

Mutation MYD88 L265P

by Steven P. Treon, MD, PhD



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Dr. Steven Treon discusses the recent discovery of a mutation present in a large majority of WM patients in his research team's study and its future implications for the diagnosis and treatment of our disease. The IWWMF is proud to have provided partial funding for this groundbreaking research.

A team of scientists led by Dr. Steven P. Treon from the Bing Center for Waldenstrom's Macroglobulinemia at the Dana-Farber Cancer Institute and Harvard Medical School announced the identification of a gene mutation that underlies most cases of Waldenstrom's macroglobulinemia. The research, which was presented at the 53rd Annual Meeting of the American Society of Hematology (ASH) on December 12, 2011, described a mutation, occurring in one single DNA molecule out of three billion DNA molecules which make up the genetic code of a cell, as the leading culprit in Waldenstrom's and a target for new diagnostic tests and drug development for this disease.

The mutation was discovered after the team performed whole genome sequencing of tumor and normal cells from thirty patients with Waldenstrom's macroglobulinemia. By lining up and comparing the DNA sequences of tumor and normal cells, Treon and his colleagues were able to detect the same recurring mutation in the MYD88 gene, a finding present in ninety percent of patients with Waldenstrom's macroglobulinemia who were tested. In most patients the L265P mutation was found in one of the 2 copies of the MYD88 gene, but in some patients with a longstanding history of WM, a reduplication of the segment of chromosome 3 where MYD88 is located resulted in 2 copies of the L265P mutation being present. The clinical significance of having an extra copy of the mutation remains to be determined.

Importantly, Treon and colleagues showed that the mutation, which results in a change in the amino acid leucine for proline at position 265 in MYD88, helps keep the malignant Waldenstrom's cells alive by activating a signal pathway which leads to activation of NF- κ B, a protein essential for the growth and survival of Waldenstrom's cells. Using either genetically engineered viruses which knock down MYD88 or drugs which shut down the MYD88 signaling pathway, the Treon team was able to induce Waldenstrom's cells to undergo a form of programmed cell death called apoptosis. Treon and colleagues are currently working on development of agents which can be used in the clinic to shut down the MYD88 pathway. "We are fortunate that the MYD88 pathway has been under study for many years by scientists interested in rheumatology diseases. As such, we have a running start with many agents that have been developed to block the MYD88 pathway, and it will not be long before we have these agents in clinical trials for Waldenstrom's patients." Treon estimates that in the next 1-2 years the first clinical trial using agents blocking the MYD88 signaling pathway will become available.

How did whole genome sequencing lead to the discovery of MYD88 L265P mutation in WM patients?

Whole genome sequencing (WGS) is a powerful new technology that enables the reading of each of the 3 billion DNA molecules that make up the 23 paired chromosomes that are found in the nucleus of a human cell. This includes the DNA molecules that make up genes that code for proteins that regulate how a cell functions, as well as the DNA molecules that string together genes, which include regions that regulate how genes function.

To perform whole genome sequencing, malignant cells (lymphoplasmacytic cells) in the bone marrows of patients with Waldenstrom's macroglobulinemia were isolated. DNA was then isolated from the purified WM lymphoplasmacytic cells and, by using enzymes, shredded into smaller fragments that were subjected to sequencing. The resulting small DNA sequence readouts were aligned with the aid of supercomputers to a "reference genome" made possible by the Human Genome Project. Since hundreds of thousands of differences in DNA molecules (polymorphisms) can exist from one person to another, the patient's own WM cell DNA readouts were compared against their own normal cells. This process, known as paired sequencing, used non-WM blood cells to supply "normal DNA" which was then aligned against "WM cell DNA." Paired analysis for 10 WM patients revealed that the MYD88 L265P mutation was present in all 10 patients. The same mutation was then found in unpaired WM cell DNA from 17 of 20 other WM patients who also underwent whole genome sequencing. The presence of MYD88 L265P was confirmed in all the positive patients using an alternative (Sanger) method of gene sequencing. In addition to the MYD88 L265P mutation, other mutations were also discovered in WM cells although the frequency for these mutations was much lower. Dr. Zachary Hunter from the Bing Center will be presenting the finding of other (non-MYD88 L265P) mutations in WM patients at the Annual Meeting of the American Society of Oncology (ASCO) in June 2012.

What is MYD88?

MYD88 is as an "adaptor" or linker protein which controls signaling through receptors found on the surface of immune cells, including Toll-like receptors and Interleukin-1 and Interleukin-18. Toll-like receptors are important for receiving signals from pathogens like bacteria or viruses, while Interleukin-1 and Interleukin-18 play roles in infections and inflammatory conditions. Following stimulation of these receptors, MYD88 becomes recruited to the activated receptor complex and then complexes with the proteins IRAK1 and IRAK4. The MYD88/IRAK protein complex then stimulates the MAPK and NF-kB pathways, both of which are known to play important roles in the growth and survival of Waldenstrom's cells.

How does the L265P mutation in MYD88 function?

The L265P mutation, which is present in 90% of Waldenstrom's patients, causes a change in the amino acid leucine for proline. This change causes a shift in the three dimensional character of the MYD88 protein. The mutation is found in a region that is highly conserved in evolution, which means that all mammalian species carry this domain of MYD88 without much difference, implying that it is critical to the function of MYD88. Waldenstrom's cell lines that carry the MYD88 L265P show activation of proteins, including the IRAK proteins, which funnel down to two important pathways that regulate cell growth – the MAPK and NF-kB pathways. Velcade (bortezomib) affects the NF-kB pathway, so the discovery of the MYD88 L265P mutation now gives us an important clue as to why this drug has been so successful in the treatment of Waldenstrom's patients.

Is there a test for the MYD88 L265P mutation?

Whole genome sequencing requires lots of purified DNA. For many patients with WM, obtaining such amounts is problematic. In addition, whole genome sequencing can take 4-6 months and, at present, can cost \$5,000 per genome (down considerably from \$100,000 just 3 years ago).

An alternative to whole genome sequencing is the use of Sanger sequencing. One limitation of Sanger sequencing is that WM cells have to be purified so as to provide enough DNA for this test; another limitation is that there have to be enough cells in the bone marrow for testing of unselected bone marrow samples. Since most clinical laboratories do not purify cells, we sought to develop a highly sensitive test using a platform called PCR (polymerase chain reaction) to selectively test for the presence of the MYD88 L265P mutation. An abstract detailing the successful use of this PCR test to detect MYD88 L265P in WM patients will be reported by Dr. Lian Xu from our center at the Annual Meeting of ASCO in June 2012. This test is currently being validated in clinical laboratories and potentially can pick up one WM cell in a background of 4,000 normal cells. This test also has the potential to be used as a blood test and to possibly assess residual disease in patients who have undergone treatment. The PCR test for MYD88 L265P should become available for commercial use in the next few months.

Are there diagnostic implications for MYD88 L265P for WM patients?

There are many overlapping disorders that have clinical and pathological features similar to Waldenstrom's macroglobulinemia. These include marginal zone lymphomas (such as splenic, nodal, and MALT lymphomas) as well as IgM multiple myeloma. The MYD88 L265P mutation is either absent or rarely expressed in these entities, allowing the MYD88 L265P mutation to be used as an aid to separate WM from these other entities. In the studies presented by Dr. Lian Xu at the 2011 Annual Meeting of ASH, the MYD88 L265P mutation was absent in almost all patients with IgM monoclonal gammopathy of unknown significance (MGUS), a precursor condition to WM. This finding may either imply that the mutation is altogether absent in IgM MGUS patients or is expressed at a very low level, far lower than the threshold of detection using Sanger sequencing. It is interesting that for one IgM MGUS patient who had the MYD88 L265P mutation, disease progression to WM occurred. A larger series of studies of IgM MGUS patients with long term follow-up will be required to clarify whether MYD88 L265P is a mutation that transforms IgM MGUS to WM.

Does the MYD88 L265P mutation distinguish those patients with familial versus non-familial forms of WM? Can it be used to identify family members at risk for WM?

Up to 27% of patients have a familial form of WM, defined as having at least one first or second degree relative with WM or another type of lymphoma, myeloma, chronic lymphocytic leukemia or MGUS. The MYD88 L265P mutation was found in WM cells from patients with both familial and nonfamilial WM and appeared at the same frequency (90%). These results suggest that MYD88 L265P is unlikely to be a predisposition gene for familial forms of WM. A search for predisposition gene(s) for familial WM is currently underway using whole genome sequencing to compare the genomes of patients with and without familial forms of WM.

Does the finding of the L265P mutation in MYD88 have implications for the treatment of Waldenstrom's patients?

The most important implication of the discovery of the MYD88 L265P mutation in WM patients is the prospect of developing novel targeted therapies for WM patients. In studies reported at the 2011 Annual Meeting of ASH, Bing Center scientists Dr. Yangsheng Zhou, Dr. Xia Liu, and Dr. Yang Cao used gene knock-in and knock-down models to show that the MYD88 pathway was essential for keeping WM cells with the MYD88 L265P mutation alive. These studies suggested that drugs blocking the MYD88 pathway could provide a targeted approach for WM therapy. The MYD88 pathway has been under active investigation for many years, primarily because of its importance in rheumatologic (autoimmune) diseases. Inhibitors for both MYD88 and the signaling proteins for MYD88 – IRAK1 and IRAK4 – have already been developed. Bing Center scientist Dr. Guang Yang reported at the 2011 Annual Meeting of ASH that both MYD88 and IRAK1/4 inhibitors induced dramatic cell death of the BCWM.1 and MCWL-1 WM cell lines which carry the MYD88 L265P mutation, as well as primary malignant lymphoplasmacytic cells purified from bone marrows of WM patients. More potent inhibitors of both the MYD88 and IRAK proteins are currently under study by the Bing Center team. It is anticipated that in the next 1-2 years the results from these laboratory studies will identify lead drug candidates for use in clinical trials for WM patients.

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