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PRESIDENT'S MESSAGE

BY JUDITH MAY



Judith May, President

The Ed Forum Review summarizes the many presentations of our 16th annual Educational Forum held in Minneapolis, Minnesota. Attendees had the opportunity to hear from renowned researchers and clinicians with expertise in the study and treatment of Waldenstrom's macroglobulinemia.

We are indebted to our editorial staff, whose names appear on page 2, and we also extend thanks to Jack Whelan, who took the photographs that appear in these pages.

You may order a set of three DVDs of these presentations using the order form on the last page. Or, if you prefer, you may purchase them online at our website www.iwmf.com/iwmf-library/dvds.aspx. The cost is \$25 to IWMF members and \$35 to non-members and includes shipping.

VARIATIONS OF WALDENSTROM'S MACROGLOBULINEMIA

ROBERT KYLE, M.D.

Mayo Clinic, Rochester, MN



Robert Kyle, M.D.

According to Dr. Kyle, approximately 5% of persons over 70 years of age have MGUS (monoclonal gammopathy of undetermined significance), either of the IgM, IgG, or IgA type, so it is not that uncommon in the older population. Also, just about everyone with WM has had IgM MGUS at one time.

Dr. Kyle described both IgM MGUS and smoldering WM, the rate of their progression to full-blown WM or related disorders, and the risk factors for progression.

There are several criteria used to distinguish WM from MGUS. Generally speaking, MGUS is characterized by a serum monoclonal protein less than 3g/dL; less than 10% infiltration of the bone marrow with lymphoplasmacytic cells; absence of symptomatic anemia, lymphadenopathy (enlarged lymph

nodes), hepatosplenomegaly (enlarged liver and spleen), and hyperviscosity; and absence of constitutional symptoms (fever, chills, night sweats, weight loss, and fatigue).

Dr. Kyle was involved in a long-term study (1960-1994) in Minnesota of 213 IgM MGUS patients that determined the rate of progression of IgM MGUS to WM, as well as to the

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related diseases of amyloidosis, chronic lymphocytic leukemia, and other types of non-Hodgkin's lymphoma. MGUS tends to progress more commonly to WM than to these other diseases. Overall the relative risk of progression of IgM MGUS to WM and other similar diseases is approximately 1.5% per year.

Risk factors for progression of IgM MGUS to WM include the size of the monoclonal spike, low serum albumin, and reduced hemoglobin level.

Smoldering WM is characterized by a serum monoclonal protein equal to or greater than 3g/dL and/or infiltration of the bone marrow with lymphoplasmacytic cells equal to or greater than 10%; absence of symptomatic anemia, lymphadenopathy, hepatosplenomegaly, and hyperviscosity; and absence of constitutional symptoms. Smoldering WM patients tend to be older, with an average age of 63. There are more men than women affected, and the occurrence of IgM with kappa light chains is more prevalent than IgM with lambda light chains. A small percentage is biclonal (two monoclonal spikes).

Risk factors for progression of smoldering WM to WM include the percentage of lymphoplasmacytic cells in the bone marrow, reduced level of hemoglobin, the size of the monoclonal spike, and reduction in uninvolved immunoglobulin levels, particularly IgA.

In Dr. Kyle's study, the relative risk of progression of smoldering WM to symptomatic WM or amyloidosis was determined to be approximately 11% per year for the first five years of follow-up, and then 2% per year for five to ten years of follow-up. This still leaves a significant percentage of patients who may not progress to WM, and Dr. Kyle emphasized that neither IgM MGUS nor smoldering WM should be treated because patients can remain stable with either one for many years. By not treating at an early, asymptomatic stage, MGUS and smoldering WM patients can thus avoid some of the toxicities associated with many types of treatment.

Dr. Kyle answered a number of questions from the audience. Several of them dealt with the presence of Bence Jones proteins in the urine. Bence Jones proteins are the light chain portion (either kappa or lambda) of the monoclonal immunoglobulin, and excess amounts of these proteins may be found in the urine of WM patients. These proteins have the potential to damage the kidneys, and patients with a high Bence Jones protein level in their 24-hour urine specimens should be monitored for kidney problems.

Dr. Kyle emphasized that he would not treat anyone on the basis of IgM level alone, unless the patient was manifesting symptoms of hyperviscosity. There is no specific IgM level or serum viscosity measurement that denotes hyperviscosity – the diagnosis of hyperviscosity is based on symptoms and findings such as blurred vision, bleeding from the nose, gum, and gastrointestinal tract, mental confusion, and dilatation and sausageing of the retinal veins of the eye.



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BONE MARROW BIOPSIES AND WHAT YOU LEARN FROM THEM

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William G. Morice, M.D., Ph.D.

Patients suspected of having WM soon learn that a bone marrow biopsy will be performed as a part of their diagnosis. Following the biopsy, they will receive a very detailed description of cells observed in their marrow, but rarely will they have the opportunity to interact directly with the person providing that description. In this presentation, WM patients at the Ed Forum learned what

to look for in their bone marrow biopsy reports from Dr. William Morice, a bone marrow specialist who has published several recent papers of particular significance to WM.

Why are biopsies performed in connection with WM? Most patients begin their WM journey when they see a doctor because of an unusual blood test result or because of unexplained symptoms, such as peripheral neuropathy or anemia. Several tests may be performed until finally a bone marrow biopsy is ordered to make a final diagnosis. Biopsies may also be required later to assess response to treatments or to evaluate why the disease is behaving in a certain way. WM is inherently a disease of cells that are produced in the bone marrow, so it makes sense to look there to assess the status of the disease.

The bone marrow sample is collected by a hematologist, pathologist, surgeon, or trained nurse, using local anesthesia in some cases or mild general anesthesia in others. The sample is almost always taken from the rear hip, which is usually a very active area of hematopoiesis (blood formation). The portion of bone selected is covered by very little skin or connective tissue and is “easy” to access from a patient lying flat on his or her stomach.

There are generally two types of samples that are prepared. One is a smear of the liquid portion of the marrow (or aspirate) and the other is an actual cored section of the marrow. Once the samples are collected, it is the job of the pathologist to choose which tests to perform. There are two primary objectives when conducting bone marrow biopsies on potential WM patients: (1) to determine if there is a malignancy; and (2) if a malignancy is present, to determine what types of cells are causing the malignancy. The pathologist must work with the

clinician to select the right tests on the marrow samples at the right time.

Dr. Morice indicated that WM cases can sometimes be particularly challenging to diagnose owing to the variability in the way the disease occurs. Several slides were presented to the audience showing what WM cells look like compared to normal cells. Normal B-lymphocytes have a large, darkly colored nucleus that is surrounded by a relatively thin, lightly colored layer of cytoplasm. Normal plasma cells are much larger than B-lymphocytes and contain a greatly expanded volume of cytoplasm surrounding the nucleus. Malignant WM cells are typically intermediate between B-lymphocytes and plasma cells, but in one patient can look more like normal B-lymphocytes, while in another patient more like plasma cells. This can make the cells difficult to distinguish from several other types of lymphoma and myeloma. WM cells from yet another patient can span the whole range from B-lymphocyte to plasma cell in appearance. This range of appearance occurs because the malignant WM cells, although derived from a single cell, can retain the capacity to undergo continued differentiation from B-lymphocyte to plasma cell.

Of course, pathologists do not rely on appearance alone to make their diagnosis. Many sophisticated tests have been developed, the most important of which is referred to as “immunophenotyping.” All cells, healthy or cancerous, contain surface proteins that are referred to as antigens. Clonal disorders, such as cancer, produce abnormally large populations of cells that all share the same characteristics and surface protein expressions. The antigens for cancers of different types vary greatly from one other. Thus, by analyzing the antigens on the surfaces of malignant cells, a pathologist can refine the diagnosis of cancer type. Immunophenotyping involves the use of antibodies that react with these surface antigens to help the pathologist determine the protein expression on the surface of the cells.

One method of immunophenotyping is referred to as “immunohistochemistry.” This method involves treating the cells on the biopsy slides with antibodies that stain specific antigens and allows cells containing those antigens to be quickly distinguished from cells that lack the antigens. Of particular importance for WM and other lymphomas are the surface antigens on the B-lymphocytes and plasma cells that are thought to be malignant. One of the many important antigens for B-lymphocytes, for example, is CD20, the same antigen that forms the basis for rituximab and ofatumumab immunotherapies. The more plasmacytic cells in WM, however, usually lose CD20 surface expression but gain CD138. CD138 can also be stained and evaluated using a different antibody treatment. By comparing distributions of CD20 and CD138 cells in a marrow biopsy, the pathologist can better evaluate the relationships between lympho- and plasma-cytic cells in the WM clone. Numerous other stains



have been developed for other surface antigens, the presence or absence of which can provide additional clues that a pathologist can use to identify and characterize the disease in the marrow.

The second commonly used immunophenotyping method is called “flow cytometry.” This method is only used on the aspirate, or liquid, fraction of the biopsy sample. In this technique, antibodies are used to attach compounds that fluoresce (glow) to antigens on cell surfaces. Instruments are then used to analyze the light emitted from these cells. Because specific wavelength (or color) of the light produced can be varied, each antigen can be marked with its own identifying color. This allows multiple surface antigens to be evaluated on the same sample at the same time. Flow cytometry requires a fresh aspirate sample, so the pathologist must work with the clinician to decide which antigens to analyze before the sample is collected.

Dr. Morice emphasized that, even with the development of these sophisticated tools, WM is a highly variable disease and requires clinicians and pathologists to act as a team in order to make a complete diagnosis. Phenotypes (physical appearances) for WM cells often look like those in other disorders, such as chronic lymphocytic leukemia and mantle cell lymphoma, even though the clinical symptoms and pathways for these diseases are unique. Furthermore, it has been found that the pathological characteristics of the marrow do not predict the clinical features of particular WM cases. A bone marrow biopsy, in effect, only provides a single sample from a single point in time for a dynamic and potentially progressive disease process. Teamwork between the clinician who is following the disease and the pathologist who understands WM is fundamental for making the best diagnosis and treatment decisions.



PERIPHERAL NEUROPATHY IN WALDENSTROM'S MACROGLOBULINEMIA

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Michelle L. Mauermann, M.D.

Peripheral neuropathy (PN) is a dysfunction or disease of peripheral nerves (nerves outside the brain and spinal cord). This problem affects about 2-3% of the general population and up to 8% of those over 55 years of age.

A peripheral nerve is an enclosed, cable-like structure that contains bundles of axons. Axons are long, slender projections that extend from spinal nerve cells (neurons) via nerve roots adjacent to the spinal cord out to peripheral portions of the body. Each axon (with the exception of those less than about one micron in diameter) is covered by individual Schwann cells whose plasma membranes form an insulating, laminated spiral sheath called myelin. Gaps between individual Schwann cells and their myelin are called nodes of Ranvier. Surrounding the myelin is delicate connective tissue called the endoneurium. A bundle of axons is surrounded by a sheath called the perineurium, and the entire peripheral nerve is composed of many such bundles separated by delicate vascular connective tissue membranes that are continuous with the outermost connective tissue layer, the epineurium, which surrounds the nerve. The insulating myelin and the nodes of Ranvier together greatly speed the transmission of electrochemical impulses between the spinal nerve cell and the far end of the axon by allowing the signals to “hop” from node to node rather than traveling as a slower continuous wave. In the case of a motor nerve, the signal passes from the spinal cord to the nerve, whereas in sensory nerves, signals travel in the opposite direction, from the periphery to the spinal cord.

EVALUATION OF PATIENTS WITH NEUROPATHY

Information obtained from the patient’s personal clinical history and family history, as well as physical examination, is very important in assessing which types of nerves are involved.

In **sensory neuropathy**, the patient’s history reveals symptoms that indicate diminished sensation, such as numbness of the feet; the feeling that socks are being worn when they are not; or in the case of cranial nerve involvement, numbness on areas of the face. There may be abnormal sensations such



as burning, shooting/stabbing pains, electrical sensations, and an abnormal sensitivity to touch (e.g., bed sheets feel uncomfortable). Five types of sensation are tested during physical examination, including pain (pinprick sensation); vibration sense (tuning fork); joint position (awareness of toe position and ability to maintain balance when standing with the eyes closed); touch; and temperature sense.

Motor neuropathy is revealed by symptoms such as foot slapping while walking; difficulty arising from a chair or toilet seat due to leg muscle weakness; decreased grip strength or difficulty in using tools; difficulty in lifting the arms above the head; and in the case of cranial nerve involvement, motor symptoms such as difficulty in chewing or swallowing. Signs of motor involvement may include visible muscle loss or twitching of involved muscles.

In **autonomic neuropathy**, the affected nerves involve bodily functions that occur automatically, without conscious intervention. If autonomic nerves are damaged (or if their function is affected by medications), symptoms of impairment of the affected organs may appear. Examples include lightheadedness upon standing due to failure of blood pressure control; dry mouth; dry eyes; digestive problems including an early satiety (feeling of fullness after eating), bloating or vomiting; lower bowel symptoms including diarrhea and constipation; lack of sweating in some parts of the body (e.g., feet not sweating after vigorous exercise); and male erectile dysfunction.

Patterns identified in the personal medical and family histories and from the physical examination provide the neurologist with clues to determine not only which nerve fibers are involved (sensory, motor or autonomic), but also which different sets of diagnoses should be considered. The following are key medical and family history questions:

- Are symptoms and physical examination findings similar on both sides of the body? Did the symptoms start in the feet and work their way upward? If so, these symptoms indicate a “length-dependent” neuropathy (one that affects the long peripheral nerves of the extremities starting at the far ends).
- What has been the pace of onset and progression of symptoms? Genetic, toxic, metabolic, medication-related, and vitamin deficiency neuropathies tend to have a gradual onset and to be length-dependent, whereas immune neuropathies secondary to inflammation, a viral illness, or a cancer tend to have rapid onset and progression, or to progress in a pattern of relapses and remissions.
- Does the patient have preexisting medical conditions such as diabetes or an autoimmune disease that may cause neuropathy?
- Do the patient’s medications include any drugs that can cause neuropathy?

- Does the patient or a close relative have high arches, hammer toes, or toes that curl under? These may be indicative of an inherited neuropathy. One third of neuropathies are inherited.

Peripheral neuropathy is really a syndrome (a set of signs and symptoms) rather than a disease. Peripheral neuropathies of many different causes may present with similar signs and symptoms. The most common category of peripheral neuropathy is **length-dependent or distal neuropathy** (mentioned above). Symptoms appear first in the feet, gradually progress up to the knees, and then involve the fingertips. Patients often have the sensation of having stockings on their feet and gloves on their hands. In this type of neuropathy, the symptoms are predominantly sensory, so that patients feel numbness more often than weakness. The differential diagnosis includes diabetes mellitus (most common); B12 deficiency; monoclonal protein-associated neuropathy; inherited neuropathy; severe kidney disease; alcohol-induced neuropathy; and hypothyroidism. Laboratory blood and urine testing is directed toward excluding or confirming the presence of one or more of these conditions.

Several types of neurological tests may be performed to help determine the diagnosis, and these tests may be aimed at large or small nerve fiber function.

Nerve conduction studies (NCS) and electromyography (EMG) are useful for detecting large fiber abnormalities. Nerve conduction studies assess the integrity of the sensory and motor nerves, and if abnormalities are present, whether it is the axons or their myelin sheaths that are damaged. If axons are involved, the responses are smaller (lower amplitude) than normal due to the presence of fewer intact signal-carrying fibers. If myelin sheaths are involved, then the responses are slower. Electromyography involves the placement by the neurologist of a small needle into muscles of the involved areas to assess the distribution of the neuropathy among motor axons and its severity. Evidence of demyelination can also be obtained.

There are several tests used for small nerve fiber function. Quantitative sensory testing utilizes specialized instruments to provide more detailed sensory testing than the simple tests performed by the neurologist during physical examination. This testing is partially subjective in that patients provide yes or no answers in response to stimuli. It is useful in assessing sensory thresholds corresponding to different fiber types: A-β fibers (vibration); A-δ fibers (cold); and C fibers (pain and heat). Because sensitivity to stimuli in the feet decreases both with age and with increasing height, normal values that vary according to age and height are used for reference. The test results are useful both for initial evaluation and for evaluating response to treatment. The autonomic reflex screen (as performed at Mayo Clinic) includes a battery of tests to evaluate sweating, heart rate response during deep breathing, heart rate and blood pressure response while attempting to



exhale against resistance, and heart rate and blood pressure response to tilt table maneuvers. A thermoregulatory sweat test gives a good picture of sweat production over the entire body. A skin biopsy involves taking a few small punch biopsies that are examined with special stains to enable the microscopic identification and counting of small, unmyelinated nerve fibers that extend from the superficial dermis into the epidermis. The number of nerve fibers should be decreased in small fiber neuropathy, but the technique is subject to sampling error and does not indicate the cause of the neuropathy. A nerve biopsy is intended for the detection of large fiber injury, and to a degree, small fiber injury and provides information regarding the cause of the neuropathy. Used in selected cases at Mayo Clinic, it usually involves removal, under local anesthetic, of a segment of the sural nerve behind the ankle or from the posterior part of the leg. The drawback of sural nerve biopsy is that it leaves part of the foot permanently numb.

WM AND PERIPHERAL NEUROPATHY

WM can be associated with peripheral neuropathy in several different ways: from the disease itself through deposition of IgM and anti-myelin antibodies, from cryoglobulinemia (particularly when associated with hyperviscosity), and from amyloidosis.

IgM-MGUS (monoclonal gammopathy of undetermined significance) has been known for some time to be associated with a gradually progressive, painless, distal neuropathy. The symptoms are predominantly sensory (numbness and tingling), but some balance problems (ataxia) and mild tremor are also noted. The neuropathy in IgM-MGUS had been thought to be identical with that in WM, primarily because it is likely that cases of IgM-MGUS had been combined with WM cases when the bone marrow biopsies needed for diagnosis had not been done. While the neuropathies of IgM-MGUS and WM are similar, a recent Mayo Clinic study demonstrated that the incidence of demyelination, as identified by slowed conduction time in nerve conduction studies, is much greater in IgM-MGUS. In this series, the incidence of demyelination was 62% in 73 patients with IgM-MGUS neuropathy and only 27% in 30 patients with WM neuropathy. Dr. Mauermann stated that in contrast to IgM-MGUS, neuropathy in WM is more likely to involve the nerve axons.

PN is the presenting symptom in up to 25% of WM patients. The median age of development of neuropathy is 65 years. It is more frequent in men (73%), and the most common symptom is numbness of the toes and feet (93%). Only 7% of patients had fine tremor with the hands outstretched as the presenting symptom.

Cryoglobulins (cold-associated antibodies) can occur in WM. PN due to cryoglobulins differs from that of most WM patients in that it is often asymmetric, multifocal, and painful. Skin lesions and internal organ involvement may be present.

Amyloid, which can occur in WM patients, is a material composed of monoclonal immunoglobulin light chains and can be deposited within nerves or small blood vessels that supply nerves. Typical symptoms are those of distal, painful PN with sensory symptoms predominating over motor weakness. Autonomic nerves are typically involved, causing symptoms as previously described above.

CHEMOTHERAPY AND NEUROPATHY

Chemotherapeutic agents used in treating WM may also cause neuropathy or may worsen existing PN.

Bortezomib (Velcade) – In multiple myeloma and WM trials, bortezomib-induced PN occurred in 23-44% of patients. Dose reductions were necessary in 12%, and dose discontinuance was necessary in 5-8%. This type of PN is a painful, sensory neuropathy that affects the hands and feet, although motor symptoms occur in a minority of cases. It frequently develops within three months of treatment, and patients who have not developed it within the first five cycles of treatment are unlikely to do so. Bortezomib can also worsen pre-existing neuropathy; however, the neuropathy usually improves within weeks of onset.

Thalidomide – There is some correlation of PN development to cumulative dose. This PN is typically associated with painful sensory symptoms in the feet and cramps in the leg muscles. The sensory neuropathy typically extends to the level of the knees and then involves the hands. There is also mild weakness. Prompt discontinuation of the drug after onset of symptoms leads to rapid recovery.

Vincristine – Symptoms develop in most patients within two months of receiving treatment. Paresthesia (tingling) develops first, frequently starting in the hands before involving the feet. Weakness and autonomic involvement can be prominent and disabling. Weakness usually improves rapidly if the drug is stopped early. Most patients are left with a mild, distal sensory loss. The drug can be received in the future if given at a lower dose.

TREATMENT OF NEUROPATHY

Treatment of the underlying disease may improve neuropathy. Some small trials of rituximab have been undertaken with modest therapeutic results for reduction of PN symptoms. As this treatment is not free of side effects, it is not recommended in patients with mild neuropathy.

The comprehensive approach to chronic pain management used at Mayo Clinic involves interdisciplinary assessment and treatment planning. The pain is first assessed. It may be continuous, paroxysmal, or may present as a painful sensation in response to a normally non-painful stimulus. The cause of the pain must be established to the extent possible, and co-morbidities such as cardiac, kidney or liver disease, or depression identified. The physician should explain the diagnosis, lay out a treatment plan, and establish realistic



expectations for the results of treatment. The goal in general is to reduce the pain by 50%.

First-line treatments may include the following:

- Soak the feet in cool water for 10 minutes at the end of the day and then dry them off (particularly for patients with very sensitive feet).
- Topical agents minimize systemic side effects and are good choices for the relief of mild symptoms of stabbing, shock-like pains and sensitivity. These may include a lidocaine patch or gel or combination gels/creams such as amitriptyline/ketamine combinations. Baclofen, clonidine, and lidocaine can also be added.
- Antidepressants (amitriptyline, nortriptyline, desipramine, Cymbalta, Effexor) and calcium channel ligands (Neurontin, Lyrica).
- Antiepileptic medications such as carbamazepine, oxcarbazepine, lamotrigine, topiramate, and valproic acid.
- Opioids may be used, especially when pain is new and severe.

WHAT IN THE TUMOR ENVIRONMENT OF WALDENSTROM'S MACROGLOBULINEMIA SUPPORTS CANCER CELL GROWTH AND FUNCTION?

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Stephen Ansell, M.D., Ph.D.

Dr. Ansell opened his presentation by describing the range of cells that are involved in WM, namely the B-lymphocytes and the plasma cells that are derived from them. The primary job of normal B-lymphocytes is to identify invaders and mount an immune response against them. A major part of the immune response involves the differentiation of B-lymphocytes into plasma cells. Normally produced plasma cells make the immunoglobulins that are used in the immune response.

Many types of immunoglobulins are made by plasma cells, including IgG, IgM, IgA, IgD, and IgE, each of which is designed to detect and react with specific antigens that are found on or produced by invaders.

WM is a disease that Dr. Ansell describes as “A Tale of Two Issues.” Issue one is that the cells produced in WM are stuck on making only one very specific kind of IgM (monoclonal IgM). While the body typically has mechanisms that carefully control the composition and amounts of immunoglobulins produced, production of the monoclonal IgM is very poorly controlled in WM patients. The resulting high levels of IgM produce many of the clinical symptoms found in WM patients. Issue two is that the malignant lymphoplasmacytic (LPL) cells can proliferate in the bone marrow and in other sites throughout the body. The LPL cells produced in WM span the cytologic spectrum from small lymphocytes to well-formed plasma cells. These can fill the marrow and congregate in other sites within the body, potentially crowding out and interfering with normal body functions.

An interesting aspect of WM is the great variability found in the disease in different patients. Even though the LPL cells may have similar genetic profiles and surface protein expressions, they can behave in very different ways in different WM patients. Just what is it that causes these differences? To help answer this question, Dr. Ansell and other WM scientists have been studying the detailed interactions that take place within the bone marrow microenvironment. Much recent attention has been focused on a particular class of proteins called cytokines.

Cytokines are proteins that allow cells to communicate with each other. They are secreted by cells when exposed to a specific stimulant and when detected by other cells, trigger a response. Types of responses may involve increasing or decreasing production of compounds needed by the body or deciding whether to grow and proliferate or whether to undergo apoptosis (programmed cell death). Understanding where the cytokines come from and why they are elevated in WM patients may lead to a better understanding of why the variations in WM exist and may also lead to better WM treatments.

According to Dr. Ansell, the concentrations of many cytokines are elevated in WM patients, but the communication network is quite complex. Although significant progress has been made, we still don't have the complete picture. Dr. Ansell described several of the most important cytokines in WM.

BLYS appears to be one of the more important ones and stands for “B-Lymphocyte Stimulator.” As the name implies, this cytokine is critical for maintaining normal B-cells and immunoglobulin production. It has been found that too little BLYS in mice results in the absence of B-lymphocytes and low immunoglobulin levels. On the other hand, too much BLYS has been shown to produce lymphoma in mice. WM patients tend to have elevated BLYS, and when BLYS is added



to WM cells in cultures, they secrete more IgM. BlyS is generated by non-WM cells in the tumor microenvironment. BlyS also collaborates with other cytokines, including IL-6, to cause production of higher levels of IgM in WM cell cultures than when BlyS is added alone.

IL-6 is an important cytokine that is produced under conditions of inflammation. IL-6 causes B-lymphocytes to proliferate and differentiate into plasma cells; it also stimulates T-cells to proliferate. IL-6 is typically elevated in the serum of WM patients compared to healthy control groups. Addition of IL-6 to WM cell cultures stimulates additional production of IgM. It is important, therefore, to determine what causes IL-6 to be elevated in WM patients.

IL-6 levels appear to be controlled, at least in part, by another cytokine: CCL5 (also called RANTES). When CCL5 is added to WM cell cultures, the production of IL-6 goes up. CCL5 is produced both by cells in the bone marrow microenvironment and by the WM cells themselves. A positive feedback loop is set up, whereby the microenvironment produces CCL5 that causes the WM cells to proliferate and produce even more CCL5. This CCL5 cycles back into the marrow promoting even more production of CCL5 from stromal cells in the marrow.

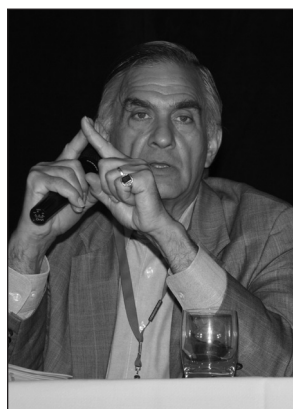
Thus, CCL5, IL-6, BlyS, and other cytokines are produced by a number of cells in the bone marrow microenvironment, including the WM cells themselves. The end result is a sort of “cytokine storm”, which Dr. Ansell likened to a situation in a room where everybody is talking at the same time. Under these conditions, the body loses control over the number of WM cells and the amount of IgM produced. One of the objectives of Dr. Ansell’s research is to find a way to quiet the cytokines or at least to interfere with a key part of the communication pathway. “This is where the opportunities lie,” remarked Dr. Ansell, when referring to the potential for manipulating BlyS, IL-6, and CCL5 to control IgM levels in WM patients.



UNUSUAL COMPLICATIONS OF WALDENSTROM’S MACROGLOBULINEMIA AND HOW TO TREAT THEM – INVOLVEMENT OF BRAIN, SPINE, EYES, AND EARS

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Fred Hochberg, M.D.

Dr. Hochberg began his presentation with a request: he appealed to the IWMF and its members to work with clinicians to help establish clear and codified criteria for nervous system involvement in WM. These criteria would govern therapy and toxicity assessments and would help to explain why WM patients have issues such as migraines, extreme fatigue, depression, lightheadedness, and neuropathies that cannot be easily diagnosed.

Bing-Neel Syndrome (BNS) is defined as WM that affects the central nervous system, which consists of the brain and spinal cord. It was first described by two Swedish physicians, Dr. Jens Bing and Dr. Axel Neel, in 1936, several years before Dr. Jan Waldenström first described the disease that bears his name. These first cases demonstrated the infiltration of lymphoplasmacytic cells in the brain and spinal cord, although the underlying disease process was as yet unknown.

BNS is a rare disorder, and Dr. Hochberg and his colleagues have studied the few case histories of Bing-Neel Syndrome described in the medical literature. Up until this time, BNS has not been well classified; however, Dr. Hochberg presented and described a new classification system for this disorder.

Type A Bing-Neel Syndrome exhibits lymphoplasmacytic cells in the spinal cord, brain, and meninges (the system of membranes that cover the spinal cord and brain), while type B does not present with lymphoplasmacytic cells but rather is the result of IgM antibody directed against the conduction cables of the brain – in other words, an autoimmune effect of IgM. Most Bing-Neel cases described to this point have been type A.



The symptoms of BNS can include headaches, personality and cognitive changes, visual hallucinations, speech impairment, seizures, gait changes, and alterations of right hand function. Rarely, loss of hearing may occur. These symptoms can occur even when bone marrow involvement by WM is stable.

In order to establish the presence of Bing-Neel and the type (which is important for the determination of appropriate treatment), certain diagnostic studies are necessary. First, the patient must have an established diagnosis of WM through blood tests, bone marrow biopsy, and other standard procedures. Then the spinal fluid must be examined for the presence of immunoglobulin or lymphoplasmacytic cells. This examination must include more sophisticated testing with flow cytometry and immunohistochemistry. Third, magnetic resonance imaging (MRI) should be performed on the brain and spinal cord to look for the presence of tumor nodules or thickening or to visualize areas of the brain that have altered conductivity.

Therapy for type A Bing-Neel Syndrome should consist of chemotherapy or localized radiation therapy. Because many types of chemotherapy are unable to cross the blood-brain barrier, Dr. Hochberg uses methotrexate in his practice and has had good results, achieving response rates of 70-75%. For patients who are resistant to methotrexate, he has used Alimta (pemetrexed) with some success. The goal of therapy is to reduce or eliminate the numbers of lymphoplasmacytic cells in the central nervous system.

Type B patients, who do not have lymphoplasmacytic cells in the brain or spinal cord, would not benefit from this therapy. Instead, they should be treated with plasma exchange to reduce the circulating IgM, as well as chemotherapy to reduce the overall disease burden and decrease IgM production.

Dr. Hochberg reiterated his belief that we need prospective studies on Bing-Neel Syndrome. Such studies have the potential to offer several advantages:

- They could identify prognostic factors for central nervous system involvement by WM.
- They could be used as a model for studying the movement of lymphoplasmacytic cells across the blood-brain barrier.
- If the Bing-Neel is type B (antibody-mediated), it might be possible to develop a blood test to detect and measure it.
- A better working definition of BNS could be developed.

PLASMAPHERESIS – WHAT IS IT?

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Jeffrey Winters, M.D.

Plasmapheresis is derived from the Greek word “apheresis” meaning “to separate,” “to take away by force,” or “to remove,” and this is the general way that any apheresis procedure works – whole blood is removed from the body, it goes into a machine where various components are separated, the desired component is removed, and then everything else is returned to the body. The component that is removed

may be infused into another person, as in the case of transfused blood products; it may be re-infused at a later date, such as a stem cell collection; or it may be discarded because it is causing disease.

Although dialysis is considered similar to plasma exchange, it is different in some important ways. Both dialysis and apheresis remove blood components and may require a central venous catheter; however, dialysis uses filters to separate blood components according to size, whereas apheresis uses centrifugation to remove larger components, such as blood cells or IgM for example. Each procedure uses different anticoagulants.

Dr. Winters said that although the term “plasmapheresis” is commonly used in the setting of WM, the correct term is actually “plasma exchange.” In plasma exchange, the removed plasma is replaced with other substances, whereas in plasmapheresis, removed plasma is not replaced but is pumped through a column to “cleanse” it before returning it to the patient.

The centrifuge in the apheresis procedure separates the blood based on the density of its various components, with plasma being the least dense. After separation, individual components can be drawn off. In the case of plasma exchange for WM, the plasma (which contains the IgM) is discarded and the remaining blood returned to the patient. The removed plasma must be replaced in order to maintain the proper blood volume, and this replacement fluid can be one of three types: purified albumin, fresh frozen plasma, or hydroxyethyl starch (a synthetic product).

In the U.S., usually the equivalent of one plasma volume is removed during a plasma exchange. Dr. Winters used an example of a patient with 5 liters of total blood volume,



approximately half of which is plasma. Therefore, during plasma exchange 2.5 liters of plasma would be removed from this patient. Because plasma contains important substances such as clotting factors, these beneficial substances are unfortunately also removed during plasma exchange.

Plasma exchange in WM is used primarily to alleviate the signs and symptoms of hyperviscosity (excessive blood thickness) caused by the size of the IgM molecule. Typical signs and symptoms include the following:

- Bleeding from the nose, mouth, gastrointestinal tract
- Retinopathy (bleeding and sausageing of the retinal vessels)
- Neurologic symptoms – headache, dizziness, vertigo, visual impairment, sleepiness, coma, seizures
- Congestive heart failure
- Respiratory failure
- Fatigue
- Peripheral neuropathy
- Loss of appetite

Symptoms of hyperviscosity are usually seen when the IgM is equal to or greater than 3 g/dL. Normal serum viscosity is 1.4-1.8 cp, and most symptoms are seen at 6-7 cp. However, symptoms of viscosity also depend on the health of the blood vessels, and some patients with a good vascular system may not have symptoms until their viscosity is higher. Dr. Winters cautioned that the necessity for plasma exchange should be based on symptoms, not on viscosity measurements.

Improvement of symptoms following plasma exchange is usually rapid, sometimes with just one treatment, and the duration of improvement depends upon the rate of IgM production by the cancer cells. Plasma exchange is temporary and is usually used in conjunction with chemotherapy, with the expectation that chemotherapy will reduce the tumor burden and result in less IgM production. Some patients who are refractory to chemotherapy can be treated with plasma exchange on a long-term basis.

Dr. Winters also noted some of the risk factors associated with plasma exchange. It is an invasive medical procedure, and reactions occur in approximately 5% of procedures. The following reactions have been noted:

- Allergy or fever due to the replacement fluid
- Reaction to the anticoagulant
- Low blood pressure
- Low blood pressure with fainting
- Reaction to fresh frozen plasma
- Complications from placing a central venous catheter

Dr. Winters also had suggestions for making a plasma exchange procedure go more smoothly. Patients should tell their physicians if they have a history of transfusion reactions or if they are taking ACE inhibitors; they should eat and drink

beforehand; they should go to the bathroom just prior to the procedure (it is about an hour-long process); and they should wear short sleeved shirts and loose fitting clothes. Dr. Winters also recommended that patients who are about to receive plasma exchange should ask if they really need a central venous catheter if one has been suggested – he personally prefers to use peripheral veins if at all possible. Patients should also ask if fresh frozen plasma is really necessary as it tends to cause more reactions than albumin. If albumin is being diluted with saline prior to replacement, it should not be diluted more than 30%.

COMPLEMENTARY AND ALTERNATIVE MEDICINE

GUY SHERWOOD, M.D.



Guy Sherwood, M.D.

Complementary and alternative medicine (CAM) is a group of diverse medical and health care systems, practices, and products, not generally considered part of conventional (Western) medicine; however, the boundaries between CAM and conventional medicine are not absolute and are evolving with time. Approximately 20-30% of Americans, many of them cancer patients, spend \$35 billion dollars a year on

CAM, indicating an increasing interest in its use and popularity.

The complementary part of CAM refers to the use of CAM **together with** conventional medicine, while alternative medicine refers to the use of CAM **in place of** conventional medicine. Dr. Sherwood emphasized that he is very much in favor of certain types of complementary medicine and, in fact, practices acupuncture to alleviate pain in his patients. However, he believes that alternative medicine is more problematic since many alternative therapies are unproven and not based on scientific methodology. Integrative medicine is another term that is used to refer to the use of both conventional and CAM treatments for which there is evidence of safety and effectiveness, and he believes that integrative medicine is the wave of the future, particularly in cancer. Several major medical centers now include integrative medicine clinics.

Dr. Sherwood explained that there are several types of CAM:

- **Natural products** – including herbal medicines, dietary supplements, probiotics.



- **Mind-body medicine** – including meditation, guided imagery, yoga, acupuncture.
- **Manipulative and body-based practices** – including spinal manipulation, massage.
- **Traditional healers** – including Native American medicine men, shamans.
- **Energy medicine** – including magnet therapy, crystals, light therapy, healing touch.
- **Whole medicine systems** – including traditional Chinese medicine, homeopathy, naturopathy, Ayurvedic medicine from India.

In the U.S., the Dietary Supplement Health and Education Act defines dietary supplements, sets product-labeling standards and health claim limits, and outlines quality and safety regulations that differ from those for drugs. The U.S. Food and Drug Administration does not review dietary supplements for safety or effectiveness.

Dr. Sherwood then explained some of the different types of CAM enumerated above, for those who may not be familiar with them.

Mind-body medicine focuses on the interactions among the brain, body, and behavior and uses the mind to affect physical functioning and promote health. For instance, meditation is a way for the user to focus attention or maintain a specific posture in order to relax the body and mind. Guided imagery enables one to visualize an image in order to bring about a desired physical response – for instance, a WM patient might imagine rituximab attacking and destroying his cancer cells. Yoga and tai chi are movement-based techniques used for meditation or relaxation. Acupuncture is the stimulation of specific points on the body by penetrating the skin with needles – this technique is used to alleviate pain, particularly musculoskeletal pain, and stress. While several of these techniques may not have direct measurable physical effects, their ability to relax the mind and alleviate stress can lead to an overall improvement in both mental and physical functioning.

Energy medicine is based on the manipulation of energy fields, such as magnetic fields or light fields, to heal the spirit and the body.

Homeopathy, which was developed in Europe, seeks to stimulate the body’s ability to heal itself by giving small doses of highly diluted substances that in larger doses would produce illness or symptoms. Dr. Sherwood expressed personal reservations about the efficacy of this type of CAM therapy, as he feels it is not well-grounded in scientific theory.

Naturopathy, which also originated in Europe, aims to support the body’s ability to heal through the use of dietary and lifestyle changes, for example, herbs, massage, and joint manipulation.

There are several arguments for and against the use of CAM therapies. Those opposed to CAM cite the lack of scientific proof for its effectiveness, the exaggerated claims made by

some CAM practitioners, the lack of regulation by the FDA, and the belief that CAM relies on a “placebo effect” (a sense of benefit derived from the fact that treatment is being given). Proponents of CAM argue that traditional medicine has failed to deal with chronic illnesses that are due in part to lifestyle issues, that the whole person and not just the disease should be treated, and that CAM helps the body to heal itself by encouraging patients to take an active role in their own health.

The medical community seems to be growing more open to the use of certain CAM modalities. Since chronic disease is responsible for 75% of all health care spending, there is a need to focus on preventing disease through modification of lifestyles, which is an important tenet of complementary medicine. There is also increasing recognition of the desirability to consider and incorporate into treatment a patient’s feelings, attitudes, and innate ability to heal.

Dr. Sherwood stated that the onus is on us, as patients considering the use of CAM, to do our own investigation and make sure we are not causing harm or being “hoodwinked.” He recommended two websites for those who are interested in learning more about CAM:

National Center for Complementary and Alternative Medicine – www.nccam.nih.gov

Natural Standard (for herbal medicines) – www.naturalstandard.com

STEM CELL TRANSPLANTATION AND WALDENSTROM’S MACROGLOBULINEMIA

RAFAT ABONOUR, M.D.

Indiana University School of Medicine, Indianapolis, IN



Rafat Abonour, M.D.

Stem cell transplantation is widely used for multiple myeloma and is also frequently used for non-Hodgkin’s lymphoma (NHL). We can extrapolate much of the information we have on transplantation for both multiple myeloma and NHL to WM.

A stem cell transplant is a way to give aggressive chemotherapy that is active against WM cells but allows us to “re-seed” or re-populate the bone marrow so that it can overcome the collateral damage caused by aggressive



treatment. The stem cells are useful because they have the capacity to continually renew themselves and because they can differentiate into the various types of blood cells – white blood cells, red blood cells, and platelets. We are able to identify stem cells by a special surface marker called CD34, and we can collect, store, and re-infuse them into the patient at a later time.

Dr. Abonour explained the two main categories of stem cell transplants: autologous and allogeneic.

Autologous transplant uses the patient's own stem cells. Its purpose is to rescue the bone marrow following marrow-lethal chemotherapy or radiation, it is generally well-tolerated with a low mortality rate (<5%), and engraftment or "re-seeding" usually occurs within 10-14 days after transplant.

Allogeneic transplant uses stem cells from a related or unrelated donor or from umbilical cord blood. It may be either myeloablative (intense chemotherapy) or non-myeloablative (less intense chemotherapy) and is associated with a higher risk of mortality.

Stem cells can be collected from several sources:

- **Peripheral blood** – the stem cells are mobilized (leave the marrow and enter the bloodstream) by using chemotherapy, growth factors such as G-CSF (granulocyte-colony stimulating factor), or a combination of the two. Peripheral stem cell collection is associated with more rapid engraftment and is more cost effective.
- **Bone marrow** – may be used if a peripheral stem cell collection fails or in the case of a pediatric transplant.
- **Umbilical cord blood** – commonly used in children but not so much in adults, primarily because the numbers of stem cells in cord blood are relatively low.

A patient undergoing a transplant procedure will require a rather extensive workup beforehand to determine his or her suitability for transplantation. The workup includes a history and complete physical examination, infectious disease workup (for example, to check for previous exposure to certain viruses), pulmonary function tests, electrocardiogram, echocardiogram, chest X-ray, and creatinine clearance. In the case of autologous transplant, the patient will then undergo mobilization with chemotherapy, growth factors, or both; have his stem cells removed by apheresis; receive a conditioning regimen of high-dose chemotherapy, radiation, or both to kill the cancer cells; receive the stem cells by infusion; be closely monitored during engraftment; and then receive long-term follow-up. The procedure for allogeneic transplant is quite similar except that the patient does not undergo mobilization or collection because he is receiving donor stem cells.

During engraftment, patients are given extensive supportive care because of the side effects from high-dose chemotherapy

and the resulting impairment of immune function. Anti-emetics are administered to control nausea and vomiting, as are pain relievers for mucositis (mouth sores). Because of immune system suppression, infection is a major concern during this period; therefore, patients receive anti-fungal, anti-bacterial, and anti-viral prophylactic medications. Most transplants occur in a hospital setting, and 24-hour monitoring by an experienced nursing staff is usual, although some transplants can be done in an outpatient setting.

A patient may elect to undergo just the stem cell collection and then store his stem cells until such time as a transplant is required. Stem cells can be stored for 20 years or more at -126° C under special conditions.

Dr. Abonour also explained the different types of allogeneic (allo) transplants. Allo transplants are more commonly used for leukemia than lymphoma.

A myeloablative allo transplant uses high-dose chemotherapy, radiation, or both – the goal is to completely eradicate the recipient's bone marrow. This type of allo transplant has a relatively high mortality rate approaching 30%. In an effort to reduce mortality, non-myeloablative allo transplants (also called reduced-intensity or "mini-allo") were developed. In this regimen, the chemotherapy or radiation is less intense because the object is not to completely wipe out the recipient's bone marrow but to "make room" for the donor's stem cells. The donor cells will then recognize the recipient's cells, including the cancer cells, as foreign and kill them. Haplo-identical transplants use a donor that is only a half match, while umbilical cord blood can also be used for allo transplant, as mentioned above. One of the major problems with any type of allo transplant is that the donor cells will see the recipient's cells as foreign and attack various tissue and organ systems of the recipient – a condition known as graft vs. host disease. Complications from this condition can be very serious, and it is a major cause of transplant mortality.

Dr. Abonour stated that autologous transplantation can be done in patients in their 70s, as their overall health is more important than age in determining suitability; however, because allogeneic transplantation is more rigorous, it is not usually done on patients who are over 55-60 years of age.

Dr. Abonour reviewed survival statistics from several U.S. and European studies on transplantation, both autologous and allogeneic, in WM. He concluded that autologous stem cell transplant is a reasonable option for patients with relapsed or refractory WM and should be carefully considered after first relapse from treatment. Allogeneic stem cell transplant is associated with high treatment-related mortality, and although it produces high rates of sustained responses, it should not be considered outside of clinical trials or for patients with early and indolent disease.



NEW AND EMERGING TREATMENTS FOR INDOLENT LYMPHOMA

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Stephanie Gregory, M.D.

In contrast to many other types of cancer, the prevalence of non-Hodgkin's lymphoma (NHL) is increasing, with approximately 4% of all cancers classified as NHL. Most NHLs are derived from B-cells, and less than 2% of NHLs are WM. Because of better treatments, primarily rituximab, about 700,000 patients are living today with NHL. The incidence of NHL peaks at around the age of

70, largely because it is a disease of the immune system – the accumulation of exposures to pesticides, fertilizers, and various bacterial and viral diseases over a lifetime can lead to de-regulation of the immune system and the development of immune system disorders such as cancer.

NHL is usually divided into two basic types: aggressive and indolent. Aggressive lymphomas, such as diffuse large B-cell lymphoma, can be cured but require rapid treatment to be effective. Indolent lymphomas, including WM, are generally incurable but slow-growing. Dr. Gregory emphasized that indolent lymphomas should not be treated unless a patient is symptomatic; there is potential harm associated with the toxicity of many current treatments, including the development of secondary treatment-related cancers such as acute leukemia.

Risk factors for NHL include the following: immune deficiency disorders (such as AIDS), autoimmune disorders (lupus, rheumatoid arthritis), organ transplantation, chemical or pesticide exposure, radiation exposure, and bacterial or viral exposure. All of these can have an adverse effect on the immune system because they can cause immune system deficiency or de-regulation.

In order to evaluate or stage a lymphoma, it is necessary to perform several tests, including a history and complete physical; laboratory tests (complete blood count with differential, serum lactate dehydrogenase and beta-2 microglobulin levels, serum electrolyte levels, tests of liver and renal function); bone marrow biopsy (BMB) with flow cytometry; and radiological scans of the abdomen, pelvis, and thorax.

The type of treatment selected for a particular patient is based on the above findings, as well as the subtype of NHL, its growth rate, the stage of the disease (localized, distant, widespread), and various prognostic factors. WM, by virtue of the fact that it is a cancer of the bone marrow, is a stage IV disease; however, staging for hematological cancers does not necessarily reflect the same prognosis as staging for solid tissue cancers. Treatments can include surgery (used primarily for diagnosis), radiation (of limited use), chemotherapy, immunotherapy, and targeted therapy. Targeted therapy is the newest type of treatment and is based on “smart” drugs designed to interact with a specific biologic target expressed on the cancer cells.

The National Comprehensive Cancer Network (NCCN), an alliance of the world's leading cancer centers, has issued practice guidelines for the indolent NHLs, but treatments still remain very individualized as there is no one treatment standard that applies in all cases. Physicians also have to be aware that each time an indolent lymphoma becomes active again there is a possibility that the disease may have transformed into a more aggressive type of lymphoma.

Emerging treatment agents can act in several ways. Dr. Gregory presented an overview of how these newer therapies work and provided examples.

Some agents affect the tumor microenvironment, which consists of the cells and chemicals that surround and “bathe” the tumor cell. One category of such treatments is the IMiDs or immune modulatory drugs such as lenalidomide (Revlimid), a derivative of thalidomide.

In the area of more traditional chemotherapy, one of the newer drugs being used is bendamustine, which is actually an old drug developed in East Germany. The combination of bendamustine and rituximab has been found to be more effective and less toxic than R-CHOP therapy.

Surface marker agents include rituximab and the newer generation of anti-CD20 antibodies, as well as anti-CD22, anti-CD40, and anti-CD37 antibodies. Rituximab targets B-cells, including the cancerous B-cell of WM, by attaching to the CD20 antigen on the surface of the B-cell at one end and recruiting the body's own immune cells to attach at the other end. These immune cells then destroy the B-cell. The newer rituximab-type therapies (ofatumumab, GA-101, veltuzumab, ocrelizumab, AME-133v, PRO-131921, IMMU-106) have been “humanized” to be more like human antibodies and appear to be more potent and do a better job of activating the body's immune system. These antibodies can also be conjugated (attached) to other substances such as radioisotopes or toxins that are taken into the B-cell and kill it more effectively. Examples of such drugs conjugated to radioisotopes include Zevalin and Bexxar. Dr. Gregory's group at Rush University has treated over 1,000 patients with Zevalin or Bexxar. Dr. Gregory also mentioned a new



anti-CD22 agent called inotuzumab ozogamicin, which is conjugated to a cytotoxic (cell-killing) agent called calicheamicin and has achieved good results in her studies on follicular lymphoma patients.

The pathway-type drugs are targeted therapies which inhibit signaling pathways in the cancer cell that cause it to grow uncontrollably. These include the proteasome inhibitors such as bortezomib (Velcade) and the newer carfilzomib; the BCL-2 family inhibitors, which include Bruton's tyrosine kinase (Btk) and SYK inhibitors; the mTOR inhibitors such as everolimus and temsirolimus; and the PI3K inhibitors such as CAL-101. Most of these drugs are in oral form and have fewer toxic side effects than the older therapies.

MOLECULAR AND FUNCTIONAL SEQUELAE OF THE PI3K/AKT/MTOR PATHWAY IN WM

ALDO M. ROCCARO, M.D., PH.D.
Dana-Farber Cancer Institute, Boston, MA



Aldo M. Roccaro, M.D., Ph.D.

Dr. Roccaro discussed the results of several studies on a series of new drugs that have been designed to correct the PI3K/Akt/mTOR molecular signaling pathway in WM cells. In WM cells, this pathway malfunctions, resulting in an increased rate of cell growth and proliferation as well as a decreased rate of programmed cell death (apoptosis). By correcting this pathway, the drugs Dr. Roccaro discussed

make it possible for the cells to die at a normal rate, thereby preventing them from forming tumors and multiplying.

Based on this evidence and our current understanding of the PI3K/Akt/mTOR signaling pathway, Dr. Roccaro and other researchers at the Dana-Farber Cancer Institute have evaluated three drugs that specifically target Akt and mTOR. These drugs are perifosine, RAD-001 and NVP-BEZ235. Perifosine is an Akt inhibitor, RAD-001 inhibits mTOR, and NVP-BEZ235 inhibits both Akt and mTOR.

Dr. Roccaro and his lab found in pre-clinical studies that perifosine was able to inhibit WM cell proliferation without damaging normal cells in the blood. In a Phase II clinical trial of patients with relapsed WM, only 11% exhibited progression of the disease. These results are promising and indicate that perifosine may be another useful tool in the fight against WM.

In pre-clinical trials, RAD-001 successfully inhibited the growth of WM cells, even when tested in an environment mimicking bone marrow. This environment is relevant because WM cells are stimulated to proliferate by cytokines secreted from bone marrow stromal cells. In a Phase II clinical trial of RAD-001, 70% of patients responded to the drug, indicating that this new medicine could be an effective new therapy for WM.

Dr. Roccaro discussed the compound NVP-BEZ235 (an inhibitor of both Akt and mTOR). Pre-clinical results indicate that the drug inhibits WM cell proliferation by apoptosis and disruption of the normal cell cycle without damaging normal cells. Furthermore, NVP-BEZ235 was also found to inhibit WM cell growth even in the context of the bone marrow microenvironment. Dr. Roccaro expects this new drug to enter Phase II clinical trials in four to five months.

Dr. Roccaro also mentioned the down regulation of PTEN in WM cells as another reason for their uncontrolled expansion. In the cell, PTEN acts as a negative regulator of both Akt and mTOR and can therefore be viewed as a tumor suppressant. WM cells, however, contain lower levels of PTEN than normal cells. These low PTEN levels were investigated by Dr. Roccaro's lab and can be partially explained in terms of microRNAs (miRNAs).

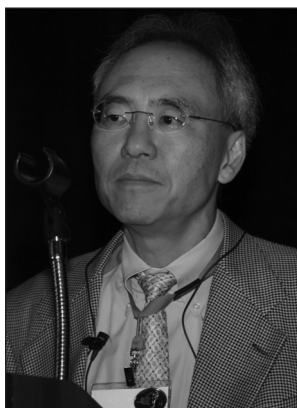
MicroRNAs are small non-coding ribonucleic acid molecules that act as negative regulators or silencers of gene expression. In WM cells, Dr. Roccaro and his lab found that miRNA-542-3p and miRNA-494 were over-expressed and that these miRNAs were down regulators of PTEN. Based on this evidence, Dr. Roccaro speculated that a compound targeting these miRNAs could eventually lead to an effective therapy for WM. This is the first time that miRNA profiling has been performed on WM cells.



THERAPEUTIC VACCINES FOR LYMPHOMAS: A TALE OF BENCH TO BEDSIDE TRANSLATION

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Houston, TX*



Larry W. Kwak, M.D., Ph.D.

Dr. Kwak's presentation discussed the theory behind human lymphoma vaccines and the remaining hurdles that these therapies must clear before they can be used to treat patients. He then went on to outline an upcoming clinical trial for WM patients and to explain the potential of the next generation of vaccines called DNA fusion vaccines.

Dr. Kwak stated that the immune system often plays an important role in cancer therapies. He cited the examples of treating chronic leukemia with lymphocyte infusions as well as a perceived correlation between the life cycle of a lymphoma tumor with the waxing and waning natural history of the patient's immune system. The idea of the cancer vaccine arises from this context by taking advantage of the immune system's powerful potential.

Cancer vaccines come in three varieties: therapeutic, secondary prevention, and prevention. A therapeutic vaccine is the most typical treatment and is used to fight off tumors when they are detected. Secondary prevention vaccines, which are the type of vaccine being studied for application in cases of WM, are used on patients whose cancer is in remission as a way to prevent the cancer from coming back. Finally, prevention vaccines are used on healthy patients to prevent a cancer from ever developing.

Next, Dr. Kwak described the general process by which a cancer vaccine is made as well as its basic molecular structure. To create a cancer vaccine, a biopsy of the patient's tumor must be taken so that specific antigens from the tumor can be isolated. An antigen is a protein or other substance that causes the body's immune system to produce antibodies against the antigen. The immune system produces these antibodies because it recognizes the antigens as foreign compounds that need to be removed. To create a vaccine, the antigen is replicated in an automated process and then paired with a carrier molecule and an adjuvant. The carrier molecule stimulates the immune system, while the adjuvant

ensures that the body's exposure to the vaccine is sustained long enough for it to be thoroughly processed.

Currently, the main research questions being pursued in the cancer vaccine field involve optimization of all of these pieces (i.e., identifying the ideal antigen(s), immune stimulant, and adjuvant). As presented by Dr. Kwak, the ideal antigens are specific or over-expressed by the tumor, are immunogenic (capable of causing an immune response), are oncogenic (essential for the tumor cell's survival), and are expressed on the surface of the cancer cells. For a WM or other cancer vaccine, these antigens are collectively called the idiotype and are comprised of distinctive protein foldings on the surface receptors of the cancerous line of B-cells. The proposed components of a WM vaccine are the idiotype, a keyhole limpet hemocyanine (derived from a marine mollusk) that acts as the immune stimulant, and GM-CSF (granulocyte/macrophage-colony stimulating factor) as the adjuvant.

Once the vaccine has been administered to the patient, the body processes the tumor antigens and ultimately produces T-cells that are specific for them. These T-cells then proceed to search the body for the antigens, killing any cells that are carrying them. In this way, the patient's own immune system can be used to eliminate tumor cells from the body.

This approach has been the subject of Phase II clinical trials for various cancer types over the last twenty years, and the results are promising. In one study, highlighted by Dr. Kwak, about half of the patients were still in their first remission nine years after receiving treatment. Based on these results, Phase III studies for many of these drugs are being undertaken.

One Phase III study by NCI/Biovest for patients with follicular lymphoma sought to determine whether the idiotype (Id) vaccine would prolong disease free survival (DFS) compared to a control population of patients who had achieved remission after standard chemotherapy. The study was also done as a way to evaluate the safety of the Id vaccine and to assess the immune response to the vaccine and the biomarkers that the vaccine exploits. The study found that the treatment prolonged DFS by an average of about 14 months, yet interestingly, in the sub-group of patients with IgM isotype (rather than IgG isotype), DFS was prolonged by an average of about 24 months. The cause of the more dramatic results in the IgM isotype sub-group is yet to be determined. The side effects of the treatment were limited to occasional redness and swelling at the injection site.

While the results of the study are promising, Dr. Kwak stressed some of the potential obstacles that this therapy faces before it can feasibly reach the bedside. The two main problems are the requirement for personalized manufacture of each vaccine (this could make the vaccine unaffordable) and the requirement for sustained and complete remission before the therapy can be given.

In the second portion of his talk, Dr. Kwak talked about the future of cancer vaccines. His goals for the future are to identify the subgroups of patients most likely to benefit



from the vaccine and to determine the biological mechanism underlying the observed clinical effects. Furthermore, Dr. Kwak hopes to improve the vaccine itself by incorporating new second generation DNA fusion vaccines.

DNA fusion vaccines improve upon existing vaccines by increasing the efficiency by which the vaccine is delivered to the immune system. In a DNA fusion vaccine, the idiotype is fused genetically, rather than chemically, to the immune stimulant. This allows the antigen presenting cells (APCs) of the immune system to present the antigen to the T-cells faster by increasing the rate at which the APC takes up the vaccine. This idea has been thoroughly tested on animal models in the laboratory. Before human studies of these vaccines can begin, however, there are bench to bedside translational issues that need to be overcome, including process development, establishing clinical-grade manufacturing, and a clinical protocol. As a result of this, Dr. Kwak estimated that a clinical trial of the DNA fusion vaccine in WM will not be possible for about six months.

DESIGNER MOUSE MODEL OF HUMAN WALDENSTROM'S MACROGLOBULINEMIA

SIEGFRIED JANZ, M.D.
University of Iowa, Iowa City, IA



Siegfried Janz, M.D.

Dr. Janz spoke about his project at the University of Iowa to develop a mouse model of WM, funded through a grant provided by the IWMF.

Important and long-standing questions about the development of WM still remain, and it is understandably difficult to pursue these answers by doing research on humans. For example, it is not yet known what specific genes may cause a genetic predisposition to WM.

There are still questions regarding the special biology of the precursor WM cell, as well as the process whereby WM can develop from IgM MGUS (monoclonal gammopathy of undetermined significance). Therefore, it is necessary to develop experimental animal models to answer these questions.

According to Dr. Janz, the laboratory mouse is clearly the most important model to use for studying cancer biology and genetics. Among the mouse advantages are small size, ease

of housing and maintenance, high proliferative ability, and the fact that mice share many physiological characteristics of tumor development in common with humans. In the past decade, methods have been developed to “humanize” mice so that they can mimic human cancer development. Laboratory mice also play an important role in pre-clinical cancer drug design and testing, i.e., assessing the efficacy of tumor cell targeting and toxicity.

The IWMF has previously participated in funding mouse models of WM, but these were xenograft mouse models, in which WM cells were engrafted into mice that were severely immunodeficient (SCID mice). In this way, WM cells could be propagated in the mice. However, xenograft assays are limited in value. They cannot be used to study tumor development because the engrafted cells are already malignant. In addition, there are limited numbers of WM cell lines that can be engrafted, they do not fully represent the diversity of WM in humans, and in fact, they may not be truly representative of human WM. Also, xenograft models cannot recapitulate the complex interaction of tumor cells with their bone marrow microenvironment.

Given the limitations of xenograft assays, it is not surprising that several cancer drugs based on promising pre-clinical results have failed to achieve these same results when tested in Phase I and Phase II clinical trials in humans.

Therefore, the IWMF has funded a new generation of mouse modeling, one in which WM-like tumors arise spontaneously in mice with normal immune systems (such as occurs in human WM). This new mouse model should be an accurate recapitulation of the clinical and histological features of human WM; it should faithfully reproduce the different stages of tumor progression; tumors should develop spontaneously in an immunocompetent host (one who has a normal immune response); it should be useful for pre-clinical studies on new treatments; and tumor development should have a high incidence rate and a reproducible tumor pattern.

Guided by our knowledge of two key players in the genetics and natural history of WM, Dr. Janz is focusing on the genes regulating interleukin-6 (IL-6) and B-cell leukemia/lymphoma (BCL-2) protein production, both of which are over-expressed in WM. Dr. Janz has crossed an IL-6 transgenic mouse strain with a BCL-2 transgenic mouse strain. Either strain by itself tends to develop B-cell and plasma cell tumors, but most of these tumors express IgG or IgA instead of IgM. Also, each strain by itself tends to have a long period before tumor onset and tumor incidence is incomplete, that is not every mouse develops tumors. By interbreeding these two mouse strains, Dr. Janz has developed a new “double transgenic” strain that solves two problems: the new strain has a shorter tumor onset, plus all the mice develop tumors. To solve the third problem, that of IgM production, Dr. Janz plans to breed this new strain, IL-6/BCL-2, with a third mouse strain called AID^{null}. The AID^{null} mouse is deficient in the production of the enzyme called activation-induced cytidine deaminase (AID). This enzyme is important in the



immunoglobulin class switching of IgM to IgG or IgA – WM tumor cells do not undergo this class switching, but instead continue to produce IgM. By using an AID-deficient mouse, Dr. Janz hopes to arrest the development of the tumor cells in the IgM-producing stage, thus producing a “triple transgenic” IL-6/BCL-2/AID^{null} mouse strain which mimics human WM in many respects.

If successful, Dr. Janz will share this mouse strain with the WM research community by donating the new strain, on behalf of the IWWMF, to the Mutant Mouse Strain Repository at the Jackson Laboratory in Bar Harbor, Maine. He also wishes to use these mice, in close association with WM clinicians and researchers, to design and test new approaches to the treatment and prevention of WM. Dr. Janz anticipates that this new strain can help to elucidate the genetic pathways of WM development. While it is likely that this new mouse strain will reproduce some features of human WM, it is possible that it may not reproduce others, such as IgM neuropathy, and Dr. Janz anticipates that further research efforts may be necessary to develop additional mouse strains that express these features.

ADVANCES IN THE GENETICS AND TREATMENT OF WALDENSTROM'S MACROGLOBULINEMIA

STEVEN P. TREON, M.D., M.A., PH.D.
Dana-Farber Cancer Institute, Boston, MA



Steven P. Treon, M.D., M.A., Ph.D.

Dr. Treon presented several updates from Dana-Farber Cancer Institute regarding different aspects of WM, including WM inheritance patterns, treatments, and genomics.

FAMILIAL DISEASE

Data from the Dana-Farber Cancer Institute (DFCI) just presented at the International Conference on Malignant Lymphoma in Switzerland reviewed the findings in 1,076 consecutive patients with the diagnosis of WM. Twenty-six percent of WM patients had a first or second degree relative with a B-cell lymphoproliferative disorder, including (in descending order of frequency) non-Hodgkin's lymphoma (other than WM), chronic lymphocytic leukemia, WM, multiple myeloma (MM), Hodgkin's disease, monoclonal

gammopathy of undetermined significance (MGUS), acute lymphoblastic leukemia, and hairy cell leukemia.

A grant from the IWWMF allowed the Bing Center at DFCI to set up the WM Familial Genomics Project. Families of patients with familial WM and sporadic WM were studied. The project involved over 800 individuals in 187 families. Three patterns were observed: **sporadic WM** in which no individuals other than the single WM patient were identified; **familial WM only**, in which at least one family member, other than the original WM patient, had WM; and **familial mixed B-cell disease**, in which family members of the original WM patient had other B-cell disorders.

The impact of familial vs. sporadic WM status on response to therapy with various rituximab-containing therapies in rituximab-naïve patients (i.e., patients never previously treated with rituximab) was evaluated. Patients with sporadic WM compared to those with familial disease had a better overall response rate (ORR) to therapy of 96% vs. 75%, major response rate of 75% vs. 56%, and very good partial/complete response rate (VGPR/CR) of 23% vs. 17%. Patients with sporadic WM also had longer progression-free survival times and longer time-to-next-treatment than did patients with familial disease.

The impact of the type of therapy on responses to treatment of familial disease was evaluated in a study involving 36 patients. In this small study, familial WM patients who received a bortezomib (Velcade)-containing regimen had a much better ORR (100% vs. 71%), major response rate (100% vs. 48%), and VGPR/CR (80% vs. 7%) than did patients who received a non-bortezomib regimen. Dr. Treon acknowledged, however, that results from a small study may not hold true in larger studies.

The impact of bortezomib-based therapy on progression free survival (PFS) in familial and sporadic WM patients was also evaluated. Progression free survival in familial WM was substantially better three years after treatment with bortezomib-based therapy than it was with non-bortezomib therapy. In contrast, patients with sporadic WM had PFS that was about the same in therapies with and without bortezomib.

THERAPEUTIC OPTIONS

Rituximab

This synthetic antibody directed against the CD20 receptor on B-lymphocytes is partly of mouse and partly of human origin. Rituximab treatment can induce a paradoxical rise in IgM levels (without any increase in disease burden) known as IgM flare. This flare can induce symptomatic hyperviscosity in some patients. For several years, the cause of this phenomenon was suspected to be related to the binding of the *Fab* (variable) portion of the rituximab antibody to CD20. When intravenous immunoglobulin (IVIG) therapy was also found to be capable of inducing an IgM flare, the possibility of a different mechanism involving the *Fc* (constant) portion of rituximab was then considered. Dr. Guang Yang (of the Bing Center at DFCI) found that neither rituximab nor IVIG could



directly stimulate WM cells to release IgM. However, when rituximab was incubated with a co-culture of WM cells and monocytes, IgM release occurred. Moreover, it was found that when rituximab or IVIG became bound to the FcγRIIA receptor on monocytes, interleukin-6 (IL-6) was released. IL-6 would in turn bind to the IL-6 receptor on WM cells, causing them to release IgM. IL-6 blood levels correlated with the rise and fall in IgM levels of patients experiencing IgM flare. IgM flare has been erroneously interpreted by physicians inexperienced in treating WM as indicating that the WM is worsening despite rituximab therapy.

Rituximab monotherapy produces an overall response rate in WM patients of 25-30% when four weekly infusions are given, and 40-45% when eight weekly infusions are given. It almost never induces complete responses. However, when rituximab is given in combination with alkylating agents such as cyclophosphamide, nucleoside analogues such as fludarabine, immunomodulatory drugs such as thalidomide, proteasome inhibitors such as bortezomib, and (in some protocols) a steroid such as dexamethasone, the ORRs are much higher and occasional complete responses are seen. For example, combinations of rituximab + cyclophosphamide + an additional drug have an ORR of 70-80% and a CR of 8-10%. Rituximab + bendamustine (a nitrogen mustard alkylating agent recently introduced to the U.S. and in clinical trials for WM) has an ORR of about 83-90%.

Transformation and MDS/AML in Nucleoside Analog Therapy

In comparison with patients who either have not been treated or have received non-nucleoside analog treatment, for patients treated with nucleoside analogs there is an increased risk of late adverse effects, including transformation to a large B-cell lymphoma (8%), myelodysplastic syndrome (MDS, a preleukemic condition with bone marrow failure), and acute myelogenous leukemia (AML). The incidence of MDS/AML following nucleoside analog therapy is about 5%.

IMiD-Based Rituximab Therapy

Thalidomide and lenalidomide (Revlimid) stimulate immune cells and are known as immunomodulatory drugs (IMiDs). They can be given with rituximab. Their ORR is 40-70%, and there are few complete responses. Peripheral neuropathy often occurs with thalidomide, and an abrupt decrease in hematocrit (average decline of about 4 percentage points) occurs in some patients given lenalidomide. Their long-term risks are minimal, but they are teratogenic (capable of causing birth defects).

Proteasome Inhibitors

These drugs block the breakdown or waste disposal of specific intracellular proteins, thereby disturbing cellular homeostatic mechanisms and causing the cells to undergo apoptosis (programmed cell death). Bortezomib (Velcade) has been studied for several years, and can produce high response rates in combination with other drugs.

As therapy for front-line use, biweekly bortezomib (1.3 mg/m² biweekly) + dexamethasone + rituximab has an ORR of 95%, a CR of 22%, and a time to progression (TTP) of >4 years. There is a 30% incidence of grade 3 peripheral neuropathy (PN). Most patients have reversal of their neuropathy, with an average time to reversal of six months. Lyrica has been of great benefit for amelioration of PN symptoms. The incidence of rituximab IgM flare is decreased with this therapy (as compared to treatment with rituximab alone). Once weekly bortezomib (1.6 mg/m² weekly) + rituximab has an ORR of 92%, a CR of 8%, 80% one year PFS, and no severe (grade 3 or 4) neuropathy.

As salvage therapy, bortezomib (weekly) + rituximab has an ORR of 81%, CR of 5%, TTP of 12 months, and a 5% incidence of grade 3 neuropathy.

Of the newer proteasome inhibitors, carfilzomib so far seems to have a much lower incidence of PN than bortezomib. DFCI is planning a trial with a combination of drugs abbreviated as CARD: carfilzomib, rituximab and dexamethasone.

Another new proteasome inhibitor, MLN4924, kills MM and WM cells in vitro. In human tests the drug does not seem to cause significant PN. There is a pending trial of MLN4924 and dexamethasone in relapsed/refractory WM.

Bendamustine

This is a chemotherapeutic drug that was developed in East Germany during the cold war. It was introduced into the U.S. only a few years ago. Bendamustine was originally thought to have both alkylating and nucleoside analog activity, but it is now believed that its activity is that of a nitrogen mustard alkylating agent (in the same general class as cyclophosphamide). Dr. Matthias Rummel has played a large role in the development of new anti-lymphoma drugs in Germany. He conducted a randomized trial that compared bendamustine + rituximab to CHOP + rituximab in the treatment of various kinds of lymphomas, including WM. The outcome was that patients who were treated with bendamustine + rituximab had PFS of about 80% after four years, in comparison to the CHOP-R group, which had a rate of about 15%.

Dr. Treon and others conducted a trial in which 30 patients with relapsed/refractory disease were treated with bendamustine (90 mg/m²) + rituximab (23 patients), bendamustine alone (5 patients who had previous intolerance to rituximab), and bendamustine + ofatumumab (2 patients). The ORR was 83% and the VGPR was 17%. Response rates were similar with or without rituximab. The estimated PFS was 13.2 months. The patients had marked reductions in IgM and also had increases in hematocrit in response to treatment.

Response to Rituximab Related to Patient's Genes

In a study of 159 rituximab-naïve WM patients treated with rituximab-based therapy, it was found that patients who achieved complete responses or very good partial responses to treatment (at least a 90% drop in their IgM level compared



to the pre-treatment level) also had longer periods of PFS than patients who had lesser responses. The response of patients to rituximab appears to be related to the gene (FCGR3A) for the FcγRIIIA receptor on cytotoxic lymphocytes (natural killer cells). This receptor is the attachment point for the *Fc* portion of the rituximab antibody. At position 158 of the receptor protein, an individual may have a valine (V) or a phenylalanine (F) amino acid. Patients inherit one of these FCGR3A genes from each parent. Rituximab binds more securely to a receptor containing a valine than one with a phenylalanine, so the valine-containing receptor better enables the cytotoxic lymphocytes to kill CD20 positive B-cells (including WM cells) that are attached to the *Fab* portions of the rituximab antibody; this indirect process of tumor-cell killing is known as antibody-dependent cell-mediated cytotoxicity or ADCC. Patients whose genes both code for valine (V/V) have a substantially better chance of having a CR or VGPR in response to rituximab than patients whose genes both code for phenylalanine (F/F). An FDA-approved test for these genetic variations is available.

GA101 is a new humanized anti-CD20 monoclonal antibody that works well with both the V/V and F/F genetic variations in laboratory experiments with WM cells. It is manufactured by Genentech and Roche, who also manufacture rituximab. This drug not only has much higher affinity for the FcγRIIIA receptor than rituximab, thereby manifesting enhanced ADCC, but also demonstrates greatly enhanced direct cell killing (apoptosis or programmed cell death). GA101 is expected to be available for use in WM clinical trials at the end of 2012 or in 2013.

Maintenance Rituximab

Although prospective studies in WM have not been conducted, a retrospective study has just been completed at DFCI in 248 rituximab-naïve WM patients who were either observed or given maintenance rituximab following initial successful rituximab therapy. This study revealed that PFS in patients on maintenance therapy was 56 months, in comparison to PFS of 29 months in patients who were under observation. The incidence of infections in the maintenance group (~38%) was nearly twice that in the observation group (20%), but those infections were mostly minor (such as sinus and upper respiratory tract infections), and the incidence of infections greater than grade 3 was about the same in both groups. Dr. Treon commented that, on balance, maintenance treatment seems to make good sense. However, further studies will be needed to determine the optimal frequency and duration of maintenance treatments.

RAD001

In a joint DFCI and Mayo Clinic study of this drug in 50 relapsed/refractory WM patients, the ORR was 72%. Dr. Treon noted that there are patients in this study who have had PFS of four years or more. Adverse effects included thrombocytopenia, pneumonitis, mucositis (mouth sores) and hyperglycemia. In a subsequent trial of RAD001 for the

primary therapy of WM (with 33 patients to date, and a total expected accrual of 60 patients), the majority experienced decreased IgM. However, seven patients who had decreased IgM were found by serial bone marrow biopsies to have disease progression, a phenomenon termed IgM discordance.

GENOMIC DRIVEN THERAPY OF WM

The aim of this approach is to rationally design drug treatments by understanding the comprehensive genetic basis for the development of WM.

Gene Expression Profiling

A joint study of WM patients involving DFCI and colleagues at the University of Arkansas evaluated arrays containing thousands of genes to see whether individual genes were turned on (expressed) or off. One target for treatment that emerged from the study was Bruton's tyrosine kinase (Btk), a protein that is an essential element of the B-cell antigen receptor (BCR) signaling pathway. Inhibitors of Btk block BCR signaling and induce apoptosis. A promising Btk inhibitory drug is PCI-32765, which in pre-clinical studies in WM cell lines was found to induce cell death by shutting down the sequential steps of phosphorylation that normally drive BCR signaling. In a recent study on the effects of PCI-32765 on B-cell lymphomas (including WM), Dr. Ranjana Advani of Stanford reported that 67% of patients who were treated responded. DFCI will soon be opening a study of PCI-32765 in relapsed/refractory WM.

Whole Genome Sequencing Project in WM

Funding for this project was supplied through an IWMF grant and the generous support of Peter Bing. Genome sequencing adds a monumental new capability to WM research. During the sequencing process, a patient's intact DNA is cut into small ribbons, the sequence of individual nucleotides in each ribbon is determined, the nucleotide sequences are reassembled into larger groups, and this information is compared to a national reference genome library called NCBI 37 to determine the entire correct sequence in the genome. Using complicated algorithms, the paired DNA sequences from each patient's normal and WM cells are compared, the differences between the two sets are determined, and then they are validated against known libraries. The preliminary finding from this study was that a single base pair out of about 3 billion has been identified that is unique to WM. This mutation was found in 90% of patients' WM cells, but not in their normal cells, and was not found in IgM-MGUS or myeloma. It stimulates WM cell growth and survival signaling via the NF-kappa B pathway. The laboratory at DFCI has been able to "knock down" this mutation (silence the gene), allowing the WM cells to undergo cell death. A drug has been developed that specifically targets this pathway and is a promising candidate for future clinical trials.



RESEARCH AT MAYO CLINIC IN WALDENSTROM'S MACROGLOBULINEMIA

A TEAM PRESENTATION
*Mayo Clinic, Rochester, MN, Scottsdale, AZ, and
Jacksonville, FL*

Dr. Stephen Ansell from the Mayo Clinic opened this team presentation by giving a brief overview of each topic. He also introduced each speaker and helped to conduct question-and-answer sessions with the audience.

DEVELOPMENT OF WM CELL LINES

Anne Novak, Ph.D.

One of the most important tools for cancer research is a cell line that models a particular disease. Historically, the development of a WM cell line has proven extremely challenging, primarily due to the low rate of WM cell proliferation and the fact that primary WM cells do not naturally grow well in a culture flask.



Anne Novak, Ph.D.

There are currently three cell lines that have been reported to model WM; however, none have genetically proven to be clonally related to the patients' tumors from which they were sampled and some have lost the ability to secrete IgM. Approximately 18 months ago, the IWMMF and the Leukemia & Lymphoma Society jointly funded a study to develop a true WM cell line.

A valid cell line should have several characteristics to be useful: it should be immortal, it should be biologically stable and not mutate over time; it should be clonally related to the original tumor cells from which it was developed; and it should retain functional characteristics of the original tumor (such as IgM production in the case of WM).

At Mayo Clinic, the work of developing a WM cell line began by taking cells from a donated bone marrow biopsy, isolating tumor cells that were CD19+/CD138+, putting them in a culture flask, and supplying nutrients. If the cells grew (and many did not grow for very long), the cells were then screened genetically to look for IgM production and appropriate CD surface antigens. Mayo Clinic has been successful in developing a new WM cell line, called Mayo Waldenstrom Cell Line 1 or MWCL1. This cell line came from a patient

diagnosed in 2009 with IgM kappa light chain and 50% bone marrow infiltration. The new cell line expresses high levels of IgM, has the typical disease markers, is genetically very similar to the patient's WM cells, and is stable. MWCL1 is now available to other researchers, and its characterization has been published in the journal *Blood*.

Dr. Novak said that cell line development is an ongoing process because it is desirable to have multiple cell lines that mimic the diversity of WM seen in patients.

THE ROLE OF THE JAK/STAT SIGNALING PATHWAY IN WM

Lucy Hodge, Pharm.D., Ph.D.

In order to grow and survive, normal cells and cancer cells need to communicate with one another; therefore, cells communicate by the release of signaling molecules called cytokines. Cytokines are produced by certain cells and then taken up by adjacent cells, producing a variety of different responses, including proliferation, apoptosis (programmed cell death), differentiation into other types of cells, and secretion of molecules (such as IgM).



Lucy Hodge, Pharm.D., Ph.D.

Research at the Mayo Clinic indicates that an important signaling pathway in WM is the JAK/STAT pathway. If part of this pathway is blocked, it has an effect downstream on WM cells. Two drugs that inhibit parts of this pathway affect specific cytokines called JAK2 and STAT5. Both drugs were tested against patient tumor cells, as well as the MWCL1 cell line described above, and both drugs decreased proliferation of WM cells, decreased viability of WM cells, and decreased IgM secretion.

The drug that blocks JAK2 is in clinical trials for other cancers but not yet for WM.

The JAK/STAT pathway is also found in normal cells, but because WM cells over-express these cell signals, it is hoped that JAK/STAT treatments will target WM cells more readily than normal cells.

GENETIC STUDIES IN WM

Esteban Braggio, Ph.D.

The genetic basis of WM remains poorly defined, and the reasons are varied. WM cells are characterized by a low proliferation rate, have few recurrent abnormalities that have been identified, and have undergone few comprehensive, high-resolution genomic studies.





Esteban Braggio, Ph.D.

Traditionally, the tools for genomic analysis have included karyotyping, which analyzes the number and structure of chromosomes, and more recently FISH (fluorescence in situ hybridization), which is a way to detect chromosomal abnormalities using a fluorescent probe.

Dr. Braggio's laboratory is using a technique called aCGH (array comparative genomic hybridization). This technique takes DNA from a patient sample and a normal sample, labels each with a different fluorescent stain, and hybridizes these to known genetic probes on a slide. The ratio of fluorescence intensity of the patient sample is compared to the normal sample, and calculations based on this intensity can determine if there are changes in the number of gene copies in the patient sample. For instance, there may be either deletions or duplications of certain genes in a patient as compared to a normal sample.

In Dr. Braggio's laboratory, aCGH analyses were performed on a cohort of WM patients, with the goals of discovering any gene copy number abnormalities present and comparing these with related low-grade B-cell malignancies such as chronic lymphocytic leukemia (CLL), marginal zone lymphoma (MZL), MGUS, myeloma (MM), and mantle cell lymphoma (MCL).

The results of the aCGH analyses demonstrated that the genomic complexity of WM is comparable to that of CLL and MZL, but significantly lower than that of MGUS, MM, and MCL. There is a great deal of overlap between WM copy number abnormalities and those of several other B-cell lymphomas, involving the genes TNFAIP3/A20 and TRAF3. Both of these genes are regulators of the NF-kappa B pathway, highlighting the importance of this pathway in WM. Also, there does appear to be a gain in copy number on chromosome 4 that is unique to WM.

Dr. Braggio concluded by saying that while whole genome sequencing is likely the wave of the future, the aCGH technique is very useful because it is less expensive and does not require such a lengthy time for analysis – typically, whole genome sequencing can take several months whereas aCGH can yield results in 2-3 days.

EPIDEMIOLOGY OF WM/LYMPHOPLASMACYTIC LYMPHOMA (LPL)

Francis Buadi, M.D.

Dr. Buadi's discussion focused on the magnitude of WM/LPL in the U.S. – its incidence, mortality and survival

rates, sex, racial, ethnic, and geographic patterns, and known and suspected risk factors.

WM comprises about 1-2% of hematologic cancers. There are approximately five cases per million diagnosed in the U.S. each year, accounting for a total of about 1,000-1,500 new cases per year. Of these, 3.4 cases per million are males and 1.7 cases per million are females. The majority of cases affect white males, followed in order by white females, black females, and black males. These rates are very similar to those found in Europe. While the incidence of myeloma is much higher in blacks than whites, the opposite is true of WM.

The median age at diagnosis of WM patients is 60-70 years, and its incidence increases with age. WM is rare in people below the age of 40 years.

There are several risk factors for WM: IgM MGUS (monoclonal gammopathy of undetermined significance); occupational exposure to leather, rubber, dyes, and paints; pesticide exposure; genetic/familial predisposition; chronic infections such as hepatitis C (HCV); and educational status (interestingly, a higher educational status seems to be a risk factor for WM). There is no known association of risk with radiation or socioeconomic status.

There is a higher incidence of B-cell disorders, including WM, among first-degree relatives of WM patients. Familial



Francis Buadi, M.D.

WM patients are more likely to have had exposure to farming, pesticides, wood dust, and organic solvents compared to unaffected family members. Familial WM patients are more likely to have had a history of autoimmune diseases and infections. It appears that WM patients with a familial history of the disease develop it sooner than the average 60-70 years of age, although the severity of the disease does not appear to be different.

Studies have suggested a 2- to 3-fold risk of developing WM in patients who have chronic immune stimulatory conditions. The Veterans Administration reported that patients with hepatitis C had an increased risk of WM; however, other studies have refuted this conclusion. Additional studies will be required to clarify this connection.

For patients who have active disease and require therapy, the median overall survival is 5-8 years; however, the median disease-specific survival (of patients who die from WM and not other causes) is higher – around 10 years. Factors adversely affecting survival at presentation include:



- age > 65 years
- anemia (hemoglobin <11.5 g/dL)
- low platelets (<100,000)
- beta 2 microglobulin >3 mcg/mL
- monoclonal protein >7 g/dL

The Mayo Clinic has been collecting epidemiology statistics on patients, and during the period 1960-2010, Mayo has seen 1,075 cases of WM; interestingly, in recent years (2009-2010), Mayo has seen a spike in WM cases, the significance of which is not yet known. In most respects, the Mayo data is very similar to the national statistics on WM.

In response to audience questions, Dr. Buadi stated that from 1960-2010 there has been an improving survival rate because of better treatments. He also said that Mayo is looking back to see whether survival statistics have changed from the “fludarabine era” to the “rituximab era.”

MONOCLONAL IGM AND KIDNEY DISEASE

Nelson Leung, M.D.

Kidney disease is common in disorders which have monoclonal gammopathies; however, it is not as common in WM as it is in myeloma. In WM, kidney disorders may be due to tumor cell infiltration or to IgM or light chain deposition.

Manifestations of kidney disease may include loss of kidney function, proteinuria (protein in the urine), electrolyte abnormalities, and systemic symptoms.

If a WM patient has cryoglobulinemia (cold-associated antibody), the cryoprecipitates can damage the kidney by forming deposits and disrupting the normal architecture of the kidneys. Other manifestations of cryoglobulinemia are systemic, including skin rashes and ulcers. Not all patients with cryoglobulinemia will develop kidney disease, but they should be closely monitored.

Several disorders of the kidney may be due to either IgM or to light chain deposition, and can affect different parts of the kidneys, such as the tubules or the glomeruli (filtering units). IgM-associated kidney disorders include intercapillary

monoclonal deposition disease and membranoproliferative glomerulonephritis. Light chain kidney disease is not as common in WM as in myeloma, but kidney disorders associated with light chains are light chain cast nephropathy, light chain deposition disease, and Fanconi syndrome (which causes the kidneys to lose electrolytes). Amyloidosis can affect the kidneys because



Nelson Leung, M.D.

the excess secreted light chains undergo a conformational change and form fibrils that interfere with kidney function.

Many of these damaging effects on the kidneys are not easily diagnosed through the use of common tests on urine, and a kidney biopsy is usually necessary to establish an exact diagnosis.

WM CLINICAL TRIALS

Craig Reeder, M.D.

Dr. Reeder presented a brief overview of clinical trials, which are undertaken to determine the benefits and side effects of new treatments. Various types of clinical trials include the following:

Phase I – to establish dosages and toxicities of new agents

Phase II – to determine efficacy and toxicities once you have established dosages

Phase III – to compare efficacy of new drug to other or standard treatments

Phase IV – to compile post-marketing data collection

Why should a patient participate in clinical trials? In clinical trials, one can have access to new treatments before they are widely available and often at no extra cost, have a focused

team of experts available to direct care, be an active participant in his or her own health care, and help others by generating knowledge through research.



Craig Reeder, M.D.

Barriers to participation in clinical trials include lack of awareness of available trials, transportation issues, money issues, and certain eligibility requirements for inclusion in trials.

In general, to participate in a clinical trial for WM treatment, a patient must have an established diagnosis of WM, be symptomatic, have measurable disease (such as total IgM, M-spike, enlarged lymph nodes, or percentage of bone marrow infiltration), and reasonable function of other organs. Some issues that might exclude patients would include comorbidities (such as heart disease or kidney disease), certain prior therapies, infection with hepatitis or HIV/AIDS, and the presence of other cancers.

Several of the newer therapeutic options in clinical trials for WM are centered on four classes of drugs:

proteasome inhibitors – bortezomib

mTOR inhibitors – RAD 001 (everolimus)

IMiDs – lenalidomide



HDAC inhibitors – LBH 589 (panobinostat)

At the various Mayo Clinic locations, there are currently several clinical trials available for WM patients:

For Previously Treated Patients

- MC0883 – Phase II trial for R-CyBorD – developed from CyBorD, a treatment for myeloma. R-CyBorD consists of rituximab, cyclophosphamide, bortezomib, and dexamethasone.
- MC0886 – Phase I-II trial for LBH589/RAD001 – both are oral agents.
- MC0981 – Phase I-II trial for lenalidomide/RAD001 – both are oral agents.
- MC0986 – Phase II trial for single agent LBH589 – this is an oral agent.

For Previously Untreated Patients

- MC0882 – Phase II trial for LR-CD – consists of lenalidomide, rituximab, cyclophosphamide, and dexamethasone. Except for rituximab, these are oral agents.

Most side effects associated with these newer therapies include lower blood counts (anemia, thrombocytopenia) and peripheral neuropathy (in the case of bortezomib).

Information on clinical trials for WM can be accessed from www.clinicaltrials.gov or from the clinical trials section of the IWMF website at www.iwmf.com/treatment/clinical-trials.aspx.

INTEGRATED CARE AT MAYO CLINIC FOR WM

Morie Gertz, M.D., M.A.C.P.

Dr. Gertz spoke about his privilege of being able to come to work at Mayo Clinic every day and associate with a gifted group of clinicians and researchers. Why has Mayo Clinic become the largest medical center in the U.S.?



Morie Gertz, M.D., M.A.C.P.

One important reason is the electronic unified medical record system at Mayo Clinic. When a WM patient sees a physician at any Mayo Clinic location, his or her physician has instant access to the patient’s medical information. This allows for meaningful cross-talk and sharing among all members of the medical team, including nationally recognized specialists in different fields. There is

integration across Mayo Clinic sites, so that a patient can have continuity of care no matter where he or she is seen. There is also standardization of diagnostic testing and therapeutic evaluation – patients with WM receive the same battery of tests. Furthermore, electronic ordering of chemotherapeutic drugs ensures that standardized protocols are followed and minimizes ordering errors.

Another important aspect of care at Mayo Clinic is its varied research program, including studies on epidemiology, clinical trials, outcomes and survivorship, prognostic factors, bleeding and thrombosis, and cancer biology. Research is an investment in the future, and publication of this research disseminates information so that other patients can benefit from new knowledge. At Mayo Clinic, the clinical trials program is strong, the biobanks are well-stocked with blood and bone marrow samples, and the young investigator pipeline is vigorous. Unfortunately, government funding for research is short, so Mayo Clinic has developed a strong program of philanthropy, dependent on patients and on advocacy groups such as the IWMF.



