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Mantle cell lymphoma: report of the 2010 Mantle Cell Lymphoma Consortium Workshop

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Abstract

Mantle cell lymphoma (MCL) is an aggressive B-cell non-Hodgkin lymphoma typically characterized by cyclin D1 overexpression as result of the t(11;14) translocation. MCL is biologically and clinically heterogeneous and frequently involves extranodal dissemination. Although MCL is incurable with current therapies, with the exception of allogeneic stem cell transplant, recent advances are improving long-term outcomes in MCL. Intensive research has continued to focus on elucidating biological mechanisms of MCL, identifying new molecular targets, and optimizing existing therapies. Most recently, researchers have begun focusing on new areas such as epigenetics and microRNAs and their potential applications to MCL therapy. Advances across a broad spectrum of MCL research were presented at a recent MCL Workshop. This report provides an overview of the scientific highlights from the meeting and a framework for future research.

Keywords

Mantle cell lymphoma; MicroRNA; Minimal residual disease (MRD); Cell cycle; Immunotherapy; Adoptive cellular therapy

Introduction

Mantle cell lymphoma (MCL) is an aggressive B-cell malignancy characterized by overexpression of cyclin D1 resulting from the t(11;14)(q13;q32) translocation. No standard

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of care has been established, and MCL is incurable with current therapies aside from allogeneic stem cell transplant. Although other intensive regimens may improve duration of remission, effects on survival are unclear. Moreover, most patients with MCL are not suitable candidates for such intensive regimens.

In 2003, the Lymphoma Research Foundation (LRF) developed a major MCL research program through the MCL Initiative and the awarding of grants specific for MCL research. Stemming from the Initiative's success, the LRF in 2005 created the Mantle Cell Lymphoma Consortium (MCLC), a working group of more than 100 laboratory and clinical investigators from North America and Europe whose research focuses on MCL. The MCLC provides a forum for sharing data, exchanging ideas, and fostering collaborations within the MCL research community.

Each year, the LRF MCLC conducts a Workshop in which lymphoma researchers from around the world are invited to report on, and discuss, their MCL research findings. The seventh MCLC Scientific Workshop convened on 24–25 March 2010 in Atlanta, Georgia. Research topics discussed included recent and ongoing clinical trials, investigations into MCL biology and pathogenesis, new targets for therapy, new vaccine and cellular therapeutic strategies, and prognostic biomarkers. This report highlights the scientific findings presented and discussed at the workshop.

Clinical trials in mantle cell lymphoma

Michael E. Williams, MD (University of Virginia) started the meeting with an overview of current priorities and challenges in MCL. He first provided a brief history of MCL research, noting the significant progress made since MCL was first recognized as a distinct clinical entity in the early 1990s [1–6]. Progress has been made in the diagnosis of MCL, in the understanding of the relevant molecular pathways and pathogenesis of the disease, and in the development of new treatment options, including chemoimmunotherapy and targeted agents. The median survival for MCL patients improved from 2.7 years in 1975–1986 to 4.8 years in 1996–2004 [7]. Survival has continued to improve as a result of several therapies that have recently become available, providing additional treatment options for patients with relapsing or resistant disease. Ongoing challenges include further elucidating the pathogenesis of MCL, developing risk-adapted therapies and robust prognostic markers, individualizing the timing and type of therapy, determining how best to combine and sequence therapies, and identifying new molecular targets for MCL therapy.

Martin Dreyling, MD (University of Munich) discussed recent and ongoing clinical trials in MCL being conducted by the European MCL Network. In 2008, Hoster and colleagues reported that the addition of rituximab to CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) improves outcomes in previously untreated MCL [8]. In 2009, Hoster and colleagues showed that both autologous stem cell transplant (ASCT) and the addition of rituximab independently prolong response duration in patients with MCL receiving CHOP [9]. Multiple strategies are being evaluated for reducing the risk of relapse. For younger patients, an ongoing trial is evaluating R-CHOP versus R-CHOP with DHAP (high-dose cytarabine, cisplatin, and dexamethasone) followed by high-dose chemotherapy and peripheral blood stem cell transplant (PBSCT). A planned trial will evaluate DHAP/R-

CHOP followed by PBSCT with or without rituximab maintenance therapy. Another will compare R-CHOP/HA followed by PBSCT with or without lenalidomide maintenance therapy. First-line strategies being evaluated or planned in older patients with MCL include R-CHOP or rituximab plus fludarabine plus cyclophosphamide (FC) followed by rituximab versus interferon, with a possible second randomization to lenalidomide maintenance versus observation. Treatments being evaluated for patients with relapsed disease include rituximab plus high-dose cytarabine with bortezomib; radioimmunotherapy; and various investigational targeted therapies. Dr. Dreyling also discussed the potential role of the MCL International Prognostic Index (MIPI) for risk stratification. In 2009, Hoster and colleagues confirmed that the MIPI independently predicts progression-free survival and overall survival in both young and elderly patients [10].

John Leonard, MD (Weill Cornell Medical College) presented an overview of clinical trials in MCL currently under way or being planned by North American cooperative groups. Until an optimal chemotherapy regimen has been identified, the focus will remain on phase II studies. For older patients who are not suitable for PBSCT, the role of bendamustine is a focus of several clinical trials. The Cancer and Leukemia Group B (CALGB), Southwest Oncology Group (SWOG), and Eastern Cooperative Oncology Group (ECOG) are planning a collaborative study of combinations of bendamustine and rituximab with bortezomib ± lenalidomide. SWOG S0601, which assessed R-CHOP plus bortezomib followed by bortezomib maintenance therapy, has completed accrual. For younger patients, trials are aiming to improve on outcomes attained with PBSCT by using aggressive induction regimens, such as rituximab plus hyper-CVAD (hyperfractionated cyclophosphamide, doxorubicin, vincristine, and dexamethasone), methotrexate, and Ara-C (SWOG 0937). CALGB trials are evaluating how best to incorporate alternative chemotherapeutic agents such as bortezomib and lenalidomide into post-transplant consolidation and maintenance regimens. Finally, correlative studies will evaluate various biomarkers in efforts to predict which patients are best suited to different therapies.

Brad S. Kahl, MD (University of Wisconsin) presented results from two trials evaluating new regimens for patients with previously untreated MCL. In 2006, Kahl and colleagues reported results of a multicenter phase II pilot study of rituximab plus a modified hyper-CVAD, followed by maintenance rituximab, in 22 patients. The regimen was associated with an overall response rate (ORR) of 77%, with 64% complete response (CR) [11]. At the MCLC Workshop, Dr. Kahl provided updated results from this study. After a median follow-up of 62 months, median progression-free survival (PFS) was 38 months and median overall survival (OS) was 70 months. Five-year PFS and OS rates were 33% and 62%, respectively. Dr. Kahl also reported results from a phase II Wisconsin Oncology Network study evaluating combination therapy with rituximab, modified hyper-CVAD, and bortezomib, followed by maintenance rituximab, in 30 patients with untreated MCL. Bortezomib and vincristine dose reductions were required due to grade 3 peripheral neuropathy developing in nine of the first 14 patients. At bortezomib 1.3 mg/m^2 and vincristine 1 mg, the incidence of painful neuropathy was reduced to one in 16 patients. Neutropenic fever or infection developed in 8% of patients. The regimen was associated with a high ORR (90%), with 77% CR. Maintenance rituximab was associated with a conversion from partial response (PR) to CR in three of four patients. Seven patients discontinued rituximab due to adverse events,

primarily infections. After a median follow-up of 34 months, 3-year PFS and OS were 60% and 85%, respectively. Dr. Kahl concluded that the role of modified hyper-CVAD is unclear, given that younger patients may benefit from more intensive regimens, and older patients need even more tolerable regimens.

Cellular therapy/stem cell transplant/vaccines

Michael C. Jensen, MD (City of Hope) discussed the feasibility of central memory T-cell immunotherapy to reduce the risk of relapse after ASCT. The strategy involves isolating T-cells prior to ASCT, using vector-mediated gene transfer to generate tumor-reactive T-cells *in vitro*, and transferring the anti-tumor lymphocytes into the patient post-ASCT. Attaining durable, high-level engraftment has presented a major challenge in immunotherapy. However, adoptive transfer of effector CD8+ T-cells derived from central memory cells has been shown to induce persistent T-cell memory in primates [12]. Conversely, effector T-cells derived from effector memory-derived T-cells cannot revert to memory T-cells and undergo apoptosis. The researchers plan to evaluate the use of central memory T-cell adoptive transfer in patients with MCL.

In another presentation focused on adoptive immunotherapy, L. Elizabeth Budde, MD, PhD (University of Washington/Fred Hutchinson Cancer Research Center) discussed a lentiviral-based gene transfer system for generating CD20-specific chimeric receptor-redirected T-cells with inducible co-expression of a caspase 9-based suicide switch. The chimeric receptor contains an anti-CD20 extracellular domain, a CD28 transmembrane domain, CD28 and CD137 costimulatory domains, and a CD3-zeta intracellular chain. Budde and colleagues showed that transduced T-cells expressed high levels of anti-CD20 chimeric T-cell receptor (TCR), that these cells had CD20-specific cytotoxicity in CD20-expressing MCL cell lines, and that the inducible caspase 9 safety switch could be activated and remove these cells effectively and specifically.

Joshua Brody, MD (Stanford University Medical Center) reviewed progress on an experimental treatment involving vaccination and immunotransplant. After induction chemotherapy, patients receive a personalized vaccine consisting of isolated MCL cells treated with CpG. The presence of CpG in combination with dying lymphoma cells induces the production of T cells with anti-tumor activity. In a phase I/II trial, the CpG-based vaccination induced anti-tumor T-cells in patients with low-grade lymphoma and induced objective clinical responses (in press, *Journal of Clinical Oncology*). Induction of T-regulatory cells inhibited anti-tumor activity. In a mouse model, Brody and colleagues showed that CpG vaccination and adoptive T-cell transfer into lymphodepleted, syngeneic recipients preferentially induced proliferation of T-effector cells over T-regulatory cells in recipients [13]. An ongoing clinical trial is evaluating this immunotransplant approach—combining vaccination, autologous transplant, and adoptive T-cell transfer—in patients with newly diagnosed MCL. The trial has had rapid accrual so far, with 10 patients enrolled within the first 8 months.

Bijal Shah, MD (H. Lee Moffitt Cancer Center) presented results from a phase II study evaluating bystander vaccine therapy in MCL. A total of 43 patients enrolled; 23 patients attained an objective clinical response to chemotherapy and received the vaccine plus

interleukin 2 (IL-2). Among seven patients who had attained a molecular and pathologic CR after chemotherapy, four maintained a CR for durations of 19–33 months. Among the 15 patients who had a PR to chemotherapy, two patients had eradication of molecular disease in the bone marrow after vaccination; both patients eventually developed disease progression, after 9 and 35 months. Median PFS from first vaccine was 9 months for patients with relapsed MCL and 13 months for patients with previously untreated MCL. Median OS from first vaccine was 25 months for relapsed patients and had not been reached for patients with previously untreated disease. The 5-year OS from diagnosis was approximately 75%. Although the vaccine did not prolong the time to progression, Dr. Shah said that according to these preliminary data, the vaccine did appear to extend survival over what would be predicted by the MIPI, even in patients with high-risk disease. A similar survival benefit has been seen in other phase II vaccine studies in prostate cancer (Prostavax) and small cell lung cancer, suggesting that vaccine therapy may direct the transition to more indololently behaving disease. As such, Dr. Shah and his team will be initiating a subsequent phase II trial testing vaccination in conjunction with immune modulating therapies with the intent of generating a more rapid and sustained immune response.

Joseph Bertino, MD (Cancer Institute of New Jersey) reviewed progress on the construction of a human immunodeficiency virus type 2 (HIV-2) lentiviral-based vector for gene therapy. The goal of gene therapy in patients with non-Hodgkin lymphoma (including MCL) would be to treat stem cells so that they carry drug resistance genes. Those treated stem cells would then be transplanted into patients during ASCT. Then, patients could receive chemotherapy after transplant to increase the likelihood of eradicating residual disease without harming transferred stem cells. The lentiviral-based system has features that may make it less likely to cause the secondary leukemias that have been associated with other gene therapy vectors. This approach has not yet been tested in humans. Bertino, Tulin Budak-Alpdogan, MD, and colleagues are currently conducting *in vitro* studies to assess requirements for cytokine stimulation and to determine levels of resistance to methotrexate and cytarabine. Mouse studies are under way to determine the degree of myeloprotection and persistence of expression in peripheral leukocytes.

Novel targets in mantle cell lymphoma

Steven H. Bernstein, MD (University of Rochester) reviewed the development of new therapies that target oxidoreduction, a process involved in multiple cellular functions. Several compounds have been shown to cause oxidative stress, causing cell death in tumors. Triterpenoids, which have demonstrated *in vitro* activity against lymphoma cells, exert direct effects on the mitochondria [14]. In 2009, Bernstein and colleagues reported that the sesquiterpene lactone parthenolide modulates cell surface protein thiols and induces cell death in diffuse large B-cell lymphoma (DLBCL) and MCL cells [15]. Parthenolide also inhibits nuclear factor κB (NF-κB) and activation of JNK. Pretreatment with thiol antioxidants blocks the cytotoxic activity of parthenolide. Dr. Bernstein also discussed how the mitochondrial Lon protease, which is up-regulated in lymphoma cells, may be a new potential target for lymphoma therapy. Bernstein and colleagues showed that the triperpenoid 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO) and its derivatives inhibit activity of both purified and cellular Lon [16]. Moreover, exposure of MCL Granta

cells to CDDO causes the formation of electron-dense inclusions in the mitochondria but not in other cellular compartments.

Shantaram S. Joshi, PhD (University of Nebraska Medical Center) presented results of animal studies investigating characteristics of therapy-resistant MCL cells. The researchers inoculated NOD-SCID (non-obese diabetic–severe combined immune deficiency) mice with the MCL cell line Granta-519 then treated the mice with CHOP and bortezomib. CHOP-resistant MCL cells were isolated from the kidneys and livers after relapse, and stable cell lines were developed. Compared with the originally transplanted MCL cells, the CHOP-resistant cells had enhanced proliferation and were more aggressive when transferred into NOD-SCID mice. Microarray and polymerase chain reaction (PCR) analysis showed up-regulation of pathways known to be involved in lymphoma progression, including phosphatidylinositol 3-kinase (PI3K)/Akt/survivin, Aurora kinase A, and sonic hedgehog (Shh) signaling. The HIV drug ritonavir has previously been shown to inhibit survivin and the NF-κB pathway and induce apoptosis in T-cell leukemias and lymphoblastoid B-cells [17]. Dr. Joshi and his colleagues therefore undertook *in vitro* studies evaluating the effects of ritonavir in MCL cells. Ritonavir appeared to reduce proliferation in MCL and treatment-resistant MCL cell lines and induced apoptosis in a dose-dependent manner. Ritonavir was also associated with reduced mRNA expression of PI3K/Akt/survivin pathway targets. Thus, these studies demonstrate potential therapeutic effects of ritonavir on MCL.

Daniel Medina, PhD (Cancer Institute of New Jersey) presented results from studies related to the oncoprotein Bmi1, which has demonstrated significant prognostic value in multiple cancer types [18–21]. Medina reported that Bmi protein expression was elevated in primary MCL cells and MCL cells lines versus normal peripheral blood monocytes (PBMCs). Moreover, mRNA levels were elevated in MCL cell lines versus PBMCs. A lentiviral shRNA-mediated knockdown of Bmi1 inhibited growth of myeloma cell lines. Ongoing experiments in conjunction with a pharmaceutical company are evaluating different small-molecule Bmi1 inhibitors as potential therapeutic agents for hematologic malignancies and other cancers characterized by high levels of Bmi1.

Shigeki Miyamoto, PhD (University of Wisconsin) discussed atypical NF-κB activity in MCL. Alterations in the NF-κB pathway have been observed in other cancers, including other non-Hodgkin lymphomas, myeloma, and breast cancer [22–25]. Miyamoto and colleagues demonstrated constitutive NF-κB activity in MCL cells lines that was resistant to bortezomib. Perillyl alcohol blocked this constitutive NF-κB activity, inducing cell death. MCL samples isolated from patients also showed constitutive NF-κB activity that was frequently bortezomib-resistant. The investigators are evaluating novel small-molecule compounds that selectively block this atypical NF-κB activity. Finally, Dr. Miyamoto described the development of a microchannel platform that enables high-throughput analysis of signaling mechanisms and cellular responses in primary MCL cells, to optimize the use of these limited samples.

Patricia Pérez-Galán, PhD (National Heart, Lung and Blood Institute) discussed molecular characteristics associated with bortezomib resistance in MCL. The investigators developed bortezomib-resistant MCL cell lines by exposing MCL cell lines to increasing

concentrations of bortezomib over a 1-year period. Acquisition of bortezomib resistance was a gradual and reversible process. Bortezomib-resistant cells had increased proteasome activity and a reduced dependence on proteasome activity for survival. In microarray analysis, bortezomib-resistant cell lines had high expression of plasma cell signatures (including high levels of IRF4, BLIMP1, CD38, and SDC1) and reduced expression of the mature B-cell signature. Flow cytometric analysis confirmed expression of plasma cell surface markers (CD38, CD138) and reduced expression of the B-cell markers CD19, CD24, CD52, and human leukocyte antigen (HLA)-DR. Reversion of bortezomib resistance was associated with loss of this plasmacytic phenotype. Moreover, the intrinsically bortezomib-resistant MCL cell lines MINO and REC showed similar plasmacytic features. In patient samples, Pérez-Galán showed that primary bortezomib-resistant MCL cells also showed a significant increase in CD38 and IRF4 expression compared with cells isolated from bortezomib-sensitive patients. She suggested that this plasmacytic phenotype in the absence of immunoglobulin production as found in multiple myeloma cells may confer resistance to bortezomib. Finally, she reported preliminary investigations into IRF4 and CD38 as potential markers for predicting responses to bortezomib in MCL. In order to validate these markers of resistance, the future project directions included expanding the analysis to larger cohorts of patients with MCL enrolled in bortezomib clinical trials.

Anas Younes, MD (M. D. Anderson Cancer Center) discussed transforming growth factor-β (TGF-β) activated kinase 1 (TAK-1) as a potential target for MCL therapy. TAK-1 overexpression was detected in a variety of lymphoma cell lines, including Hodgkin disease, MCL, and non-Hodgkin lymphoma (NHL) lines. The investigational TAK-1 inhibitor AZ4248 induced apoptosis via the mitochondrial pathway, causing cell death in MCL cell lines and in primary MCL cells. The compound appeared to inhibit multiple cell survival pathways, including p38, Akt, and ERK. Ongoing studies using siRNA are investigating on-target and off-target effects of AZ4248.

Robert Baiocchi, MD, PhD (The Ohio State University) discussed protein arginine methyltransferase (PRMT) enzymes as targets in MCL. PRMT enzymes are major regulators of protein function, transcriptional control, RNA metabolism, and DNA repair. PRMT5 associates with repressive chromatin remodeling complexes (SWI/SNF; switch/sucrose non-fermentable), working in concert with histone deacetylation and DNA methylation to silence key regulatory genes [26]. PRMT5 is overexpressed in MCL and other types of NHL [27]. Moreover, PRMT5 overexpression is associated with more aggressive malignancies, and is a negative prognostic factor in hematologic malignancies and in solid tumors [28]. *In vitro* experiments using PRMT5 knockdowns with siRNA suggest that PRMT5 could be a therapeutic target. Baiocchi and his colleagues, in collaboration with the Molecular Targeted Therapeutics Group at The Ohio State University, have designed an *in silico* model of the human PRMT5 catalytic site to allow high-throughput screening of potential PRMT5 inhibitors. The researchers identified and synthesized a compound that showed selective PRMT5 inhibition, antiproliferative activity, and induction of cell death similar to siRNA controls. Second-generation and third-generation inhibitors showed more potent activity and demonstrated synergy with histone deacetylase (HDAC) inhibition in MCL cells.

Ari Melnick, MD (Weill Cornell Medical College), on behalf of Samir Parekh, MD (Albert Einstein College of Medicine), discussed progress in the development of epigenetic therapy in MCL. Using genome-wide promoter methylation analysis, Parekh and colleagues investigated differential gene methylation in samples from 22 patients with MCL versus 10 naive B-cell samples. On a global scale, significant hypomethylation was observed in the MCL cells versus normal B-cells. Overall, 4110 genes were differentially methylated. The investigators found correlations with gene expression data for 1413 genes, and selected eight genes relevant to MCL or cancer biology for further study. These included four hypermethylated genes (PCDH8, MLF1, HOXD8, and CDKN2B) and four hypomethylated genes (CD37, HDAC1, NOTCH1, and CDK5). Gene and protein expression was significantly higher in the hypomethylated genes compared with the hypermethylated genes. Treating MINO cells with low-dose decitabine caused demethylation of aberrantly hypermethylated genes. Moreover, the histone acetylation inhibitor suberoylanilide hydroxamic acid (SAHA) enhanced decitabine-induced induction of tumor suppressor genes and cytotoxicity in MCL cells. These data suggest the feasibility of targeting aberrantly methylated genes for therapeutic benefit in MCL.

Mantle cell lymphoma pathogenesis

Carlo Croce, MD (The Ohio State University) delivered the meeting's keynote address, discussing the emerging role of microRNAs in cancer development. In 2002, Croce and colleagues first reported the association between microRNAs and human cancer, showing that miR15 and miR16 were deleted or down-regulated in approximately 68% of cases of chronic lymphocytic leukemia (CLL) [29]. Subsequent studies have shown that microRNAs are involved in the development of many different cancer types, including hematologic malignancies and solid tumors. In fact, levels of 21 microRNAs are commonly elevated in six solid cancers (breast, colon, lung, pancreas, prostate, and stomach cancer). MicroRNAs exert effects on many downstream proteins, acting as suppressor genes or as oncogenes depending on the cellular context. In 2005, Croce and colleagues reported a microRNA signature associated with prognosis and progression in CLL [30]. Subsequent studies have shown that micro-RNAs also predict outcomes in other tumor types, including lung cancer, gastric cancer, and liver cancer. In a murine model, reintroduction of microRNAs in tumors characterized by loss of microRNA has demonstrated significant antitumor activity. Dr. Croce suggested that microRNAs may not only be potential targets for therapy, but could possibly be used as anticancer agents themselves.

Elliot Epner, MD, PhD (Penn State Hershey Cancer Institute) discussed his research into the epigenetics of MCL and the rationale for epigeneticstargeting therapy. The purine analogs cladribine and fludarabine have been shown to inhibit DNA methyltransferase *in vitro* [31]. In patients with CLL, cladribine treatment can induce global demethylation and up-regulation of microRNA 195, a microRNA that has demonstrated tumor suppressor characteristics [32]. In patients with MCL, cladribine treatment was associated with global hypomethylation [33] Moreover, cladribine inhibited histone methylation both *in vitro* and *in vivo* [34] Several clinical trials have investigated combination therapy with cladribine and rituximab. In a 2006 phase II trial of 29 patients (median age 70 years), Inwards and colleagues reported a 52% CR rate [35]. Given that HDAC inhibition may have an additive

effect when used with DNA hypomethylation, several trials are evaluating cladribine with SAHA (vorinostat) and rituximab in hematologic malignancies.

Kai Fu, MD, PhD (University of Nebraska Medical Center) discussed the functional role of microRNA (miR)- 17~92 in MCL. Although the t(11;14)(q13;q32) translocation is a hallmark of MCL, secondary genetic changes are also needed for malignant transformation. One common alteration that has been detected in MCL lines and in primary tumor samples involves the amplification of 13q31–32 [36]. The *c13orf25*, located at 13q31–32, is the target locus; within *c13orf25* lies the miR-17~92 cluster [37]. Expression of miR-1~92 is induced by MYC; overexpression accelerates the development of MYC-induced lymphoma in mice [38,39]. However, miR-17~92 is essential to B-cell development [40,41]. Mice with miR-17~92 overexpression develop a lymphoproliferative disorder and autoimmunity and have shortened survival [42]. In patients with MCL, *c13orf25* overexpression is a poor prognostic factor. *In vitro* studies show that overexpression of miR-17~92 down-modulates PTEN and PHLPP2, thereby activating the PI3K/AKT pathway and protecting MCL cells from apoptosis. Over-expression of miR-17~92 also down-modulates the proapoptotic protein BIM, which further inhibits apoptosis. Knockdown of miR-17~92 inhibits xenograft MCL tumor growth *in vivo*.

Randy D. Gascoyne, MD (BC Cancer Agency) reviewed results of a retrospective study evaluating the role of the microenvironment in MCL. Flow cytometry was used to characterize the non-malignant lymphoid cells present in biopsy samples from 122 patients with MCL who received treatment at the BC Cancer Agency between 1989 and 2007 [43]. The study excluded patients who were too frail to receive chemotherapy or who had flow cytometry performed only on the bone marrow or peripheral blood. Treatment was variable over the nearly 20-year period, with 41% of patients receiving rituximab. The median survival of the patients was 2.2 years; 21% were alive at 5 years. The investigators reported that the frequency of CD8+ T-cells in the biopsy sample was significantly predictive of survival in a univariate analysis. Among rituximab-treated patients, the frequency of CD8+ T-cells in the biopsy was independently predictive of outcome, with a survival advantage among those with <8% CD8+ T-cells in the biopsy (p=0.009). Gene expression profiling in 20 cases stratified by CD8+ cell count identified a T-helper cell type 1 (Th1) T-cell/M1 macrophage signature in those with elevated CD8+ cells. Immunohistochemistry analyses of tissue microarrays (n=185) of MCL samples also showed a significant association between overall survival and macrophage frequency, as assessed by CD68 and CD163 [44]. MCL-associated macro-phages had prognostic value independent of MIPI, proliferation, and p53 expression. Dr. Gascoyne noted that the prognostic effect of the microenvironment may be altered by the addition of rituximab.

Elena M. Hartmann, MD, PhD (Institute of Pathology, University of Wuerzburg, Germany) presented a genome-wide characterization of chromosomal copy number and gene expression alterations in MCL. Analyses were performed on nine cell lines and 77 primary cases. Copy number alterations (CNAs) were common in primary MCL, with an average of 15 CNAs per sample, determined by visual inspection. An integrated analysis of single nucleotide polymorphism (SNP) and gene expression array data (using an algorithm modified from [45]) was used to identify the most relevant CNAs. Deletions of the *ATM*

(ataxia telangiectasia mutated) region were detected in approximately 50% of cases, and correlated significantly with the tumor genomic complexity. Moreover, the integrated analysis revealed multiple CNAs that were significantly associated with survival. Large regions of copy number-neutral loss of heterozygosity (CNN-LOH) were detected in approximately 35% of cases. The investigators noted a high similarity between the 72 cyclin D1-positive cases and the five cyclin D1-negative cases. Potential target genes of CNAs identified in the integrated analysis of SNP and gene expression array data are being evaluated in ongoing studies.

Timothy Greiner, MD (University of Nebraska Medical Center) discussed methylation analysis in primary samples analyzed from 68 patients with MCL. The researchers found that global hypomethylation occurred in MCL cells affecting about 3000 genes. Hypomethylation was associated with increased expression of several genes involved in regulation of cell proliferation or cell cycle control, reflected by an increased proliferation signature [46]. A comparison of methylation and gene expression patterns in five patients with good survival and five patients with poor survival found a correlation between greater hypomethylation in the genes of the proliferation signature and poor survival.

Prognostic markers and minimal residual disease

Elias Campo, MD (University of Barcelona) presented progress his group has made in understanding the biology of indolent MCL. Some cases of MCL show an indolent clinical course, characterized by a stable disease and an absence of generalized lymphadenopathy. An important question has been whether these indolent cases are truly MCL or another subtype, and whether they can be identified prospectively. Campo and colleagues conducted genomic and gene expression profiling of 12 patients with indolent MCL who were followed for a median of 6.4 years without being treated with chemotherapy and 15 patients with conventional MCL who were followed for a median of 15 months [47]. There was a significant difference in immunoglobulin gene mutational status between the two groups ($p < 0.04$), with indolent MCL showing primarily hypermutated immunoglobulin genes (70% vs. 20% of conventional MCL samples). Indolent and conventional MCL shared a gene expression profile that differed from other leukemias. However, 13 genes were highly expressed in conventional MCL but not in indolent MCL. Among these was SOX11, which was further evaluated in protein studies. In an analysis of 112 patients with MCL, survival was significantly superior in the 15 patients with SOX11-negative tumors than in the 97 patients with SOX11-positive tumors (5-year OS 78% vs. 36%; p=0.001). Lack of SOX11 expression could help identify patients with indolent MCL who may not require immediate chemotherapy.

Dr. Campo also reviewed studies investigating the potential of SOX11 expression as a biomarker for cyclin D1-negative MCL [48]. The investigators analyzed a microarray database of 238 mature B-cell neoplasms, including cyclin D1-positive MCL (n=76), cyclin D1-negative MCL (n=12), and other lymphoid malignancies (n=209). SOX11 mRNA was highly expressed in both cyclin D1-positive and cyclin D1-negative MCL, and was detected in 33% of cases of Burkitt lymphoma, but was not present in any mature lymphoid neoplasms. SOX11 nuclear protein was detected in 93% of conventional cases of MCL,

100% of cyclin D1-negative cases, six lymphoblastic lymphomas, two of eight Burkitt lymphomas, and two of three T-prolymphocytic leukemias. Clinical characteristics and outcomes were similar in patients with cyclin D1-negative MCL and conventional MCL. These findings suggest that SOX11 expression specifically identifies MCL, including cyclin D1-negative MCL.

Thomas M. Habermann, MD (Mayo Clinic) discussed the association between genetic variations in MCL cells and overall survival in 98 patients with MCL. The median age of patients with genotype data was 64 years; 38% had a low-risk simplified MIPI score, 36% were intermediate-risk, and 26% were high-risk. The median follow-up of living patients was 47 months (range, 23–85 months). The most common initial treatment was anthracycline-based chemotherapy (55%) in combination with rituximab followed by autologous stem cell transplant, nucleoside analog-based chemotherapy (20%), and other treatments. The researchers used a high-throughput Oligo Pool Assay to identify candidate SNPs for subsequent analysis that included 1536 SNPs, focusing on variations in cell cycle and NF-κB pathways. Of 84 SNPs from the NF-κB pathway that were evaluated, one SNP in *TRAF5* was replicated and was significantly associated with risk of recurrence.

In another line of investigations, Dr. Habermann and colleagues have been evaluating the role of clinical and lifestyle factors in outcomes in patients with MCL and other NHLs. Parameters being assessed include pre-diagnosis smoking, obesity, and physical activity. In a recent publication, Habermann and associates reported that in 1286 patients with NHL, factors associated with poorer overall survival included former and current smoking, drinking more than 43.1 g/day of alcohol, and obesity [49]. Lifestyle data have been collected from 65 patients with MCL and adjusted for MIPI, treatment, sex, and education. Preliminary data suggest similar trends that were observed in the overall NHL cohort, with links between clinical outcomes and body mass index (BMI) in this small series of patients.

Pedro Jares, PhD (IDIBAPS; University of Barcelona) presented studies investigating associations between methylation of genes associated with clinicopathological features and prognosis in MCL. Following an initial large-scale screening of potentially methylated genes, the investigators evaluated methylation levels in 25 genes in seven MCL cell lines. Overall, 20 of the 25 genes were highly methylated (>65%) in at least one MCL cell line. Jares and colleagues then focused on eight of those 20 genes, testing methylation levels in 48 DNA samples from patients with MCL and normal B-lymphocytes. Seven of those genes were found methylated in primary MCL, and the methylation status and/or gene expression levels of five of them correlated with multiple clinicopathologic tumor characteristics, including cell proliferation, aneuploidies, cytological variants and survival. Gene methylation tended to occur concordantly, and a significant association was observed between survival and number of key genes methylated (0 vs. 1–3 genes vs. 43 genes).

On behalf of Christiane Pott, MD (University Hospital of Schleswig-Holstein), Dr. Martin Dreyling presented results of studies attempting to improve on the detection of minimal residual disease (MRD). Dr. Pott and her colleagues have evaluated the presence of MRD in patients with MCL who have received induction chemotherapy followed by high-dose chemoradiotherapy and ASCT or maintenance treatment [50]. Of 190 patients who received

induction therapy, 56% (106 patients) had molecular remission, while 45% (84 patients) had detectable MRD. There was a significant association between residual disease in the blood or the bone marrow and a shorter duration of remission. In fact, MRD status appeared to be a better predictor of remission duration than clinical response. Significant independent prognostic factors included MIPI score (hazard ratio, 3.2; 95% confidence interval [CI], 1.8–5.6; $p < 0.0001$) and molecular response (hazard ratio, 0.4; 95% CI, 0.1–0.9; $p=0.0279$) but not CR. MRD also predicted remission duration in patients receiving ASCT or maintenance treatment. The researchers are working to develop a six-color flow cytometric assay for MRD detection.

Summary

The 2010 MCLC Workshop demonstrated the continued progress in improving the understanding of MCL pathogenesis, identifying potential therapeutic targets, and developing novel therapeutic strategies. Newer areas such as epigenetics and microRNAs are gaining increasing attention as their role in MCL biology is better understood. Together, these scientific advances may lead to new therapeutic strategies and greater individualization of therapy that could ultimately benefit patients with MCL as well as other lymphomas and cancer types.

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