

Recent Advances and Future Directions in Mantle Cell Lymphoma Research:

*Report of the 2021 Mantle Cell
Lymphoma Consortium Workshop*

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Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma characterized by the t(11;14) chromosomal translocation. This translocation most often results in overexpression of cyclin D1 in the pre-B cell population, which leads to extensive proliferation of the B-cells and block in differentiation originating in the mantle zone. MCL is clinically heterogeneous, outcomes are often poor, and optimal therapy has not yet been defined for patients with high-risk disease, in particular for relapsed/refractory disease. Though the number of possible therapies has increased in recent years, sequencing and prioritization of these therapies is complex, with no clear optimized treatment pathway. Beyond targeted molecular therapies, the recent introduction of anti-CD19 chimeric antigen receptor T-cell (CAR-T) therapies for relapsed disease represents both an effective treatment option and platform for development of additional therapies, as well as another layer of complexity in disease management. Resistance to both first- and second-line treatment and the heterogeneity of MCL presents an unmet need for multiple novel treatments which target unique pathways. Detailed study of MCL biology, exploration and development of novel agents, and clinical trials aimed at understanding the impact of these agents (and resistance to them) remains critical. As therapies advance, measurement and impact of minimal residual disease (MRD) on patient outcomes and treatment personalization is increasingly relevant and emerging as an important consideration in treatment selection and monitoring.

At the Lymphoma Research Foundation's 14th MCL Workshop, researchers gathered virtually to discuss pre-clinical, translational, and clinical research findings, treatment personalization, minimal residual disease and other molecular tools for disease monitoring, and the impact of COVID-19, as part of a comprehensive workshop aimed at furthering understanding of MCL and its biology and identifying emerging research imperatives. This report includes a summary of each presentation and is designed to serve as an overview of the broad range of MCL research presented during the workshop.

Introduction

Mantle cell lymphoma (MCL) is a rare and aggressive B-cell lymphoma typically associated with the t(11;14) translocation, which results in overexpression of cyclin D1. MCL is a clinically and biologically heterogeneous malignancy. Over recent years, the number of available therapies has increased, and brought to light the need for personalized treatment, development of prognostic measures, and new treatments which take into account the molecular underpinnings of heterogeneity. Diligent exploration of basic disease biology not only supports a basic understanding of MCL, but also informs intelligent clinical trial design, treatment planning, and patient monitoring. This progress is supported by technologic advances, including minimally invasive methods for evaluating tumor sensitivity to individual treatments, highly specific technologies for examining individual patient MCL genetics, and the computational power to dissect how each of these factors changes in response to therapeutic exposure. These advances must dovetail with clinical studies at various stages which seek to establish the optimal use of currently available therapies and efficacy and safety of novel agents.

The Lymphoma Research Foundation has been a driving force in accelerating advances in MCL research through provision of MCL-specific research grants and development of the Mantle Cell Lymphoma Consortium (MCLC), a working group that includes both scientists and clinical researchers from North America and Europe. Since 2003, the MCLC has met regularly to share research findings, a rare opportunity for researchers with a range of expertise—from laboratory-based research to clinical studies—to come together and exchange the most recent research on MCL. This type of cross pollination and collaboration drives the field of MCL research forward by eliminating many of the barriers that commonly exist between knowledge of basic scientific research, treatment development, and optimized clinical care. The 2021 MCL Scientific Workshop, held virtually on April 6th, 7th, and 8th, included sessions on MCL biology, novel pathways and targets, therapy personalization, minimal residual disease (MRD), therapeutic resistance, outcomes research, prognostic and predictive biomarkers, and an international overview of clinical trials. In addition, Foundation grantees reported on their research projects and Dr. Michael Williams, The University of Virginia, was awarded the inaugural MCL Leadership Award.

Keynote Address

To open the meeting, Elaine S. Jaffe, MD (National Cancer Institute) gave the keynote address. Dr. Jaffe shared a history of lymphoma and MCL disease classification, including how the current classification system came to be and how it may evolve in the future. In 1994, the Revised European-American Classification of Lymphoid Neoplasms' (REAL) [1] criteria were published in a pivotal paper that attempted to define the lymphoid malignancies in a way that could be recognized with then current immunologic, morphologic, and genetic techniques with integration of clinical features as an important parameter. Prior to this time, several systems had been proposed, raising the need for resolution and consensus for clinical classification and diagnosis. Many of these systems were based on theoretical approaches to disease definition, but without validation in the clinical setting. Further, when each of these 6 available systems was systematically applied by pathologists to >1,000 non-Hodgkin's lymphoma samples, no single system was superior at predicting OS [2]. Compounding this ambiguity was the rapidly changing technology and understanding of how to characterize lymphoid neoplasms.

In 1991, the international Lymphoma Study Group (ILSG) formed, with the goal of uniting international efforts at disease classification to facilitate diagnosis, treatment, development of therapies and clinical studies [3,4]. Over the next three years, the group would continue to work towards the development of the REAL criteria [5]. For the development of the REAL criteria, each of the ILSG members reviewed the literature, presented their experience,

and proposed conclusions for recognizing distinct entities with the broad categories of precursor lymphoid, low Grade B-cell, high Grade B-cell, and mature/ peripheral T-cell lymphomas, as well as Hodgkin lymphoma. The classification was unique in that it was a result of a consensus built through input from 19 expert hematopathologists and was informed by published data. The classification included diseases as distinct entities based on morphology, immunophenotype, genetic features, clinical presentation and course, as well site of involvement and clinical presentation. Initially, the reaction to the REAL criteria was negative, and even hostile. Criticisms were primarily focused on the applicability of what seemed to be a granular disease classification system not easily applied by community pathologists or interpreted by physicians. Indeed, one critic expressed the anxiety that, “with tools and methods to precisely dissect each patient’s cell of origin, genetic abnormality, level of differentiation every patient’s lymphoma might be different,” [6] a challenge that we embrace today as a tenant of personalized medicine. However, a subsequent clinical study to test its utility concluded that the precise definitions for each disease entity made it easier to implement, with improved reproducibility and accuracy, as well as clinical validity [7].

Since the publication of the REAL criteria, understanding of molecular drivers of disease has advanced, as well as technologies for immunostaining of biopsies. The process of disease discovery and definition remains iterative and must be adjusted to reflect differences in prognosis and treatment response. Today, molecular biology and clinical research has expanded diagnostic flow charts, recognition of disease subtypes, and sought to define the relationship between these definitions and clinical response to increasingly precise treatments. Today, gene expression profiling, mutational profiling, tumor microenvironment biology, and metabolic profiling are at the forefront of our understanding of MCL disease biology and are part of driving the development of innovative therapies and optimal treatment for patients.

Mantle Cell Lymphoma (MCL) Biology (I)

Shannon Buckley, PhD (University of Nebraska Medical Center) discussed the role of mutations in the E3 ubiquitin ligase UBR5 C-terminal HECT domain in the control of B-cell maturation. Across several types of cancer, UBR5 is overexpressed, however, mutations in the UBR5 HCT domain are unique to MCL and are found in 15.8% of cases [8-10]. To better understand the impact of the HECT domain mutation on MCL biology, researchers created Ubr5 Δ HECT mice using a CRISPR/Cas9 conditional mouse model. These mice have altered splenic, but not marrow, B-cell populations. Within the spleen the major B-cell population is follicular B-cells (the MCL tumor initiating population), both B1 and marginal B-cell subsets are reduced, and pre-germinal center B-cells in the spleen are phenotypically abnormal and functionally impaired. The UBR5 Δ HECT protein is stabilized, which in turn leads to stabilization of the U5 spliceosome, both of which are highly expressed in MCL [10]. The increased activity of the U5 spliceosome results in aberrant splicing events, primarily intron retention and skipped exons. Skipped exons were observed in proteins important for regulation of DNA-templated transcription, chromatin modification, cell shape, and mRNA processing. Retained introns were found in proteins important in many of these same processes, as well as the DNA damage response pathway. Importantly, the loss of the UB5 HECT domain in a mouse model of MCL indicated that this mutation leads to a pre-lymphoma phenotype. These findings provide insights into MCL transformation and progression and identify potential therapeutic targets and pathways for further study and possible treatment development.

Elias Campo, MD, PhD (Hospital Clinic Barcelona – IDIBAPS) presented results of an integrated genomic and epigenomic study aimed at understanding the origin, pathogenesis, and clinical behavior of MCL. While CCDN1 rearrangement and Cyclin D1 overexpression is characteristic of MCL, there are two molecular subtypes of the

disease, conventional (cMCL) and leukemic non-nodal (nnMCL), which have differing biology and clinical behavior. To better understand the molecular basis for this divergence, next generation sequencing (NGS), microarrays (copy number and expression arrays), and methylation arrays (EPIC) were carried out for the whole-genome (n=61) and exome (n=21) sequence in MCL samples (74% cMCL, 26% nnMCL). The CCND1 rearrangement was found primarily in B-cell precursors and was found to be largely mediated by RAG (54 IGH and 1 IJK mutations were identified in 60 Cyclin D1+ patients) in both cMCL and nnMCL. Of note, the origin of the Cyclin D1 t(11;14) in 8% of MCL cases occurred in mature B cells and was mediated by AID and somatic hypermutations or class switch recombination mechanisms. This translocation in mature B-cells occurred in both cMCL and nnMCL and did not appear to have other clinical or biological differences compared to MCL in which t(11;14) is acquired in precursor B-cells. A high level of structural complexity was observed in MCL, and these complex structural arrangements are linked to oncogene activation, as they lead to gene amplifications and remodeling of regulatory regions. Structural variations include chromotripsis, breakage-fusion bridge (BFB) cycles, and translocations. The profile of these structural changes is different in cMCL and nnMCL: cMCL carried a significantly higher number of copy number alterations, and driver changes than nnMCL. Further alterations of ATM were exclusively found in cMCL, whereas in nnMCL TP53 and TERT alterations were enriched. Genome wide DNA methylation analysis identified several changes in heterochromatin and transcriptionally silenced regions in both types of MCL. Importantly, only MYC and TP53 added prognostic value in the absence of genomic complexity. Future studies will seek to understand how methylation changes related to proliferative cell history and structural changes can be used to stratify patients with distinct clinical outcomes.

Johannes Hellmuth, MD (University Hospital LMU Munich) reviewed an MCL case study in which long-read sequencing was used to follow a patient with accelerated nnMCL and an aggressive course. A detailed molecular analysis to characterize the disease was undertaken to identify drivers of this unusual clinical phenotype. Whole-genome, cDNA, and direct RNA Nanopore sequencing was used for analysis of the MCL genome, epigenome and transcriptome. Importantly, nanopore sequencing resulted in 26x mean coverage and a median read length of 5.2kb empowering detection of numerous complex structural aberrations including translocations in repetitive regions which typically cannot be resolved with conventional Illumina sequencing. A breakpoint cluster upstream of CCDN1, was identified and CCDN1 translocation to the IgH locus at chr14q32 was observed. This particular translocation is commonly found in MCL, making this finding an expected one. In this instance, close proximity to the I_H transcriptional enhancer increases transcription. However, a second breakpoint in the CCDN1 3'-UTR and translocation to chr9p21 was uncovered, a novel finding. This second translocation would lead to the replacement of the CCDN1 3'-UTR with IgH 3' UTR, would further increase overexpression by increasing the stability of the CCDN1 mRNA. Together, these two translocations lead to increased CCDN1 expression though increasing both mRNA quantity and stability. In addition, allele-specific methylation at hallmark genes revealed additional changes in imprinting patterns for MEG3 (lncRNA that inhibits proliferation and regulates gene expression) in the cells with the double translocation, which may further accelerate disease.

Pedro Jares, PhD (Hospital Clinic Barcelona - IDIBAPS) finished the first session by describing research on Cyclin D1 transcriptional programs. While Cyclin D1 is recognized as a major cell cycle regulator, it also has a role in transcription. To better understand the impact of Cyclin D1 overexpression on transcription, genome-wide expression analysis in cyclin D1-silenced and overexpressing cells was combined with cyclin D1 chromatin binding profiles to identify a cyclinD1-dependent transcriptional program in MCL cells. The identified cyclin D1-dependent transcriptional signature included 295 genes, 182 of which were cell cycle genes from a range of phases. Accordingly, cyclin D1 interacted with key cell cycle transcriptional regulators such as E2F4 and FOXM1 in MCL cells.

Overexpression of this genetic program in primary MCL was directly proportional to Cyclin D1 levels. Evaluation of 106 MCL tissue and 53 MCL blood samples revealed that clinically, expression of this Cyclin D1 profile is present in MCL and is associated with a poor prognosis. A simplified transcriptional program signature composed of 37 genes was used to validate the association with poor outcome in an independent cohort of 53 MCL blood samples containing conventional and non-normal MCL cases. Further research revealed that the cyclin D1-dependent transcriptional program is also present in subsets of multiple myeloma and breast cancer that are characterized by cyclin D1 overexpression. These findings suggest that cyclin D1 oncogenic effect in MCL, and other cancers, is based not only in its canonical cell cycle function but also in its role as a transcriptional activator.

Mantle Cell Lymphoma (MCL) Biology (II)

Alexander Shestov, PhD (University of Pennsylvania) opened the second session on MCL biology by sharing his research on the metabolic changes indicative of tumor response to kinase inhibitors. To evaluate metabolic flux, metabolomic studies using ¹H magnetic resonance spectroscopy (MRS) and liquid chromatography mass spectrometry (LC-MS) were used to identify metabolic signatures of early therapeutic response, while fluxomics studies using ¹³C metabolic flux analysis (MFA) were used to provide information on intracellular fluxes through various metabolic pathways. Quantitative analysis allowed for examination of glycolysis, TCA cycle and glutaminolysis (among other pathways) in ibrutinib (IBR) sensitive (MCL-RL) and resistant (JeKo-1) cells upon response to IBR. Glutamine contribution to energy production was 56% in IBR-resistant and 61% in IBR-sensitive cells, a significant contribution to cellular energy. Following IBR treatment, ATP production decreased only in IBR-sensitive cells, a change accompanied by a significant decrease in glutaminolysis flux. In IBR-resistant cell lines, inhibition of glutamase with the novel compound CB-839 results in a profound metabolic response via changes in energy production, glutamine metabolism, and metabolic activity of approximately 20 other pathways (e.g., aspartate metabolism, pyruvate, TCA cycle). Future studies may be aimed at further characterizing the activity of CB-839 as well as identifying metabolic targets which may be used to overcome drug resistance.

Vu Ngo, PhD (City of Hope) continued the discussion by presenting findings detailing the role of B-cell receptor (BCR) signaling regulation by the CEACAM1 type-1 transmembrane protein. CEACAM1 was detected by a genome-wide CRISPR library screen as a novel potential target in MCL and is expressed at a significantly higher level in MCL as compared to normal B cells or other B-cell malignancies [11]. CEACAM1 is a known regulator of BCR [12], however the current study sought to better understand whether CEACAM1 contributes to oncogenic BCR signaling. In vitro cell based assays and animal studies revealed that CEACAM1 is essential in MCL, and that its cytoplasmic tail, which contains two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), is required for BCR signaling. Mechanistic studies revealed that CEACAM1 recruits actin binding filament A to the membrane and is required for the reorganization of the cytoskeleton, which is required for activation of the BCR-proximal kinase LYN upon antigen binding and recruitment of FNLA to lipid rafts. With the necessity of CEACAM1 established and a clearer understanding of the mechanism in place, CAR-T cells against this protein were developed and found to eliminate MCL tumors while sparing other lymphomas, making it a potential therapeutic approach. CEACAM1 was also found to be a potential biomarker for ibrutinib response in MCL. Together, these findings provide insights into lymphoma development and may represent the first step toward developing a novel immunotherapeutic target in MCL.

Mariusz Wasik, MD (Fox Chase Cancer Center) continued, presenting research on identification of 21 core mutations in MCL. These core mutations are maintained, even as other mutations within a single tumor arise and shift over

time. Four MCL biopsies were obtained over the course of an 8-year period from the same patient with the patient's normal cells serving as control. The 21 core mutations identified were maintained at all four time points, while the shifting mutational landscape included between 25 and 209 mutations unique to each time point. Maintained mutations of interest include missense mutations of CCND1 and ATM as well as non-sense mutation of KDM5C H3K4 demethylase. Of note, in later stage disease, a missense mutation of KMT2D H3K4 methyltransferase was acquired, leading- in conjunction with the KDM5C loss- to hypermethylation of H3K4me3 and altered expression of genes involved in cell proliferation, adherence/movement, and invasiveness. Importantly for future research, a cell culture line was established with cells from late-stage disease. These cultured cells share genome-scale DNA methylation patterns present in primary MCL-RL cells, including the CpG site methylation found in promoter regions.

Birgitta Sander, MD, PhD (Karolinska Institutet) presented data showing that cannabinoids reduce the number of malignant B-cells and non-malignant leukocytes (B and T cells and platelets) in the blood of patients with indolent B-cell leukemia. The cannabinoid receptor type 1 (CB1) and type 2 (CB2) are overexpressed in most MCL cases and half of CLL cases compared to normal B-cells [13-16]. Low CB1 expression has been correlated with better survival in CLL [17] and higher lymphocytosis in MCL [18]. In this study, patients were given a sublingual spray with a mixture of tetrahydrocannabinol and cannabidiol (each actuation THC 2.7mg, CBD 2.5mg, patients were given up to 7 actuations as a single dose at 9AM) and monitored for changes in blood leukocytes by flow cytometry. One hour following administration, the absolute numbers of malignant B-cells in the blood were significantly reduced and was higher in cells not expressing CB1 (10% reduction of lymphoma cells in CB1 positive cases vs. 15% in CB1 negative cases). The reduction of lymphoma cells remained for 6h after administration. T-cell numbers in blood decreased at 4 hours after administration (35% reduction). However, the effect on lymphoma cells or T-cells was not present 24-hours post treatment. CXCR4 upregulation has previously been described in response to THC [19]. In the present study, THC/CBD increased expression of CXCR4 in both malignant B cells and T cells 4 hours after administration. Thus, the early reduction of lymphoma cells in blood at 1-2 hours was not driven by upregulation of CXCR4 and neither by apoptosis (no caspase-3 activation was detected by flow cytometry). The results suggest a redistribution of cells, potentially through CB2-induced egress from blood to tissues where malignant B-cells may receive survival signals.

MCL Novel Pathways and Targets

To open the session on novel pathways and targets in MCL, Michael Jain, MD, PhD (Moffitt Cancer Center) presented research on how the tumor microenvironment (TME) stromal cells contribute to development of acquired resistance to immune checkpoint blocker (ICB) therapies, and how manipulation of stromal cellular signaling reprogramming may introduce vulnerability to ICBs. When stromal cells are present, CAR-T therapy is less effective. The stromal cells increase MCL expression of PD-1 and Myc expression, expression of genes in several oncogenic pathways, expression of inflammatory cytokines, and phosphorylation of RNA polymerase II. When stromal cells are lacking cyclin-dependent kinase 7 (CDK7), however, tumor volume is reduced, growth is slowed, and response to PD-1 therapy is increased. Further, T-cell memory and infiltration into lymphnodes is improved. By inhibiting CDK7 with pharmacologic agents (i.e., THZ1) in stromal cells, the vulnerability of the MCL tumor is increased. This research shows that the TME and stromal cell influence on MCL behavior is a potential avenue for treatment development or for promoting sensitivity to existing treatments.

Raguveer Ranganathan, MD (University of Southern California) discussed a potential, new CAR-T strategy for addressing B-cell aplasia due to CD19-directed CAR-T therapy, which eliminates both normal and malignant B-cells

during treatment. By identifying the surface immunoglobulin light chain as a more selective CAR target, patient susceptibility to serious infection may be mitigated and the consequences of indiscriminate B-cell depletion avoided. In most MCL cases, the light chain of cell surface immunoglobulin is expressed, while in the non-malignant B cells of an MCL patient, the -light chain is expressed. The -CAR construct selectively kills the malignant Ig + MCL in vitro, while sparing the non-malignant cells that express the -light chain. In vivo, -CAR showed equivalency to CD19-CAR in tumor cytotoxicity against Ig+ MCL tumor cells regardless of CAR-T dose as well as equivalent survival in preclinical murine models. Additionally, as part of this research, a humanized murine model was developed by reconstituting human B-cells within NSG mice post-irradiation using CD34+ umbilical cord blood cells, thereby replicating a humanized humoral immune system with a full complement of humanized B-cells. Within this humanized mouse model, -CAR selectively eliminated Ig + B cells while sparing Ig + B cells, further illustrating the ability of the -CAR to properly kill the appropriately targeted -light chain expressing B-cells in vivo and spare the reciprocal light chain containing B-lymphocytes. Presently, development of a -CAR clinical trial protocol is underway with the aim of initiating the study in 2023-2024.

Hilka Rauert-Wunderlich, PhD (University of Wuerzburg Institute of Pathology) discussed findings of a single-cell analysis of MCL response to IBR. Following exposure to IBR, single cell RNA-seq (scRNA-seq) of >28 000 cell transcriptomes with an average of 2358 genes per cell identified six clusters of transcriptional changes present in >1% of cells [20]. One cluster in particular contained altered expression of CXCL10 (encoding a chemokine), SPP1 (encoding an extracellular matrix protein), and LGALS1 (encoding Galectin-1), making this cellular subtype one with aggressive features (i.e., promotion of angiogenesis, higher risk for metastasis and immune escape). Other clusters contained overlapping, but distinct expression patterns. All but one subpopulation survived 48 hours following IBR treatment, and each cluster had a rapid downregulation of NF- κ B associated genes (NF- κ B target genes, NF- κ B regulating genes, and genes of NF- κ B subunits) as well as upregulation of B-cell receptor subunit genes, signaling genes, and genes of surface antigens. In addition, cell subgroups shared a metabolic reprogramming which included increased glycolysis/hypoxia or OXPHOS-based metabolism across ibrutinib treatment as well as increased OCR/ECAR (oxygen consumption rate/ extracellular acidification rate) ratios after 3 days of ibrutinib, indicating that survival of cells was associated with increased dependence on oxidative phosphorylation. While the relative contribution of each cell population to IBR resistance is unknown, there is a potential for crosstalk between these distinct populations and the TME.

To continue the discussion of transcriptional programming and response to treatment, Bijal Shah, MD (Moffitt Cancer Center) presented work on the drivers of IBR resistance (IR) in MCL identified using genomic, chemical proteomic and drug screen profiling. Resistance is complex, and is known to be related to kinome remodeling, making transcriptome rewiring a likely underlying driver of this change [21]. By inhibiting the transcriptional machinery, specifically cyclin-dependent kinase 9 (CDK9) catalytic subunit of the positive transcription elongation factor b (P-TEFb) of RNA polymerase II (RNAPII), the transcriptional remodeling that drives adaptive change in MCL required for IR is blocked. Importantly, this reprogramming is also blocked-in patient samples when CDK9 is inhibited. Currently, trials are underway in MCL and DLBCL with the CDK9 inhibitors, VIP152 and AZD4573 [22]. Accompanying discovery of this novel treatment strategy is the capability to use ex vivo modeling of primary patient samples in the context of the tumor microenvironment to better predict responsiveness to treatment in patients and identify MCL vulnerabilities that can be targeted to disable the evolution of IR.

Harriet Walter, MD, PhD (University of Leicester) presented work on immunodeficiency caused by prolonged inhibition of BTK, which results in a B-cell differentiation block. In three patients with a complete and durable response to tirabrutinib 480mg OD, who have been on study drug for >5 years, 2 have complete absence of peripheral

blood CD19+ B cells with a block in B-cell differentiation at early B-cell precursor stage [23,24]. By examining the features of these patients, researchers hope to better understand why they have responded to treatment in an exceptional way and further, if patients may feasibly stop therapy. Flow cytometry studies revealed that exceptional response correlates with an X-linked agammaglobulinemia (XLA) phenotype, which in one patient manifested as <1% CD 19+ B cells detected in peripheral blood. Remaining questions include whether the development of the XLA phenotype is predictive of long-term response.

MCL Novel Targets and Therapies and Personalization of Therapy

To begin talks on novel targets and therapy personalization, Vivian Jiang, PhD (MD Anderson Cancer Center) presented results from a study on tumor heterogeneity and evolution in refractory MCL. scRNA-seq of MCL specimens from 21 patients collected across a spectrum of therapy, including ibrutinib and ibrutinib+venetoclax responders and non-responders was carried out alongside an examination of clonal evolution. This analysis confirmed that 17q gain, but not 12p gain, occurred at disease progression, a change that was detected in multiple IBR-resistant patients. Overexpressed genes at 17q include BIRC5, an apoptosis inhibitor known to be associated with poor outcomes and drug resistance. By targeting BIRC5 in cell lines and PDX models, YM155, a clinically tested BIRC5 inhibitor was able to overcome IBR+ venetoclax dual resistance. In addition, CD8 T-cell reprogramming in non-responders was observed, and non-responder CD4 and CD8 T cells expressed significantly higher levels of the T-cell exhaustion marker CD69 and CXCR4. By identifying these cancer hallmarks, drivers of clonal evolution, and genetic alternations associated with disease progression and therapeutic resistance, drivers of clonal evolution and resistance may be targeted in therapeutic development.

Adam Lin, MD, PhD (Northwestern University) discussed a nanoparticle delivery system for CpG oligodeoxynucleotides (CpGs), which are DNA strands that activates toll-like receptor 9 (TLR9), which enhances the innate immune system anti-lymphoma effect and has direct cytotoxicity toward MCL. Because TLR9 resides in the endosomes, nanoparticles naturally accumulate there, makes them an appealing solution for targeted delivery of CpGs, which have poor biodistribution and are rapidly degraded. Therefore, a tri-ethylene modified CpG conjugated gold nanoparticle (tmCpG NP) platform was developed as a delivery system [25]. In addition, they were also able to synthesize tmCpG NPs for both class B and class CpGs. In MCL cell lines, tmCpG NPs were cytotoxic to MCL, including MCL that is TP53 mutated, with a 5-day exposure showing the highest degree of killing [26]. This finding was recapitulated in a MCL patient peripheral blood sample, where tmCpG NPs of both classes reduced the viability of lymphoma cells. In a mouse dual tumor flank model, an intratumoral injections of combination of class B and class C tmCpG NPs had the greatest effect on local and systemic tumor suppression. Furthermore, in a pilot study, combination tmCpG NPs delivered intravenously significantly reduced tumor burden both in the flank and in the liver. In each of these settings the tmCpG NPs were more effective than unconjugated CpGs. Future studies will be focused on understanding the immune profile after treatment with tmCpG NPs and will include studying the effects of combination treatment with other immune-oncologic agents.

Yixin Yao, PhD (MD Anderson Cancer Center) discussed a novel approach to evaluate patient response to targeted therapy and to overcome ibrutinib resistance in MCL. The deletion of 5-methylthioadenosine phosphorylase (MTAP) and accumulation of metabolite methylthioadenosine, a selective inhibitor of the catalytic activity of the protein arginine methyltransferase 5 (PRMT5), represents a passenger vulnerability to PRMT5 inhibition in MCL. PRMT5 is a protein important in regulating mRNA splicing and consistently found to be overexpressed in MCL cells, where it

not only produces suppressive histone modifications to downregulate tumor suppressive genes, but also methylates important proteins that promote DNA damage repair and cell cycle progression. PRMT5 expression is inversely correlated to patient's response to ibrutinib treatment and inhibition of PRMT5 results in cell cycle arrest and decreased proliferation of MCL tumors in PDX models, thereby its arginine methyltransferase activity is required. Blocking methylation activity may result in reversal of PRMT5-catalyzed epigenetic markers and induce aberrant RNA splicing, including alternative splicing of *mdm4*, therefore possibly restoring the expression of tumor suppressive genes such as TP53. In summary, upregulated PRMT5 and MTAP deletion in MCL confers synthetic lethal dependence on PRMT5, making an attractive therapeutic target that will be further exploited for the treatment of patients with MCL.

To close the session, Shalin Kothari, MD (Yale University) discussed pre-clinical work on the effects of proteasome inhibitors (PI) in combination with venetoclax (V) in MCL. To overcome V resistance, upregulation of the proteasome inhibitor-mediated Noxa leads to interaction with Mcl-1 and release of BIM (sequestration is the mechanism of venetoclax resistance). Venetoclax exhibits synergistic activity with proteasome inhibitors, especially carfilzomib (in vitro) or ixazomib (in vitro and in vivo) likely due to increased expression of Noxa and more efficient cleavage of Mcl-1, both of which were shown to be selectively upregulated in V+PI treated cells. In support of this mechanism, combination treatment was shown to increase MCL-1 interaction with Noxa. Markers of cell viability are synergistically suppressed by V+PI combination and late markers of apoptosis dramatically elevated. Finally, the V+ IXZ combination prolongs survival in MCL pre-clinical models. Together, these data support clinical evaluation of venetoclax in combination with readily available novel PIs (ixazomib or carfilzomib) in MCL.

Minimal Residual Disease in MCL

To begin talks on minimal residual disease (MRD) Christiane Pott, MD (University Hospital Schleswig-Holstein) presented research on novel approaches to MRD monitoring in MCL, which included digital droplet PCR (ddPCR) and amplicon next-generation sequencing (NGS). Standardized allele-specific real-time quantitative PCR (qPCR) of clonal immunoglobulin gene (IG) rearrangements is the gold standard for MRD quantification, however the technique has limited sensitivity and is less specific below the quantitative range. A total of 207 blood/marrow samples from 44 patients in the EMCL study cohort were analyzed using qPCR, ddPCR, and NGS. In developing this novel method, it is important to consider whether different methods identify different risk groups, as well as the applicability of the results to future clinical research. Steps in methodological development included establishment of standards and evaluation of agreement between methods. MRD quantification correlated well among NGS and ddPCR ($r^2=0.867$). NGS MRD data were concordantly positive in 74% of samples and ddPCR MRD were concordant in 65% of samples. In 71 qPCR negative cases, NGS detected MRD in 10 and ddPCR detected 9 cases. MRD quantification by NGS demonstrated reliable results comparable to established methods and enables a more specific readout compared to qPCR and a higher sensitivity than ddPCR. Both ddPCR and NGS remain to be confirmed in prospective clinical trials with respect to identification of prognostic subgroups.

Zachary Epstein-Peterson, MD (Memorial Sloan Kettering Cancer Center) described a study aimed at characterizing an intensive front-line, high-dose therapy/autologous stem cell rescue (HDT/ASCR)-sparing immunochemotherapy approach incorporating lenalidomide, as well further clarifying the role for MRD assessment in MCL. Patients were treated with lenalidomide + R-CHOP x 4 cycles followed by R-HiDAC x 2 cycles, and then R-lenalidomide x 6 months. While the overall primary endpoint of the study was not met, among TP53-wildtype/retained patients, this treatment program was efficacious, even for patients with elevated ($\geq 30\%$) Ki-67 (historically an adverse marker in MCL).

The 3-year progression free survival (PFS) was 69%, which is improved compared to a historical benchmark and treatment was associated with a high overall response rate. There was a high rate of MRD after induction chemoimmunotherapy (Len-R-CHOP + R-HiDAC) at 1×10^{-5} sensitivity (97%) and at 1×10^{-6} (80%), and the latter predicted remission duration. Additionally, MRD at 6 months following end of treatment predicted response duration and this later conversion to MRD- suggests that maintenance therapy may be advantageous. MRD at 1×10^{-6} sensitivity post-R-HiDAC and at 6 months post-and of treatment predicted remission duration and may represent an optimal cut off for stringent MRD determination, in particular for dictating treatment de-escalation. The treatment regimen was less effective for patients with TP53-aberrant MCL, a finding that may prompt earlier use of CAR-T cell therapy or other novel agents earlier in the care of these patients. While the study is small (N=49), these findings can inform therapy selection in clinical practice and pave the way for future research.

Reem Karmali, MD (Northwestern University) provided an overview of interim data on response, MRD, and safety for IBR maintenance (I-M) in MCL following front-line intensive induction. Following an intensive approach (eg, autoSCT + rituximab maintenance [4 year PFS ~80%]) [27], the optimal duration of rituximab maintenance remains undetermined, and the potential role for IBR-M in this setting undefined [27,28]. In this Phase II study, the 3-year PFS rate and MRD was measured for patients receiving IBR-M for up to 4 years, following induction with R-CHOP (with or without cytarabine-containing cycles), R-HyperCVAD, or bendamustine + rituximab (could be consolidated with auto SCT). For the 36 patients enrolled, at a median follow up of 41.1 months, 11 (31%) remain on maintenance, 6 (17%) had completed therapy, and 15 (42%) had discontinued for TRAEs (median cycles=24, most common grade 3/4 TRAEs were infection, neutropenia, and HTN). At the last assessment, 34 patients were in CR. Once data mature at 3 years, analyses will include PFS, overall survival (OS) rates to address primary and secondary endpoints, and correlation of MRD with intensity of front-line therapy, among others.

To continue the discussion, Rahul Lakhotia, MD (National Cancer Institute), Michael Williams, MD (Abstract Award Winner) discussed an NGS-based assay for circulating tumor DNA (ctDNA) in the measurement of tumor responsiveness and prediction of clinical relapse. Serum from 53 untreated MCL patients was collected pre-treatment, after Cycles 1 and 2 of bortezomib based induction chemotherapy, and during surveillance. Various clones were identified in baseline tumor tissue and tracked in peripheral blood using ctDNA. Baseline ctDNA levels were significantly elevated in patients with a higher MIPI score. The clearance rate of ctDNA increased with progression of therapy and was associated with improved PFS after induction, however those who cleared ctDNA after 1 cycle had the best prognosis. ctDNA negativity as early as after 2 cycles was also associated with higher median OS, which was not reached for ctDNA negative patients but was 3.9 years for patients with detectable ctDNA. Of 40 patients with negative ctDNA after induction treatment, 6 were without progression at the end of surveillance. All 6 were persistently ctDNA negative during entire follow up demonstrating high specificity of this assay. Of the 34 patients who progressed during follow-up, 21 (62%) were ctDNA positive at the time of progression or prior to clinical relapse. These patterns indicate that early identification of treatment failure is possible and may allow for response-adaptation. Prospective studies that incorporate ctDNA assays during and after induction will determine the role of ctDNA role in individualizing management for MCL.

Mitchell Smith, MD, PhD (Member, ECOG-ACRIN) continued the discussion of MRD by presenting data from a Phase 2 trial (E1411) in 373 patients randomized to induction BR±bortezomib, then consolidation R±L. In this study, MRD was assessed by NGS and flow cytometry after cycle 3, the end of induction, and after cycle 4 consolidation. Utilization of these methods is unique, as most studies use real-time quantitative PCR (RQ-PCR). At restaging, end of induction, and after 4 months of maintenance, flow cytometry and NGS with cut-off at 1×10^{-4} yielded similar results. Marrow and blood samples at the end of induction are generally concordant. By cycle 3 of induction, most

patients become MRD- (92% by NGS and 95% by flow cytometry). Post cycle 3, MRD is also associated with PFS, however few patients were MRD+ at the 10^{-4} cut-off and patients who do achieve a MRD- response continue to experience treatment failure. When using a lower cut-off for NGS (1×10^{-5}) MRD+ there is an increase in MRD positivity, raising the question of whether this threshold is better at identifying either MRD+ or MRD- samples. In clinical practice, the threshold used may be guided by the treatment goal. If the goal is “cure,” the threshold would be lower than if MRD negativity is for prognostic value. Importantly, the high MRD rate for this treatment regimen, supports use of BR as a platform in ongoing trials. Future studies will include longer follow up, comparison of MRD with PET imaging, further exploration of NGS cut offs, serial monitoring, and eventual evaluation of novel therapies at MRD progression to prevent treatment failure.

MCL Therapeutic Resistance, Prognostic and Predictive Biomarkers, and Epidemiology and Outcomes

To begin the session Samir Parekh, MD (Icahn School of Medicine at Mount Sinai) presented an update on SOX11 inhibitors. SOX11, a transcription factor, is overexpressed in a majority of nodal MCL. B-cell specific overexpression of SOX11 is known to promote MCL pathogenesis by increasing BCR signaling and promoting B-cell proliferation, and conversely, SOX11 silencing inhibits MCL growth, making it an attractive target for MCL therapy [29,30]. Though transcription factors are generally considered “undruggable”, SOX11 activity relies on a unique distortion of DNA. A SOX11 inhibitor (SOX11i) was identified by sequential computational and in-vitro screening that has anti-MCL cytotoxicity as well as inhibition of downstream BCR signaling and genes upregulated by SOX11 expression. Importantly, fluorescence resonance energy transfer showed that SOX11i inhibits the SOX11-DNA interaction, while additional analysis showed that SOX11i does not interchelate with DNA. SOX11i caused transcriptional changes similar to those observed in the SOX11 knockdown systems [31]. Furthermore, SOX11i has cytotoxic synergy with IBR specifically in SOX11-positive Ibrutinib-resistant MCL patient samples and patient-derived xenograft mouse models. SOX11i also has synergistic activity with venetoclax in BTKi/ BCL2-resistant MCL cell lines. Taken together, these results provide a foundation for therapeutically targeting SOX11 in MCL by a novel class of small molecules and suggests that use with established therapies may bolster activity.

Yucai Wang, MD, PhD (Mayo Clinic, Rochester) continued, presenting findings on the impact of recent advances in MCL care, specifically rituximab and high dose cytarabine [HiDAC]-containing induction regimens in young, fit, transplant-eligible patients and bendamustine-rituximab [BR] in elderly, unfit, or transplant-ineligible patients in front-line MCL. To characterize the current pattern of care, 343 newly diagnosed patients from the Molecular Epidemiology Resource (MER) cohort study of the University of Iowa/Mayo Clinic Lymphoma SPORE from 2002-2009 (Era 1) and 2010-2015 (Era 2) were studied. In patients <65, the use of Nordic or R-CHOP/R-DHAP was increased in Era 2, while use of R-HyperCVAD and R-CHOP/R-CHOP-like induction was decreased. Event-free survival (EFS) and overall survival (OS) were significantly improved between Era 1 and Era 2 in patients ≤ 65 . In patients >65, the use of BR was increased in Era 2, while use of R-CHOP/R-CHOP-like and non-standard systemic therapy was decreased. A shift from R-CHOP/R-CHOP-like regimen to R-Benda resulted in improved EFS. Future studies will include a larger patient population from multiple institutions and will further examine the impact of changes in care in the front-line and be expanded to examine effects of treatment in the relapsed/refractory setting on EFS and OS.

Chengfeng Bi, MD, Ph.D. (University of Nebraska Medical Center) shared results from a North American Mantle Cell Lymphoma Project (NAMCLP) study of 587 MCL cases from 23 institutions, aimed at evaluating the clinical,

biological, and molecular markers that affect clinical outcome. For these MCL patients, the overall median OS was 5.5 years (40.6% survival at 10 years). For young patients, both MIPI and MIPI-C scores were predictive of survival; however, this was not the case for older patients. This may be due to the large contribution of age and the lack of pathological markers in these scores. Univariate analysis of the lab and pathological parameters in this cohort of patients identified several additional variables (multi-system involvement, Ki67 status, LDH, and anemia, among others) that may improve the predictive value of clinical scoring. The resulting modified prognostic scoring system includes age, LDH, growth pattern, Ann Arbor Stage, and cytological subtype. When applied to 109 MCL cases, this system was a more effective predictor of survival than MIPI and MIPI-C systems, not only for the OS but also for the PFS ($P < 0.0001$ vs $P=0.012$ and $P=0.025$), as well as for the old patients (OS, $P < 0.0001$ vs $P=0.016$ and $P=0.025$). Future works will establish a mathematical algorithm based on the clinical and pathological parameters and compare this new prognostic tool with MIPI systems.

Yang Liu, PhD (MD Anderson Cancer Center) discussed the use of molecular profiling and preclinical drug efficacy to identify pathways important for precision medicine in relapsed MCL. The two-pronged platform included whole exome sequencing (WES) and a companion NanoString sequencing (190 gene panel) followed by in vitro drug screening (23 agents acting on 8 distinct pathways) and assessment of a PDX mouse model. In this pilot study, 7 treatment naïve patients and 9 relapsed MCL patients (8 relapsed after chemotherapy, 6 after ibrutinib or zanubrutinib, and 1 after CAR-T therapy), were studied. Importantly, frequent somatic alterations in TP53 (69%), ATM (25%) and NSD2 (25%) were observed in both treatment naïve and relapsed patient groups, a significant finding given there are no clinical drugs which target these pathways. For samples in which the apoptosis pathway was dysregulated, BCL-2 and MCL-1 inhibitors were shown to be more potent in vitro, a finding verified by the PDX mouse model, where a BCL-2 inhibitor (venetoclax, 50mg/kg) strongly inhibited tumor growth and significantly prolonged the survival of the treated mice ($P < 0.0001$). Similarly, in a PDX mouse model with dysregulated cell-cycle pathway genes, a PLK1 inhibitor (volasertib, 10mg/kg) was significantly better at reducing tumor size compared with vehicle mice ($P < 0.001$). With the ability to differentiate the expression of targetable pathways in MCL, this platform holds promise as an approach to assessment for precision medicine in relapsed MCL.

MCL Clinical Trials (I)

To begin the session on MCL clinical trials, Juan P. Alderuccio, MD (Sylvester Comprehensive Cancer Center, University of Miami) presented final results from a single-arm phase II clinical trial in 44 patients on frontline R-MACLO-IVAM followed by maintenance in MCL. Patients were chemotherapy-naïve MCL patients, age 18-75 years, with ECOG PS: 0-2, adequate organ function and no history of HIV/other cancer. For R-MACLO-IVAM, most patients achieved CR after an initial two cycles ($n=41$ [93%]), indicating that a shorter immunochemotherapy induction may be required than previously thought, a valuable finding for planning future clinical trials. Maintenance with rituximab had no significant toxicity, while thalidomide maintenance was associated with 35% grade 3-4 neutropenia and 40% grade 3-4 peripheral neuropathy. At a median follow up of 7.2 years (range 0.04 to 16) median PFS for thalidomide was 7.7 years (10-year overall survival 63.6%) compared 8 years (10-year overall survival 63.7%) for rituximab. The study established R-MACLO-IVAM as an effective regimen to induce long-term remission in MCL and established no significant difference in PFS or OS for rituximab vs. thalidomide maintenance therapy.

Martin Dreyling, MD, PhD (University Hospital LMU Munich) presented findings from a study aimed at differentiating the impact of high-risk MCL biology on MCL outcomes. High-risk MCL was determined based on cytology (classical

type vs. blastoid variant), cell proliferation (Ki-67 expression), and p53 alterations [32]. In a total of 1183 MCL patients in front line treatment, 294 had at least one of the biological parameters indicating a high-risk biology, including blastoid cytomorphology (10% of analyzed cases), increased Ki-67 > 30% (30%), or p53 overexpression in immunohistochemistry indicating a mutated p53 status (16%). These patients, when compared to standard-risk MCL patients, had a lower median failure-free survival (2.2 years v.s 6.3 years ($p < 0.001$)) irrespective of whether they were initially treated with conventionally dosed chemotherapy (median PFS: 1.3 vs. 3.8 years) or a cytarabine-containing induction with autologous transplantation (median PFS: 3.7 vs. 7.5 years) across all age groups. The presence of only one risk factor was sufficient to have a major impact on outcome and outcomes in patients with multiple indicators of high-risk biology were only slightly worse. Within these risk cohorts, MIPI retains prognostic value. These dramatic differences lend support to the treatment of MCL with blastoid morphology and/or TP53 mutation as a distinct entity, a consideration for therapeutic development and clinical studies.

Daniel Guy, MD (Earle Chiles Research Institute, Providence Cancer Institute) provided results from a pilot study (NCT03623373) on acalabrutinib with bendamustine/rituximab followed by cytarabine/rituximab (R-ABC) pre-transplant. This study sought to further improve the high response rates and MRD negativity observed with regimen of R-bendamustine (R-B) followed by R-cytarabine (R-C) observed in previous trials with acalabrutinib. At the time of this presentation, 12 young patients with previously untreated MCL have completed the treatment protocol: OR is 83%, CR is 75% (67% MRD-), and progressive disease (PD) is 17%. At a median follow up of 11 months, no relapse has occurred in responding patients. The median CD34+ stem cell collection 3.84×10^6 cells/kg (range 2.77-5.9) indicates no negative impact on mobilization. No unexpected AES were observed: there were no significant bleeding events, no treatment related mortality, and most non-hematologic AEs were low-grade. However, 2 patients discontinued therapy due to persistent thrombocytopenia. This study will be expanded to a larger patient group and to include three treatment arms in the activated intergroup trial EA-4181.

Preetesh Jain, MD, PhD (MD Anderson Cancer Center) presented results from an efficacy/safety analysis of a phase II clinical trial using IR in previously untreated elderly patients with MCL (NCT01880567). Earlier studies of patients >70 years showed that IR is effective in relapsed MCL [33-35]. As elderly patients are not suitable for intensive therapy or stem cell transplant, the activity of IR in first-line therapy is of interest, however, has not been tested. This study examined front-line IR in patients ≥ 65 years ($PS \leq 2$ and normal organ function, non-blastoid/pleomorphic MCL, Ki-67% < 50%, largest tumor size < 10 cm). For 71% of patients best response was CR and for 25% best response was PR. At a median follow up of 45 months median PFS and OS have not yet been reached. Four patients have progressed: 2 had Ki-67 $\geq 30\%$, one was TP53 mutated with additional FAT1 and SF3B1 mutations. While longer term follow up will reveal relapse patterns and second cancers, at present arrhythmia (34%) has been identified as an important AE in this group (median time to onset 9.5 months).

To complete the first update on recent clinical trials, Michael Wang, MD (MD Anderson Cancer Center) presented results from the phase II ZUMA-2 study on anti-CD19 CAR therapy (KTE-X19) in patients with relapsed/refractory MCL. In the primary efficacy analysis of ZUMA-2 (N=60), the ORR was 93% (67% CR rate) after a median follow-up of 12.3 months. After 1 year (median follow-up of 17 months, range, 12.3-37.6) ORR was 84% with a CR rate of 59% with 48% remaining in ongoing responses and 70% of those who achieved CR remaining in response. For the first 28 patients treated, the median follow up is higher at 32.3 months (range, 30.6- 37.6 months), and for these patients 39% remained in continued remission with no further therapy. The medians for DOR, PFS, and OS were not reached after a median follow-up of 17.5 months. No new safety signals were observed with additional follow-up. A robust expansion of CAR-T cells is required to achieve a response, evidenced by the fact that peak CAR T-cell expansion was higher in patients with an ongoing response at 12 months or those who relapsed at 12 months compared to

nonresponding patients. Among patients with ongoing responses at 12 months, recovery of B-cells increased over time and gene-marked CAR-T cells decreased over time, a finding in contrast to earlier findings that most relapsed patients had detectable CD19 at relapse [36]. This substantial and durable clinical benefit in R/R MCL and the manageable safety profile at extended follow up is promising for CAR-T therapy in MCL.

Update on International Clinical Research Efforts in MCL

To conclude the meeting, two leading MCL investigators provided updates on planned and ongoing MCL trials. Brad Kahl, MD (Washington University in St. Louis) spoke about the coordination between 5 separate groups, including SWOG, ECOG-ACRIN, ALLIANCE, BMT CTN, and NCIC CTG in the National Clinical Trials Network (NCTN) initiatives. Initially, initiatives were intended to replicate the coordination in studies carried out by the European MCL consortium and included two coordinated intergroup trials in younger (S1106) and older (E1411) patients. These efforts have resulted in identification of acalabrutinib as a novel agent to test as part of hyperCVAD, which will be tested in the study EA4181 in younger patients prior to ASCT. This study is partially enrolled and will help to better understand if high-dose cytarabine can be safely eliminated as well as whether acalabrutinib improves CR/MRD- rate. Another study, EA 4151, aims to develop a risk-adapted approach based on MRD for intensive therapy and to better define the impact of MRD on survival. To facilitate enrollment the study design is flexible and allows a range of auto-HCT conditioning and local administration of maintenance rituximab. In older patients, E1411 aims to analyze the impact of bortezomib and lenalidomide on PFS. For this study enrollment is complete, however events have occurred at a slower rate than expected, prolonging time to read out. Finally, another study currently enrolling, PrE0405, is a phase II study of bendamustine and rituximab plus venetoclax in untreated MCL in patients >60 years, a design which considers a chemo-free approach and assesses continuous vs. intermittent therapy. Together, these studies represent examples of collaborative and coordinated endeavors which further clinical care of MCL in younger and older patients.

Martin Dreyling, MD (University Hospital LMU Munich), spoke about current generation of European MCL Network Studies. Studies address MCL in younger, elderly, and relapsed patients, and increasingly consider blastoid (+/- TP53) and plieomorphic MCL as distinct diseases. In younger patients, the TRIANGLE trial, which is taking place in 14 different countries with 861 patients randomized is comparing three different approaches to treatment that addresses both induction and maintenance therapy: R-CHOP/R-DHAP induction followed by ASCT, R-CHOP+I/R-DHAP induction followed by ASCT plus 2 years of ibrutinib maintenance, and R-CHOP+I/R-DHAP induction followed by 2 years of ibrutinib maintenance (skipping ASCT).

In patients >60 years old, the MCL ELDERLY R2 trial compares an alternating induction of R-CHOP/R-HAD to R-CHOP/Ara-C, and patients who respond to either treatment will then receive either combined rituximab-lenalidomide maintenance or rituximab only for 2 years. This study has completed recruitment of 620 patients for the induction phase and 494 patients for the maintenance phase, with enrollment nearly complete. Additionally, a phase II study of rituximab and bendamustine with low-dose cytarabine (RBAC500) is underway to examine this highly effective combination, adding a venetoclax maintenance in high risk patients. Another study, (IMCL-2015) examines the efficacy of ibrutinib in indolent MCL. The OASIS study on IBR, obinutuzumab, and venetoclax with obinutuzumab maintenance has 100% OS at 1 and 2 years, and PFS of 93.3% . While some studies (SHINE) explore the combination of chemotherapy and targeted approaches, future trials including OASIS-2, ENRICH, and MCL- elderly II address the question whether first line treatment can be chemo-free, and optimal duration of therapy.

Covid-19 and Mantle Cell Lymphoma

In the following set of presentations, the challenges faced by lymphoma patients due to COVID-19 were discussed. Hematologic malignancies are known to pose an increased risk of complications and death from COVID-19, a risk compounded by the immunomodulatory activity of lymphoma treatments. Furthermore, the availability of a vaccine raises the question of how specific therapies may impact vaccine response and protection. On March 1, 2020, the LRF convened a panel to review the latest research on epidemiology, vaccine science, and COVID-19 in lymphoma and to develop recommendations regarding SARS-CoV2 vaccination in lymphoma patients. Andrew D. Zelenetz, MD, PhD (Memorial Sloan Kettering Cancer Center), LRF Scientific Advisory Board Chair, provided an overview of that panel's discussion. The American Society of Hematology and Memorial Sloan Kettering Cancer Center recommendation document are helpful, however there remains a need to develop evidence-based patient and provider guidelines. This should include directions and support for patients to retain records and information related to their inoculation. The panel also discussed how to communicate to patients the importance of getting vaccinated while being realistic about low efficacy in this population and the hazards of relaxed distancing. In addition, tables for general practitioners to guide timing of vaccination with different treatments are needed, and these tables as well as more general guidance must be continually updated as data emerge. Finally, in order to build vaccination best practices and understand the details of vaccine response, it is critical that clinical studies be developed in an expedited, coordinated fashion, with the clear goal of generating information sufficient to predict how the lymphoma population will react to vaccination. The panel will likely be ongoing and convene at regular intervals to provide timely and evidence-based information to the lymphoma community.

The clinical and immunological impact of B-cell depletion in the context of COVID-19 is unclear. Oliver Weigert, MD (University Hospital LMU Munich) and colleagues conducted a prospectively planned analysis of COVID-19 in patients who received B-cell depleting anti-CD20 antibodies and chemotherapy for B-cell lymphomas. The control cohort consisted of age- and sex-matched patients without lymphoma who were hospitalized because of COVID-19. They performed detailed clinical analyses, in-depth cellular and molecular immune profiling, and comprehensive virological studies in 12 patients with available biospecimens.

B-cell depleted lymphoma patients had more severe and protracted clinical course (median hospitalization 88 vs 17 days). All patients actively receiving immunochemotherapy (n=5) required ICU support including long-term mechanical ventilation. Neutrophil recovery following G-CSF stimulation coincided with hyperinflammation and clinical deterioration in 4 of the 5 patients. Immune cell profiling and gene expression analysis of PBMCs revealed early activation of monocytes/macrophages, neutrophils, and the complement system in B-cell depleted lymphoma patients, with subsequent exacerbation of the inflammatory response and dysfunctional interferon signaling at the time of clinical deterioration of COVID-19. Longitudinal immune cell profiling and functional in-vitro assays showed SARS-CoV-2-specific CD8+ and CD4+ T-effector cell responses. Finally, they observed long-term detection of SARS-CoV-2 in respiratory specimens (median 84 vs 12 days) and an inability to mount lasting SARS-CoV-2 antibody responses in B-cell depleted lymphoma patients. In summary, they identified clinically relevant particularities of COVID-19 in lymphoma patients receiving B-cell depleting immunochemotherapies.

The 2021 MCL Virtual Scientific Workshop covered recent advances in our understanding of MCL biology as well as the recent technological advances that have allowed more detailed study and quantification of tumor response, relapse, and interpatient variation. Continuing efforts are needed for the ongoing development of basic research tools and translation of findings into clinical studies and patient care. Future studies will inform treatment

optimization with currently available agents as well as integration of novel agents as they become available and identify the molecular underpinnings of unmet need in refractory disease. Another emerging key area of MCL research include application of less invasive monitoring techniques for prognosis, adaptive care, or therapy cessation. With the cooperative approach to research and clinical trials, these research questions can be addressed more efficiently than before, accelerating the process of clinical development and furthering patient care.

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