

SIXTH INTERNATIONAL WORKSHOP ON WALDENSTRÖM'S MACROGLOBULINEMIA

SUMMARY OF ABSTRACTS AND PRESENTATIONS

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Dr. Sherwood's report is written for the lay reader with an interest in the most recent research directed toward understanding and treating the disease Waldenström's macroglobulinemia.

The Sixth International Workshop on Waldenström's Macroglobulinemia (IWWM-6) was held October 6-10 in Venice, Italy. This premier scientific conference for WM was attended by close to 200 individuals from all over the world. The 3-day workshop consisted of 15 lecture sessions and a total of 80 presentations from over 90 speakers, including 14 young investigators, 5 special guest presentations, 5 debates, and 2 consensus panel discussions.

The IWWM-6 workshop sessions were very well organized but extremely busy with rapid-fire exchange of the latest information on the pathogenesis, genetics, immunology, and molecular biology of WM, as well as the clinical features, treatments, and future directions in the treatment of WM. I have attempted here to briefly summarize the highlights from the Workshop and to give personal observations regarding presentations that struck me as particularly illuminating

More information at: www.wmsummit.org/wmwkshop/Venice-2010/Overview.htm

Workshop abstracts at: www.wmsummit.org/wmwkshop/Venice-2010/Abstracts.htm

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PART 1: BASIC BIOLOGY OF WM

Session I: Pathological Challenges in WM Diagnosis

- Dr. Harris (USA): **Pathological Features of LPL**
- Dr. Ocio (SPAIN): **Immunophenotypic Differences Between IgM MGUS and WM**
- Dr. Morice (USA): **Novel Immunophenotypic Features of WM**
- Dr. De Tute (UK): **Development of WM and SMZL**
- Dr. Kyrtsonis (Greece): **CD138 possibly distinguishes IgM MM from WM**
- Dr. Mikhael (USA): **Differentiating IgM MM from WM**
- All Session I Speakers: **Panel Discussion**

The first session focused on the common challenges faced in the pathological diagnosis of WM. WM is defined as a lymphoplasmacytic lymphoma (LPL) with bone marrow involvement and an IgM monoclonal gammopathy of any concentration. The characteristic cells found in tissues infiltrated by WM cells are small lymphocytes, plasma cells, and plasmacytoid lymphocytes, plus an increased amount of mast cells. Typically the ratio of B-cells to plasma cells in the bone marrow of a WM patient is 9:1. Establishing bone marrow involvement is fundamental in diagnosing WM.

There are several diseases similar to WM, including multiple myeloma (MM), IgM multiple myeloma (IgM MM), IgM monoclonal gammopathy of undetermined significance (IgM MGUS), and splenic marginal zone lymphoma (SMZL). The ability to further differentiate between these diseases and WM relies not only on clinical features but also on immunophenotypic differences, differences established by immunophenotyping, the technique used to identify cells based on the identification of proteins on their surface. When distinguishing WM from MM, the examination of the morphology (form and structure) of the plasma cell is the preferred technique. In WM, the more normal the plasma cells appear to be in the plasma cell component of the tumor mass, the better the prognosis. Distinguishing between IgM MM and WM is critical as management is significantly different for initial therapy (selection for autologous stem cell transplant and choice of long-term maintenance). IgM MM patients have a much shorter survival span than WM patients. The distinction between IgM MGUS and WM is based on two main features: the presence of bone marrow infiltration by lymphoplasmacytic lymphoma and signs or symptoms attributable to the disease. When making the difficult distinction between WM and its close relative splenic marginal zone lymphoma (SMZL), one notes that: SMZL has much more abdominal adenopathy and splenomegaly; 27% of SMZL patients are positive for the hepatitis C virus (versus 9% in WM); mast cells are relatively unimportant in SMZL; and, finally, WM presents with increased CD138 expression when compared to SMZL. It is therefore reasonable to suggest that the development of a specific WM immunophenotypic profile will improve diagnosis and permit more accurate identification of complete remissions.

Session II: Genetic Predisposition to WM

- Dr. Kyle (USA): **IgM MGUS and Smoldering WM**
- Dr. Morra (ITALY): **Factors Predicting Evolution in IgM MGUS and IGM-Related Disorders**
- Dr. Lynch (USA): **Familial Predisposition in Plasma Cell Disorders**
- Dr. Ogmundsdottir (ICELAND): **Familial paraproteinemias**
- Dr. Landgren (USA): **Risk Factors for LPL/WM**
- Dr. McMaster (USA): **Environmental Exposures in Familial WM**
- Dr. Hunter (USA): **GWAS Studies in WM**
- Dr. Varettoni (ITALY): **Associated Cancers in WM**
- All Session II Speakers: **Panel Discussion**

The second session highlighted genetic predispositions to WM. **Dr. Robert Kyle** presented results from a long-term follow-up study of patients with IgM monoclonal gammopathy of undetermined significance (IgM MGUS). Approximately 14% of the patients developed non-Hodgkin's lymphoma (NHL); of these, 3% developed WM. The probability of progression to NHL, WM included, was approximately 1.5% per year. Smoldering Waldenström's macroglobulinemia (SWM) is defined as a serum IgM ≥ 3 g/dL and/or $\geq 10\%$ bone marrow infiltration but with no evidence of end-organ damage and symptoms that can be attributed to the disease. According to another study of Mayo Clinic patients with SWM, 71% had progressed to WM within a median of 4.6 years. The serum IgM level, hemoglobin value, and bone marrow infiltration were noted risk factors for progression.

A study of Italian patients with asymptomatic IgM MGUS revealed that approximately 10% progressed to WM after a median of 75 months. Of interest is the subset of IgM-related disorders (IgM-RDs) which are defined as IgM monoclonal gammopathies characterized by the specific properties of the IgM in question—cryoglobulinemia and activities characterized as anti-red blood cell, anti-platelet, or anti-nerve — without any evidence of lymphoma. IgM-RDs are thus similar to IgM MGUS because in both an underlying lymphoma is absent and both have a similar probability of transformation into a malignant disease such as WM. The probability of progression to a malignant lymphoproliferative disorder at 5 years was 15%. One can therefore state that although IgM-RDs frequently require treatment in view of their IgM related symptoms, the risk for malignant transformation is similar to IgM MGUS.

The familial (hereditary) predisposition in plasma cell disorders such as WM and MM is felt to be due in large part to genetic factors. Nonetheless, a chronic autoimmune response has been identified in MM as well as in WM. The existence of environmental risk factors for WM appears to imply chronic immune stimulation as well. In fact, studies examining familial MM and WM suggest common genetic and environmental factors in the etiology of both MM and WM. These factors, in turn, merit future intensive genetic and environmental investigation. In summary, increased familial risks of developing WM, NHL, CLL (chronic lymphocytic leukemia), and MGUS, as well as a personal history of certain autoimmune diseases (for example, Sjögren syndrome and autoimmune hemolytic anemia) and infectious conditions (pneumonia, septicemia, pyelonephritis, sinusitis, herpes zoster, and influenza) were strongly associated with increased risk of WM. Furthermore, familial WM patients were also more likely to report exposure to farming, pesticides, wood dust, and organic solvents compared to unaffected family members. Further evaluation of individuals who have a disproportionate number of family members with plasma cell disorders similar to WM has linked abnormalities in the biology of the B-cell to the development of disorders affecting IgG, IgA, and IgM. A prevalence of B-cell disorders is seen in up to 20% of patients with WM.

Finally, an increased incidence of second cancers has been reported in WM patients: 22 % of WM patients in a population study developed second cancers. WM patients were at increased risk for diffuse large B cell lymphoma (DLBCL), myelodysplastic syndrome or acute myeloid leukemia (MDS/AML), brain cancer, and prostate cancer. The age, sex, or clinical and hematologic features of WM patients at presentation did not influence the risk of developing a second cancer.

Session III: Genomic and Epigenomic Alterations in WM

- Dr. Braggio (USA): **High Resolution aCGH Analysis in WM**
- Dr. Poulain (FRANCE): **Genomic/SNP Studies in WM**
- Dr. Nguyen-Khac (FRANCE): **Cytogenetic Abnormalities in WM**
- Dr. Sahota (UK): **Probing B-Cell Receptor in WM**

- Dr. Dunn-Walters (UK): **Immunoglobulin repertoire Analysis of B-Cell Populations: Clues to the Origins of WM**
- Dr. Zhou (USA): **Ets-Family Dysregulation in WM**
- All Session III Speakers: **Panel Discussion**

The third session dealt with the very complex topic of genetic and epigenetic abnormalities in WM. Genetic abnormalities refer to changes in DNA sequences; epigenetic abnormalities refer to changes in appearance or gene expression caused by mechanisms other than changes in DNA sequences. Using very sophisticated and cutting-edge technology researchers are able to identify genetic aberrations, both those shared with other low-grade B-cell lymphomas and others that are distinct in WM. As an example, the genetic factors associated with the NF- κ B pathway (a protein complex that controls the transcription of genes involved in cellular responses to stimuli such as stress and regulating the immune response to infection) were observed in around 70% of WM patients, but only in 20-30% of other common NHL disease types. Cytogenetic abnormalities in WM differ from those commonly reported in other B-cell cancers and confirm the originality of this disease (the 6q deletion is the most frequent reported cytogenetic abnormality in WM).

In addition to genetic abnormalities, epigenetic mechanisms that contribute to the inactivation of tumor suppressor genes by mutations have also been noted. It seems evident that there is progressive genetic instability in WM patients. WM tumor cells have variable rates of differentiation resulting in failure to fully undergo plasma cell differentiation. Analysis of genes involved in B-cell differentiation reveals the presence of factors that repress plasma cell differentiation while promoting tumor cell survival. Adding to the biological complexity of WM is the significance of the B-cell receptor in WM. The B-cell receptor (BCR) is a protein located on the outer surface of B-cells that binds with a specific antigen and causes the cell to proliferate and differentiate into a population of antibody (such as IgM) secreting cells. Recent studies from England have revealed that the WM tumor cells have an active and functional BCR, which can in turn be a potential therapeutic target with the newer targeted drug therapies.

Completing this very complex series of lectures was a special talk by **Dr. Steve Bogen**, Medical Director, Clinical Chemistry Laboratory, Tufts Medical Center, Tufts University. We are now well aware that chronic antigenic stimulation (and genetics) contributes to the development of WM; what is of particular interest is the role of the monoclonal IgM in WM. Is this IgM production in response to an infection or just a simple error in genetics and cellular machinery? Research is now being conducted in the identification of the target of the WM IgM. Although for some the target of the IgM is the nerve coating leading to painful peripheral neuropathy, or perhaps the red blood cell leading to anemia, very early studies suggest that WM patients may actually share a common target (or targets) for the WM IgM such as an infectious agent. In fact, published experimental data has suggested that gammopathies (such as MM) may be associated with chronic exposure to an inflammatory and infectious stimulus such as the herpes virus.

Session IV: Immunological Dysregulation in WM

- Dr. Joshua (AUSTRALIA): **Clonal Expansion of CTLs in WM**
- Dr. Gorochov (FRANCE): **Impaired Regulatory T-Cell Function in WM**
- Dr. Munshi (USA): **Role of TH17 Pathway in WM**
- Dr. Jiang (USA): **Monocytic Dysregulation in WM**
- Dr. Grass (GERMANY): **Hyperphosphorylated Paratag-7 is a Frequent Target in WM**
- Dr. Rawstron (UK): **Humoral Immune Suppression in Indolent B-Cell Malignancies**
- All Session IV Speakers: **Panel Discussion**

The fourth session focused on immunological abnormalities in WM. One of the most interesting

developments in immunology has been the study of T-cells in B-cell cancers. The regulatory T-cell (Treg, sometimes known as suppressor T-cell) is a specialized subpopulation of T-cells that acts to suppress activation of the immune system. Treg function was found to be frequently impaired in WM; this finding supports the contention that immune regulation defects may be responsible for the transition from MGUS to WM or MM. Furthermore, a newly identified CD4 cell population, the T_H17 cells, important in the development of anti-tumor immunity and auto-immunity, were decreased in numbers in WM. The associated pro-inflammatory cytokines (molecular messengers) are elevated, once again supporting the role of immune dysfunction in WM.

Other cellular elements of the immune system in WM patients such as peripheral monocytes (a type of white blood cell that can elicit an immune response) demonstrate a distinct genetic profile that is characterized by abnormalities in up genes affecting immunity, inflammation, and apoptosis (cell death). The antigenic targets of the IgM paraprotein in MGUS, MM and WM may play a role in these diseases. A recently identified protein of unknown function (paratarg-7) was identified as the antigenic target of a large proportion of familial WM patients. This protein and its associated gene that is dominantly inherited may indeed induce auto-immunity and contribute to the development of familial WM.

One of the more striking lectures of the entire workshop for me was the presentation by **Dr. Andy Rawstron**, St. James Institute of Oncology, Leeds, UK, on the progressive humoral immune suppression in indolent B-cell malignancies. We know very well that recurrent infections are a major issue for WM patients. Simply put, it appears that in early B-cell malignancies, including WM, it is possible to detect normal B-cells in the majority of cases at presentation but subsequently there is a progressive depletion of normal B-cells over time. The depletion is independent of whether the B-cell disorder is stable or progressive – irrespective of IgM level. The depletion of immunoglobulins IgA and IgG (the condition called hypogammaglobulinemia) is also a relatively late event occurring approximately 2-3 years after normal peripheral B-cells are depleted. Measuring the depletion of normal peripheral B-cells may provide a better indicator for prognosis in patients with B-cell malignancies.

Session V: Molecular Pathways in Growth and Survival of WM

- Dr. Ansell (USA): **Jak Stat Pathway in WM**
- Dr. Terpos (GREECE): **Angiogenic Support in WM**
- Dr. Roccaro (ITALY): **MicroRNAs in WM Pathogenesis**
- Dr. Cao (USA): **Aberrant MicroRNA Regulation of IRS-PI3K Pathway in WM**
- Dr. Ken Anderson (USA): **Special Lecture – Role of Plasmacytoid Dendritic Cells in Plasma Cell Growth and Survival**
- All Session V Speakers: **Panel Discussion**

The final session relating to the basic biology of WM focused on the complex and sometimes bewildering topic of the molecular pathways involved in the growth and survival of WM. Appropriately **Dr. Stephen Ansell** from the Mayo Clinic, Rochester, Minnesota, led off the session with his lecture on the important cytokine IL-6 and its associated regulatory pathways. IL-6 significantly stimulates IgM production by WM cells. Inhibition of the regulatory pathway associated with IL-6 production may provide a valuable target in future therapies for WM. The production of new blood vessels (angiogenesis) also represents an important step in the progression of WM. Serum levels of MIP-1 α , a potent chemical attractant for macrophages and mast cells, which in turn contribute to increased angiogenesis, are elevated in WM. WM cells have been found to produce MIP-1 α and may therefore present important implications for the treatment of WM.

Future therapeutic targets were also identified in this session. MicroRNAs are short non-coding forms of RNA that regulate gene expression and are in turn key regulators of WM progression. Studies have shown that the aberrant expression of regulatory miRNAs provides support for the development of targeted drug therapy in WM.

The session closed with a special lecture from **Dr. Kenneth Anderson** from the Dana-Farber Cancer Institute, Harvard University. Dr. Anderson is a world recognized expert in MM and has a keen interest in WM as well. His special guest lecture highlighted the recent advances in the biology of MM research and its potential applications to WM. Dr. Anderson discussed the peculiar and very important biology of the bone marrow microenvironment and the interaction of cancer cells (principally MM cells) and normal cells in this microenvironment. Elegant studies have demonstrated a key role for plasmacytoid dendritic cells (pDCs: specialized white blood cells that initiate a primary immune response by activating lymphocytes and secreting cytokines) in the growth, migration, and survival of MM cells. Dr. Anderson suggests that researchers may wish to evaluate the role of pDCs in WM.

This final lecture concluded the sessions on the basic biology of WM. The research in the basic biology of WM is expanding at an ever-increasing rate and represents the best hope for future targeted therapies in the treatment of WM. Although these topics are incredibly complex, and while many unknowns remain, researchers are making continued inroads into the understanding of this very challenging disease.

PART 2: RECENT ADVANCES IN CLINICAL AND THERAPEUTIC ASPECTS OF WM

In this part I will focus on Sessions VI – XV and describe the recent advances in the clinical and therapeutic aspects of WM, including some personal observations regarding the evolution of research and treatments, as well as possible future directions.

Session VI: Prognostic and Predictive Factors in WM

- Dr. Morel (FRANCE): **Can WM ISS Be Used to Make Treatment Decisions?**
- Dr. Thomas (USA): **ISSMM and ISSWM as Prognosticators in WM**
- Dr. Kastiris (GREECE): **Prognostication of the High Risk WM Patient**
- Dr. Kristinsson (SWEDEN): **Survival in Patients with LPL/WM**
- Dr. Treon (USA): **Fcy Polymorphisms and Depth of Response as Predictors of Outcome to Rituximab Based Therapy in WM**
- All Session VI Speakers: **Panel Discussion**

Prognostic and predictive factors in WM were the subject of this session. **Dr. Pierre Morel**, Centre Hospitalier de Lens, Lens, France, is a leader in the development of an international staging system for WM (ISSWM). Using 5 characteristics (age > 65 years, hemoglobin <11.5 g/dL, platelet count <100 x10⁹/L, β2-microglobulin >3 mg/L, and M-protein >7.0 g/dL) the ISSWM was designed for predicting overall survival after initiation of first-line therapy. In patients with advanced WM, the prognostic significance of previous treatment(s), the quality or the duration of previous response(s), and quality of life (QOL) issues—which are difficult to assess—should also be evaluated. For this reason, the ISSWM should be limited to the design of prospective first-line trials.

Dr. Sheeba K. Thomas, M.D. Anderson Cancer Center, Houston, Texas, compared the multiple myeloma staging (ISSMM) system to the aforementioned ISSWM. She noted that the much simpler ISSMM using only albumin and β2-microglobulin levels and stages 1 to 3, reveals interesting results: “stage 1” WM patients had excellent overall survival, and “stage 2” WM patients had overall survival of 116 months! Using the much more comprehensive ISSWM, high-

risk patients still had greater than 5 year overall survival. The author did conclude that the ISSWM was the preferred tool for assessing prognosis in WM patients. Not surprisingly, age remains the most important prognostic factor.

Dr. Efstathios Kastritis, University of Athens School of Medicine, Athens, Greece, studied the prognosis of the high-risk WM patients who died from WM within 2 years of diagnosis. This subset of patients were older (median age 74 years), had lower hemoglobin (median 9.1 g/dL), lower serum albumin (serum albumin ≤ 3.5 g/dL) and lactate dehydrogenase (LDH) that was elevated (≥ 250 IU/L). The $\beta 2$ -microglobulin levels as well as IgM levels at diagnosis did not seem to make much of a difference in prognosis when compared to lower risk or “standard risk” patients. Dr. Kastritis did note that the detection of elevated LDH levels was quite helpful in identifying these high-risk patients. He also added that high-risk patients who do not respond to initial therapy should strongly consider a clinical trial as second-line therapy. Lower risk or standard risk patients were easily salvaged with second-line therapy even if they did not respond well to initial treatment.

Dr. Sigurdur Kristinsson, Karolinska University Hospital Solna, Stockholm, Sweden, studied survival in 1,555 lymphoplasmacytic lymphoma/ Waldenström macroglobulinemia patients diagnosed in Sweden from 1980 to 2005. The author concluded that survival of patients with LPL/WM has improved significantly over time, that older age at LPL/WM diagnosis was consistently associated with a poorer survival, and that females had a better survival than men.

Finally, **Dr. Steven Treon**, Bing Center for Waldenstrom’s Macroglobulinemia, Dana Farber Cancer Institute, Harvard University, Boston, described elegant studies on the impact of genetic factors in rituximab-based therapy responses. The use of rituximab in various therapeutic regimens has improved response in WM patients. However, patients who have the “correct” genetics, meaning in this case the polymorphism in the Fc γ RIIIA rituximab CD-20 receptor (V/V; as opposed to V/L or L/L) had better responses, and better responses translated to improved progression free survival. Newer therapeutic agents are now being studied that will overcome the lower rituximab-based therapeutic responses of WM patients with “unfavorable” genetics.

Session VII: Genetic Predisposition to WM

- Dr. Mitsiades (USA): **Novel Proteasome Inhibitors**
- Dr. Yang (USA): **Preclinical Studies of GA101 Antibody in WM**
- L. Xu (USA): **Hypomethylation Agents in WM**
- All Session VII Speakers: **Panel Discussion**

Novel therapeutic approaches to WM were presented in this interesting session. **Dr. Constantine Mitsiades**, Jerome Lipper Multiple Myeloma Center, DFCI, Boston, discussed newer proteasome inhibitors. Bortezomib (Velcade) has been used in WM for some time now, but its use is limited by potentially serious side effects, notably neuropathy. Bortezomib, alone and in combination with rituximab, is quite active in WM, has been found to rapidly decrease IgM levels, and is, therefore, well-suited for the management of IgM hyperviscosity-related symptoms and rituximab-associated IgM flares. Studies with the second generation proteasome inhibitor carfilzomib in MM have suggested less neuropathy for patients, but similar observations in WM remain to be demonstrated. Other questions that remain unanswered are the efficacy of carfilzomib in previously bortezomib-treated WM patients, neuropathy in WM patients, and use with other drugs in combination.

Dr. Guang Yang of the Bing Center for Waldenstrom’s Macroglobulinemia, DFCI, presented fascinating research on the new anti-CD20 monoclonal antibody (MAb) GA101. This novel humanized anti-CD20 MAb appears to be 2-3 times more effective than rituximab in WM and

may be of particular benefit for WM patients with “unfavorable” genetics with respect to FcγRIIIA CD-20 receptor polymorphisms (see Dr. Treon’s report above, at the end of session VI). Future research and clinical trials using this exciting new monoclonal antibody in WM will hopefully be forthcoming in the very near future.

Dr. Lian Xu, Dr. Yang’s colleague at the Bing Center for Waldenstrom’s Macroglobulinemia, reported on the complex involvement of lipogenic genes in 5-azacytidine-induced cytotoxicity in WM. The drug 5-azacytidine, used in the treatment of myelodysplastic syndrome (MDS), appears to reactivate epigenetically-silenced tumor-suppressor genes leading to the observed increase in apoptosis (cell death) in WM cells. Future research may lead to new therapies targeting WM.

Session VIII & IX: Treatment of WM

- Dr. Owen (UK): **Phase III Study of Chlorambucil vs. Fludarabine as Initial Therapy in WM**
- Dr. Tedeschi (ITALY): **Multicenter Study of FCR in Untreated & Previously Treated WM**
- Dr. Rummel (GERMANY): **Primary Therapy of WM with Bendamustine and Rituximab**
- Dr. Hoster (GERMANY): **Frontline Treatment Options in WM: German Experience**
- Dr. Treon (USA): **Update on Frontline Treatment Studies in WM**
- All Session VIII Speakers: **Panel Discussion**
- Dr. Laszlo (ITALY): **Phase II Study of Rituximab and Subcutaneous 2CDA in WM**
- Dr. Rossi (FRANCE): **Phase I Study of Atacept in Relapsed/Refractory MM and WM**
- L. Ioakimidis (USA): **Maintenance Rituximab in WM**
- Dr. Ghobrial (USA): **Update on Salvage Treatment Studies in WM**
- All Session IX Speakers: **Panel Discussion**

Presentations on the treatment of WM comprised the next few sessions. **Dr. Roger Owen** of the St. James Institute of Oncology, Leeds, UK, reported on a Phase III study of chlorambucil versus fludarabine as initial therapy for WM patients. This international study was a prospective, international, randomized, open-label study that included patients with symptomatic and previously untreated WM, MZL (marginal zone lymphoma) and LPL. The aim of the study was to compare the efficacy of oral chlorambucil (10 days every 28 days to a maximum of 12 cycles) with oral fludarabine (5 days every 28 days to a maximum of 6 cycles). Oral fludarabine was significantly better than oral chlorambucil as a single agent for WM in all parameters evaluated (response, progression-free survival, time to retreatment, and overall survival). Side-effects and toxicity were comparable although hematological toxicity (neutropenia) was more pronounced with fludarabine than with chlorambucil.

Dr. Alessandra Tedeschi, Department of Hematology, Niguarda Ca' Granda Hospital, Milan, Italy, presented yet another large clinical study in WM. This multicenter Phase II trial evaluated the combination of fludarabine, cyclophosphamide, and rituximab (FCR) in untreated and previously untreated WM patients; 43 WM patients were enrolled (median age of 65 years) and of those patients 65% received FCR as first line treatment. The treatment consisted of rituximab on day 1 and intravenous fludarabine and intravenous cyclophosphamide on days 2 to 4. Courses were repeated every 28 days for a maximum of 6 cycles; 39 patients (87%) completed 4 or more courses of therapy; the remaining 4 patients were considered treatment failures. The primary reason for discontinuation of therapy was persistent low white blood cell count requiring supportive treatment with growth factors in 9 patients. The overall response rate was 79% with 12% considered as complete responses and 21% as near-complete responses. The β 2-microglobulin level was the only predictor for the achievement of a major response. Of note are the continued significant decreases in IgM levels seen in patients up to eighteen months following discontinuation of therapy. The presenter concluded that the FCR regimen produced

rapid responses and could be considered an effective salvage regimen particularly in patients with adverse prognosis. In younger patients FCR should be avoided based on the possible difficulty in future stem cell collection.

Dr. Mathias Rummel, Clinic of the Justus-Liebig University, Giessen, Germany, reported for the German study group on indolent lymphomas on a multicenter, randomized Phase III study comparing a maximum of 6 cycles of bendamustine plus rituximab (B-R) versus R-CHOP as first-line treatment in various indolent lymphomas including WM. The current standard of care for first-line treatment in patients with advanced indolent lymphoma and WM is rituximab plus chemotherapy (such as R-CHOP). At a median follow-up of 35 months, median progression free survival was significantly prolonged with B-R compared with R-CHOP. Decreases in IgM levels were better with B-R, as were increases in hemoglobin levels. The median progression free survival for 22 WM patients randomized to B-R was not yet reached at time of presentation, whereas the median PFS for 19 WM patients randomized to R-CHOP was 35 months. Four relapses (18%) have occurred in the B-R group and 11 relapses (58%) in the R-CHOP group as of time of the presentation. The B-R regimen was better tolerated than R-CHOP with far less toxicity. The author concluded that B-R should be considered the new standard of care for treatment in WM. Of interest was the well-received announcement that this group is now undertaking a rituximab maintenance study.

Dr. C. Buske, University Hospital, Ulm, Germany, discussed the German experiences with front-line treatment options in WM. Given the rarity of WM (1 % of all NHLs) and that WM is predominantly a disease of the elderly (median age of 68 years), thus limiting aggressive treatments, large Phase II/III trials are difficult to perform. A randomized clinical trial comparing R-CHOP versus CHOP in previously treated patients with WM clearly demonstrated the superiority of rituximab/chemotherapy regimens compared to chemotherapy alone. Newer regimens such as rituximab and bendamustine (B-R), or rituximab with oral cyclophosphamide and dexamethasone (DRC), have offered comparably efficacious options to clinicians weary of treating fragile elderly WM patients with R-CHOP.

Dr. Steven Treon followed with a comprehensive report on treatment advances in WM. As is well known, Dr. Treon and his colleagues at the DFCI are very active in clinical trials as well as development of newer therapeutic options for WM patients. With his trademark measured delivery and his capacity to inform attendees on a large amount of data, Dr. Treon reiterated that nucleoside analogues (such as fludarabine) and oral alkylators (chlorambucil) should be avoided in younger patients; that plasmapheresis be considered for symptomatic hyperviscosity and as a prophylactic measure prior to rituximab administration in patients with high IgM levels in order to avoid the IgM flare phenomena; that cyclophosphamide-based therapy in combination with steroids and rituximab be considered as an appropriate first line treatment; that the use of vincristine (the "O" in CHOP) be avoided due to the risk of neuropathy; that the use of bortezomib (Velcade) in combination with steroids and rituximab may be useful for patients with symptomatic hyperviscosity as well as patients requiring rapid disease control; that the active combination of thalidomide and rituximab can produce prolonged remissions; and, finally, that the newer agents bendamustine, everolimus (RAD001), and pomalidomide hold particular promise for the treatment of WM.

Dr. Daniele Laszlo, Department of Hematology, European Institute of Oncology, Milan, Italy, reported on a Phase II trial that assessed the efficacy of the nucleoside analogue 2-chlorodeoxyadenosine (2-CdA) given subcutaneously in combination with rituximab in the treatment of newly diagnosed and previously treated WM patients. The 29 patients enrolled in the study were also evaluated for the expression of genes involved in 2-CdA metabolism. After a median follow-up of 50 months the overall response rate (ORR) was 89.6% with 7 complete responses (CR) and no difference between newly diagnosed and previously treated patients. Median time to retreatment was 60 months and no patients developed transformation to high-

grade NHL or myelodysplastic syndrome. The expression of the gene hCNT1 was felt to be a potentially useful biomarker in assessing clinical response to this particular treatment.

Dr. Jean François Rossi, Le Centre Hospitalier Universitaire, Montpellier, France, reported on a Phase I study of atacept in relapsed or refractory multiple myeloma (MM) and WM. Atacept is a biomolecule that blocks the binding of two cytokines (BLys and APRIL) on B-cells, thus inhibiting the growth of malignant cells. In this small Phase I study, 4 WM patients received one cycle of five once-weekly injections of atacept subcutaneously. Patients with stable disease after one cycle were given either 2 additional cycles or 15 consecutive weekly injections of atacept. Of these 4 WM patients, 3 were progression-free after cycle 1. At the end of the extension study, 2 patients were progression-free, 1 patient had a minimal response, and the other had stable disease. No progression of the tumor mass was observed in the 2 patients who had lymph node involvement at the beginning of the trial. Polyclonal immunoglobulins and total B-cells were reduced. The biological effect of atacept was more pronounced in patients with WM than MM. Atacept is well tolerated in patients and may represent an option for the treatment of WM in the near future.

Thea Ioakimidis, Bing Center for Waldenstrom's Macroglobulinemia, DFCI, reported on a study of maintenance rituximab (MRx) in rituximab naïve WM patients who initially responded to a rituximab containing regimen. A total of 248 rituximab naïve WM patients who responded to a rituximab based induction therapy, including 86 (35%) patients who then received MRx, were evaluated. The median progression free survival for MRx treated patients was 56.3 versus 28.6 months for non-MRx patients irrespective of previous treatment status, front-line treatment with rituximab alone or combination therapy. IgM levels were much lower in MRx patients, and hemoglobin levels were higher. Platelet levels were similar in MRx vs. non-MRx patients. No difference in progression free survival and overall survival was observed between patients who received MRx as 1 infusion every 3 months versus 4 weekly infusions every 6 months. More sinus and pulmonary infections were noted in the MRx patients but none of these infections were severe in nature. The presenter concluded that MRx is well tolerated and associated with improved clinical outcomes in WM patients responsive to rituximab-based treatment.

Dr. Irene Ghobrial, DFCI, reported on her ongoing Phase II trial of single agent panobinostat (LBH589) in relapsed or relapsed/refractory WM patients. Panobinostat is a novel biological agent that inhibits one or more of the histone deacetylase enzymes, leading to apoptosis (cell death) of WM cells. At the time of this presentation, 27 patients were participating in the study. The median number of cycles received was 4 (range 1 - 12). Fifteen (60%) patients achieved minimal response or better; 9 (36%) patients achieved stable disease; and 1 patient had disease progression. Response to therapy was quite rapid (median time to first response was 2 cycles), and the median decrease in IgM was 1020 mg/dL. Four patients halted the study due to side effects; most of the serious side effects were hematological (anemia, low platelets) and 20% of patients developed asymptomatic pulmonary changes which were reversible. The author concluded that panobinostat is active in patients with relapsed or refractory WM with an overall response rate of 60%. Studies combining panobinostat with rituximab or bortezomib are being considered.

Dr. Ghobrial continued with a report on the first-line therapy of WM with weekly bortezomib (Velcade) and rituximab. Twenty-six WM patients with no prior therapy and symptomatic disease were treated with weekly bortezomib IV (1.6 mg/m² on days 1, 8, 15 every 28 days x 6 cycles) in combination with rituximab weekly on cycles 1 and 4. Minor responses or better were seen in 23/26 patients (88%) with 1 complete response, 1 near complete response, 15 partial responses, and 6 minor responses. The median time to progression was not yet reached at the time of the presentation (at least >12 months). No significant (grade 3 or 4) treatment related neuropathy occurred. Dr. Ghobrial concluded in patients with untreated WM, the combination of

weekly bortezomib and rituximab is an efficacious treatment and exhibits minimal treatment related neuropathy.

The session concluded with a special lecture from **Dr. Stephanie Gregory** of the Rush University Medical Center, Chicago, Illinois, on novel treatment options for B-cell malignancies. Dr. Gregory summarized very nicely many of the new agents that have been developed recently in the treatment of incurable indolent lymphomas. She noted that patients experience frequent relapses, survival requires good responses to newer drugs, and that combinations are usually more effective than single agents. Of the newer monoclonal antibodies (MAbs), the humanized GA101 appears to be the best to date. Complete responses in up to 20% of follicular lymphoma patients have been seen with the MAb veltuzumab. Combinations of MAbs such as rituximab and epratuzumab have also been very effective with high complete responses. Dr. Gregory also commented on newer agents that have specific activity in the complex biological pathways in lymphomas, essentially highlighting the continued expansion of targeted therapy. Continued improvement in treatments and responses in WM is directly related to the support of research and clinical trial participation.

Session X: Response Assessment in WM

- Dr. Owen (UK): **Challenges With Response Assessment in WM**
- Dr. Garcia-Sanz (SPAIN): **Flow Cytometric Evaluation of WM Response**
- Dr. Leleu (FRANCE): **sFLC in Response Assessment in WM**
- All Session X Speakers: **Panel Discussion**

The tenth session focused on the—at times controversial—response assessment in WM. As is appropriate in the opinion of this writer, the session began with a presentation by **Dr. Roger Owen**. Dr. Owen cautioned that the usual method of assessing response to WM treatment by serial measurements of serum IgM levels is no longer appropriate and can lead to false assumptions given the newer targeted therapies now being developed and used in WM. Specifically, Dr. Owen pointed out that: changes in IgM do not always result in the improvement of symptoms; responses to some drugs (alkylating agents, purine analogues and monoclonal antibodies) are typically slow whereas responses to other drugs (Velcade) can be rapid; great bone marrow responses may be seen in patients with small reductions in IgM due to the selective depletion of B cells and persistence of the plasma cell component of the disease; and, finally, rapid drops in serum IgM levels may be seen with poor bone marrow and lymph node responses in some patients treated with bortezomib (Velcade). Dr. Owen concluded his presentation by suggesting that the assessment of residual bone marrow disease by flow cytometry is a highly effective way to define response independent of IgM levels. In other words, more bone marrow biopsies should be performed in order to truly evaluate ongoing therapy and end-of-therapy response.

Dr. Ramon García-Sanz, Hospital Universitario de Salamanca, Spain, presented a very elegant talk on the contribution of flow cytometry analysis of residual disease in the bone marrow of WM patients. Flow cytometry is a technique used to measure the number of cells and determine certain characteristics of cells, most notably the presence of tumor markers by using fluorescent markers on the cell surface. In this study Dr. García-Sanz noted that the use of bone marrow biopsies and flow cytometry analysis in WM patients, and not serum IgM levels, is by far the best way to determine response to treatment.

Dr. Xavier Leleu, formerly of the DFCI and now at Hôpital Huriez-CHRU, Lille, France, presented a lecture on the controversial topic of the measurement of serum immunoglobulin free chains (sFLC) in the response and progression of WM. Immunoglobulin light chains, together with heavy chains, are produced by WM cells to form immunoglobulins like IgM. Some of these

light chains, produced in excess of heavy chain, can be measured in the serum. This sFLC assay has been used often in multiple myeloma. Dr. Leleu discussed the usefulness and limitations of using this test in WM. This controversial topic elicited a fair amount of questions from the expert WM clinicians and researchers present at the meeting. It was recognized that sFLC assays have a very limited role in WM given the existence of the two cell populations in WM (B-cells and plasma cells). In essence, the measurement of sFLC is almost equivalent to the measurement of serum IgM levels and does not always truly represent the actual status of the disease. The measurement of sFLC does not add any additional useful information in the treatment or assessment of WM.

Session XI: Treatment Challenges in WM

- Dr. Munshi (USA): **Challenges in the Management of the WM Patient**
- Dr. Morra (ITALY), Dr. Leleu (FRANCE): **Debate: Is There a Role for Nucleoside Analogue Therapy in WM?**
- Dr. Rummel (GERMANY), Dr. Leblond (FRANCE): **Debate: Should Benda-Rituximab Be Considered the Standard as Frontline Therapy in WM?**
- Dr. Advani (USA), Dr. Kimby (SWEDEN): **Debate: Should Maintenance Rituximab Be Used for Rituximab Responders in WM?**
- Dr. Kyriakou (UK), Dr. Femand (FRANCE): **Debate: Should ASCT Be a Frontline Option for WM?**
- Dr. Maloney (USA), Dr. Barlogie (USA): **Debate: Should Allogeneic Transplant Represent a Standard of Care Option in WM?**

This session can best be described as a series of five debates on treatment challenges in WM between world-renowned WM experts in a “Yes versus No” format. Following a brief introduction by the debate moderator **Dr. Nikhil Munshi**, DFCI, the debates began.

THE FIRST DEBATE: “Is there a role for nucleoside analogue therapy in WM?”

Dr. Enrica Morra, a very knowledgeable and experienced clinician from the Department of Hematology, Niguarda Ca' Granda Hospital, Milan, Italy, argued that nucleoside analogues (NAs) do indeed have a role in WM therapy given their record of efficacy as single agents or in combinations, with good responses and long duration of responses, even in heavily pre-treated WM patients.

Dr. Xavier Leleu argued that the risk of myelosuppression, together with the risk of stem cell damage (and hence difficulty in possible future stem cell harvest) and risk of transformation (4-7% according to a study published earlier by Dr. Leleu), should give great pause to clinicians considering the use of NAs in the treatment of WM. Both debaters agreed that the use of nucleoside analogues should be limited in younger WM patients, that consideration be given to reduction of doses or cycles in order to minimize the associated side effects of these very effective agents, and that clinicians should be allowed to consider their use in limited, yet appropriate, circumstances. Both debaters also agreed that the use of nucleoside analogues in WM should be limited to clinicians very familiar with the potential hazards of these agents.

THE SECOND DEBATE: “Should the combination bendamustine and rituximab (B-R) be considered the standard as frontline therapy in WM?”

Dr. Mathias Rummel, who had previously presented the very favorable results of a German clinical trial on B-R in WM, argued that the results of the Phase III randomized clinical trial had indeed elevated B-R as standard treatment in WM versus the previous choice of R-CHOP. B-R use in WM resulted in better results in virtually all parameters as well as fewer side effects attributable to therapy.

Dr. Veronique Leblond, who is a widely respected and experienced clinician from the Groupe Hospitalier Pitié Salpêtrière, Paris, France, had the unenviable task of arguing the opposing view. She focused her arguments on the relatively low numbers of WM patients studied and the

relative paucity of data in the optimal dosing schedule as well as the use of B-R in patients older than 70. She suggested that patients preferred oral therapy versus the B-R intravenous therapy and that the cost of B-R was quite high. Unfortunately for Dr. Leblond, who was admittedly finding it difficult to argue against B-R in support of R-CHOP, it appeared that most of the audience sided with Dr. Rummel.

THE THIRD DEBATE: “Should maintenance rituximab (MRx) be used for rituximab responders in WM?”

Beginning debate on this controversial topic, **Dr. Ranjana Advani** of the Stanford Advanced Medicine Cancer Center, Stanford University, argued that Dr. Treon’s recent retrospective study on MRx and WM, coupled with the large trial in follicular lymphoma showing definite advantages to MRx, clearly indicated the positive benefits of MRx in WM.

However, **Dr. Eva Kimby** of the Karolinska Institute, Stockholm, Sweden, pointed out that there is no completed Phase II or Phase III trial in MRx and WM. Furthermore, long-term use of rituximab results in immune compromise, leads to an increased incidence in infections, and may impair vaccination efficacy. Dr. Kimby went on to ask why bother with MRx when WM patients have access to many efficacious options after relapse?

Dr. Advani countered by stating that rituximab has never been fully evaluated in a randomized Phase III clinical trial and is nonetheless used as single agent and in combinations without any hesitation in the treatment of WM. Dr. Advani also brought up the often ignored quality of life issues that WM patients face every day and argued that these issues should not always hold second place to absolute scientific validation, particularly in terms of rigorous Phase III randomized clinical trials that are so difficult to do in WM, given the relatively small number of patients. The questions and comments from the audience certainly mirrored the ongoing controversial nature of the MRx debate.

THE FOURTH DEBATE: “Should autologous stem cell transplant (ASCT) be a frontline option for WM?”

Dr. Charalampia Kyriakou of the Royal Free Hampstead Trust in the UK, a recognized expert in transplants and WM, argued in the affirmative. Once again, due to the low number of WM patients involved in Phase III trials, it is difficult to interpret data on the use of ASCTs in WM. Furthermore, ASCTs have been used as “last resort” for many WM patients, leading to a bias in the outcomes. Not only do high risk WM patients have a very poor prognosis, but the duration of response for most non-ASCT treatments is lower than with ASCTs (66-82 months). Dr. Kyriakou also noted that newer ASCT protocols have high efficacy, reduced toxicity, lead to prolonged survival, and favor quality of life issues for the WM patient. It is better to go early in ASCT than as a last resort, and young WM patients should definitely consider harvesting stem cells in the event that an ASCT is chosen as treatment.

Dr. Jean-Paul Femand, Hôpital Saint-Louis, Paris, France, did not disagree that ASCT was an efficacious option for WM patients but did argue against using it as a frontline option, preferring to reserve it for second line treatment for the young relapsed high-risk patient. He did state that WM patients should choose stem-cell sparing frontline treatments such as his preferred dexamethasone plus rituximab plus cyclophosphamide (DRC) combination to preserve the option of an ASCT in the future. Dr. Femand also questioned if ASCT may eventually be replaced by lower intensity non-myeloablative allogeneic transplants in the near future.

THE FIFTH DEBATE: “Should allogeneic transplant represent a standard of care option in WM?” was debated by two of the most respected experts in transplants and WM.

Dr. David Maloney of the Fred Hutchinson Cancer Center in Seattle, Washington, remarked that in autologous transplants the efficacy of the treatment is due to the high dose chemotherapy given prior to the stem cell infusion and that it is very difficult to completely “sanitize” the bone marrow of a WM patient prior to stem cell infusion. This contrasts to the newer “mini-allo” or low-intensity non-myeloablative allogeneic transplants used in ever increasing frequency, where

the chemotherapy regimen is reduced and the donor stem cells produce a graft versus tumor effect that may actually lead to a complete eradication of the disease—a cure. Furthermore, the mini-allo transplants are less toxic and can be used in older patients. Dr. Maloney did go on to say that the conventional allogeneic transplants were no better than ASCTs.

Dr. Bart Barlogie, Myeloma Institute for Research and Therapy of the University of Arkansas, refused to limit himself to any single option in the treatment of WM, preferring to use the most appropriate treatment for the individual patient. However, for the sake of the debate, Dr. Barlogie did refer to his considerable experience in autologous and allogeneic transplants in both WM and MM. He reiterated that the data do demonstrate a higher mortality in allogeneic transplants compared to ASCTs without any apparent increase in length of remission. He did not disagree that low-intensity non-myeloablative allogeneic transplants may hold considerable promise, but he maintained that the protocols for these mini-allo transplants still need considerable refinement and are not quite ready for “prime time” yet. Dr. Barlogie finally alluded to the current WM treatment recommendations that list ASCTs as second line options and agreed that allogeneic transplants still remain an option to be used in a clinical trial setting.

Session XII: Young Investigator Presentations

- **Section I:**
- Dr. Novak (USA): **Development of WM Cell Lines**
- M. Lewicki (CANADA): **Assessment of Cytokines and Chemokines in Patients with WM**
- Dr. Koulieris (GREECE): **Quantification of IgM kappa and IgM lambda in WM**
- S. Guidez (FRANCE): **SM-IgC as Response Criteria in WM**
- Dr. Greco (ITALY): **Factors Predicting Malignant Evolution of IgM-MGUS and IgM Related Disorders**
- **Section II:**
- Dr. Trojani (ITALY): **Gene Expression of Malignant and Microenvironment Cells from WM Patients**
- Dr. Fulciniti (USA): **Sp1 Trans-Activation in WM**
- Dr. Liu (USA): **Metalloprotease Inhibitors and CD27 in WM**
- A. Sacco (USA): **NVP-BE2235 Exerts Anti-Tumor Activity in WM**
- **Section III:**
- Dr. Maintezas (GREECE): **Clinical Impact of Sflcs or FLCR on WM**
- L. Compain (FRANCE): **Rituximab, Fludarabine and Cyclophosphamide in WM**
- Dr. Chitta (USA): **Induced Resistance to Bortezomib and its Association with BCL-2 Up-Regulation**
- Dr. Azab (USA): **Role of Hypoxia in the Dissemination of WM**
- Dr. Ricci (ITALY): **Impact of Advanced Age in WM at Diagnosis**
- Dr. Hospital (FRANCE): **Anti-MAG Neuropathy**

This session, a personal favorite of Dr. Treon, consisted of 15 presentations by young investigators.

Dr. Anne Novak, Mayo Clinic, Rochester, Minnesota, presented her experience with the development of WM cell lines. Working closely with such distinguished researchers as Dr. Morie Gertz and Dr. Stephen Ansell, Dr. Novak identified some of the challenges encountered in the development of WM cell lines. This difficult task is complicated by the fact that the proliferative rate of WM cells is much less than most lymphomas; the rate is, however, similar to MM cells. This young investigator cited as a primary objective the development and characterization of WM cell lines that are biologically stable and immortalized but functionally retain characteristics of the original tumor. To date she has used cells from 16 WM patients and has cultured 4 potential cell lines. Her work is supported by a research grant from the IWMPF.

Megan Lewicki, Bing Center for Waldenstrom's Macroglobulinemia, DFCI, presented her important studies on the comprehensive assessment of cytokines and chemokines in patients with WM. Noting that the dysregulation of plasma cytokines and chemokines has been described in related malignancies, she evaluated the levels of 20 cytokines, 27 chemokines, and soluble CD27 (sCD27) from 54 patients with WM, 31 patients with MM, and 37 patients with monoclonal gammopathy of unknown significance (MGUS). Using complex biological analytical instruments, she demonstrated that significant cytokine, chemokine, and soluble CD27 dysregulation in WM, MM, and MGUS corresponded to important clinical correlations in WM. Furthermore, not only are there impacts on B-cell regulation but also on T-cell regulation. Comprehensive studies in the role of T-cell biology in WM would therefore aid in the understanding of WM.

Dr. Efstathios Koulieris from the National and Kapodistrian University of Athens, Athens, Greece, presented a study on the quantification of serum IgM kappa (IgM κ) and IgM lambda (IgM λ) in WM patients. Using automated nephelometric assays to quantify serum concentrations of IgM κ and IgM λ and the subsequent calculation of IgM κ / IgM λ ratios to identify monoclonal IgM, the author was able to discriminate normal IgM from WM-cell IgM as well as identify patients with more aggressive disease.

Dr. Stephanie Guidez, Centre Hospitalier Schaffner in Lens, France, reported on the prognostic value of percentage reduction in serum monoclonal immunoglobulin concentration as a response criterion for the overall survival in patients with symptomatic WM. Using data from the follow-up of 83 symptomatic WM patients followed at their institution between 1993 and 2009, the researchers noted that reductions of at least 25% and 50% in serum monoclonal immunoglobulin concentration after first line therapy were associated with a significant prognostic value for overall survival.

Dr. Antonino Greco, Niguarda Ca' Granda Hospital, Milan, Italy, presented a study on factors predicting malignant evolution of IgM MGUS and IgM-related disorders (IgM-RDs). Reviewing a number of studies on IgM MGUS and IgM-RDs, a higher risk of transformation correlated with monoclonal IgM level, albumin levels, hemoglobin level, abnormally low levels of neutrophils in the blood (neutropenia), abnormal increases in the number of lymphocytes in the circulating blood (lymphocytosis), the presence of Bence Jones proteins in the urine, and a high erythrocyte sedimentation rate (ESR). The authors conclude that IgM MGUS and IgM-RDs are distinct clinical entities with similar probability of transformation to lymphoid malignancy.

Dr. Alessandra Trojani, also of the Niguarda Ca' Granda Hospital, Milan, Italy, reported on a study of the distinct gene expression signature of malignant and bone marrow microenvironment cells from WM patients. When the author analyzed the gene expression profiles of CD19+, CD138+ and CD19-, CD138- cells obtained from the bone marrow of WM patients and compared their profiles to the same cell counterparts from normal individuals and to the CD19+ cells from CLL patients, a distinct gene expression profile was identified in WM patients compared with normal and CLL individuals.

Dr. Mariateresa Fulciniti, DFCI, presented a study on a novel therapeutic option in WM: targeting the Sp1 transcription factor expression in WM with terameprocol, a small biomolecule. The Sp1 transcription factor plays an important role in regulating expression of cell differentiation, cell cycle, and apoptosis related genes affecting the growth and metastatic potential of tumor cells. Terameprocol was found to regulate cell cycle and apoptosis-related genes aberrantly expressed in WM and MM, significantly inhibiting WM and MM cell growth in a dose-dependent fashion, and was able to overcome the protective effects of bone marrow stromal cells.

Dr. Xia Liu, DFCI, described a complex study on the effects of metalloprotease inhibitors on the release of soluble CD27 in WM cells. The soluble CD27 (sCD27)-CD70 signaling plays an

important role in the homeostasis of both WM cells and tumor supporting mast cells. Some of the known metalloprotease inhibitors may be useful in the study of sCD27-CD70 signaling and therefore potential therapeutic reagents for WM.

Antonio Sacco, DFCI, presented a study examining therapeutic agents that target the PI3K/Akt and mTOR pathways in WM. The PI3K/Akt/mTOR pathways play an important role in the initiation and progression of malignancies. The researchers tested the dual PI3K/Akt and mTOR inhibitor NVP-BEZ235 in WM cells and found that this molecule induced cytotoxicity and inhibited DNA synthesis in primary WM cells. These findings represent yet another promising potential targeted therapeutic option for the treatment of WM.

Dr. Dimitrios Maltezas, Laikon University Hospital, Athens, Greece, presented a study on the clinical impact of increased serum free light chains (sFLC) or their ratio on WM at diagnosis and during disease course. sFLC and their ratio are useful for monitoring plasma cell dyscrasias (myeloma, amyloidosis), and in WM increased sFLC correlates with adverse prognostic factors such as anemia, thrombocytopenia, increased β 2-microglobulin, and with time to treatment. The researchers found that sFLC levels greater than normal at presentation, and in particular greater than 60mg/L, correlated with parameters of disease severity and with time to first line treatment. Levels greater than 60mg/L at diagnosis also correlated with overall survival in WM.

Dr. Laetitia Compain, of the Service d'Hématologie Clinique, Groupe Hospitalier Pitié-Salpêtrière, Paris, France, reported on a retrospective study of 55 WM patients who received fludarabine plus cyclophosphamide plus rituximab. This study reaffirmed the role of this combination in relapsed or refractory WM patients. However, the choice of this combination must be used cautiously for first-line treatment because of increased incidence of disease transformation and potential impairment of stem cell harvest.

Dr. Kasyapa Chitta, Roswell Park Cancer Institute, Buffalo, New York, presented an interesting study on the induced resistance to bortezomib (Velcade) in a preclinical model of WM associated with BCL-2 pathway upregulation. The retained BCL-2 mediated pathway in bortezomib resistant cells could therefore potentially be targeted by inhibitors of BCL-2.

Dr. Abdel Kareem Azab, DFCI, discussed the role of hypoxia (oxygen deprivation) in the metastasis of WM. Using a hypoxic laboratory-created environment, the researchers showed that hypoxia plays a major role in the interaction of WM cells with the bone marrow microenvironment, reducing the adhesion of WM cells and increasing cell migration (metastasis). These findings provide the basis for yet another potential new therapeutic target in the treatment of WM.

Dr. Francesca Ricci, Niguarda Ca' Granda Hospital, Milan, Italy, performed a retrospective analysis of 242 WM patients reviewing the impact of advanced age on the outcome of WM irrespective of clinical and laboratory features at diagnosis. The researchers found that although younger and older patients did not differ at diagnosis in clinical and laboratory features, median overall survival was longer in younger patients. A significantly shorter time to first treatment and a higher number of disease-related deaths was noted in younger patients, likely reflecting a more aggressive disease progression. The lower survival rate of patients older than 65 years was felt to be likely due to the higher number of non-WM disease related deaths.

Dr. Marie-Anne Hospital, Hôpital Pitié-Salpêtrière, Paris, France, presented a retrospective study of 61 WM patients with anti-MAG (IgM anti-myelin-associated glycoprotein) peripheral neuropathy. The researchers evaluated the response of these WM PN patients treated with either chlorambucil or rituximab or rituximab-based combination. The researchers were able to determine that WM anti-MAG patients treated first line with rituximab alone or in combination had higher response rates than those treated with chlorambucil. Patients who relapsed after chlorambucil responded favorably to subsequent treatment with rituximab. The serum IgM level was noted to be an important prognosis factor with respect to clinical response to treatment. In

order to better define the efficacy of rituximab in WM anti-MAG patients, a French clinical trial comparing rituximab versus placebo is pending.

A special lecture followed: “Update in Indolent Non-Hodgkin’s Lymphoma: Paradigm for WM.” **Dr. Umberto Vitolo** of the San Giovanni Battista Hospital and University, Torino, Italy, discussed some of the newer therapeutic strategies used in the treatment of indolent lymphomas. The improved outcomes seen in the patients in this report may help guide researchers and clinicians in the future treatments of WM.

The second special lecture of this session was delivered by **Dr. Mario Boccadoro**, Professor of Medicine and Director, Section of Hematology, University of Torino, Italy. Drawing from his considerable experience in the management of multiple myeloma, Dr. Boccadoro touched on the newer treatment strategies and drugs used in WM and on the increasing interest in maintenance treatment supported by favorable preliminary results of several large clinical trials in MM. Treatments in WM have long been influenced by MM clinical trial experiences.

Session XIII: Focus on WM-Related Peripheral Neuropathy

- Dr. Nobile-Orazio (ITALY): **Antigenic Determinants in IgM-Related PN**
- Dr. Levine (USA): **Treatment of Painful Neuropathies in WM**
- Dr. Steck (SWITZERLAND): **Rituximab for Anti-MAG Neuropathy**
- Dr. Karlin (FRANCE): **Combination Therapy of WM-Related PN**
- NP. Sheehy (USA): **Natural History and Treatment Outcome of WM-Related PN**

The organizing committee of the IWWM-6 acknowledged the importance of WM-related peripheral neuropathies (PN) by dedicating an entire session on this issue that is so vexing for many WM patients.

Dr. Eduardo Nobile-Orazio of the University of Milan, Italy, discussed antigenic determinants in IgM-related neuropathies. The overwhelming majority of WM patients with PN have a chronic progressive, symmetric, and predominantly distal neuropathy. Such neuropathy is most frequently caused by the reactivity of the IgM with a number of antigens present on the nerve surface, the most common of which is the anti-myelin associated glycoprotein (anti-MAG). Other less common antigens have been described, but more importantly the current therapies, predominantly immune-based (i.e. rituximab), although modestly effective to a certain extent in relieving symptoms of WM PN, do not always impact significantly the long-term outcome of the neuropathy itself. Further research in the pathological and clinical aspects of the various neural antigens in WM PN will lead to more efficacious treatments and perhaps reverse long-term damage to nerves of WM patients.

Dr. Todd Levine, well-known WM PN expert from Phoenix, Arizona, discussed the treatment of painful neuropathies in WM patients. Dr. Levine stressed the importance of a correct diagnosis coupled with the identification of the actual location of the painful nerve(s) in question and the mechanism(s) of injury that cause the unpleasant symptoms of PN. It is important to note that a very painful neuropathy may be present even with a relatively normal exam and nerve conduction studies. The absence of autoantibodies directed against neural antigens (i.e. anti-MAG) does not preclude painful WM neuropathy that can be very responsive to immunomodulatory therapy. Improvement in WM PN can be very slow, usually 2-3 years, and in many cases the damage is irreversible if therapy has been delayed too long. Dr. Levine recommends skin biopsies that permit direct visualization of the affected small nerve fibers. In the absence of a specific therapy for PN, clinicians may use pharmacological agents to alleviate symptoms. Various drug therapies were discussed including non-pharmacological therapies such as alpha lipoic acid and N-acetyl carnitine which have some evidence of efficacy.

Dr. Andreas Steck, Department of Neurology, University Hospital, Basel, Switzerland, focused on the use of rituximab for anti-MAG neuropathy. The anti-myelin-associated glycoprotein (anti-MAG) neuropathy is a demyelinating neuropathy, characterized by a distal and symmetric, mostly sensory, neuropathy. IgM anti-MAG antibodies are believed to be the causal agent. Dr. Steck reported on a study of nine patients with anti-MAG-associated IgM PN who received rituximab in four weekly infusions. The number of B-cells in the blood was undetectable in all patients following therapy; the IgM levels decreased between 35% and 82% (median, 58%); reduction of anti-MAG antibody titers of more than 52% was observed in eight patients; and, finally, six patients improved, two remained stable, and one worsened. The same patients received a double dose of rituximab in a follow up study (750 mg/m² instead of 375 mg/m²). Over half of the patients improved symptomatically, and nerve conduction studies demonstrated improvement in all but one patient. IgM levels fell by a median of 38% and anti-MAG antibody titers by a median of 16%. Duration of symptomatic response was 6–9 months and retreatment will likely be necessary to maintain a satisfactory response.

Dr. Lionel Karlin, Department of Immuno-Hematology, Hôpital Saint-Louis, Paris, France, reported on a retrospective study of 15 patients with IgM anti-MAG PN who were treated with rituximab, fludarabine, and alkylating agents. Twelve patients received a combination of rituximab plus chemotherapy (7 patients received rituximab plus fludarabine plus cyclophosphamide; 4 patients received rituximab plus cyclophosphamide or chlorambucil; 1 patient received rituximab plus fludarabine) while 3 patients received rituximab alone. Significant and durable improvement occurred in 7 patients treated with rituximab plus chemotherapy. No responses were noted in patients receiving rituximab as single agent. The author of this study concluded that in IgM anti-MAG PN, rituximab plus chemotherapy appeared to be more efficacious than single agent rituximab.

Dr. Steven Treon, Bing Center for Waldenstrom's Macroglobulinemia, DFCI, reported on the clinical characteristics and treatment outcome of disease-related peripheral neuropathy in 900 patients with WM. Of these, 199 (22.1%) patients had disease related PN; PN patients had lower serum IgM, β_2 -microglobulin, and bone marrow infiltration. Among 122 PN patients tested for neuropathic antibodies, 24.5% were positive for anti-MAG antibodies, 1.64%, had GM1 antibodies (a type of anti-ganglioside antibody), and 0.81% had sulfatide antibodies. The median time to treatment for all PN patients was 9 months. Dr. Treon's impressive WM patient numbers enabled him to conclude that PN is common in WM patients, that plasmapheresis is effective in the interim (except for patients with amyloid-related PN), and, finally, that improvement in symptomatic neuropathy occurs in half of PN patients following chemotherapy. Dr. Treon cautioned that clinicians should evaluate WM PN patients for amyloidosis as these patients may require more aggressive treatment altogether. WM PN patients who receive earlier therapy, who receive combination therapy with rituximab, and who achieve a major response are more likely to see improvements in PN symptoms.

Sessions XIV & XV: Focus on WM-Related Morbidities in WM

- Dr. Merlini (ITALY): Diagnosis and Therapy of Amyloidosis
- Dr. Gertz (USA): Management of IgM-Related Amyloidosis
- Dr. Femand (FRANCE): Renal Manifestations of WM
- Dr. Asli (FRANCE): Physiopathology of Schnitzler's Syndrome
- Dr. Stone (USA): Pathogenesis and Morbidity of Autoantibody Syndromes in WM
- Dr. Berentsen (NORWAY): Management of Cold Agglutinin Disease
- Dr. Dammacco (ITALY): HCV-Positive Macroglobulinemia
- Dr. Owen (UK): Histologic Transformation of WM
- Dr. Hochberg (USA): Bing Neel Syndrome

The last formal lecture sessions focused on the morbidities, or medical complications, arising from WM. Dr. **Giampaolo Merlini**, WM expert clinician from the Amyloidosis Research and Treatment Center, University of Pavia, Italy, discussed systemic amyloidosis and WM. In amyloid light chain (AL) amyloidosis, tissue depositions of monoclonal immunoglobulin light chain undergo conformational changes and aggregate in fibrils. The aggregation and tissue deposition of these fibrils cause dysfunction of the organs involved, and death is possible if this process is not stopped by therapy. Early diagnosis is crucial in order to establish effective treatment before irreversible organ damage occurs. The treatment of AL amyloidosis is aimed at reducing the concentration of the amyloid light chain and thus improving organ function and prolonging survival. Patients with AL amyloidosis are more susceptible to treatment toxicity due to organ dysfunction. Involvement of the heart is the most important prognostic determinant.

Dr. Morie Gertz of the Mayo Clinic, Rochester, MN, another well-known WM expert with an interest in AL amyloidosis, emphasized that IgM AL amyloidosis is a rare but distinct entity. IgM AL amyloidosis occurs in 4.7% of patients with AL. Patients with IgM amyloidosis are on the average six years older than non-IgM patients and have a 32% incidence of neuropathy. The incidence of non-IgM AL amyloidosis with neuropathy is, in contrast, 11%. Dr. Gertz suggests that IgM amyloid proteins have a greater affinity for peripheral nervous system tissues and a lower affinity for cardiac tissues.

Dr. Jean-Paul Fermand discussed the renal manifestations of WM. He noted that in WM and in other IgM-secreting monoclonal proliferation diseases renal lesions are less frequent than in other monoclonal gammopathies such as MM. Nonetheless, IgM AL amyloidosis can injure the kidneys, and WM patients with very high serum levels of IgM and corresponding serum viscosity can get deposition of IgM in the kidney, resulting in acute injury. There are other causes of renal injury in WM patients that are not related to WM itself, and Dr. Fermand underscored the importance of kidney biopsies when faced with such issues.

Dr. Bouchra Asli, Hôpital Saint Louis, Paris, described the physiopathology of Schnitzler syndrome. Schnitzler syndrome is a rare acquired auto-inflammatory syndrome defined by the association of a non-itchy rash (non-pruriginous urticaria) with a monoclonal IgM gammopathy. Other clinical features of this syndrome may also include fever, bone pain, adenopathy (enlarged lymph nodes), and hepatosplenomegaly (enlarged liver and spleen). The treatment of Schnitzler syndrome was, until recently, the antibiotic pefloxacin, which offered only symptomatic relief and was frequently unsatisfactory. Dr. Asli commented on the recently demonstrated dramatic symptomatic effect of the interleukin 1 receptor antagonist called anakinra. A novel agent, anakinra was quite effective in lowering inflammatory markers (particularly C reactive protein) but had no effect on IgM production per se. This observation does pose the question: are the symptoms and signs of Schnitzler syndrome due to IgM infiltration or to the release of inflammatory mediators? Further review of the long term effect of anakinra in controlling the symptoms of the disease is ongoing.

Dr. Marvin Stone, Baylor Sammons Cancer Center, Baylor University, Dallas, Texas, discussed the pathogenesis and morbidity of autoantibody syndromes in WM. Autoimmune disorders in WM include cold agglutinin disease, mixed cryoglobulinemia, and immune-mediated polyneuropathy (better known as PN to most WM patients). Dr. Stone noted that 10-20% of WM IgMs have definable antibody activity but that this may indeed be an underestimation as population-based studies suggest that infection and inflammation are often associated with chronic antigenic stimulation in some patients who develop WM and other plasma cell dyscrasias. The clinical presentation and natural history of WM patients with autoimmune-mediated IgM-related syndromes are distinct, and rapid recognition of signs and symptoms leads to correct diagnoses and subsequent initiation of appropriate treatment.

Dr. Sigbjorn Berentsen, Institute of Medicine, University of Bergen, Bergen, Norway, reported on a study of patients with cold agglutinin disease treated with the combination rituximab and fludarabine (R+F). The results of this prospective, uncontrolled, multi-center trial suggest that R+F is a very effective combination in patients with cold agglutinin disease – 76% of patients responded to therapy, including 21% obtaining complete responses; 24% of patients were non-responders; and median time to response was 4 months. The author did note that toxicity is more prevalent with R+F versus rituximab as single agent therapy; nonetheless, combination therapy resulted in high response rates and durable responses in chronic cold agglutinin disease.

Dr. Franco Dammacco, University of Bari Medical School, Bari, Italy, discussed the association between herpes C virus (HCV) infection and WM. Although an association between WM and HCV infection has been suggested in past studies, no definitive evidence has ever been demonstrated. Nonetheless, patients with WM mixed cryoglobulinemia have a very high prevalence of HCV infection, and people with HCV infections have a 3-fold higher risk of developing WM. Dr. Dammacco concluded his talk by recommending that WM patients with HCV mixed cryoglobulinemia be treated with the antivirals interferon and ribavirin as well as rituximab.

Dr. Roger Owen presented an interesting talk on histological transformation to other lymphomas that can occur in WM patients. We now know that treatment with nucleoside analogues (e.g. fludarabine) carries a 5-10% risk of WM transforming to a more aggressive disease. Although histological transformation was initially thought to occur as a result of accumulated genetic abnormalities, sometimes secondary to repeated chemotherapy, new research suggests that WM transformation can arise from a variety of scenarios, including spontaneous development of diffuse large B-cell lymphoma (DLBCL) independent of the WM tumor itself; infection from the Epstein Barr virus (EBV) leading to DLBCL, Hodgkin's lymphoma, and a newly described EBV-positive mucocutaneous ulcer entity; a second unrelated WM tumor discovered during evaluation of DLBCL; and other less common factors. Dr. Owen, one of the world's top experts in WM pathophysiology, recommends that future studies are essential to understanding the complex processes involved in WM transformation events.

The last lecture of the workshop was presented by **Dr. Fred Hochberg**, Massachusetts General Hospital, Boston, Massachusetts, on the very interesting and worrisome Bing-Neel syndrome (BNS). Although WM produces peripheral neurologic complications in over half of patients, rarely do we see involvement of structures in the brain and spinal cord. Dr. Hochberg and his colleagues reviewed their clinical experiences with BNS together with cases reported by others to accumulate 31 examples of BNS. They subsequently classified these cases as either Group A with evidence of lymphoplasmacytoid (LMP) cells within the central nervous system or Group B with LMP cells absent but symptoms and/or signs explained by an autoimmune mechanism. Of the patients studied, 61% had BNS in the setting of progressive WM when BNS developed subsequent to a diagnosis of WM. A median of 36 months separated a diagnosis of BNS from the initial diagnosis of WM. However, 26% of the patients reviewed had coincident occurrence of BNS and WM at diagnosis. Neurologic symptoms attributable to BNS include cortical dysfunction (memory deficits and behavioral changes), visual field deficits, optic nerve lesions, cranial nerve sensation changes, and amnesia. Spinal cord changes were noted in 67% of the BNS WM patients studied. The treatment of WM in the setting of BNS does offer some hope as 42% of responders sustained a response from 6 months to 4 years while three non-responding patients succumbed within 8 months. Dr. Hochberg commented that BNS may be both over-diagnosed and under-diagnosed, and in order to increase the accuracy of BNS diagnoses he proposed a registry for WM BNS using criteria provided for the diagnosis of BNS Group A and Group B. These proposed criteria would include cerebrospinal fluid flow cytometry and/or immunohistochemistry and light chain quantification, and contrast-enhanced MRI of the central nervous system and the spine.

Conclusion and Thanks to the Organizers

As the tired reader can easily surmise, the amount of information presented at these meetings can be overwhelming. Having attended a few of these conferences, I usually am struck by a few presentations that stand out from the rest because of importance or sheer “wow” factor. In no particular order of significance, here are the “take home messages” from Venice as I see it:

1. **Dr. Roger Owen** from the UK presented a very convincing argument regarding the need for more frequent bone marrow biopsies (BMBs). Given that we have more and more new targeted therapies that are so specific to certain pathways (and as a result such therapies generally result in fewer side effects), it is imperative to get BMBs prior to starting any new treatment and sometimes even during the actual course of a treatment plan in order to assess the response exactly. Measuring serum IgM levels exclusively is a fool’s errand.
2. **Dr. Mathias Rummel** from Germany presented a well-executed Phase III randomized trial comparing the standard R-CHOP versus bendamustine and rituximab (B-R) for WM. It is clear that B-R is the new standard: better results, far fewer side effects.
3. **Dr. Andy Rawstron**, colleague of Dr. Owen, delivered a fascinating yet quite sobering lecture on the progression of disease in indolent lymphomas. Drawing predominantly on his experience with CLL, he noted that over time normal B-cells are replaced with abnormal B-cells, even in the setting of an asymptomatic disease state. These findings, which Dr. Rawstron plans to study in detail in WM, give pause to individuals who prefer to watch and wait for extended periods of time without a periodic and accurate gauging of the status of their disease. Ignore appropriate periodic bone marrow biopsies at your own peril.
4. **Dr. Guang Yang**, monoclonal antibody “expert” from the prolific research department at the Dana Farber Cancer Institute, presented a stunning report on the new monoclonal antibody GA101, basically a “super rituximab” that is able to overcome unfavorable genetics that play a large role in treatment failure with rituximab. One can only hope that clinical trials with GA101 in WM are soon to begin and that WM patients line up to participate in this trial testing a very exciting new option in the treatment of WM.
5. **Dr. Fred Hochberg**, relative newcomer to the WM research community but a very experienced and distinguished clinician in neuro-oncology at Massachusetts General Hospital, has truly started to unravel some of the mysteries of Bing-Neel Syndrome (BNS), a devastating complication seen in ever-increasing frequency in WM patients. I believe that BNS poses a serious threat to WM patients as we push the survival curve beyond 15 and even 25 years!
6. The young investigators who presented at the Venice IWWM-6 conference hold the promise of better futures for WM patients worldwide. These bright individuals must be supported and encouraged to continue their amazing research in WM. We are seeing more and more young (and nervous) investigators present at conferences, and the sophistication of their work is only eclipsed by the obvious interest they have in the pursuit of their research. The IWWMF is proud to help sponsor these young investigators: is there a better investment in the future and prolonged health of WM patients?
7. Finally, **Dr. Steven Treon**, WM researcher and clinician extraordinaire, has the uncanny ability to remember an unbelievable amount of data, synthesize all of it, and come out with prescient statements. One of the many statements made, echoed by numerous WM experts at the Venice conference, is the increasing evidence that aggressive treatment for WM may be a better overall strategy than more timid approaches as it results in more complete responses (CR) or very good partial responses (VGPR), and these translate into improved overall survival.

The success of the Sixth International Workshop on Waldenström's Macroglobulinemia owes a great deal of thanks to the tireless efforts of Dr. Steven Treon, Christopher Patterson, and the entire team at the Bing Center for WM, DFCI. The IWWMF is proud to have been a sponsor of this event.

As a trustee of the IWWMF, and as a 10 year+ WM patient, I am very grateful for the incredible amount of work and dedication that everybody involved demonstrated on behalf of WM patients and caregivers worldwide. I very much look forward to IWWMF-7 to be held in Newport, Rhode Island, USA, and I anticipate many more years of fruitful cooperation between the IWWMF and their close friends at the Bing Center for WM, DFCI. Well done!

Donate and participate.

Dr. Guy Sherwood is IWWMF Trustee and Member of the IWWMF Board of Directors, Chair of the IWWMF International Committee, and Member of the IWWMF Research Committee.