2024

Current Research Projects

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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

The IWMF partnered with the Leukemia & Lymphoma Society (LLS) in 2015 to sponsor an annual Strategic Research Roadmap Summit to help determine the specific areas in which additional research on the biological basis of WM is needed. From this collaboration of the most important global researchers in WM, the IWMF's research strategy and research funding program were updated and expanded. The IWMF's flagship research grants fall under a section of its funding program called the IWMF-LLS Strategic Research Roadmap Initiative and consist of two-year awards from the IWMF in the maximum amount of US $480,000. Following the success of this Initiative, additional types of grants have been added to the IWMF's funding program.

The IWMF's research strategy continues to be modified as new developments expand our knowledge of WM. The following are the pillars of the current research strategy:

- **WM Cell Biology:** Includes research into signaling pathways driving the growth and survival of WM. Also includes preclinical testing of novel therapeutic strategies and research into signaling mechanisms related to recurrent somatic mutations such as MYD88, CXCR4 and ARID1A as well as WM specific dysregulation of novel and established pathways such NF-kB, ERK, and PI3K/AKT.

- **T-Cell Based Therapeutics:** Includes development and preclinical testing of CAR-T and bi- and tri-specific antibodies, as well as the development of novel therapeutic strategies that promote immune anti-WM engagement. Also includes proposals to overcome T-cell anergy and counter the immunosuppressive effects associated with myeloid derived suppressor cells and similar mechanisms.

- **Microenvironmental Research:** Like many indolent lymphomas, WM cannot survive without the support of its local environment. This pillar supports research into identifying these dependencies and assessing their therapeutic potential. It also supports the development of organoids and xenograft models that can support long-term primary WM cultures for research and testing.

- **Genomic, Epigenomic, and Transcriptional Research:** Supports bulk, single cell, and spatial studies to better understand the biology of WM evolution from MGUS to symptomatic disease, as well as mechanisms driving primary and acquired therapeutic resistance. This pillar further supports related research into identification of high-risk features, novel target identification, and biological

characterization of WM disease manifestations such as Bing-Neel Syndrome and transformation.

- **Proteomics:** This pillar is distinct from the previous one as it focuses on protein properties and modifications that impact key WM signaling pathways or can be used for prognostic testing in a manner distinct from the underlying genetics. It also supports protein-based research into the causes and treatment of cryoglobulinemia, cold agglutinins, amyloid, demyelinating neuropathy, and other complications associated with the IgM paraprotein.

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

Project Period 09/01/23 – 09/01/25	**Investigator**: Stephen Ansell, MD, PhD
US $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
US $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.

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 – 10/16/25	**Investigator**: Dr. Signy Chow
US $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
US $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 – 01/31/24	**Investigator**: Bruno Paiva
US $90,000 over one ½ 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.

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
US $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 – 07/01/25	**Investigator**: Jithma Prasad Abeykoon, M.D.
US $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
US $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
US $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.
US $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
US $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
US $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
US $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.

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

Project Period 12/01/23 – 12/01/2025	**Investigator**: Marcel Spaargaren, PhD
US $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.

WM-NET

Project Period 09/01/23 – 08/31/2028	**Investigator**: Jorge Castillo, MD
US $2,500,000 over five years	**Institution**: Dana-Farber Cancer Institute
Sponsored in part by:	**Hamberg Family Research Fund of the IWMF**

Clinical trials are essential to evaluate new drugs or combinations of drugs for treating WM. Meaningful clinical trial results depend on evaluating a number of patients. Currently, clinical trials in the US are typically designed and conducted only at a single institution, making it difficult and slow to recruit sufficient patients for a rare disease such as WM in a reasonable timeframe. If multiple institutions could cooperate on the same trial, using a single, unified protocol, a larger number of patients could be recruited more rapidly. This would lead to considerably more rapid development of new drugs or drug combinations. Additionally, if institutions throughout the country could offer patients access to the same clinical trial, it would reduce the travel, time, and financial commitment from participants, making clinical trials more accessible. The WM-NET is designed to collaboratively identify critical clinical questions and design and administer multi-institutional studies to answer them. Funding by IWMF and other donors supports a sustainable infrastructure to share information and coordinate and administer unified study design among institutions. It is expected that WM-NET will activate clinical trials concurrently in multiple centers, with the goal of facilitating more rapid translation of study results of new WM drugs or drug combinations into clinical practice.

PATIENT PREFERENCES REGARDING TREATMENT OPTIONS FOR WALDENSTROM'S MACROGLOBULINEMIA: AN INTERNATIONAL CHOICE EXPERIMENT

Project Period 02/06/24 – 02/06/2026	**Investigator**: Josephine Vos, MD, PhD
US $105,5011.60 over two years	**Institution**: AMC Medical Research B.V.

The WM-VOICE study is scientific research about Waldenström macroglobulinemia (WM). The study focuses on what patients with Waldenström value most in their treatment. Researchers from the hematology department of Amsterdam UMC are conducting this study, in collaboration with Waldenström experts and patient organizations from the United Kingdom, the United States of America, Australia, Canada, and the Netherlands. Treatment options for Waldenström macroglobulinemia (WM) are expanding rapidly. However, it remains an incurable disease with no consensus on one preferred treatment. WM treatments vary regarding efficacy, side effects, administration, and duration. An improved understanding of patient preferences may guide tailoring treatment to the individual and set priorities in drug development and trial design in the future.

DISSECTING THE TUMOR MICROENVIRONMENT IN WALDENSTROM MACROGLOBULINEMIA

Project Period 10/01/24 – 10/01/2026	**Investigator**: Aldo M. Roccaro, MD, PhD
US $480,000 over two years	**Institution**: ASST-Spedali Civili di Brescia

The bone marrow microenvironment plays a pivotal role in the development and progression of B-cell malignancies, including WM. In his project, Dr. Roccaro proposes to do a deep study of bone marrow stromal cells, also known as mesenchymal stromal cells (MSCs), which, he suggests, have the ability to influence the growth and behavior of WM cells. He intends to identify which of these bone marrow stromal cells interact with WM cells, with the overarching goal to identify and develop therapies that target WM by halting the vital interactions within the stromal cells that nourish WM cells and guide their growth and disease progression. Specifically, Dr. Roccaro will characterize the types of bone marrow stromal cells found in IgM MGUS and WM patients that are different those in from healthy individuals by sequencing the transcriptome (RNA) in individual cells, and he will determine how differences in the transcriptomes influence the progression of IgM MGUS to WM. He will identify which WM cell-to-bone marrow environment interactions at the individual cell level have significant influence on the behavior of WM cells, and he will further define the functional role of those significant interactions by studying their behavior in WM cell lines. This project falls under the IWMF-LLS Strategic Research Roadmap Initiative.

THE APPLICATION OF A DUAL IRAK4 AND IMID SUBSTRATE DEGRADER, KT-413, IN WALENSTROM'S MACROGLOBULINEMIA

Project Period 10/04/24 – 09/31/2025	**Investigator**: Jennifer Kimberly Lue, MD
US $90,000.00 over one years	**Institution**: Memorial Sloan Kettering Cancer Center

The MYD88 L265P mutation has been identified in over 90% of WM patients and has been identified in patients with IgM MGUS (monoclonal gammopathy of undetermined significance). This suggests that the mutation has an early role in malignant progression. An important downstream pathway from MYD88 is the protein called IRAK4. MYD88 itself is a difficult protein to target for treatment, but small molecule inhibitors of IRAK4 are in early development. In this IWMF Research Seed Money grant, Dr. Lue suggests that the most effective IRAK4 targeting therapy is not an IRAK inhibitor,

but rather an IRAK degrader that breaks down the protein and may lead to more effective tumor cell killing. To this end, Dr. Lue will evaluate a drug in early development called KT-413, as both a single agent therapy and in combination with venetoclax in preclinical models of WM, including mouse models.

UNCOVERING THE MOLECULAR UNDERPINNING OF WALDENSTROM MACROGLOBULINEMIA FROM TUMOR CELLS TO IMMUNE MICROENVIRONMENT

Project Period 02/26/25–02/25/2028	**Investigator**: Zachary Hunter, PhD & Patrizia Mondella, MD Phd HSC
US $1,500,000 over 3 years	**Institution**: Dana-Farber Cancer Institute, Inc. & Mayo Clinic
Sponsored in part by:	**The Rosen Family Foundation Research Fund of the IWMF** **Donald and Alison Weiss and Family Research Fund of the IWMF**

This represents a new IWMF grant type called the IWMF-LLS Enhanced Research Roadmap Initiative, intended to provide greater funding for proposals that require more effort, time, or resources than the two-year IWMF-LLS Strategic Research Roadmap Initiative grants. In this grant, Drs. Hunter and Mondello point out that the current classification of WM does not take into account all the clinical differences that occur with this disease or the underlying biology responsible for these differences. In previous work, Dr. Mondello identified three distinct molecular WM disease clusters called C1, C2, and C3, while a previous analysis by Dr. Hunter identified a common unusual clone prevalent in asymptomatic/smoldering WM that evolves into one of two mature subtypes, referred to as B cell-like (BCL) and plasma cell-like (PCL). In this new collaborative study, Drs. Hunter and Mondello hypothesize that each WM cluster represents a different biologic entity with an origin from a different progenitor (ancestor) and that each has unique genetic and microenvironment features which dictate how the disease behaves clinically and responds to therapy. Previous work identified alterations in a gene called *TNFAIP3*, predominantly seen in the WM C1 cluster, that may cooperate with mutated *MYD88* to cause malignant cell development. Aim 1 of the current project will attempt to confirm this hypothesis and further define the role of *TNFAIP3* alterations in affecting WM cells and their bone marrow microenvironment. Aim 2 will identify if and how the bone marrow microenvironment plays a role in driving the WM molecular clusters, particularly whether a T cell clone is prevalent in each cluster and how it may facilitate WM cell development and growth. Aims 3 and 4 will determine the evolutionary relationship between the early subclone associated with asymptomatic/smoldering WM and the dominant clone seen as the disease progresses to active WM. The researchers will determine if this early subclone persists in relapsed/refractory WM and is potentially responsible for disease relapse; based on this information, they also intend to screen 3-4 candidate drug compounds that target the early subclone.

TRACKING THE EVEOLUTION OF IBRUTINIB RESISTNAT PROTEOME IN WM

Project Period 10/01/24 – 09/30/2026	**Investigator**: Dr. Tina Bagrtuni
US $157,500 over 2 years	**Institution**: National and Kapodistrian University of Athens / Special Account for Research Grants

This is a Robert A. Kyle Career Development Award grant intended to foster a new generation of talented WM researchers. Development of drug resistance is currently one of the main challenges plaguing anti-cancer therapy and is often responsible for cancer progression and treatment failure. Drug resistance is generally considered to evolve by means of already-present or acquired genetic alterations of cancer cells but is also heavily influenced by the tumor microenvironment. Even though our understanding of WM has grown significantly over the last few years, the protein profile (the proteome) of WM cells and immune cells in patients undergoing ibrutinib therapy is unclear, specifically in

terms of clinical responses to the drug. Tracking the evolution of cell proteins that develop during resistance to ibrutinib treatment is particularly important, as it could lead to the identification of biomarkers useful for monitoring ibrutinib therapy and detecting early resistance to the drug. Dr. Bagratuni intends to explore the evolution of the ibrutinib-resistant proteome in WM patients undergoing ibrutinib therapy by combining proteomic data with single cell transcriptome (RNA) data. She will also perform screening of blood plasma proteins before and after ibrutinib therapy to detect biomarker proteins associated with the development of ibrutinib resistance, with the goal of developing a less invasive way than bone marrow biopsies to monitor ibrutinib treatment in WM patients.

DISSECTING CANCER CELL-INTRINSIC AND MICROENVIRONMENTAL ROLES OF MYD88 MUTATIONS AND CHROMOSOME 6Q DELETION IN WALDENSTROM MACROGLOBULINEMIA

Project Period 09/01/24 – 08/31/2026	**Investigator**: Filip Garbicz, MD
US $157,500 over 2 years	**Institution**: Dana-Farber Cancer Institute, Inc.

Although the MYD88 L265P mutation is seen in a certain type of diffuse large B cell lymphoma called ABC-type and is especially common in WM, this mutation by itself is not sufficient to drive cancer transformation in mouse models of lymphoma, and it appears that additional mutations are necessary. Notably, deletions in the long arm of chromosome 6, called 6q deletions, are observed in almost half of WM cases but are not seen in those who have unmutated MYD88 (also called wild-type WM). Dr. Garbicz suggests that 6q deletions are a critical step in the development of MYD88 L265P-mutated WM and in the ABC type of diffuse large B cell lymphoma, and he proposes to investigate his hypothesis. He will introduce these deletions in mouse cells to study the resulting pathways involved in malignant transformation, and he plans to develop genetically engineered mouse models with these deletions that

DECIPHERING TUMOR HETEROGENEITY IN WALDENSTRÖM'S MACROGLOBULINEMIA THROUGH THE GENOMIC AND IMMUNE PROFILING

Project Period no contract yet	**Investigator**: Cristina Jiménez, PhD
US $157,500 over 2 years	**Institution**: University Hospital of Salamanca / Biomedical Research Institute of Salamanca (IBSAL)

This is a Robert A. Kyle Career Development Award grant. Disease progression in WM is a complex and dynamic process characterized by extensive genetic variation and the development of multiple subclones, leading to the diversity of the cancer cell population. The immune tumor microenvironment (iTME) is a complex network of immune cells interacting with both the cancer clone and each other and has been shown to profoundly impact cancer development. In WM, alterations in the iTME precede the expansion of the malignant clone. Moreover, exhaustion of CD8 T cells and dysregulation of natural killer (NK) cells show strong correlation with cancer infiltration in the microenvironment. However, the contributions of the different components of the iTME to disease progression remain poorly explored. By using DNA and RNA sequencing, along with immunophenotyping, Dr. Jimenez intends to characterize the genomic alterations and the tumor microenvironment of WM cells in symptomatic patients and correlate them with the patients' clinical characteristics, treatment response, and prognosis.

SPATIAL IMMUNE PROFILING TO DEFINE BIOMARKERS FOR DISEASE PROGRESSION IN WALDENSTROM'S MACROGLOBULINEMIA

Project Period 10/01/24 – 09/31/2026	**Investigator**: Yoshinobu Konishi, MD, PhD
US $157,500 over 2 years	**Institution**: Dana-Farber Cancer Institute, Inc.
Sponsored in part by:	**The Poh Family Research Fund of the IWMF**

WM is consistently preceded by two precursor conditions, IgM monoclonal gammopathy of undetermined significance (IgM MGUS) and smoldering WM (SWM). Together, Dr. Konishi refers to IgM MGUS and SWM as asymptomatic WM (AWM). Currently, patients with AWM are not treated until they progress to overt, symptomatic WM because of concerns over the short-and long-term complications of treatment. There needs to be better understanding about which patients with AWM are at the greatest risk of disease progression and might benefit from early treatment intervention. Dr. Konishi's overarching hypothesis is that spatially mapping the bone marrow immune microenvironment will provide a better understanding of how WM develops. With the advent of technologies such as spatial transcriptomics and new imaging techniques based on mass cytometry, he will be able to characterize the majority of immune cell types in large groups of WM patients. He will use these state-of-the-art techniques to investigate the microenvironment and gain a detailed picture of how the immune system works to support or fight against WM cells. This will allow him to develop immune biomarkers that may indicate those most at risk of disease progression. Dr. Konishi's project is funded by a Robert A. Kyle Career Development Award grant.

SINGLE CELL SUBCLONAL CHARACTERIZATION AND EVOLUTION OF THE GENOME AND TRANSCRIPTTOME IN IGM MONOCLONAL GAMMOPATHY

Project Period no contract yet	**Investigator**: David F. Moreno, MD
US $157,500 over 2 years	**Institution**: Fundació de Recerca Clínic Barcelona - IDIBAPS

The cell of origin of WM is still under investigation, and new technologies may help pinpoint when malignant transformation occurs. In this Robert A. Kyle Career Development Award grant, Dr. Moreno suggests that whole genome and transcriptome (RNA) characterization of WM subclones in single cells of patients with IgM MGUS (monoclonal gammopathy of undetermined significance) will identify new genetic variants as drivers for disease progression to symptomatic WM. To accomplish this, he will use a newer technology called primary template-directed amplification (PTA). This technology enables single cells in subclones to be amplified so that whole genome and transcriptome sequencing can be performed with fewer errors. After obtaining this information, Dr. Moreno intends to link the genomic sequencing with the transcriptome to reconstruct the history of the subclones for a better understanding of how they develop and lead to disease progression.

SELF-ORGANIZING STEM CELL-DERIVED BONE MARROW ORGANOID SYSTEM FOR STUDYING THE TUMOR MICORRENVIRONMENT IN WALDENSTROM'S MACROGLOBULINEMIA

Project Period 11/01/24 – 10/31/2025	**Investigator**: Erna Magnusdottir, Associate professor
US $90,000 over one year	**Institution**: The University of Iceland

Although the advent of new cell lines derived from WM tumors has been tremendously useful for modeling several aspects of the disease and recent mouse models have shed important light on the origin of WM, there are still significant gaps in our knowledge of WM that need new models to address. In particular, the study of how the bone marrow microenvironment influences tumor biology, and vice versa, how the tumor affects the bone marrow microenvironment would be greatly facilitated by new models of the WM microenvironment. Dr. Magnúsdóttir proposes to use pluripotent stem cells (PSCs) to develop living models of the bone marrow, called bone marrow organoids, that can be seeded with WM cells and support their growth, thereby shedding light on the molecules and pathways that could serve as novel

targets for WM treatment. This is a grant under the IWMF Research Seed Money Initiative, which supports one-year funding to give investigators the chance to test new ideas in preparation for larger grant applications through the IWMF-LLS Strategic Research Roadmap Initiative.

INVESTIGATING THE ROLD OF CD34+ CELLS IN THE PATHOGENESIS OF WALDENSTROMS MACROGLOBULINEMIA

Project Period 01/13/25 – 01/12/2026	**Investigator**: Maria Luisa Guerrera, MD
US $90,000 over one year	**Institution**: Dana-Farber Cancer Institute, Inc.

This IWMF Research Seed Money grant builds upon a recent study by the Dana-Farber research team suggesting the existence of clonal B cells in WM that carry the early hematopoietic stem cell surface marker CD34 and may re-activate stem cell programming early on before being replaced by clonal cells bearing more typical B cell makers as the disease progresses. Traditionally, mature lymphomas such as WM are thought to arise from differentiated mature lymphocytes. But there is growing evidence pointing toward an earlier, stem cell origin for some lymphomas. In this proposal, the central hypothesis is to explore whether WM can be traced back to an altered stem cell or early lymphoid progenitor (ancestor) in at least some cases of WM. To Dr. Guerrera's knowledge, this is the first study exploring such a topic in WM. To test her hypothesis, Dr. Guerrera will use multiple technologies to study the morphology and genomic and transcriptomic (RNA) features of WM cells that have both CD19 and CD34 surface markers.

DECIPHERING THE DYNAMICS OF THE MUTATIONAL LANDSCAPE IN PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA TREATES WITH TARGETED THERAPY IN PROSPECTIVE CLINICAL TRIALS OF THE EUROPEAN CONSORTUIM FOR WALDENSTROM'S MACROGLOBULINEMAI (ECWM)

Project Period no contract yet	**Investigator**: Christian Buske, MD
99.200 €	**Institution**: University Hospital Ulm

This is a grant under the IWMF-LLS Companion Initiative, which is a one-to-two-year award to support a basic research project associated with an ongoing clinical trial of WM patients. The introduction of targeted chemotherapy–free treatments such as BTK inhibitors has revolutionized the clinical management of WM. Despite the progress seen with these treatments, they are not able to cure patients, rarely induce complete remissions, and, ultimately, cannot prevent relapse. This demonstrates that, at least at a subclonal level, there is treatment resistance. The underlying mechanisms for this are poorly understood. Dr. Buske believes that an analysis of these mechanisms, embedded into prospective clinical trials and accompanied by a pre-specified biosampling program, would offer a unique opportunity to understand the shifts in clonal composition of WM during therapy, until relapse or progression occurs. Dr. Buske proposes to use for his project a Phase 2 clinical trial currently being conducted by the European Consortium of Waldenström's Macroglobulinemia (ECWM) to evaluate the efficacy of bortezomib/ibrutinib/rituximab in treatment naïve patients. This trial is now fully recruited, with 53 participants, and Dr. Buske has been obtaining multiple sample sites from each, including cheek cell, bone marrow, peripheral blood, serum, and plasma. By analyzing prospectively collected samples during therapy, he aims to investigate therapy response and analyze gene mutations contributing to disease.