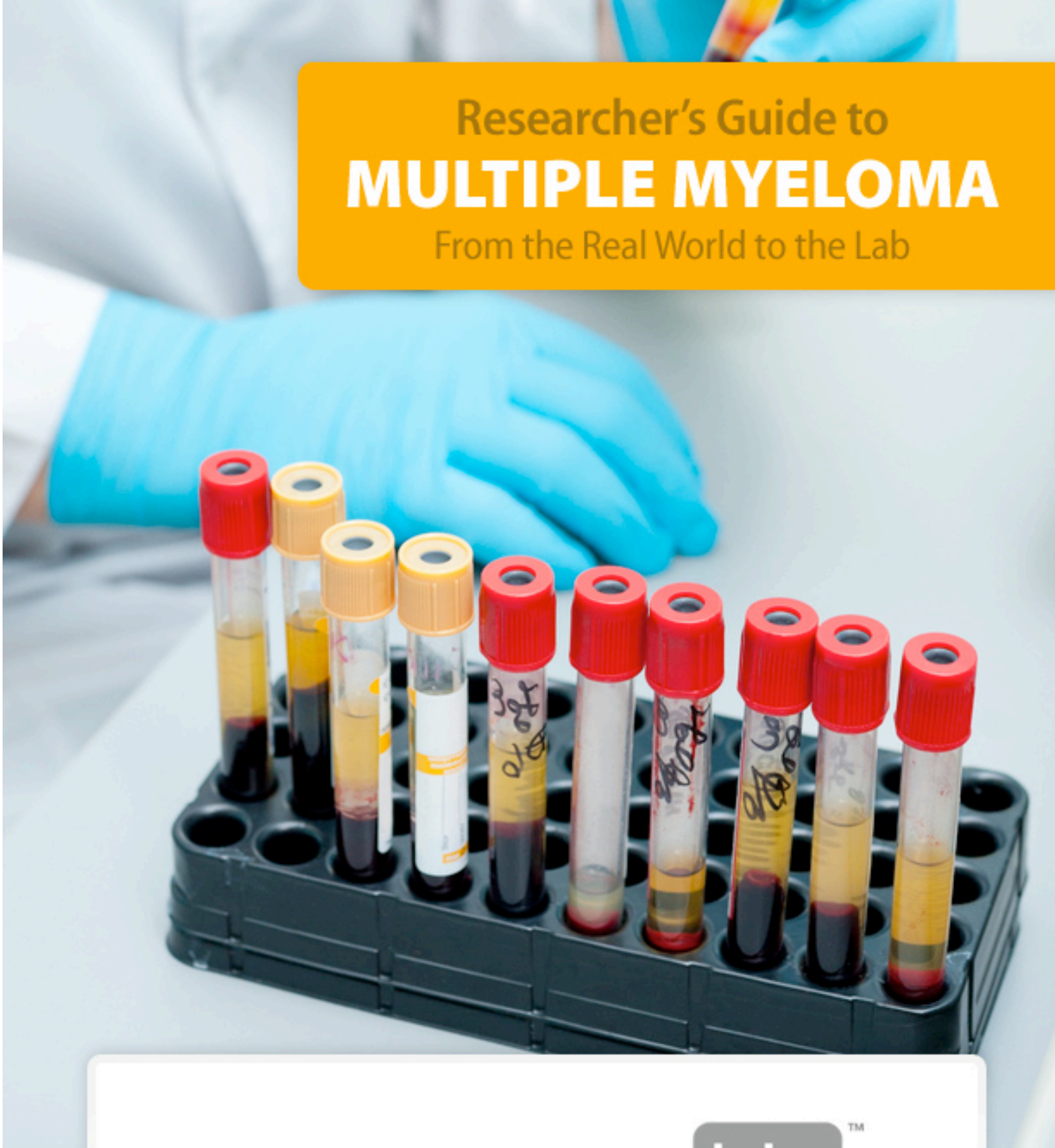

Researcher's Guide to
MULTIPLE MYELOMA
From the Real World to the Lab

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Multiple Myeloma

Accelerate Your Research With Conversant Bio

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Introduction

Blood and bone marrow both play a significant role in the search to find a cure for multiple myeloma. Researchers are discovering that there are a number of ways for bone marrow to be used, including studies in toxicology, cell isolation, tissue regeneration, and genetics. Additionally, they are used in drug discovery to rule out compounds for future in vivo testing and in biomarker identification and validation. The type of study being performed will determine which sample type researchers will use. Fresh bone marrow, bone marrow mononuclear cells (BMMCs), or isolated cells such as CD34+ or CD138+ are a few of the available bone marrow derivatives from bone marrow that can be used in research. Whole blood and blood derivatives such as peripheral blood mononuclear cells (PBMCs), plasma, and serum are also widely used in the fight to find a cure for multiple myeloma.

Section 1:

From the Real World to the Lab

Multiple myeloma is a hematologic cancer that develops in the bone marrow of the long bones. It is nearly twice as common in the non-Hispanic black population as compared to the white population with a higher incidence among men. It is important to note that the lowest incidence of cases is found among the Asian population, an anomaly that is actively being studied in the research community.

Multiple myeloma is much less common than other forms of cancer. The American Cancer Society estimates that 1 in 149 people in the United States will be diagnosed with multiple myeloma over the course of their lifetime. This equates to a 0.67% chance.[1]

The Multiple Myeloma Research Foundation (MMRF) says that 96% of diagnosed cases occur in patients over the age of 45, over 60% of whom are above the age of 65.[2]

Diagnosing Multiple Myeloma

The process of diagnosing multiple myeloma often begins when doctors are performing routine exams on their patients and the results reveal a need for further testing. There are a number of tools used to diagnose multiple myeloma, but it generally requires a combination of testing for doctors to reach a conclusion. Three categories of testing are used for diagnosis:

1. Laboratory Tests

Blood and Urine Testing – Doctors will run a **Complete Blood Count (CBC)** test. The CBC measures the number of red blood cells, white blood cells, and the number of platelets present in the blood. The chart below indicates the normal range of blood cell counts:

[1] http://www.cancer.org/cancer/multiplemyeloma/detailedguide/multiple-myeloma-key-statistics
[2] http://www.themmrf.org/living-with-multiple-myeloma/newly-diagnosed-patients/what-is-multiple-myeloma/

Normal Range of Blood Cell Counts

Erythrocytes (RBCs)	**Definition:** Number of red blood cells in the blood. Red blood cells bring oxygen from the lungs to the various tissues in the body and carry carbon dioxide back to the lungs. Low numbers of red blood cells, low hemoglobin, or hematocrit indicate anemia can cause physical and mental fatigue	**Normal Range*** **Female** 4.1-5.1 x 1012/L **Male** 4.5-5.3 x 1012/L
Hemoglobin (Hb)	**Definition:** Oxygen-carrying substance in red blood cells.	**Normal Range*** **Female** 12 – 16 g/dL **Male** 13 – 18 g/dL
Hematocrit (HCT)	**Definition:** Percentage of red blood cells in the blood.	**Normal Range*** **Female** 36 – 46% **Male** 37 – 49%
Leukocytes (WBCs)	**Definition:** Number of white blood cells in the blood; counts or percentages of the individual types of blood cells are also provided. White blood cells help fight infection and remove harmful substances from the body. A low number of white cells can increase the possibility of infection.	**Normal Range*** Total 3.5 - 10.5 x 109/L Neutrophils 1.7 - 7.0 Monocytes 0.3 - 0.9 Lymphocytes 0.9 - 2.9 Basophils 0.0 - 0.3 Eosinophils 0.05 - 0.5
Platelets	**Definition:** Number of platelets in the blood. Because platelets help blood to clot, low counts can lead to excessive bleeding.	**Normal Range*** 150 – 450 x 10^9/L

**Normal ranges may vary.*
Table adapted from The Multiple Myeloma Research Foundation[3]

[3] http://www.themmrf.org/living-with-multiple-myeloma/newly-diagnosed-patients/what-is-multiple-myeloma/diagnosis-and-staging.html

Erythrocyte Sedimentation Rate (ESR) is sometimes used as part of the diagnosis since a raised rate may indicate an abnormally high protein or raised calcium level.[4] More commonly, the **Serum Protein Electrophoresis (SPEP)** test is used to measure abnormal proteins in the blood. These abnormal proteins are also known as paraproteins, monoclonal immunoglobulin, M protein, and M spike. The test that measures abnormal proteins in urine is known as the **Urine Protein Electrophoresis (UPEP).** It requires collecting a urine sample over the course of 24 hours to determine the level of abnormal (light chain, Bence-Jones) proteins.[5]

A full **metabolic panel** can be run to find levels of albumin, blood urea nitrogen (BUN), creatinine, and calcium.

- A low level of albumin found in the blood can indicate a more advanced myeloma.
- BUN and creatinine levels indicate kidney function. Higher levels are often found in people with impaired kidney function, including some multiple myeloma patients.
- Symptoms such as weakness, confusion, and fatigue are often the result of high levels of calcium in patients with advanced multiple myeloma.

2. Bone Marrow Exams

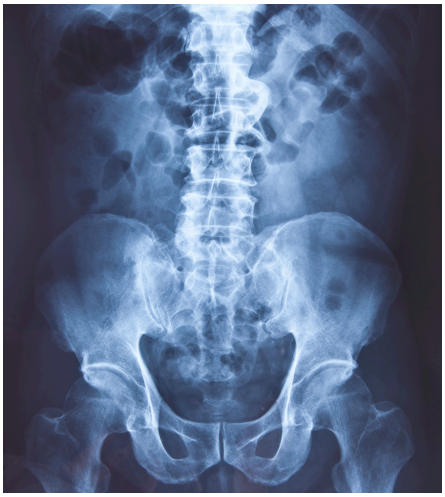
Bone marrow biopsies and aspirations are often used as diagnostic tools to detect high plasma cell counts in bone marrow, a feature of multiple myeloma. Biopsies require surgery with an approximately 1/16 x 1 inch piece of bone marrow tissue being removed. This tissue will be observed in a lab under a microscope to determine if the cells are myeloma cells. If plasma cells account for more than 10% of the cells, it is likely that there is multiple myeloma in the marrow.[6] Bone marrow aspirations can be performed as part of a bone marrow exam or used alone to test for the presence of multiple myeloma in a patient. If the bone marrow aspiration is performed as part of the bone marrow exam, it will usually be extracted before the larger section of tissue is removed.

Often the aspirates will be sent for other tests including **immunochemistry and flow cytometry** which help identify plasma cells that may support a multiple myeloma diagnosis. **Cytogenetic testing** is used to identify chromosome mutations. **Fluorescent in Situ Hybridization (FISH)** is also used to look at chromosomes. In this test, a dye attaches to specific parts of the chromosome, such as the location of a transmutation or deletion.

[4] http://www.news-medical.net/health/Multiple-Myeloma-Diagnosis.aspx
[5] http://www.cancer.org/acs/groups/cid/documents/webcontent/003121-pdf.pdf
[6] http://www.themmrf.org/living-with-multiple-myeloma/newly-diagnosed-patients/what-is-multiple-myeloma/diagnosis-and-staging.html

A **Fine Needle Aspiration (FNA)** biopsy is sometimes used to obtain samples of bone marrow tissue. The procedure does not require surgery but the sample acquired can be too small for doctors to reach a conclusive decision about diagnosis. This test is more often used to determine if cancer has spread to other organs. The core needle biopsy uses the same process but requires a larger needle that collects a larger tissue sample.

3. Imaging Studies



Doctors will do a series of bone x-rays, often referred to as a **bone or skeletal survey**, to determine if skeletal lesions have formed as a result of multiple myeloma. These x-rays generally include the skull, long bones, and spine.

Computed Tomography (CT) scans, also known as **Computerized Axial Tomography (CAT)** scans, are used to tell if the bones have been damaged by multiple myeloma. These scans create a 3D rendering of a cross section of the bone.

A **Magnetic Resonance Imaging (MRI)** is also used to provide detailed images of the bone. The MRI varies from the CT scan in that it can also produce an image of a parallel cross section the length of the patient's body. Because these are detailed, they are often helpful in locating plasmacytomas (malignant plasma cell tumors).

Positron Emission Tomography (PET) scans are used to locate multiple plasmacytomas, and are sometimes ordered after an MRI locates a plasmacytoma. Radioactive glucose is injected into a patient's vein. Radioactivity gathers in the cancerous cells and can be seen with the PET scan.[7]

Diagnosis is generally based on the presence of high levels of plasma cells in bone marrow and the presence of excess paranormal proteins in the blood or urine. Doctors will use this initial set of testing as a starting point to determine the stage and classification of multiple myeloma.

[7] http://www.cancer.org/cancer/ultiplemyeloma/detailedguide/multiple-myeloma-diagnosis

Subtypes of Multiple Myeloma: Staging and Diagnosis

There are two common staging methods that doctors use: The International Staging System (ISS) and the Durie-Salmon staging system. Many doctors have begun using the newer International Staging System because it tends to be more accurate and easier to interpret. In the ISS, there are three stages.

ISS Multiple Myeloma Staging

Stage I	Serum beta-2 microglobulin < 3.5 (mg/L) **AND** Albumin is > 3.5 (g/dL)
Stage II	The beta-2 microglobulin level is between 3.5 and 5.5 (g/dL) (with any albumin level) **OR** The albumin < 3.5 (g/dL) while the beta-2 microglobulin < 3.5 (mg/L)
Stage III	Serum beta-2 microglobulin > 5.5 (mg/L)

Table adapted from Cancer.org[8]

There is no cure for multiple myeloma. The course of treatment a patient receives depends on which of the three categories he or she falls into: Monoclonal Gammopathy of Undetermined Significance (MGUS), Asymptomatic (Smoldering or Indolent Myeloma), or Symptomatic.

Monoclonal Gammopathy of Undetermined Significance (MGUS) is identified by the presence of a monoclonal M-protein or paraprotein in the blood that is produced by plasma cells. About 1% of the population is affected by MGUS. The highest incidence of patients with MGUS occurs in patients over the age of 80. As individuals age, the chances of having MGUS increases. About 3% of individuals over the age of 70 are diagnosed with MGUS; however, it is relatively harmless until it progresses to a malignant cell disorder.[9] Patients will have the serum M-protein monitored at 3 months, 6 months, and yearly to establish, confirm, and monitor the diagnosis of MGUS.

[8] http://www.cancer.org/cancer/multiplemyeloma/detailedguide/multiple-myeloma-staging
[9] http://www.themmrf.org/living-with-multiple-myeloma/newly-diagnosed-patients/what-is-multiple-myeloma/diagnosis-and-staging.html

Monoclonal Gammopathy of Undetermined Significance (MGUS)	**Criteria:** Serum M-protein <3.0 g/dL and bone marrow plasma cells <10% Absence of anemia, renal failure, hypercalcemia, lytic bone lesions

Asymptomatic Myeloma means that a patient is not showing signs of having the disease. Asymptomatic myeloma can be classified as Smoldering Multiple Myeloma or Indolent Multiple Myeloma. These patients require no immediate treatment, but instead will be monitored on a three to six month basis to determine if symptoms are advancing. While some patients will be started on a course of bisphosphonates, most simply remain under observation until the disease progresses.

Smoldering Multiple Myeloma (SMM)	**Criteria:** Serum paraproteins >3.0 g/dL and/or bone marrow plasma cells ≥10% Absence of anemia, renal failure, hypercalcemia, lytic bone lesions No myeloma related organ or tissue damage
Indolent Multiple Myeloma (IMM)	**Criteria:** Stable Serum/ urine M-protein Bone marrow plasmacytosis Mild anemia or few small lytic bone lesions Absence of symptoms

Symptomatic Multiple Myeloma (MM) requires individuals to undergo immediate treatment.

Symptomatic Multiple Myeloma (MM)	**Criteria:** M-protein in either serum or urine Bone marrow plasma cells >30% Evidence of anemia, renal failure, hypercalcemia, lytic bone lesions

Treatment and Side Effects

There are several treatment options for individuals with multiple myeloma. When deciding on a treatment regimen, patients and doctors must consider factors such as the stage of multiple myeloma, a patient's age, and their susceptibility to infection from invasive surgeries.

Treatment Options

Autologous Stem Cell Transplant

A more aggressive form of treatment, known as induction, begins with a patient receiving a high-dose of chemotherapy. The chemotherapy destroys both the unhealthy, disease causing plasmas and the normal plasmas. Chemotherapy is then followed by a stem cell transplant using the patient's own stem cells (autologous). These implanted cells proliferate to replace the unhealthy cells. While the stem cells provide no "anti-cancer" effects, according to one study cited on Medscape.com, they allow doctors to use a dose of chemotherapy that is 10-20 stronger than the dose used for standard therapy.[10] Many doctors recommend that the initial transplant be followed up by another stem cell transplant 6-12 months later. While it often causes more negative side effects for the patient, patients who receive the second transplant tend to live longer.

Allergenic Stem Cell Transplant

Doctors can also choose to transplant stem cells that are not the patient's own in a process known as allogeneic transplantation. The transplanted cells usually come from a family member; however, they can also come from a matched but unrelated donor. This method is less commonly used since most multiple myeloma patients are older, and patients receiving this type of treatment must be healthy and relatively young to endure this particular transplant. While these transplants are more dangerous in nature, they have proved to be an especially effective method of treating multiple myeloma.

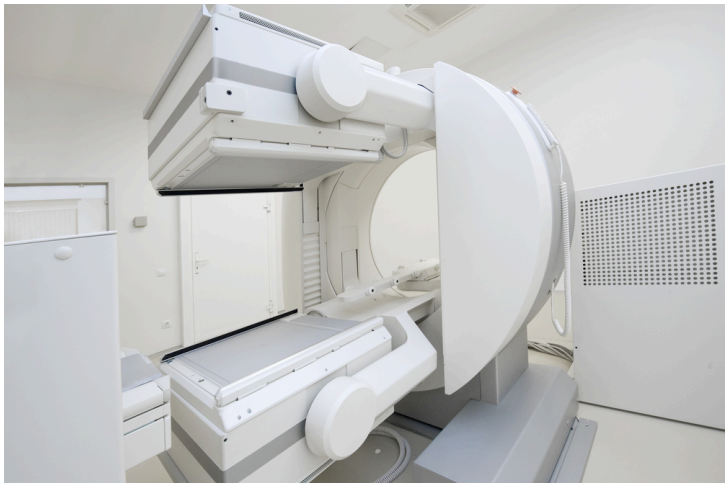
Chemotherapy

The most common form of chemotherapy administered to patients is Melphalan. Melphalan can be administered orally or intravenously. Like other alkylating agents, it works by interfering with cells as they divide. Since cancer cells divide more quickly than healthy cells, this helps stop the spread of cancerous cells. Unfortunately, these agents do not target only the cancerous cells, so healthy cells are depleted as well.

[10] http://emedicine.medscape.com/article/204369-treatment#aw2aab6b6b3

Interferons

Interferons are often used for patients who are in remission. They slow the growth of multiple myeloma cells and are often given after chemotherapy.

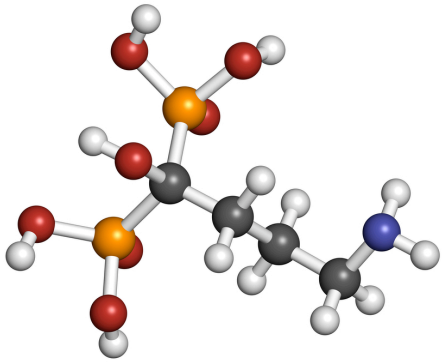


Radiation

Radiation therapy is used to treat patients who have not responded to other treatments, such as chemotherapy. Radiation is used to treat specific areas of multiple myeloma or single plasmacytomas. External beam radiation treatment (EBRT) is the most common form of radiation used. Just like it sounds, an external beam is aimed at the plasmacytoma to deliver radiation to the cancerous cells; however, the process may also damage the healthy cells as well.

Bisphosphonates

Bisphosphonates are administered intravenously to patients but their use must be monitored closely because they can cause osteonecrosis of the jaw (ONJ). This is a rare but damaging side effect where the jaw bone dies, often leading to open sores and tooth loss. Patients will usually begin by getting bisphosphonates once a month, but it can be administered less often to avoid the aforementioned side effects.



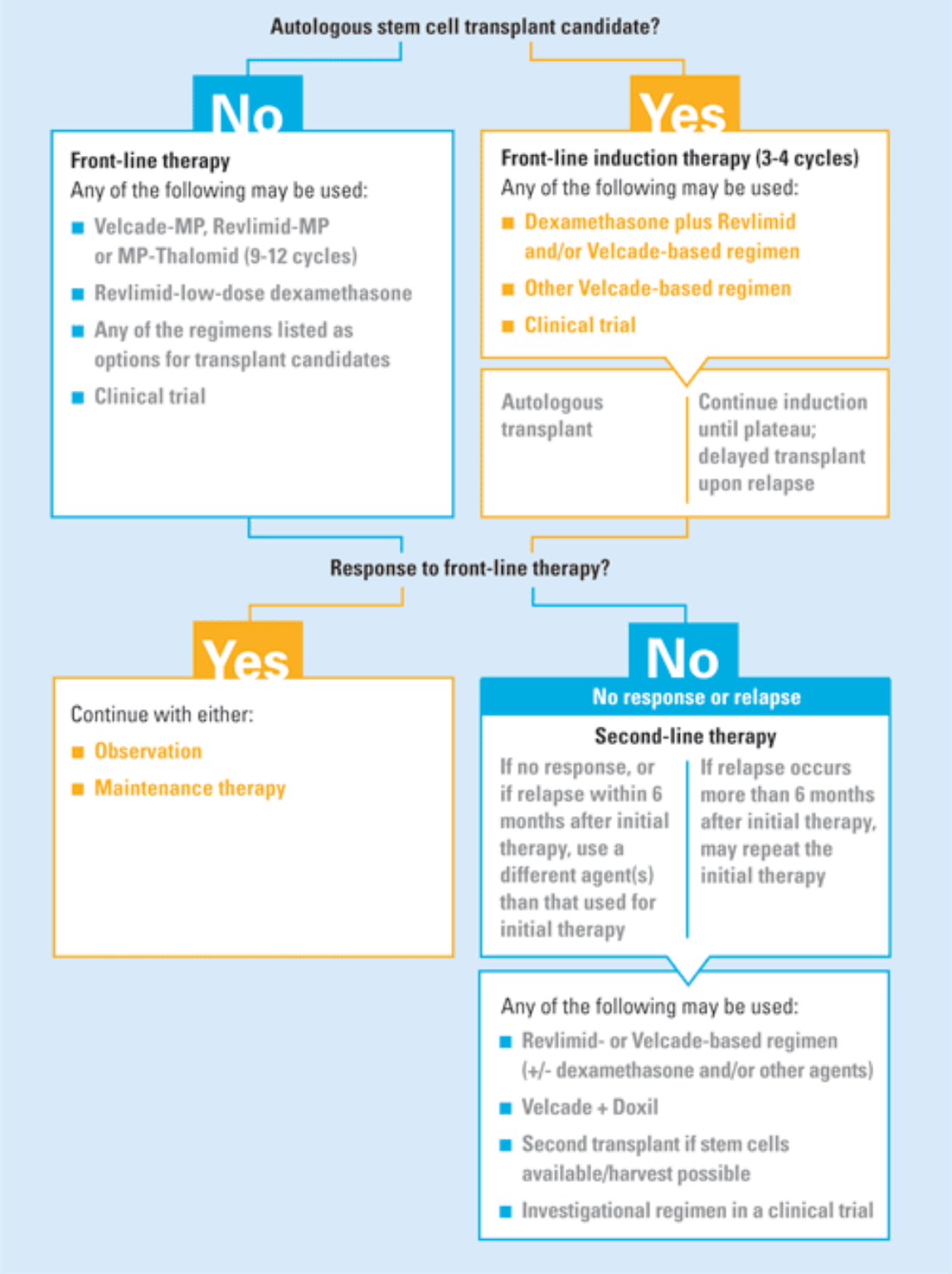
Bisphosphonates help combat bone loss by blocking the activity of osteoclasts to prevent further bone damage.

Surgery

Surgery is rarely used as a treatment option in multiple myeloma cases; although, single plasmacytomas are sometimes removed using surgery. Surgery may also be required as a result of the side effects associated with multiple myeloma. For instance, surgery may be performed to relieve spinal cord compression which can result in muscle weakness, numbness, and even paralysis over time. Additionally, elective surgery is sometimes performed to implant rods or plates to strengthen bones where the disease has weakened them and to prevent further fracturing. [11]

[11] http://www.cancer.org/cancer/multiplemyeloma/detailedguide/multiple-myeloma-treating-biologic-therapy

The flow chart included below details the path multiple myeloma patients might follow as they move through different treatment options. The chart does not indicate effectiveness of treatment, but merely potential flow. [12]



Note: MP = Melphalan and Prednisone

[12] http://www.themmrf.org/living-with-multiple-myeloma/patients-starting-treatment/treatment-options/

Common therapies for multiple myeloma are often categorized as chemotherapy, targeted therapies, or supportive therapies. The chart below details some of the more common therapies used in treating multiple myeloma.

Therapies for Multiple Myeloma

Therapy	Description
Velcade® (bortezomib, Millennium: The Takeda Oncology Company) for Injection	Proteasome inhibitor approved for use across the entire spectrum of myeloma disease
Revlimid® (lenalidomide, Celgene)	Oral agent that is an improvement over Thalomid and is effective across the spectrum of myeloma disease; approved for use in combination with dexamethasone in individuals who previously received treatment
Thalomid® (thalidomide, Celgene)	Oral agent show to effective across the spectrum of myeloma disease; approved in combination with dexamethasone as front-line therapy
Doxil® (doxorubicin HCl liposome injection, Ortho Biotech)	Chemotherapy agent approved for use in combination with Velcade for individuals who previously received therapy other than Velcade
Steroids (corticosteroid)	May be used alone or in combination with other therapies
Conventional (standard dose) chemotherapy	The use of drug(s), administered alone or in combination, to kill cancer cells. Low-dose melphalan (Alkeran®, Celgene, GlaxoSmithKline) is a chemotherapy agent used frequently for the treatment of myeloma
High-dose chemotherapy and stem cell transplantation	The use of higher doses of chemotherapy drugs followed by transplantation of stem cells to replace those damaged by the chemotherapy
Radiation therapy	The use of high-energy rays to damage cancer cells and prevent them from growing
Supportive therapy	Therapies that alleviate symptoms and manage complications of the disease and its treatment, such as bisphosphonates for bone disease, low-dose radiation therapy and analgesics for pain relief, growth factors, antibiotics, intravenous immunoglobulin, orthopedic interventions, anticoagulants, antiemetics, and drugs to prevent and reduce the severity of neuropathy (nerve damage)

Table adapted from Multiple Myeloma Research Foundation[13]

[13] http://www.themmrf.org/living-with-multiple-myeloma/patients-starting-treatment/treatment-options/

Side effects of treatment

Patients receiving treatment for multiple myeloma will generally suffer from some of the following side effects: hair loss, fatigue, mouth sores, low blood counts, loss of appetite, and nausea or vomiting. More serious side effects, such as the previously mentioned ONJ, can result from prolonged use of bisphosphonates. If kidney problems occur, bisphosphonate treatment may be suspended until they are resolved. Patients receiving radiation to the lower pelvic region may experience issues with infertility including: loss of sperm production, early menopause, permanent infertility, or scarring and fibrosis of the uterus.

Potential Treatment Outcomes

Type of Response	M Protein	% Plasma Cells in Bone Marrow	Skeletal Disease (On X-ray)
Complete Response (CR)	No longer detectable in blood and/or urine; negative immunofixation test	<5%	Stable
Near complete response(nCR)[1]	No longer detectable in blood and/or urine, but positive immunofixation test	<5%	Stable
Very good partial response (VGPR)[2]	No longer detectable in blood and/or urine, but positive immunofixation test, or 90% decrease	N/A	Stable
Partial response (PR)	=50% decrease	N/A	Stable
Minimal response (MR)[3]	25%-49% decrease	N/A	Stable
Stable disease (SD)	Not meeting the definition of minimal response or progressive disease		
Progressive disease (PD)	>25% increase	>25% increase	New bone lesions or increase in size of existing lesions

These outcomes are based on criteria developed by the EBMT (European Group for Blood and Marrow Transplant), IBMTR (International Bone Marrow Transplant Registry), and ABMTR (Autologous Blood and Marrow Transplant Registry; Bláde criteria), and the International Myeloma Working Group (IMWG Uniform Response Criteria).

[1]Some clinical trials modify the EBMT criteria to include the nCR category.

[2]Only defined in the IMWG criteria.

[3]Only defined in the EBMT criteria.

Table adapted from Multiple Myeloma Research Foundation[14]

[14] http://www.themmrf.org/living-with-multiple-myeloma/patients-starting-treatment/treatment-options/#What-are-the-potential-outcomes-of-treatment

Section 2:

Research Challenges

Multiple myeloma is not often staged in physician notes, so staging is often determined using the criteria described earlier.

The amount of bone marrow drawn from each patient varies greatly. This makes guaranteeing a volume for perspective collections difficult to determine since it is uncertain going in to the procedure.

Obtaining fresh, pre-treatment bone marrow samples is difficult. Bone marrow draws from patients suspected of having multiple myeloma are collected but do not have confirmation of disease and the accompanying documentation for several weeks. Viable, cryopreserved bone marrow mononuclear cells (BMMCs) can be used for research after definitive diagnosis of the disease.

Fresh pre-treatment blood and formalin fixed, paraffin embedded (FFPE) blocks from core biopsies are more easily obtained than fresh bone marrow. Blood can be drawn in the timeframe after diagnosis but prior to the beginning of treatment. Archival FFPE blocks are released years after confirmed diagnosis.

Blood and bone marrow are drawn more frequently when a patient is actively being treated to assess disease status and possible progression. While a patient is being actively treated, plasma cell counts will be minimal.

Section 3:

How Conversant Bio Can Help

Access a wide variety of donors from our cryopreserved inventory. Our hyper-annotated samples include information about patient demographics, medical history, and physical condition.

Match normal donors by age to compare to diseased samples. Our age matching capabilities ensure that you receive samples relevant to your target population.

Bone Marrow

Supply fresh marrow, BMMCs, and isolated CD34+ and CD138+ from normal and diseased bone marrow.

Deliver fresh normal bone marrow within 24 hours of collection and active-treatment multiple myeloma bone marrow within 48 hours of collection.

Blood Products

Supply fresh whole blood, PBMCs, plasma, serum, and isolated cells from normal and diseased blood samples.

Deliver fresh whole blood within 24 hours of collection.

Section 4:

Case Studies

Detection of circulating Multiple Myeloma Cells (CMMC) by flow cytometry is an indicator of active disease. In addition, circulating plasma cells can be detected in earlier stages of disease, including MGUS and Smoldering Multiple Myeloma, and appear to correlate with prognosis. The capture and characterization of these circulating plasma cells from peripheral blood may provide novel biomarkers for the management of Multiple Myeloma patients, particularly in monitoring minimal residual disease and in progression from MGUS or Smoldering Multiple Myeloma to active disease.

Case Study One: CTCs from Varying Treatment Statuses

In less than two years, Conversant Bio successfully collected blood from 160 unique multiple myeloma patients for a study of circulating multiple myeloma cells. The study participants represented patients from varying treatment statuses. We used our unique targeting methods to select patients for the study, and nearly 80% of these patients tested positive for circulating tumor cells (CTCs).

Case Study Two: Custom Collections and Processing

For this particular project, a large biotech firm needed Conversant Bio to collect matching sputum and bone marrow mononuclear cells from 20 MGUS patients and from 20 normal donors. Because of their project's specific needs, they needed the sputum collected in a specific kit that would accommodate DNA analysis. Additionally, the client asked us to isolate CD 138s from the BMMCs. Conversant Bio successfully completed this project in the allotted time frame designated by the customer.

Section 5:

Conversant Bio Company Information

Company Information

Take Your Research Further. Faster.

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