard treatment approach. Thus, there remains a significant need for more research on MCL biology, including those molecular mechanisms underpinning treatment response or lack thereof, so that novel agents may be identified and/or the use of existing agents may be optimized. At the Lymphoma Research Foundation’s 20th MCL Workshop, researchers gathered to discuss recent developments in both basic scientific and clinical research, so that together, we can continue to develop our understanding of MCL and to improve outcomes for patients. This report, which includes a summary of each presentation, aims to review the findings presented at the workshop and to highlight opportunities, open questions, and areas for future study that would pave the way for a cure of this disease in the coming decades.
Introduction

Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma most often associated with the t(11;14) translocation, leading to overexpression of cyclin D1. MCL is clinically heterogeneous, and as a result, there is no single therapeutic approach or standard of care. Furthermore, the determinants of these varying clinical phenotypes (e.g., indolent disease versus more aggressive disease), are unclear and thus far, there is no way to predict treatment response. Importantly, research has revealed that it is not just the genetic or epigenetic profile of individual tumor cells which affect treatment response, but also the tumor microenvironment (TME). Thus, in recent years, research has not only taken advantage of an improved ability to monitor genetic/epigenetic changes and gene expression on the single cell level to evaluate MCL tumor biology, but has expanded to include analysis of the cross talk between the tumor cells and the cells of the TME and analysis of the resultant impact on treatment response. In addition, the introduction of several new agents which may be used to treat MCL has amplified the need to clearly define molecular response and clinical outcomes, so that patient response may be rapidly and accurately assessed, and the use of existing agents optimized. As clinical trials continue to be developed, it will be important to include biomarkers to better stratify patients and assess response in clinical trials so that these markers may eventually be used to guide clinical care.

Recognizing the need for accelerated MCL research and collaboration between clinical and scientific researchers, The Lymphoma Research Foundation has provided MCL-specific research grants and developed the Mantle Cell Lymphoma Consortium (MCLC), a working group that includes both basic scientists and translational/clinical researchers from North America and Europe. Since 2003, the MCLC has met regularly to allow researchers to share their work and offers a unique opportunity for collaboration between investigators across a wide range of MCL areas of interest. Through this type of exchange, thoughts on the current and future direction of MCL research are shared, and researchers are provided with a unique opportunity to develop collaborations needed to continue to drive MCL research forward. The 20th MCL Scientific Workshop, held on May 2nd and 3rd, 2023, in Chicago, IL, included sessions on MCL genetics, mechanisms of resistance or response to treatment, personalization of therapy and prognostics using biomarkers, the TME, and an international overview of clinical trials and an open forum to establish a roadmap toward the CURE of MCL in the next 20 years.
Mantle Cell Lymphoma (MCL) Genetics, Epigenetics and Genomics

In MCL, ongoing research is critical for identifying not only novel therapeutics, but also for better understanding MCL disease biology and how it relates to clinical behavior so that treatment approaches may be tailored and outcomes optimized. In the workshop proceedings described here, the very latest research and advances within these areas are shared.
To open this session, Preteesh Jain, MBBS, MD, DM, PhD (MD Anderson Cancer Center), discussed the impact of mutation profiling on MCL prognosis and outcome. To date, there are no established biomarkers which are routinely used clinically to predict outcome or to plan treatment; however, there are a number of studies which have identified associations between mutational status of individual genes and patient outcomes. A 162-gene panel was used as part of routine care for 227 MCL patients and outcomes assessed. Within the cohort, genomic DNA was isolated from a range of sources: bone marrow (BM) aspirate, formalin-fixed paraffin embedded (FFPE) blocks, fine needle aspirate (FNA), and peripheral blood (PB). Patients were either treatment naive (130) or pre-treated (97). Of the pre-treated patients, 47 had received a BTKi and had refractory disease and 23 had been treated with CAR-T. The mutations present in patient samples included ATM (51.1%), TP53 (29.5%), KMT2D (21.1%), CCND1 (19.3%), BIRC3 (16.2%), NSD2 (11.4%), SMARCA4 (10.1%), UBR5 (9%), NOTCH1 (8.3%), CARD11 (7%), SAMHD1 (7%), NFKB1E (6.6%), SP140 (6.6%), S1PR1 (6.6%), DNMT3A (6%), NOTCH2 (6%), IGLL5 (6%), TRAF2 (5%), TET2 (5%). For these patients, there was no single constellation of mutations predictive of outcome; however, patients with >3 mutations had significantly worse survival (p<0.0001).

Brian Li (Washington University School of Medicine) then presented a survey of the genomic landscape of MCL. Whole exome sequencing (WES) on 27 tumor-normal tissue pairs (lymph node and skin, respectively) was carried out and single-nucleotide variants (SNV), insertions/deletions (indel), structural variants, and copy-number variants were compared. For a subset of samples, a combined analysis of whole-genome sequencing (WGS), structural analysis, and RNA fusion detection were carried out. Study findings confirmed the presence of recurrent variants canonical to MCL, including t(11;14) translocation (8/10 WGS samples) as well as TP53 (6/27), CCND1 (4/27), NSD2 (3/27), and NOTCH1 (3/27). CCND1 and TP53 mutations were found to be co-occurring in the patient cohort (p<0.05) and were associated with shorter PFS (p<0.05). Several novel mutations were also uncovered, including ATM (7/27; missense variants, frameshift variants, deletions, and duplications), ZNF804B (3/27), DAZAP1 (3/27), and ASXL1 (2/27). Of note, the mutational profile observed in patients following CAR-T therapy was unique, highlighting the need to continue research on mechanisms of relapse in this setting. Importantly, the use of multiple methods for assessment of molecular changes within this cohort of MCL patients permitted an integrated analysis across variant types and provided some insights into the differing mechanisms by which genes are mutated. As our understanding of biomarkers expands, it will be important to include them in clinical trials for validation and in order to support their incorporation into clinical care.

In the next talk, Sundandini Sharma, PhD (University of Nebraska Medical Center), presented research on the impact of tumor mutations on interactions with the TME, and how a combination of MCL genetics and TME composition may be used to identify prognostic subtypes in MCL. Initial research findings revealed that MCL tumors with high proliferative indices not only have different constellations of mutations, but also are associated with differing populations of immune cells within the TME, when compared to MCL tumors with low proliferative indices. To better understand this dynamic, and how individual tumors regulate their immune landscapes, this study of samples from 153 patients used imaging mass cytometry (IMC), a time-of-flight analysis that circumvents the need for complexing inherent in other single-cell analysis methods. The study identified at least two prognostic MCL subtypes dependent on ATM or TP53 mutational status. TP53-positive tumor cells (which were also found to express high levels of p-STAT3, p-NFKB, and HLA) were associated with a diminished T-cell population (CD8+ and CD4 +) compared to ATM-positive tumor cells, suggesting that the clinical outcomes for these subtypes may also hinge upon TME dynamics. By including information on the TME in stratification, accuracy may be improved beyond that achievable with tumor mutational analysis alone. Future research will include assessment of expression in nearest neighbors as well as expansion of analysis to include established tumor genetic markers of disease severity and their association with effects within the TME.
Mantle Cell Lymphoma Mechanism(s) of Resistance/Response

To open the session on mechanisms of MCL therapeutic resistance and response, Jianguo Tao, MD, PhD (University of Virginia), discussed both the genetic heterogeneity and plasticity of MCL, and how these features give rise to therapeutic resistance and clinical progression. In MCL, plasticity manifests as adaptive remodeling of the kinome, which limits the efficacy of even combination approaches with targeted kinase inhibitors. To overcome MCL resistance to BTKi therapeutics, adaptation of the global kinome must be blocked. To understand the keystone activities which permit plasticity in MCL, molecular assessment of venetoclax-tolerant MCL persister and expander cells was carried out. Results of the analysis suggest that chromosomal 18q21 deletion and concomitant super enhancer remodeling drive venetoclax resistance. While expression levels of over 100 genes were shown to either increase or decrease, all downregulation observed is 18q21 related, indicating pressure and selection for this mutation following BTKi exposure. Importantly, persister cells from both MCL cell lines and primary patient samples are sensitive to transcription inhibition (CDK7): Thus, co-targeting CDK7 transcription reprogramming and BCL-2 may prevent and/or overcome drug resistance achieved through adaptive remodeling. In the case of CAR-T resistance, which eventually occurs in MCL, in vitro targeting of CDK7/9 transcription also overcomes TME-mediated CAR-T therapy resistance and enhances CAR-T therapy efficacy against lymphoma growth through reshaping of TME evasion, thereby enhancing CAR-T cell trafficking and efficacy. Because residual disease is the eventual cause of relapse, this research provides important insight into how MCL cells may be treated to reduce the likelihood of persistence and expansion and also highlights possible therapeutic strategies to improve the efficiency of or prevent resistance to existing therapies.

Next, Tycel Phillips, MD (City of Hope), provided an update on bispecific antibodies in MCL. For NHL, the first data on bispecific agents using a T-cell engaging strategy was generated with blinatumomab. In the original blinatumomab dose-escalation study, therapy was administered as a continuous infusion with mandated hospitalization (due to short drug half-life), using doses as high as 90 µg/m2/d. The study was halted due to high rates of neurological toxicity, and a step-up dosing with a reduced target dose of 60 µg/m2/d was implemented to reduce complications. While blinatumomab was shown to be effective, the need for inpatient infusion has limited use to ALL. Thus, newer bispecifics have been modified to improve half-life and reduce the need for intensive infusion. In MCL, several CD20/CD3 bispecific studies have attempted to enroll patients with R/R MCL. To date, significant results have only been reported with two agents. The first was mosunetuzumab (n=13, ORR 30.8%, CR 23.1%). Another agent, glofitamab, has shown promising results in Phase I studies in patients with relapsed and refractory (R/R) MCL (NCT03075696) with prior BTKi exposure. Thus far, glofitamab response has been positive, with most responses to therapy achieved early. Responses have also been durable, with a median duration of complete response of 10.0 months (95% CI: 4.9- NE), and at the time of data cut-off, 74.1% of patients remained in remission. The most common glofitamab-related adverse event is cytokine release syndrome (73.0%). Of note, MCL has been a more difficult space for bispecific antibodies than other lymphomas, likely due to the presence of circulating tumor cells, which increases risk of CRS/ICANS. Beyond the efficacy of these agents in MCL, open questions include whether bispecifics are able to cross the blood-brain barrier as well as how these agents will be used within a landscape where CAR-T is a treatment option.

Next, Selina Chen-Kian, PhD (Weill Cornell Medicine), discussed how CDK4/6i control of T cell surveillance may deepen or prolong the clinical response to BTKi. In MCL, overcoming drug resistance remains a significant hurdle. Due to the suspected role of transcriptional control in ibrutinib resistance, a Phase I clinical trial exploring the effect of cyclin D1/CDK4 inhibition, using palbociclib, on ibrutinib response in recurrent MCL was conducted in 27 patients. A complete response (CR) rate of 42% was observed, and 5 patients (2 CR and 3 PR) have maintained their treatment regimen for approaching 9 years. To understand the molecular underpinnings of these clinical results, a comprehensive longitudinal analysis was performed using single-cell RNA sequencing (scRNA-seq) on sequential tissue and blood samples collected from patients.
before, during, and upon progression. This exploration led to the identification of four transcriptomically distinct clusters (Cs) within MCL cells; C1 mirroring quiescent normal B cells, C2 exhibiting characteristics of hyper-activated B cells with enhanced B-cell receptor (BCR) and cytokine signaling, C3 representing non-dividing, long-lived MCL cells, and C4 comprising highly proliferative cells. Primary resistance or progression on treatment of MCL was found to correlate with a significant expansion of either C3 or C2 cell populations. The latter fuels the proliferating C4 cells. This shift was accompanied by a substantial decrease in the expression of both MHC I and MHC II on MCML cells. As disease progressed, a sharp reduction in both CD8+ and CD4+ T cells was observed, which apparently arose from two independent mechanisms. This suggests that T-cell surveillance is required for maintaining a prolonged response to ibrutinib. In one patient, progression on palbociclib + ibrutinib in the clinical trial was specifically associated with expansion of long-lived C3 MCL cells. Treatment with venetoclax with ibrutinib restored ibrutinib sensitivity, leading to recovery of CD4+ and CD8+T cells and a complete response for ~ 3 years and continuing. These findings highlight the importance of T-cell mediated immunity in treatment response in MCL as well as the potential for leveraging these pathways and shifts in MCL cell cluster dynamics to improve outcomes for patients.

Marcus Messmer, MD (Fox Chase Cancer Center), then presented a case study which lends insight into the mechanism of acquired resistance to zanabrutinib. An 81-year-old male patient, given zanabrutinib following prior treatment with R-CHOP, BR, and acalabrutinib, was assessed using next-generation sequencing (NGS) on PB samples. The patient achieved a near-complete response on zanabrutinib, but within 2 years developed a rising lymphocyte count with flow cytometry consistent with MCL. At this time in the treatment course, newly emerged mutations, BTK C481S and TP53 F134L, were uncovered. Of note, BTK C481S is rare in ibrutinib-treated MCL, and so may be unique to zanabrutinib resistance. At the time of relapse, the patient was initiated on venetoclax with normalization of lymphocyte count within 3 weeks. Circulating primary lymphoma cells were later evaluated using a novel ex vivo model incorporating the TME, which was able to accurately predict that the MCL cells were resistant to ibrutinib, zanubrutinib, and pirtobrutinib, and sensitive to venetoclax. This is of particular interest because it lends further support to the use of this ex vivo model to assess drug sensitivity, which could reduce patient exposure to therapies against which their MCL is resistant. Additionally, further studies are needed to determine if there is a relationship between the BTKi used and efficacy of pirtobrutinib at relapse.

Hilka Rauert-Wunderlich, Dr. rer. nat. (University of Wuerzburg), discussed research on the role of CD52 and OXPHOS in MCL. The adaptations of the transcriptome in response to ibrutinib may be assessed via time-resolved scRNA-seq to reveal key features of ibrutinib-surviving cells, and was used in the presented study to identifying pathways which may be targeted by therapeutics. Following exposure of ibrutinib sensitive MCL cells, cells which survive undergo a metabolic reprograming to reliance on oxidative phosphorylation (OXPHOS) and undergo a decrease in glycolysis and NF-κB signaling, with a concomitant increase in CD52 and CD37 expression and decrease in CD40 expression. By combining ibrutinib with the OXPHOS-inhibitor IACS-010759, MCL toxicity was significantly increased, compared to ibrutinib monotherapy. Targeting CD52 using a CD52 mAb following ibrutinib pretreatment was also effective, leading to a complement-dependent cytoxicity in an ibrutinib-sensitive cell line. Thus, the use of anti-CD52 therapy may be considered as consolidation therapy after ibrutinib treatment in primary BTKi-responsive patients to achieve MRD negativity and prolong PFS. In addition, targeting oxidative phosphorylation using ibrutinib + IACS-010759 as a co-administered combination therapy is of interest for future development.

Next, Mariusz Wasik, MD (Fox Chase Cancer Center), continued the discussion of MCL mechanisms of resistance to BTKis. While in chronic lymphocytic leukemia (CLL) BTK or PLCG1 mutations are drivers of resistance, these mutations are rare in MCL, suggesting a different mechanism of resistance. Additionally, MCL patients can exhibit varying degrees of resistance, indicating a wide range of tumor biologies underpinning resistance. In MCL, the receptor tyrosine kinase-like orphan receptor 1 (ROR1), which is absent in normal hematopoietical cells, is frequently seen, both in MCL primary cells and cell
lymphoma.org

lines, where it is associated with the CD19 receptor. CRISPR-mediated disruption of ROR1 revealed that ROR1-dependence leads to resistance to BTKi ibrutinib through BCR- and BTK-independent but CD19-dependent activation of intracellular signaling pathways PI3K-AKT and MEK-ERK. ROR1 also sustains activity of MCL cell metabolic pathways, with both glycolysis and glutaminolysis remaining unaffected by BTK inhibition in ROR1-expressing MCL cells but not BCR/BTK-dependent MCL cells. Thus, ROR1 expression is able to promote MCL cell growth in an BCR/BTK-independent fashion rendering them insensitive to ibrutinib. Together, these findings raise the question of whether ROR1 monitoring in clinical practice would be of value and also suggests a novel approach to MCL therapies which may be effective in treating BTKi-resistant MCL or even preventing development of ROR1-dependent BTKi resistance.

Fangfang Yan, PhD (University of Texas MD Anderson Cancer Center), continued the discussion of resistance by presenting findings from a single-cell RNA sequencing analysis of 66 samples from 25 patients treated with BTKi and/or CAR-T therapy. While CAR-T is effective for overcoming ibrutinib resistance in MCL, many patents relapse following this treatment. The presented analysis aimed to understand how tumor cell-intrinsic features shape resistance to BTKi therapy and CAR-T therapy after BTKi. Single-cell RNA was collected pre- and post-BTKi treatment and pre- and post-CAR-T treatment following ibrutinib failure. Analysis revealed that increasing MYC activity correlates with increasing, sequential resistance, relative to normal controls and BTKi-R samples. To permit identification of early drivers of resistance, trajectory analysis was used to order cells based on transcriptome similarity and to evaluate continuous transitions. Both heat shock protein 90 alpha family class B member 1 (HSP90AB1) and cyclin-dependent kinase 9 (CDK9) expression, each of which are correlated with MYC activity levels, were identified as early drivers distinct from those active in ibrutinib resistance. Further, HSP90AB1 also appears to be an early driver of CAR-T resistance and CDK9 was significantly upregulated in CAR-T and ibrutinib dual-resistant samples. Importantly, co-targeting of HSP90 and CDK9 synergistically diminished MYC activity, decreasing MCL cell viability and inducing apoptosis. Collectively, the study revealed the HSP90-MYC-CDK9 network is the primary driving force of therapeutic resistance and uncovers a possible avenue for reducing the impact of dual ibrutinib and CAR-T resistance.

Pradeep Kumar Gupta, PhD (University of Pennsylvania), discussed methods for the detection of metabolic biomarkers indicative of ibrutinib response in MCL. The primary means by which ibrutinib is able to successfully treat MCL is through inhibition of key cell proliferation pathways; however, these same pathways also affect cell metabolism. Thus, ibrutinib-induced metabolic changes may be a valuable tool for the assessment of MCL sensitivity/response. To investigate the predictive value of these changes, MCL metabolism analysis and high-resolution 1H MRS (magnetic resonance spectroscopy; performed at 9.4T vertical bore spectrometer) were carried out on patient-derived cell lines of varying sensitivity to ibrutinib ([MCL-RL [high responder in in vitro cell growth assays], REC-1 [moderate], JeKo-1 [poor], and MCL-SL [non-responder]]. 1H MRS is a non-invasive modality capable of dynamic assessment, making an evaluation of tumor response using this approach of interest for supporting adaptive and personalized treatment approaches. Imaging studies revealed that ibrutinib affects glycolysis (lactate), amino-acid metabolism (alanine), and membrane metabolism (choline), most strongly in ibrutinib-responsive MCL-RL, followed by REC-1, and borderline in IBR poorly responsive JeKo-1. In addition, ibrutinib markedly inhibited lactate accumulation in MCL-RL and REC-1 cell lines, much less so in JeKo-1, and essentially not at all in MCL-SL cells, an effect which directly correlates to ibrutinib impact on cell growth of these MCL cell lines. These findings indicate the potential of lactate, alanine, and choline to serve as early and sensitive signatures of effective BTK inhibition.

Tumor Microenvironment in MCL

To open this session, Prateesh Jain, MBBS, MD, DM, PhD (MD Anderson Cancer Center) discussed findings which describe
TME subtypes and their impact on BTKi response and subsequent clinical outcomes. The study used multiomic profiling of the TME in tissues from MCL patients treated with a BTKi (ibrutinib, acalabrutinib, or zanubrutinib). Sequencing methods include WES \( n = 42 \) and RNA-seq \( n = 42 \) in combination with an analysis of a previously published 122-patient MCL cohort. Based on the transcriptomic profiles obtained from patient samples, four distinct groups based on TME were identified: normal lymph node \( n = 27 \), immune cell-enriched \( n = 46 \), mesenchymal \( n = 44 \) and immune cell-depleted “D” \( n = 51 \). In patients with primary BTKi resistance, the “D subtype” was most common, compared to responders and those with acquired resistance. Within the D subtype, a high tumor proliferation gene signature was observed and Ki-67 > 50% from tissue biopsies had a linear correlation with group proliferation rate signature. Somatic mutations previously reported in ibrutinib resistant MCL and/or in refractory high-risk MCL patients (gene mutations in \( TP53 \), \( SMARCA4 \), \( NOTCH1 \), \( NSD2 \)) were also predominant in group “D.” Clinically, the “D” TME group had significantly shorter survival compared with other TME groups \( p < 0.001 \). These findings may have prognostic/predictive value and suggest that the MCL-TME may have a dominant role in pathogenesis of MCL immune suppression and BTKi resistance.

Virginia Amador, PhD (Institut d’Investigacions Biomèdiques August Pi i Sunyer), presented research which uncovered CD70 as a possible novel target for immunotherapy in MCL. SRY-related HMG-box gene 11 (SOX11) transcription factor has an established role in MCL oncogenesis. In cMCL, SOX11 is overexpressed, whereas in nnMCL, SOX11 is either not present or minimally expressed. In this study, NanoString immune cell panel-based transcriptome analysis, along with immune-cell phenotyping by immunohistochemistry of both SOX11+ and SOX11− nodal MCL, was carried out to better understand the interactions between SOX11 expression in MCL cells and their surrounding TME. These analyses showed a downregulation of most of the specific immune cell subtype markers, suggesting an immunosuppressive microenvironment in SOX11+ nodal MCL. Differential expression profile analyses resulted in the identification of CD70 as a target gene of SOX11 and revealed that in SOX11+ nodal MCL, CD70 expression is activated. CD70 upregulation in MCL cells associated with worse patient prognosis and was accompanied by TME changes, including significantly higher infiltration of effective regulatory T (eTreg) cells and lower GrancymeB+ and CD8+ T cells in nodal MCL. Furthermore, CD70 upregulation in MCL and higher infiltration of eTreg cells in nodal MCLs are associated with worse survival of patients. Additionally, in a preclinical 2D coculture model of MCL and allogeneic CD3+ activated T cells, CD70-blocking antibodies increased IFN secretion and MCL cell death. Thus, expression of CD70 promotes immune evasion through inhibition of T-cell anti-tumor toxicity and supports increased viability and proliferation. Taken together, these findings suggest that CD70 and CD19 dual CAR-T cell therapy may be a promising therapeutic approach for MCL.

Next, Mamta Gupta, PhD (George Washington University), continued the discussion of the MCL TME by sharing research on the role of macrophages in the crosstalk between malignant MCL cells and intra-tumoral immune cells. In preclinical models, evaluation of lymphoma-associated macrophages (LAMs) showed polarization of F4/80+ LAMs into CD206+ M2 and CD80+ M1 phenotypes. Similarly, in an analysis using human MCL cell lines, co-culturing monocyte-derived macrophages with MCL cells induced a M2-like phenotype by elevated CD163+ and IL-10 expression, while M1 markers CD80 and IL-12 were not altered. In the presented research, these findings were expanded by demonstrating that CCR1, which has established pro-inflammatory effects, is highly expressed in monocyte (Mo) and macrophages (M), and that pharmacologic inhibition or genetic deletion of CCR1 is able to block chemotactic Mo/M migration and a reprogramming of M toward an MHCII+/TNF + immunogenic phenotype. Interestingly, MCL tumors raised in CCR1-null (CCR1 KO) mice showed significantly smaller tumors with decreased infiltration of peritoneal-M, compared to wild-type CCR1. In addition, CCR1 KO mice exhibited increased T-cell infiltration in MCL-TME and an anti-tumor CD8+ T cell response. Collectively, these data highlight the importance of LAMs reprogramming in MCL progression and CCR1 antagonists as a potential therapeutic strategy against MCL.

To continue the discussion of the MCL TME, Dylan McNally (Weill Cornell Medicine) discussed findings from a study on TME
structural and compositional patterns. First, multiparameter imaging of 44 proteins in 155 treatment-naïve tumor samples from the prospective Lymphoma Epidemiology of Outcomes (LEO) cohort study was carried out. A total of 5.5 million single cells were analyzed for established B-cell, NK, stromal, myeloid, T-cell, and mitotic markers. This analysis identified 46 cell types in MCL TME, including 5 malignant MCL states, 12 T cell populations, 8 monocyte/macrophage populations, and 6 stromal populations. Next, composition-based clustering of protein expression revealed distinct TMEs, or structural patterns. "Cold” TMEs were largely depleted of infiltrating immune and stromal cells; "Follicular" TMEs presented extensive follicular dendritic cell networks intermixed with malignant cells; "T cell regulated" were highly enriched for CD4+ T cells, including FoxP3+ T-regs and only residual myeloid cell infiltration; "Inflammatory" TMEs were enriched with plasma cells, neutrophils, cytotoxic lymphocytes, and CD163+S100A9+ macrophages; and "Atypical myeloid” TMEs were enriched with CD16+ monocytes and CD16+CD206+ macrophages. These 5 MCL TME subtypes were subjected to a survival analysis, which revealed that "Follicular” TME was significantly associated with superior outcome (OS; log-rank p < 0.01). Importantly, the imaging technology used in this study allows for assessment of spatial interactions and community analysis, which are important next steps.

How I Treat Mantle Cell Lymphoma

To open the second day of the workshop, Michael Wang, MD (MD Anderson Cancer Center), presented clinical pearls for treating MCL along with several case studies illustrating preferred approaches to disease management. Dr. Wang began by discussing considerations for approaching the newly diagnosed MCL patient, including features of both typical and atypical patient presentation, approaches to risk stratification, and important clinical and non-clinical factors for treatment selection. He then discussed his treatment approach for patients > 65 years and < 65 years, including his approach to incorporating trial therapies and novel off-trial therapies and the rationale for considering or selecting each option. The presentation also included a discussion of ibrutinib withdrawal, and a review of open questions in MCL with regards to treatment selection and clinical management. Dr. Wang also provided a review of recent scientific findings which suggest alternative pathways in MCL for which novel therapeutics may be developed, including those which have been recently demonstrated to be critical for metabolic reprogramming and ibrutinib resistance. For example, the 17q gain and BIRC5 may cause clonal evolution and disease progression in the ibrutinib-venetoclax non-responders. Additionally, TIGIT overexpression has been recently shown to be a critical part of T-cell exhaustion and CAR-T resistance in MCL.

Advances in Mantle Cell Lymphoma Epidemiology; Prognostications, Predictive Biomarkers and Precision Medicine

To begin the session, Max Gordon, MD (MD Anderson Cancer Center), presented a validated comorbidity score associated with survival in MCL patients. First, to efficiently and accurately assess patient risk in CLL, the Three-factor Risk Estimate Scale (TRES) comorbidity score was developed in CLL from the Cumulative Illness Rating Scale.18 In the presented study, the utility of the TRES scale was investigated for MCL. A multicenter retrospective cohort of patients with MCL from 4 US sites (n=413, median age 63 years [range 37-86]) was used. Of these patients, 361 were previously untreated and most treated patients received bendamustine-rituximab (n=120) and 173 received autologous stem cell transplant. Findings were then validated using a 1565-patient cohort from SEER-Medicare. The TRES score grades risk based on 3 categories to generate a measure of risk: low (score=0), intermediate (1) and high (2-3). A single point is given based on the presence of 1) vascular comorbidities (any CIRS grade; e.g., venous insufficiency/lymphedema, aortic stenosis, DVT or PE, symptomatic atherosclerosis, etc.); 2) upper GI comorbidities (documented OUD, acute or chronic pancreatitis, melena, history of
perforated ulcer, or gastric cancer); and 3) moderate to severe endocrine disorders (e.g., diabetes with oral agents or insulin, hyperthyroidism, obesity, adrenal insufficiency, etc.). In this study’s primary cohort, the TRES comorbidity risk score was low in 51% of patients, intermediate 31%, and high in 18%. Median EFS was 77, 56, and 42 months (p=0.019) and 5-year OS was 81%, 78% and 61% in low, intermediate and high TRES, respectively. TRES was also associated with EFS (p=0.004) and OS (p=0.002) in front-line MCL. Following a similar pattern, in the validation cohort, from time of diagnosis, 5-year OS was 52%, 41% and 31% (p<0.001) in low, intermediate and high TRES, respectively. Because TRES is an easy-to-use comorbidity score independently associated with survival in older patients with MCL, it may be used in addition to age to stratify patients for clinical trials or in clinical practice to identify high-risk patients. Importantly, some of the identified comorbidities are treatable and/or modifiable, highlighting the importance of proactive management of non-MCL comorbidities as a part of clinical care.

Yucai Wang, MD, PhD (Mayo Clinic), continued, presenting findings from a prospective cohort study on treatment patterns and associated outcomes in patients with R/R MCL in the Mayo/Iowa MER prospective cohort treated with second-line (2L) therapy. Study patients were diagnosed with MCL between 2002 and 2015. Within this time, the landscape of frontline therapy evolved, and several new agents for both first-line (1L) and 2L therapy became available. To understand if these changes have given rise to changes in 2L treatment choice, response, and/or associated survival, a total of 183 patients with R/R MCL who received 2L therapy were analyzed. Sixty-one patients from Era 1 (2003-2009), 73 from Era 2 (2010-2014), and 49 from Era 3 (2015-2021) were included in the analysis. There were no statistical differences in age, sex, stage, or simplified MIPI between eras. Second-line treatment was clearly different between eras, indicating that 2L MCL therapy use has evolved over time. For example, 2L BTKi use was largely limited to Era 3 (44%), due to availability. Second-line bendamustine-rituximab use was minimal in Era 1 (3%) and less common in Era 3 (17%) than in Era 2 (37%). Second-line stem cell transplant use was similar across Eras, ranging from 9% to 14%. Observed treatment advances appear to be associated with improved outcomes. The ORR to 2L therapy was 56%, 80%, and 88%, respectively (CR 31%, 54%, and 53%, respectively). The 2-year EFS was 21% in Era 1, 39% in Era 2, and 58% in Era 3. The 5-year OS was 31%, 38%, and 69%, for Era 1, 2, and 3, respectively.

Kavindra Nath, PhD (University of Pennsylvania), continued the discussion by reviewing data on in vivo detection of BTK inhibition using MCL xenograft models. Currently, MCL and other lymphomas are staged by FDG PET/CT imaging, and therapeutic response is measured primarily by changes in tumor volume. However, BTKi activity is more cytostatic than cytotoxic, and changes in tumor burden are delayed. In contrast, metabolic changes indicative of BTKi response may occur within hours. Thus, in order to rapidly distinguish between responders and non-responders, alternative methods are needed. In the presented study, MCL-derived cell lines REC-1 (ibrutinib-responsive in in vitro studies), JeKo 1 (poorly responsive) and SL (resistant) were xenotransplanted into mice and were examined for metabolic changes induced by treatment with ibrutinib using 1H MRS on day 0, 2, and 7 following initiation of BTKi therapy. In this model, ibrutinib produced an early and profound reduction in REC-1 tumor concentrations of lactate (biomarker of glycolysis), alanine (biomarker of amino-acid metabolism) and, to a lesser degree, choline (biomarker of membrane metabolism) tumors. In JeKo-1 tumors, this reduction was less profound. Conversely, ibrutinib resistant tumor cells showed no significant reduction of the above-listed metabolic biomarkers. Thus, inhibition of metabolic pathways and resultant reduction of intra-tumoral concentrations of lactate, alanine and, possibly, choline measured by 1H MRS hold potential as early biomarkers of BTK inhibition in MCL.

**Mantle Cell Lymphoma Clinical Trials**

To begin the day’s discussion of MCL clinical trials, Timothy Fenske, MD, MS (Medical College of Wisconsin), discussed
minimal residual disease (MRD). MRD has been used in MCL to assess prognosis, guide direction of therapy, as a clinical trial endpoint, as a sensitive measure for response to treatment, and for MCL surveillance. There are a range of methods for assessing MRD, including multi-color flow cytometry, quantitative PCR, immunoglobulin gene high throughput sequencing (Ig-HTS), and other NGS approaches (CAPP seq, PhasED seq). To date, most 1L and 2L clinical studies which have included MRD have used a PCR-based measures, and most often the endpoint used is the rate of MRD. In these studies, as well as in studies using Ig-HTS (clonoSEQ) MRD is consistently shown to be predictive of PFS. In spite of its potential to accelerate clinical trial read out, thus far, there have been no MRD-driven trials. To meet this need, the ECOG-ACRIN 4151 trial was designed to assess the benefit from auto-HCT in patients achieving MRD-negative CR 1L, with a primary outcome of OS. At the time of this presentation, 573 patients have been enrolled (target 689 patient) across 4 arms and results are pending. Dr. Fenske also presented data on MRD as a surveillance tool that may be considered to identify high-risk patients for early interventions.

In the next presentation, Jia Ruan, MD, PhD (Weill Cornell Medicine), presented the results of a phase 2 trial assessing acalabrutinib-lenalidomide-rituximab (ALR) with real-time monitoring of MRD in 24 patients with treatment-naïve MCL (NCT03883184). Given the past success of lenalidomide + rituximab in 1L MCL, as well as the positive effect of acalabrutinib, this study of ALR was not only designed to assess the viability of this triplet therapy, but also to explore the feasibility of MRD response-adapted strategy during maintenance. Patients received 12 cycles of ALR, followed by maintenance with real-time MRD using adaptive ClonoSeq of peripheral blood. At the end of induction (12 cycles), 83.3% of patients had a CR and there was a 100% ORR. After 24 cycles, therapy was de-escalated in those who achieved MRD negativity, and at the time of the presentation, 10 patients in MRD-negative CR had discontinued acalabrutinib and lenalidomide. At a median follow-up of 26 months, all 22 patients had completed induction and remained in remission and 2 patients had progressed during maintenance. The 2-year OS was 100%, and 2-year PFS was 90%. PB-MRD was undetectable (<10^-6) in 50% patients after 6 cycles, 67% after 12 cycles, 80% after 24 cycles. Overall, preliminary data demonstrated that ALR is well tolerated, highly effective, and produces high rates of MRD-negative CR as initial treatment, even in patients with TP53 mutations. In addition, these findings suggest that real-time MRD has the potential to guide response-adapted treatment de-escalation, especially during maintenance where it is critical to minimize toxicity.

Nikesh Shah, MD (Tampa General Hospital Cancer Institute), discussed recent data on frontline treatment approaches in TP53-aberrant MCL. MCL patients with TP53 alterations (present in ~15% of cases) have poor outcomes, even when they receive intensive front-line chemotherapy. Lenalidomide + rituximab (R2) has been shown to have activity in MCL; however little data are available on activity specifically in TP53 mutated MCL. Dr. Shah presented findings from a retrospective review of 89 patients with MCL with TP53 mutations, deletions, or both, diagnosed between 2005 and 2019 at two academic cancer centers in Florida. In the study cohort, TP53 aberration was detected either at diagnosis (54) or at relapse/progression (35). Compared to non-TP53 mutated MCL, TP53-altered MCL was associated with more aggressive disease at presentation and worse overall survival. Of the TP53-altered patients, 83.1% had ≥1 high-risk feature and 58.3% had a high MIPI score. Frontline therapies received by these patients included bendamustine + rituximab (BR) or R-CHOP (n=28), a high-intensity regimen (cytarabine-based or autologous stem cell transplant consolidation; n=23), R2 (n=14), BTK inhibitor + rituximab (n=2), palliation (n=14), or observation (n=8). There was no significant difference in EFS among patients with TP53 mutation, deletion, or both (p=.86). For patients with TP53 alteration detected at diagnosis, median EFS was higher for those receiving R2 vs R-CHOP/BR or high-intensity regimens (85 vs 7.5 vs 19 months; p < .001), respectively. Median OS was not significantly different among regimens (R2 [74], R-CHOP/BR [27.5], high-intensity [28.5 months]; p=.24). Performance status was associated with better survival across treatment lines. Together, these data support consideration of R2 as a chemotherapy-free 1L option for TP53-mutated MCL, in particular in patients unfit for transplant and/or cytotoxic chemotherapy. There remains a substantial need for prospective trials to further explore immunomodulatory therapies for TP53-mutated MCL as well as CAR-T and bispecific antibodies.
To close the session on clinical trials, Yucai Wang, MD, PhD (Mayo Clinic), shared the US Lymphoma CAR-T consortium experience of bresucabtagene autoleucel (brexu-cel) for R/R MCL in standard-of-care practice. Brexu-cel was approved by the FDA for R/R MCL based on the pivotal ZUMA-2 trial, which showed a 91% ORR and 68% CRR and durable responses, with a median PFS of 25.8 months and a median OS of 46.6 months at 3-year follow-up. After FDA approval, there is a need to continue to evaluate outcomes in real-world patients and to compare this experience with trial data. In the presented retrospective study, 189 patients who underwent leukapheresis between August of 2020 and December of 2021 at 16 US institutions with an intent to manufacture commercial brexu-cel were included and data analyzed for treatment response, outcome, and toxicities. Of the 189 enrolled patients, 168 (89%) received brexu-cel infusion. Of the 189 leukapheresed patients, 86% were BTKi refractory, and 68% received bridging therapy. Of note, 79% would not have met ZUMA-2 eligibility criteria (61% would have been ineligible due to disease status). After a median post-infusion follow-up of 14.3 months, 6-month PFS was 69% and 12-month PFS was 59%. Six-month OS was 86% and 12-month OS was 75%. Cytokine release syndrome and neurotoxicity occurred in 90% (8% ≥ Grade 3) and 61% (32% ≥ Grade 3), respectively. CRS and ICANS incidences were similar to the clinical trial experience. In univariable analysis, high-risk simplified MIPI, high Ki-67, TP53 aberration, complex karyotype, and blastoid/pleomorphic variant were associated with shorter PFS after brexu-cel infusion. Patients with bendamustine exposure within 24 months prior to leukapheresis had shorter PFS and OS in intention-to-treat univariable analysis. Taken together, these data show that efficacy and toxicity of brexu-cel in standard-of-care practice is consistent with that reported in the ZUMA-2 trial. Importantly, the study highlights that tumor-intrinsic features of MCL, and possibly recent bendamustine exposure, may be associated with inferior efficacy outcomes.

To close the session on MCL clinical trials, Brad Kahl, MD (Washington University School of Medicine), and Martin Dreyling, MD, PhD (University of Munich-Grosshadern), shared an update of US and European clinical trials, respectively. In the US, the National Clinical Trials Network (NCTN) continues to make significant contributions in MCL treatment, supporting multiple inter-group studies involving SWOG, ECOG-ACRIN, ALLIANCE, and BMT CTN. These trials are aimed at optimizing treatment approach specifically for older/less fit patients, younger/fit patients and relapsed/refractory disease, or include novel drug combinations. Dr. Kahl presented information on several studies. First, EA4181, which compares three chemotherapy regimens consisting of bendamustine, rituximab, high-dose cytarabine, and acalabrutinib in newly-diagnosed MCL was presented. Trial parameters were shared, and Dr. Kahl provided the update that the trial is now fully enrolled and primary endpoint analysis is expected by the end of 2023. Next, developments in EA4151, a phase III trial of rituximab after stem-cell transplant comparing rituximab alone in MRD-negative MCL in first complete remission, were also presented. While more than the 412 patients needed to randomize have been enrolled, almost 10% of patients have refused treatment assignment and the study has enrolled additional patients to compensate. For the phase 2 E1411 trial, data confirm BR as effective induction in older patients and show that B(V)R + R-based consolidation yields a median PFS of > 5 years. Another study, PrE0405, a phase II study of bendamustine and rituximab plus venetoclax in untreated MCL in patients > 60 years of age, has been fully enrolled since March 2022, and data are expected to be presented at ASH in 2023. Finally, A052101, a randomized phase 3 trial comparing continuous vs intermittent maintenance therapy with zanubrutinib as upfront treatment in patients aged > 70 years or > 60 years with comorbidities, is expected to enroll this year.

Dr. Dreyling continued, discussing updates to European MCL studies. The talk began with a discussion of the SHINE trial, a phase III study of ibrutinib in combination with BR and R maintenance as a 1L treatment for older patients with MCL in patients aged > 65 years. This study demonstrated that ibrutinib + BR with R maintenance significantly improves PFS compared with standard chemoimmunotherapy, with a median PFS of 6.7 years. TRIANGLE, a phase III trial of ibrutinib with SOC or without autologous transplantation in first-line MCL, was also reviewed. Adding ibrutinib improved PFS and OS, begging the question of whether or not autologous transplant is needed IL in MCL. The final analysis is currently underway and open questions remain about the how these findings will impact SOC. Finally, building on results of the phase II AIM
study of ABT-199 (venetoclax) + ibrutinib in MCL, the phase II OASIS II trial NCT02558816, investigates if adding venetoclax to ibrutinib + obinutuzumab will result in improved outcomes in relapsed MCL patients.

Together, these studies not only evaluate a range of new therapeutic approaches in MCL, but also contribute to the development of biomarkers, disease stratification, and present an opportunity to better understand the molecular, cellular, and tumor microenvironment changes which occur during treatment response and/or development of resistance.

Roadmap to the Cure of Mantle Cell Lymphoma in the Next Twenty Years: Opportunities and Challenges Panel Discussion

In the final session of the workshop, a panel of MCL expert academic researchers and industry representatives from various pharmaceutical companies discussed opportunities and challenges in MCL clinical trials. As our understanding of molecular drivers of disease continues to evolve, collaboration between regulatory and industry stakeholders will be critical for rapidly incorporating biomarkers and other molecular measures of response into clinical trials as endpoints or stratification tools. It is important to be aware that adoption of surrogate endpoints can drastically reduce the costs of clinical trials, accelerate readout, and lead to improved patient care. Especially in an era of incremental progress, surrogate measures are needed because effect sizes are smaller, and therefore trials must include more patients. Furthermore, as more MCL subgroups are recognized, trials specific to disease subsets will be increasingly harder to enroll. In addition to innovation in the execution of clinical trials such that trials are smaller and smarter, use of biomarkers to further reduce time to read out and the number of patients needed will be critical.

Another challenge facing research in MCL is low enrollment and a lack of diversity in clinical trial populations. While this represents a complex and multifaceted issue, one preliminary step could be to adjust exclusion criteria so that real-world patients may be more easily enrolled. Physicians emphasized that ongoing partnership and communication between industry and physicians throughout clinical trial planning can improve feasibility (especially around enrollment criteria and endpoint selection). As trials are designed, it is critical to involve clinicians who care for patients, as they are better able to identify designs which are unnecessarily burdensome to patients (e.g., frequent follow up for long periods). In addition, adequate support for sites (e.g., study coordination) and industry support for rapid enrollment are critical. Clinicians agreed that enrolment is bolstered by including a comparative arm that includes a regimen appealing to patients. MCL patients are well educated and savvy and are largely uninterested in enrolling in trials where the comparator is clearly going to underperform, compared to the experimental arm.

In addition to traditional safety measures, there is a need to use efficient PRO tools to assess quality of life, as well as short and long-term outcomes. Measures should also be age-adjusted so that outcomes are relevant for the given age group.

Panelists agreed that the poor performance of patients with TP53 mutations, even with immunotherapy, highlight that there is some aspect of disease pathology which remains to be uncovered and targeted and that will improve outcomes. Incorporating exploratory measures which facilitate investigation into these mechanisms is needed.

Finally, the impact of out-of-pocket costs for patients continues to grow. These increasing costs are problematic for all patients, but also worsen access and increase healthcare disparity. The cost of care is especially burdensome for patients who may not be able to take time off work, live some distance from the center, or are under- or uninsured.
**Summary**

The 2023 MCL Scientific Workshop covered recent advances in our understanding of MCL biology and how these advances have uncovered several potential avenues for drug development. Continuing efforts are needed to best understand the basic biology of MCL so that novel therapeutics can be developed to target MCL, especially BTKi-resistant MCL. As our ability to evaluate disease metabolism, molecular changes, and the TME continues to improve, it will be critical to incorporate these findings into clinical studies and treatment paradigms so that adjustments to therapy may be made in real time. As clinical trials continue to be developed, it is important that biomarkers of disease risk be applied for stratification and novel measures of response be incorporated as endpoints in order to increase the efficiency of clinical trials and to decrease time to read out.

**Acknowledgements**

This meeting, in addition to several projects presented, was supported by grants from the Lymphoma Research Foundation Mantle Cell Lymphoma Initiative and the MCL Consortium. Each presenter whose work is included herein reviewed and approved the summary of that work.

Meeting support----

Potential conflict of interest: ----

**References**


