MYD88, CXCR4 and the Genetics of WM

Zachary Hunter
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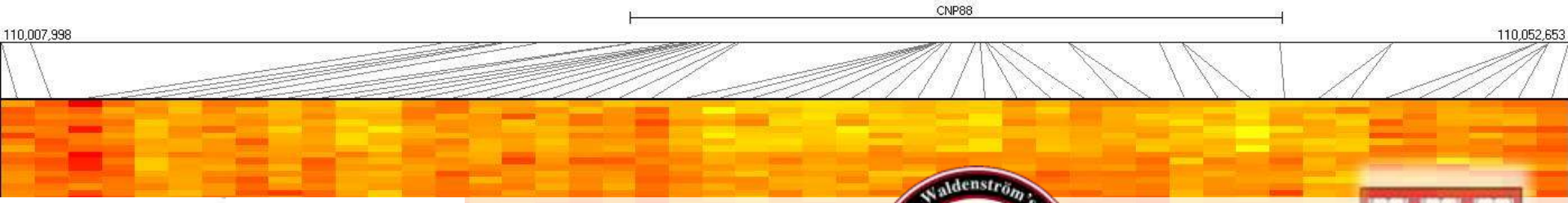
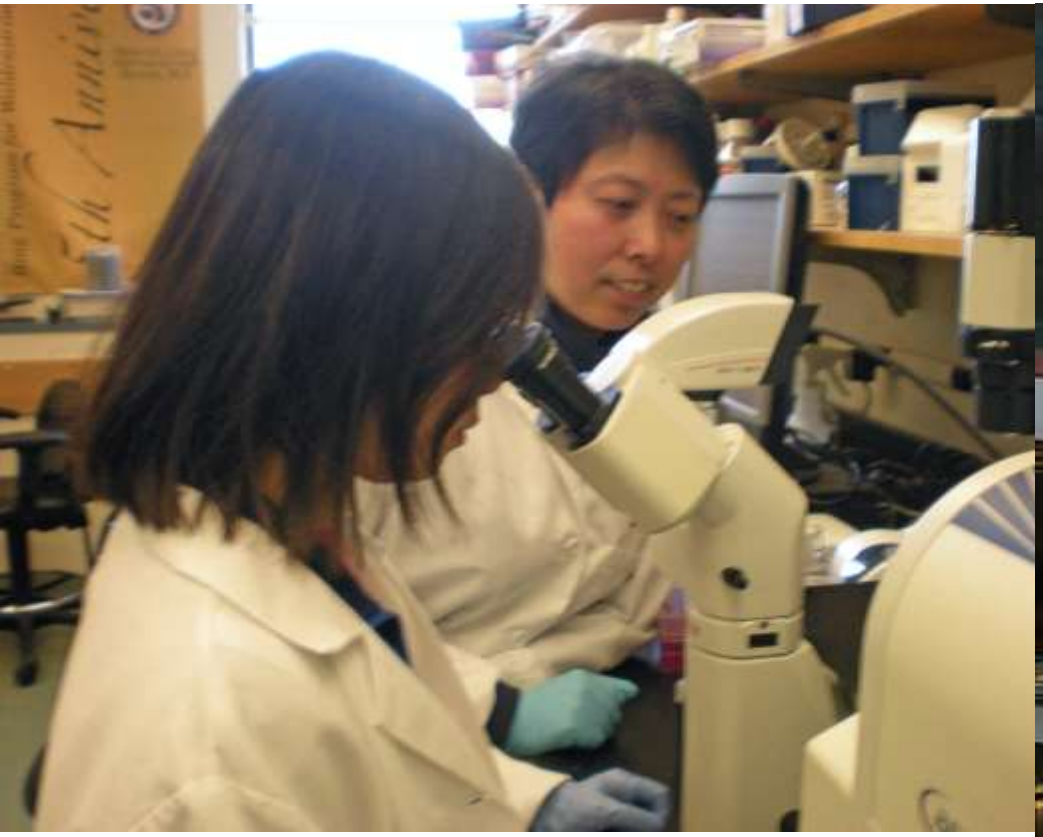


DANA-FARBER CANCER INSTITUTE

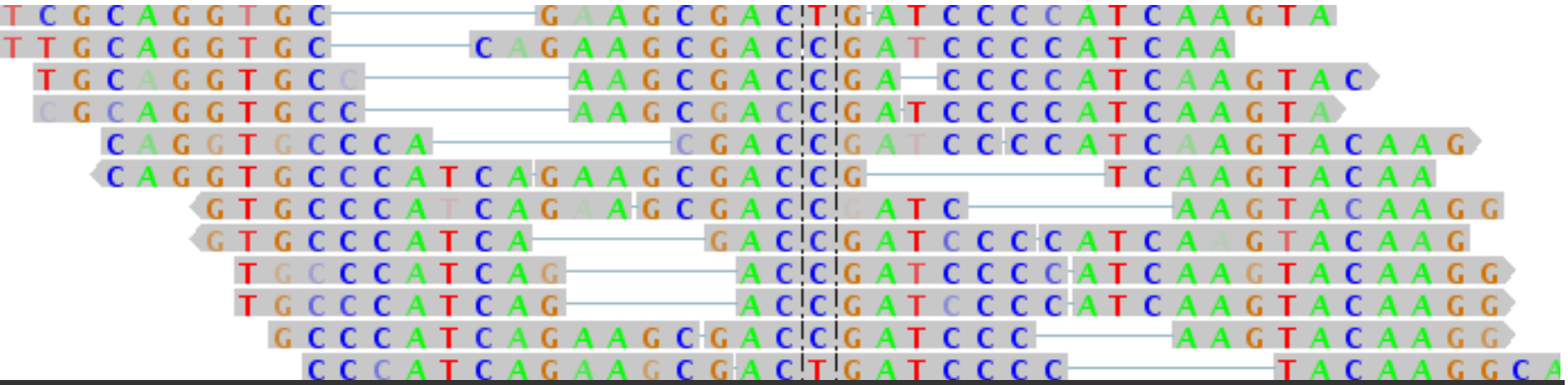
Bing Center for WM Research at the Dana-Farber Cancer Institute

Harvard Medical School



DANA-FARBER
CANCER INSTITUTE

Bing Center for WM Research at the Dana-Farber Cancer Institute



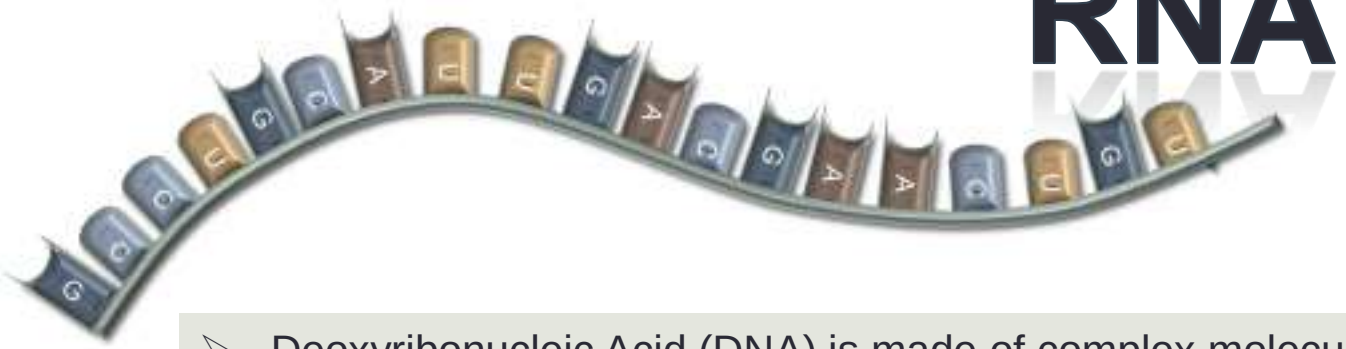
Understanding Genetics

If you only had four letters to work with, what kind of story could you tell?

So What is DNA Anyway?

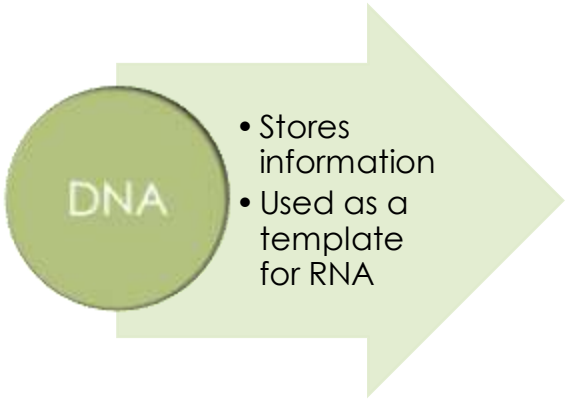
DNA

RNA



- Deoxyribonucleic Acid (DNA) is made of complex molecules called nucleotides. There are 4 types abbreviated A,T,C,G.
- Nucleotide bases form stables pairs: A-T and C-G. Two complimentary strands of these bases form DNA.
- DNA is broken into 23 long strands known as chromosomes.
- The sections of the DNA that contain instructions on how to build proteins are called genes.
- Genes in the DNA are transcribed into a single strand of similar nucleotides called Ribonucleic Acid (RNA) and this “message” is processed by the cell and turned into protein.

DNA – RNA – Protein



- ***Genes are regions of the DNA that are transcribed into RNA***
- ***RNA carries the DNA code out into the rest of the cell where it can be used as instructions to make protein***

Identical Twin Studies

While identical twins share many things in common, they also have very different lives, unique personalities, get different diseases and after a number of years can look quite different from each other.

theguardian | TheObserver

Why do identical twins end up having such different lives?

Their genes are exactly the same, so why don't identical siblings' lives follow more similar patterns? The scientist behind a pioneering 21-year study believes he has the answer

Robin McKie

The Observer, Saturday 1 June 2013

Jump to comments (0)

Your DNA is a blueprint for building proteins, NOT a blueprint for who you are

You are much more than the sum of your genes

If People are Genetically Unique, How do we Make Comparisons?

- There are over 3 billion base pairs in the human genome. We receive a complete copy from each parent.
- To create a common basis for comparison, a reference genome was created. This reference is not a single person but a mosaic of 13 different individuals.

The Human Reference Genome (Print Edition)

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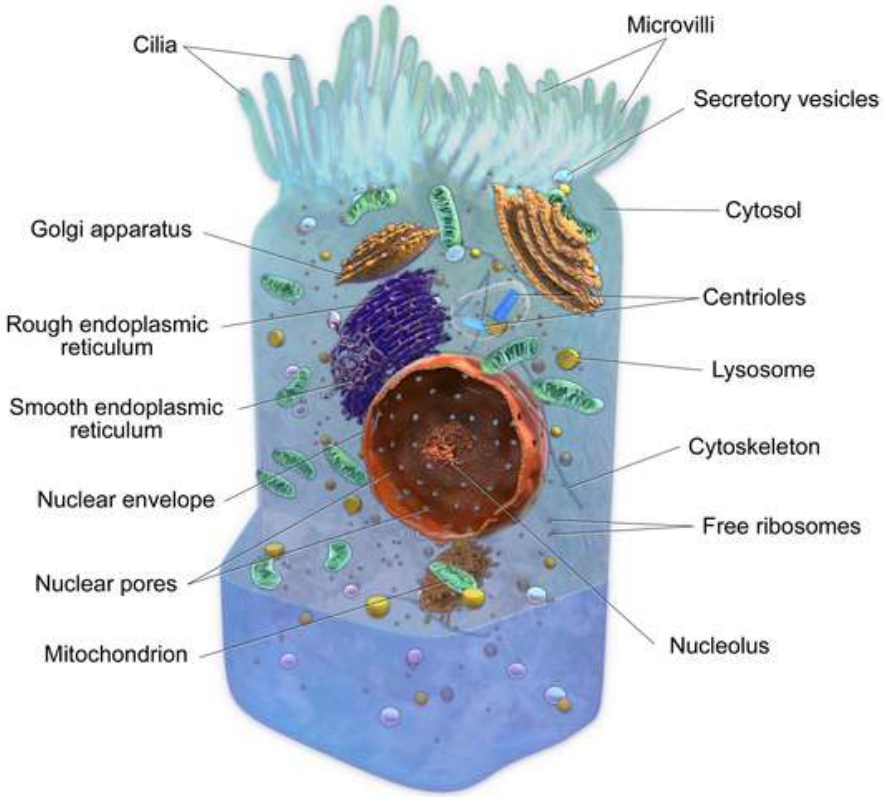
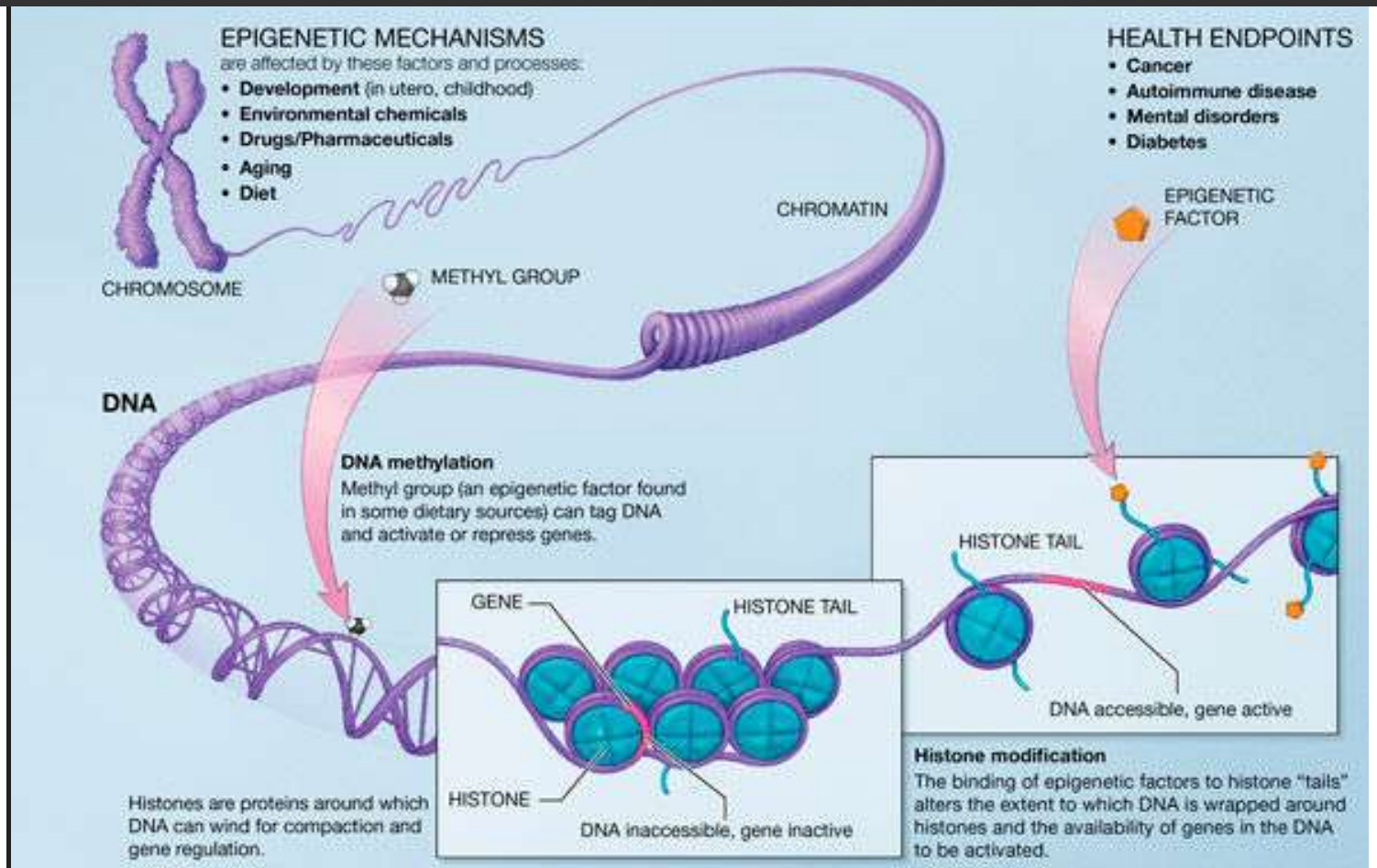
How to make it all fit…



Anatomy of a Cell

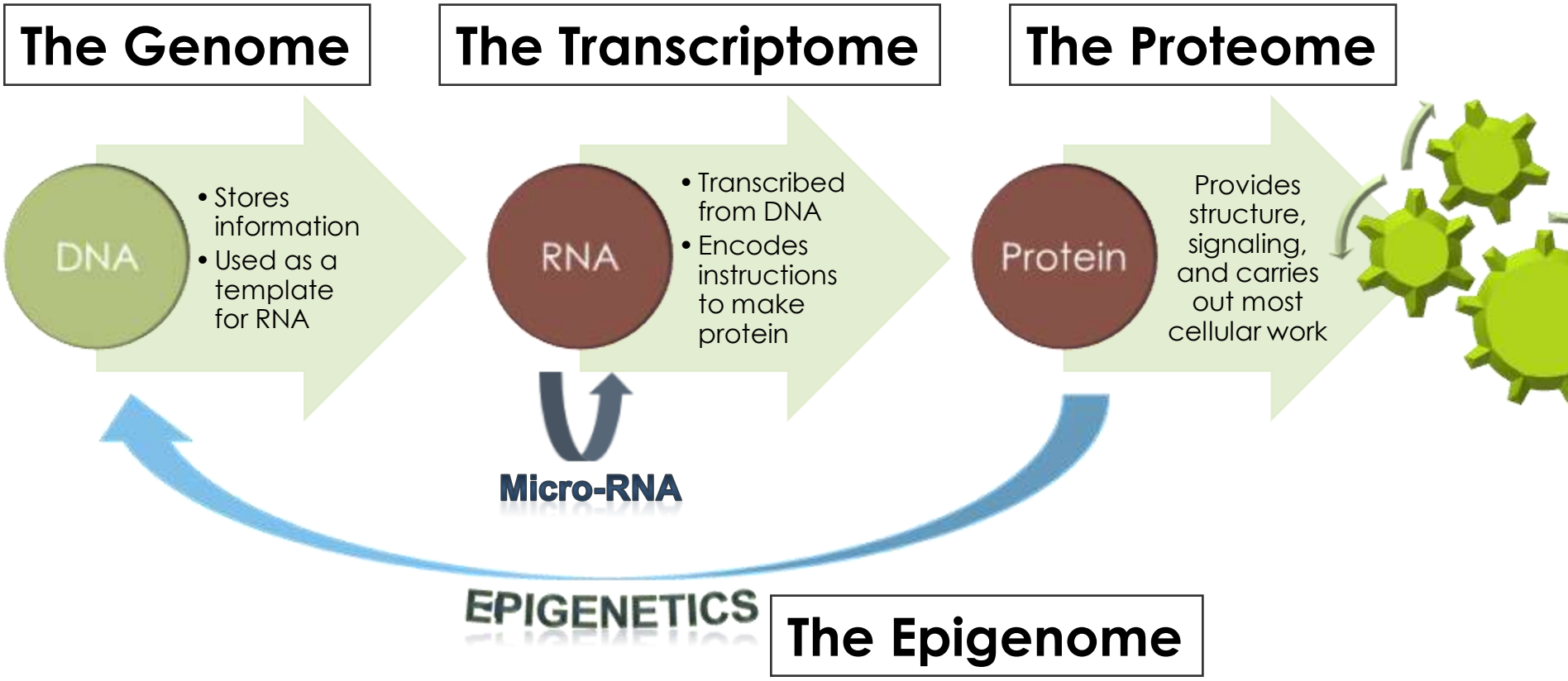
- 3 billion base pairs strung end to end is about 40 inches in length.
- This quantity of DNA resides inside the nucleus of every cell in your body
- Needless to say, this is a tightly controlled process.

Our apologies, the DNA you are looking for is temporarily unavailable…



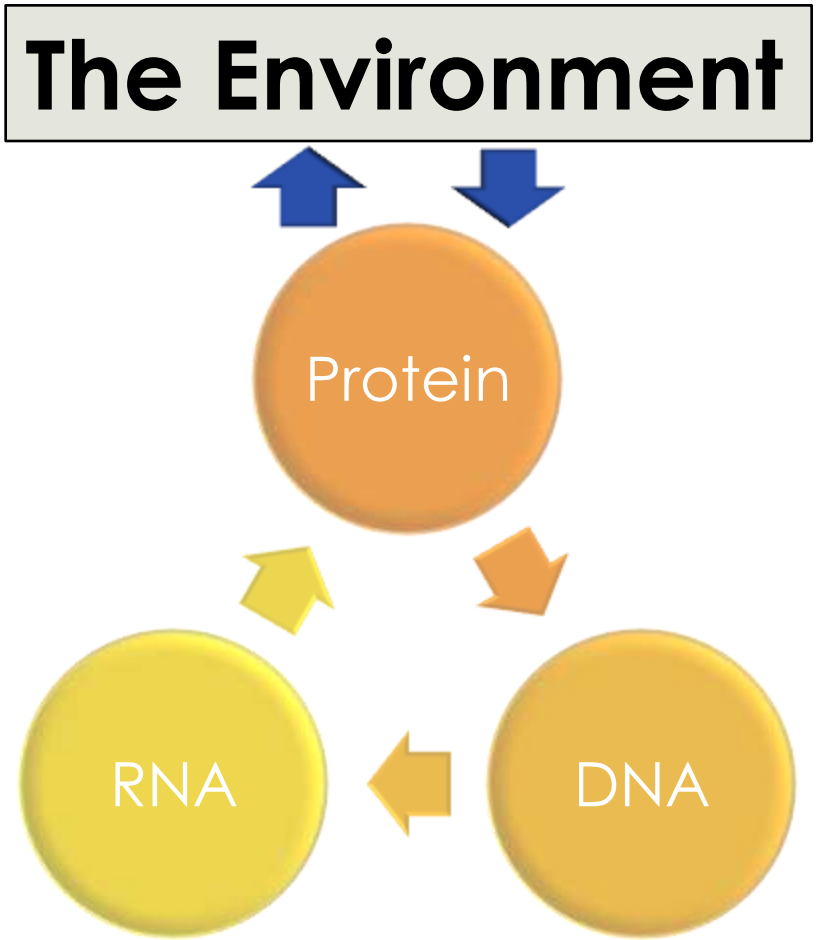
Laura, B. (2008) Epigenomics: The new tools in studying complex disease. Nature Education

Introducing the "omes"

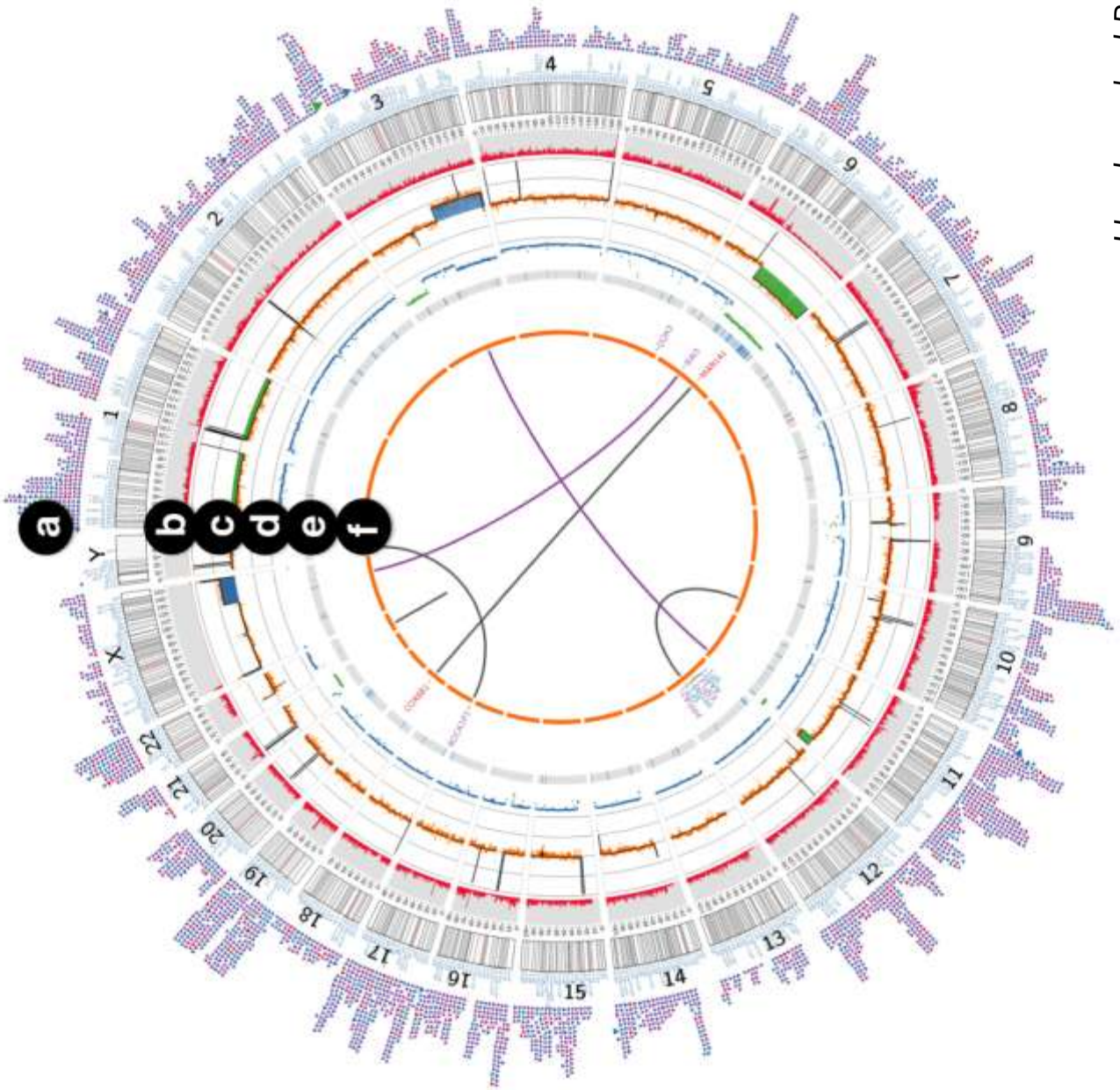


The genome as the cell's operating system

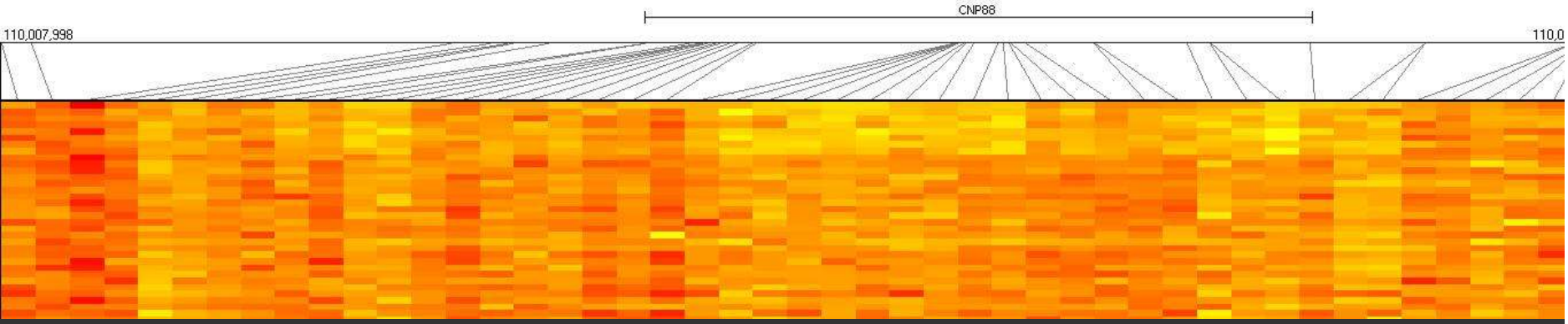
The operating system of your computer (Windows/Max OSX/Linux) provides a set of tools to allow programs to be loaded and run. Every laptop may have the same operating system, but no one uses their laptop in exactly the same way



Whole Genome Integrative Data Visualization



Hunter et al Blood 2013



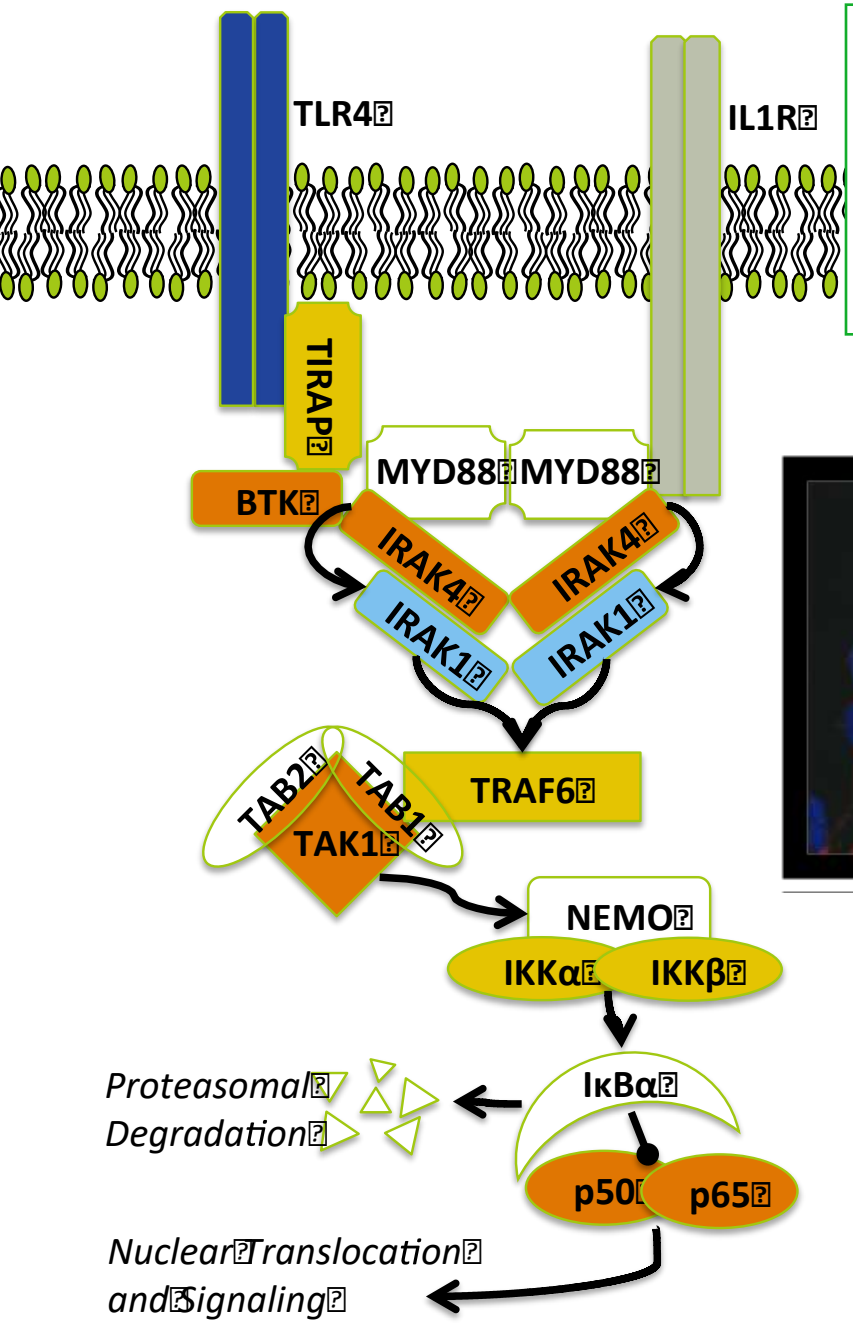
The Genomics of WM

Understanding the WM "Operating System"

Mutation and Cancer

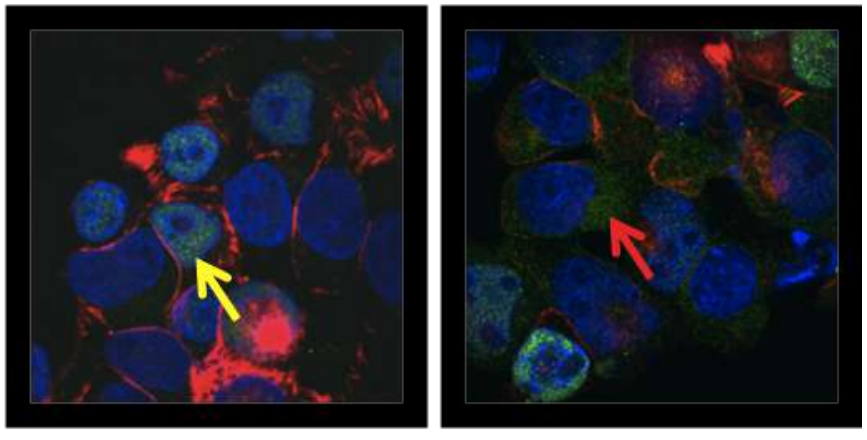
- Cancer can be caused by accidental changes in the genome known as mutations
- As these accumulate, genes start to gain new functions, or lose old ones
- At the point that the cells start to multiply in an uncontrolled way, we call it cancer

MYD88 Signaling



A single base pair mutation the gene MYD88 is found in over 90% of WM

NFKB Nuclear Translocation in The BCWM.1 Cell Line



Control | MYD88 Inhibitor

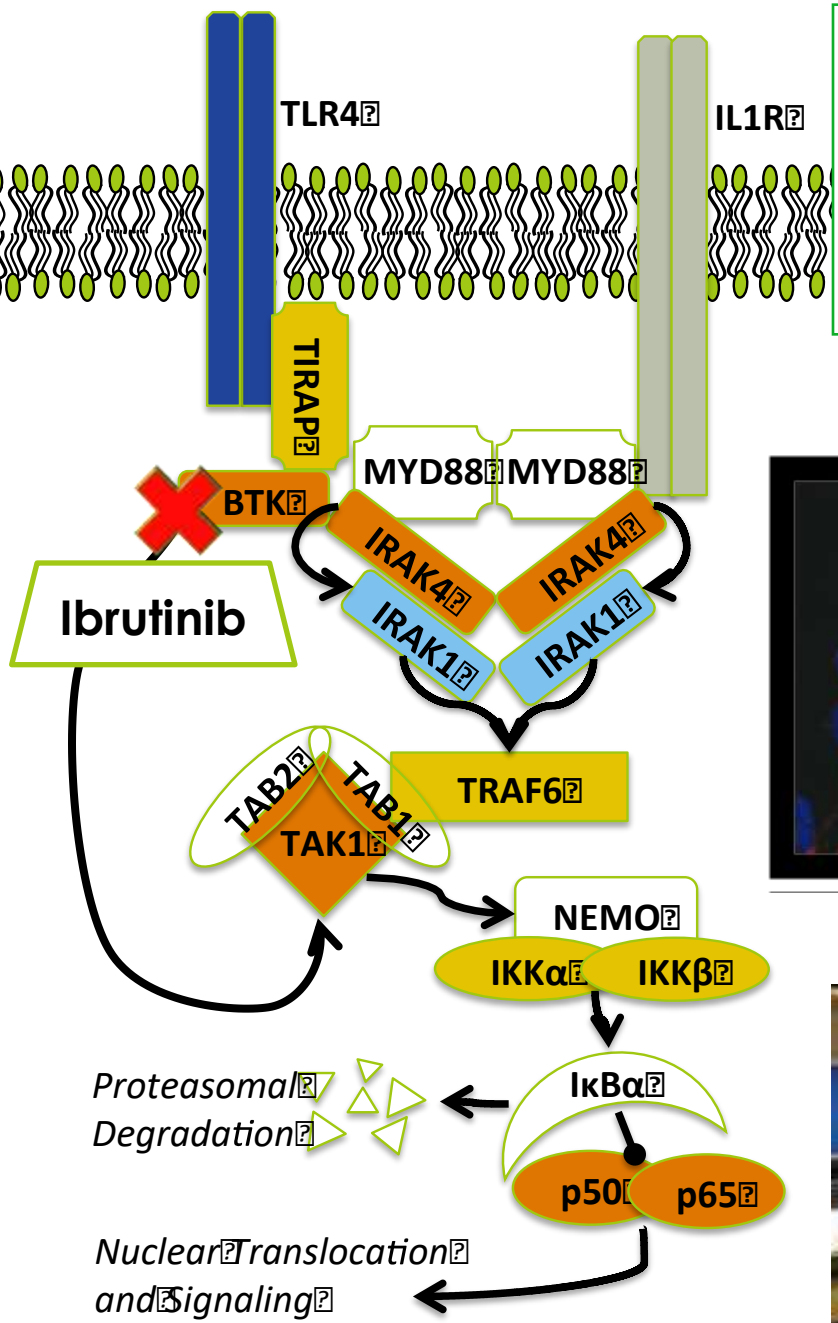
Treon et al NEJM 2012

MYD88 L265P in WM/IGM MGUS

	METHOD	TISSUE	WM	IGM MGUS
Treon	WGS/Sanger	BM CD19+	91%	10%
Xu	AS-PCR	BM CD19+	93%	54%
Gachard	PCR	BM	70%	
Varettoni	AS-PCR	BM	100%	47%
Landgren	Sanger	BM		54%
Jiminez	AS-PCR	BM	86%	87%
Poulain	PCR	BM CD19+	80%	
Argentou	PCR-RFLP	BM	92%	1/1 MGUS
Willenbacher	Sanger	BM	86%	
Mori	AS-PCR/BSiE1	BM	80%	
Ondrejka	AS-PCR	BM	100%	
Ansell	WES/AS-PCR	BM	97%	
Patkar	AS-PCR	BM	85%	

Updated from Treon and Hunter. Blood. 2013;121(22):4434-6.

MYD88 Signaling



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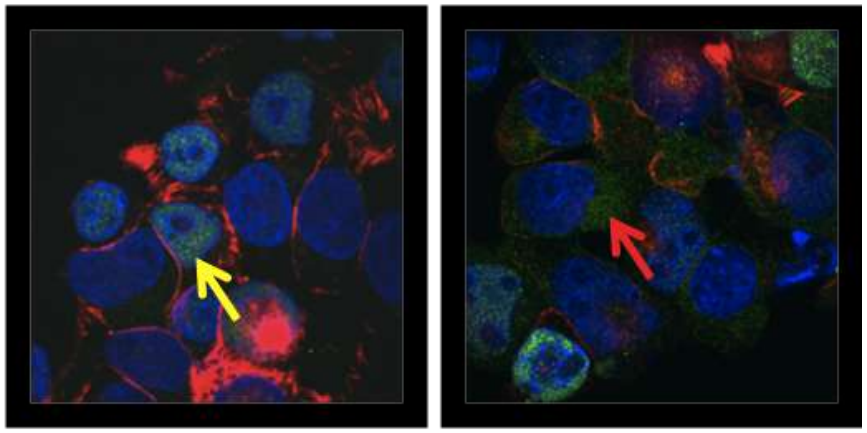

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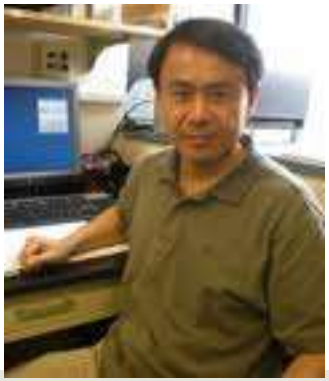
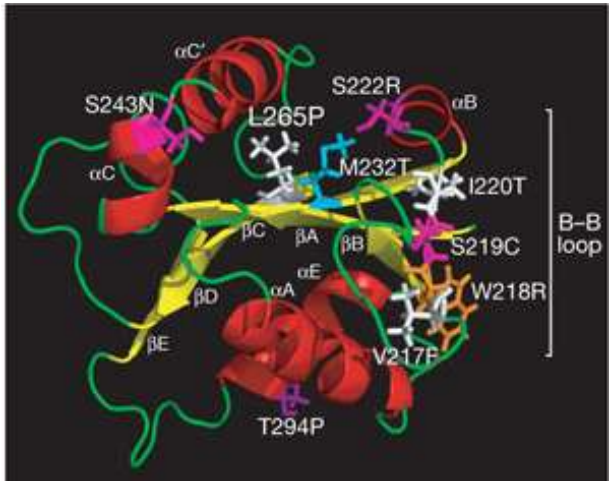
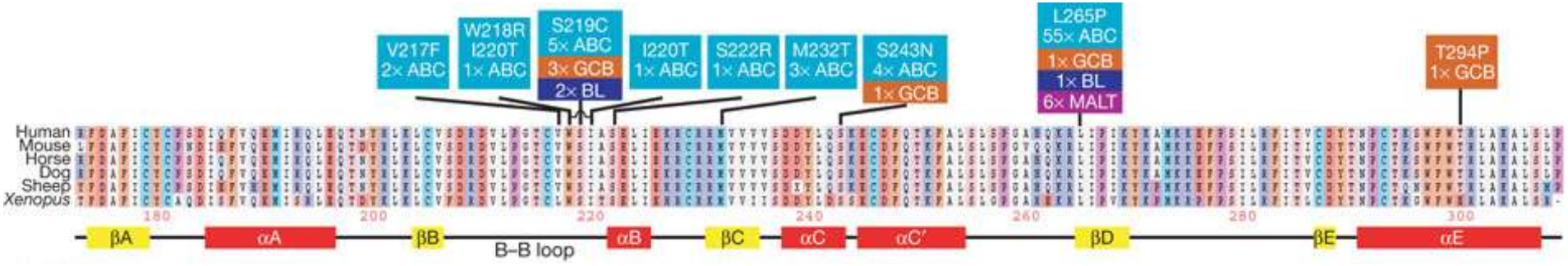


Guang Yang, PhD
Yang et al. Blood 2013

MYD88 Mutations in Diffuse Large B-Cell Lymphoma

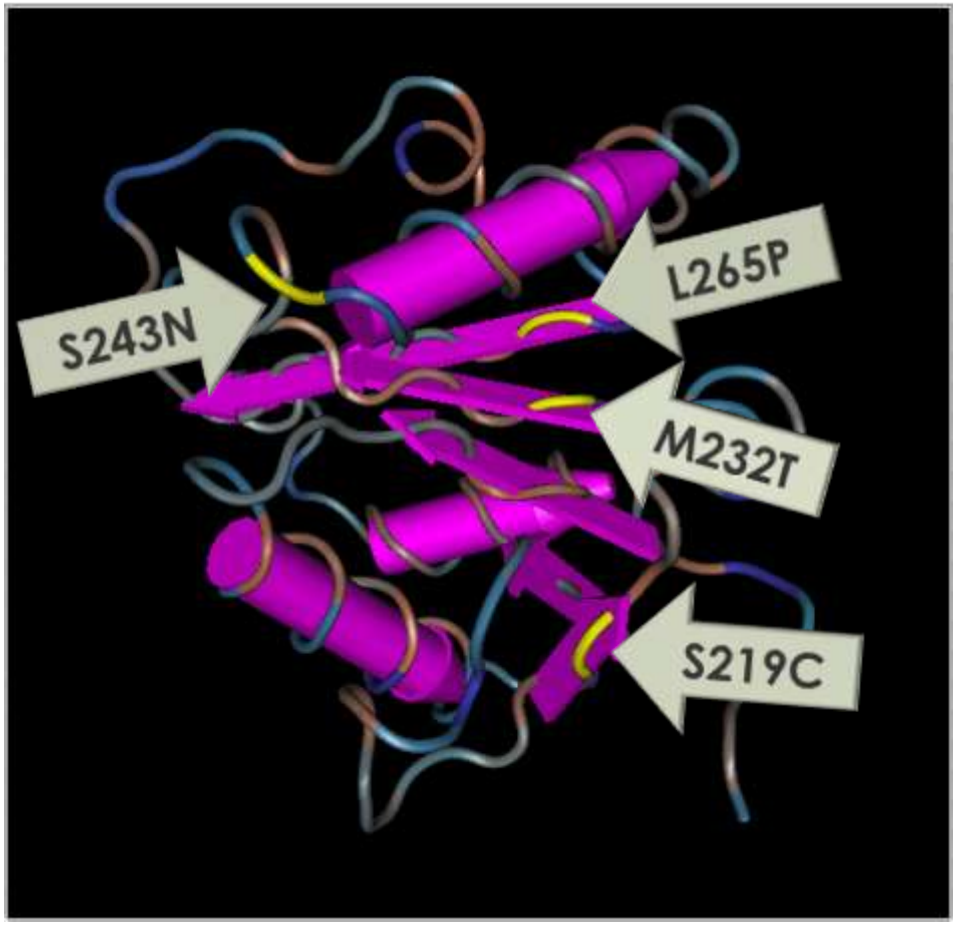




Ngo et al. Oncogenically active MYD88 mutations in human lymphoma. Nature 2011

Mutations observed in WM

- L265P
- S219C (subclonal)



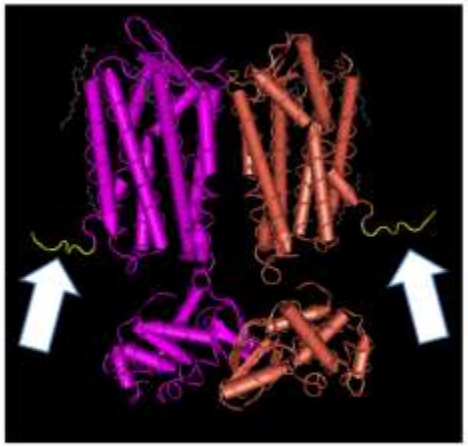
WHIM Syndrome Like Mutations in CXCR4 Found in 51/177 (28.8%) of WM Patients

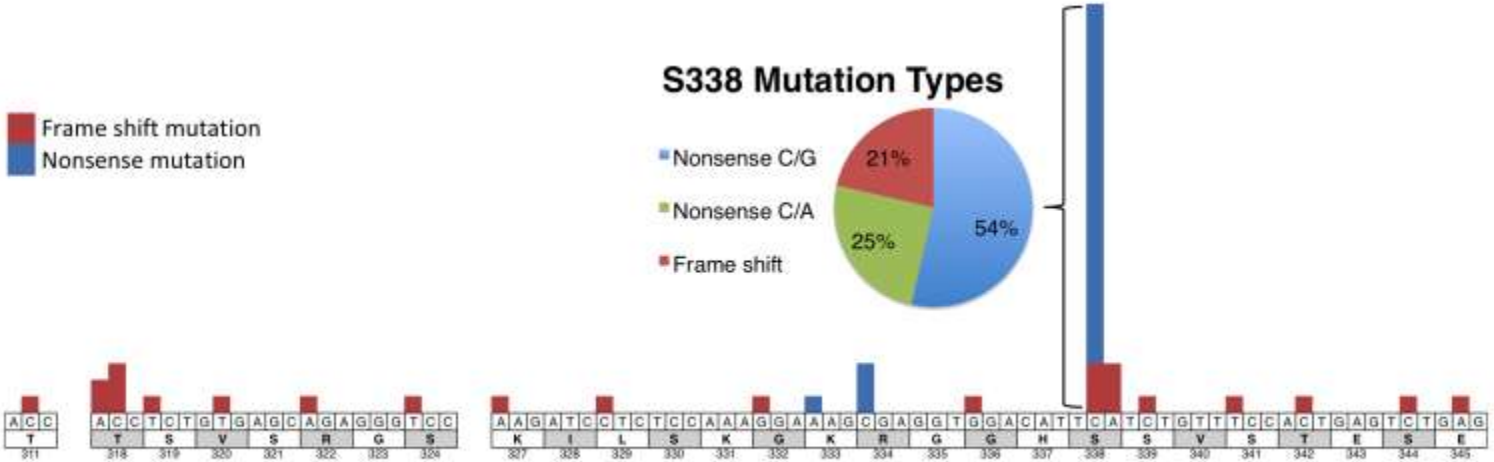
MEGISIYTSDNYTEEMGSGDYDSMKEPCFREENANFNK***IFLPTI***
YSIIFLTGIVGNGLVILVMGYQKKLRSMTDKYR***LHLSVADLLFVI***
TLPFWAVDAVANWYFGNFLCK***AVHVIYTVNLYSSVLILAFI***SLD
RYLAIVHATNSQRPRKLLAEK***VVYVGVWIPALLLTIPDFIF***ANVS
EADDRYICDRFYPNDLW***VVVFQFQHIMVGLILPGIVIL***SCYCIIIS
KLSHSKGHQKRKALKTT***VILILAFFACWLPYYIGISI***DSFILLEIIK
QGCEFENTVHK***WISITEALAFFHCCLNPILY***AFLGAKFKTSAQH
ALTSVSRGSSLKILSKGKRGGHSSVSTESESSSFHSS

LEGEND

A - Germline variant in WHIM syndrome ***A*** - Transmembrane helix

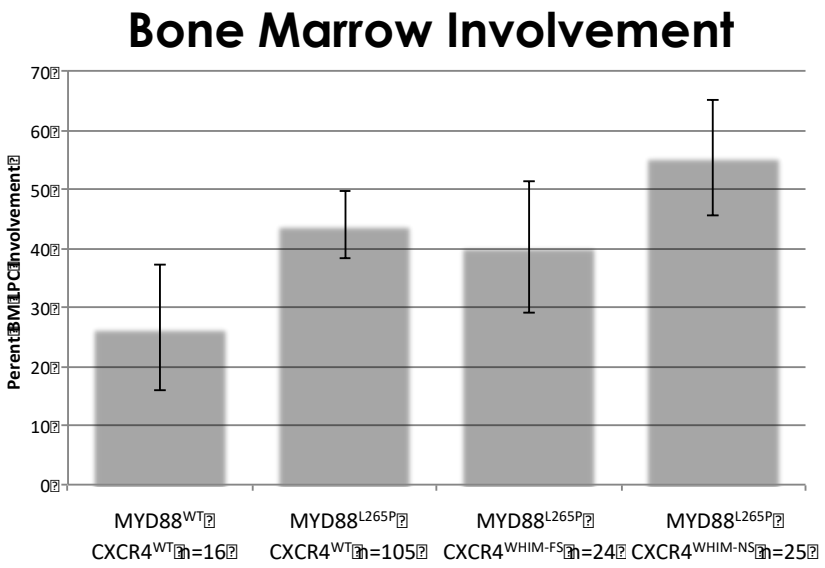
▯ - Somatic frame shift or nonsense WM variant

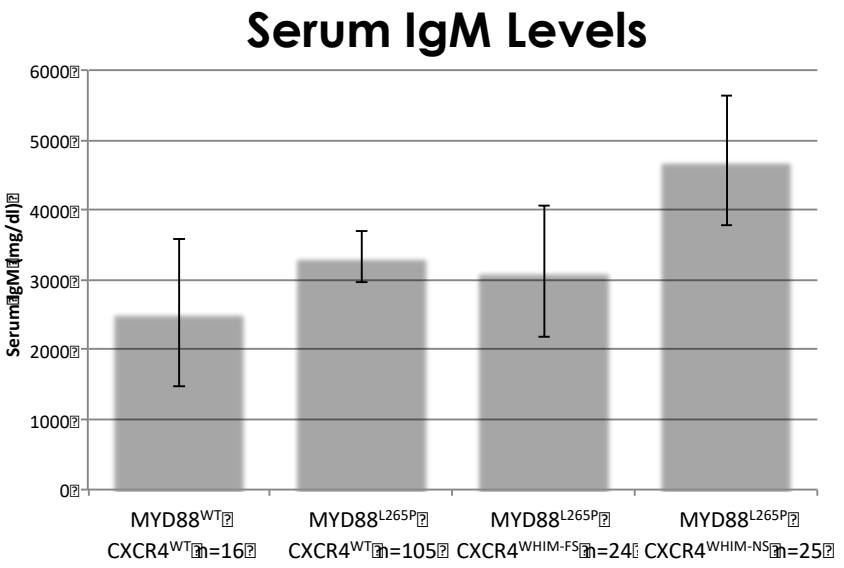




Hunter et al, Blood 2013

Marrow and Serum IgM by Genotype in 174 WM Patients

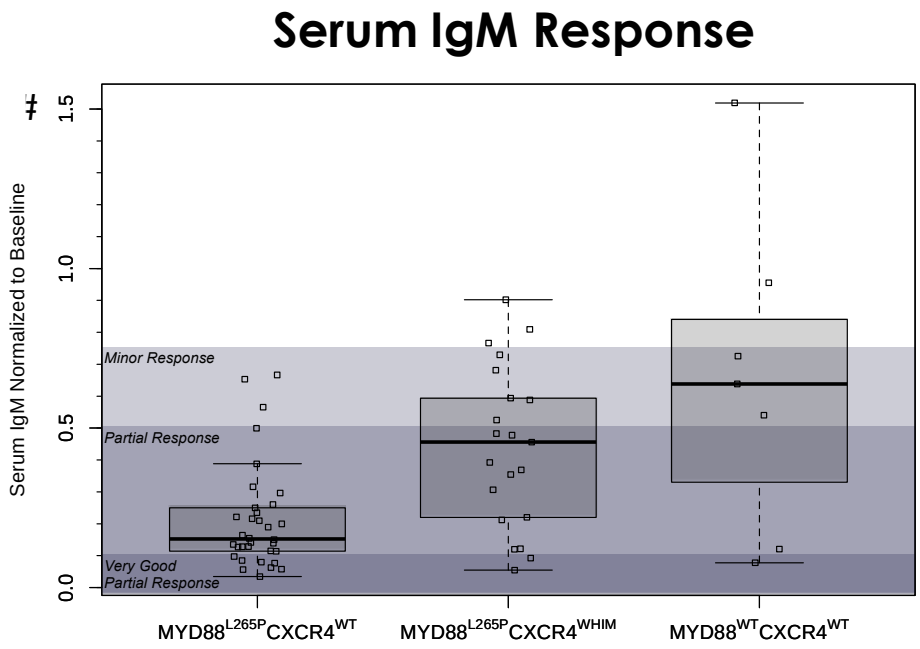


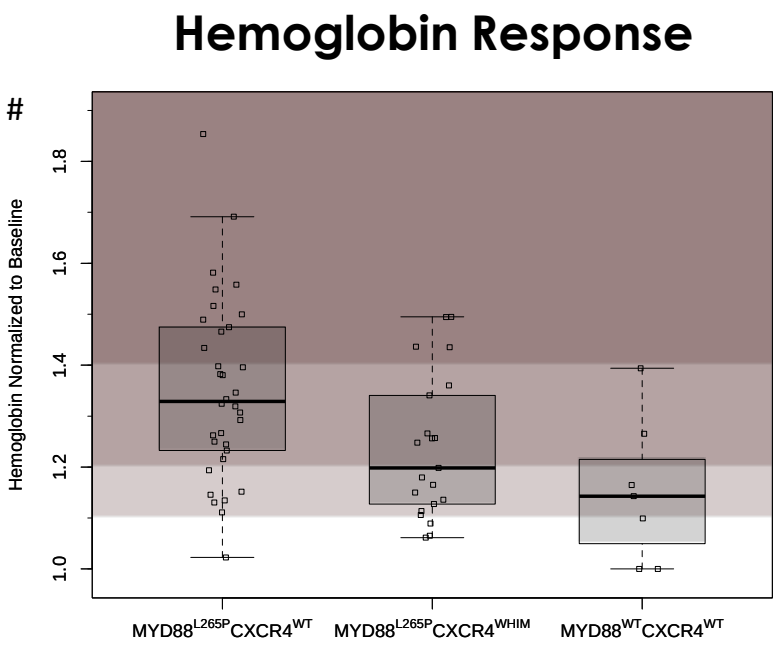


MYD88	**Wild Type**	**L265P**		
CXCR4	**Wild Type**		**WHIM**	**WHIM-NS**
Genotype	$MYD88^{WT}CXCR4^{WT}$	$MYD88^{L265P}CXCR4^{WT}$	$MYD88^{L265P}CXCR4^{WHIM}$	$MYD88^{L265P}CXCR4^{WHIM\text{-}NS}$
Prevalence	~10% of patients	~60% of patients	~30% of patients	~50% of $CXCR4^{WHIM}$
	Poor prognosis/Overall Survival		Low adenopathy	High BM
	Low serum IgM		Therapeutic resistance	High IgM
	Low BM involvement			Hyperviscocity syndrome
				More symptomatic

Treon and Hunter et al, Blood 2014

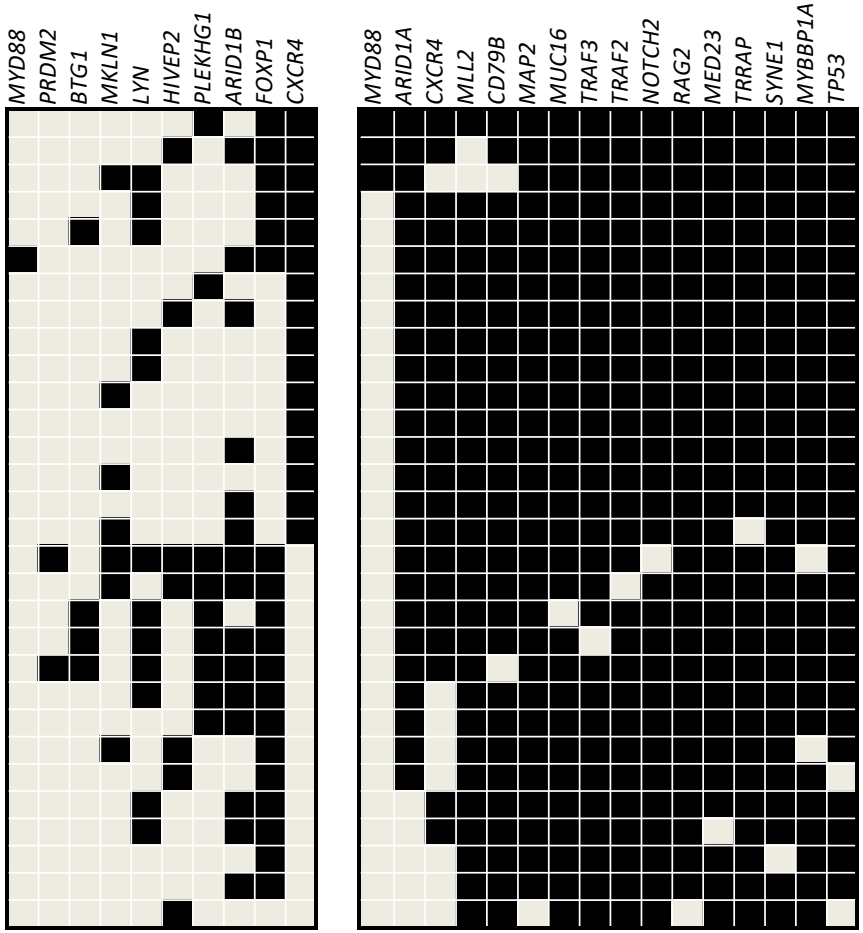
Using Mutation Status to Understand Responses to Ibrutinib



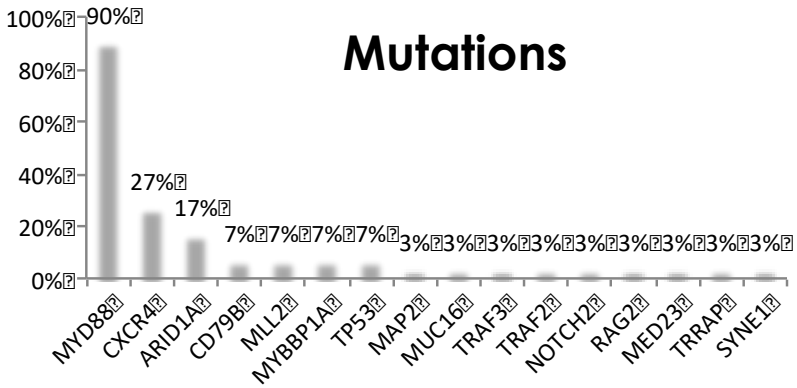


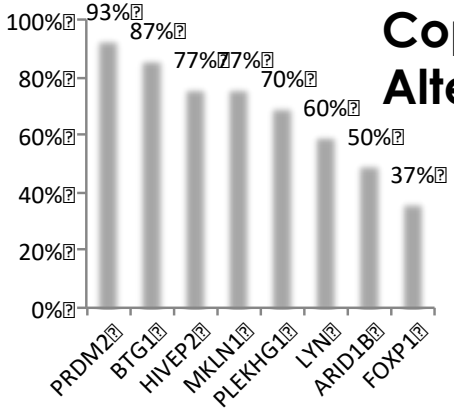
Treon et al, NEJM 2015

The WM Genome So Far…



Somatically deleted/mutated
Consistent with germline





Hunter et al, Blood 2013

New Findings from the WM transcriptome

	Median/Count	Range/Percent
Gender (Female)	21 / 57	36.8%
Age in Years	60	(40 - 78)
B2 Microglobin at Diagnosis > 3 (mg/L)	19 / 46	41.3%
Previous Treatment	20 / 57	35.1%
Familial B-Cell Disorders	23 / 57	40.4%
Splenomegaly	13 / 57	22.8%
Lymphadenopathy	30 / 57	52.6%
Bone Marrow Involvement (%)	60	(5 - 95)
Hematocrit (%)	32.8	(23.4 - 42.2)
Serum IgM (mg/dL)	3750	(416 - 8320)
Serum IgG (mg/dL)	517	(82 - 3890)
Serum IgA (mg/dL)	40	(6 - 516)
MYD88 Mutations	52 / 57	91.2%
CXCR4 Mutations	23 / 57	40.0%
ARID1A Mutations	5 / 51	9.8%
CD79B Mutations	4 / 51	7.8%
Deletion 6q	24 / 53	45.3%
Amplification 6p	6 / 53	11.3%
Amplification 3q	11 / 52	19.2%
Amplification Chr4	10 / 52	21.1%

RNA from 57 $CD19^+$ selected patient bone marrow samples were collected for sequencing.

For comparison, paired B-cells ($CD19^+CD27^-$) and memory B-cells ($CD19^+CD27^+$) were selected from 9 healthy donor peripheral blood samples.

Paired end samples were run two per lane run for 50 cycles using an Illumina HiSeq. Data analyzed using the voom/limma bioconductor package in R. Isoform specific expression was estimated using the cufflinks analysis pipeline.

False discovery rate of 10% use to determine significance.

Significant Differentially Expressed Genes by Category

Cytogenetic Changes

- Chromosome 6q Deletions: 131 genes
- Chromosome 6p Amplification: 65 genes
- Chromosome 4 Amplification: 776 genes
- Chromosome 3q Amplification: 11 genes

Healthy Donor Comparisons

- $MYD88^{L265P}CXCR4^{WT}$ vs. Healthy: 12,937 genes
- $MYD88^{L265P}CXCR4^{WHIM}$ vs. Healthy: 12,178 genes
- $MYD88^{WT}CXCR4^{WT}$ vs. Healthy: 11,059 genes

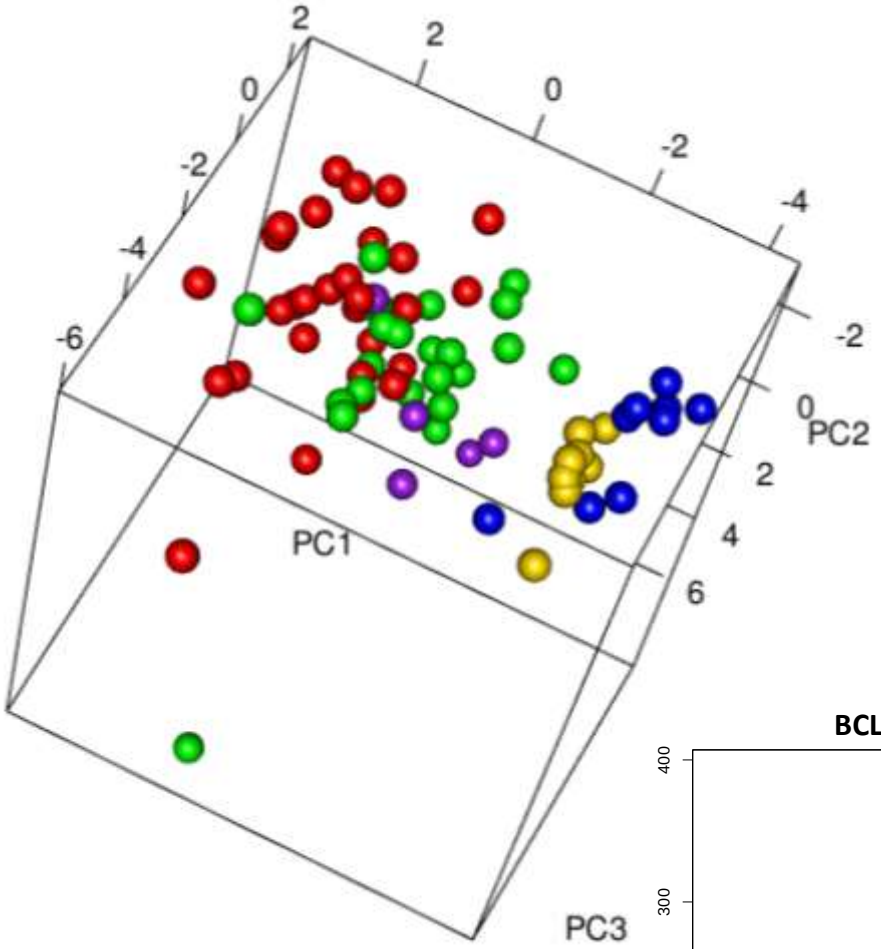
Predisposition

- Familial History of WM: 263 genes

Affects of Somatic Mutations

- $MYD88^{L265P}CXCR4^{WT}$ vs. $MYD88^{L265P}CXCR4^{WHIM}$: 3,103 genes
- $MYD88^{L265P}CXCR4^{WT}$ vs. $MYD88^{WT}CXCR4^{WT}$: 1,155 genes
- ARID1A: 16 genes

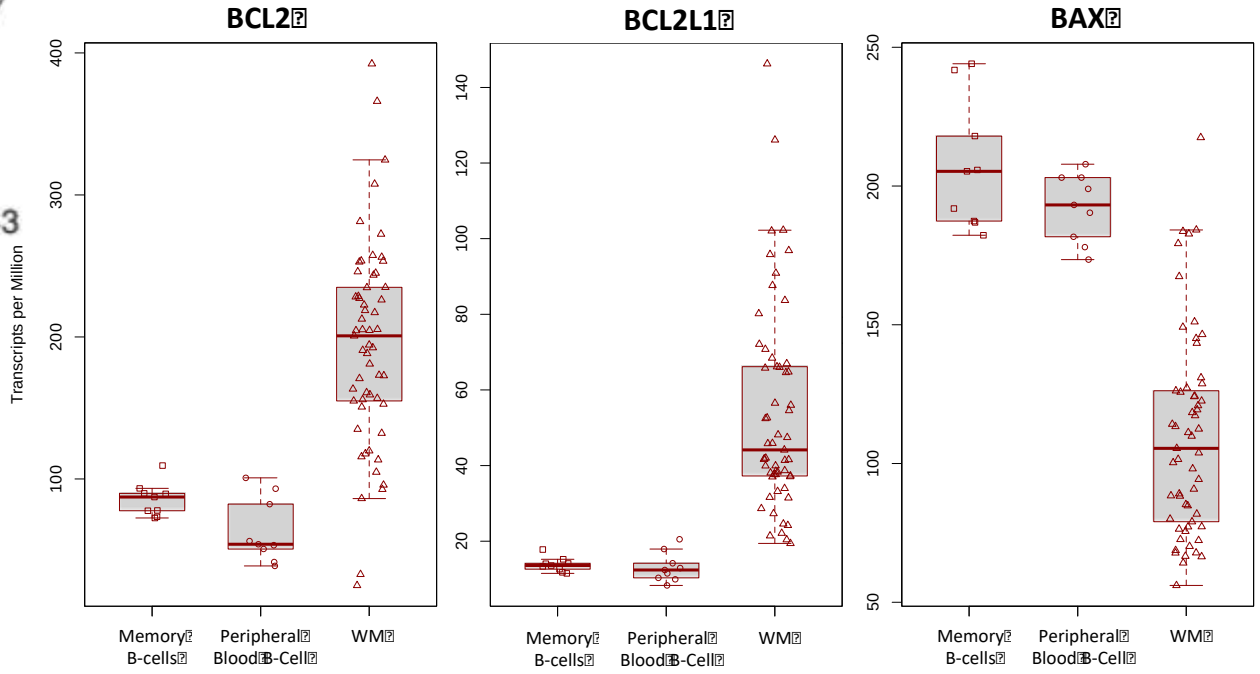
Expression of BCL2 Family Members in WM



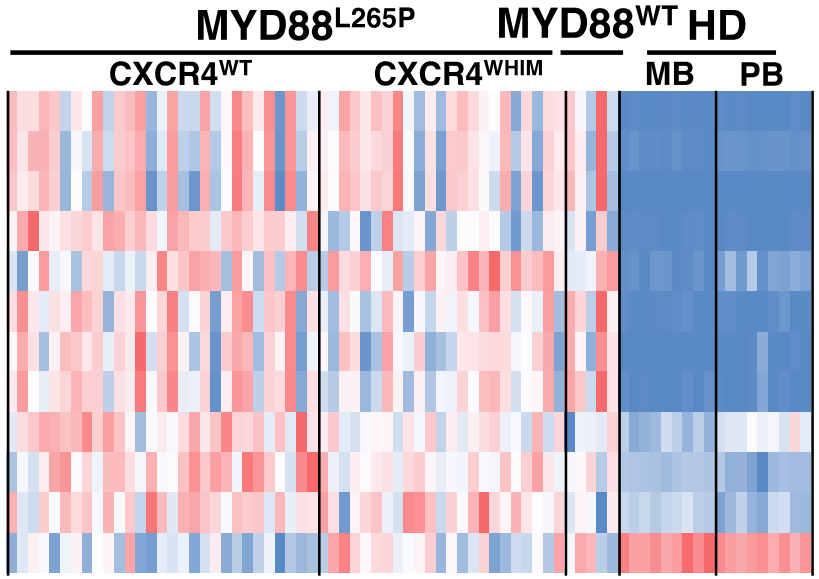
- MYD88^{L265P}CXCR4^{WT}
- MYD88^{L265P}CXCR4^{WHIM}
- MYD88^{WT}CXCR4^{WT}
- Memory B-Cell
- Peripheral Blood B-Cell

62% of Variation Accounted For in Components 1-3

WM versus HD Expression $p < 0.0001$



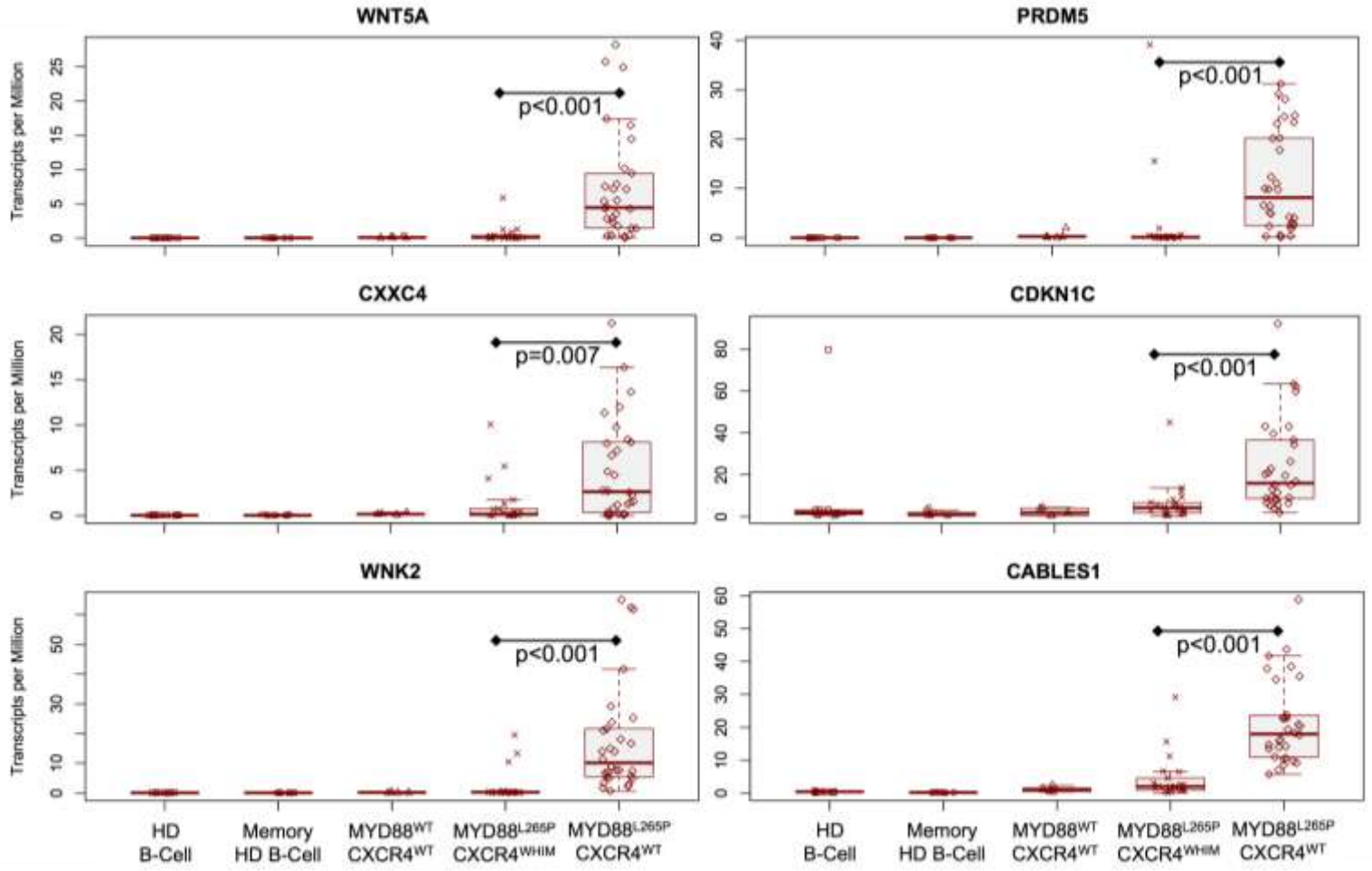
Is CXCR4 Important to all WM patients regardless of mutation?



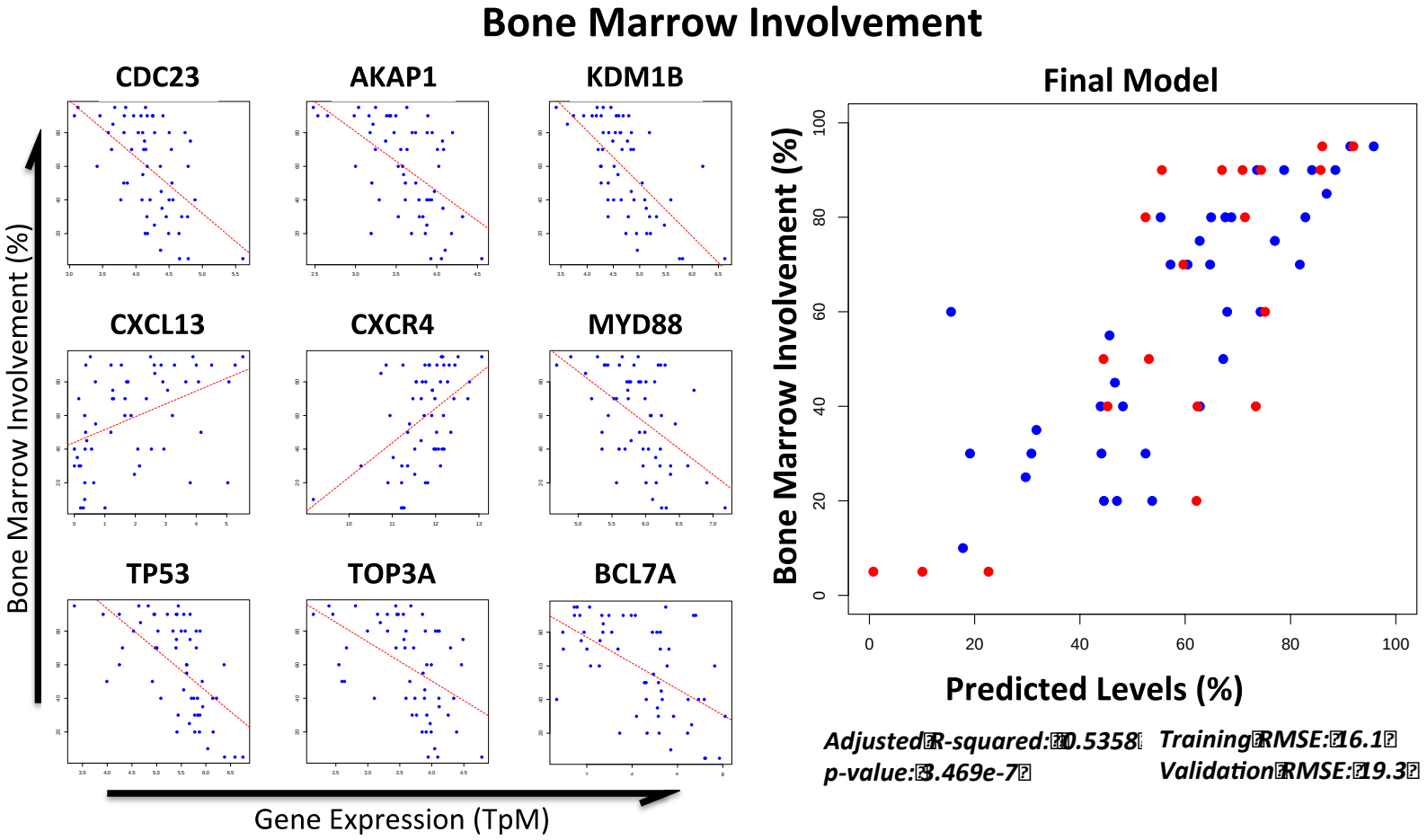
	WM (All Samples)	Healthy Donor - MB	p-value
DNTT	71.146 (0.5-2884.9)	0.023 (0-0.1)	0.0005
RAG1	7.0669 (0.2-255.5)	0.038 (0-0.1)	0.0004
RAG2	5.1208 (0-163.3)	0 (0-0)	<0.0001
IGF1	3.2971 (0.1-28.5)	0.018 (0-0)	<0.0001
BMP3	21.157 (0.5-269.5)	0.127 (0-0.3)	<0.0001
CD5L	8.1506 (0.3-372.6)	0.021 (0-0.1)	0.0001
CXCL12	3.44 (0.1-168.5)	0 (0-0)	0.0001
VCAM1	7.2862 (0.1-383.3)	0 (0-0.1)	0.0001
CXCR4	3607.9 (577.9-8578.8)	1160 (913.8-1955)	<0.0001
B2M	11916 (5501.6-18419.1)	5563 (4920.4-5862.5)	<0.0001
BCL2	200.75 (25-392.3)	87.24 (72.4-109.4)	<0.0001
BAX	105.47 (56.1-217.5)	205.3 (182.3-244.1)	<0.0001

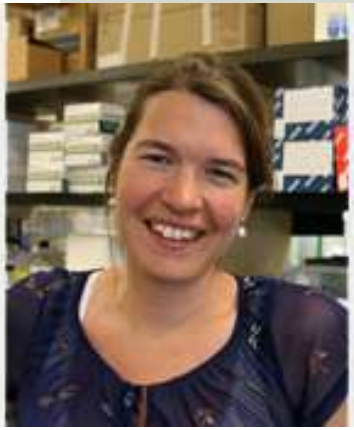
Relative gene expression differences between WM patients and healthy donors (Red indicates high expression, blue low expression)

Differentially Expressed Genes Between MYD88^{L265P}CXCR4^{WT} and MYD88^{L265P}CXCR4^{WHIM} WM Samples



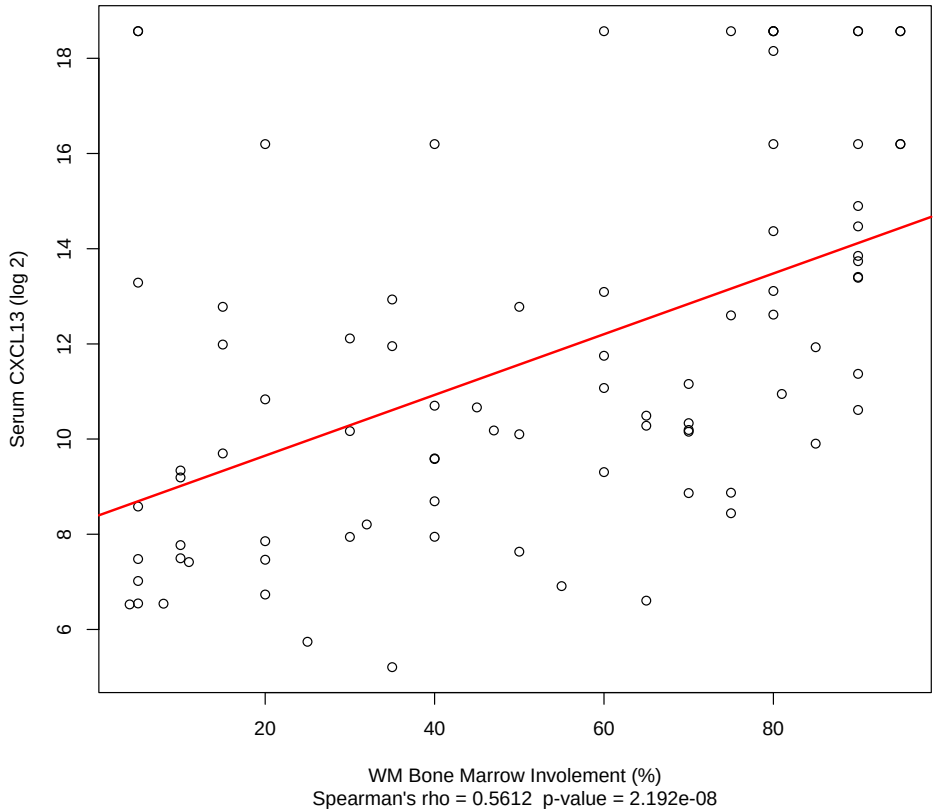
Gene Expression Associated with Bone Marrow Involvement in WM

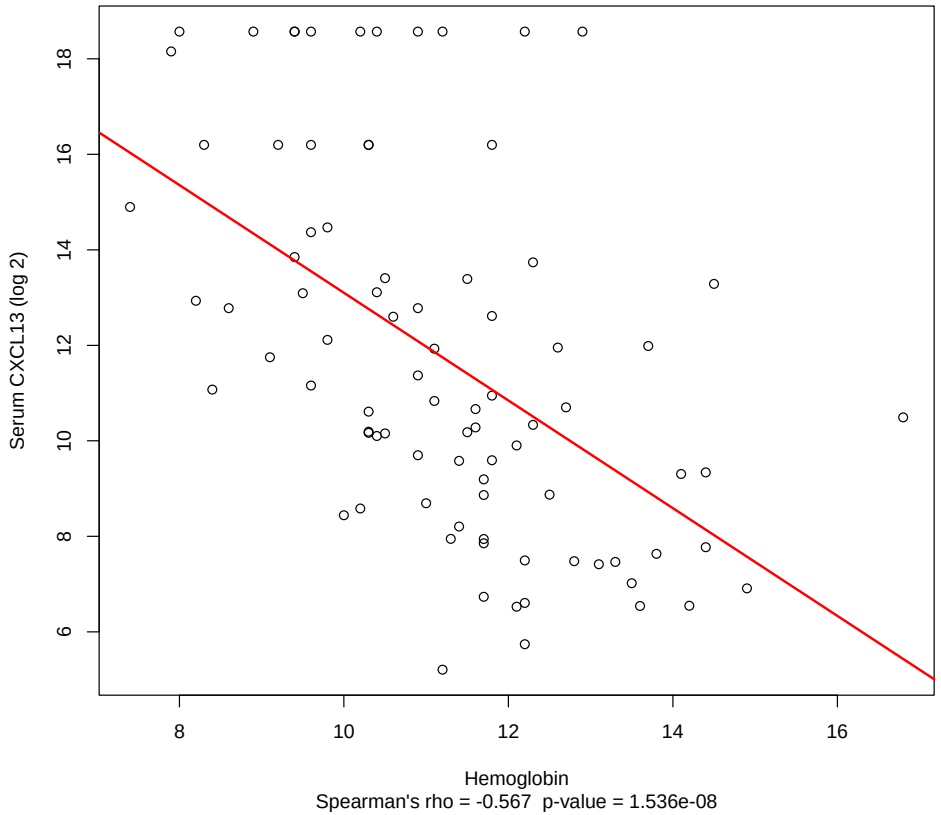




Validation of CXCL13 Correlation with Bone Marrow and Hemoglobin in 86 WM Patients

Josephine Vos, MD





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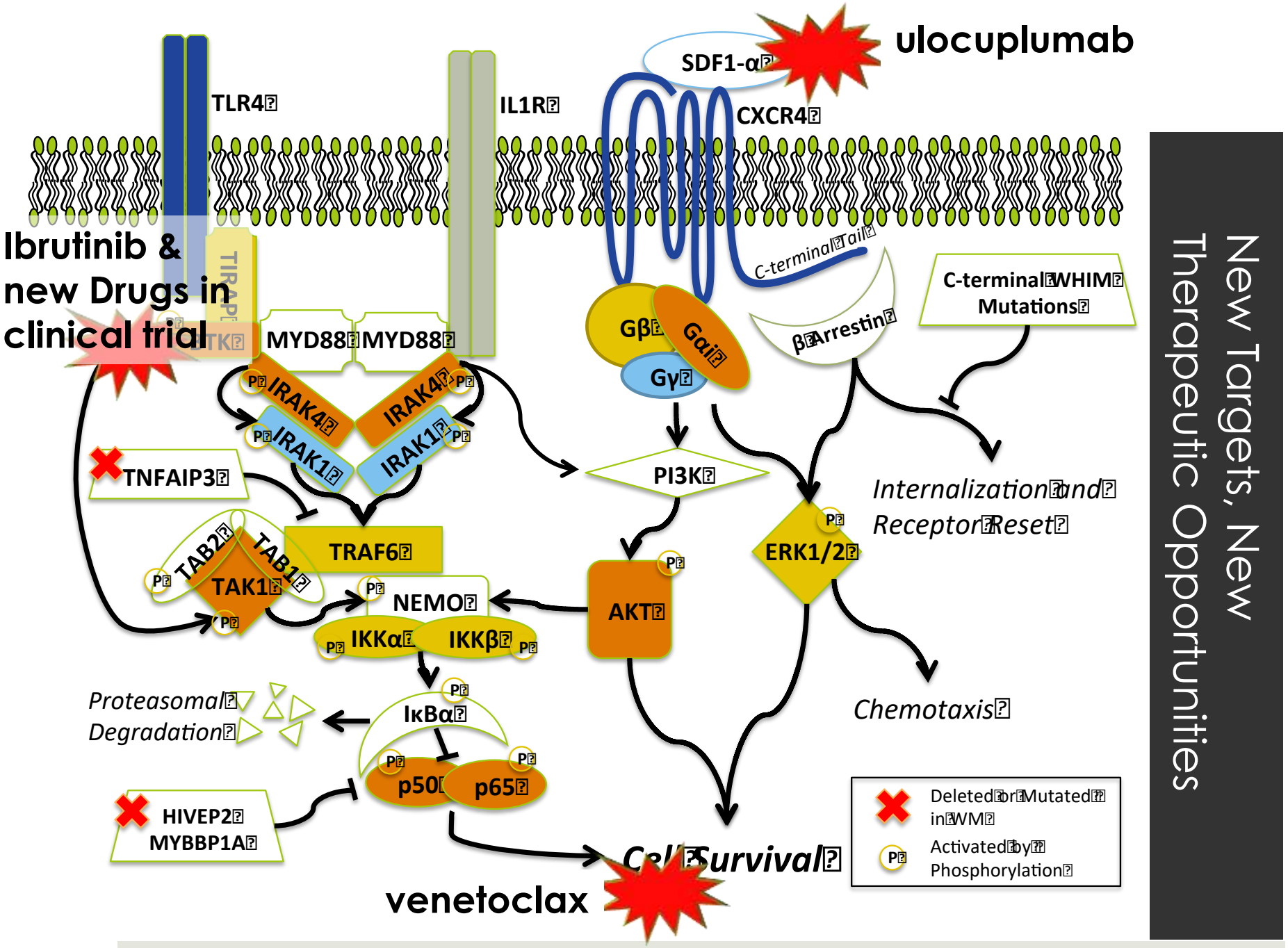
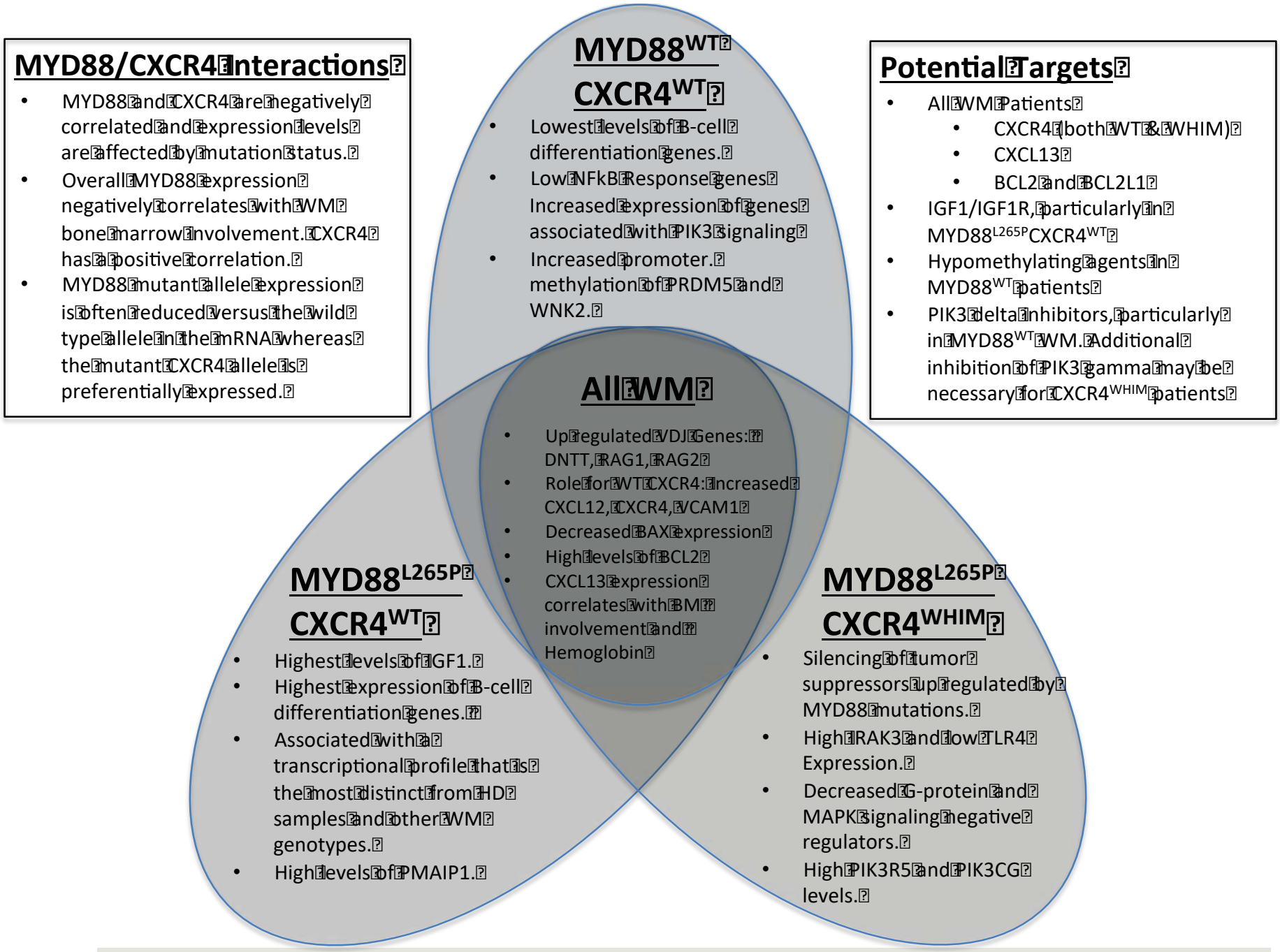

ulocuplumab
SDF1-α
TLR4
IL1R
CXCR4
Ibrutinib &
new Drugs in
clinical trial
C-terminal Tail
C-terminal WHIM
Mutations
β-Arrestin
Gβ
Gαi
Gγ
TIRAP
BTK
MYD88
MYD88
IRAK4
IRAK4
IRAK1
IRAK1
TNFAIP3
PI3K
Internalization and
Receptor Reset
TRAF6
TAB2
TAB1
TAK1
NEMO
IKKα
IKKβ
AKT
ERK1/2
Proteasomal
Degradation
IκBα
Chemotaxis
p50
p65
HIVEP2
MYBBP1A
Cell Survival
venetoclax
Deleted or Mutated
in WM
Activated by
Phosphorylation
New Targets, New
Therapeutic Opportunities


MYD88/CXCR4 Interactions
MYD88 and CXCR4 are negatively correlated and expression levels are affected by mutation status.
Overall MYD88 expression negatively correlates with WM bone marrow involvement. CXCR4 has a positive correlation.
MYD88 mutant allele expression is often reduced versus the wild type allele in the mRNA whereas the mutant CXCR4 allele is preferentially expressed.
MYD88WT CXCR4WT
Lowest levels of B-cell differentiation genes.
Low NFkB Response genes
Increased expression of genes associated with PIK3 signaling
Increased promoter. methylation of PRDM5 and WNK2.
All WM
Up regulated VDJ Genes: DNTT, RAG1, RAG2
Role for WT CXCR4: Increased CXCL12, CXCR4, VCAM1
Decreased BAX expression
High levels of BCL2
CXCL13 expression correlates with BM involvement and Hemoglobin
Potential Targets
All WM Patients
CXCR4 (both WT & WHIM)
CXCL13
BCL2 and BCL2L1
IGF1/IGF1R, particularly in MYD88L265P CXCR4WT
Hypomethylating agents in MYD88WT patients
PIK3 delta inhibitors, particularly in MYD88WT WM. Additional inhibition of PIK3 gamma may be necessary for CXCR4WHIM patients
MYD88L265P CXCR4WT
Highest levels of IGF1.
Highest expression of B-cell differentiation genes.
Associated with a transcriptional profile that is the most distinct from HD samples and other WM genotypes.
High levels of PMAIP1.
MYD88L265P CXCR4WHIM
Silencing of tumor suppressors up regulated by MYD88 mutations.
High IRAK3 and low TLR4 Expression.
Decreased G-protein and MAPK signaling negative regulators.
High PIK3R5 and PIK3CG levels.

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