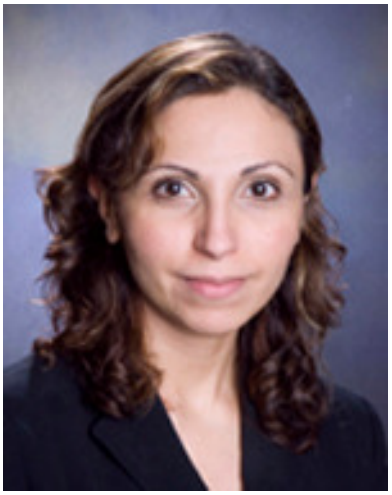
Dana-Farber Cancer Institute | May 2010

The Michele and Steven Kirsch Laboratory for Waldenström's Research

The Kirsch Laboratory: Home to an Orphan Disease

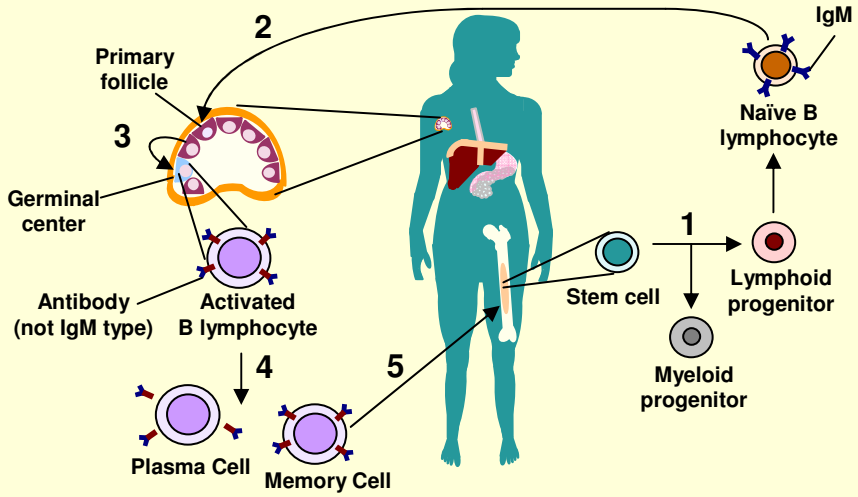
Waldenström's macroglobulinemia (WM) is a rare blood cancer that arises when immune cells called ***B lymphocytes*** develop abnormal properties and become malignant (see figure below). The disease is incurable and responds to few available treatments. Because WM strikes only about 1500 people in the US each year, pharmaceutical companies and government funding agencies have shown little interest in developing drugs to fight it. Philanthropy is helping to fill the void, driving crucial research on this "orphan" disease.

Under the direction of Irene Ghobrial, MD, the Michele and Steven Kirsch Laboratory for Waldenström's Research is spearheading efforts to transform basic scientific discoveries into new therapies for WM patients as quickly as possible. Established in 2008, the laboratory is a key component of Dana-Farber's Bing Center for Waldenström's Research—the only center in the world devoted exclusively to studying and treating this unusual malady. What follows is an update on recent progress Dr. Ghobrial and her colleagues have made thanks to your generous support.



Your support is helping Dana-Farber's Irene Ghobrial, MD, and her colleagues in the Kirsch Laboratory identify new therapies for Waldenström's macroglobulinemia.

B cell development and WM



1. Stem cells in the bone marrow give rise to lymphoid and myeloid progenitor cells. Lymphoid progenitors can develop into one of three main cell types, including ***B lymphocytes***. Newly formed B lymphocytes are considered "naïve" because they have not yet encountered ***antigens***. Naïve B lymphocytes produce a particular type of ***antibody*** called IgM, but at this stage, the antibody remains lodged in the cell membrane. **2.** B lymphocytes migrate to sites known as primary follicles found in lymph nodes throughout the body. **3.** Once they are exposed to antigens and become activated, the cells move to so-called "germinal centers" in the lymph node. Activated B lymphocytes begin to produce antibodies that are distinct from IgM; however, the antibodies are still, for the most part, sequestered in the cell membrane. **4.** In germinal centers, some activated B lymphocytes develop into plasma cells, which export antibodies through the membrane and outside of the cell. Others become memory cells, whose function is to help the immune system remember invading pathogens so the body can respond more quickly to future attacks. **5.** Memory cells and some plasma cells migrate back to the bone marrow where they can survive for long periods. In WM patients, a population of B cells—all derived from a single parent cell—grow out of control. These cells can mature into plasma cells, but unlike their normal counterparts, they continue to produce IgM.

B lymphocytes—components of the immune system that have the unique capacity to produce antibodies.

Antibodies—proteins produced by the body to fight off disease-causing pathogens. Each antibody binds specifically to a unique ***antigen.***

Antigen—a molecule that the body recognizes as foreign.

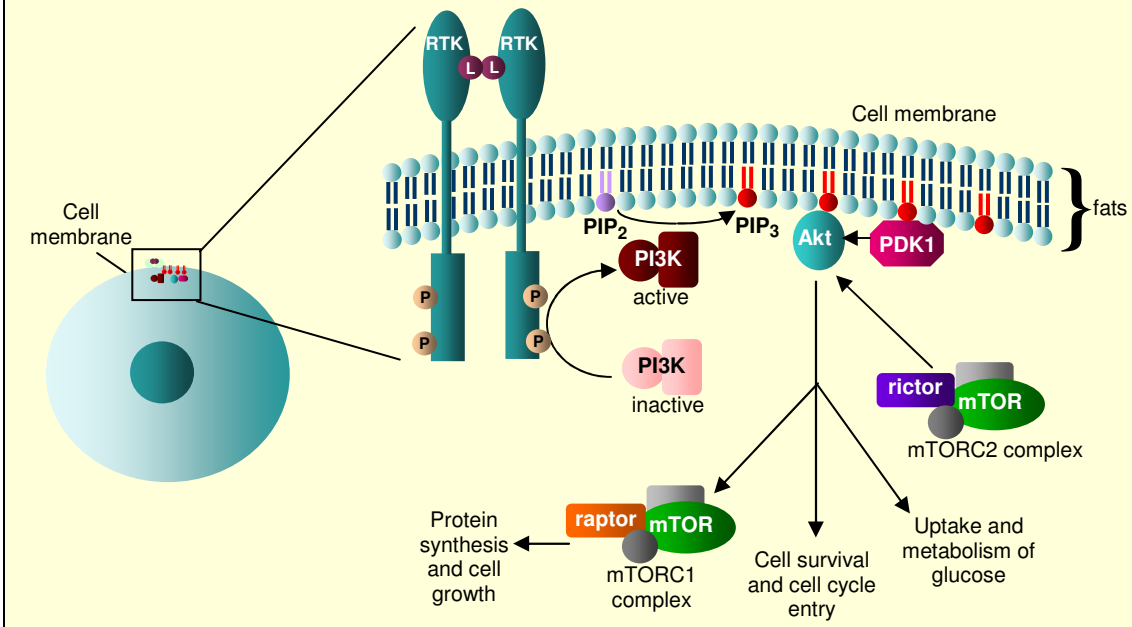
NVP-BEZ235: A Potential Multi-Targeted WM Therapy

In many ways, a cell is like a fortress. Its membranous barrier holds internal cellular components together while excluding unwanted molecules found in the surrounding environment. Yet the cells in our bodies cannot live in complete isolation. Just as the inhabitants of a walled city must communicate with the outside world to form alliances or defend against hostile invaders, a cell must coordinate its activities with its neighbors and respond appropriately to environmental conditions. One way it does that is through a cellular communication network known as the PI3K/Akt/mTOR pathway (see figure below), which seems to play a pivotal role in cancer.

When switched on, this system promotes cellular growth and survival. Its activity is normally controlled very tightly so that it is only switched on when specific signals are present in the external environment. However, in many types of cancer, including WM, the PI3K/Akt/mTOR switch is stuck in the "on" position, allowing malignant cells to grow all the time. Previous research in the Kirsch Laboratory suggested a drug called rapamycin (see sidebar) might help turn the switch back off. The compound inhibits a specific protein in the PI3K/Akt/mTOR pathway and has shown

Rapamycin was originally discovered in a soil sample from Easter Island. The compound helps block cellular proliferation by partially inhibiting the PI3K/Akt/mTOR pathway and is being tested as a treatment for various forms of cancer.

The PI3K/Akt/mTOR Pathway



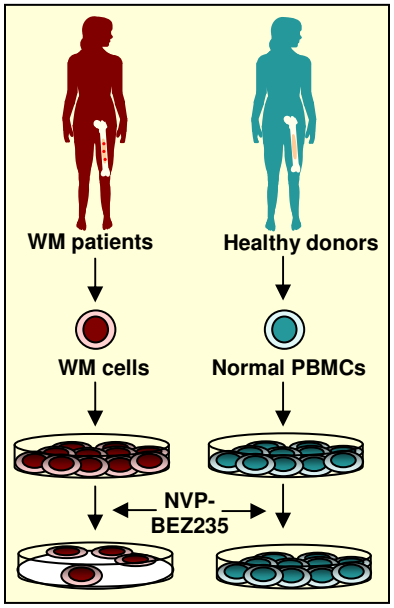
When bound by external activating molecules known as ligands L, RTK proteins jutting out of the membrane modify one another by adding chemical groups called phosphates P at specific sites along their flanks. These alterations enable the RTKs to transform a protein called PI3K from its inactive form to an active state. Active PI3K facilitates the chemical conversion of one particular type of fat in the cell membrane, PIP_2, into a slightly different fat, PIP_3. PIP_3 attracts the proteins PDK1 and Akt to the cell membrane where PDK1 activates Akt. In addition, the protein mTOR can team up with rictor and other proteins inside the cell to form the mTORC2 complex, which further activates Akt. Once activated, Akt sets in motion a cascade of events that promotes protein synthesis, cell growth, cellular survival, sugar metabolism and cell division. One key component of this cascade is a group of proteins called the mTORC1 complex, which includes mTOR and raptor.

significant clinical activity in WM patients (see figure below). Nevertheless, WM patients treated with rapamycin do not achieve full remissions, perhaps because the drug cannot fully block this crucial signaling network.

Recently, Dr. Ghobrial's team decided to test a new drug called NVP-BEZ235 as a potentially more effective alternative to rapamycin in treating WM. Unlike rapamycin or PI3K inhibitors such as LY294002, NVP-BEZ235 is designed to block multiple components of the PI3K/Akt/mTOR signaling cascade (see figure below) and is therefore predicted to shut down the pathway more fully than single-target inhibitors. Supporting this hypothesis, Kirsch Laboratory investigators found that NVP-BEZ235 is a more potent inhibitor of the PI3K/Akt/mTOR pathway in WM cells than either LY294002 or rapamycin.

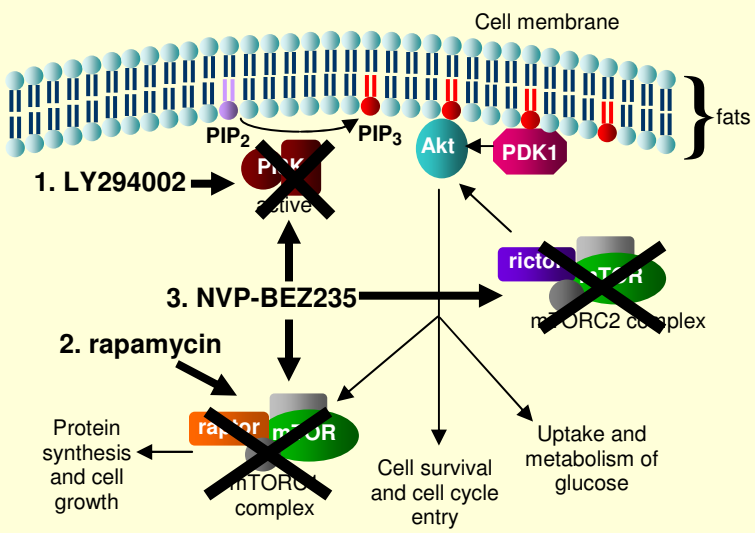
Additional analyses by the Kirsch team demonstrated NVP-BEZ235's anti-WM effects. The drug prevents patient-derived WM cells from growing and encourages them to self-destruct (see sidebar). In contrast, identical doses of the compound yield no toxic effects on normal blood cells taken from healthy volunteers, indicating the drug may one day help fight WM in patients without causing harmful side effects.

Despite these encouraging results, prior studies have shown that a drug's ability to kill WM cells in isolation is not necessarily a good



Dr. Ghobrial and her colleagues found that NVP-BEZ235 kills WM cells isolated from WM patients but has no toxic effect on normal peripheral blood mononuclear cells (PBMCs) derived from healthy donors. Further study revealed that NVP-BEZ235 kills WM cells by triggering a self-destruct program called apoptosis.

Drugs that inhibit components of the PI3K/Akt/mTOR Pathway

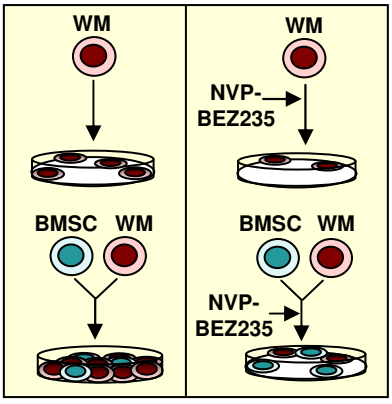


1. LY294002 directly blocks PI3K activity. 2. Rapamycin inhibits mTOR associated with raptor as part of the mTORC1 complex but has no inhibitory effect on mTORC2. 3. In contrast, NVP-BEZ235 is a potent inhibitor of PI3K as well as the mTORC1 and mTORC2 complexes.

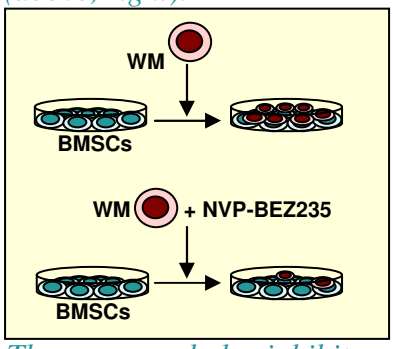
predictor of how well it will work as a treatment in WM patients. This is due, in part, to the artificiality of the system: Outside of the laboratory, WM cells live not as a homogeneous population but as members of a diverse cellular community in the bone marrow. There WM cells can form alliances with their neighbors to evade anti-WM medicines.

To test NVP-BEZ235 under more realistic conditions, researchers in the Kirsch Laboratory examined its effects on WM cells grown together with bone marrow stromal cells (BMSCs), which are known to stimulate WM cell growth and induce resistance to anti-WM compounds. The investigators found NVP-BEZ235 destroys WM cells even in the presence of BMSCs (see sidebar, top panel). The compound also prevents WM cells from sticking to BMSCs (see sidebar, middle panel) and inhibits WM cell migration toward molecules normally found in the bone marrow (see sidebar, bottom panel). The results suggest NVP-BEZ235's therapeutic benefits may extend beyond the compound's direct toxic effects on WM cells. The drug may also help destroy WM cells by preventing them from finding sanctuary in the bone marrow—an assertion further supported by Dr. Ghobrial's studies in mice (see figure below).

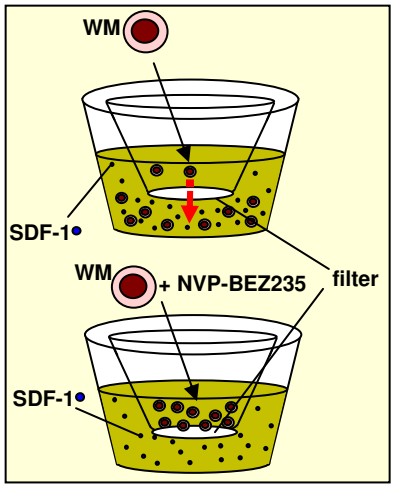
These discoveries make a strong case for NVP-BEZ235's potential as a therapy for WM patients; however, the drug is unlikely to defeat WM all on its own. Even the most effective anti-cancer



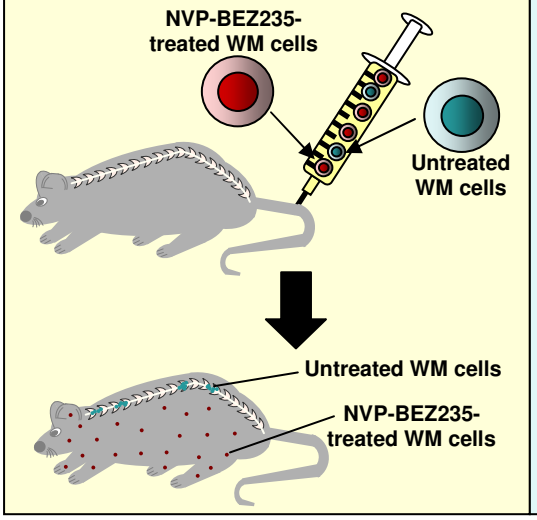
The growth-promoting effects of BMSCs on WM cells (above, left) are completely blocked by NVP-BEZ235 (above, right).



The compound also inhibits WM cell adherence to BMSCs (above) and prevents WM cells from migrating through a filter toward SDF-1—a molecule known to attract WM cells (below).



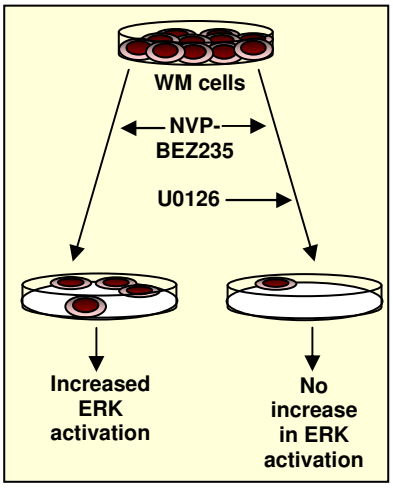
NVP-BEZ235 prevents WM cells from homing to the bone marrow



Dr. Ghobrial and her colleagues labeled NVP-BEZ235-treated WM cells with a red fluorescent tag while marking untreated WM cells with a green fluorescent tag. The investigators mixed treated and untreated WM cells together, injected them into mice and then used the fluorescent tags to determine where each cell population ended up at various time points. Untreated WM cells migrated to the bone marrow, whereas NVP-BEZ235-treated WM cells circulated freely in the bloodstream and did not home to the bone marrow.

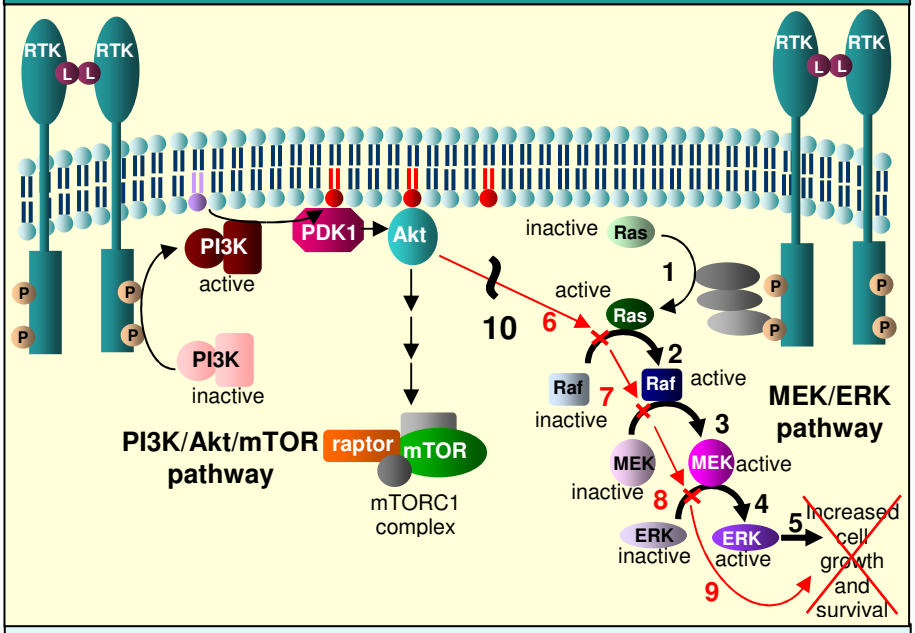
drugs, when used one at a time, cannot prevent the inevitable development of drug-resistant cancer cells. In fact, some anti-cancer drugs can actually promote specific mechanisms of drug resistance due to their unforeseen effects on molecules peripherally connected to the drug target.

For example, Dr. Ghobrial and her colleagues discovered that even as NVP-BEZ235 blocks the PI3K/Akt/mTOR pathway, it also indirectly stimulates another growth-promoting cellular communication network called the MEK/ERK pathway (see figure below). This creates an escape hatch for some WM cells, allowing them to withstand NVP-BEZ235 treatment. By adding an inhibitor of the MEK/ERK pathway to the mix, the researchers effectively walled off the escape hatch (see sidebar). The results hint that a therapy combining both drugs may be more effective and stave off drug resistance far better in WM patients than either drug alone.



NVP-BEZ235 indirectly stimulates the MEK/ERK pathway. To combat this potential mechanism of drug resistance, Kirsch investigators combined NVP-BEZ235 with the MEK1/2 inhibitor U0126 in WM cells. The researchers found that the drug combination blocks ERK activation and kills WM cells more effectively than NVP-BEZ235 alone.

Cross-talk between PI3K/Akt/mTOR and MEK/ERK pathways



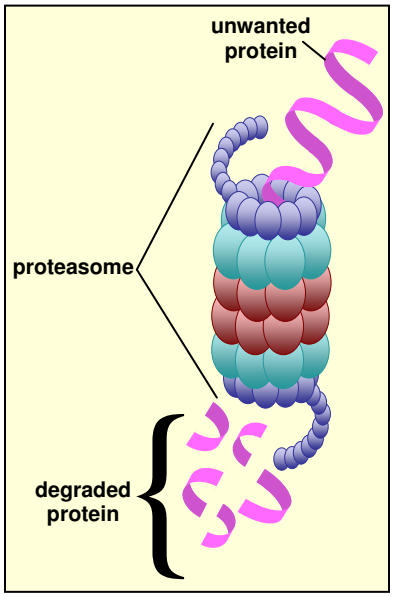
Normally, the MEK/ERK pathway is activated when the Ras protein is switched on (**1**) by proteins associated with ligand-bound RTKs. In its active form, Ras promotes Raf activity (**2**), which switches on MEK (**3**), which activates ERK (**4**), which promotes cell growth and survival (**5**). However, this straightforward cascade of events is complicated by the activities of proteins in other signaling pathways via a process known as "cross-talk." For example, Dr. Ghobrial's team uncovered evidence that in WM cells, a component of the PI3K/Akt/mTOR signaling pathway (Akt) inhibits the activity of Raf, which is a key protein in the MEK/ERK signaling pathway (**6**). This inhibition affects other proteins further along in the MEK/ERK signaling cascade: Because there is less active Raf around, fewer MEK molecules are activated (**7**), which means ERK is less active (**8**) and therefore less apt to promote cell growth and survival (**9**). Thus, activation of the PI3K/Akt/mTOR pathway can dampen signaling in the MEK/ERK pathway. By blocking PI3K (and therefore Akt) activity, NVP-BEZ235 severs the inhibitory connection (**10**) between these two pathways and promotes activation of ERK—a finding with important implications for the use of PI3K inhibitors as potential therapies for WM (see sidebar).

The Immunoproteasome: A New Anti-WM Drug Target

Communication networks represent one important class of anti-WM drug targets, but there are undoubtedly many others. One that was originally identified as a cancer drug target in the WM-related blood cancer multiple myeloma is the proteasome—a structure made up of many different proteins that work together to degrade unstable, dysfunctional or unneeded cellular proteins (see sidebar). The novel drug bortezomib, which is a remarkably effective treatment for multiple myeloma, was designed to block proteasomes. In so doing, bortezomib causes abnormal or unwanted proteins to accumulate and interfere with myriad cellular functions, including cell growth, gene expression and cellular survival. Multiple myeloma and WM cells are more sensitive to bortezomib's effects than normal cells and often die when exposed to the drug.

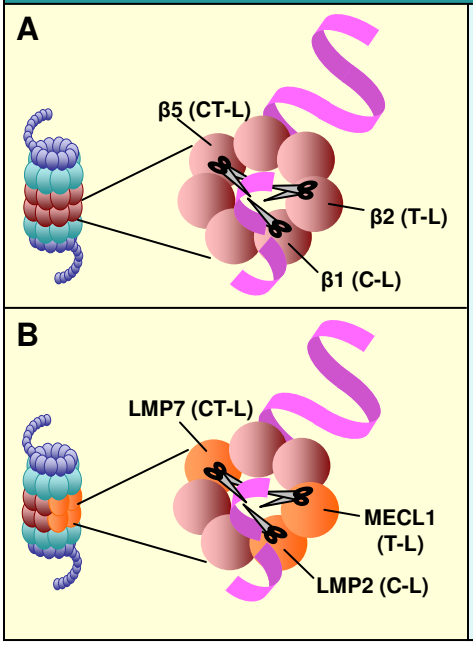
Nevertheless, bortezomib is no silver bullet. It can cause debilitating side effects due, in part, to its occasional "off-target" inhibition of other cellular proteins not involved in proteasome function. Furthermore, bortezomib does not work for all patients, and even those who do respond to the medicine inevitably develop resistance to it over time.

These obstacles point to the need for the development of next-generation proteasome inhibitors capable of targeting WM cells more specifically and effectively. One strategy for designing such compounds is to target the immunoproteasome (see below)—a



The proteasome is like a cellular garbage disposal system for unwanted proteins. Its outer ring-like structures guide unwanted proteins into its central core where specific components of the proteasome's inner rings chop the unwanted proteins into smaller pieces. These protein snippets are exuded from the other end of the proteasome.

Constitutive proteasomes and immuno-proteasomes: similarities and differences

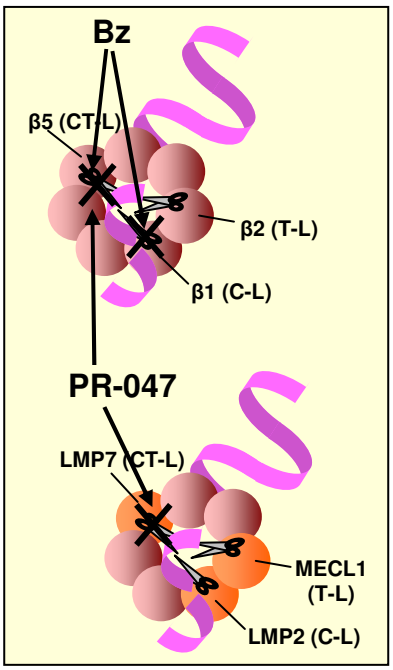


A. The proteasome's two inner rings (shown in red) are responsible for cutting unwanted proteins into smaller pieces. Each inner ring contains seven protein subunits, and some of these subunits have the ability to cut the unwanted protein in specific ways. The β5 subunit, for instance, has a chymotrypsin-like (CT-L) activity, which means it cuts the unwanted protein in a manner that resembles the cutting activity of a cellular enzyme called chymotrypsin. The β2 subunit has a trypsin-like (T-L) activity, so it behaves more like the protein-cutting enzyme trypsin, whereas the β1 subunit exhibits a caspase-like (C-L) cutting activity. **B.** The immunoproteasome also has CT-L, T-L and C-L activities, but the protein subunits performing these functions (shown in orange)—LMP7, MECL1 and LMP2—are distinct from the β subunits of regular (also known as "constitutive") proteasomes. These alternative subunits target different portions of unwanted proteins for cutting.

special kind of proteasome that is particularly prevalent in immune cells. Immunoproteasomes are thought to be especially important in the normal functioning of immune cells, so it stands to reason that these specialized structures could represent an Achilles' heel in cancerous immune cells, including those responsible for the development of WM.

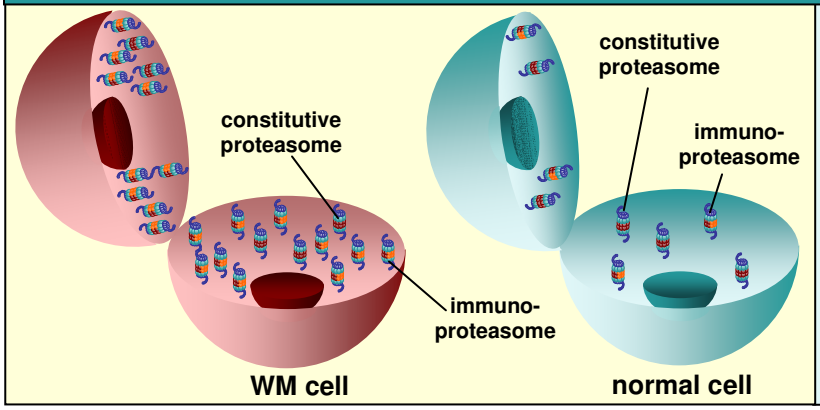
Dr. Ghobrial and her colleagues began to explore this possibility by measuring the abundance of regular proteasomes (often referred to as "constitutive" proteasomes) and immunoproteasomes in WM cells and in normal immune cells. The researchers found higher immunoproteasome levels in WM cells than in normal cells, and they also discovered that WM cells have higher levels of immunoproteasomes than constitutive proteasomes (see figure below). Their greater abundance in WM cells suggests immunoproteasomes may, indeed, play an important role in WM cell survival.

To directly test this prediction, Kirsch Laboratory investigators turned to a novel compound known as PR-047. This chemical inhibits both constitutive proteasomes and immunoproteasomes; however, it targets only one specific activity in both proteasome types and is therefore a more precise inhibitor than bortezomib, which blocks more than one protein-cutting activity within the proteasome (see sidebar). In both constitutive proteasomes and immunoproteasomes, PR-047 blocks the so-called CT-L activity, which appears to have special significance in blood cancers. (Previous studies have shown that blocking only the CT-L activity of proteasomes and immunoproteasomes is sufficient to combat malignancy in T-cell leukemia, Burkitt's lymphoma and multiple myeloma). Thus, Dr. Ghobrial and her colleagues used this



Bortezomib (Bz) primarily inhibits the proteasome's CT-L and C-L activities. In contrast, PR-047 inhibits only the CT-L activity of both regular proteasomes and immunoproteasomes.

Immunoproteasomes are more prevalent in WM cells

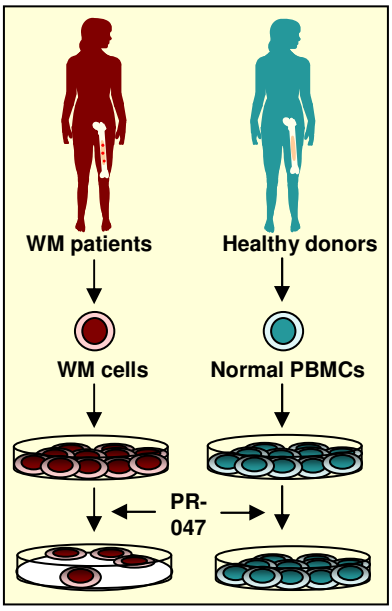


Dr. Ghobrial's team discovered that WM cells (left) have higher proteasome levels than normal cells (right). The investigators also found higher levels of immuno-proteasomes than constitutive proteasomes in WM cells.

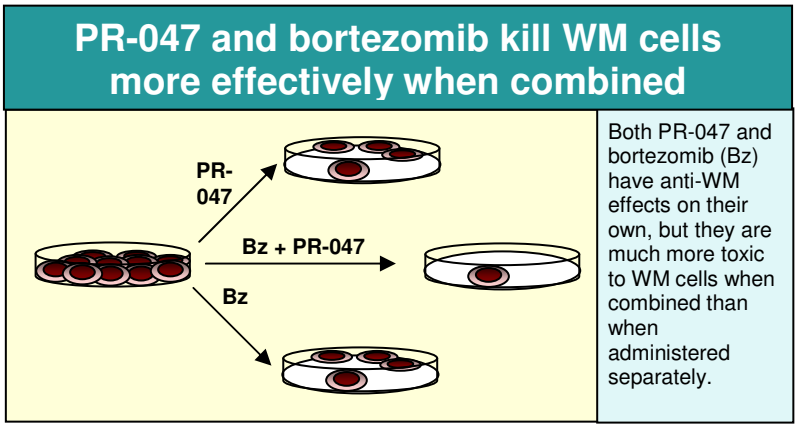
compound to study the effectiveness of CT-L proteasome/immunoproteasome inhibition as a potential therapeutic strategy for WM.

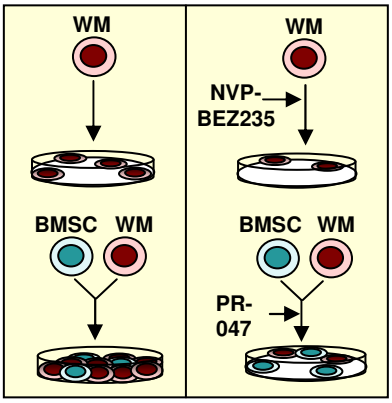
The results were extremely encouraging. The investigators found that PR-047 kills patient-derived WM cells but is not harmful to normal blood cells (see sidebar, top). Additional studies uncovered some of the mechanisms of PR-047's anti-WM effects: The drug rapidly triggers cell death in some WM cells, and in the survivors, the compound causes an accumulation of unfolded proteins and prolonged cellular stress, which leads to a second wave of WM cell death. Furthermore, PR-047's interference with proteins controlling gene expression and the cellular life cycle discourages the proliferation and survival of WM cells. The compound also prevents BMSCs from stimulating WM cell growth in the laboratory (see sidebar, bottom)—a finding that bodes well for PR-047's effectiveness against WM cells in the real-life context of human bone marrow.

The drug also appears to have anti-WM effects distinct from those of bortezomib. Kirsch investigators found that PR-047 and bortezomib kill WM cells much more effectively when combined than when used separately (see figure below). This suggests that even though both compounds target the proteasome's CT-L activity, they each have other unique anti-WM properties and can complement one another. Such findings encourage future pre-clinical and clinical testing of a potential bortezomib/PR-047 combination therapy for WM patients.



Dr. Ghobrial and her colleagues found that PR-047 kills WM cells isolated from WM patients but has no toxic effect on normal PBMCs derived from healthy donors. Further study revealed that PR-047 kills WM cells by triggering apoptosis.





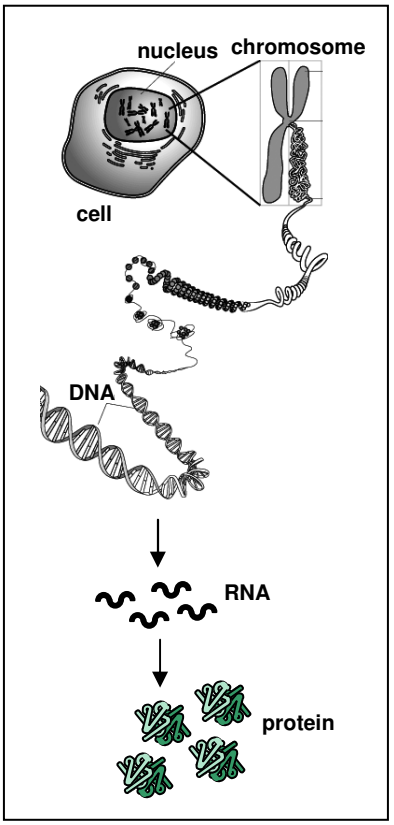
The growth-promoting effects of BMSCs on WM cells (above, left) are blocked by PR-047 (above, right).

Investigating the Role of MicroRNAs in WM

As the cell's molecular movers and shakers, proteins play a pivotal role in promoting and maintaining malignancy. However, a host of other cellular factors influence cancer-associated cellular behaviors by controlling gene expression—a process that helps determine how much of a particular protein is present within a cell at any given time (see sidebar). Over the past decade, scientists have identified a new class of molecules called microRNAs that help govern gene expression (see figure below), and more recent studies indicate cancer cells subvert the normal activities of some microRNAs to alter the expression of certain critical proteins thereby promoting abnormal cell growth. Last year, Dr. Ghobrial and her colleagues in the Kirsch Laboratory implicated a specific group of microRNAs in the development of WM.

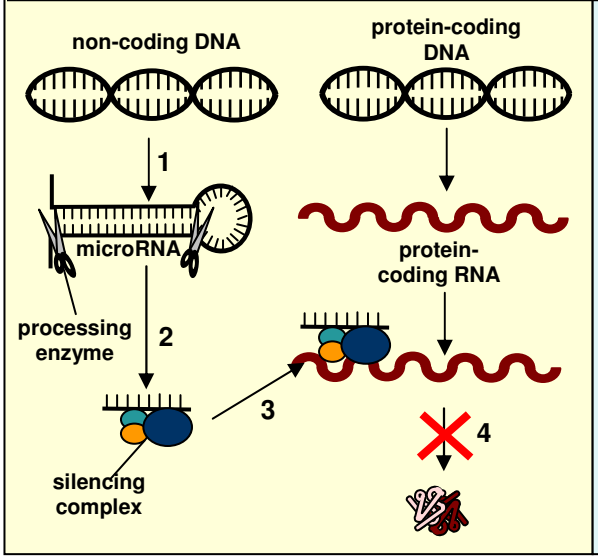
MicroRNAs usually work by switching off the expression of their target genes, and each microRNA has unique properties allowing it to target one specific gene or set of genes. This means that for any given microRNA, the higher the microRNA level, the lower the expression of its target gene(s) and vice versa. If the same microRNA is produced at much higher levels in one cell type compared to another, one would predict potentially significant differences in gene expression between the two cell populations. Dr. Ghobrial looked for such differences to identify microRNAs involved in WM development.

She and her team measured the abundance of hundreds of microRNAs in patient-derived WM cells and in normal immune



Genes are encoded in DNA, which is compressed and packaged into chromosomes inside the nucleus of a cell. When a gene is expressed, cellular factors "read" the genetic information on the DNA and use it as a blueprint to build RNA molecules that relay this information to the rest of the cell. The cell's machinery, in turn, builds proteins according to the specifications laid out in the RNA.

MicroRNAs: regulators of gene expression



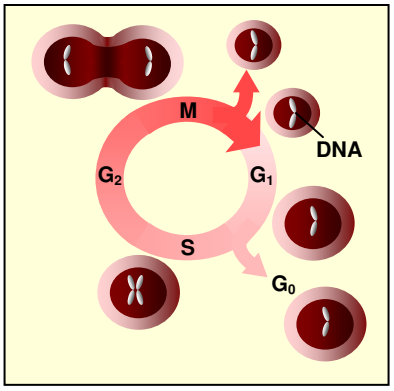
1. Certain DNA regions that do not encode proteins give rise to small RNA molecules called microRNAs. **2.** After being processed by other cellular factors, microRNAs bind to a group of proteins called "silencing complexes." **3.** MicroRNAs guide the silencing complexes to specific protein-coding RNAs. **4.** The complex prevents the cell from translating the information on the RNA molecule into a protein.

cells isolated from healthy volunteers. The investigators found seven microRNAs that correlate with disease status: six are consistently more prevalent in WM cells than in normal immune cells and one is produced at higher levels in normal cells than in WM cells (see table below). More detailed inspection revealed that the six "high-prevalence" microRNAs are even more abundant in high-risk WM patients than in low-risk patients, further supporting the idea that these microRNAs play an important role in promoting malignancy.

One of these molecules, microRNA-155, has already been implicated in a number of other blood cancers, so the researchers decided to focus on it during their subsequent analyses. They found that a molecule designed to block microRNA-155 significantly inhibits WM cell growth by forcing WM cells into a dormant phase of the cellular life cycle (see sidebar). The anti-microRNA-155 molecule also renders laboratory-grown WM cells less adherent to factors present on supportive cells in the bone

microRNAs	9	155	184	206	363	494	542 -3p
1 WM cells → WM patients (high or intermediate risk)	↓	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑
2 WM cells → WM patients (low risk)	↓	↑	↑	↑	↑	↑	↑
3 normal cells → Healthy donors	↑	↓	↓	↓	↓	↓	↓

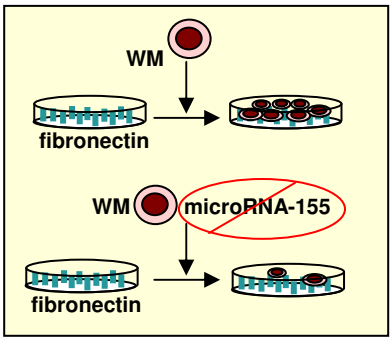
Kirsch Laboratory investigators isolated WM cells from high- to intermediate-risk WM patients **(1)** and from low-risk WM patients **(2)**. For comparison, the researchers used normal immune cells derived from healthy volunteers **(3)**. In each cell population, the scientists measured the levels of more than 300 microRNAs, searching for specific microRNAs that are more prevalent in WM patients than in healthy donors or vice versa. The team found that six microRNAs (-155, -184, -206, -363, -494 and -542-3p) are present at higher levels in WM cells compared to normal immune cells, whereas one microRNA (-9) is less prevalent in WM cells than in normal immune cells. Furthermore, WM patients classified as high or intermediate risk based on a number of prognostic factors (age, hemoglobin and immunoglobulin levels, platelet counts, etc.) have significantly higher levels of microRNA-155, -184, -363, -494 and -542-3p in their WM cells than low-risk WM patients.



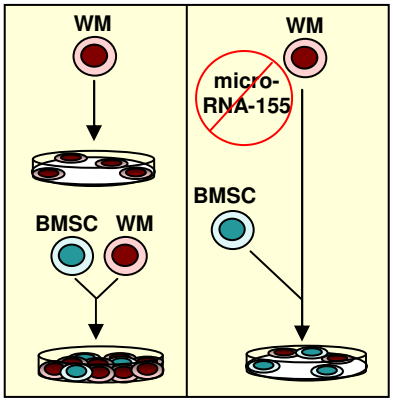
A dividing cell goes through several distinct phases as part of its life cycle. After passing through an initial growth phase (G_1), the cell makes an exact copy of its DNA during S phase. It then enters a second growth phase (G_2) and subsequently splits into two daughter cells during the M phase. When a population of cells reaches a certain critical mass, its constituent cells normally exit the cell cycle just before the G_1-S transition, entering a dormant phase known as G_0. In contrast, WM cells tend to blow right past this important checkpoint and continue growing regardless of population size. Dr. Ghobrial and her team discovered they could force WM cells into the G_0 phase by blocking microRNA-155.

marrow (see sidebar, top), and it blocks the growth-promoting effects of BMSCs (see sidebar, middle). Furthermore, microRNA-155 blockade prevents WM cells from migrating toward attractants normally found in the bone marrow (see sidebar, bottom) and impedes WM cells from homing to the bone marrow in mice. These findings strongly suggest that microRNA-155 is an important factor in the development of WM, and drugs or molecules that reduce its levels in WM cells have great potential as new treatments for this disease.

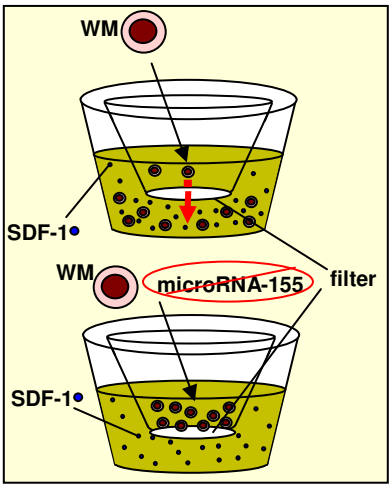
Additional support for this prediction came from studies examining the effects of existing WM therapies on microRNA levels. Kirsch investigators found that drugs already used to treat WM, including rituximab, perifosine and bortezomib, significantly reduce the levels of microRNA-155, -184, -494 and -363. Although the drugs are known to combat WM cells by a number of different mechanisms, their effects on microRNA levels may account for at least some of their anti-WM properties. The results further validate therapeutic strategies in which researchers target specific microRNAs to fight cancer cells in WM patients.



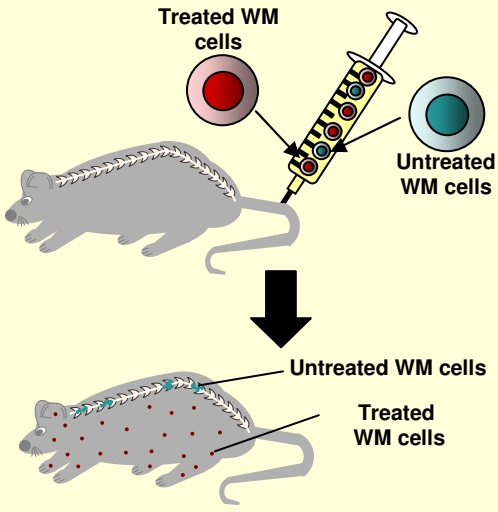
A molecule designed to block microRNA-155 impedes WM cell adherence to fibronectin (above) and inhibits the growth-promoting effects of BMSCs on WM cells (below).



MicroRNA-155 blockade also prevents WM cells from migrating through a filter toward SDF-1—a molecule known to attract WM cells (below).



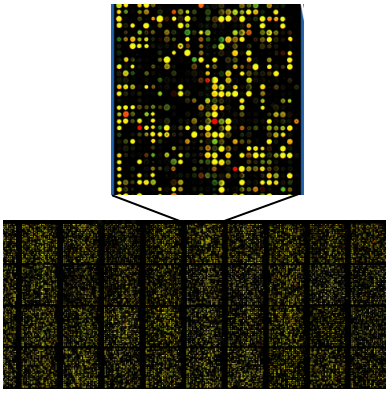
Blocking microRNA-155 prevents WM cells from homing to the bone marrow



Dr. Ghobrial and her colleagues used a green fluorescent tag to track untreated WM cells and a red tag to track WM cells treated with a molecule that blocks microRNA-155. The investigators mixed treated and untreated WM cells together, injected them into mice and then used the fluorescent tags to determine where each cell population ended up at various time points. Untreated WM cells migrated to the bone marrow, whereas treated WM cells circulated freely in the bloodstream and did not home to the bone marrow.

Mining the Genome to Uncover the Genetic Roots of WM

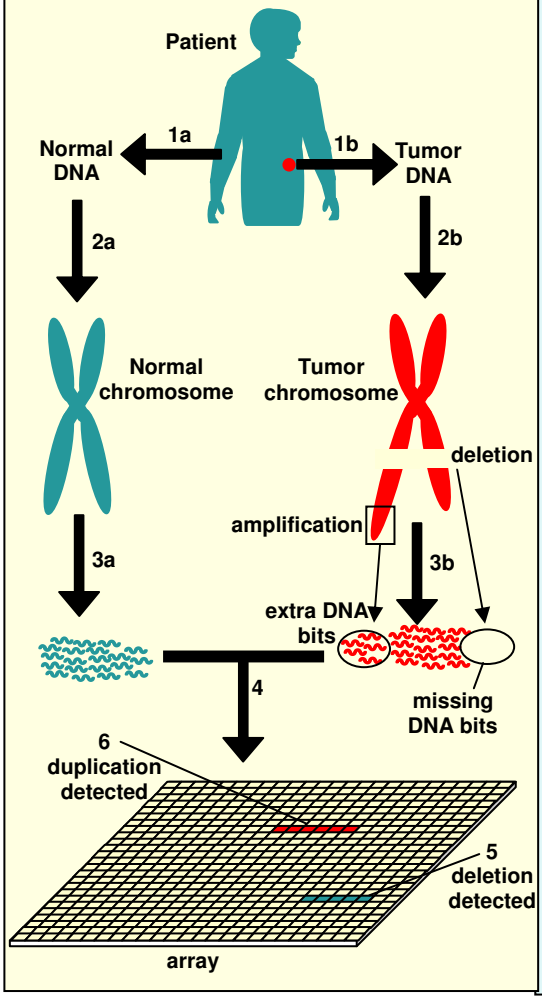
Cancer is characterized by the ill-timed production and inappropriate activities of many different molecules in the cell, including proteins and microRNAs; however, these abnormally-behaving molecules do not represent the root causes of disease. Like foot soldiers on the battlefield, they follow the genetic orders specified by their commander, DNA. The true masterminds of malignancy, therefore, are genetic mutations—aberrations in the DNA that instruct key molecules in the cell to take on abnormal properties. If we are to strike at the heart of WM, we must find the DNA aberrations that are ultimately responsible for orchestrating cancerous cellular behaviors in WM cells.



DNA microarrays allow researchers to monitor all of the genes in the human genome simultaneously. Each dot on the array corresponds to a snippet of DNA at a specific chromosomal address within the genome.

To do this, Kirsch Laboratory investigators have turned to genome-scanning technologies such as aCGH (see figure below) that can rapidly locate and identify potential cancer-causing abnormalities in DNA extracted from malignant cells. Dr. Ghobrial and her

Array comparative genome hybridization (aCGH)



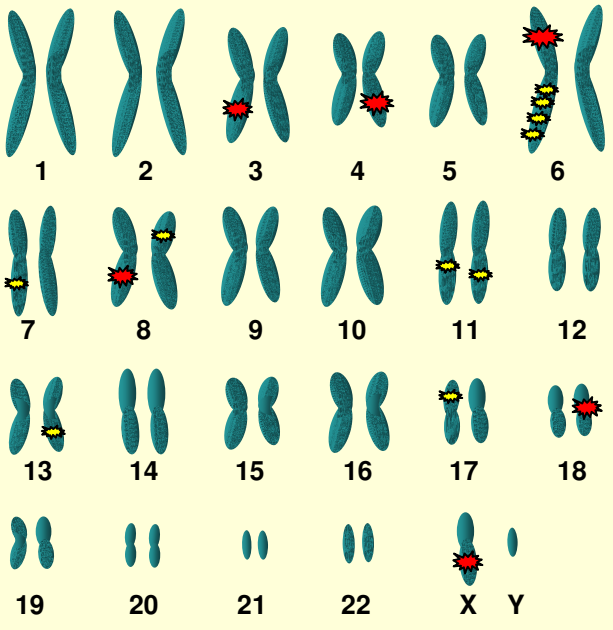
Scientists extract DNA samples from a cancer patient's normal **(1a)** and tumor tissues **(1b)**. Researchers color each DNA sample with a unique fluorescent marker, shown here as green for normal DNA and red for tumor DNA **(2a&b)**. They chop the DNA samples into smaller bits **(3a &b)**, mix the normal and tumor DNA bits together and add the bits to an array **(4; see sidebar)**. Each bit comes from a particular place on the chromosome, and the array is designed in such a way that every DNA bit sticking to each spot on the surface of the array can be matched to its specific chromosomal address. At most spots, an equivalent number of green and red DNA bits bind (this is depicted as yellow squares on the array). However, if some tumor DNA bits are missing due to chromosomal deletions, those spots on the array corresponding to the deleted regions appear green **(5)** because only green DNA bits are around to bind to those spots. Conversely, if there are extra tumor DNA bits due to a chromosomal amplification, the spots on the array corresponding to the duplicated region of the chromosome appear red **(6)** because there are more red DNA bits than green ones. Thus, by simply detecting green and red spots on the array, researchers can pinpoint the precise locations of chromosomal abnormalities in tumor cells.

collaborators used this technology to identify 187 genetic aberrations (110 deletions and 77 amplification) in WM cells taken from 42 WM patients. In some cases, WM cells from three or more patients harbored DNA abnormalities that mapped to the same chromosomal address. The scientists identified 16 such recurrent regions, which are illustrated in the figure below.

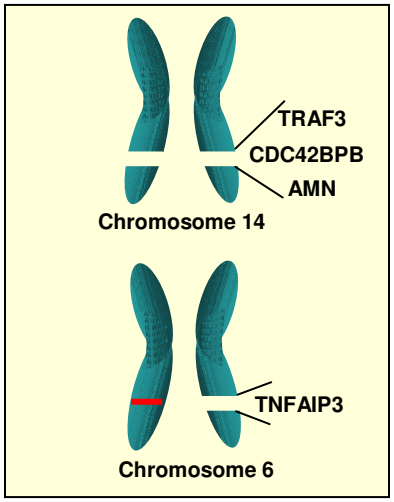
One recurring chromosome 6 deletion encompasses a gene called *TNFAIP3* whose protein product helps regulate the NF-κB pathway (see figure on page 14)—a regulatory system that has been implicated in WM cell growth and survival. Dr. Ghobrial and her colleagues more carefully analyzed the *TNFAIP3* gene in WM patients and discovered that one patient who had a ***monoallelic*** deletion of the gene (see definitions box) also bore a smaller genetic aberration in *TNFAIP3* on the other copy of chromosome 6 (see sidebar). WM cells in this patient would not be able to produce the normal protein product of *TNFAIP3*—a finding that implies *TNFAIP3* mutations may help contribute to the development of WM in some patients. The researchers also found one patient with a ***biallelic*** deletion of another gene (*TRAF3*) involved in the NF-κB pathway (see sidebar), further validating NF-κB's role in WM.

These are just a few examples of the important clues uncovered by this study. The findings lay the groundwork for future analyses aimed at more precisely defining which specific genes and mutations drive malignant behaviors in WM cells.

WM-related chromosomal abnormalities



Dr. Ghobrial and her collaborators searched all 23 pairs of chromosomes in the human genome for abnormalities associated with WM. The researchers found several anomalies that occurred in malignant cells taken from not just one but three or more WM patients. These recurring anomalies clustered to 16 specific regions, which are illustrated in this figure. Ten represent chromosomal regions commonly deleted in WM patients (depicted in yellow) and six chromosomal regions are frequently amplified (red). In almost all patients, each of these anomalies was ***monoallelic*** (see definitions box).

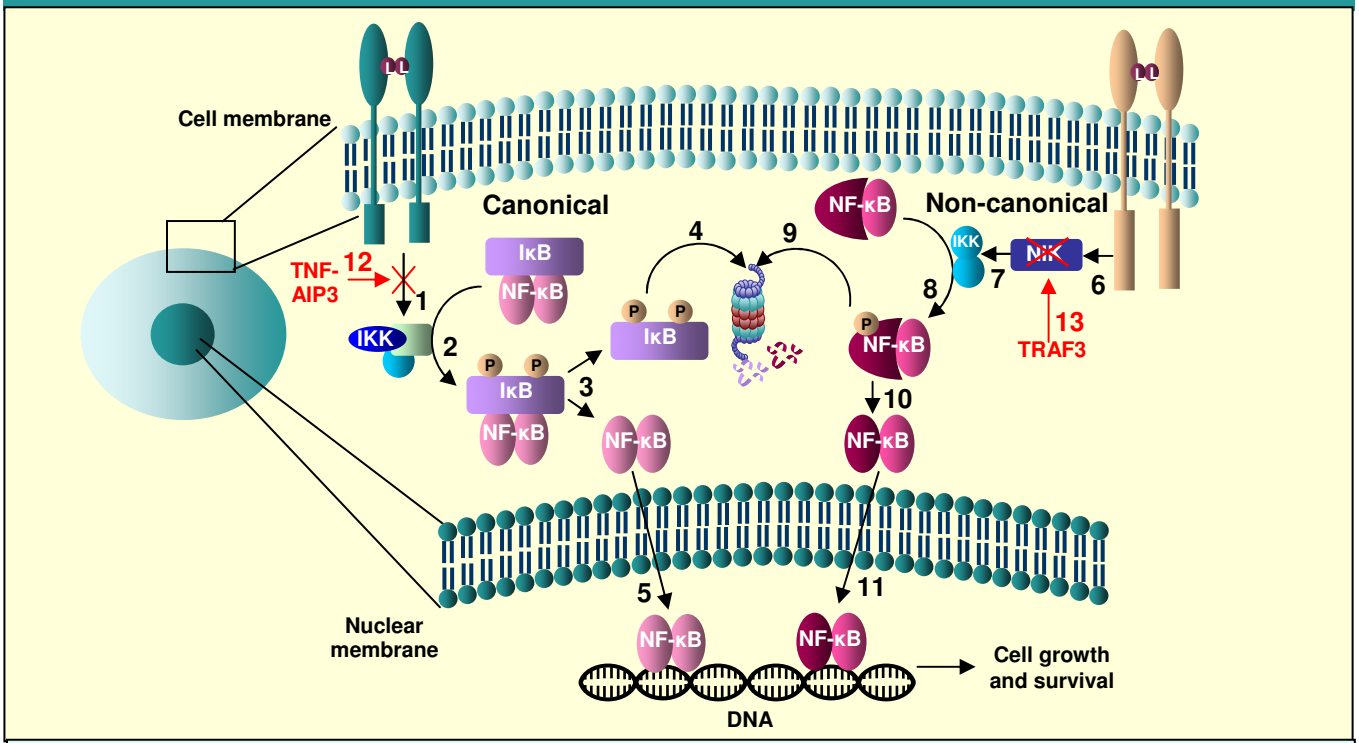


One patient in this study had WM cells with a **biallelic deletion** *on chromosome 14; therefore, the genes within the deleted region, including* TRAF3, *were entirely missing. Another patient's WM cells had a deletion on one copy of chromosome 6 (the deletion spanned the region containing the TNFAIP3 gene) and a much smaller anomaly in the TNFAIP3 gene on the other copy of chromosome 6 (shown in red).*

Definitions

The human genome is comprised of 23 pairs of chromosomes. This pairing creates redundancy: If a gene is deleted on one chromosome within a chromosomal pair (a so-called **monoallelic** deletion), there is still another backup copy on the other chromosome in the pair. The anomalies depicted in the figure to the left are all monoallelic. However, if both chromosomes of a chromosomal pair bear deletions within the same region (referred to as a **biallelic** deletion) there is no backup copy of the deleted genes. A biallelic deletion is shown on chromosome 14 in the sidebar figure above.

The NF-κB Pathway



Proteins in the NF-κB family are important regulators of gene expression, and the cell has more than one way to activate them. **1.** In the "canonical pathway," receptors in the membrane respond to environmental signals by activating a complex of proteins called IKK. **2.** Active IKK adds chemical groups to another protein, IκB, which normally binds to and inhibits NF-κB. **3&4.** In its modified form, IκB releases NF-κB and is degraded by the proteasome. **5.** Unbound NF-κB passes through the nuclear membrane and binds to specific sections of DNA inside the nucleus. This, in turn, switches on the expression of neighboring genes whose protein products promote cellular growth and survival. The non-canonical pathway works by a similar mechanism. **6.** Certain receptors in the membrane activate a protein called NIK in response to particular signals in the external environment. **7&8.** Working through a slightly different IKK complex than the one involved in the canonical pathway, activated NIK chemically modifies a specific member of the NF-κB family (again, this particular variant differs from NF-κB involved in the canonical pathway). **9.** When modified in this way, portions of the NF-κB protein complex become vulnerable to degradation by the proteasome. **10&11.** The non-degraded portions take on new properties that allow NF-κB to pass through the nuclear membrane. As with the canonical pathway, when NF-κB binds to the DNA, it activates expression of genes whose protein products encourage cell growth and survival. **12.** Recent evidence indicates that TNFAIP3 can inhibit the canonical NF-κB pathway by preventing the activation of IKK. **13.** Furthermore, TRAF3 can block NIK, thereby shutting down the non-canonical NF-κB pathway.

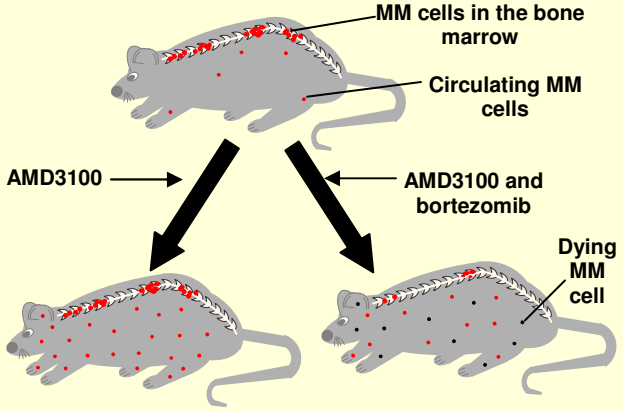
Future Directions

The anti-WM compounds described in this report hold great promise as treatments for WM patients, but such drugs may never reach their full therapeutic potential as long as they are forced to fight on WM cells' home turf. Even anti-WM drugs that can work in the bone marrow would undoubtedly kill WM cells much more effectively without interference from WM's many allies in the bone marrow. With this in mind, Dr. Ghobrial and her colleagues have identified a new type of drug that may level the playing field.

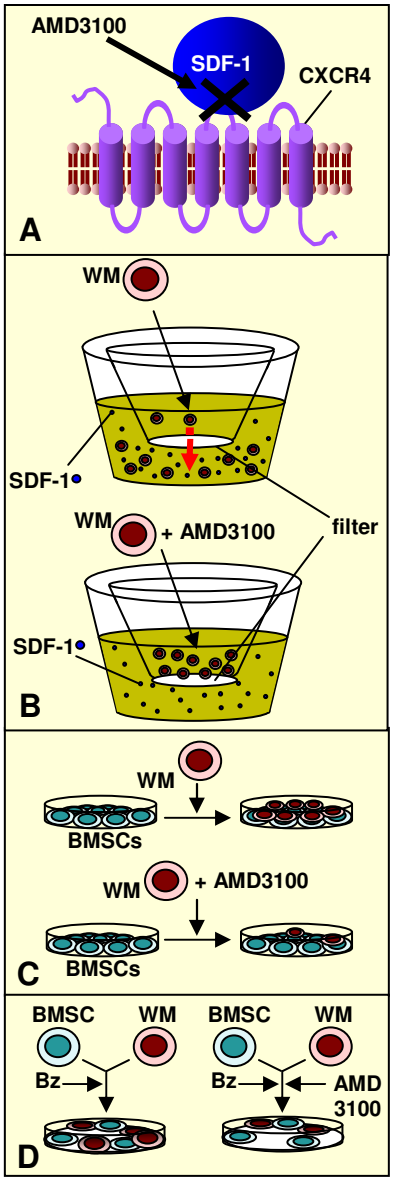
AMD3100 is a chemical designed to block the interaction between the WM-attracting molecule SDF-1 and a protein on the surface of WM cells called CXCR4 (see sidebar). Kirsch investigators previously demonstrated that this compound impedes WM cell migration toward SDF-1, reduces WM cell adherence to BMSCs and enhances bortezomib's anti-WM effects in the presence of BMSCs. Dr. Ghobrial has observed similar effects on multiple myeloma (MM) cells, suggesting that the compound may work similarly in both diseases.

Last year, she and her team examined the effects of AMD3100 on MM in mice (see figure below). The researchers discovered that the drug forces MM cells out of the bone marrow and into the circulatory system where the cells are much more vulnerable to attack by anti-MM compounds like bortezomib. When combined with bortezomib, AMD3100 shrinks MM tumors even more than bortezomib alone, encouraging clinical investigations of this combination therapy. Scientists in the Kirsch Laboratory are now testing whether AMD3100 has similar effects against WM in mice. If so, AMD3100 and other compounds like it may represent an entirely new strategy for treating both WM and MM.

Forcing MM cells out of their hiding places



Dr. Ghobrial and her colleagues discovered that AMD3100 forces multiple myeloma (MM) cells out of the bone marrow and into the circulatory system in mouse models of the disease. The compound also enhances bortezomib-induced tumor regression and renders circulating MM cells vulnerable to bortezomib-mediated cell death.



*AMD3100 prevents a membrane protein called CXCR4 from binding to SDF-1 (**A**). The compound also blocks WM cell migration toward SDF-1 (**B**), inhibits WM cell adherence to BMSCs (**C**) and renders WM cells more sensitive to bortezomib (Bz) in the presence of BMSCs (**D**).*

The Kirsch Laboratory: Leading the Fight Against WM

Despite many hurdles and a widespread dearth of government funding, our understanding of WM has greatly expanded over the past year thanks to the work of Dr. Ghobrial and her colleagues in the Kirsch Laboratory at Dana-Farber's Bing Center for Waldenström's Research. These advances could not have happened without philanthropic support. By bringing state-of-the-art technologies and genomic tools to bear on this orphan disease, your gift has paved the way for rapid progress in a field that has been neglected by the scientific community for far too long. Ultimately, these discoveries promise to help WM patients live longer, healthier lives.

On behalf of Dr. Ghobrial and her colleagues in the Michele and Steven Kirsch Laboratory for Waldenström's Research, thank you for making this critical work possible.