WMFC
Waldenstrom's Macroglobulinemia Foundation of Canada

December 2019 Update

I would like to thank the membership for their enthusiastic response to our recent request for donations to the WMFC. We are delighted and feel very encouraged that we will be able to continue supporting Canadians with WM and funding cutting edge research. Our most recently funded research project is partnering with Dr. Carrasco at the Dana Farber Cancer Institute. Attached is his 12 month report to the WMFC that we received in November.

For those who have not yet made a contribution to the WMFC this year, it is certainly not too late. Any contribution that is either post-marked or made on line by December 31st, will be eligible for a 2019 tax receipt. We are counting on most of our membership making a donation by the end of the year.

I apologize that my letter accompanying our mail out in November said there would be a stamped, self-addressed envelope included; unfortunately, the stamps were missing from the envelope. (Inadvertent cost control!)

In addition to the membership being very supportive, a number of individuals and organizations have helped us in various ways over the last year. They include:

Advocate Printing of Pictou, NS
Dr. Neil Berinstein
Stu Boland, Calgary Support Group Leader
Estate of Arlene Carsten
Dr. Jorge Castillo, Dana Farber Cancer Institute
Janet Cherry-Parcher, Ottawa Support Group Leader
David Derooey
Scott Ferguson, AV
Cam Fraser, Calgary Support Group Leader
Jennifer Gleason, Jennifer Gleason Art
Stan and Jeannette Hrescak
Dr. Zack Hunter, Dana Farber Cancer Institute
Janssen Pharmaceutical
Johnson & Johnson
Janelle Jones, Ottawa Support Group Leader
Leukemia and Lymphoma Society of Canada
Charles T. Low
Lymphoma Canada
Jim Mason, Halifax Support Group Leader
Kristy McKay
RBC
Kit Schindell, Vancouver Support Group Leader
Ron Ternoway, Halifax Support Group Leader
Dr. Steve Treon, Dana Farber Cancer Institute

One of the goals of the 2019-2025 WMFC Strategic Plan is to increase our membership numbers. Firstly, we have removed the requirement of making a contribution to become a WMFC member. If you have a support person, a friend with WM or a family member who would like to hear regularly from us, we would love to include them as members at no cost. Down the road, when we need to advocate for WMers in Canada, a larger membership will give the WMFC greater impact. (What a great Christmas present for a loved one!!)

Here`s wishing everybody a happy and relaxing holiday season.

All the best. Thank you again for all the support that you have offered the WMFC through your kind notes and contributions.

Sincerely,

Paul Kitchen, WMFC Board Chair

Project Title: Generation of mice with conditional chromosome 10q deletion and development of a mouse model of Waldenström Macroglobulinemia

Principal Investigator: Ruben D. Carrasco, Department of Oncologic Pathology, Dana-Farber Cancer Institute

Preparation date: 11/29/2019

Progress report: We have previously shown that, although the *MYD88*^{L265P} mutation might be indispensable for the LPL/WM phenotype, it is insufficient by itself to drive malignant transformation in B cells. We also demonstrated that the murine findings are recapitulated in the human setting, corroborating their similarity and providing clinical correlations and insight into the pathogenesis of lymphoplasmacytic lymphoma/Waldenström macroglobulinemia (LPL/WM).

To strengthen our findings, increase the reliability and reproducibility, and enhance the impact of our work, we expanded our immunohistochemical and flow cytometric analysis by incorporating more human cases and murine samples. These improvements led to publishing our manuscript in an open access journal of American Society of Hematology – *Blood Advances* (please see the pdf document attached).

Title: Human *MYD88*^{L265P} is insufficient by itself to drive neoplastic transformation in mature mouse B cells

Authors: Tomasz Sewastianik, Maria Luisa Guerrera, Keith Adler, Peter S. Dennis, Kyle Wright, Vignesh Shanmugam, Ying Huang, Helen Tanton, Meng Jiang, Amanda Kofides, Maria G. Demos, Audrey Dalgarno, Neil A. Patel, Anwesha Nag, Geraldine S. Pinkus, Guang Yang, Zachary R. Hunter, Petr Jarolim, Nikhil C. Munshi, Steven P. Treon, Ruben D. Carrasco

Abstract: *MYD88*^{L265P} is the most common mutation in lymphoplasmacytic lymphoma/Waldenström macroglobulinemia (LPL/WM) and one of the most frequent in poor-prognosis subtypes of diffuse large B-cell lymphoma (DLBCL). Although inhibition of the mutated MYD88 pathway has an adverse impact on LPL/WM and DLBCL cell survival, its role in lymphoma initiation remains to be clarified. We show that in mice, human *MYD88*^{L265P} promotes development of a non-clonal, low-grade B-cell lymphoproliferative disorder with several clinicopathologic features that resemble human LPL/WM, including expansion of lymphoplasmacytoid cells, increased serum immunoglobulin M (IgM) concentration, rouleaux formation, increased number of mast cells in the bone marrow, and proinflammatory signaling that progresses sporadically to clonal, high-grade DLBCL. Murine findings regarding differences in the pattern of MYD88 staining and immune infiltrates in the bone marrows of *MYD88* wild-type (*MYD88*^{WT}) and *MYD88*^{L265P} mice are recapitulated in the human setting, which provides insight into LPL/WM pathogenesis. Furthermore, histologic transformation to DLBCL is associated with acquisition of secondary genetic lesions frequently seen in de novo human DLBCL as well as LPL/WM-transformed cases. These findings indicate that, although the *MYD88*^{L265P} mutation might be indispensable for the LPL/WM phenotype, it is insufficient by itself to drive malignant transformation in B cells and relies on other, potentially targetable cooperating genetic events for full development of lymphoma.

Acknowledgments: The authors thank Clyde Bongo and Madison L. O'Donnell of the Dana-Farber Cancer Institute (DFCI) Molecular Pathology Core Laboratory for help with imaging analyses, and members of the DFCI Flow Cytometry Core for assistance with cell sorting.

This work was supported by research grants from the Leukemia Lymphoma Society, the International Waldenstrom's Macroglobulinemia Foundation, **the Waldenstrom's Macroglobulinemia Foundation of Canada**, and a grant from the National Institutes of Health, National Cancer Institute (R01CA196783).

The generous support of the Waldenstrom's Macroglobulinemia Foundation of Canada made our important and unexpected findings available to the scientific and patient communities. We are certain that this article will spark more research effort aimed at better understanding of the pathogenesis of LPL/WM.

Since the last progress report, we were also extensively breading our mice to:

- Generate homozygous *Chr10q*$^{Fl/Fl}$ lines, which bear all four modified alleles, to:
    - ensure proper segregation of two modifications on one chromosome during offspring generation. This is necessary because of the length of chromosome 10q deletion and the fact that modifications at both ends are far enough to be separated independently during breeding.
    - facilitate the following breading steps. Mating mice will be paired so all the progeny will have experimentally useful genotypes, which will expedite the project and reduce the number of mice according to the ethics of using animals for scientific research.
- Cross out the enhanced flippase (FlpE) recombinase transgene, which was used to excise selection markers at the modified loci.

We successfully generated litters of *Chr10q*$^{Fl/Fl}$ mice (**Figure 1**) that are already being mated with mice carrying the transgene for Cre recombinase, which will generate mice with conditional deletion of chromosome 10q to be used for aging and functional experiments.

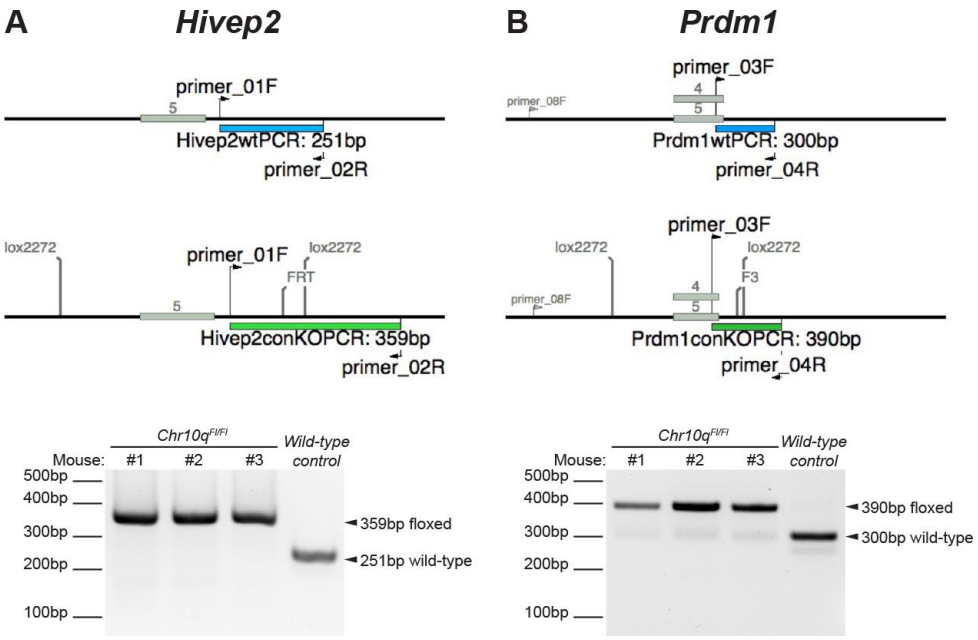

Figure 1.

Top: Schematic representation of wild-type and genetically modified (floxed) **(A)** *Hivep2* and **(B)** *Prdm1* loci flanking chromosome 10q deletion and the PCR detection strategy.

Bottom: Genotyping PCR showing presence of only floxed band for **(A)** *Hivep2* and **(B)** *Prdm1* in the examined litter illustrating generation of homozygous mice.

Brief layperson-friendly progress report

We have previously shown that *MYD88*^{L265P} mutation, while exceptionally recurrent in lymphoplasmacytic lymphoma/Waldenström macroglobulinemia (LPL/WM) cells (90-100%), is insufficient by itself to cause LPL/WM development. It does however, promote a prelymphomatous condition with clinicopathologic features of human LPL/WM and diffuse large B-cell lymphoma (DLBCL) transformation. Moreover, using our murine model, we found new insight into the pathogenesis of human LPL/WM. To strengthen our findings, increase the reliability and reproducibility, and enhance the impact of our work, we have recently expanded our analysis by incorporating more human cases and murine samples. These improvements led to publication of our manuscript in an open access journal of American Society of Hematology, which wouldn't be possible without the support of the Waldenstrom's Macroglobulinemia Foundation of Canada. Our somewhat unexpected findings will stimulate the research community to increase the effort to better understand the pathogenesis of LPL/WM.

Deletion of a large fragment of chromosome 10q requires modifying murine DNA at two distant positions that can be inherited separately. Therefore, precautions are needed during mouse breading to ensure proper segregation of genetic modifications in the offspring. We have generated mice carrying only the modified DNA sequences, which will ensure correct genetics as well as make the future breading more efficient. These mice are already being mated with a strain carrying a gene that will induce the deletion of chromosome 10q in a specific type of white blood cells called B lymphocytes, which give rise to LPL/WM. Upcoming litters will be characterized functionally and aged to induce tumor development.