

MYD88, CXCR4 en de genetica van WM

*Zachary Hunter
Bing Center for WM
Dana-Farber Cancer Institute
Harvard Medical School*

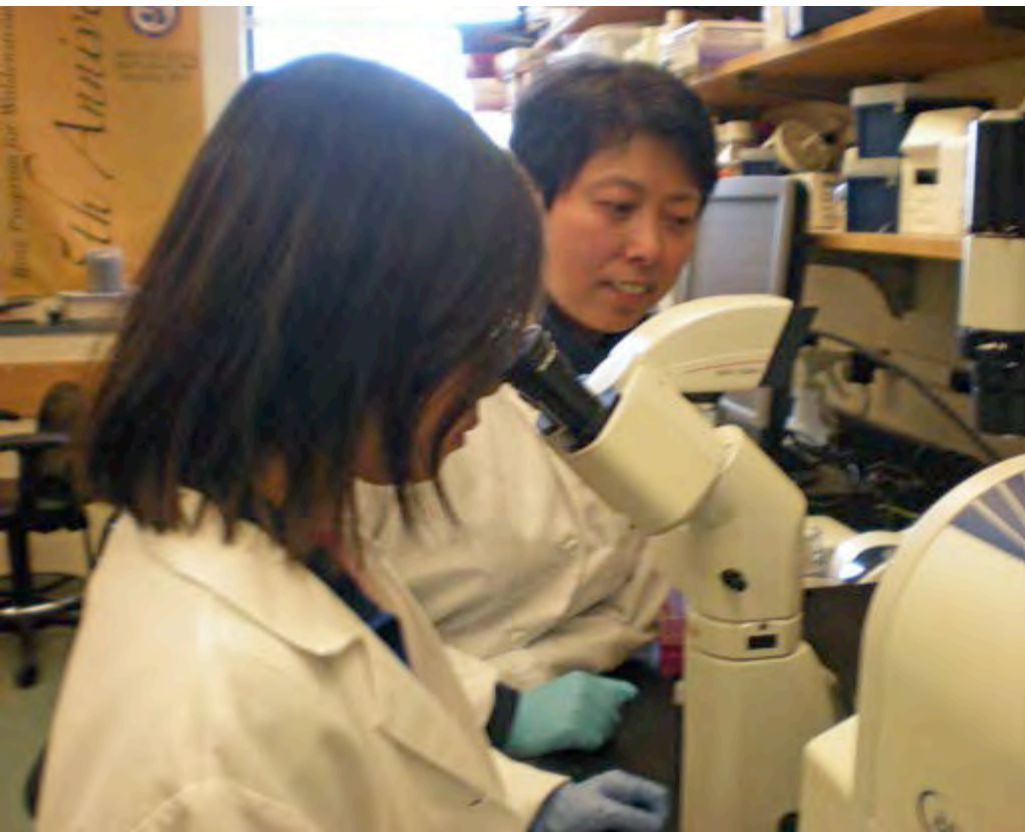


Bing Center for WM
Research at the
Dana-Farber
Cancer Institute



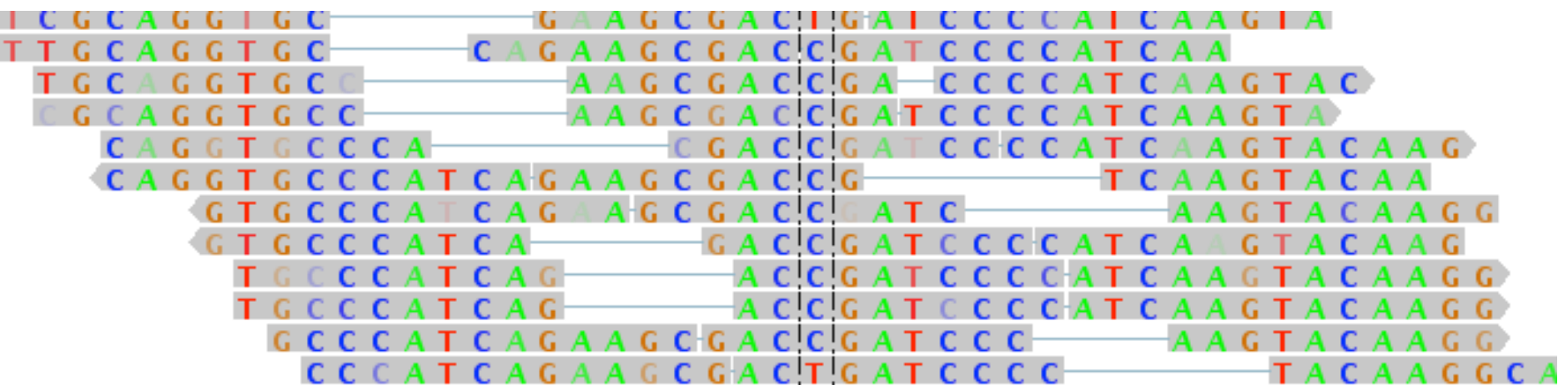
Harvard
Medical
School





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





Genetica begrijpen

Als je maar 4 letters to je beschikking had, wat zou je dan voor een verhaal vertellen?

Wat is DNA eigenlijk?

DNA

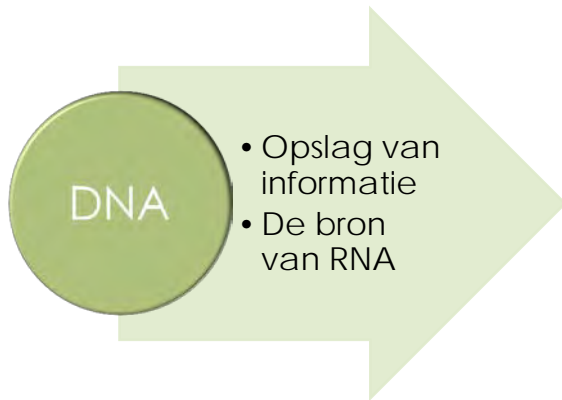


RNA



- Deoxyribonucleic Acid (DNA) bestaat uit complexe moleculen genaamd nucleotiden. Er zijn 4 types: A, T, C, G.
- Nucleotiden vormen vaste paren: A-T en C-G. Twee complementaire stroken van deze basen vormen DNA.
- DNA is verdeeld over 23 lange fragmenten die chromosomen genoemd worden. I
- De delen van DNA waarin de instructies staan om eiwitten te bouwen, worden genen genoemd.
- Genen worden dan omgezet in enkele fragmenten van vergelijkbare nucleotiden, dat heet RNA, dit wordt dan door de cel verwerkt en omgezet in eiwit.

DNA – RNA – Eiwit



- *Genen zijn regio's in het DNA die worden vertaald in RNA*
- *RNA draagt de DNA code naar de rest van de cel waar het gebruikt wordt als recept voor het maken van eiwit*

1-eiige tweelingen

Hoewel 1-eiige
tweelingen veel
gelijkenissen
hebben, zijn ze ook
heel verschillend
(andere leven,
persoonlijkheid,
andere ziektes, en
op een gegeven
moment ook een
ander uiterlijk.

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 \(0\)](#)



Je DNA is het recept voor het
bouwen van je eiwitten, NIET
het recept voor jou!

Je bent veel meer dan de som
van je genen.

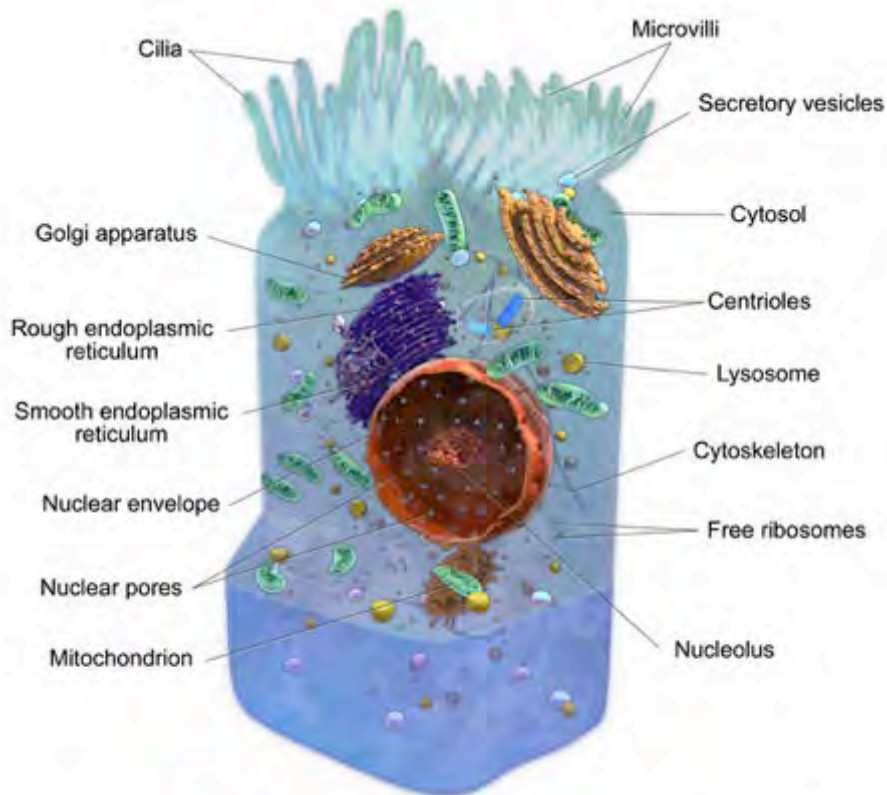
Als mensen genetisch uniek zijn, hoe kunnen we ze dan vergelijken?

- Er zijn > 3 biljoen basenparen in het menselijk genoom. We krijgen een complete set van beide ouders.
- Er is een "referentie genoom" gemaakt voor vergelijkingen. Het is niet van 1 individu maar een combinatie van 13 personen.

The Human Reference Genome (Print Edition)



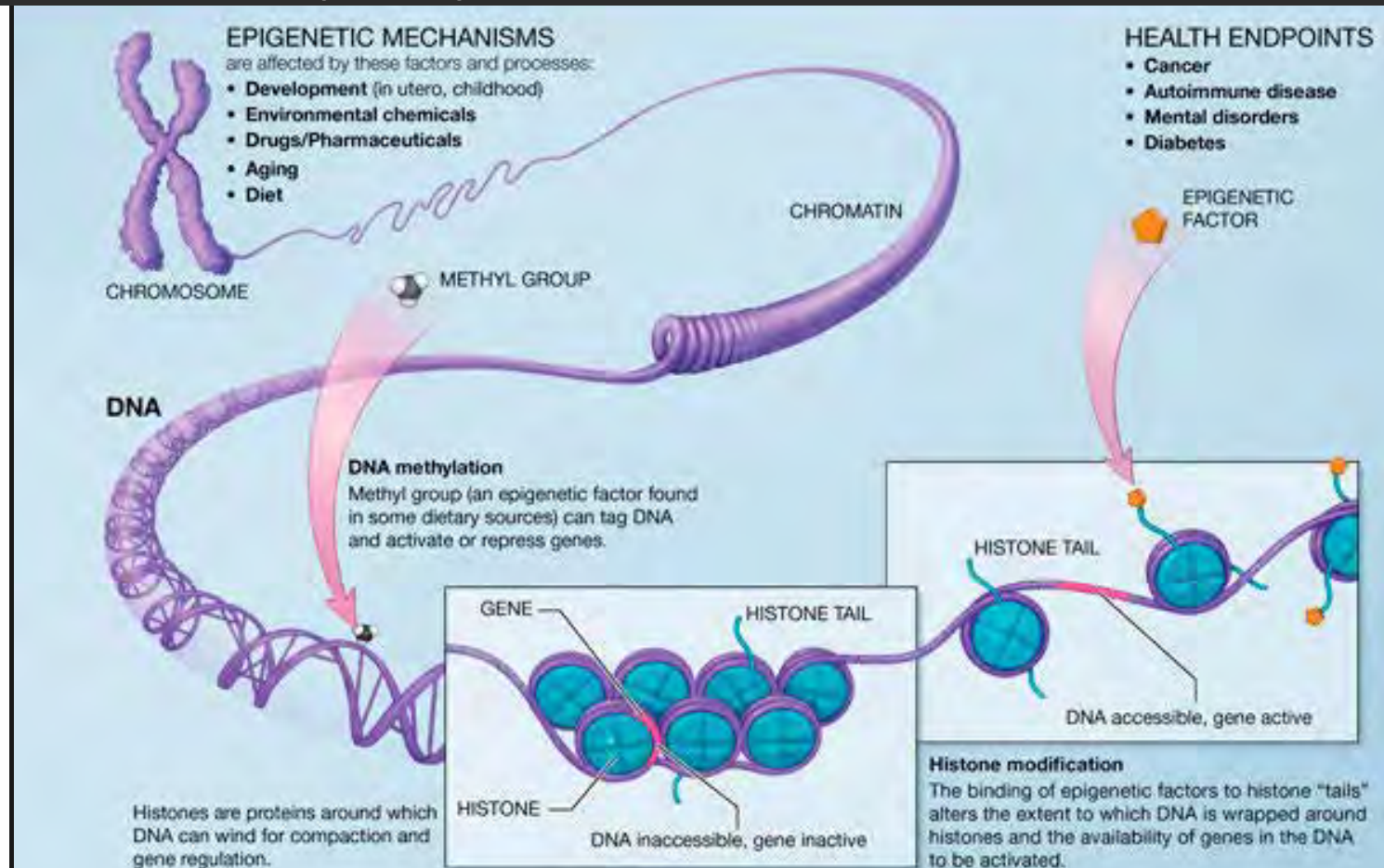
Hoe past het er allemaal in...



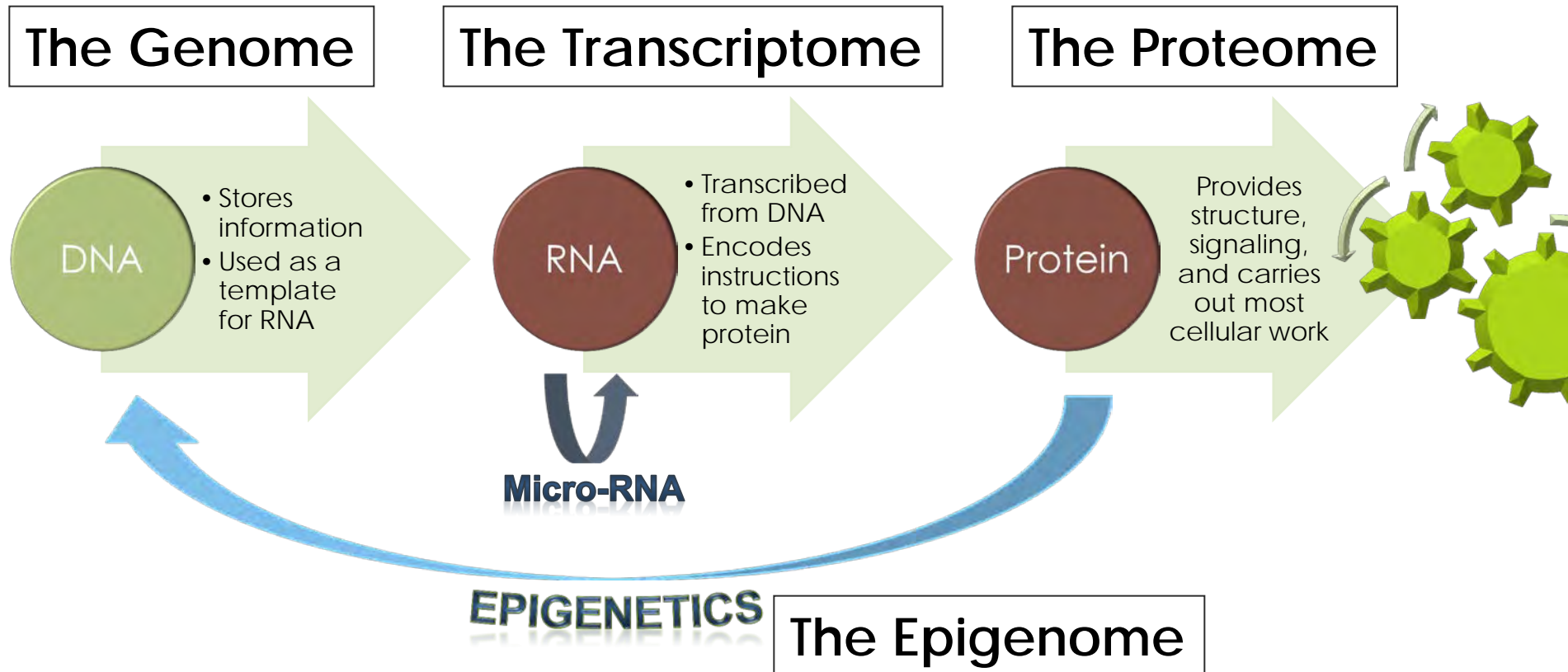
Anatomy of a Cell

- 3 biljoen basenparen aan elkaar is ongeveer 1 meter lang.
- Deze hoeveelheid DNA zit in de kern van elke cel in het lichaam!
- Dit is uiteraard een streng geregeld proces.

Excuses, het DNA waar u naar op zoek bent is tijdelijk niet beschikbaar....



Introductie van de "omes"

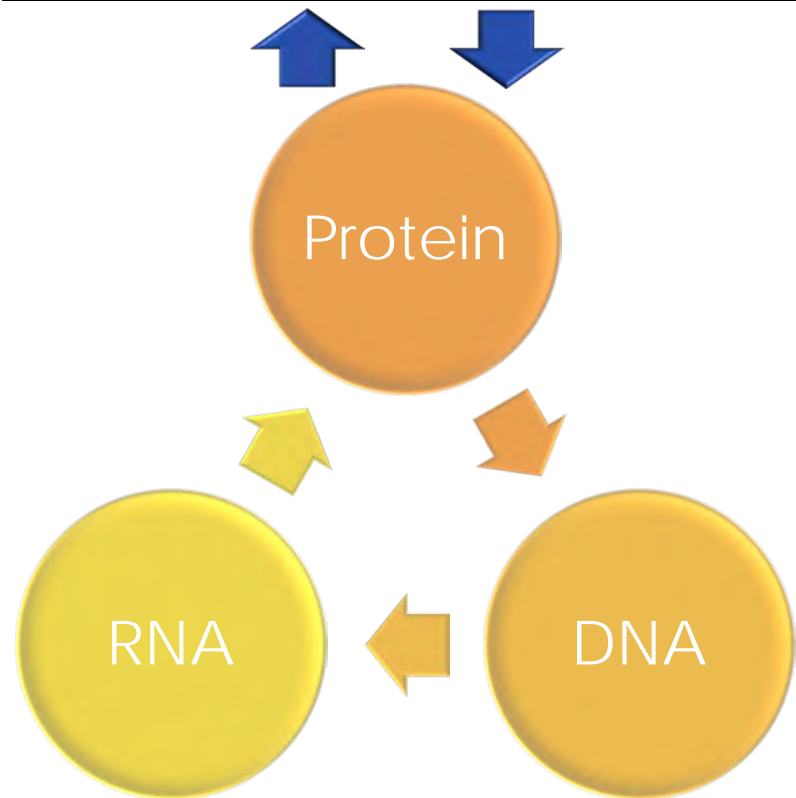


Het genoom is als het besturingssysteem van de cel

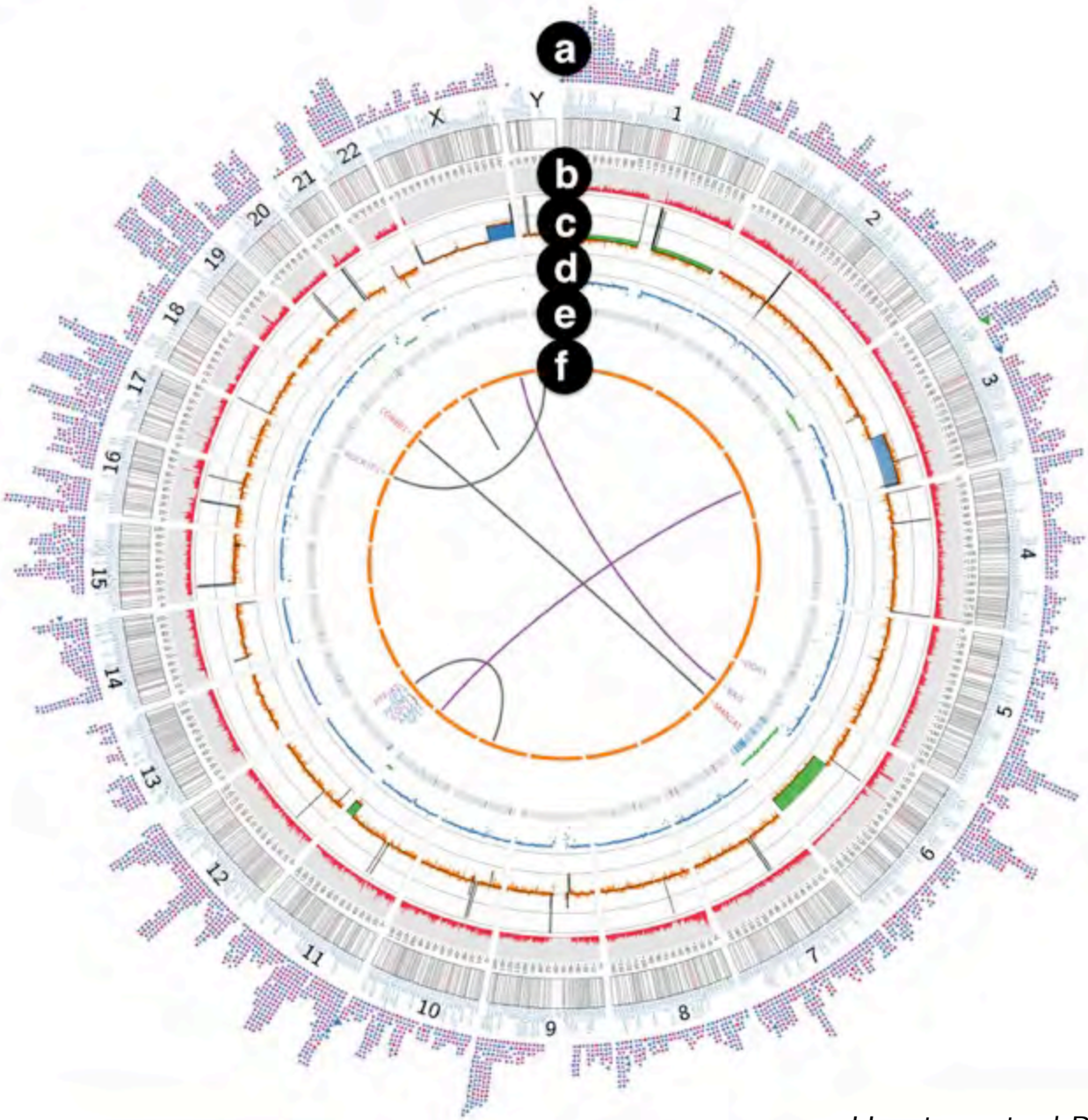


The operating system of your computer (Windows/Mac OSX/Linux) provides a set of tools to allow programs to be loaded and run. Every laptop may have the same operating system, but no one uses their laptop in exactly the same way

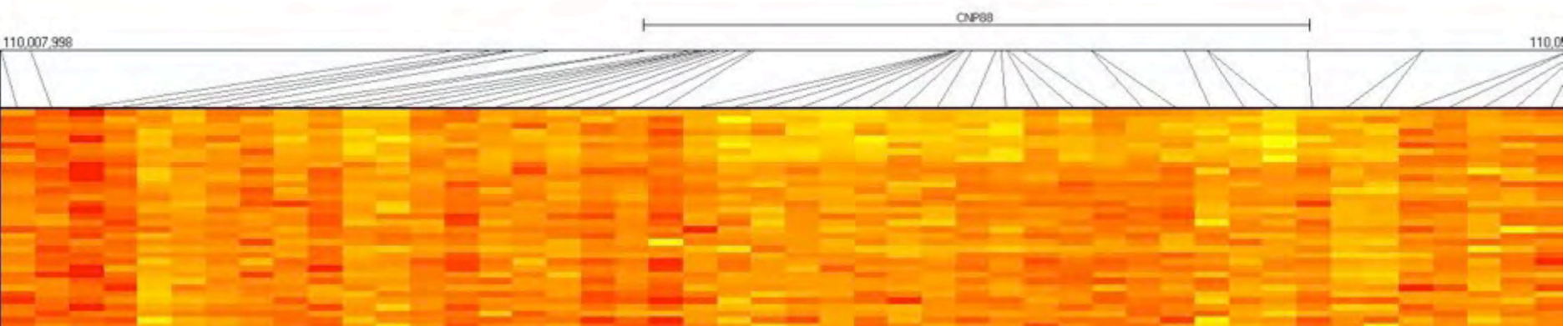
The Environment



Whole Genome Integrative Data Visualization



Hunter et al Blood 2013



De genetica van WM

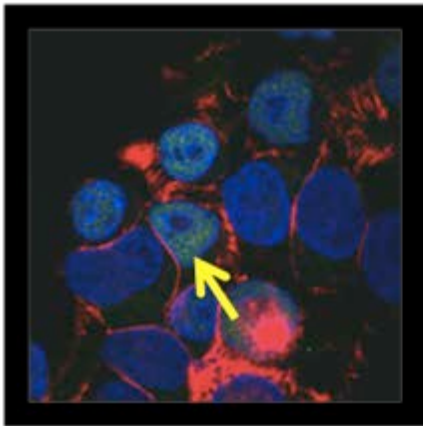
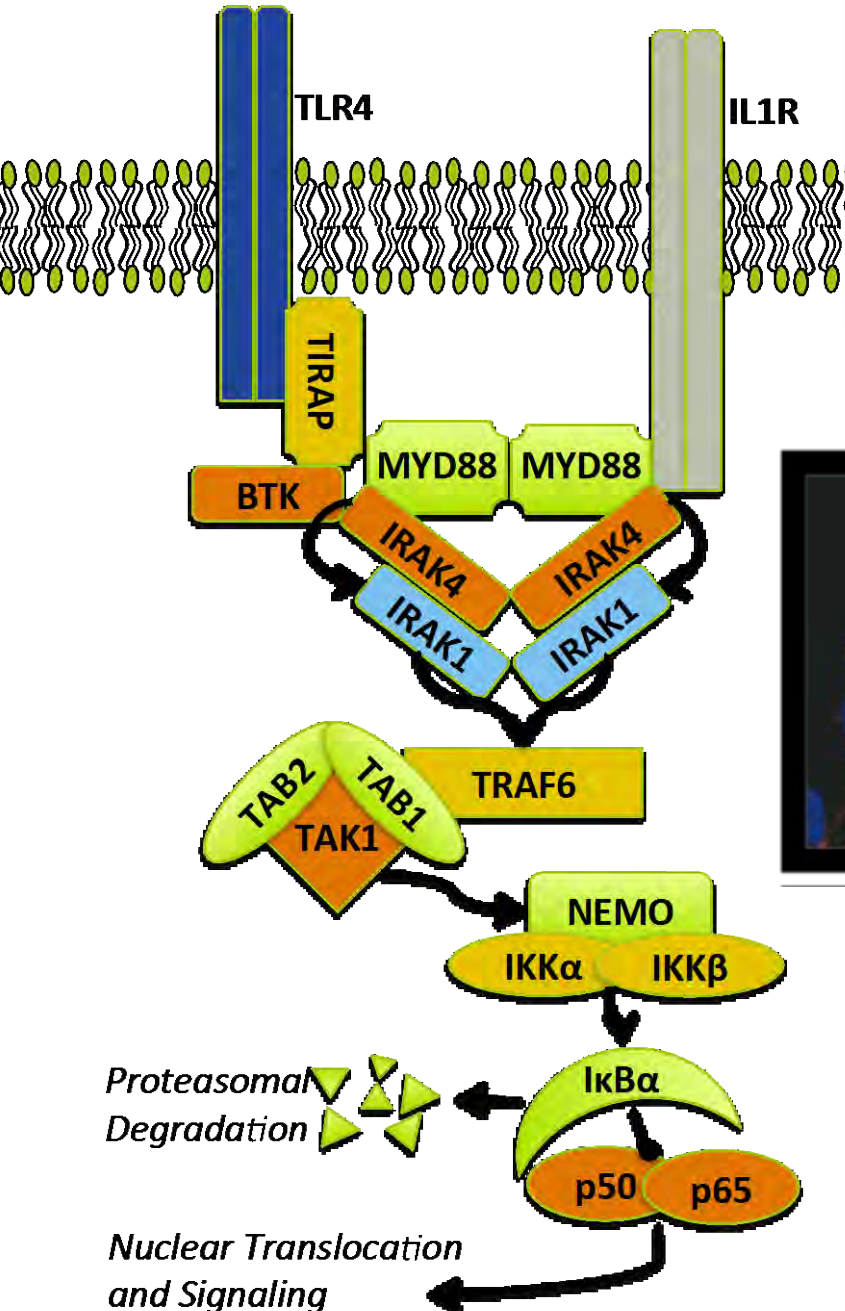
Inzicht in het "Operating System" van WM

Mutatie en kanker

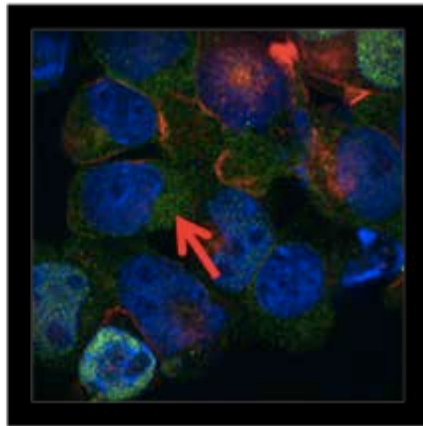
- Kanker kan veroorzaakt worden door foutjes in het genoom, die worden mutaties genoemd.
- Naarmate er meer foutjes ontstaan, krijgen genen nieuwe functie of verliezen functies.
- Als cellen ongecontroleerd gaan delen, noemen we dat kanker.

Er is een mutatie in 1 enkel basepaar van het MYD88 gen bij 90% van WM patienten

NFKB Nuclear Translocation in The BCWM.1 Cell Line
















Control



MYD88 Inhibitor

Treon et al NEJM 2012

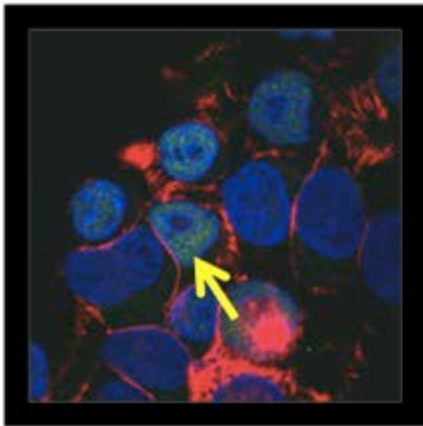
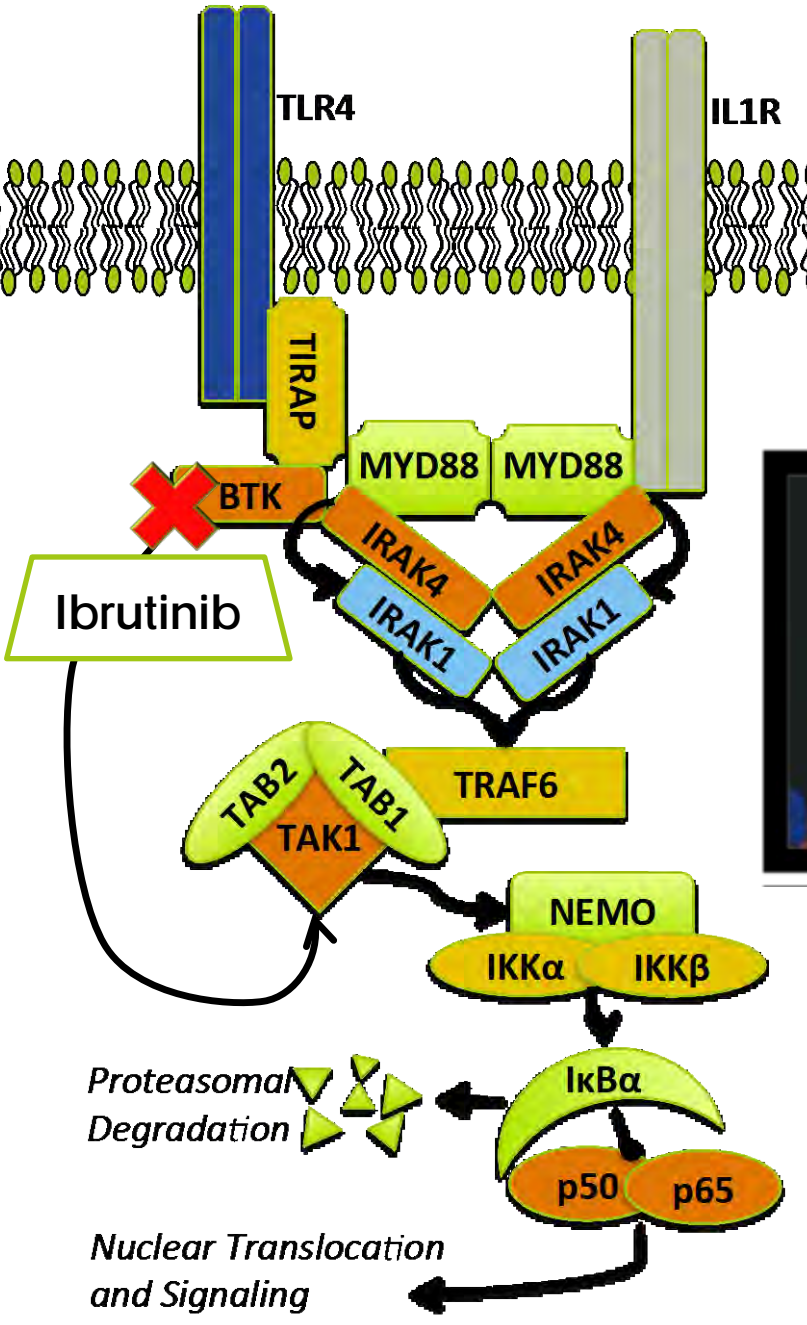
MYD88 L265P in WM/IGM MGUS

		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%	

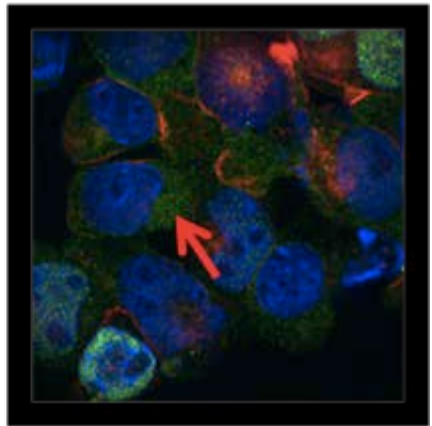
Updated from Treon and Hunter. Blood. 2013;121(22):4434-6.

Er is een mutatie in 1 enkel basepaar van het MYD88 gen bij 90% van WM patienten

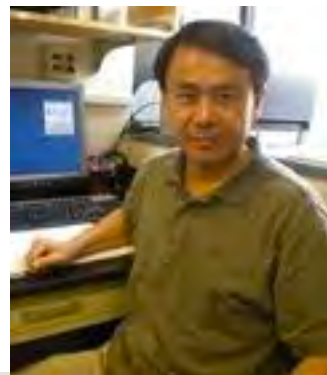
The BCWM.1 Cell Line



Control



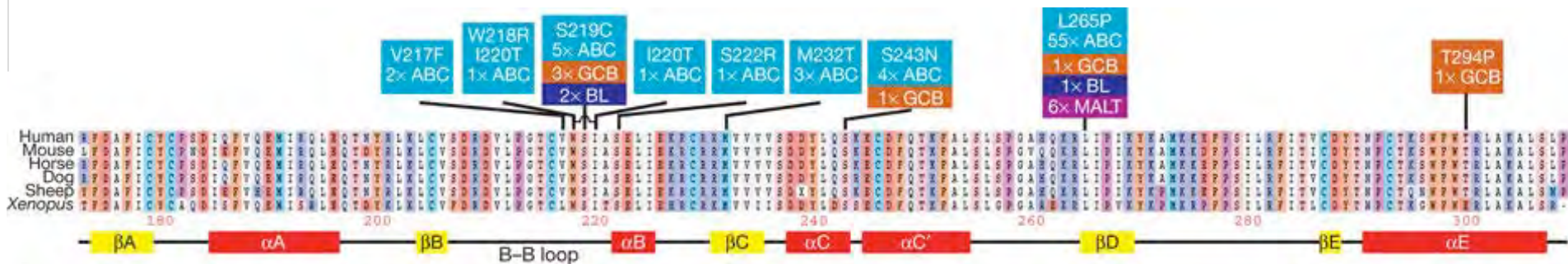
MYD88 Inhibitor



Guang Yang, PhD
Yang et al. Blood 2013

Denk je dat er mutaties zijn op andere plekken in MYD88?

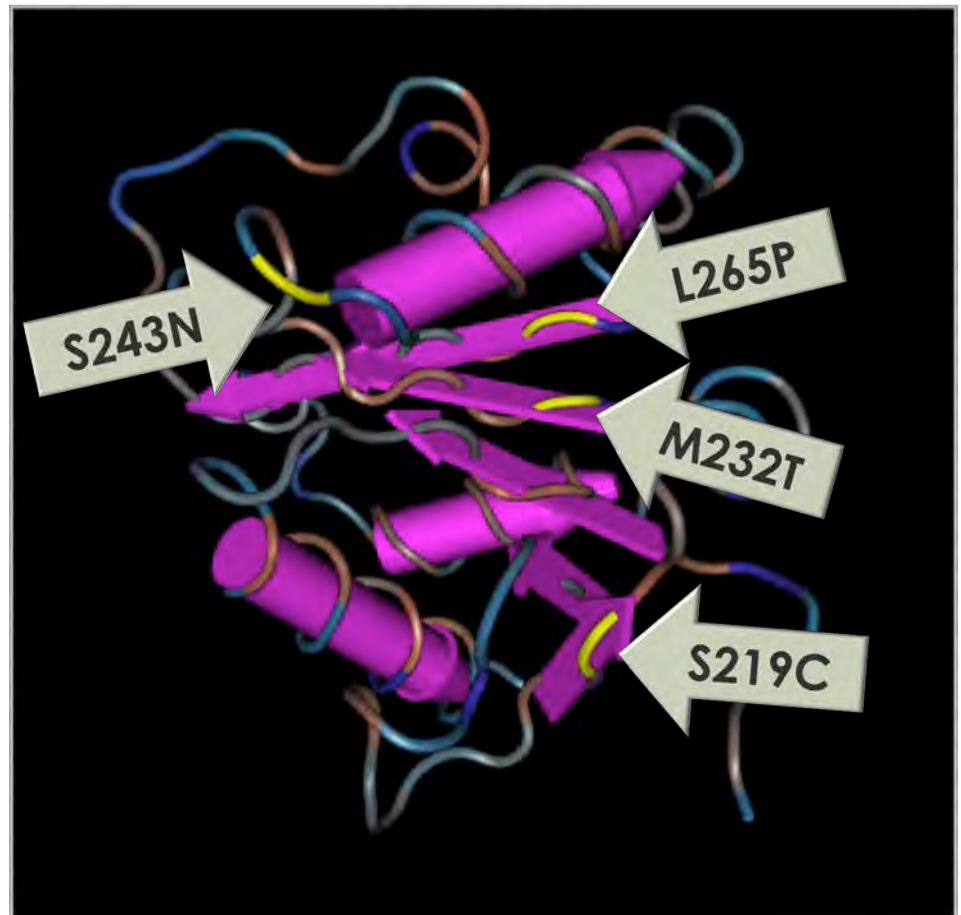
MYD88 Mutations in Diffuse Large B-Cell Lymphoma



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



Denk je dat er MYD88 mutaties zijn in andere aminozuren?

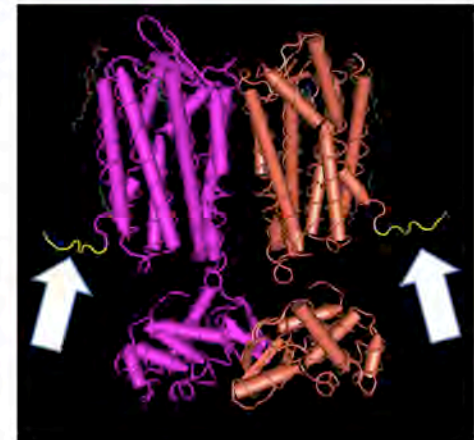


Mutations in WM

- L265P
- S219C (subclonal)

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

MEGISIYTS DNYTEEMGSGDYDSMKEPCFREENANFNK**IFLPTI**
YSIIFLTGIVGNGLVILVMGYQKKLRSMTDKYR**LHLSVADLLFVI**
TLPFWAVDAVANWYFGNFLCKAVHVIYTVNLYSSVLILAFISLD
 RYLAIVHATNSQRPRKLLAEK**VVYVGVWIPALLLTIPDFIFANVS**
 EADDRYICDRFYPNDLW**VVVFQFQHIMVGLILPGVILSCYCIIS**
 KLSHSHKGHQKRKALKTT**VILILAFFACWLPYYIGISIDSFILLEIK**
 QGCEFENTVHK**WISITEALAFFHCCLNPILYAFLGAKFKT**SAQH
 ALT**SMSRGS**SLK**L**SK**CKR**GGH**SS****SV****ST****ES**SSSFHSS

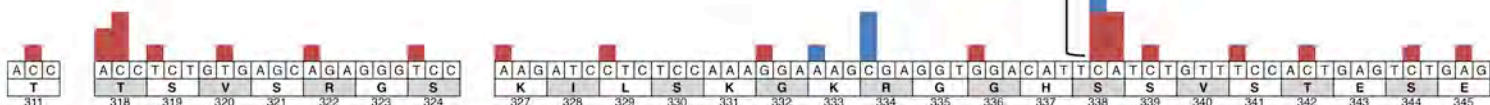


LEGEND

- A** - Germline variant in WHIM syndrome **A** - Transmembrane helix
- - Somatic frame shift or nonsense WM variant

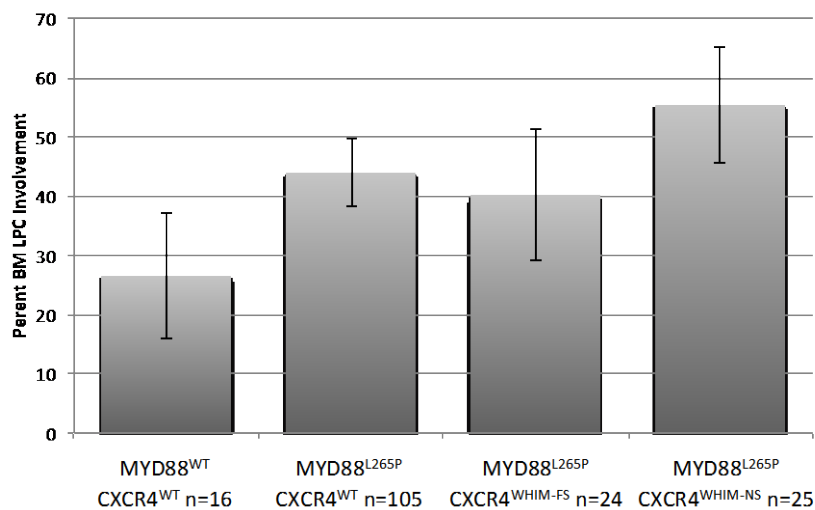
- Frame shift mutation
- Nonsense mutation

S338 Mutation Types

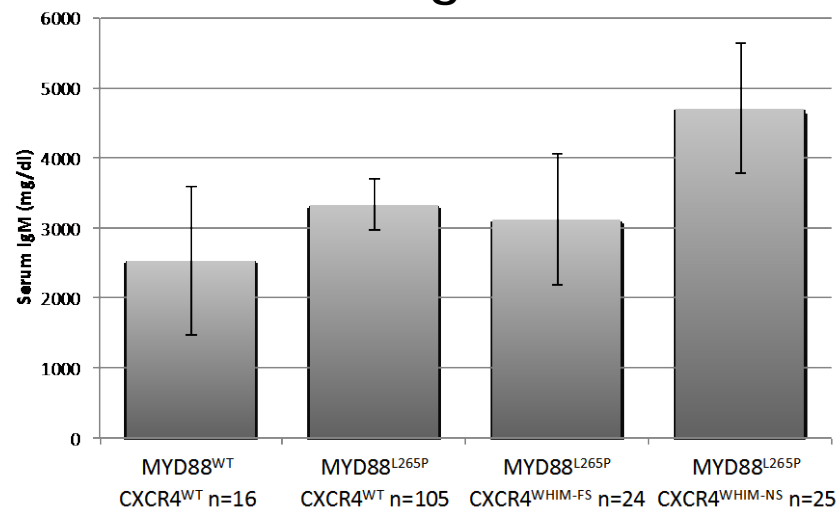


Beenmerg en Serum IgM per Genotype in 174 WM Patienten

Bone Marrow Involvement



Serum IgM Levels

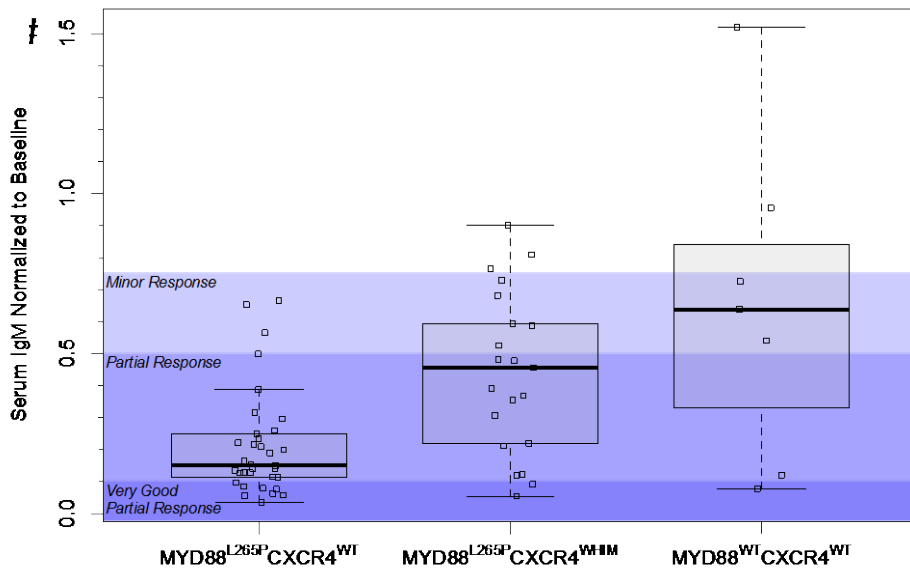


MYD88
CXC4
Genotype

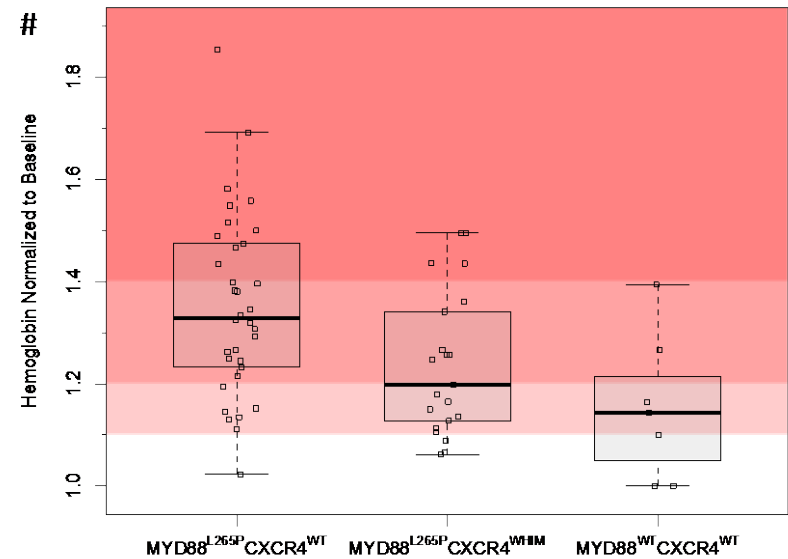
			omatic

Mutation Status gebruiken om meer inzicht te krijgen in de reactie op Ibrutinib.

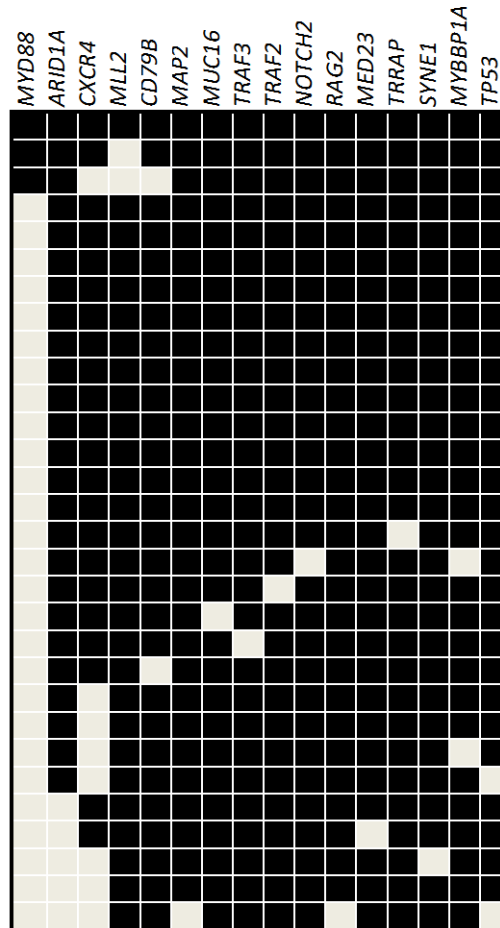
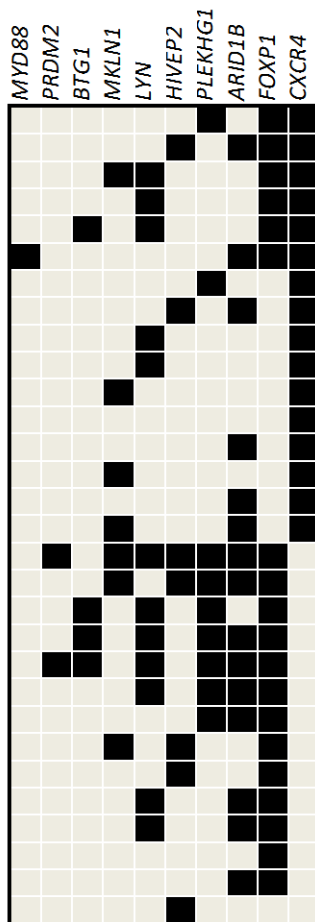
Serum IgM Response



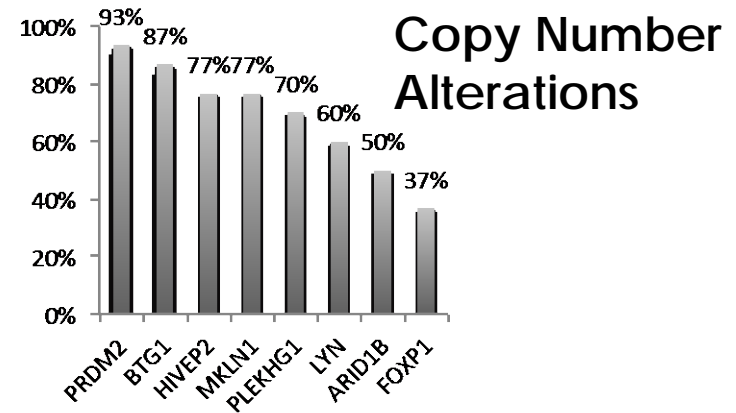
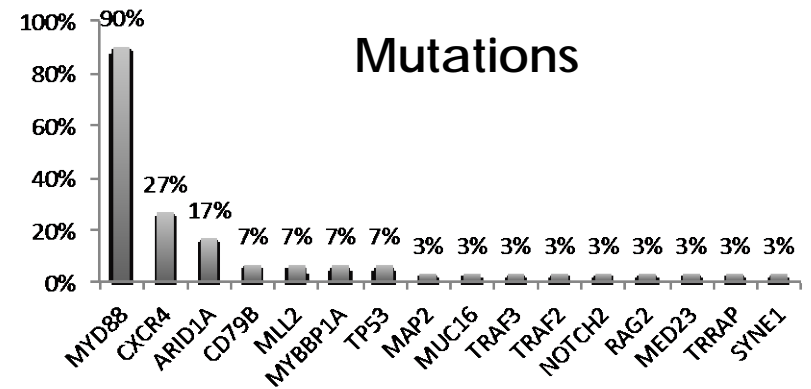
Hemoglobin Response



Het WM Genome ...



Somatically deleted/mutated
 Consistent with germline



Nieuwe bevindingen in het WM transcriptome

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

Cytogenetic Changes

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

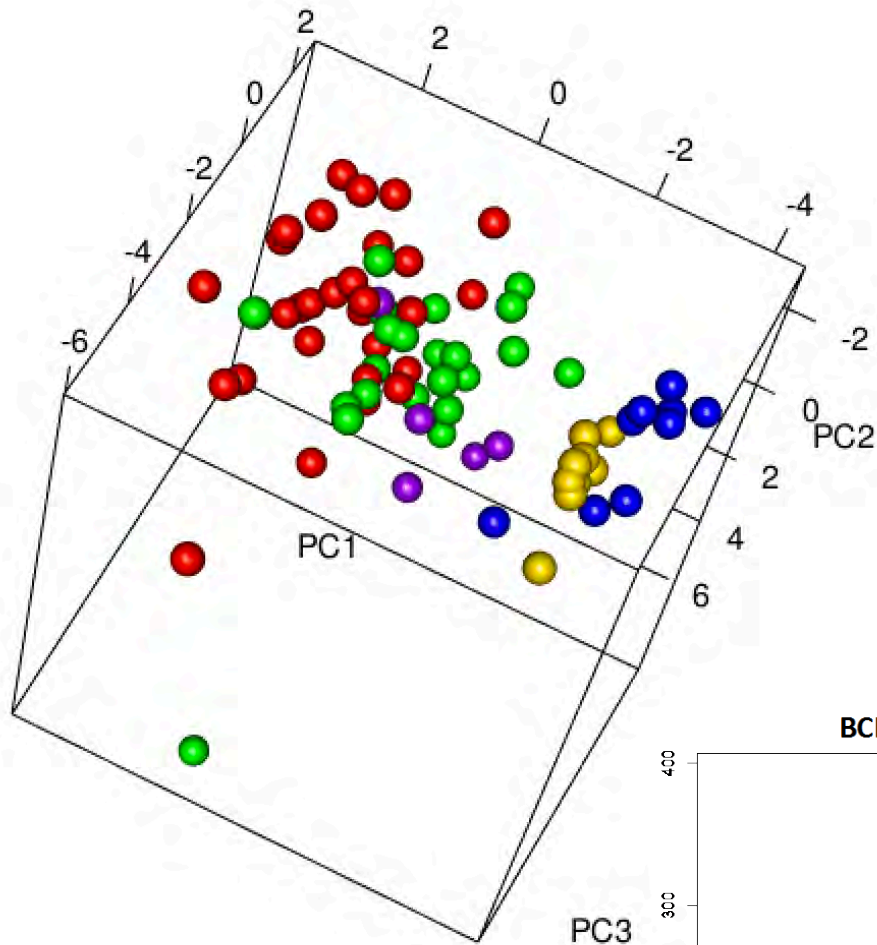
Healthy Donor Comparisons

- 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

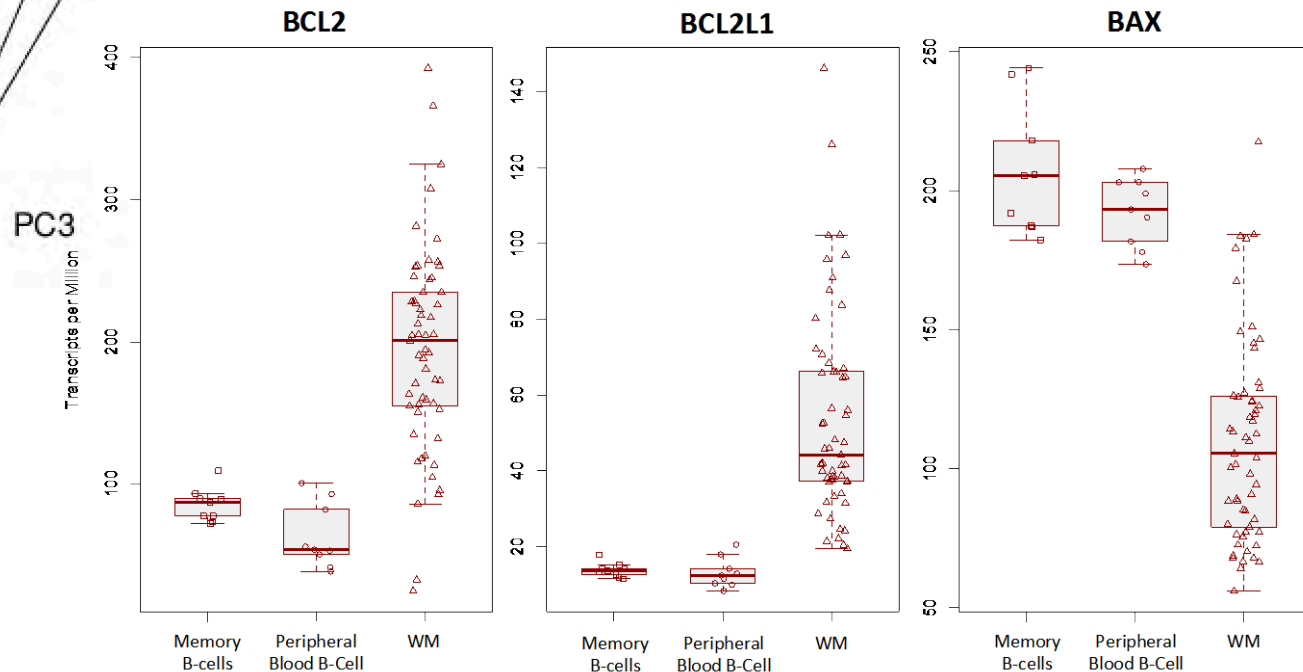
Expressie van BCL2 Familie genen in WM



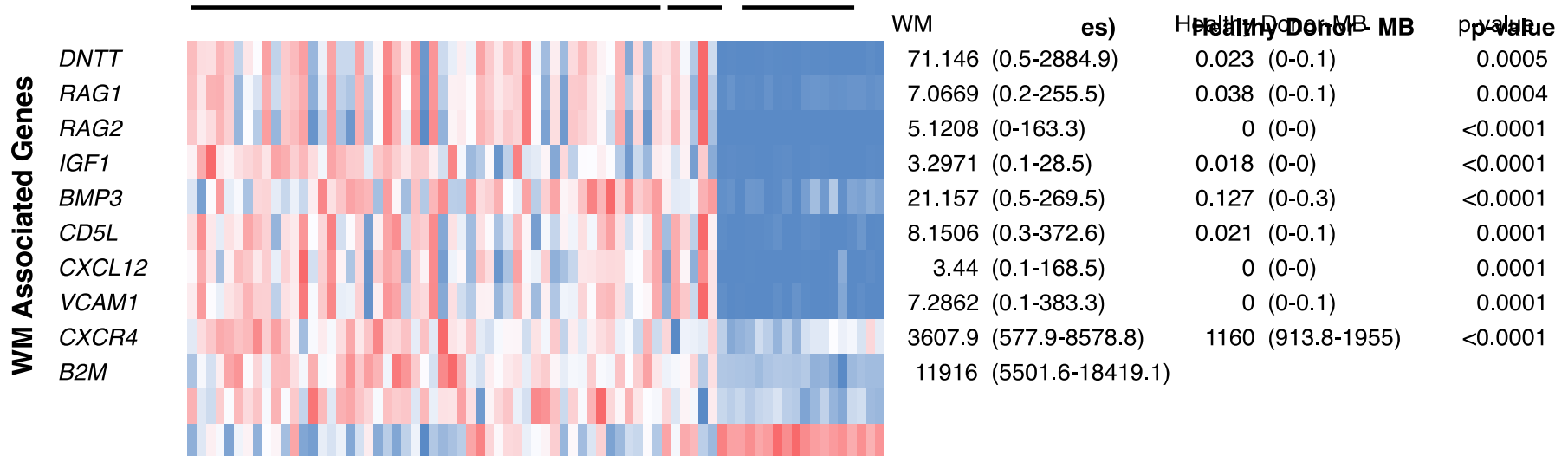
WM versus HD Expression $p < 0.0001$

- 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

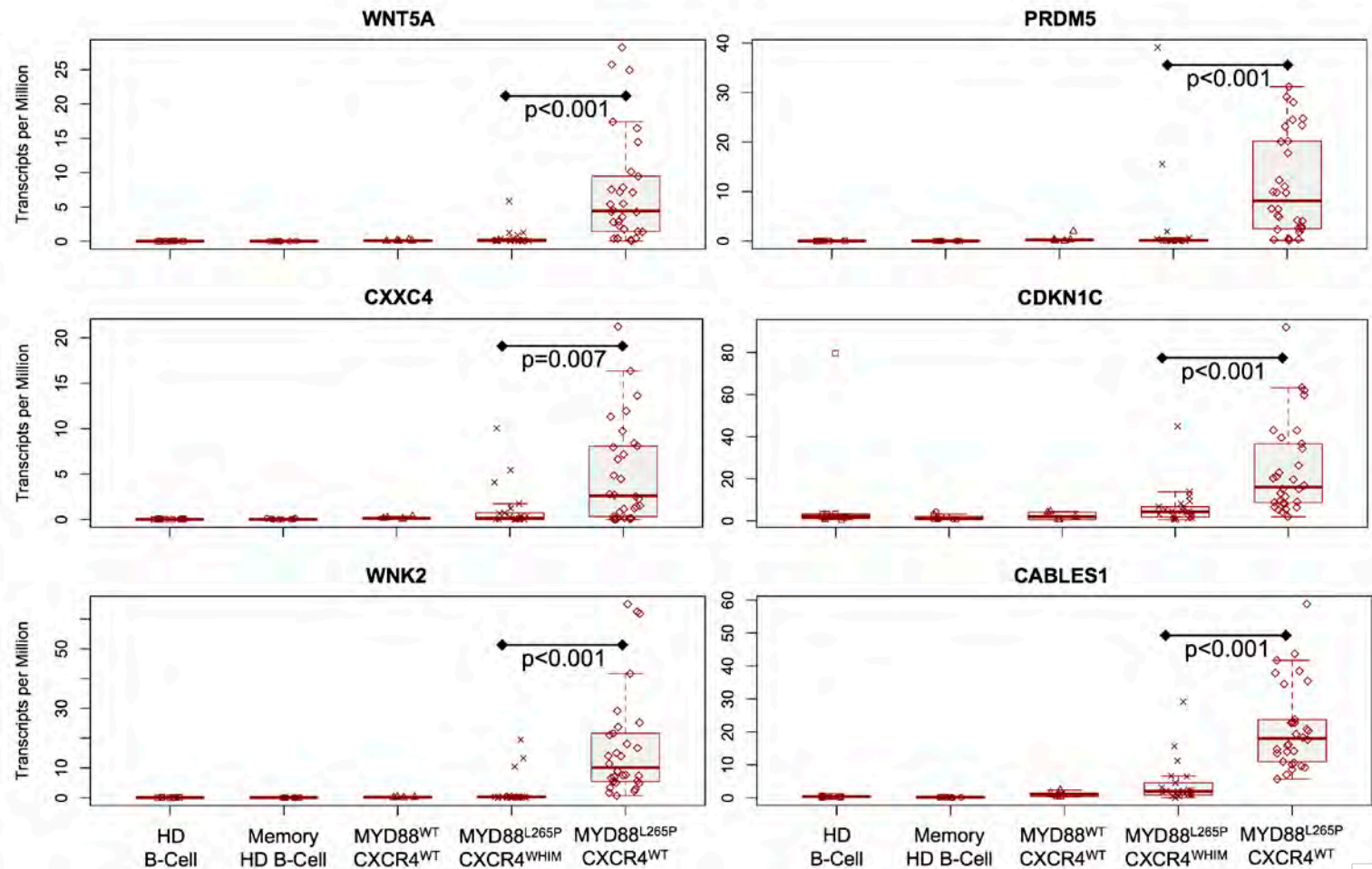


Is CXCR4 belangrijk voor alle WM patienten ongeacht de mutation?



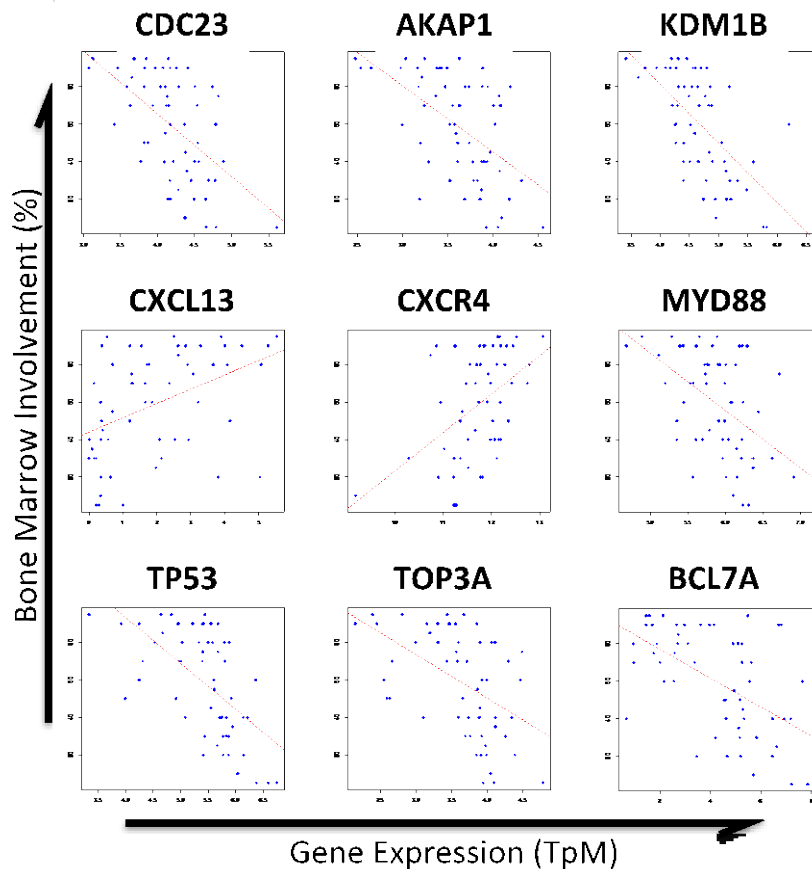
Relative gene expression differences between WM patients and healthy donors (Red indicates high expression, blue low expression)

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

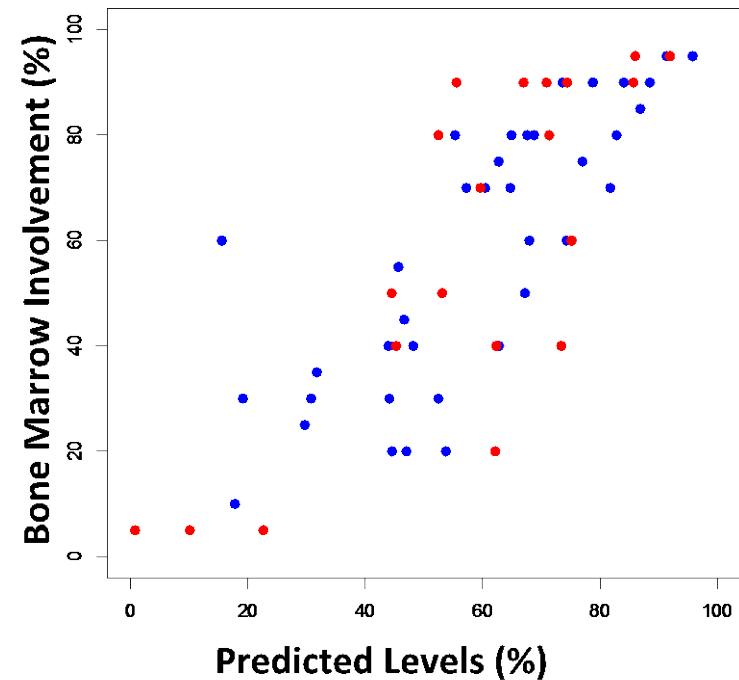


Gene Expression Associated with Bone Marrow Involvement in WM

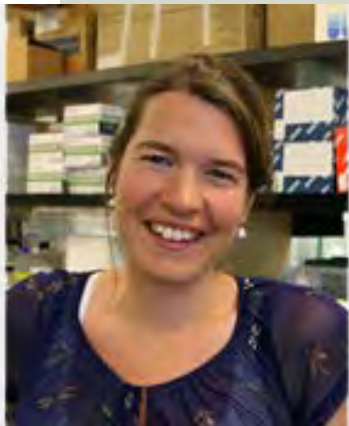
Bone Marrow Involvement



Final Model

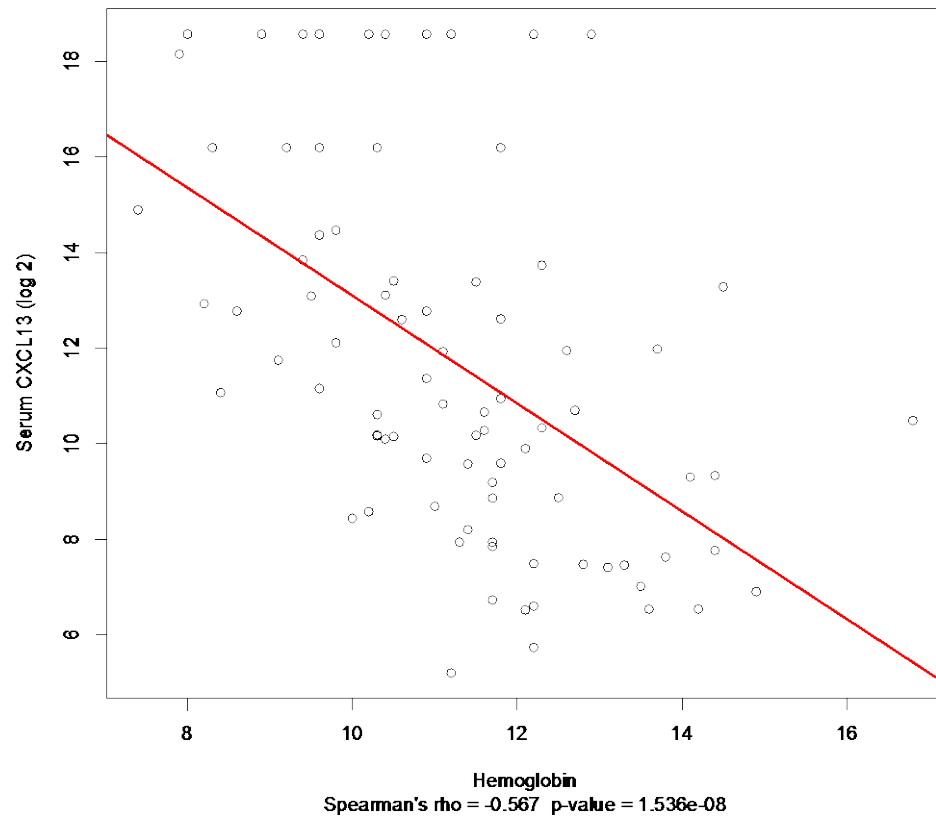
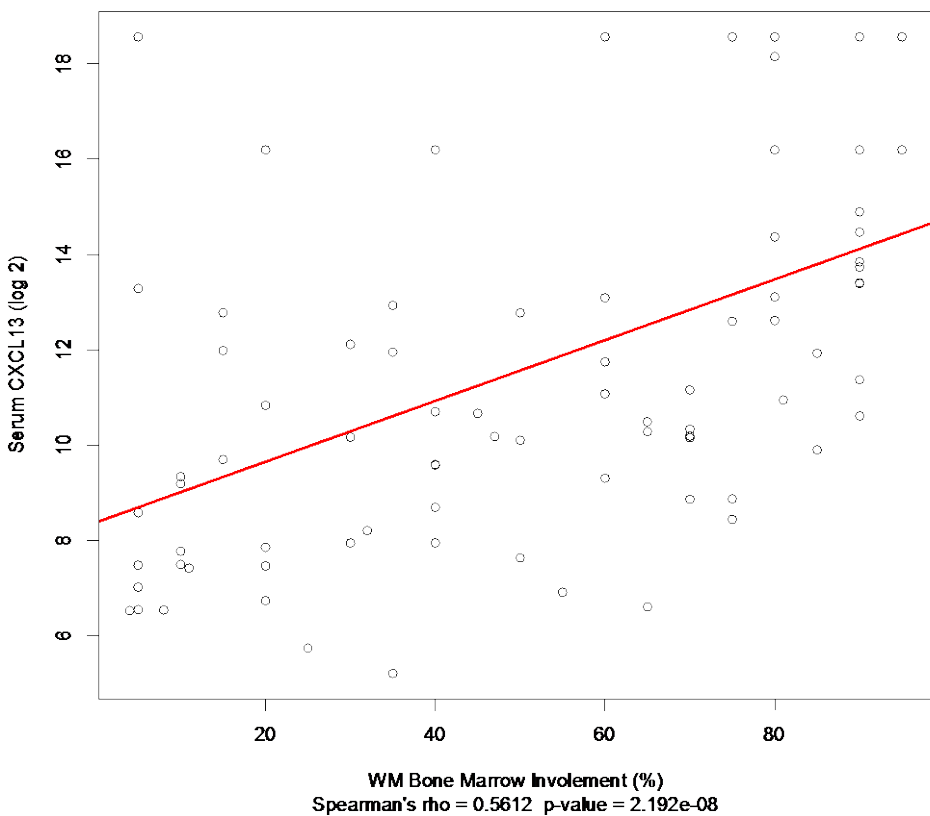


Adjusted R-squared: 0.5358 *Training RMSE: 16.1*
p-value: 3.469e-7 *Validation RMSE: 19.3*



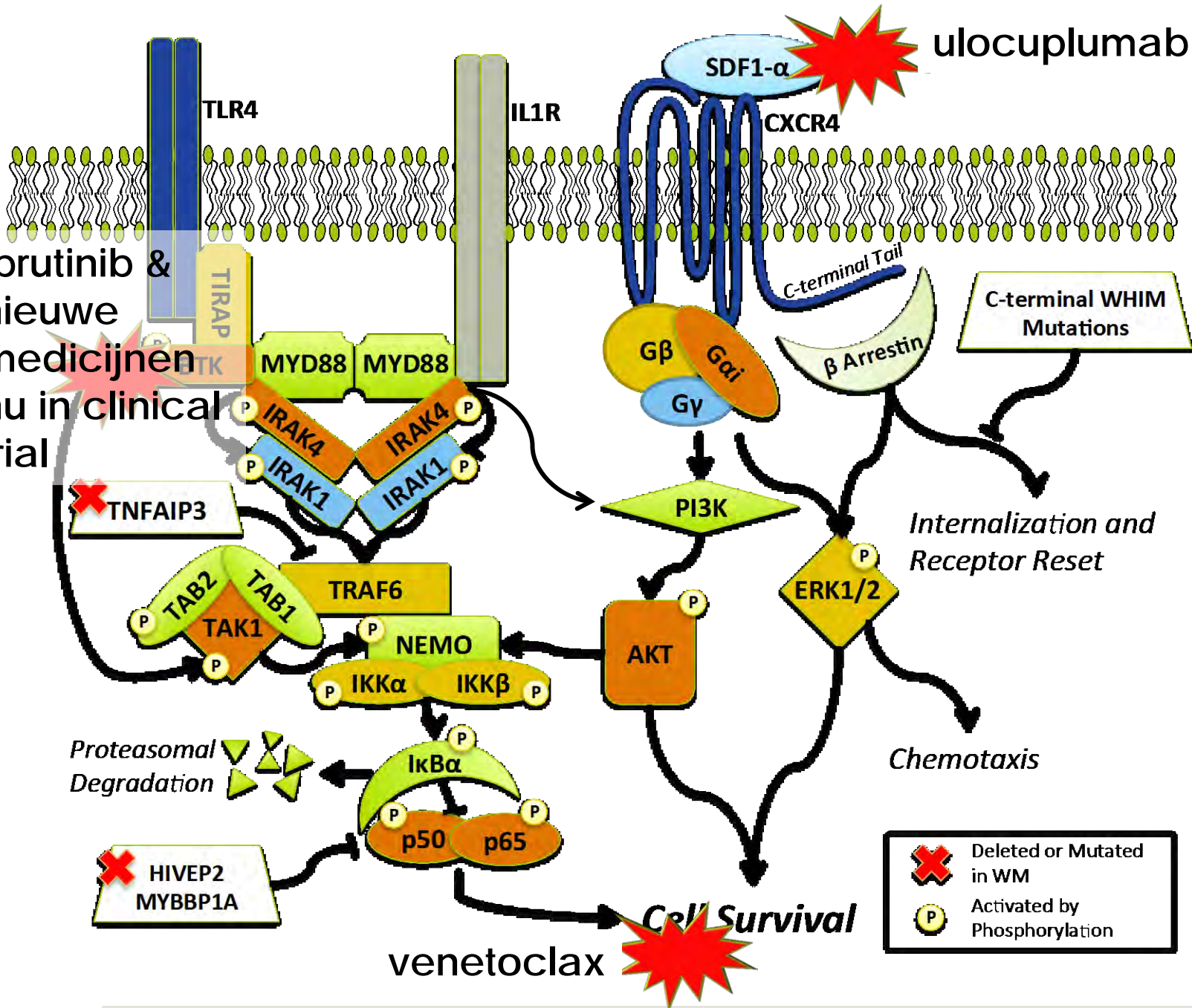
Validatie van CXCL13 Correlatie met Beenmerg en Hemoglobine in 86 WM Patienten

Josephine Vos, MD



Ibrutinib & nieuwe medicijnen nu in clinical trial

ulocuplumab



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.

MYD88^{WT} CXCR4^{WT}

- 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.

Potential Targets

- All WM Patients
 - CXCR4 (both WT & WHIM)
 - CXCL13
 - BCL2 and BCL2L1
- IGF1/IGF1R, particularly in MYD88^{L265P}CXCR4^{WT}
- Hypomethylating agents in MYD88^{WT} patients
- PIK3 delta inhibitors, particularly in MYD88^{WT} WM. Additional inhibition of PIK3 gamma may be necessary for CXCR4^{WHIM} patients

All WM

- Up regulated VDJ Genes: DNMT, 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

MYD88^{L265P} CXCR4^{WT}

- 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.

MYD88^{L265P} CXCR4^{WHIM}

- 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.

Dankwoord

Funding and Support

- The Bing Fund for WM
- International Waldenstrom's Macroglobulinemia Foundation
- Coyote Fund for WM
- Bailey Family Fund for WM
- D'Amato Family Fund for Genomic Discovery
- Orzag Family Fund for WM Genomics
- Leukemia and Lymphoma Society
- NIH Spore Development Award



Bing Center for WM

Steven P. Treon, Lian Xu, Guang Yang, Xia Liu, Jorge Castillo, Robert Manning, Christopher J Patterson, Philip Brodsky, Kirsten Mied, Jie Chen, Nicholas Tsakmaklis, Jiaji Chen, Maria Demos, Toni Dubeau, Joshua Gustine, Gloria Chan.

The Bing Center



Jerome Lipper Myeloma Center

Kenneth Anderson, Nikhil Munshi



Yaoyu Wang
John Quakenbush



And the support of all the WM patients who made this study possible!