The Bone Marrow and Waldenstrom's Macroglobulinemia (Beyond Basics) Fact Sheet

INTRODUCTION

Waldenstrom macroglobulinemia (WM) is a rare mature B cell lymphoproliferative disorder characterized by the presence of detectable serum monoclonal immunoglobulin M (IgM) in the blood and associated with bone marrow infiltration by small lymphocytes, plasma cells, and lymphoplasmacytic cells. Because demonstration of bone marrow infiltration by WM cells is a necessary part of the WM diagnosis, it is important to have an understanding of the bone marrow, how it is affected by WM, and the bone marrow tests used for making the diagnosis.

For a more basic understanding of the bone marrow and Waldenstrom's macroglobulinemia, go to https://iwmf.com/publications/ and scroll down to the booklet**, A Basic Guide to Understanding Your Bone Marrow and Waldenstrom's Macroglobulinemia.**

BONE MARROW ELEMENTS

In adults the cells circulating in the bloodstream originate from the bone marrow. The bone marrow is the soft, spongy material that is found inside the bones, underneath the hard, outer portion of the bone, called the compact or cortical bone. Much of the bone marrow in humans is found within the bones of the pelvis, the sternum (breastbone), and the vertebrae (backbones). Since the cells and immunoglobulins circulating in the bloodstream originate in the bone marrow, this is where investigations of hematologic (blood-related) issues typically begin. All of the immature blood cells or precursor cells can be found in the bone marrow.

Within the bone marrow are stem cells, the cells that have the potential to develop into a variety of different cell types. Depending on the type of stem cells, they can develop into mature blood cells, blood vessels, fat, cartilage, or other structures. The hematopoietic stem cells can be divided into myeloid and lymphoid stem cells. These stem cells will ultimately become the circulating red blood cells, platelets, and white blood cells (*Figure 1*).

The myeloid stem cells can mature into erythroid precursors (immature red blood cells) and then ultimately into erythrocytes (mature red blood cells). Within healthy red blood cells, there is a protein called hemoglobin that is responsible for holding oxygen within the blood cell and then releasing it into different tissues as the red blood cell circulates around the body. The lab value of hemoglobin is often used as a surrogate marker for the amount of effective red blood cells circulating around the body.

The myeloid stem cells can also develop into megakaryocytes. Megakaryocytes are the largest cells found within the bone marrow. Small pieces of megakaryocytes separate from the cell and are released into the circulation as thrombocytes, otherwise known as platelets. Platelets play a large role in forming blood clots and preventing bleeding.

There are many types of leukocytes (white blood cells) which all play a role in the function of the immune system. White blood cells are formed from both myeloid and lymphoid stem cells. Myeloid stem cells can develop into eosinophils, basophils, monocytes, and neutrophils, which play important roles in protecting against infection. Lymphoid stem cells develop into cells that also make up a central

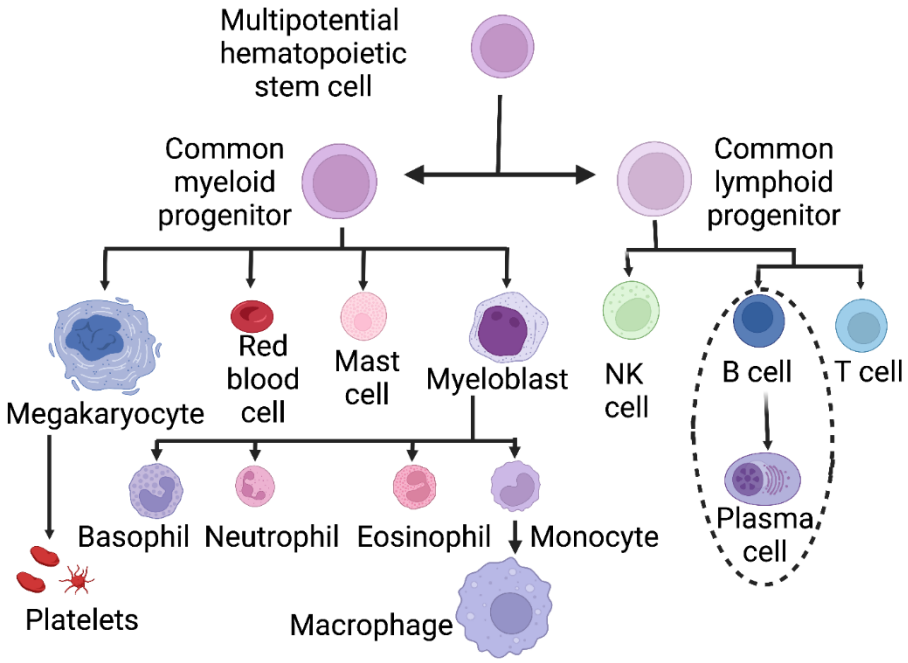

Figure 1: Blood cell development (created with Biorender.com).
Malignant WM cells form from cells in the pathway between B cells and plasma cells (dotted oval).

part of the immune system, including B lymphocytes, T lymphocytes, plasma cells, and natural killer cells. B lymphocytes are precursors for plasma cells, and the normal pathway for their development into plasma cells is affected in WM. In WM, a mutation generally occurs within this pathway and a clonal population of cells (formed from the mitotic division of a single somatic cell) develops. Hence the abnormal clone in WM may contain any of the cells in this pathway, including B lymphocytes,

lymphoplasmacytic cells (cells that have characteristics of B lymphocytes and plasma cells), and plasma cells.

Plasma cells make antibodies (also called immunoglobulins), which are then released into the bloodstream Each antibody is composed of four proteins, including a pair of large proteins called heavy chains and a pair of small proteins called light chains (see Figure 2). There are many varieties of heavy chains and two categories of light chains, called kappa and lambda. Under normal circumstances, each antibody-producing cell would select either kappa or lambda light chains, and all the antibodies produced by that particular cell would use either kappa or lambda light chain. Normally, there is a diversity of different plasma cells, some producing antibodies with kappa light chain and others producing antibodies with lambda light chain. The result is that blood normally contains a mixture of antibodies with different heavy chains and light chains. There are also different classes of antibodies. In WM, the predominant class is called IgM. IgM antibodies consist of 5 identical antibodies, all linked together in a circle to form one large molecular complex (*Figure 2*).

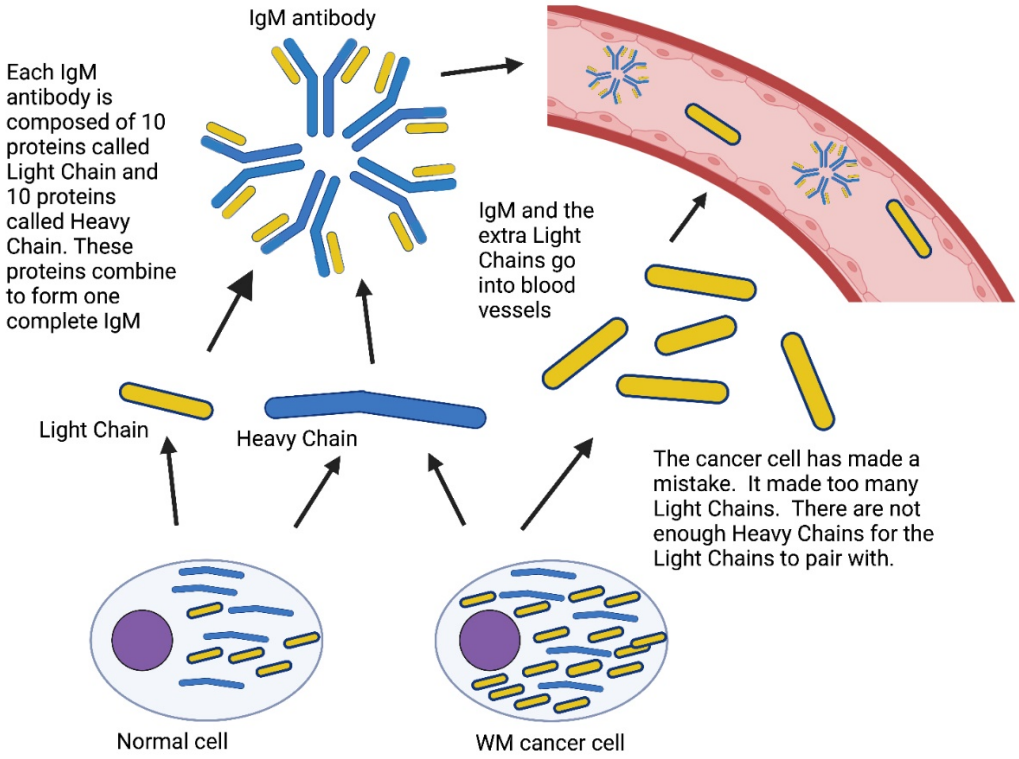

Figure 2: Light Chain Proteins in Blood (created with Biorender.com)
Proteins called Light Chain and Heavy Chain come together to form antibodies, including IgM. Antibodies are composed of equal numbers of Light Chains and Heavy Chains. Accordingly, cells normally produce these two proteins in approximately equal amounts (actually, a slight excess of Light Chain). In some people with WM, the WM cancer cells produce Light Chain in considerable excess. Light Chain proteins that do not attach to Heavy Chain proteins to form antibodies can be found free in the blood.

Cells in the bone marrow are not arranged haphazardly. If a scientist looked closely at the bone marrow with a microscope, they would see what is called the "microenvironment." The microenvironment is the arrangement of cells and structures that allow for signaling between the cells of the bone marrow. The microenvironment plays an important role in the growth of both malignant and healthy cells. Much research is currently being performed to better understand the role of the microenvironment on the development and growth of malignant cells, as well as effect of the microenvironment on treatment responses.

One question about WM is why the malignant WM cells preferentially reside in bone marrow and not as much elsewhere in the body. Clues to this may reside in the bone marrow microenvironment. Interactions between the WM cells and other cells and structures of the bone marrow environment may make the bone marrow a more hospitable place for growth of WM cells. One mechanism by which WM cells home to the bone marrow is a protein on the surface of WM cells called CXCR4, which interacts with signals in the bone marrow microenvironment. CXCR4 mutations cause an accumulation of CXCR4 on the surface of WM cells, which may facilitate their retention and growth in the bone marrow. There are likely other signals as well that help WM cells to remain and grow in the bone marrow, and how to disrupt these signals is an area of active scientific investigation.

Another question about WM is why the population of WM cells, despite having mutated MYD88 and other alterations that make them grow and survive better than normal cells, expand so slowly (or not at all!) in asymptomatic (watch-and-wait) patients and even in some symptomatic patients. The answer to this is under investigation, but a key may lie with the immune cells in the bone marrow microenvironment. These immune cells likely play an important role in keeping the growth of the WM cells under control. Researchers are trying to better understand the body's immune response to WM cells, with the hope of using specific drugs to improve certain critical aspects of the immune response.

BONE MARROW BIOPSY AND ASPIRATE

A bone marrow biopsy and aspirate can be performed for many reasons, including investigation into the cause of cytopenias (low blood cell counts) or evaluation for the presence of a hematologic malignancy. When a thorough investigation of the bone marrow is required, two samples are typically obtained: a bone marrow aspirate and a bone marrow core biopsy. The bone marrow aspirate is a liquid sample of the bone marrow, and the bone marrow core biopsy is a solid portion of the marrow.

Typically, the bone marrow samples are obtained while the patient is lying on their side or on their stomach. The procedure may occur in a range of locations, including a clinic, interventional radiology suite, or an operating room. A local anesthetic (numbing medication), similar to that for dental procedures, is used during the bone marrow biopsy and aspirate. Additionally, dependent on patient or

practitioner preference, conscious sedation may be used to allow for patient comfort. This is not standard practice at many institutions, and in most cases the procedure can be easily performed with only local anesthetic. After the local anesthetic is administered, the proceduralist will use a hollow needle and syringe to collect the bone marrow samples. Generally, a separate needle is utilized to collect each sample, although the original anesthetized location on the skin and bone can be utilized to obtain both samples. After completion of the bone marrow biopsy, pressure is held over the site of the biopsy to prevent any bleeding and then a bandage is applied. Patients are generally able to return to their usual activities on the same day that the biopsy is completed, although some temporary soreness at the site can be expected.

INTERPRETING BONE MARROW REPORTS IN WALDENSTROM MACROGLOBULINEMIA

Depending on the reason for obtaining the biopsy, bone marrow samples can be sent for multiple different tests. Many of the following tests are commonly performed in patients with WM, and the typical findings in WM are described below.

BONE MARROW CORE BIOPSY

The bone marrow core biopsy is generally used to assess the structure of the bone marrow, the cellularity, the degree of cell maturation, and the proportion of different cell types.

Cellularity

The cellularity (number of cells present in the bone marrow) varies with age. The number of cells in the bone marrow decreases with age while the amount of fat increases. In general, the amount of fat in the bone marrow should be similar to a patient's age (i.e., a 70-year-old patient would likely have 60-80% fat in the bone marrow). Often the first evaluation performed by a pathologist when looking at the bone marrow is to evaluate the bone marrow cellularity to determine if the quantity correlates with the patient's age. There are some conditions, such as leukemia and lymphoma, in which the fat has been replaced by malignant cells, and in this case the number of cells is increased. It is common in WM for the patient to have a hypercellular marrow (a marrow with more cells than would be expected for the patient's age) because of the malignant WM cells filling the bone marrow.

Cell identification

Pathologists can easily identify types of cells that are present in the core biopsy by looking at the shape, size, and structure of the cells. Pathologists study the biopsy to determine if there is an excess of or a substantial decrease in the number of healthy cells. Special stains referred to as immunohistochemical stains can help identify each type of cell. Often in WM, an excess of plasma cells, lymphocytes, or lymphoplasmacytic cells will be present in the bone marrow. These abnormal cells, or those of other malignancies, are often found in clusters rather than evenly distributed throughout the bone marrow.

The number of abnormal cells in WM is generally reported as the percent of bone marrow infiltration by lymphoplasmacytic cells and this is the quantity of malignant cells that is generally referred to when discussing results with patients.

In the case of suspected WM, once an excess of plasma cells, lymphocytes, and/or lymphoplasmacytic cells is detected, pathologists can determine if these cells are monoclonal (related malignant cells) by examining if all of the cells have the same antibody light chain expression using *in situ* hybridization (see above, **Bone Marrow Elements**, for a description of antibody light chains). *In situ* hybridization is a laboratory technique that allows a pathologist to assess the mRNA (genetic material) within a cell to determine which light chain a cell will produce.

A normal population of many cells would show a mixture of cells that use kappa and other cells that use lambda light chains. In the case of WM, either a kappa or a lambda light chain will be expressed uniformly by the malignant clone, resulting in an abnormally high amount of that particular light chain. The pathologist can correlate the clone of cells in the bone marrow with the light chain type seen on blood tests, specifically by tests such as the serum immunofixation electrophoresis (SIFE) or the serum free light chain (FLC) assay.

BONE MARROW ASPIRATE

The bone marrow aspirate is the liquid sample that is generally used to evaluate the amount of each cell type as well as the cell morphology (shape/form). The aspirate is also required in most cases to obtain detailed genetic testing.

Aspirate smear

The aspirate smear is a thin layer of cells taken from the liquid portion of the bone marrow that a pathologist can review to analyze individual cells. Ideally a bone marrow aspirate would contain spicules (small pieces of bone) which indicate that the specimen is adequate and was collected from the correct location within the bone marrow. The aspirate smear is important in the evaluation of many bone marrow disorders, such as myelodysplasia (a bone marrow disorder that can also lead to decreased production of cells or production of abnormally shaped cells).

Flow cytometry

Flow cytometry is also performed on the bone marrow aspirate and can identify specific types of cells (i.e., plasma cells or B lymphocytes) based on markers found on the outside of the cells. Identification of specific cell types by flow cytometry is called immunophenotyping. With this test, a pathologist can identify if an abnormal (clonal) population(s) of cells is present. It is important to note that not all cells

will survive the processing for this test. This is especially true for plasma cells, so the percentage of abnormal cells detected on this test may be lower than that reported from the core biopsy.

Karyotyping and FISH

Karyotyping is a process that is performed to closely evaluate the overall size, shape, and number of chromosomes. Chromosomes are structures within the nucleus of cells, including those of the bone marrow, that carry DNA (the genetic material of the human body). A karyotype can give a general idea about any additions or deletions of the chromosomes, as well as any translocations (swaps of material between different chromosomes). In-depth testing of the chromosomes may also include fluorescence in situ hybridization (FISH) testing, which can look for specific chromosomal changes that may be associated with other bone marrow disorders such as multiple myeloma or follicular lymphoma. Although we do not routinely use this information to determine treatment or confirm the diagnosis of WM, there are some mutations found on these tests that are often seen in WM, such as a TP53 mutation and deletion 6q.

Mutational analysis

When the aspirate is obtained, a portion of this sample should also be sent for additional testing to evaluate for specific genetic mutations typically associated with WM. Thorough testing for an MYD88 mutation and a CXCR4 mutation should be performed as part of an initial comprehensive work-up for WM. This testing can be performed through many different procedures, including next generation sequencing, polymerase chain reaction (PCR) testing, or Sanger sequencing. Knowing if there is a MYD88 mutation or a CXCR4 mutation is important when deciding about treatment options.

Diagnosis of WM requires the presence of lymphoplasmacytic lymphoma in the bone marrow. A bone marrow aspiration and biopsy that include immunophenotyping and genetic analyses will help establish the diagnosis of WM by distinguishing it from IgM multiple myeloma, and other IgM-secreting disorders, such as marginal zone lymphoma and chronic lymphocytic leukemia.

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ABOUT THE IWMF

The International Waldenstrom's Macroglobulinemia Foundation (IWMF) is a patient-founded and volunteer- led, nonprofit 501(c)(3) organization with an important vision, "A World Without WM," and a mission to "Support and educate everyone affected by WM while advancing the search for a cure."

More information about Waldenstrom macroglobulinemia and the services and support offered by the IWMF and its affiliate organizations can be found on our website, www.iwmf.com.

The IWMF relies on donations to continue its mission, and we welcome your support. The Foundation maintains a Business Office at 6144 Clark Center Ave., Sarasota, FL 34238. The Office can be contacted by phone at 941-927-4963, by fax at 941-927-4467, or by email at info@iwmf.com.

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