2023

Current Research Projects

Updated: November 4, 2023

Current IWMF Research Projects

IWMF grant funding for research projects has helped to provide an understanding of the basic biology and genetics of WM. This research in turn has played a significant role in the development of treatments and treatment guidelines in current use, as well as potential new drugs still in the pipeline. The goal of our research program is to improve quality of life for WM patients and, ultimately, discover a cure.

The Foundation has a rigorous process in place for all research grant proposals, which includes review by an independent committee composed of selected members of the IWMF Scientific Advisory Committee and other experts in the field. The awarding of research grants is ultimately subject to IWMF Board of Trustees approval. Researchers who receive grant awards must submit periodic progress reports, including a layman's summary, to the volunteer IWMF Research Committee for review and comment.

IWMF / LLS Strategic Research Roadmap Initiative

Because of exciting advances in our understanding of the biological basis of WM, the IWMF decided in 2014 to update its research strategy and enlist the cooperation of many of the major players in the WM research community. To this end, the IWMF partnered with the Leukemia & Lymphoma Society (LLS) to sponsor an annual Strategic Research Roadmap Summit, with the agenda is divided into five major topics:

Signaling – How do we find and block the pathways that WM cells use for communication?

Immunology/immunotherapy – How can we boost our immune system to fight WM?

Tumor microenvironment – How do we manipulate the bone marrow/tumor environment to kill WM cells?

"**Omics**" – What else can we learn about genomics, epigenomics, and mutations in WM cells that will improve the lives of WM'ers?

IgM Monoclonal Gammopathy of Undetermined Significance (MGUS) - How can understanding changes in the genome, transcriptome, and epigenome that accompany IgM MGUS progression to WM identify patients at risk of progression and interventions that may prevent or suppress progression?

All research projects that are funded by the Strategic Research Roadmap Initiative are marked accordingly.

CRISPR-BASED FUNCTIONAL CHARACTERIZATION OF WM CELLS: INSIGHTS INTO THERAPEUTIC VULNERABILITIES AND STRATEGIES TO OVERCOME RESISTANCE

Project Period 10/01/19 – 11/01/23	**Investigator**: Constantine Mitsiades, MD, PHD
$400,000 over four years	**Institution**: Dana-Farber Cancer Institute, Boston, USA

This project falls under the IWMF-LLS Strategic Research Roadmap Initiative. The research takes advantage of new technologies, including the gene editing tool CRISPR, improved and powerful computational approaches, and innovative new mouse models. The researchers will conduct a broad, genome-wide search to identify specific genes that are required to allow Waldenström's macroglobulinemia (WM) cells to thrive. Additionally, the researchers will attempt to identify genes that allow WM cells to resist established therapies. The key is to identify specific gene targets that cause death of WM cells, but do not alter normal body cells. Any genes identified will be further tested in laboratory cells and then evaluated in mouse models. This research will hopefully identify new, previously unsuspected molecular targets for WM therapy.

FACTORS REGULATING IMMUNOGLOBULIN-PRODUCING B-CELLS IN PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA – PART VII

Project Period 09/01/23 – 08/01/25	**Investigator**: Stephen Ansell, MD, PhD
$525,807.00 over two years	**Institution**: Mayo Clinic, Rochester, MN, USA

WM cells live primarily in bone marrow. The bone marrow is not merely a hollow cavity in which WM cells grow. Instead, bone marrow is a complex environment with many cell types. Collectively, the bone marrow forms a hospitable place for WM cells to survive, grow, and secrete IgM. Dr. Ansell thinks there may be a way to change the bone marrow, to make it less hospitable to WM cells. The bone marrow of WM patients differs from normal bone marrow, making it an even better place for survival and growth of WM cells. Dr. Ansell and his group hypothesize that one feature that makes WM patients' bone marrow such a good place for WM cells is that in the bone marrow, WM cells are protected from the body's normal immune system. In previous IWMF-funded research work, Dr. Ansell's group found specialized cells in the bone marrow of WM patients that prevent the body's normal immune system from killing WM cells. If these specialized cells, called myeloid-derived suppressor cells (abbreviated MDSCs), could be inhibited with appropriate drugs, perhaps the body's immune system would be free to better attack the WM cells in the bone marrow. Moreover, the MDSCs may not only suppress immune killing of WM cells, but may also directly send positive growth signals to the WM cells. Drug therapy in the future could be a two-pronged, combining drugs such as ibrutinib or rituximab to kill WM cells, together with drugs that inhibit MDSCs to make the bone marrow environment less hospitable to WM and allow the body's immune system to attack the WM cells. This project falls under the IWMF-LLS Strategic Research Roadmap Initiative.

TARGETING MYD88 SIGNALING IN WALDENSTROM'S MACROGLOBULINEMIA

Project Period 07/01/23 – 06/30/25	**Investigator**: Principal Investigator Steven Treon, MD, PhD
$600,000 over three years	**Institution**: Dana-Farber Cancer Institute, Boston, MA, USA
Sponsored in part by:	**David and Janet Bingham Research Fund of the IWMF** **Yang Family Research Fund of the IWMF** **Robert and Nadeline White Family Research Fund of the IWMF**

This is a continuation of previous projects proposed by Dr. Treon and funded by the IWMF. In previous research partially funded by the IWMF, Dr. Treon and his team discovered the highly recurring mutation in the MYD88 gene that occurs in more than 90% of WM patients and showed that mutated MYD88 promoted growth and proliferation of WM cells through the downstream signaling pathways BTK and IRAK1/IRAK4. These findings enabled the pivotal clinical trial that led to approval of the BTK inhibitor ibrutinib (Imbruvica) for the treatment of WM in the US, Europe, and Canada. Resistance to ibrutinib is an emerging problem in WM patients, and Dr. Treon's team has identified mutations in BTK that disrupt ibrutinib-BTK binding in samples from half of WM patients whose disease progressed on ibrutinib. His group has sought novel strategies to overcome the most common type of BTK mutation-related ibrutinib resistance in WM. His group is also working on uncovering the importance of other MYD88 downstream signaling pathways, including HCK, which triggers AKT, ERK1/2, and BTK itself. For this project, Dr. Treon has three principal Aims: 1) to delineate the importance of IRAK signaling to ibrutinib resistance and develop selective IRAK inhibitors based on this work, 2) to clarify whether HCK inhibition can suppress mutated BTK-acquired ibrutinib resistance in WM and develop selective HCK inhibitors, and 3) and to validate these inhibitors alone and in combination using animal models for future translation to clinical trials.

MYD88L265P SIGNALING-ASSOCIATED MULTIPLEX CHARACTERIZATION OF THE BONE MARROW MICROENVIRONMENT IN WM PATIENTS FOR CLINICAL APPLICATION

Project Period 11/01/20 – 11/12/23	**Investigator**: Ruben Carrasco, MD, PhD
$400,000 over three years	**Institution**: Dana -Farber Cancer Institute, Boston, Ma, USA

This project falls under the IWMF-LLS Strategic Research Roadmap Initiative. Dr. Carrasco will harness new and powerful digital pathology and artificial intelligence technology for better diagnostics and understanding of WM using two approaches. (1) More than 90% of WM patients have a mutation of the MYD88 gene, resulting in an altered protein, called MYD88 L265P. The altered MYD88 drives abnormal signaling which is key to survival and growth of WM cells. Detection of the MYD88 mutation by PCR is one of the cornerstones for making the diagnosis of WM. Dr. Carrasco has detected a feature of the mutant MYD88 protein, in which it forms microscopic aggregates inside cells. He proposes to detect these aggregates of mutant MYD88 protein with a technique called immunohistochemistry to speed up and simplify the diagnosis of WM. (2) WM cells live primarily in bone marrow, where they interact with other bone marrow cells. Some of the bone marrow cells help maintain a favorable environment for the WM cells to grow, while other bone marrow cells attack the WM cells and prevent excessive growth. Dr. Carrasco's group, in collaboration with Drs. Treon, Hunter, and other state-of-the-art labs at Harvard, will examine bone marrow samples using automated digital pathology to identify WM cells and the specific cell types in their immediate proximity which may be interacting with the WM cells. He hypothesizes that the local interactions of WM cells with other bone marrow cells changes during disease progression and if disease resistance develops. If scientists could understand these local interactions between cells better, it may be possible to treat patients with drugs to make the bone marrow less supportive of WM cell growth or to enhance immune attack on the WM cells.

GENOMIC AND IMMUNE BIOMARKERS OF PROGRESSION FROM IGM MGUS TO WALDENSTROM'S MACROGLOBULINEMIA

Project Period 06/01/21 – 12/31/23	**Investigator**: Ramonas Sklavenitis-Pistofidis, MD
$157,500 over two years	**Institution**: Dana -Farber Cancer Institute, Boston, Ma, USA

A common benign precursor to WM is called IgM-MGUS (monoclonal gammopathy of undetermined significance). Of all the people with IgM-MGUS, a small proportion will progress to WM. Many patients with WM are first detected before they show significant disease symptoms. The disease at this stage is called "asymptomatic WM" or "smoldering WM." The patients are monitored without being treated, a strategy called "watch-and-wait." Some IgM-MGUS or watch-and-wait WM patients never progress to symptomatic WM or only progress after many years. Others progress more rapidly to active, symptomatic disease that requires treatment. Dr. Sklavenitis-Pistofidis's project is designed to more accurately predict which patients will progress to active disease. He will build upon a predictive model that his group published earlier, which used clinical biomarkers such as the degree of bone marrow infiltration and the level of abnormal serum IgM. In the present proposal, Dr. Sklavenitis-Pistofidis will use advanced technologies to characterize patients' tumor cells and immune cells. He will then incorporate genomic and immune information into an improved, next-generation predictive model. The model could be used as the foundation for a clinical trial to test whether the patients predicted to progress rapidly would benefit from earlier drug treatment. This is a Kyle Young Investigator Award project.

ISOLATION AND SPATIAL CHARACTERIZATION OF 6Q DELETIONS AND CXCR4 MUTATIONS USING NOVEL BIOMARKERS IN WM

Project Period 07/01/21 – 07/01/23	**Investigator**: Maria Luisa Guerrera, MD
$157,500 over two years	**Institution**: Dana -Farber Cancer Institute, Boston, Ma, USA

Cell division isn't a perfect process. When an original cell duplicates its DNA in order to divide into two new cells, it sometimes makes mistakes. Some of these mistakes, called mutations, can be passed on to the next generation of cells. As cells with the mutation continue to divide and make new cells, eventually, there are a large number of cells that share the same mutation. These are called clones. But cells in a clone keep dividing. Eventually, one of the cells in a clone makes a mistake in a different area of DNA and develops a new mutation. Soon, some of the cells from the original clone—but not all of them—have the new mutation in addition to the original mutation. This is called a subclone. The existence of different subclones makes WM complicated. In most WM patients, the MYD88 gene is mutated. However, if a scientist analyzes every one of the WM cells in a patient's body, they may find some WM cells that also have a second mutation, such as a mutation in a particular part of the CXCR4 gene. In some patients, there are subclones of different CXCR4 mutations. Other MYD88 subclones may have other DNA modifications, such as deletions of a certain area of DNA called "6q" which contains a large number of genes. Dr. Guerrera's project is to study subclones in WM patients. If a patient has multiple subclones, each with different mutations or other DNA alterations, how does that affect the disease? Does one subclone of WM cells influence the growth of another separate subclone of WM cells? How can different subclones be separately identified in the best way, when they are all mixed together in the same patient's body? One of Dr. Guerrera's goals is to identify new biomarkers that laboratories can use to identify and isolate different subclones within a patient. She will also investigate treatment—if a drug kills WM cells from one subclone, what about the other subclones? This work may lead to combination treatments, tailored to individual patients and intended to kill multiple subclones at the same time. This is a Kyle Young Investigator Award project.

HARNESSING EPIGENETIC SIGNATURES FOR NEW INSIGHT INTO THE MECHANISMS AND CLASSIFICATION OF WALDENSTOM MACROGLOBULINEMIA

Project Period 08/01/21 – 08/01/23	**Investigator**: Christopher Oakes, Ph.D.
$400,000 over two years	**Institution**: The Ohio State University / James Comprehensive Cancer Center

DNA contains a specific code. When the code is read, it enables cells to make proteins. However, all the DNA in a cell is not read at any one time. Modifications—called "epigenetics"—regulate which parts of the DNA are available to the cell and which parts are not. Epigenetic modifications can make regions of DNA "open," meaning that the genes in those regions are available to encode proteins for the cell, while other regions of DNA are "closed," meaning that the genes are unavailable to the cell. Cancer cells often have unique patterns of epigenetic changes. This can cause cancer cells to express many proteins in an abnormal way, which can drive the cells to divide and grow more than they should. Dr. Oakes previously discovered two types of epigenetic changes in WM cells. Some WM patients had WM cells with a epigenetic pattern resembling a normal cell type called a plasma cell. Other WM patients had WM cells with a pattern resembling another normal cell type, called the memory B cell. In the present grant, Dr. Oakes will dig more deeply into understanding the epigenetic changes of WM cells, including the two types he previously identified. He will also try to better understand exactly what leads to the characteristic epigenetic changes seen in WM cells. It is well known that most WM patients have a mutated (altered) protein called MYD88. It is possible that specific signals from the abnormal MYD88 cause the epigenetic changes of WM cells. If scientists could better understand the epigenetic changes that regulate WM cells, it may be possible to use epigenetics-modifying drugs to restore normal regulation and control WM. This project falls under the IWMF-LLS Strategic Research Roadmap Initiative.

CHARACTERIZATION OF GENOMIC ALTERATIONS IN TREATMENT NAIVE PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA THROUGH A COURSE OF TARGETED TREATMENT AND DISEASE PROGRESSION

Project Period 09/01/22 – 08/31/24	**Investigator**: Dr. Signy Chow
$157,700 over two years	**Institution**: Sunnybrook Research Institute
Sponsored in part by:	**Waldenstrom's Macroglobulinemia Foundation of Canada** **The Poh Family Research Fund of the IWMF**

Recently, the IWMF established the Robert A. Kyle Career Development Award to encourage and fund research from young investigators, with the goal of generating the next generation of WM researchers. Signy Chow, MD is a staff hematologist at the Odette Cancer Centre, Sunnybrook Health Sciences, at the University of Toronto, Ontario, Canada with considerable experience in multiple myeloma and its underlying DNA abnormalities. Here, she is applying her scientific skills in DNA analysis (called genomics) to WM. Specifically, she is examining how the genes of WM cells change, starting with patients before they have ever been treated, and then during treatment. By sampling the same people repeatedly during the course of a 1 ½-year period, she can trace the genetic changes in patients who do not respond well to drug treatment or who become resistant to drug treatment, in comparison with those who respond well. This analysis can identify if there are new mutations arising during the course of treatment. Another genomic change that sometimes occurs in cancers is duplication of genes, including even genes without mutations. Increased copies of particular genes that may drive cell proliferation is a key feature of some cancer cells that would not show up if analysis were confined only to looking for mutations. She will also test techniques to obtain WM DNA directly in blood samples ("cell-free DNA") so that bone marrow biopsies would not be needed for DNA analysis. To do this work, her study is linked to an ongoing multicenter clinical trial in Canada, the BRAWM trial, in which

previously untreated patients with WM are treated with Bendamustine and rituximab, in combination with the BTK inhibitor acalabrutinib. The goal of her project is better understanding of why patients respond or do not respond to treatment and why certain patients become resistant to the drugs they are receiving. With this knowledge, better therapies can be targeted to individual patients. This is a Kyle Young Investigator Award project.

A MULTI-OMICS APPROACH FOR DECIPHERING THE MECHANISMS OF PROGRESSION IN PREMALIGNANT IGM GAMMOPATHIES: NEW INSIGHTS FROM THE FIL "BIO-WM" TRIAL

Project Period 01/15/23 – 01/14/25	**Investigator**: Dr. Simone Ferrero
$157,700 over two years	**Institution**: Fondazione Italiana Linfomi Onlus

Recently, the IWMF established the Robert A. Kyle Career Development Award to encourage and fund research from young investigators, with the goal of generating the next generation of WM researchers. Simone Ferrero, MD, is an Assistant Professor of Hematology at the University of Torino in Italy. In this project, he will investigate the progression to symptomatic WM. To do this, he will leverage samples already collected in the joint Italian-Spanish FIL BIO-WM clinical trial, in which several hundred people with IgM-Monoclonal Gammopathy of Uncertain Significance (IgM-MGUS), a common, non-neoplastic disorder, or smoldering WM (watch-and-wait WM) were repeatedly sampled over time to see what changes occurred in their cells and how those changes correlated with progression to overt WM. While some gene mutations such as MYD88 and CXCR4 have already been characterized, Dr Ferrero will investigate other possible gene mutations. He will also evaluate RNA markers that might be diagnosed from blood samples, which would reduce the need for bone marrow biopsies. The third part of the project is to study clonal hematopoiesis of indeterminate potential (CHIP), a common occurrence in older people, in which non-cancerous cells from the bone marrow harmlessly accumulate in the body. Dr Ferrero will investigate genetic changes in these cell populations that might tip them over the edge to become harmful. By making good use of the large number of patient samples that have already been collected from the FIL BIO-WM study, Dr Ferrero's project may provide new insights into the underlying molecular changes that initially lead to development of WM. This could open new routes of therapy. This is a Kyle Young Investigator Award project.

SINGLE-CELL MULTIOMICS FOR MINIMALLY INVASIVE ASSESSMENT OF TREATMENT EFFICACY IN WALDENSTROM'S MACROGLOBULINEMIA

Project Period 09/01/22 – 08/31/23	**Investigator**: Bruno Paiva
$90,000 over one year	**Institution**: Clinica Universidad de Navarra

In an effort to encourage creative, innovative thinking, the IWMF initiated a new grant called the "Research Seed Money Initiative Grant." This is a one-year grant that allows a scientist to test a new idea. If the project is successful, the scientist is then able to use the results to support a larger, multi-year grant to do a more in-depth investigation. Bruno Paiva, PhD, at the Clinical University of Navarra, in Pamplona, Spain, is a widely published scientist with experience in WM. Currently, he is the Director of the Flow Cytometry Core laboratory and Co-Director of the Monoclonal Gammopathies research laboratory. In this project, he will evaluate whether it is possible to use ultra-sensitive techniques to obtain useful information about WM from blood samples. His laboratory has already found new markers, proteins on the outside of cells that enable researchers to identify the cell. He will test cells from more WM patients to see if his earlier findings were correct. Then, he will combine two existing technologies, called immunomagnetic enrichment and flow cytometry, into a technique that can be performed on a single blood sample from WM patients. He has already adapted this technique to patients with multiple myeloma and shown that it is extraordinarily sensitive at detecting even small numbers of tumor cells in the blood. If successful with WM, this may mean that patients could avoid bone marrow biopsies and that doctors could follow the disease course more accurately and thoroughly, without significant discomfort to the patient. This is a Research Seed Money Initiative project.

ANALYSIS OF MOLECULAR AND MICROENVIRONMENTAL LANDSCAPE AND ITS ROLE IN DRUG RESISTANCE IN WALDENSTRÖM MACROGLOBULINEMIA

Project Period 01/02/23 – 01/01/24	**Investigator**: Damien Roos-Weil, MD, PhD
$90,000 over one year	**Institution**: French National Institute for Health and Medical Research

In an effort to encourage creative, innovative thinking, the IWMF initiated a new grant called the "Research Seed Money Initiative Grant." This is a one-year grant that allows a scientist to test a new idea. If the project is successful, the scientist is then able to use the results to support a larger, multi-year grant to do a more in-depth investigation. Damien Roos-Weil, MD, PhD is a clinical professor of hematology at the Pitié-Salpêtrière Hospital in Paris, France who has published some novel and highly impactful WM research recently. WM cells mainly live in the bone marrow. An emerging theme in WM research is that the bone marrow is not just a location where WM cells happen to grow. Instead, different kinds of normal bone marrow cells associate and communicate with WM cells, forming a complex interaction that is necessary to allow WM cells to live and grow in the bone marrow. Scientists are just beginning to unravel how different types of normal bone marrow cells encourage the growth of WM cells and what types of signals they use to do so. Collectively, this complicated relationship is called the tumor microenvironment. In this project, Dr Roos-Weil proposes to use bone marrow samples from several hundred WM patients from an on-going multinational European clinical trial and to apply new technologies to better understand the tumor microenvironment that allows WM cells to grow. Drugs targeted against specific signaling pathways or cell types in the bone marrow present an opportunity for better combination WM drug therapy in the future.

TARGETING AN IRF4/CXCR4 AXIS TO REVERSE DRUG RESISTANCE IN WALDENSTROM'S MACROGLOBULINEMIA

Project Period 10/01/22 – 09/30/23	**Investigator**: Dr. Leslie Crews
$90,000 over one year	**Institution**: The Regents of the University of California, San Diego

In an effort to encourage creative, innovative thinking, the IWMF initiated a new grant called the "Research Seed Money Initiative Grant." This is a one-year grant that allows a scientist to test a new idea. If the project is successful, the scientist is then able to use the results to support a larger, multi-year grant to do a more in-depth investigation. Leslie Crews, PhD, is an Assistant Professor of medicine at the University of California, San Diego with an interest in stem cell biology and blood cancers. Dr Crews hypothesizes that abnormal expression inside cells of a regulatory protein called IRF4 (interferon regulatory factor-4) is involved in WM. IRF4 is a key protein that drives early B cells to develop into more mature B cells and plasma cells. When expressed in excessive amounts, IRF4 is important in cell survival in multiple myeloma, a related type of blood cancer. If multiple myeloma cells express large quantities of IRF4, they can become drug-resistant. Moreover, high levels of IRF4 can increase expression of CXCR4, a key protein in many people with WM. Dr Crews will try to bridge these findings in multiple myeloma to WM, by asking if IRF4 is involved in WM cell proliferation, drug resistance, and CXCR4 over-expression. Importantly, if she shows that IRF4 is involved in WM, her findings could quickly translate from the laboratory to human clinical trials. A drug targeting IRF4 has been developed and is currently in clinical trials for multiple myeloma. If Dr Crews Seed Money project successfully shows that IRF4 is involved with WM, she will test the effects of the new IRF4 drug in a mouse WM model. Positive findings could lead to clinical trials of this novel drug in WM patients.

ANALYSIS OF THE CHROMATIN ACCESSIBILTY LANDSCAPE AND REGULATORY NETWORKS OF IGM MONOCLONAL GAMMOPATHIES: TOWARDS A BETTER UNDERDTANDING OF PROGRESSION MECHANISMS

Project Period 10/ 26/22 – 10/25/23	**Investigator**: David Fernando Moreno Fajardo, MD
$79,140 over one year	**Institution**: Fundació Clínic per a la Recerca Biomèdica

In an effort to encourage creative, innovative thinking, the IWMF initiated a new grant called the "Research Seed Money Initiative Grant." This is a one-year grant that allows a scientist to test a new idea. If the project is successful, the scientist is then able to use the results to support a larger, multi-year grant to do a more in-depth investigation. While many impactful studies have been performed to identify mutations in key genes that lead to WM, there has been less attention to underlying gene regulation. David Moreno, MD, is a hematologist at the Clinical and Provincial Hospital of Barcelona in Spain. In this project, Dr Moreno will use new, state-of-the-art technologies to investigate what determines whether the expression level of genes is increased or decreased. Cells use regulatory proteins, which attach to DNA, to determine when genes should be active or expressed. However, DNA is arranged in a tightly packed conformation, and only certain areas of the DNA are exposed to the regulatory proteins. This 3-dimensional packing varies from one cell type to another, resulting in different types of cell regulation, depending on the cell. Specifically, using B cells and plasma cells, Dr Moreno will examine long stretches of DNA and determine where the DNA is exposed and accessible to regulatory proteins. These techniques will be applied to cells from people with IgM MGUS (a precursor to WM), smoldering WM (also called asymptomatic or watch-and-wait WM), and symptomatic WM. Results will be analyzed by computer to better understand the network of abnormal gene expression associated with progression to WM. Better understanding of why cells progress from a benign precursor condition, IgM MGUS, to symptomatic WM may reveal new therapeutic targets for treatment.

USING MUTOGRAPHS TO DEFINE THE MOLECULAR LANDSCAPE AND CELL OF ORIGIN OF WALDENSTROM'S MACROGLOBULINEMIA

Project Period 01/01/23 – 01/01/2025	**Investigator**: Dr. Gareth Morgan
$400,000 over two years	**Institution**: New York University Grossman School of Medicine
Sponsored in part by:	**Leukaemia Foundation of Australia** **Elting Family Research Fund of the IWMF**

While substantial attention has been given to mutations of DNA that encode genes such as the well-known MYD88 or CXCR4, less work has been done on non-coding genome sequences, the sections of DNA located in-between genes. These non-coding sequences are often involved in gene regulation. Dr Gareth Morgan is a well-established Professor at the New York University Grossman School of Medicine with considerable experience in understanding the genetic basis of multiple myeloma. He has accumulated a large set of DNA sequence data from WM patients, through multiple collaborations with other WM researchers. He will use this data to look for variations in the non-coding regions. He will combine these results with another test that can determine which areas of DNA are physically open and available to the cell machinery and which areas of DNA are covered up and inaccessible. He will also use the DNA information to trace the multiple routes by which WM cells developed in the body. Most gene mutations are harmless and do not cause cancer or other illnesses. As a person gets older, these harmless "passenger mutations" accumulate as cells divide and form new cells, leaving an indelible history in the cells. These mutational patterns are called "mutographs". Dr. Morgan will use computer systems that he and his group originally developed for the study of related cancers such as multiple myeloma. With these computer tools, he can assemble and trace cells' mutographs to reveal alternative pathways by which normal cells developed into WM cells. It is increasingly obvious that there is no

one treatment that will work on all people with WM. Better understanding of the different types of WM, together with understanding the routes by which normal cells develop into WM, will hopefully lead to more precise, individualized WM treatments. This project falls under the IWMF-LLS Strategic Research Roadmap Initiative.

DEFINING THE PROGNOSTIC SIGNIFICANCE OF TP53 ALTERATIONS IN WALDENSTROM EXPLOITING THEM FOR THERAPEUTIC BENEFIT

Project Period 06/01/23 – 06/01/25	**Investigator**: Jithma Prasad Abeykoon, M.D.
$157,000 over two years	**Institution**: Mayo Clinic

Research work in the past 15 years has enabled scientists to understand key gene mutations in WM, including genes called MYD88 and CXCR4. More recently, mutations in another gene, called TP53, have been identified in WM patients. The prevalence of this mutation in WM has not yet been definitively determined, due to few studies that included small numbers of patients. TP53, called the 'guardian of the genome', is essential because it prevents cancer. Usually, TP53 can tell when cells have damaged DNA or other genomic aberrations, stopping those abnormal cells from growing further and causing cancer. If the TP53 gene is mutated in certain key places, then TP53 can no longer prevent cancer or survey the genome of the cell for errors. With the loss of TP53, a critical control is removed, and abnormal cells can multiply quickly and form cancers. With some types of cancer, if there is loss of TP53, the cancer is more aggressive and can resist standard treatment. With this two-year Robert A. Kyle Career Development Award, Dr. Abeykoon, a talented and productive Assistant Professor of Hematology and Medical Oncology at the Mayo Clinic, will test cells from a large number of WM patients to better understand how common TP53 mutations are, whether TP53 mutations are associated with more aggressive disease, and how TP53 mutations affect response to the standard treatments used in WM. In the second part of the project, Dr. Abeykoon will investigate a new form of therapy for WM patients with TP53 mutations. Using new genetic information and computer tools developed at the Broad Institute at MIT and Harvard, Dr. Abeykoon identified a potential weakness in cells that have mutated TP53. If WM cells lose their normal TP53 function as 'guardian of the genome', they are more prone to proliferate rapidly and to have damaged or broken DNA. The WM cells with broken DNA may need to rely more on DNA damage repair mechanisms in order to survive. Dr. Abeykoon hypothesized that this increased reliance on DNA damage repair mechanisms is a critical weakness, making the WM cells with mutated TP53 especially sensitive to drugs that target DNA damage repair pathways. If the WM cells cannot faithfully repair their broken DNA, they will likely die. In this project, Dr. Abeykoon will test WM cells in the laboratory and in mice to see if drugs targeting DNA damage repair pathways could be used in the future to treat the subset of WM patients with TP53 mutations.

GENOMIC CHARACTERIZATION OF IBRUTINIB-RESISTANT WM

Project Period 07/01/23 – 07/01/2025	**Investigator**: Dr. Tina Bagratuni and Dr. Meletios Dimopoulos
$400,000 over two years	**Institution**: National and Kapodistrian University of Athens

A major dilemma in treating WM patients is the eventual development of resistance to therapy. After initially responding well to ibrutinib treatment, a substantial number of patients eventually develop ibrutinib resistance. Underlying the development of resistance is the ability of the initial population of WM cells in the body to develop or evolve in a number of different directions. As the initial population of WM cells grow, one WM cell might develop an additional mutation. If this mutation allows the cell to grow faster or to avoid the immune system, it will produce daughter cells more rapidly than the other WM cells. Eventually, the descendants of that cell, called a "clone" may become a large proportion of the total WM cells in a patient's body. Meanwhile, another WM cell might develop another mutation. The descendants of the cell with this new mutation may develop another clone in the body. WM researchers have become increasingly aware that individual WM patients often harbor multiple different clones of WM cells, each with somewhat different characteristics. To

unravel this complexity and be able to study multiple clones within the same patient, Dr. Bagratuni, a Senior Researcher at the School of Medicine at the National and Kapodistrian University of Athens, and her team will take a large number of WM cells from each WM patient and analyze the cells one by one, in what is called single-cell analysis. They will compare results from before treatment with results after ibrutinib therapy. They hypothesize that as patients are treated with ibrutinib, multiple clones arise, each with perhaps different ways to avoid being killed by ibrutinib. With this type of specific, cell-by-cell analysis, the investigators hope to better understand the development of ibrutinib resistance and perhaps uncover new therapeutic targets for drug treatment.

IMPACT OF MYD88 AND CXCR4 MUTATION ON AGE ASSOCIATED B CELLS AT STEADY STATE AND IN THE COURSE OF WALDENSTROM'S MACROGLOBULINEMIA

Project Period 07/01/23 – 07/01/2025	**Investigator**: Dr. Marion Espeli
$90,000 over two years	**Institution**: L'Institut National de la Sante et de la Recherche Medicale

IWMF Research Seed Money Initiative Grants provide one year of funding to allow scientists to test a new idea. If the project is successful, the scientist is then able to use the results to support a larger, multi-year grant to do a more in-depth investigation. Using a newly developed mouse model of WM which combines a MYD88 mutation and a CXCR4 mutation, Dr. Espéli, a senior scientist at the Institut de Recherche Saint Louis in France, observed an unusual subset of B cells that accumulated before the onset of WM disease. These B cells were seen in mice that had both the MYD88 and CXCR4 mutations, like many WM patients. This cell subset has the ability to become IgM-secreting cells, so this raises the possibility that they could be important in WM. In this Seed Money project, Dr. Espéli and her team will investigate the significance of this B cell population, its role in WM development, and how these cells are affected by drug treatment.

IDENTIFYING THE ONCOGENIC COOPERATION BETWEEN IRF4 AND MYD88 AND THEIR IMPACKT ON THE TUMOR MICROENVIRONMENT OF WALDENSTROM MACROGLOBULINEMIA.

Project Period 08/21/23 – 08/20/2025	**Investigator**: Patrizia Mondello, M.D. Ph.D.
$480,000 over two years	**Institution**: Mayo Clinic
Sponsored in part by:	**Waldenstrom's Macroglobulinemia Foundation of Canada**

It is increasingly recognized that WM is not one disease. New research findings from several groups point to the existence of several subtypes. In this project, Dr. Patrizia Mondello, an Assistant Professor at Mayo Clinic, will investigate how heritable, pre-existing mutations in a gene called IRF4 may predispose patients to develop one of the subtypes of WM, called plasma cell-like WM. She hypothesizes that pre-existing mutations in IRF4 work together with the acquired mutation in MYD88 gene to drive growth of WM tumor cells and also to reduce the body's immune response to WM cells. This combination of two mutations that work together is called "oncogenic cooperation." Dr Mondello and her team will use genetically-engineered WM cell lines and mouse models that combine either too much IRF4 or too little IRF4, together with the MYD88 mutation. They will examine the effects of these mutations on the growth of WM and the immune response to WM in the mice. They will then study the specific ways in which different immune cells and their signals are altered, making the bone marrow environment more hospitable to growth of the WM cells. Importantly, they will ask if their findings in mice apply to human WM patients. This will be done by examining cells from a large number of WM patients who have donated their bone marrow cells for research projects at Mayo Clinic. In collaboration with Dr. Zachary Hunter, these findings will be validated in an independent group of WM patients from the Dana-Farber Cancer Institute. In future studies, if IRF4 is confirmed as a critical factor for progression to WM, the investigators propose to introduce a genetic screening test of IRF4 for IgM MGUS patients, to help identify patients at risk of developing WM. They also propose to work together with pharmaceutical companies to

evaluate drugs targeting IRF4 in their mouse models, with the ultimate goal to identify a novel and more effective therapeutic approach for WM patients.

STUDY OF IMMUNE MICROENVIRONMENT AND BCR SIGNALLING IN WM-LIKE MOUSE MODEL

Project Period 09/01/23 – 08/31/2025	**Investigator**: Dr. Christelle Vincent-Fabert
$157,000 over two years	**Institution**: Laboratoire CRIBL UMR CNRS 7276 / INSERM 1262

A challenge that WM researchers have faced in testing new therapies is the lack of good animal models. Dr Vincent-Fabert, an innovative WM researcher at the University of Limoges In France, has been working to create genetically engineered mice that develop WM-like disease, as tools for WM researchers. With this two-year Robert A. Kyle Career Development Award, she will use the mice that she and her collaborators have created to investigate how WM cells interact with the immune system. Normally, the body's immune system controls the growth of many types of cancer cells. In WM, the tumor cells have developed ways to escape from immune control, enabling them to grow in the bone marrow environment. Scientists and pharmaceutical companies are discovering and developing many different drugs to prevent tumor cells from escaping the immune system. The exact drugs that are used in any particular type of cancer depend on understanding the specific mechanisms in that type of cancer. Using her mice that develop WM-like disease, Dr. Vincent-Fabert will study the tumor immune microenvironment and then test a variety of drugs to see if they alter the immune response to WM and help control disease. In the second part of her project, Dr Vincent-Fabert will focus on a hallmark of WM, the production of excessive IgM in the blood and on the surface of WM cells. She wants to understand why WM cells produce so much IgM, and not other related proteins, such as IgG. If IgM is so important, she hypothesizes that a role of IgM on the surface of WM cells is to send signals to the inside of the cell. These signals could enable the WM cells to grow and to avoid the immune system. Better understanding of the pathways involved in this signaling could lead to selection of specifically targeted drugs in the future.

CHARACTERIZING THE ROLE OF THE ERK1/2 REGULATOR WNK2 AS A NOVEL TARGET IN THE DISEASE PROGRESSION OF MYD88 MUTATED WM

Project Period 07/01/23 – 07/01/25	**Investigator**: Maria Luisa Guerrera, MD
$157,000 over two years	**Institution**: Dana-Farber Cancer Institute

Tumor suppressors are proteins that act by preventing cells in the body from becoming cancerous. A mutation in a key tumor suppressor can "remove the brakes" and allow small, early cancers to progress. Dr. Guerrera, a creative and innovative Instructor in Medicine at Harvard Medical School and Dana-Farber Cancer Institute, has been working in the laboratory of Dr. Steven Treon. In a previous 2-year Robert A. Kyle Career Development Award, she brought attention to a previously little-known tumor suppressor protein called WNK2 that may be important in controlling WM. With this renewal of her Kyle Award, she will continue to investigate the role of WNK2 in WM. WNK2 abnormalities are surprisingly common in WM. Among WM patients, there are a number of different ways that normal WNK2 can go awry. In some WM patients, the WNK2 gene is improperly regulated, and there is too little WNK2 available. This may remove a key restraint and allow WM cells to grow. In other WM patients, however, there is plenty of WNK2, but it is improperly formed, so it doesn't function properly. Incorrectly formed WNK2 can also result in too much WM cell growth. Dr. Guerrera's project is to better understand the many different ways WM cells improperly regulate WNK2 and how WNK2 improper regulation can change the WM cells' behavior in the body and impact their growth. Her long-term goal is to develop drugs to correct these defects, restore the ability of WNK2 to suppress WM, and prevent WM from progressing.

CHARACTERIZATION OF ISOFORM USAGE, NOVEL ISOFORMS, AND TUMOR EVOLUTION IN WALDENSTROM'S MACROGLOUBULINEMIA

Project Period 07/01/23 – 07/01/25	**Investigator**: Zachary Hunter, PhD
$480,000 over two years	**Institution**: Dana-Farber Cancer Institute
Sponsored in part by:	**Waldenstrom's Macroglobulinemia Foundation of Canada**

In previous, groundbreaking work, Dr. Zachary Hunter and his team at Dana-Farber Cancer institute have analyzed a large number of genes from WM cells and found a complex variety of abnormalities. Normally, cellular DNA (a gene) is copied into a molecular messenger, called mRNA, which encodes proteins that a cell needs. But mRNA is not an exact copy of a DNA. First, the DNA is copied into a large RNA strand called a transcript. Then specific pieces of the large RNA transcript are cut out and spliced together to create whatever mRNA the cell needs. Dr. Hunter' group discovered that many of the mRNA's made in WM are improperly assembled, due to incorrect splicing. These RNA's are called "alternative isoforms." Sometimes, cells interpret the alternative isoform as gibberish and fail to make a required protein. In other cases, an alternative isoform, created by improper splicing, results in an mRNA that encodes a new protein different from the original one. In some cases, the new protein can be harmful. The discovery of so many alternative isoforms in WM cells led Dr. Hunter to ask what the underlying problem is. Why are WM cells prone to making splicing mistakes and improperly assembled RNAs? Is there a way to correct the problem? Additionally, the pattern of alternative isoforms in different kinds of WM cells may be important to know. Dr. Hunter's team has already identified different subtypes of WM, and now they are finding evidence that each subtype has its own pattern of alternative isoforms. By understanding the abnormal proteins that the alternative isoforms encode and how these abnormal proteins interact with each other, it may be possible to identify new targets for novel WM drugs.

WARDS A RATIONAL TARGETED COMBINATION THERAPY FOR WALDENSTRÖM'S MACROGLOBULINEMIA BY VENETOCLAX SENSITIZER CRISPR SCREENS

Project Period	**Investigator**: Marcel Spaargaren, PhD
$476,000 over two years	**Institution**: Amsterdam UMC, University of Amsterdam

Dr. Spaargaren and his team at the Amsterdam UMC, University of Amsterdam, have been working for a number of years to discover what they call the "Achilles heels" of B cell malignancies, including WM, the weak points that might be exploited by a targeted drug. They try to identify particular proteins which, if inhibited, could significantly keep the cancer cell from growing, avoiding immune cells, or resisting drug therapy. Using a screening technique they developed in their lab, this approach has been successful in a previously IWMF-funded project in identifying several proteins that could be new drug targets for WM. Some of the proteins they found are important for enabling WM cells to attach and live in the bone marrow. Others are important for enabling WM cells to resist being killed by ibrutinib treatment. With this knowledge, researchers and physicians can develop better combinations of drugs to avoid ibrutinib resistance. In the present project, Dr. Spaargaren and his team are focusing on the drug venetoclax. Venetoclax is emerging as a useful drug which reduces the survival of WM cells. A number of clinical trials are in progress. Not all WM patients respond to venetoclax, however, and sometimes, patients respond initially but then become venetoclax-resistant later. Dr. Spaargaren and his team will work on discovering "sensitizers," drugs that can be given in combination with venetoclax to help improve how well venetoclax works for WM patients.