

SUMMARY OF LECTURES AND SCIENTIFIC POSTERS FROM THE 5TH INTERNATIONAL WORKSHOP ON WALDENSTRÖM MACROGLOBULINEMIA

STOCKHOLM, SWEDEN, OCTOBER 15-19, 2008

The IWWM5 conference was organized in ten formal sessions: Incidence and Predispositions to WM; Genetic Basis and Pathogenesis of IgM and WM Related Disorders (two sessions); Micro-environment and Immune Regulation in WM; Prognostic, Predictive and Response Markers in WM; Treatment of WM; Novel Agents for Treatment of WM; Transplant Therapy in WM; Therapy Related Complications in WM; Disease Related Morbidities in WM. Each session consisted of a series of 15 minute lectures followed by a panel discussion (much like the popular “Ask the Doctor” session at IWWMF Education Forums). There were three additional special guest lecture presentations, and eight young investigator presented research results in poster format.

The following is a summary of the 59 lectures (and 8 posters) I attended in Stockholm. Relevant comments from the panel discussions have been added to the summaries. These summaries are not intended to replace the actual lectures, abstracts, or future medical and research journal articles from the original authors. I have sought to present what I consider to be the salient points from each lecture and poster in the most layperson-friendly language that I could muster, without altering the content of the lectures or posters. A glossary of terms is appended to this document, and words that are in blue text and hyperlinked have been reviewed in this glossary. The glossary does not replace a good medical and scientific dictionary—there are many excellent Internet-based glossaries, and several of the IWWMF publications include glossaries as well.

I hope and expect that my WM friends and colleagues will correct any inadvertent mistakes I may have made; I do apologize in advance for any of these mistakes. I wish to thank the IWWMF, especially IWWMF President Judith May and the IWWMF Board of Trustees, for having given me the opportunity to attend this very exciting and wonderfully educational conference. Special thanks as well to all the researchers throughout the world who are actively pursuing the common goal of a cure for WM and, last, but certainly not least, to the brilliant young investigators who are the future of WM research. I am very grateful for your interest in this bizarre and rare disease and I wish you continued success in your future endeavors.

Guy Sherwood MD, FCFP, FAAFP
December 2008

Editor’s Note: additional information regarding the 5th workshop and prior workshops can be found at the IWWM website: <http://www.wmworkshop.org>

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Session I - Incidence and Predispositions to WM

- Dr. Michael Wang: **Trend and geographic variations in the incidence of Waldenström's macroglobulinemia in the USA.**

This ambitious [epidemiological](#) study utilized Surveillance, Epidemiology, and End Results (SEER) tumor registry data from 1988 to 2004 to identify 1463 patients with WM out of 78,558 patients with non-Hodgkin's lymphoma (NHL). Various statistical tools were then used to analyze the data. This study concluded that the overall incidence of WM (2% of NHL) did not vary significantly from 1988 to 2004. The incidence of WM was higher in male Caucasians, and there appeared to be geographical variations in the incidence of WM in the United States. This last observation was quite interesting, but further clarification was not possible since the lead author was unfortunately not able to present his research at the conference.

- Dr. M. Iwanaga: **Prevalence of MGUS and Incidence of WM in Japan.**

It is well known that the prevalence of [Monoclonal Gammopathy](#) of Undetermined Significance (MGUS) is twice as high in African-Americans in the US compared to US Caucasians. The 2-fold higher prevalence of African-Americans over Caucasian Americans is confirmed by a study of Ghanaians. US Asians similarly have a lower prevalence of MGUS than US Caucasians. This study evaluated whether the incidence of MGUS and WM in Asians resident in Asia is higher or lower than the incidence in Caucasians and African-Americans in the US. The authors also studied the effect of radiation exposure on MGUS prevalence in 52,781 atomic bomb survivors diagnosed between 1988 and 2004. The prevalence of MGUS was noted to be 2.4% at age 50+ years, which is lower than in Caucasians and Africans. The effect of radiation exposure on MGUS prevalence was significantly higher (3.6–4.2%) in men exposed to a higher radiation level, particularly if they were exposed at younger than 20 years of age. Between 1996 and 2003, the prevalence of WM in Japan was noted to be 0.053 per 100,000 person-years (0.089 for men, 0.028 for women), which is much lower than in the US (0.63 per 100,000). The median age at diagnosis was similar to that in the US at 73 years (ranges 26–96), and the age-specific incidence rates increased sharply with age, as is the case in the US.

- Dr. Robert A. Kyle: **IgM Monoclonal Gammopathy of Undetermined Significance (MGUS) and Smoldering Waldenström's Macroglobulinemia (SWM).**

Dr. Robert Kyle is the world's leading expert on MGUS. This succinct lecture described the risk of progression from MGUS to a more severe condition, as well as the risk of progression from smoldering WM (SWM) to symptomatic WM. Dr. Kyle defined the terms MGUS and SWM in his lecture, and these certainly bear repeating in this summary: "IgM monoclonal gammopathy of undetermined significance (IgM-MGUS) is defined as a serum IgM monoclonal protein < 3 g/dL, bone marrow [lymphoplasmacytic](#) infiltration < 10%, and no evidence of anemia, constitutional symptoms, hyperviscosity, [lymphadenopathy](#), or [hepatosplenomegaly](#). Smoldering Waldenström's macroglobulinemia (SWM) is defined as a serum IgM monoclonal protein > 3 g/dL and/or > 10% bone marrow lymphoplasmacytic infiltration but no evidence of symptomatic anemia, constitutional symptoms, or hyperviscosity." Dr. Kyle studied 213 Mayo

Clinic patients diagnosed with MGUS who progressed to NHL (14%). Of these 17 patients who progressed, 6 developed WM (35%). In other terms, the relative risk (RR) of a MGUS patient developing NHL was 15 fold greater than a non-MGUS patient; the RR of a MGUS patient developing WM was 262 times greater than a non-MGUS patient. The risk of progression from MGUS to NHL was 1.5% per year. In another study, 48 patients with SWM at Mayo Clinic from 1974 to 1995 were evaluated. The risk of progression to symptomatic WM was 53% at 5 years (10.5% per year). Risk factors for progression from SWM to symptomatic WM included the degree of bone marrow lymphoplasmacytic cell infiltration, serum IgM level, hemoglobin level, and reduction in serum IgA. Dr. Kyle did state that the risk of WM patient's child developing WM is extremely small: "Frankly, why even bother to worry them, the incidence is so low".

- Dr. H. Ögmundsdóttir: **Familial predisposition to MGUS, WM and MM.**

This very interesting study of an Icelandic family with multiple cases of MGUS, WM, and MM highlights the importance of studying the natural history and hereditary predisposition of rare diseases in order to better understand how they develop in susceptible individuals. Family members of WM patients are more likely to develop WM, establishing a stronger genetic link in WM compared to multiple myeloma (MM). The family in question had individuals who tended to produce higher levels of IgG, IgA and IgM in response to antigen stimulus ("hyper-responders"). The antigen-stimulated B-cells of these individuals also lived longer than normal and had higher levels of Bcl-2 (an anti-[apoptotic](#) molecule that prolongs cell survival). Levels of CD-27 (B-cell marker) and CD-95 were also elevated. Family studies can play an important role in identifying the genetic and biologic causes of immunoglobulin-producing cell diseases.

- Dr. M. McMaster: **The relationship between Waldenström macroglobulinemia and IgM-MGUS: studies of high-risk families.**

This well-know scientist from the National Institutes of Health has been studying WM patients and their families in order to identify genetic markers or abnormalities that will help understand the biology of WM, identify patients at risk of developing WM, and help prevent the development of WM in susceptible patients. Dr. McMaster defines "WM families" as families with at least 2 cases of WM. We are now aware of the link between MGUS and NHL, and further studies have suggested that there appears to be a genetic link between IgA/IgG-MGUS and MM, as well as a link between IgM-MGUS and WM (there does not seem to be a link between IgA/IgG-MGUS and WM, nor between IgM-MGUS and MM). Greater than 60% of patients with IgM-MGUS who went on to develop WM have a familial link. There is evidence of 4 chromosomal regions (1q, 3q, 4q, 6q) that link WM familial cases. Dr. McMaster states that there are multigenic (multiple genes) influences in familial WM, and she adds that environmental factors may indeed play an important role as well.

- Dr. S. Kristinsson: **Risk of lymphoproliferative disorders among first-degree relatives of lymphoplasmacytic lymphoma/Waldenström’s macroglobulinemia patients.**

In this population-based study from Sweden, 2,144 LPL/WM patients (1,539 WM [72%] and 605 LPL [28%]) diagnosed between 1966 and 2005 were identified. The mean age at diagnosis was 71, and men comprised 60% of the patients. First-degree relatives of LPL/WM patients had a 20-fold, 3.0-fold, 3.4-fold, and 5.0-fold increased risk of developing LPL/WM, NHL, CLL, and MGUS respectively. There was no evidence of an increased risk of developing multiple myeloma or Hodgkin lymphoma. This large study further reinforces the finding of increased genetic predisposition of developing LPL/WM, NHL, CLL, and MGUS among first-degree relatives of LPL/WM patients.

- Z. Hunter: **Increased prevalence of monoclonal gammopathy, abnormal immunoglobulin levels, and recurrent infections in family members of patients with Familial Waldenström’s macroglobulinemia.**

Many IWMM Ed Forum participants are familiar with this ongoing IWMM-funded study from Dr. Treon’s WM research lab at the Dana-Farber Cancer Institute in Boston. Using collected blood samples and cheek cell DNA from first and second degree family members of WM patients, the investigators evaluated the familial predilections for WM. Among family members of patients with WM and a family history of B-cell disorders (familial prevalence of B-cell disorders is seen in up to 20% of patients with WM), higher incidence of recurrent sinus infections were noted, abnormally low IgA and elevated IgM levels were observed, as well as a 10-fold increase in the incidence of [monoclonal gammopathies](#) (undiagnosed MGUS was noted in 23% of WM families). Further molecular studies evaluating the underlying genetic basis for these observations are currently underway in the Dana-Farber laboratory. As a result of this research (and other ongoing research in many other labs throughout the world), there now appear to be potentially different heritable factors for what are now tentatively introduced as WM “subtypes”.

Session II – Genetic Basis and Pathogenesis of WM and IgM Related Disorders.

- Dr. Linda M. Pilarski: **Origins of Waldenström’s macroglobulinemia.**

The genetic factors resulting in WM likely involve inherited genes and [alleles](#) and may also include genetic mutations acquired during the life of an individual that lead to the development of WM. Dr. Pilarski’s lab at the University of Alberta, Canada (funded in part by the IWMM) is studying the hyaluronan synthase 1 (HAS1) gene in malignant and normal cells from patients with WM. The HAS1 gene synthesizes hyaluronan, a large structural molecule involved in cancer spread and growth. Abnormal HAS1 gene replication in WM has been identified, and the resulting abnormal HAS1 gene variants produce a biologically active molecule that appears to be involved in the transformation of a normal cell into a malignant WM cell. 100% of the WM B-

cells have the HAS-1 mutation. Acquired HAS1 mutations are also found in malignant cells as well as nonmalignant CD34+ progenitor cells (stem cells). This suggests that the HAS1 gene abnormalities precede the development of malignant cells and contributes to the initial transforming events in WM as well as to the disease progression. Evaluating the presence of HAS-1 mutations may enable clinicians to identify which MGUS patients may go on to develop WM. In order to study the cancer stem cells in WM, Dr. Pilarski's team also developed a 3-D culture system that mimics the WM bone marrow microenvironment. WM cells are subsequently able to grow in this novel 3-D culture. This fascinating 3-D model permits the study of the growth of WM cells and to makes the mysterious WM cancer stem cell accessible. Further development and refining of this model will enable researchers to determine the sensitivity of WM to new drugs and treatments.

- Dr. S. Sahota: **CD27 in defining B-cell memory origins in WM.**

Mutations in the immunoglobulin variable gene (VH) regions following exposure to an antigen are necessary in the development of normal memory B-cells. There is a high frequency of these VH mutations in WM cells. Some WM cells from different individuals have been recently found that have no VH mutations. It is now currently suggested that there are two subsets of WM: 95% of them have mutated VH genes, 5% unmutated. In the mutated WM type, which is significantly more common, there now appear to be continuous, on-going mutations in some of the daughter cells. This results in genetic differences, or intraclonal variations, within the specific tumor cell population itself. Furthermore, some of these genetically different daughter cells can also switch from IgM to IgG/IgA production, an event that was not thought possible until recently. The molecular marker CD27, implicated in B-cell activation, is thought to be found on the surface of all memory B-cells. However, Dr. Sahota and his team were able to identify WM cell lines that traced back to CD27+ve or CD27-ve memory B-cells. Genetic analysis of this distinct IgM+CD27-ve B-cell memory population revealed low levels of mutations. These IgM+CD27-ve cells, given the right circumstances and subsequent *oncogenic* mutations, could in turn give rise to WM. These findings question whether all WM tumors actually arise from the typical normal memory B-cells (CD27+ve). This complex genetic research essentially demonstrates that the mutational status of the VH gene is a much more important marker of WM origin than CD27 expression, since some memory B-cells may simply shed the CD27 marker as the WM tumor develops. The mutational status of the VH gene may also suggest a different pathway of origin than the memory B-cell lineage for some WM tumors. The definite origins of the WM cell(s) remains elusive.

- Dr. F. Nguyen-Khac: **Cytogenetic abnormalities in a cohort of 120 untreated patients enrolled in the WM1 trial; trisomy 4 prevalence.**

120 untreated WM patients enrolled in a clinical trial in France were studied using genetic research techniques. The average age of the patient was 66 years of age, the sex ratio was 2M/1F, and the average bone marrow infiltration was 53%. Among the 45% of individuals evaluable who had abnormalities in their chromosomal analysis, the most common abnormality (27%) was a missing piece of chromosome 6 (6q deletion), followed by 14% who had *trisomy* 4 (three

copies of chromosome 4 instead of the normal two) or partial trisomy 4, and other abnormalities. Using more refined techniques such as [FISH](#), specific deletions of 6q21 were observed in 26% of individuals. The 6q deletion is the most frequent reported genetic abnormality in WM. Trisomy 4 is also noted in 14% of these WM patients (but not seen in other B-cell malignancies). Chromosomal abnormalities in WM are therefore specific to this disease and are different than those found in other B-cell neoplasms.

- Dr. M. Eurelings: **Cytogenetic aberrations in polyneuropathy associated with IgM monoclonal gammopathy.**

Patients with monoclonal gammopathies who also have neuropathy are more prone to developing WM than those without neuropathy. In this small study from the Netherlands, 12 patients with IgM-MGUS and 10 patients with indolent WM, all of them with neuropathy, were evaluated. No chromosomal abnormalities were found in the 12 patients with IgM-MGUS. Of the 10 patients with WM, four patients had genetic abnormalities in their immunoglobulin genes. In WM, the immunoglobulin genes (VH) are implicated in neuropathy. Of particular interest is the fact that many of these mutated VH genes are related to the immune response to bacterial infections. This suggests the possibility of an antigenic trigger (a bacterial infection for example) in the development of the neuropathy-producing IgM seen in some 30% of WM patients.

- Dr. E. Braggio: **NF- κ B abnormalities in Waldenström's Macroglobulinemia.**

In this IWMF-funded study, genes involved in the production of nuclear factor kappa B (NF- κ B) were analyzed. NF- κ B is involved in the production of hundreds of biomolecules involved in inflammation, immunity, cell growth and cell death ([apoptosis](#)). NF- κ B has also been implicated in the development of WM, although its exact role is poorly understood. Using sophisticated genetic research techniques, Dr. Braggio (Mayo Clinic, Scottsdale Arizona) noted that two important genes, TNF receptor-associated factor 3 (*TRAF3*) and tumor necrosis factor alpha-induced protein 3 (*A20/TNFAIP3*), were inactivated in WM patients, leading to the increased production of NF- κ B. This in turn results in the activation of complex biochemical pathways and interactions in the WM bone marrow microenvironment. The identification of genetic mutations affecting NF- κ B pathways can possibly identify a subset of WM patients who may benefit from treatment with Velcade (Velcade is a strong inhibitor of the NF- κ B pathways). This would be an example of patient-individualized therapy.

Session III – Genetic Basis and Pathogenesis of WM and IgM Related Disorders.

- Dr. R. Garcia-Sanz: **Immunoglobulin gene rearrangements in Waldenström's macroglobulinemia.**

This large Spanish research study of the rearrangements in VDJH and DJH segments of the immunoglobulin gene(s) in 56 symptomatic WM, 36 asymptomatic WM and 14 IgM-MGUS patients highlights important genetic mutations found in these related disorders. The most frequently examined gene segments in WM were VH3 and VH3-23, which markedly differs from normal B-cells. The VH3-23 segment in particular is quite different from CLL but similar to MM, reinforcing the proposed similarities between WM and MM as opposed to studies suggesting that WM and B-CLL are closely related diseases. Of interest is the observation that the presence of the VH3-23 mutated segment is generally found in WM patients with high IgM levels, normal platelet levels, lower bone marrow infiltration, and generally more indolent disease. The absence of the VH3-23 mutated segment seems to indicate worse prognosis. As noted in an earlier study presented at this conference, it appears that we can now state that class-switching (IgM to IgG/IgA), long felt to be impossible in WM, is possible. This study also suggests that intracлонаl variation (different IgM producing WM cells within a WM tumor), also thought to be absent in WM, is now possible.

- Dr. S. Adamia: **High-throughput microRNA profiling: Identification of MicroRNAs with potential pathogenetic roles in Waldenström's macroglobulinemia.**

This cutting-edge research from Dr. Adamia, formerly of Dr. Pilarski's lab at the University of Alberta, Canada, and now at Dana-Farber Cancer Institute in Boston, MA, is evaluating the role of microRNAs in WM. MicroRNAs (miRs) are small RNA molecules that regulate the production of proteins. MicroRNA assays were performed in 13 WM, 79 MM, and 13 healthy patients. Results indicate significant up-regulation as well as down-regulation of specific miRs in CD19+WM cells of WM patients compared to healthy patients. It appears that some of these miRs in question are implicated in critical pathways responsible for apoptosis (cell death), hematopoietic cell differentiation, as well as cell proliferation and survival in WM. MicroRNAs have the potential to be good targets for future drug therapy.

- Dr. L. Xu: **5-Azacytidine inhibits the mammalian target of rapamycin complex 1 signal And induces apoptosis in Waldenström's macroglobulinemia**

The drug 5-Azacytidine (5-AzaC) has been approved by the FDA for the treatment of [*myelodysplastic syndrome \(MDS\)*](#). Dr. Lu, a member of Dr. Treon's research team at Dana-Farber Cancer Institute in Boston, investigated the effect of (5-AzaC) in WM cells. 5-AzaC was

very effective in the WM laboratory cell line BCWM1. 5-AzaC also induced cell death in samples of WM cells from patients but demonstrated no significant side-effects in white blood cells from healthy donors. 5-AzaC inhibits the mTORC1 pathway (involved in the regulation of cell growth, cell proliferation, cell motility, cell survival, and protein synthesis) . The combination of the drug 5-AzaC with the experimental drug RAD001 (currently in clinical trials in WM) has the potential to be a very effective combination in WM.

- **Dr. S. Ansell: Role of CCL5 and interleukin-6 in the Biology of Waldenström's macroglobulinemia.**

This important IWMF-funded research from Dr. Ansell of the Mayo Clinic in Rochester, MN, studied the roles of interleukin-6 (IL-6) in WM. [Interleukins](#) are a group of [cytokines](#) (messenger molecules) that are released by white blood cells . Dr. Ansell has confirmed that IL-6 is significantly elevated in WM patients compared to healthy patients, and has also found that the levels of the cytokine CCL5 (which regulates IL-6 secretion) are elevated in WM. Connective tissues cells (stromal cells), and WM cells from the bone marrow of WM patients were tested in order to evaluate their ability to secrete IL-6. All cell types secreted IL-6, with stromal cells secreting the most. CCL5 was also found to significantly increase IL-6 secretion by stromal cells and WM cells. IL-6 appears to have a minor effect on cell proliferation but has no effect on cell viability. However, IL-6 does increase IgM secretion by WM cells in a dose-dependent manner. Drugs that target CCL5, IL-6 secretion, or the IL-6 signaling pathway may one day be proven useful for WM patients.

- **Dr. T. Giordano: Role of hepatitis C in Waldenström's macroglobulinemia.**

Approximately 2% of the world's population is infected with the Hepatitis C virus (HepC). This chronic viral infection is the most common cause of cryoglobulinemia, especially mixed cryoglobulinemia. The HepC virus can infect B-cells and can subsequently increase the risk for non-Hodgkin lymphoma by 20%. Although chronic HepC infection is neither necessary nor sufficient for the development of WM (despite a 3 fold higher risk), further research into the putative link between chronic HepC infection and WM may help explain the steps leading to the development of WM.

- **Dr. O. Landgren: Chronic immune stimulation and subsequent Waldenström's macroglobulinemia.**

As noted in the previous study, certain autoimmune and infectious conditions are associated with increased risk of non-Hodgkin's lymphomas (NHL). Some have suggested that chronic inflammation may increase the risk for developing WM. This study evaluated the role of a wide range of chronic immune stimulatory conditions and WM in four million U.S. veterans. 361 WM cases with up to 27 years of follow-up were identified. In this largest epidemiological study of WM risk factors to date, an increased risk of WM was found among persons with a history of

autoimmune diseases (particularly Sjogren's disease, 13x increase), auto-antibodies (2-3x), hepatitis B (5.5x), HIV (12x), and the infectious disease rickettsiosis (3.4x). The age-standardized incidence of WM was 0.34/100,000 person-years. As has been suggested in other studies, exposure to immune modulating drugs, chronic antigenic stimulation of the immune system and perhaps even certain vaccinations can increase the risk of WM.

Session IV – The Micro-environment and Immune Regulation in WM

- Dr. E. Hatjiharissi: **Gene expression profiling of malignant and microenvironment cells in Waldenström's macroglobulinemia: therapeutic implications.**

The bone marrow in WM is infiltrated by a malignant population of small lymphocytes and lymphoplasmacytic cells (CD19+CD138-), as well as mature plasma cells (CD19-CD138+). This study of [gene expression profiles](#) in WM bone marrow cells and normal bone marrow cells revealed a number of over-expressed genes in the WM tumor cells. Many of these over-expressed genes (such as BCL-2, TACI, CD-40, and others) are implicated in cell survival, cell proliferation, and cell death pathways. The WM bone marrow microenvironment also includes stromal cells (non-cancerous connective tissue cells such as fibroblasts, mast cells, etc.) that assist the growth and proliferation of WM tumor cells. Not surprisingly, these stromal cells have gene expression profiles that demonstrate over-expressed genes implicated in cytokine production, cell proliferation, and others. These findings underline the very important role the microenvironment plays in WM tumor growth and survival. Many of these over-expressed genes may someday become targets for therapeutic drugs.

- Dr. E. Terpos: **Angiogenesis in Waldenström's macroglobulinemia.**

The formation of new blood vessels (angiogenesis) is an important feature of many hematological malignancies. An earlier study found that increased blood vessel density (intermediate or high grade angiogenesis) in 30% of WM patients had a weak correlation with bone marrow infiltration percentage and more importantly had no impact on patients' survival. This research group from Greece evaluated the serum levels of angiogenic cytokines (messenger molecules that stimulate blood vessel formation) in WM patients in various stages of the illness. Patients with either WM or IgM-MGUS had increased levels of angiogenic cytokines (angiogenin, angiopoietin-1, angiopoietin-2). Angiogenin levels correlated with disease status: increased levels (compared to healthy patients) in IgM-MGUS and untreated WM; reduced in WM patients in remission, and increased again in relapsed/refractory WM. The angiogenic cytokine levels also correlated to albumin levels, beta-2-microglobulin levels, hemoglobin and degree of lymphadenopathy. The authors report that elevated levels of a potent macrophage chemokine (white blood cell attractant) also results in increased angiogenesis in several malignancies, including WM. In effect, WM patients with high levels of the chemokine MIP-1alpha also had high levels of macrophages in the bone marrow: this in turn leads to increased angiogenesis. (Note: the drugs thalidomide and lenalidomide (revlimid) are anti-angiogenesis agents).

- Dr. E. Ocio: **Immunophenotypic comparison of IgM MGUS and Waldenstrom's macroglobulinemia.**

The differences in the *immunophenotype* (presence of protein markers on cell membranes, i.e. CD20, CD52,) in bone marrow samples from 210 WM patients and 60 IgM-MGUS were studied. As expected, a higher infiltration by B-lymphocytes and lymphoplasmacytic cells was noted in the bone marrow of WM patients. Plasma cells only comprised about 2% of the cell population in WM and IgM-MGUS, and mast cells were increased in WM. With respect to the immunophenotypic analysis, the expression of CD22, CD25, and CD27 on B-lymphocytes was highest in symptomatic WM, followed by asymptomatic WM, and lowest in IgM-MGUS. Plasma cell analysis on the other hand showed opposite results for CD19 and CD45. Of interest was the presence of the CD20 marker on plasma cells in symptomatic WM (40%), asymptomatic WM (17%), and IgM-MGUS (9%). Plasma cells in healthy individuals are usually negative for CD20. B-lymphocytes in the IgM-MGUS patients displayed higher expression of CD20, CD38 and CD5.

- Dr. N. Munshi: **TH17 pathway and associated pro-inflammatory cytokines in Waldenström's macroglobulinemia (WM).**

Immune dysfunction is a characteristic of WM and MM. Hypogammaglobulinemia (low levels of normal immunoglobulins/antibodies), inadequate vaccine responses, as well as T-cell and B-cell dysfunctions are common in WM. TH17 cells are a subset of T helper cells that produce interleukin 17 and are thought to be involved in the development of normal immunity as well as autoimmune diseases. TH17 cells are decreased in WM, as opposed to MM. The levels of the more common T helper cells TH1 and TH2 were also reduced in WM, compared to healthy patients. The levels of important immune-related cytokines (IL-2 IL-15 and, GM-CSF) were elevated in WM as were certain TH17 cell-associated cytokines (IL-1-beta and IL-17). The TH17 cells in WM patients seem less responsive to normal immune-related stimulus. Immune dysfunction in WM appears to be quite a different process than that seen in MM.

- Dr. D. Joshua: **T cell immunity in Waldenström's macroglobulinemia and myeloma.**

The immune system's T-cells (a subset of white blood cells) help keep WM tumors indolent. Transformation to a more aggressive lymphoma is sometimes seen in WM after these T-cells are depleted by chemotherapeutic agents such as cladribine and fludarabine. The transfer of cell membrane material between T- cells (for example, the transfer of T-cell receptors such as CD80 and CD86) is called *trogocytosis*. This fascinating communication mechanism between helper T-cells and cytotoxic killer T-cells may be a method whereby tumor cells can escape detection by the body's immune system. Trogocytosis may lead to the suppression of cytotoxic killer T cells and thus to decreased tumor suppression. Treg cells (cells that have undergone trogocytosis) can be quite common in MM (30% of MM patients) and indicate a worse prognosis. In this study there was no detectable trogocytosis in the blood samples from 11 patients with WM. These results help support the concept of T cell disease control in WM.

Session V: Prognostic, Predictive and Response Markers in WM

- Dr. P. Morel: **International Waldenström macroglobulinemia prognostic index project.**

Researchers and clinicians need an effective WM disease staging system which can predict prognosis in the various stages of the disease process. The International Staging System for WM (ISSWM) was originally developed for WM patients who were initially treated with chlorambucil or fludarabine mainly. With the advent of new therapies, in particular the monoclonal antibodies such as rituximab, this staging system was re-evaluated and subsequently determined to be valid in identifying high-risk patients in advanced phases of the disease. The ISSWM staging system is currently defined as follows. Using the combination of adverse characteristics of age >65 years, Hgb <11.5 g/dL, platelet count <100,000, B2M >3 mg/L and M-protein >7.0 g/dL, **low risk** is defined by a patient of age 65 years or more who has at most one other adverse characteristic; **high risk** is defined by the presence of more than 2 adverse characteristics; remaining patients with 2 adverse characteristics plus age >65 years were classified as at **intermediate risk**.

- Dr. P. Chang: **Prognostic relevance of 6q deletion in Waldenström's macroglobulinemia.**

The presence of the common WM cytogenetic chromosomal abnormality on the long arm of chromosome 6 (6q deletion) was noted in 41.6% of the 77 WM patients in this Canadian study. There were no links between patients with the 6q deletion and age, gender, hemoglobin level, platelet count, serum viscosity, beta-2 microglobulin, albumin, IgM level and degree of bone marrow infiltration. Patients with 6q deletion had higher C-reactive protein levels. 93% of the patients with 6q deletion received treatment, whereas 80% of the patients without the deletion were treated. There was no significant difference in time to initial treatment or overall survival in either group of patients. This study suggests that the clinical outcome in WM is not influenced by the presence or absence of the 6q deletion.

- Dr. J. Feuillard: **Plasma cell differentiation in indolent lymphomas originated from marginal zones.**

This very complex study from France sought to elucidate the similarities and differences between two closely related indolent lymphomas: WM and marginal zone lymphomas (MZL). These two types of lymphomas are often very difficult to separate one from the other, and the pathological diagnosis is at times quite challenging. It is, however, very important to differentiate them, particularly insofar as treatment and prognosis are concerned. Indolent lymphomas such as WM, MZL, and especially those originating from the spleen (S-MZL), are thought to derive from the marginal zone of secondary lymphoid organs (such as the spleen, lymph nodes, etc.). The marginal zone is a region between the white pulp (predominantly white blood cells) and the red pulp (predominantly red blood cells) of the spleen. It is comprised

mostly of T-dependant memory B-cells and two other B-cell types: naive B-cells that respond to T-dependent antigens and T-independent B-cells. Using very sophisticated techniques, the physical characteristics, DNA gene transcription products, gene segments of the much studied IgHV immunoglobulin genes, and stage/type of plasma cell differentiation of these tumor types were evaluated. It has been suggested that chronic antigen stimulation is likely implicated in the development of MZLs of secondary lymphoid organs. The results from this study seem to indicate that WM and S-MZL not only have different mechanisms of transformation but also originate from B-cell compartments with two different antigen exposure histories.

- Dr. E. Kastiris: **Prognostication in young and elderly patients with WM.**

Although the median age at WM diagnosis is 70 years, the disease-related features and treatment outcomes in young (< 50 years of age) or in the very elderly (>75years) patients with symptomatic WM are not well known. Advanced age (age > 65 years) is a recognized adverse prognostic feature in WM. This study analyzed data from 240 previously untreated, symptomatic WM patients who subsequently underwent treatment: 9% were younger than 50 years and 20% older than 75. The clinical and laboratory features were similar among all patients in the study, as was the initial treatment used (chlorambucil, nucleoside analogs or rituximab-based regimens). Partial responses were observed in 68% of the young, 58% of the middle-aged (50-75 years of age) and in 56% of the older patients; median overall survival was not reached in young patients, whereas it was 113 months for the middle-aged and only 53 months for patients older than 75 years. The researchers recognized that older patients were subject to increased mortality from other non-disease related causes (stroke, heart attacks, etc.), and adjusted accordingly for this. Nonetheless, despite similar clinical and laboratory features and response to treatment for all age groups, the survival of older (>75 years) patients is significantly shorter than that of middle aged (50-75) or very young (< 50) patients. Older patients were treated with Rituxan more often than the other age groups, yet remained more susceptible to treatment-related mortality.

- Dr. R. Owen: **Complexities of assessing response in WM.**

The majority of oncologists rely on the measurement of serum IgM level to assess the response of treatment in WM patients. Unfortunately, we are now well aware that IgM levels are not representative of the overall tumor burden. Furthermore, maximum responses are often not seen until 6 months following completion of therapy (18 months when treated with purine analogs, i.e. fludarabine). The WM tumor comprises B-cells and plasma cells, (and even the intermediate “lymphoplasmacytic” cells). The B-cell component dominates in the majority of patients and has a typical immunophenotypic signature of sIgM+ CD20+ sCD79+ CD52+ CD138- COX2-; the plasma cell component is cIgM+ CD20- cCD79+ CD52- CD138+ COX2+. Monoclonal antibody therapy with either alemtuzumab (Campath - CD52) or rituximab (Rituxan – CD20) results in the depletion of the B-cells but does not appear to kill plasma cells. Patients treated with fludarabine (or other nucleoside analogs) also show selective depletion of B-cells in bone marrow studies. Some of these patients may potentially have been considered non-responders if only short-term serum IgM levels were used as a marker of disease burden; IgM levels do drop significantly in

many cases however 6-12 months later. Serum IgM levels are therefore not always a good indication of treatment response, and repeat bone marrow biopsies remain the gold standard for confirmation of actual disease status (particularly when treated with monoclonal antibodies and/or purine analogues). The serum free light chain (SFLC) assay is a helpful test as the majority of patients with symptomatic WM have abnormal results. MM studies have shown that the SFLC assay is a valuable indicator of early treatment response but not a reliable indicator of complete response. The persistence of plasma cells in the WM patient following treatment may also impair the use of this test in this disease. Sophisticated [flow cytometry](#) assays that are able to detect low levels of circulating tumor cells may also be helpful in determining early response to treatment.

- Dr. G. Yang: **Soluble CD27 is a faithful marker of disease burden and is unaffected by the rituximab-induced IgM flare, or by plasmapheresis in patients with Waldenström's macroglobulinemia.**

Serum IgM levels are routinely used in WM to assess disease status as well as response to treatment. The accurate assessment of treatment response may be complicated by an “IgM flare” phenomenon, often seen in the use of the monoclonal antibody rituximab. Removal of serum IgM by plasmapheresis may also impact the clinical assessment of the WM patient. Researchers from Dr. Treon’s lab at the Dana-Farber Cancer Institute in Boston have evaluated the soluble CD27 (sCD27) protein as a marker of WM disease status. The sCD27 protein is a member of the very important tumor-necrosis-factor (TNF) family of cytokines secreted by WM cells. This biomolecule, elevated in patients with WM, supports WM tumor growth in the bone marrow micro-environment by the induction of CD40L on mast cells (sCD27, CD70, April, CD40L, CD40, TACI, BCMA pathways). Using specifically timed blood tests in various WM patients including patients with stable or progressive disease, patients experiencing rituximab-induced flare, or patients pre- and 48-hours post plasmapheresis, the level of sCD27 was evaluated. Results of these observations revealed that the change in sCD27 levels closely followed changes in IgM levels in patients with stable, progressive, or treatment-responsive disease. Furthermore, sCD27 levels were actually reduced in patients who experienced an IgM-flare, and were essentially unchanged in a patient undergoing plasmapheresis. This study suggests that the sCD27 level is an accurate marker of disease status and is unaffected by patients undergoing either rituximab treatment or plasmapheresis.

Session VI: Treatment of Waldenström’s Macroglobulinemia

- Dr. M. Dimopoulos: **Rituximab based therapies in Waldenström macroglobulinemia.**

The monoclonal anti-CD20 antibody rituximab is effective either as a monotherapy or in combination with other chemotherapy in WM patients, whether previously treated or untreated. The combinations of rituximab and nucleoside analogues (fludarabine, cladribine, etc.) produce high response rates, and are very useful when rapid disease control is needed. The addition of

rituximab to two standard courses of cladribine (2Cda) may double remission time. When combined with agents such as dexamethasone and cyclophosphamide, an overall response rate (ORR) of 83% is seen. The frequently used combination regimen R-CHOP results in an ORR of 91%. These non-stem cell toxic first-line treatments are useful in patients where stem cell collection may be an option. The immunomodulatory drug thalidomide combined with rituximab, resulted in an ORR of 78% and median time to progression (TTP) for evaluable patients of 35 months. Dose reduction or discontinuation of thalidomide was required in all patients due to side-effects. Similarly, the combination of the immunomodulatory drug lenalidomide and rituximab resulted in an ORR of 67% and a median TTP of 15.6 months. Once again, side-effects, particularly significant anemia, question the appropriateness of this combination in WM. The combination of rituximab and bortezomib (Velcade) +/- others are currently being actively studied in clinical trials. Rituximab, bortezomib and dexamethasone in the primary therapy of WM patients resulted in an ORR of 96% including 17% of complete responses. Although median response rate was rapid (1.1 months), the development of peripheral neuropathy was significant. As a result, the combination of rituximab with reduced doses of bortezomib administration is being evaluated.

- Dr. M. Ghilmini: **Maintenance rituximab in indolent lymphoma.**

Treatment-related toxicity has always been a limiting factor in earlier studies involving maintenance chemotherapy. The primary objective in maintenance treatment is prolonged remission. The development of rituximab has now permitted the resurgence of maintenance treatment in selected indolent lymphomas. Rituximab immunotherapy appears safe, has minimal side-effects for the most part, and is able to prolong remission and overall survival. Given after chemotherapy, immunotherapy, or stem cell transplantation, maintenance therapy improves the quality of response whether it is given over a short period of time (rituximab consolidation), or over a period of years (rituximab maintenance). The most common initial maintenance schedule of 4 weeks of rituximab every 6 months for 2 years had a significant effect on event-free survival. Further scheduling refinements, based on pharmacokinetic properties of rituximab, lead to the administration of one single infusion of rituximab every 2-3 months for the duration of 1-2 years. This newer schedule has also proven effective in prolonging event-free survival and overall survival in a European study. Most clinicians will agree that a serum level of 25µg/ml is optimal; however there is controversy whether a 375 mg/m² single dose of rituximab every 2 or 3 months is needed to maintain the ideal serum concentration. The principal concern in long-term maintenance treatment is the prolonged depletion of normal B-cells and the decrease in circulating immunoglobulins. An increased incidence of infections or secondary tumors has not been demonstrated, however. Rituximab maintenance has been studied predominantly in follicular lymphoma and has demonstrated efficacy in consolidation or as long-term therapy. There is increasing evidence that maintenance treatment is efficacious in other indolent lymphomas, including WM. A long-overdue clinical trial of Rituxan maintenance in WM would appear to be the next logical step.

- Dr. E. Morra: **Fludarabine-based Combination therapies for Waldenström's macroglobulinemia**

The nucleoside analog fludarabine is commonly used in WM, whether as a single agent or in combination with other active agents. Fludarabine (F) was compared to a combination of cyclophosphamide (C), doxorubicin, and prednisone, and was shown to produce a higher response rate as well as a longer duration of response. The combination of fludarabine and cyclophosphamide (FC) however, known to act synergistically, resulted in increased efficacy. The addition of rituximab to FC (FCR) further increased the overall RR to 80% - 90%. In one study, the FCR combination produced a high percentage of good quality responses, with 42% of patients achieving near complete responses (complete resolution of symptoms, regression of adenopathy and/or organomegaly, and absence of bone marrow infiltration). Responses occurred within 3-6 months and improved up to 6-12 months after end of treatment. The major side-effects of fludarabine combinations are myelosuppression and immunosuppression (prophylaxis with antibiotics and antivirals is recommended). Other fludarabine-related risks are transformation to a high grade lymphoma, development of myelodysplastic syndrome (MDS), and potential stem-cell collecting difficulties (nucleoside analogs should generally be avoided in patients who may be considered candidates for future autologous stem cell transplant). Fludarabine-based combinations regimens are nonetheless a very effective option for use in first-line treatment in patients with aggressive and/or advanced disease as well as for second-line treatment in patients with short remission or resistance to first-line therapy.

- Branagan: **Long term responses to fludarabine and rituximab in Waldenström's macroglobulinemia.**

This study evaluated the long term outcome of the combination of fludarabine and rituximab in a clinical trial comprising 43 WM patients. Eligibility parameters included less than 2 prior therapies, and no previous nucleoside analog or rituximab treatment. The regimen consisted of 6 cycles of fludarabine and 8 infusions of rituximab over 31 weeks. The overall response rate was 95.3%; 2 patients (5%) had a complete response; at best response (19.2 months post-cessation of therapy), median bone marrow infiltration was reduced to 5% from 55%, serum IgM decreased from 3,840 to 443 mg/dL and hematocrit increased from 31.2% to 38.0%. The median time to progression for all patients was 51.2 months; longer for untreated versus previously treated patients (77.6 vs. 38.4 months). Severe (grade 3) treatment-related side effects included markedly low white blood cell count (neutropenia) in over 50% of patients, pneumonia in 14% of patients (including 2 patients who died of interstitial pneumonia), neuropathy in 5% of patients, and one case of central nervous system disease (limbic encephalitis). Transformation to aggressive disease (median follow-up of 40.3 months) was seen in 3 patients (7%) who developed a non-indolent lymphoma, two patients developed [*acute myelogenous leukemia \(AML\)*](#) and one patient developed [*myelodysplasia \(MDS\)*](#). The combination of fludarabine and rituximab is an effective regimen in WM. The presence of important short and long term side-effects with this regimen requires a careful assessment of the risk-vs.-benefit issues in individual patients.

- Dr. C. Chen: **Bortezomib in relapsed/refractory Waldenström's macroglobulinemia.**

Proteasomes are intra-cellular structures that help dispose of numerous proteins including proteins that are involved in the regulation, activation and inhibition of various important cellular pathways (including cell proliferation, cell growth, cell death, etc.). Bortezomib (Velcade) is a potent and reversible proteasome inhibitor. Recent studies have evaluated the efficacy of single agent bortezomib in WM (50% drop in IgM in 48%-60% of patients). Dr. Chen and her colleagues from Toronto, Canada, evaluated single-agent bortezomib in WM using similar dosing schedules but the number of cycles was not fixed and therapy was continued until disease progression. 27 previously treated and untreated patients received a median of 8 cycles of bortezomib (range 2-74). After a median follow-up of 48 months, IgM dropped in 78% of patients (50% reduction in 44%, and 25% reduction in 33% of patients). The reduction in IgM levels was rapid (median 2 cycles), and a decrease in lymphadenopathy was somewhat slower (median 4 cycles). In those patients who responded, the median duration of response was 13.2 months, with stable disease noted for 14.3 months. These studies all demonstrate only moderate activity with single-agent bortezomib in WM. The development of peripheral neuropathy (PN) remains a contentious issue for WM patients; 70% of WM patients treated with bortezomib develop PN (only 5-10% of MM and NHL patients develop PN with bortezomib). Laboratory evidence of the synergistic activity of bortezomib with rituximab, perfosine and steroids in WM cell lines has led to the ongoing clinical study of bortezomib combination therapy in WM. Maintenance therapy with bortezomib may also be of potential therapeutic benefit.

- Dr. A. Rohatiner: **Bortezomib alone and in combination with rituximab in patients with Waldenström's macroglobulinemia.**

This study of 17 relapsed WM patients evaluated bortezomib (Velcade) alone (two different dosing schedules) and the combination of bortezomib and rituximab. Partial response was observed in 71% of patients; 4/7 treated with bortezomib alone and 8/10 who received the combination. A median reduction of 78% in the IgM level and significant improvements in the patient's CBC was noted in the combination group; 2 of these patients no longer required transfusions. One in every four patients developed severe (grade 3/4) hematological toxicity (neutropenia and thrombocytopenia); fatigue was seen in 76% of patients, nausea in 56%, diarrhea in 56%, and (reversible) neuropathy developed in virtually half of the patients. Dose reduction was common, and many patients stopped treatment because of side-effects or disease progression. Bortezomib was found to be active as a single agent in WM for 57% of patients, but the combination bortezomib and rituxan was more effective at 80% response rate. Furthermore, a once-weekly dose of bortezomib (1.6 mg/m²) was more convenient, less toxic, and equally as effective as the standard twice-weekly dose (1.3 mg/m²). A new clinical trial using the combination bortezomib, rituximab, and cyclophosphamide is now under way.

- S. Treon: **Primary therapy of Waldenström's macroglobulinemia with bortezomib, dexamethasone and rituximab: results of WMCTG Clinical Trial 05-180.**

The combination of bortezomib, dexamethasone, and rituximab (BDR) in symptomatic WM patients was studied in this clinical trial. Treatment consisted of IV bortezomib at 1.3 mg/m² and IV dexamethasone 40 mg on days 1, 4, 8, and 11, and IV rituximab at 375 mg/m² on day 11. Patients received 4 consecutive cycles initially, followed by 4 more cycles, given three months apart. Twenty-three patients received a median of 7 cycles of therapy; median bone marrow infiltration declined from 55% to 10%, serum IgM levels dropped from 4,830 to 1,115 mg/dL, and hematocrit increased from 29.8% to 38.2%. The overall and major (>50% decrease in IgM) response rates were 96% and 83% respectively. Rapid responses were seen at a median of 1.4 months. With a median follow-up of 22.8 months, 18/23 patients remain free of disease progression. Peripheral neuropathy was the most common toxicity in 13/16 patients at a median of 6.0 months, but mostly resolved after six months. Prophylaxis for herpes zoster (shingles) was required. The addition of rituximab and dexamethasone to bortezomib extends progression free survival or time to re-treatment by a significant amount, and complete responses were seen. The IgM flare phenomenon associated with rituximab monotherapy (seen in 40-60% of patients) was reduced to just 9% in these patients receiving combination treatment.

Session VII: Novel Agents for Treatment of Waldenström's Macroglobulinemia

- Dr. C. Buske: **CHOP versus R-CHOP in Waldenström's macroglobulinemia.**

Rituximab is a monoclonal antibody (MAb) that targets the CD20 cell surface receptor molecule found on some WM cells. Despite the abundance of CD20 on most of these cells, single agent rituximab therapy only provides overall response rates (ORR) of up to 48%. Complete response (CR) rates are low, and the responses are typically of short duration in the majority of patients. A German clinical trial compared rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) to CHOP alone in previously untreated WM patients: 34 patients received R-CHOP, and 30 patients received CHOP alone. Following treatment, the majority of patients received interferon-alpha maintenance as post-induction therapy, and 8 patients underwent autologous stem cell transplantation. R-CHOP resulted in a significantly higher ORR (91% vs. 60%). R-CHOP patients benefited from a significantly longer time to treatment failure (median of 63 months) compared to CHOP patients (22 months). No major difference in treatment associated side effects were noted between groups. The addition of rituximab to first-line CHOP chemotherapy significantly improves treatment outcome in patients.

- Dr. M. Rummel: **bendamustine plus rituximab (B-R) versus R-CHOP in the first-line-treatment of Patients with Waldenström's macroglobulinemia.**

Bendamustine (Treanda in the US) is an older chemotherapy agent developed in the former East Germany over 30 years ago in order to provide an inexpensive agent for the treatment of hematological cancers. The drug bendamustine (B) can roughly be described as a combination alkylating agent (such as cyclophosphamide) and purine analog (such as fludarabine). A German phase-III clinical trial compared the efficacy and safety of the combination B-R versus R-CHOP as first-line therapy for WM: 40 WM patients (median age 64 years) received rituximab 375 mg/m² (day 1) plus either bendamustine 90 mg/m² (days 1+2) every 28 days or the standard CHOP regimen every 21 days for a maximum of 6 cycles. The ORR with B-R was the same as with R-CHOP (96% vs. 94%). After a median follow-up time of 26 months, progressive or relapsed disease was seen in 2 B-R and 7 R-CHOP patients (9% vs. 41%). IgM levels were lower following B-R (530) compared to R-CHOP (990). The B-R regimen also had fewer side-effects: lower rates of alopecia (hair loss was 0% with B-R vs. 89% with R-CHOP) and lower number of infections (9% with B-R group vs. 47% with R-CHOP). There was greater risk of neuropathy with R-CHOP (3 patients vs. 1), and serious leukocytopenia (low WBC's) was more common in R-CHOP patients. In this study, the combination of bendamustine plus rituximab seemed to be equally if not better than the standard R-CHOP regimen, and fewer side-effects were noted as well with B-R than with R-CHOP therapy.

- Dr. I. Ghobrial: **Regulation of the PI3K/mTOR pathway in Waldenström's macroglobulinemia.**

The PI3K/Akt/mTOR and NF-κB pathways are currently the subject of intense study in numerous medical research laboratories all over the world. These complex biochemical cellular pathways are implicated in the regulation of cell death (apoptosis), cell division, tumor blood vessel growth, and tumor proliferation in WM (as well as in numerous other hematological cancers such as MM). Dr. Ghobrial and her team at the Dana Farber Cancer Institute in Boston are actively studying a number of new drugs (perifosine, enzastaurin, and RAD001) in WM. Perifosine is a Akt inhibitor, enzastaurin is a PI3/Akt inhibitor, and RAD001 (Everolimus) is a mTOR inhibitor. These drugs have inhibited tumor growth in both laboratory and early clinical studies (the phase II clinical trial of perifosine in relapsed WM, and the phase II clinical trial of RAD001 in relapsed WM). Perifosine was noted to cause significant gastrointestinal problems, resulting in a lower dose and better tolerability. RAD001 thus far has demonstrated an overall response rate (ORR) of 72%. The phase II clinical trial of weekly bortezomib (Velcade) and rituximab as first-line therapy or in relapsed/refractory WM patients has produced an ORR of 90%, including a complete response (CR) in one patient. Severe neuropathy was considerably reduced with the once-weekly Velcade dosing regimen. The use of these novel biological agents has measurably advanced the understanding of the biology and therapy of WM as well as mechanisms of drug resistance. Future clinical trials will feature combination therapies (these drugs have been proven to be more efficacious when combined with biological agents) such as perifosine + Velcade + Rituxan and RAD001 + Velcade + Rituxan.

- Dr. I. Grewal: **Antibody-based therapeutics targeting the TNF super-family member CD70.**

The cell surface receptor molecule CD70 is implicated in the expansion and differentiation of immune system cells. CD70 is the ligand that binds CD27 and subsequently activates NF- κ B related tumor necrosis family pathways (involving B-LYS, APRIL for example). It is expressed after immune activation on about 20% of normal T cells and on about 70% of normal B cells. CD70 is not present on resting (non-activated) white blood cells but is however very commonly expressed in hematological cancers. It is widely expressed on WM mast cells and WM tumor cells. CD70 is rarely found in normal cells, as well as non-activated lymphocytes, and is thus an excellent target for monoclonal antibody therapy. A new investigational drug was developed, the humanized anti-CD70 antibody SGN-70, which significantly reduces tumor burden and also prolongs survival in animal model studies. Furthermore, the addition of a very potent synthetic cytotoxic drug, MMAF, to the CD70 antibody has resulted in a very effective monoclonal antibody-conjugate drug (SGN-75). Preclinical laboratory studies strongly suggests that targeting CD70 with either an un-conjugated antibody (SGN-70) or with an antibody-drug conjugate (SGN-75) may offer an effective therapeutic option in the treatment of WM.

- Dr. R. Advani: **Targeting CD40 in Waldenström's macroglobulinemia.**

The cell surface receptor molecule CD40 (a member of the TNF receptor family, as is CD70) is found in all stages of B cell development and in many of B cell malignancies, including WM. CD40 is implicated in the survival, proliferation and differentiation of normal B cells but may paradoxically cause activation-induced cell death in WM. The CD40 antigen expression in WM bone marrow tumor cells is $\geq 50\%$ according to flow cytometry analysis. The anti-CD40 monoclonal antibodies (MAbs) SGN-40 and HCD122 are being studied in MM, NHL and CLL. HCD122 is a human, IgG1 monoclonal antibody while SGN-40 is a humanized IgG1 antibody. Both MAbs target the CD40 receptor and are potent effectors of antibody-dependent cellular cytotoxicity (ADCC). Both MAbs appear to be well tolerated, with no immunogenicity (allergic reactions similar to ones seen with rituximab). HCD122 has anti-tumor activity against multiple myeloma cell lines, and SGN-40 is a potent inhibitor of cell proliferation, and also induces cell death and ADCC in high grade B-cell lymphoma lines. SGN-40 has also shown results similar to rituximab in animal models. Early studies suggest that both MAbs have activity against WM cells, with HCD122 demonstrating increased efficacy on WM patient cells rather than in cell lines. As previously mentioned, CD40 is involved in the NF- κ B pathway and the combination of these two CD40 MAbs (SGN-40 and HCD 122) with other agents that target the NF- κ B pathway such as lenalidomide (Revlimid) may provide an effective and targeted treatment in WM.

- Dr. A. Roccaro: **Novel proteasome inhibitor in Waldenström's macroglobulinemia.**

The production of abnormal amounts of the large IgM protein is a cardinal feature of WM. The intra-cellular structures that are involved in protein degradation (catabolism) and recycling are called [*proteasomes*](#). Bortezomib (Velcade) is a widely used proteasome inhibitor active in

numerous hematological malignancies, including WM. The new proteasome inhibitor NPI-0052 also inhibits cell proliferation and induces cell death (apoptosis) in WM cells. This study evaluated the combination of NPI-0052 and bortezomib in WM. This combination acted in a synergistic fashion in WM models, effectively killing tumor cells. The combination regimen inhibited the NF- κ B and Akt pathway, inhibited the migration and adhesion of WM cells to the bone marrow microenvironment, and overcame drug resistance. This basic research focused on the cellular biology of WM and the specific effects of proteasome inhibitors, which in turn not only adds greatly to the understanding of this disease but also provides the requisite rationale for the clinical use of proteasome inhibitor combination therapeutic regimens in WM.

Session VIII: Transplant Therapy of Waldenström's Macroglobulinemia

- **Dr. B. Barlogie: Fludarabine for Waldenström's macroglobulinemia**

Dr. Barlogie, a well known oncologist from the University of Arkansas, spoke briefly on the results of a 10 year follow-up study on both untreated and previously treated WM patients who were treated with fludarabine. Important prognostic factors were identified: age ≥ 70 yr, prior non-protocol therapy, hemoglobin < 10.5 g/dL, beta-2-microglobulin (B2M) ≥ 3 mg/dL, IgM < 3.8 g/dL. Serum lactate dehydrogenase (LDH) \geq upper limit of normal was noted to be an additional independent prognostic factor. Using age, prior therapy, B2M, IgM, LDH and hemoglobin, 3 distinct risk groups with vastly different 8-year survival estimates were identified (62%, 46% and 6%). Dr. Barlogis is one of the most experienced MM and WM transplant oncologists in the U.S., and he spent some time discussing transplant therapy in WM. In his own inimitable style, he stated that he did not worry too much about bone marrow contamination (i.e. % tumor infiltration) prior to transplant. "Don't kill someone before you transplant them" was Dr. Barlogie's amusing yet pointed retort to an audience member's question.

- **Dr. H. Kyriakou: Hematopoietic stem cell transplantation for Waldenström's macroglobulinemia patients.**

Dr. H. Kyriakou of the European Group for Blood and Marrow Transplantation, London, U.K., reviewed 202 WM patients treated with an autologous stem cell transplant (ASCT) and 106 treated with an allogeneic transplant (allo-SCT). For ASCT, the median age at transplant was 53 years and median time from diagnosis to transplant was 18 months. With a median follow-up of 26 months: 112 patients are alive and free of disease, 73 patients relapsed after a median of 14 months (1 - 110) post ASCT. For the 106 allogeneic patients the median age at transplant was 49 years, the median time from diagnosis to allo-SCT was 34 months, 44 patients had a conventional myeloablative and 62 a reduced intensity conditioning (RIC) allo-SCT. With a median follow up of 31 months: 59 patients are alive and free of disease, 48 patients developed acute graft versus host disease (GVHD), 16 patients developed limited and 11 extensive chronic GVHD. The overall survival for ASCT patients was 70% vs. 40% for the allo-SCT. More extensive statistics are available in the published study. Citing an extensive review of

conventional therapy vs. transplants by Dr. J.D. Shaughnessy, Dr. Kyriakou noted that transplants offered complete responses (CR) in 18% of WM patients, whereas non-transplant therapies had a CR rate of 7%. Survival was also noted to be better for WM patients receiving transplants, leading her to conclude that ASCT and allo-SCT have to be considered therapeutic options in WM patients.

- Dr. D. Maloney: **Evidence for GVWM following mini-allo in WM.**

Dr. David G. Maloney from the Fred Hutchinson Cancer Research Center in Seattle, WA, has been very active in the study of high-dose therapy and autologous hematopoietic stem cell transplantation (HCT) in WM. Dr. Maloney believes, however, that the use of allogeneic HCT provides healthy stem cells that have not been exposed to chemotherapy and also eliminates the theoretical risk of re-infusion of WM tumor cells. More importantly, the donor stem cells may provide anti-tumor activity, also known as graft-vs.-tumor effect (GVT). Allogeneic HCT has typically been used only in younger, medically fit patients capable of tolerating the intense regimen; treatment-related mortality (TRM) is still responsible for about 30-40% of deaths. In order to reduce this high TRM, reduced intensity regimen allogeneic transplants (RIC-allo) have been used in ever-increasing numbers. The rationale for the RIC-allo is the realization that most of the tumor activity is due to the GVT. Dr. Maloney reported on a series of 13 patients treated in Seattle with a RIC-allo regimen: median age was 58 (44-65) years, patients were at a median time from diagnosis of 5.5 years, and had a median of 5 prior treatment courses. 54% of patients had acute graft-vs.-host-disease (GVHD) and over half developed extensive chronic GHVD. Three patients died from relapsed aggressive NHL, host derived [MDS/AML](#) and refractory hemolytic anemia (all present prior to allogeneic HCT). The 4 year overall and progression-free survival is 60%. Patients with extensive chronic GHVD had longer survival. Only one patient was noted to have progression of WM. Dr. Maloney firmly believes that RIC-allo regimen offers less risk to the WM patient than traditional allogeneic transplants, provides the best anti-tumor effect through GVD, and is one of the best options for cure.

- Dr. M. Gertz: **Stem cell transplantation for IgM amyloidosis and IgM multiple myeloma.**

Not every hematological malignancy that features elevated IgMs is WM: amyloidosis and IgM myeloma are rare diseases not frequently encountered. Dr. Gertz reported on patients with amyloidosis that underwent stem cell transplants. Of these 374 patients, 17 (5%) had a monoclonal IgM serum protein. Of the patients with IgM amyloidosis, most were older (64 vs. 57), cardiac involvement was less common, (25% vs. 50%), and peripheral neuropathy was more common, (30% vs. 10%). Only 4 of the 17 patients have died. Among 882 multiple myeloma patients who underwent a stem cell transplant, only 8 had IgM myeloma, and the IgM myeloma patients were older (65 vs. 58). Of the 8 patients, only 3 of the 8 patients have relapsed (the time to progression for IgM myeloma was 22.5 months versus 17.5 months for MM). The clinical outcome for IgM myeloma patients and non-IgM myeloma patients appears to be similar, despite the older age for the IgM myeloma patients.

Session IX: Therapy Related Complications in Waldenström's Macroglobulinemia

- **Dr. X. Leleu: Increased incidence of transformation and myelodysplasia/acute leukemia in patients with Waldenström's macroglobulinemia treated with nucleoside analogues.**

The nucleoside analogs (NA) fludarabine and cladribine (2CdA) have long been considered effective and appropriate therapy for the treatment of WM despite the well recognized risks of myelosuppression, immune suppression, and potential difficulties in stem cell collection. Dr. Leleu and members of Dr. Treon's team at Dana-Farber Cancer Institute in Boston evaluated the incidence of transformation to more aggressive lymphomas (typically diffuse large B-cell lymphoma -DLBCL) and myelodysplastic syndrome (MDS) and/or acute myelogenous leukemia (AML) in a large population of WM patients. After a mean follow-up of 5 years, 439 WM patients (193 of whom were previously treated with a NA) were reviewed: 9 patients (4.7%) either transformed, 3 developed MDS/AML (1.6%). Only 1 patient (0.4%) transformed in the non-NA treated group, and no such events occurred among untreated patients. Transformation and MDS/AML occurred in 12 (6.2%) of all patients treated with NAs, occurring at a median of 5 years from onset of NA therapy. The median survival of NA treated patients who transformed (to DLBCL) was the same as other NA treated patients. All patients who developed MDS/AML died at a median time of 5 months. Perhaps one of the more positive aspects of this sobering review was the recognition that effective treatment is available in the form of R-CHOP for patients transforming to DLBCL; the outlook for those WM patients developing MDS/AML is considerably worse. Newer guidelines for the recommendation of NA therapy in WM patients may need to be updated in view of these findings.

- **Dr. J. Seymour: The pro's and con's of initial treatment with purine-nucleoside analogue based combination therapies in patients with Waldenström macroglobulinemia.**

The chemotherapy drugs cladribine (2CdA) and fludarabine are purine nucleoside analogs (PNAs) known to be very effective agents in the treatment of WM. Fludarabine is used much more frequently in WM than 2CdA. When PNAs are used in combination with rituximab (R) and cyclophosphamide synergistic cytotoxicity is observed and thus combination therapies are superior to single agent therapy. PNA combination therapies achieve overall response rates ranging from 78 – 90%, among the highest of any WM treatments. The hematological responses are durable, and median time to progression is 3 – 5 years; these can be even longer in previously untreated patients. Recently a number of studies have suggested the sobering possibility of a number of undesirable potential adverse effects with PNA based therapies. Patients with kidney disease can experience greater toxicity with fludarabine as this drug is eliminated via the kidneys. Patients receiving PNA combination therapy can experience prolonged bone marrow toxicity, leading to decreased white blood cell and platelet production – severe infections or bleeding problems are not frequent however. PNAs can cause prolonged immunosuppression, particularly decreased levels of CD4+ lymphocytes, which has minimal clinical consequences for infection unless other risk factors are present (the use of corticosteroids in particular). The author

notes that the addition of rituximab to fludarabine does not increase the risk of infection during treatment or during the first year following therapy. Successful stem cell harvest can be achieved in about 40% of patients treated with PNA (70% if aged ≤ 50 years). The use of stem-cell growth factors and twice daily G-CSF leads to greater successes in stem cell harvest (63% if aged ≤ 50 years, 58% among pts aged >50). The risk of secondary myelodysplasia (MDS /AML) after fludarabine combination therapy was 11.6%, less than 5% in previously untreated patients. The risk of disease transformation to a more aggressive lymphoma or the development of Epstein - Barr virus driven disorders after PNAs has been mentioned in the literature but this author has not observed any cases of transformation among 25 WM patients treated with fludarabine combination therapy. PNA use does not influence transformation risk in CLL. In conclusion, PNA combination therapy is very effective in WM, specific treatment-related risks have been identified, and most are manageable. Nonetheless, the toxicity risks of PNA combination therapies need to be weighed against the consequences of suboptimal disease control.

- **Dr. S. Thomas: Success rates of autologous stem cell collection in patients with Waldenström macroglobulinemia.**

Dr. Thomas, University of Texas M. D. Anderson Cancer Center, evaluated autologous peripheral blood stem cell collection (ASCC) in 21 symptomatic WM patients: median age of the patients was 55 (46-70), 15 were male. The patients underwent ASCC at a median interval of 9.5 months (2.8-24 months) from the start of chemotherapy. The chemotherapy regimen was rituximab (R) + bortezomib (Velcade) in 10 patients, cladribine (2CdA) + cyclophosphamide (Cy) +/- R in 6 patients, solo R in 3 patients, and modified R-hyperCVAD in 2 patients. Successful ASCC was defined as harvest of $> 2 \times 10^6$ CD34+ cells per kg in ≤ 4 consecutive collection procedures (apheresis). The granulocyte colony stimulating factor filgrastim (Neupogen, G-CSF) was used alone in 3/6 patients treated with 2CdA and 8/15 patients treated with non-2CdA based regimens; 9 patients received cyclophosphamide-based mobilization therapy + filgrastim, and 1 patient received ifosfamide (alkylating agent – also known as Mitoxana/ Ifex) + filgrastim. ASCC was successful after 1st aphaeresis in 14/15 patients who received non-2CdA based induction vs. 2/6 patients who received 2CdA. In the patients treated with 2CdA, the median number of CD34+ cells/kg collected in successful ASCCs was 6.9×10^6 ($5.6-8.1 \times 10^6$) vs. 7.7×10^6 ($4.6 \times 10^6-15.8 \times 10^6$) in those treated with non-2CdA regimens. White blood cell count, absolute neutrophil count, absolute lymphocyte count and hemoglobin prior to mobilization were not predictive factors. This study suggests that there may be some difficulty in ASCC post-nucleoside analog therapy (2CdA, fludarabine) and that ASCC with filgrastim is possible in patients who have not received 2CdA as prior therapy. This small study also reinforces the recommendation that treatment with nucleoside analogs be avoided in patients where ASCC and potential autologous stem cell transplant may be a consideration.

Session X: Disease Related Morbidities in Waldenström's macroglobulinemia

- Dr. F. Hochberg: **Waldenström's and the nervous system: "Bing Neel" revisited.**

Dr. Hochberg, from the department of Neuro Oncology, Massachusetts General Hospital, Boston, MA, USA, based his report on a review of the medical literature as well as his clinical experience with WM patients. The term "Bing-Neel Syndrome", named after Drs. Bing and Neel (neurologists in Copenhagen in the 1930's) is used to describe a variety of rare WM-associated neurologic syndromes that are in large measure distinguishable from the typical peripheral neuropathy that may afflict up to 30-50% of WM patients. The mechanisms for WM-associated encephalopathy (degeneration of brain function) or neuropathy appear to be due to direct lymphoplasmacytic involvement of brain or spinal fluid and IgM deposition in brain blood vessels and nerve cells. The resultant complications include WM meningitis, transformation into DLBCL, and other IgM antibody-associated brain and spinal cord processes. Treatment strategies may involve plasmapheresis and chemotherapy in order to reduce the IgM levels and WM tumor cells. Dr. Hochberg did go on to state that many of the Bing-Neel symptoms are reversible, and that IgM reduction seems to be key as WM infiltration itself is not a major issue: "It is like PN of the brain".

- Dr. E. Nobile-Orazio: **Antigenic determinants in IgM paraproteinemic neuropathies.**

Neuropathies are known to affect up to 30-50% of WM patients and only 50% of these patients are actually symptomatic. Many different forms of these IgM associated neuropathies have been identified and likely reflect various mechanisms of the disease process itself. Not only can IgM directly affect the nerves, but direct infiltration of the WM cells in the nerves, amyloid deposition, complications from cryoglobulinemia, as well as other pathological processes are also seen in WM. By far the most common condition is a chronic progressive, symmetric and predominantly distal neuropathy (commonly known as peripheral neuropathy – PN). The accumulation of a specific type of IgM that reacts to the myelin-associated glycoprotein (anti-MAG) of nerves is believed to always result in neuropathy symptoms. A much more serious, and thankfully rarer, consequence of the accumulation of this anti-MAG is the distal sensory demyelinating sensory neuropathy syndrome (DADS); 24% of these afflicted with DADS are totally disabled at 10 yrs, and 50% at 15 yrs. It is interesting to note that these anti-MAG IgM antibodies can sometimes be the result of an immune reaction to an antigen (from a bacterial infection for example). Other components of the nerve myelin surface may also be targets for the WM IgM: the anti-sulfatide IgM is seen in 4-6% of WM PN patients and generally results in worse PN than seen with anti-MAG; the anti-GQ1b ganglioside IgM is seen in up to 2.6% of WM PN patients and as a result is not considered specific to WM. The reduction of serum IgM below a critical level in some patients may be necessary in order to achieve clinical improvement. It is important to note that although PN may be reversible in some instances, some patients may need to have much lower IgM levels after therapy than others. The progressive nature of the neuropathies may also highlight the importance of individually tailored and at times aggressive treatment. Treatment with rituximab improves symptoms in 2/3 of patients; in this

case, patients with anti-MAG IgM antibodies seem to have better responses. Patients with anti-GQ1b ganglioside IgM antibodies respond very well to IVIG infusions. Timely plasmapheresis treatments may be very helpful to many WM PN patients.

- Dr. T. Levine: **Treatment of peripheral neuropathies (PN) associated with IgM monoclonal gammopathies.**

The IgM protein may cause PN in up to 45% of WM patients, half of which are asymptomatic. These IgM antibodies may target specific nerve antigens, and in turn cause distinct symptoms. The absence of any known identifiable IgM nerve antibodies does not in itself rule out the presence of PN; careful clinical evaluation is the key to diagnosis. Demyelinating features on nerve conduction studies suggests that the IgM protein is responsible for the PN. Nerve biopsies are often very helpful and can reveal IgM deposition in the nerves or the surrounding blood vessels. IgM deposition in superficial epidural (skin) nerves can be quite painful. This type of small fiber neuropathy responds well to rapid reduction of IgM. Axonal neuropathy, where the IgM (usually anti-sulfatide) attacks the actual body of the nerve itself as opposed to the myelin sheath, is worse: relatively aggressive treatment may be warranted. Treatment with the most appropriate therapy, whether it be chemotherapy, IVIG (particularly if there is demyelination), rituximab, and plasmapheresis can be used either individually or in combination in many cases. Dr. Levine has used a rituximab regimen which consists of 2 weekly doses followed by one dose every ten weeks for one year. He notes that PN treatment with rituximab will help with extremity muscle strength and improve one's gait (less frequent falls), and may take up to two years to actually see results. Unfortunately, little change is seen in sensory symptoms; numbness and pain typically often do not improve.

- Dr. J.P. Ferman: **Cutaneous manifestations of Waldenström's macroglobulinemia.**

WM can produce many different dermatological (skin) lesions. These lesions may be caused by: the direct infiltration of WM cells into skin structures; the secretion of abnormal cytokines (molecular messenger molecules) by WM cells (POEMS syndrome); the deposition of IgM along various distinct anatomical regions of the skin structures; the aggregation of IgM in skin structures (amyloidosis); the formation of crystals/microtubules (type I cryoglobulinemia); the deposition of IgM-antibody complexes in the skin (type II mixed cryoglobulinemia, bullous lesions, xanthomas); and finally as a result of the hyperviscosity syndrome. Schnitzler's syndrome is perhaps one of the more well-known skin manifestations in WM; the exact mechanism of the formation of these lesions is still poorly understood. Schnitzler syndrome may be characterized by an urticarial rash, intermittent fever and fatigue, night sweats, bone and muscle pain, osteosclerosis (hardening or abnormal increased bone density) and of course elevated IgM levels. Laboratory findings may include an increase in white blood cell count, increased platelet count, anemia, elevated red cell sedimentation rate, as well as other elevated inflammatory markers such as CRP and fibrin. The treatment of Schnitzler's syndrome is difficult: systemic corticosteroids may be helpful; the use of the antibiotic pefloxacin (fluorinated 4-quinolone) may rapidly improve urticarial and systemic symptoms, and can sometimes be used on a prophylactic basis. The newer biological drug Anakinra (interleukin-1

receptor antagonist) has demonstrated very good results in the clinical and laboratory manifestations of Schnitzler's syndrome; no effect on the serum IgM level is seen however.

- Dr. S. Berentsen: **Cold Agglutinin mediated autoimmune hemolytic anemia in Waldenström's macroglobulinemia.**

Cold agglutinin disease (CAD) is an autoimmune disease affecting 3% of WM patients; conversely, over 50% of CAD patients have WM. Most primary forms of CAD have an IgM-kappa circulating antibody which binds to red blood cells (RBC) at low temperatures (usually 28-31°C). Monoclonal CD20+IgM-kappa+ cells are detected in the bone marrow of 90% of the patients. CAD is a form of autoimmune hemolytic anemia where most patients have chronic hemoglobin levels in the 8-9 gm/dL range. Cold-induced problems such as Raynaud's phenomena are very common, 75% of patients have significant flares of the disease during febrile illnesses, and over 50% of patients require routine transfusions. The autoimmune process in CAD is triggered by the agglutination of IgM tagged RBCs and the subsequent activation complement (C3b) mediated hemolysis. The C3b labeled RBCs are cleared from the circulation by the liver. Most conventional therapies are ineffective with less than 20% of the patients responding to corticosteroids such as prednisone. Rituximab as single agent therapy has demonstrated a 50-55% partial response rate with a median duration of response of 11 months. Combination therapy with fludarabine and rituximab is being evaluated. Cold agglutinin disease is not an indolent disease for the WM patient: clinical symptoms and quality of life often require aggressive treatment.

- Dr. M. Stone: **Hyperviscosity syndrome and cryoglobulinemia.**

Hyperviscosity Syndrome (HVS), first described by Dr. Jan Waldenström in 1944, consists of mucous membrane bleeding, retinopathy with visual disturbances, and a variety of neurological disorders. A serum IgM level greater than 4 gm/dL causes the serum viscosity (resistance to blood flow, stickiness) to rise exponentially. Although symptoms vary between patients depending on different serum viscosity levels, most patients will typically experience individual reproducible symptoms at a certain viscosity level. The simple and reliable method of serum viscosity measurement is made using the Ostwald tube. HVS can be diagnosed from fundoscopic eye examinations, and prompt recognition is important since it is usually reversible by plasmapheresis. Cryoglobulinemia can dramatically influence serum viscosity; many of these patients present earlier than typical WM patients and may also be noted to have HVS. Cryoglobulins precipitate at temperatures less than 37°, and cryoprecipitation is often concentration-dependent in most monoclonal IgM cryoglobulinemias (type I). In type II cryoglobulinemia, also known as mixed cryoglobulinemia, the cryoglobulins are usually mixed immune complexes of monoclonal IgM and polyclonal IgG with rheumatoid factor activity. The IgM is a monoclonal antibody to the Fc portion of the rheumatoid-like IgG. Cryoprecipitation is due to the reversible temperature-sensitive insolubility of the IgM-IgG immune complex. Patients with mixed cryoglobulinemia are often noted to have hepatitis C liver disease; 10% develop non-Hodgkin's lymphoma, especially WM. Interferon therapy may result in regression of disease in some lymphoma patients. Dr. Stone suggests that plasmapheresis should be used

more often in WM, particularly if the serum viscosity level is greater than 3-3.5 prior to rituximab infusions.

- Dr. M. Menke: **Hyperviscosity related retinopathy in Waldenström's macroglobulinemia.**

WM hyperviscosity syndrome (HVS) can often lead to [retinopathy](#). Some of the earliest changes seen in the retina in HVS affected individuals are peripheral hemorrhages and venous dilation. There appears to be a good correlation between venous diameter and IgM level, as documented in retinal hemodynamic assessments. Plasmapheresis is effective in reversing HVS-related retinopathy and in reducing abnormal venous dilatation. Dr. Menke evaluated 46 WM patients using ophthalmoscopic techniques, retinal photography, laser Doppler retinal blood flow measurements, and serum IgM and serum viscosity (SV) measurements. WM patients with HVS were evaluated before and after plasmapheresis treatment. For the purposes of this study a retinopathy severity scale was developed. As was expected, patients experienced peripheral hemorrhages and venous dilation with increasing SV and IgM values, with central retinal changes associated with significantly higher SV values. The diameter of the retinal blood vessels increased with increasing serum IgM and SV levels. Patients with the earliest retinal changes were noted to have mean IgM levels of 5442 mg/dL and mean SV levels of 3.1 cp. Following plasmapheresis the IgM levels dropped by $46.5\% \pm 18.0\%$ and SV levels by $44.7\% \pm 17.3\%$. The venous diameter decreased in each patient by an average of $15.3\% \pm 5.8\%$, and the decrease in SV was directly related to the decrease in venous diameter. All patients had improvement in their HVS-related retinopathy after plasmapheresis. Dr. Menke notes that retinal manifestations of HVS can occur at lower serum IgM and SV levels than previously reported. WM patients should all consider obtaining ophthalmoscopic examinations (including scleral depression) along with retinal vessel diameter measurements in order to detect early HVS-related retinal complications. Dr. Menke also suggests (together with Dr. Stone) that WM patients should obtain plasmapheresis treatment before serum viscosity levels climb above 3.0 cp in order to avoid any retinal hemorrhages.

Special Guest Lecture Presentations

- Dr. O. O'Connor: **The emerging role of histone deacetylase inhibitors in Waldenström's macroglobulinemia.**

Gene expression in a cell is controlled by the coiling or uncoiling of DNA around structures known as histones. The histone acetylase enzymes assist the cell in uncoiling the compact DNA, making it more accessible for gene transcription. Histone deacetylases (HDAC) conversely lead to the formation of condensed DNA and therefore can regulate gene expression. Abnormal histone acetylation has been linked to cancer. The use of "histone deacetylase inhibitors", one of the fastest growing classes of new drugs, forms the basis for a sophisticated new anticancer treatment, particularly hematological cancers. The inhibitors do not add or delete DNA, but instead normalize existing DNA, forcing the cells to behave normally. Abnormal behavior of

cancer cells can be targeted and reversed with seeming minimal toxicity to normal cells. Currently, five of these drugs have been studied in phase 1 and 2 clinical trials in lymphoma. The rare T-cell lymphomas are one of the first lymphomas where HDAC inhibitors appear to have shown effectiveness, and one of these drugs also appears effective in Hodgkin's lymphoma. Recent studies in multiple myeloma have shown that the combination of HDAC inhibitors and bortezomib (Velcade) is quite effective. Results of these studies would seem to suggest that the HDAC inhibitors alone, or in combination with other drugs such as bortezomib, may have activity in WM.

- Dr. K. Anderson: **Applying the lessons learned from the treatment of multiple myeloma to Waldenström's macroglobulinemia.**

Dr. Kenneth C. Anderson, head of the Hematological Malignancies Department at the Dana Farber Cancer Institute (Harvard University, Boston, MA, USA), delivered an excellent lecture on the potential application of knowledge obtained in the study of multiple myeloma (MM) to the study and treatment of WM. Dr. Anderson reiterated his belief, based on his extensive experience in MM research, that oncogenomics (the study of cancer genes/DNA/RNA) as well as the continued investigation of the bone marrow (BM) microenvironment are the two key areas of study that will lead the most important information on the biology and subsequent future treatments of WM. Basic research in the fundamental interactions of WM and supporting cells (BM mast cells for instance) in the BM microenvironment will permit specific targeted therapies that will help overcome drug resistance and ultimately lead to improved patient outcomes. The immunomodulatory drugs (IMiDs) thalidomide and lenalidomide (Revlimid) as well as the proteasome inhibitor bortezomib (Velcade) are examples of BM microenvironment targeted therapy in MM that have been used successfully in WM. The continued focus on oncogenomics will also identify new combination therapies that will be based on the individual patient's genetic makeup. The anti-CD40 monoclonal antibodies (MAbs) SGN-40 and HCD122, already in clinical trials in MM, as sole agents or in combinations with other agents to enhance effectiveness, will surely be evaluated in WM in the near future. The molecular messenger molecules (cytokines) are also an area of active clinical research in MM. Targeting the signaling pathways that affect the growth and survival of MM cells as well as reducing the influence of non-malignant "helper" cells in the BM (e.g. mast cells) is possible using MAbs and other biological agents (small molecule inhibitors). Signaling pathways within the malignant cells themselves are an important and complex area of investigation. The drugs perifosine, enzastaurin, and Velcade are examples of novel agents that target PKC, Akt, NF- κ B, and proteasomes. Many other investigational agents too numerous to name target these and newly identified complex pathways. Combination therapy with Velcade, perifosine, IMiDs, histone deacetylase inhibitors, and/or other agents holds great promise. Dr. Anderson was quick to note however that with biological agents, more was not always necessarily better. Newer second generation agents, such as the proteasome inhibitors NPI0052 and Carfilzomib, have been shown to overcome Velcade resistance, and have fewer side-effects for the patient such as the neuropathy so often seen in WM patients. Dr. Anderson's lecture touched on many other new developments in the treatment of MM and the possible crossover into the treatment of WM. His main message, repeated often throughout, was that advances in MM and WM therapeutics lies in

the continued study of basic research such as oncogenomics (“DNA analysis is the future”) and bone marrow microenvironment biology.

- Dr. G. Merlini: **Biology and therapy of amyloidosis associated with IgM monoclonal protein.**

The amyloid diseases are serious hematological disorders characterized by the abnormal folding of proteins. These abnormally folded proteins aggregate as insoluble fibrous deposits in critical areas of the body resulting in organ failure and subsequent death. In immunoglobulin light-chain (AL) amyloidosis, toxic light chains (LC – kappa or lambda) are produced that can cause multiple organ damage. In Dr. Merlini’s study of 862 AL amyloidosis patients, 52 (6%) had an underlying IgM monoclonal (MC) abnormality: these patients were older (67 vs. 63 yrs), had higher rates of lymphadenopathy (30% vs. 4%), and had less severe kidney involvement. Kappa light chains were more common in IgM MC than in the general AL patients (39% vs. 25%); however, there was a clear predominance of lambda clones in IgM MC as in AL. The main prognostic factors were heart involvement and response to chemotherapy. The IgM MC patients had similar survival rates as the general AL patients. The most effective treatments were autologous stem cell transplantation and chemotherapy with purine analogs (e.g. fludarabine). Aggressive treatment of AL amyloidosis appears to significantly improve survival.

Young Investigator Poster Session

- Dr. X. Jia: **The novel hydroxamic acid-derived HDAC inhibitor, LBH589, induces in vitro antitumor activity in Waldenström’s macroglobulinemia.**

Histone deacetylase (HDAC) inhibitors are exciting new agents in hematological malignancies (see Session VI: Treatment of WM: The Emerging Role of Histone Deacetylase Inhibitors in WM, by Dr. O. O’Connor). Dr. Jia and colleagues at Dr. Ghobrial’s lab at the Dana Farber Cancer Center, Boston, MA, evaluated the novel HDAC inhibitor LBH589 in existing WM cell lines as well as in bone marrow primary CD19+ cells and bone marrow stromal cells (BMSC) obtained from WM patients. LBH589 significantly decreased cell proliferation and resulted in cell death in cell lines as well as in bone marrow primary CD19+ WM cells. LBH589 was effective in WM bone marrow cells despite the presence of BMSC, IL-6 and IGF-1, which are all known to induce resistance to conventional therapies. LBH589 did not harm healthy donor peripheral blood mononuclear cells. LBH589 exerts its effects primarily in the apoptotic pathways in a dose-dependent manner. There is significant up-regulation of a proapoptotic transcription factor and down-regulation of antiapoptotic proteins. LBH589 also inhibited important NF- κ B pathways. When used in combination with rituximab, fludarabine, bortezomib and perifosine, LBH589 significantly increases the cytotoxic effectiveness of these treatments on WM cells. These results support the development of clinical trials evaluating LBH589 as a new therapeutic agent in patients with WM.

- Dr. J. Sun: **Histone deacetylase inhibitors demonstrate significant preclinical activity as single agents and in combination with bortezomib in Waldenström's macroglobulinemia.**

Histone deacetylases (HDACs) are involved in DNA transcription regulation and signal transduction of cells (see Session VI: Treatment of WM: The Emerging Role of Histone Deacetylase Inhibitors in WM, by Dr. O. O'Connor). Dr. Sun and colleagues at Dr. Treon's lab at the Dana Farber Cancer Institute, Boston, MA, demonstrated the up-regulation of HDACs in WM cells by [gene expression profiling](#) techniques. The activity of HDAC class I, II, III inhibitors was studied using the HDAC inhibitors Vorinostat (Class I), Trichostatin A (Class II), and Sirtinol (Class III) as mono-therapy and in combination with bortezomib (Velcade) in WM cell lines. Not only was a dose-dependent increase in tumor cell killing demonstrated for all 3 classes of HDAC inhibitors, but synergistic tumor cell killing was also seen when all 3 classes of HDAC inhibitors were used in combination with sub-lethal doses of bortezomib. Similar studies are now being conducted in SCID-hu mice WM tumor models to further elucidate the mechanism of action of combined HDAC and proteasome inhibition in WM. Results of these studies may support the development of new clinical trials evaluating HDAC inhibitors, with and without bortezomib, in the treatment of WM.

- Dr. H. Ngo: **SDF-1/CXCR4 and VLA-4 interaction regulates homing in Waldenström's macroglobulinemia.**

The WM tumor cells are continuously migrating, or homing, from the circulation to the bone marrow, which in turn leads to significant infiltration of the bone marrow in most patients. Dr. Ngo of the Dana Farber Cancer Institute, Boston, MA, investigated the complex mechanisms whereby WM cells navigate and find their way to the bone marrow. It appears that WM cells express high levels of the chemokine (chemical attractant) and adhesion receptors [CXCR4](#) and VLA-4. The inhibition of WM cell migration by blocking the effect of CXCR4 using the inhibitor AMD3100 was demonstrated. CXCR4 or VLA-4 inhibition also led to decreased adhesion to stromal cells in the bone marrow, resulting in increased sensitivity of these cells to bortezomib. The migration and adhesion of WM cells in the bone marrow microenvironment was further elucidated by the study of downstream signaling pathways such as the CXCR4/[SDF-1](#) axis interaction with VLA-4.

- Dr. B. Hivert: **Acquired von Willebrand syndrome and von Willebrand factor abnormalities in Waldenström's macroglobulinemia.**

[Von Willebrand disease \(VWD\)](#) is the most common hereditary coagulation abnormality described in humans, although it can also be acquired as a result of other medical conditions. The [acquired von Willebrand syndrome \(AVWS\)](#) coagulation disorder seen in WM appears to be the result of multiple pathogenic mechanisms. The selective and pathologic adsorption of von Willebrand factor (VWF: a protein that is required for platelet adhesion) on WM tumor cells, increased VWF protein destruction, and the presence of neutralizing or non-neutralizing anti-

VWF antibodies can all contribute to AVWS. Dr. Hivert and colleagues from the Centre Hospitalier Schaffner, Lens, FRANCE, sought to determine the frequency of AVWS in WM patients. The two most common laboratory tests in VWD are ristocetin cofactor (VWF:RCo) activity and VWF antigen (VWF:Ag). These two tests were performed in 58 WM patients as well as 25 patients with IgM-MGUS or other non-WM lymphoproliferative disorder. The mean serum IgM level was 1800 vs. 700 g/L, and 7 WM patients were identified with AVWS. There appeared to be a significant relationship between serum IgM, VWF:RCo, and VWF:Ag. The reduction of serum IgM levels, either by chemotherapy and/or plasmapheresis resulted in increases in VWF:RCo and VWF:Ag and less frequent bleeding problems. Of the 7 WM patients with AVWS, 5 patients developed hemorrhagic complications, principally nose bleeds, but also bleeding complications in procedures such as bone marrow biopsies, cataract surgery, and insertion of plasmapheresis catheters. The authors suggest that VWF activity should be evaluated in WM patients routinely before initial diagnostic procedures since WM patients may have coagulation abnormalities related to AVWS.

- Dr. S. Poulain: **Is SDF-1 (-801 GA) polymorphism a new genetic prognostic for survival after treatment initiation in Waldenström's macroglobulinemia?**

The chemokine [SDF-1](#) (stromal cell -derived factor -1) and its interaction with the chemokine receptor CXCR4 influences the movement (or homing) of WM cells to the bone marrow microenvironment. A [polymorphism](#) on the SDF-1 gene may result in the overproduction of the WM cell attractant SDF-1. This study from Dr. Poulain, of the Centre Hospitalier de Valenciennes, FRANCE, evaluated the clinical implications of the specific SDF-1 (-801GA) polymorphism in WM patients. The SDF-1 genotype of 114 WM patients (M/F=0.65, median age: 68 years, range 39 to 91) was determined using PCR RFLP assay. 33 % of the WM patients were heterozygous for the SDF-1 (SDF-1-801GA) allele. These patients were not noted to have any significant biological and clinical features at diagnosis compared to the remainder of the WM patients studied. Patients with the SDF-1 (-801GA) or SDF-1 (-801AA) genotype had a 94% 5-year survival after treatment initiation compared to the 5-year survival after treatment initiation of 59% for WM patients with the SDF-1 (-801GG) genotype. There were no differences in the incidence of disease transformation, myelodysplastic syndrome or acute leukemia, and secondary cancers between each genotype subgroup. The authors conclude that a WM patient's prognosis for survival after treatment initiation may be related to the presence of a specific polymorphism on the SDF-1 gene.

- Dr. L. Vallat: **Toward a proteomic specific WM entity.**

Proteomics is the scientific study of the proteins produced by the genes of cells. It encompasses the identification, quantification, structure, and function of proteins (particular those proteins involved in metabolic pathways). A number of genetic research studies have already identified abnormalities in chromosomes and gene expression in WM patients compared to other [lymphoproliferative](#) disorders (e.g. CLL, MM). Dr. Vallat and colleagues from the Pitie-Salpetriere Hospital, Paris, France, sought to further evaluate these genetic abnormalities by determining the proteomic differences between untreated WM, Marginal Zone Lymphoma

(MZL), frontier MZL with monoclonal IgM and Chronic lymphocytic leukemia (CLL) patients. Using sophisticated laboratory techniques, 129 proteins were noted to be statistically differentially expressed in WM compared to CLL or MZL. Some of the identified proteins are implicated in the growth and survival of WM cells. The researchers are continuing to define the proteomic signature of WM in a larger number of patients by identifying and validating 12 up-regulated and 39 down-regulated proteins. This proteomic signature may one day serve as WM diagnostic and prognostic markers.

- Dr. T. Ioakimidis: **Comparative outcomes following CP-R, CVP-R and CHOP-R in patients with Waldenström's macroglobulinemia.**

The improved outcomes in non-Hodgkin's lymphoma patients treated with Rituxan (R) combination chemotherapeutic regimens has led to questions surrounding the need for the potentially toxic drugs Adriamycin and Vincristine in the treatment of WM. Dr. T. Ioakimidis and colleagues from the Dana Farber Cancer Institute, Boston, MA, reviewed 60 WM patients who were treated at their institution either with Cytoxan + Prednisone + Rituxan (CP-R, n=20), Cytoxan + Adriamycin + Prednisone + Rituxan (CVP-R, n=17), or Cytoxan + Adriamycin + Vincristine + Prednisone + Rituxan (CHOP-R, n=23). Median age, IgM and β -2M for patients was: 65, 2620, 2.3 (CP-R); 60, 2220, 3.3 (CVP-R); 54, 5150, 3.6 (CHOP-R). Overall response rate, complete response or near-complete response, and median decrease in serum IgM was: 90%, 0%, -54% (CP-R); 88%, 12%, -67% (CVP-R); 83%, 17%, -63% (CHOP-R). The study's results demonstrates comparable overall response rates but higher complete response or near-complete response rates for patients treated with CVP-R and CHOP-R. Of significant importance was the increased rate of toxicity in patients treated with CVP-R and CHOP-R compared to CP-R (neutropenic fever, treatment related neuropathy). The authors suggest that in comparable patients, the use of CP-R may confer equally efficacious overall response rates and minimize treatment related toxicity.

- Dr. S. Peinert: **Fludarabine based combinations are highly effective as first-line or salvage treatment for patients with Waldenström's macroglobulinemia.**

It is becoming increasingly clear that combination therapies are more effective than single agent therapy in the treatment of WM. Dr. Peinert and colleagues from the Peter MacCallum Cancer Centre, East Melbourne, Australia, reviewed data from 24 patients who were given intravenous fludarabine (F) combination therapy. Combinations used were: F + cyclophosphamide (C); FC + rituximab (R); F + mitoxantrone (M); F + R. Patients completed at least two treatment cycles, median age was 57yrs (range; 36-89), 84% were male, median time from diagnosis was 22 months, and baseline IgM was 3100 mg/L. A median 4 (2-6) cycles was administered per patient; no life-threatening complications were experienced; 3 heavily pre-treated patients subsequently developed [AML/MDS](#) at 52, 61 and 99 months post-treatment; 78% of the patients achieved a median IgM reduction of 90%, of which one patient had an immunofixation-negative complete response. The median time-to-progression was 57 months for all patients; the 5 and 10 year survival rates are $85 \pm 8\%$ and $68 \pm 13\%$ respectively (median follow-up of 63 months); all 6 previously untreated patients remain alive and progression-free (median follow-up of 50

months). The authors conclude that fludarabine-based combination therapy is highly active in treated and untreated WM patients. High response rates and prolonged remissions must be balanced however with the cumulative risk of treatment-related AML/MDS in heavily pre-treated patients.

GLOSSARY OF SELECTED TERMS

Allele: One member of a pair of genes occupying a locus, or specific spot, on a chromosome. As an example, in a pair of alleles that controls eye color, one allele codes for blue eyes, and another allele for brown eyes. Some alleles are dominant over other alleles, as in the case of heterozygous pairings (where paired alleles are different, in contrast to homozygous pairings where alleles are the same). In humans, simple traits such as eye color may be caused by the interaction of only one pair of alleles, but complex proteins such as immunoglobulins, or proteins involved in complex pathways, are usually caused by the interactions of series of alleles.

Apoptosis: A normal cellular process that leads to programmed cell death. Apoptosis is a tightly regulated and encoded genetic event that causes a cell to die at a certain time. Some cancerous cells are unable to experience the normal apoptosis-driven natural cell death process because of genetic mutations. Apoptosis can be triggered by various stimuli from outside or inside the cell: by activation of cell surface receptors (e.g. CD20), by DNA damage as a cause of defects in DNA repair mechanisms, by treatment with cytotoxic drugs or irradiation, by a lack of survival signals, etc.

Apoptotic: adjective, relating to apoptosis.

Chemokine: Proteins secreted by cells that are able to chemically attract and activate white blood cells. Chemokines are involved in a wide variety of processes including acute and chronic types of inflammation, infectious diseases, and cancer. Chemokines may lure cancer cells and help determine the sites to which cancer cells spread by metastasis. Most chemokines belong to one of two major sub-families, the CXC and CC subfamilies.

Chemotaxis: The phenomenon in which cells, bacteria, and other organisms direct their movements according to certain chemicals in their environment. Chemotaxis is critical to early (e.g. movement of sperm towards the egg during fertilization) and subsequent phases of development (e.g. migration of neurons or lymphocytes) as well as in normal function (movement of white blood cells to sites of infection). Chemotaxis is involved in the movement of cancer cells from one area of the body to another (e.g. cancer metastasis, bone marrow homing).

Cytokines: Any of several regulatory proteins, such as the interleukins, lymphokines, chemokines that are released by cells of the immune system and act as intercellular mediators in the generation of an immune response.

CXCR4: (CXC chemokine Receptor) A chemokine receptor specific for stromal-derived-factor-1 (SDF-1), a molecule endowed with potent chemotactic activity for lymphocytes. CXCR4's ligand SDF-1 is known to be important in hematopoietic stem cell homing to the bone marrow. Because the interaction between SDF-1 and CXCR4 plays an important role in holding hematopoietic stem cells in the bone marrow, drugs that block the CXCR4 receptor appear to be capable of "mobilizing" hematopoietic stem cells into the bloodstream as peripheral blood stem cells.

Epidemiology: The study of factors affecting the health and illness of populations, identifying risk factors for disease, and determining optimal treatment approaches.

FISH: (Fluorescence in Situ Hybridization) FISH is a special molecular genetics technique that enables the identification of chromosome abnormalities that result from genetic variations. FISH technology complements routine chromosome analysis by utilizing chromosome-specific DNA probes. A mutated gene segment in a chromosome can be made to “light up” or fluoresce when it is bound by a special probe. Genetic changes in some cancers can be detected using this method.

Flow cytometry: A method of sorting and measuring types of cells by fluorescent labeling of markers on the surface of the cells.

Gammopathy: Abnormal increase in the synthesis of protein immunoglobulins having antibody activity in the blood.

Gene expression profiling: The measurement of the activity (gene expression profiles) of multiple genes to create a global picture of cellular function. This research method measures messenger RNA made from genes and can distinguish between cells that are actively dividing or show how the cells react to a particular treatment. Gene expression profiling studies show which genes show significant differences under experimental conditions. These studies are also being used as a diagnostic test to help predict which patients may respond to treatment and which patients may be at increased risk for cancer relapse.

Genotype: The inherited genetic code of an organism for a specific trait.

Heterozygous: Possessing two different forms of a particular gene, one inherited from each parent. Heterozygous is in contrast to homozygous, the possession of two identical copies of the same gene.

Hepatosplenomegaly: Abnormal enlargement of both the liver and the spleen, as may be seen in cases of lymphoma, and other diseases.

Immunophenotyping: A technique used in basic science research and diagnostic laboratory to study the protein expressed by cells (the immunophenotype, e.g. CD19+CD20-). It involves the labeling of white blood cells with antibodies directed against surface proteins on their membrane. The identification of cells can be accurately determined by choosing appropriate antibodies. The labeled cells are processed by flow cytometry, using a laser-based instrument capable of analyzing thousands of cells per second. This procedure can be performed on cells from the blood, bone marrow or spinal fluid.

Interleukins: (IL): A group of messenger protein molecules made by white blood cells (primarily) and other cells in the body that help regulate immune responses.

Ligand: A molecule such as a cytokine, hormone, or neurotransmitter, that binds to a specific receptor.

Lymphadenopathy: Disease or swelling of the lymph nodes

Lymphoplasmacytic: Consisting of lymphocytes and plasma cells

Lymphoproliferative: Referring to the proliferation of the bone marrow cells that give rise to lymphoid cells (such as lymphocytes and plasma cells) and other related cells (such as macrophages). The term lymphoproliferative is in contrast to myeloproliferative which refers to proliferation of bone marrow elements including red cells, granulocytes, and platelets.

Lymphoplasmacytic lymphoma: An indolent type of non-Hodgkin lymphoma marked by abnormal levels of IgM antibodies in the blood and an enlarged liver, spleen, or lymph nodes; also called Waldenström macroglobulinemia. (National Cancer Institute, U.S. National Institutes of Health Dictionary of Cancer Terms).

MDS/AML: Myelodysplastic syndromes is a condition where the bone marrow does not produce enough healthy blood cells; often used interchangeably or together with acute myeloblastic leukemia which is a rapidly progressing disease where an excessive amount of immature white blood cells are found in the blood and bone marrow.

Monoclonal Gammopathy: Abnormal increase in the synthesis of protein immunoglobulins from a single clone of cells (a single cell and the progeny of that cell), having antibody activity in the blood.

Oncogene: A gene that normally directs cell growth. If altered, an oncogene can promote or allow the uncontrolled growth of cancer. Alterations can be inherited or caused by an environmental exposure to carcinogens.

PCR RFLP assay: A polymerase chain reaction is a key technique in molecular genetics that permits the analysis of any short sequence of DNA by reproducing (amplifying) selected sections of DNA. PCR is so highly efficient so that untold numbers of copies can be made of the DNA, and PCR done in test tubes takes only a few hours. A restriction fragment length polymorphism (RFLP) is a local variation in the DNA sequence of an individual. The basic technique for detecting RFLPs involves fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA whenever it recognizes a certain highly-specific short sequence (or restriction site) within the DNA. The PCR RFLP assay is a technique utilized in genetic analysis/fingerprinting.

Polymorphism: A naturally occurring variation in DNA sequence. Polymorphisms are useful as genetic markers because they allow researchers to distinguish between DNA of different origins. Forms of a gene (alleles) that are too prevalent in the population to be called mutations or to be

regarded as abnormal are called polymorphisms. Polymorphisms may confer an advantage in some circumstances (e.g. response to rituximab).

Proteomics: A field of study in molecular biology encompassing the identification, quantification, structure, and function of proteins and the effect of modifications, interactions, and activities of these proteins in the cell. Proteins are vital parts of living organisms as they are the main components of the physiological metabolic pathways of cells.

Proteasome: A protein degradation machine within the cell that can digest a variety of proteins into short polypeptides and amino acids. It is hollow and has openings at both ends to allow entry of the protein to be digested. A human cell contains about 30,000 proteasomes. These barrel-formed structures can break down practically all proteins to 7-9-amino-acid-long peptides.

Retinopathy: Any disease of the retina, the light-sensitive membrane at the back of the eye.

SDF-1: (stromal cell-derived factor -1): A small cytokine belonging to the chemokine family that is officially designated chemokine ligand 12 (CXCL12). SDF-1 is the only known ligand for CXCR4 (CXC chemokine Receptor) . CXCL12 is strongly chemotactic for lymphocytes. The SDF-1 gene is on chromosome 10q11.1.

Trisomy: The presence of three copies of a chromosome rather than the normal two. The most well known common trisomy is trisomy 21 (Down's syndrome).

Trogocytosis: The transfer of cell membrane material (of T-cell receptors such as CD80 and CD86 for example) between T- cells; often used as a communication mechanism between helper T-cells and cytotoxic killer T-cells and may be a method whereby tumor cells can escape detection by the body's immune system.

Von Willebrand's disease: (vWD): The most common hereditary coagulation abnormality described in humans, it can also be acquired as a result of other medical conditions. The various types of vWD present with varying degrees of bleeding tendency, usually in the form of easy bruising, nosebleeds and bleeding gums. When suspected, blood plasma of a patient needs to be investigated for quantitative and qualitative deficiencies of von Willebrand Factor (vWF). This is achieved by measuring the amount of vWF in a vWF antigen assay, and measuring the functionality of vWF with a ristocetin cofactor activity (VWF:RCo).

Acquired von Willebrand syndrome: (AVWS): AVWS can occur in patients with auto-antibodies, such as in WM. In this case the function of vWF is not inhibited but the vWF-antibody complex is rapidly cleared from the circulation. See **Von Willebrand's disease**.

Many more helpful definitions can be found at: <http://www.cancer.gov/dictionary/>