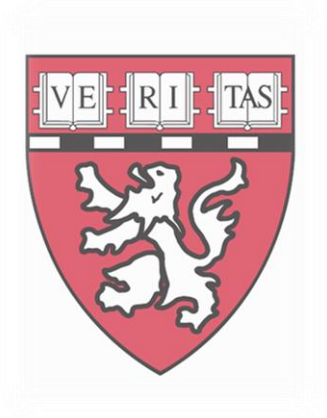
Translating Genomic Advances into Novel Therapies for Waldenström Macroglobulinemia



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Bing Center for Waldenstrom's Macroglobulinemia

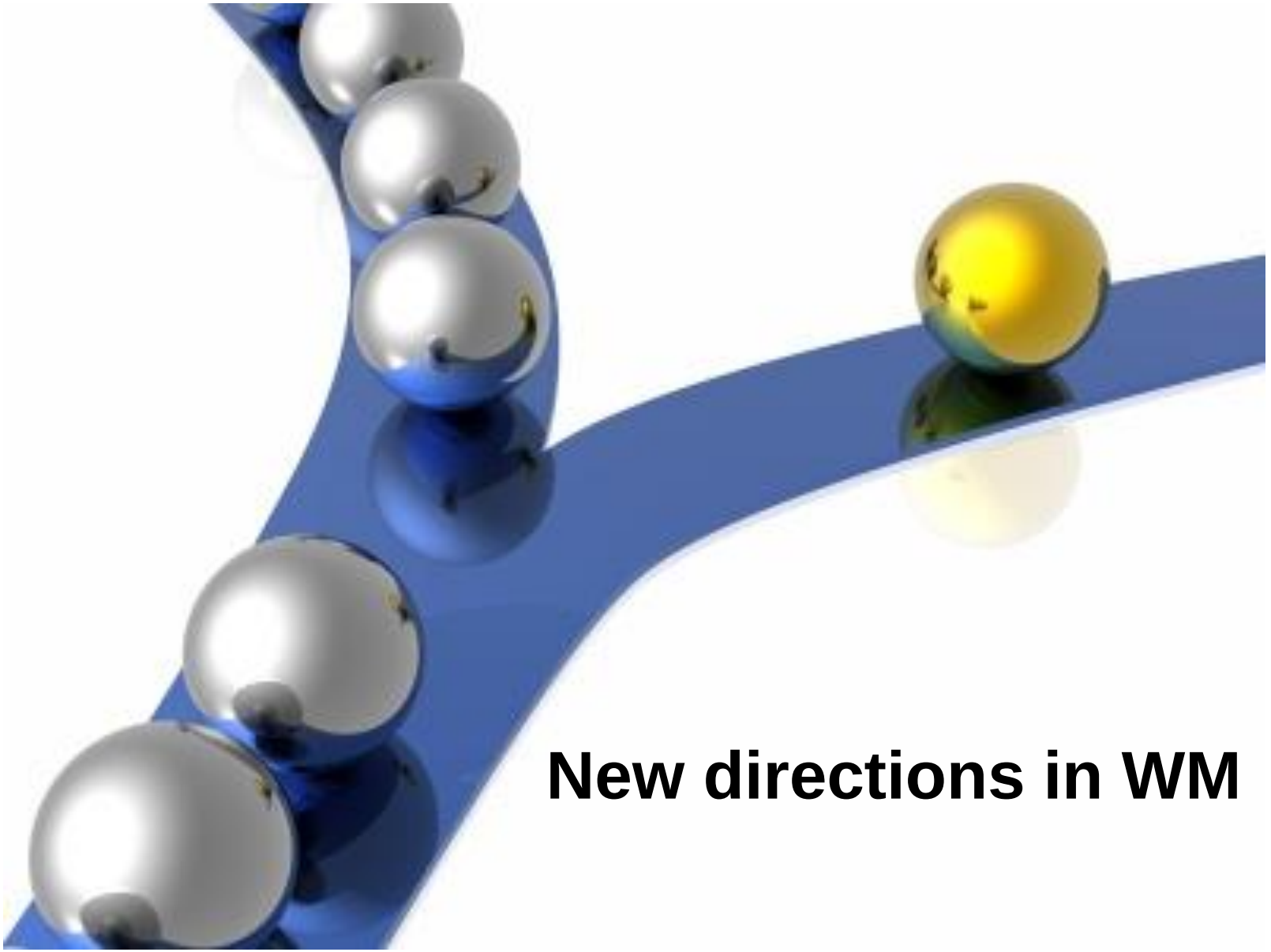
Dana-Farber Cancer Institute

Harvard Medical School

WM Treatment Approach

WM–centric toxicities with commonly used therapies

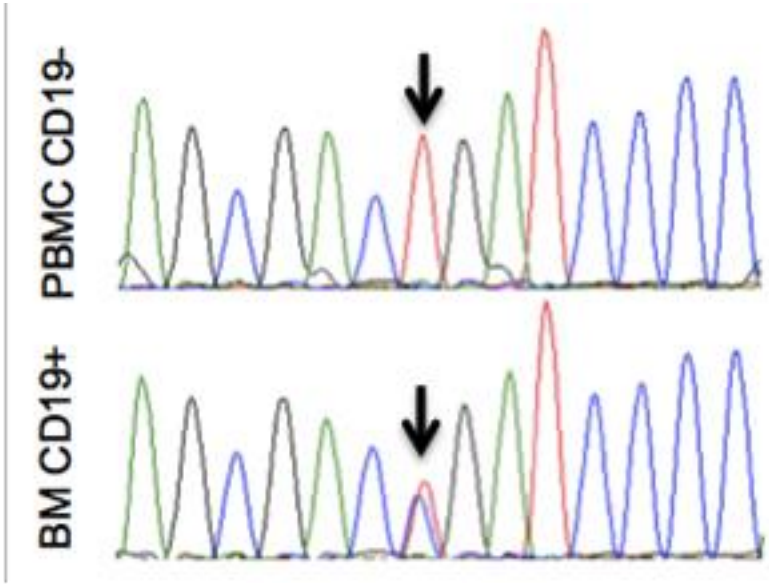
Agent	WM Toxicities
Rituximab	• IgM flare (40-60%)-> Hyperiscosity crisis, Aggravation of IgM related PN, CAGG, Cryos. • Hypogammaglobulinemia-> infections, IVIG • Intolerance (15-20%)
Nucleoside Analogues	• Hypogammaglobulinemia-> infections, IVIG • Transformation, AML/MDS (15%)
IMIDS	• Peripheral Neuropathy (60% >grade 2 with Thalidomide) • Aggravated IgM flare (Revlimid and Pomalidomide) • Severe anemia (Revlimid)
Bortezomib	• Grade 2+3 Peripheral neuropathy (60-70%); High discontinuation (20-60%)



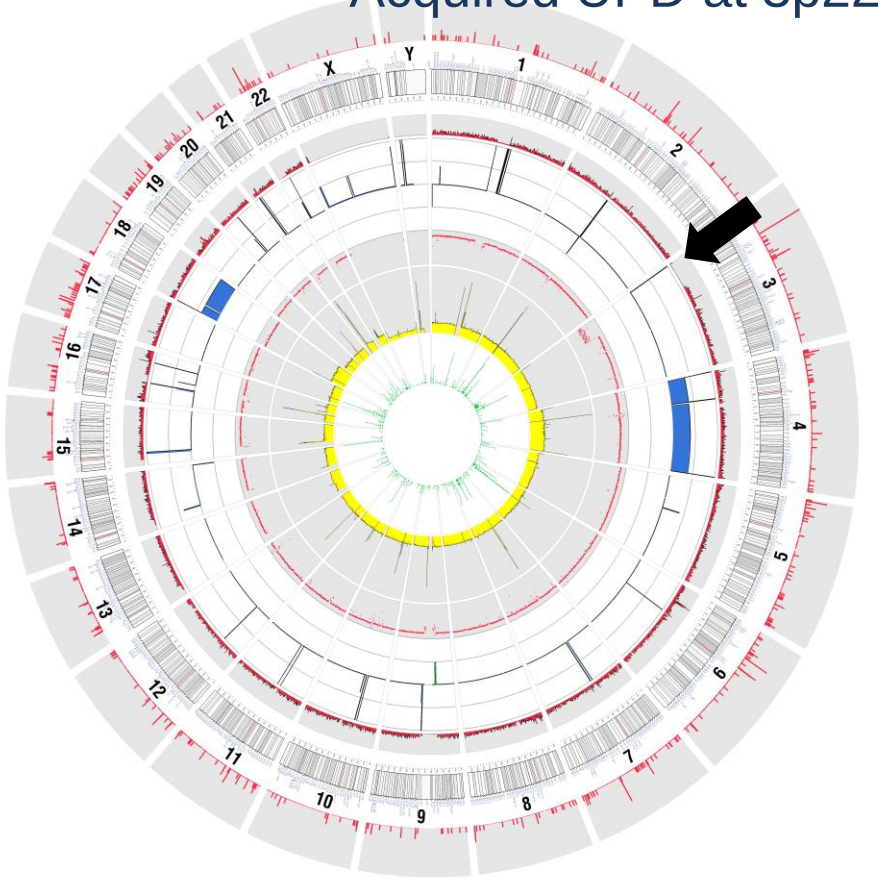
New directions in WM

MYD88 L265P Somatic Mutation in WM

C to G at position 38186241
at 3p22.2



Acquired UPD at 3p22.



MYD88^{L265P} confirmed by AS-PCR in 95% WM patients, 50-80% IGM MGUS.

Treon et al, NEJM 367:826, 2012

MYD88 L265P Somatic Mutation in WM

		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%	

> 50 confirmatory studies worldwide!!

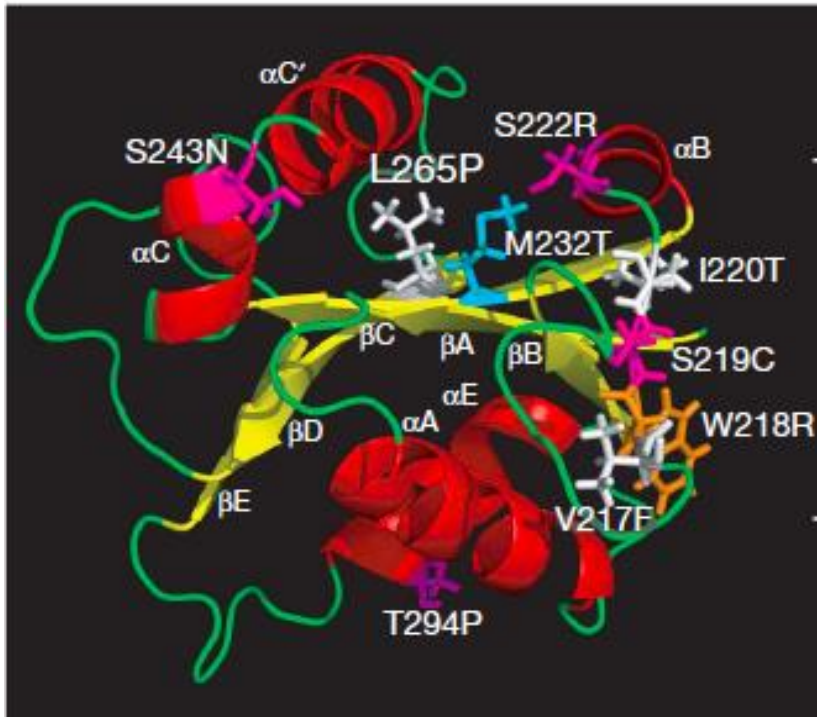
Lymphoproliferative Disorders driven by mutated MYD88

Waldenstrom's Macroglobulinemia (95-97%)
IGM MGUS (50-80%)
Lymphoplasmacytic Lymphoma (non-IGM) (100%)
Primary CNS Lymphoma (80-90%)
Immune Privileged Lymphomas (80-90%)
ABC DLBCL (20-40%)
Marginal Zone Lymphoma (6-10%)
CLL (4-8%)

Ngo et al, Nature 2011; Puente et al, Nature 2011; Montesinos-Rongeri et al, Acta Neuropathl 2011; Pasqualucci et al, Nat Gen. 2011; Treon et al, 2012;Varetonni et al, Blood 2013; Xu et al, Blood 2013; Martinez et al, Leukemia 2014;

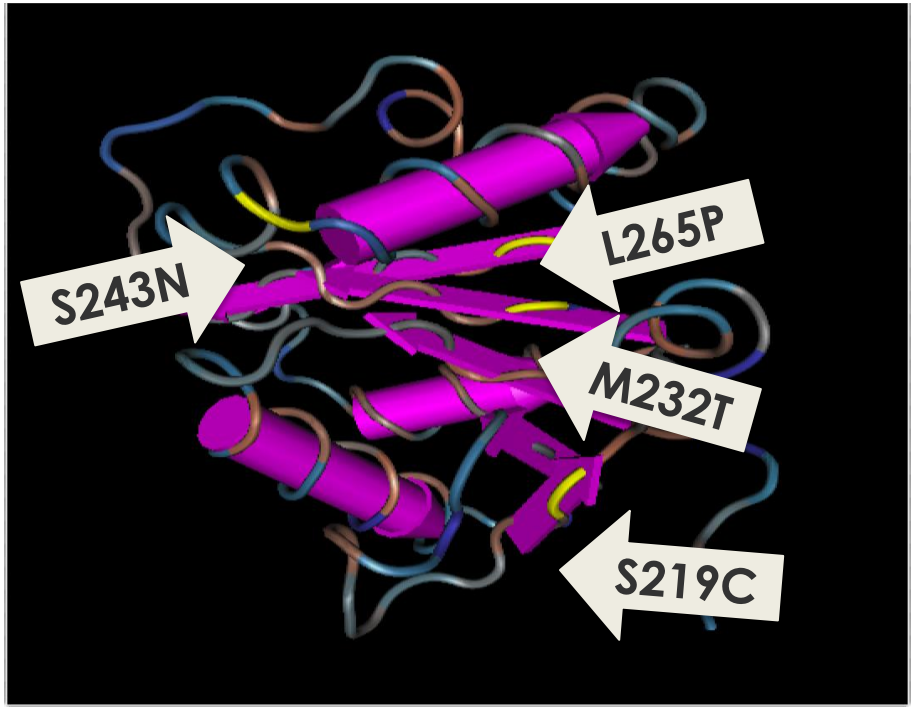
MYD88 Mutations in B-cell Malignancies

ABC DLBCL



29% MYD88 L265P
10% Non-L265P MYD88

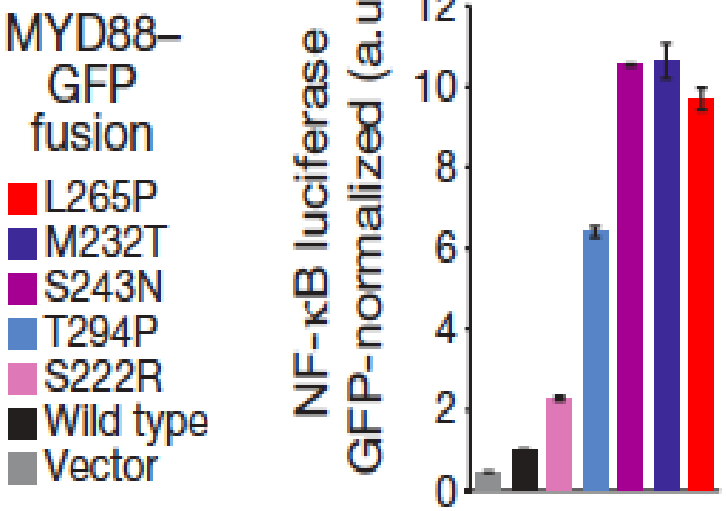
WM



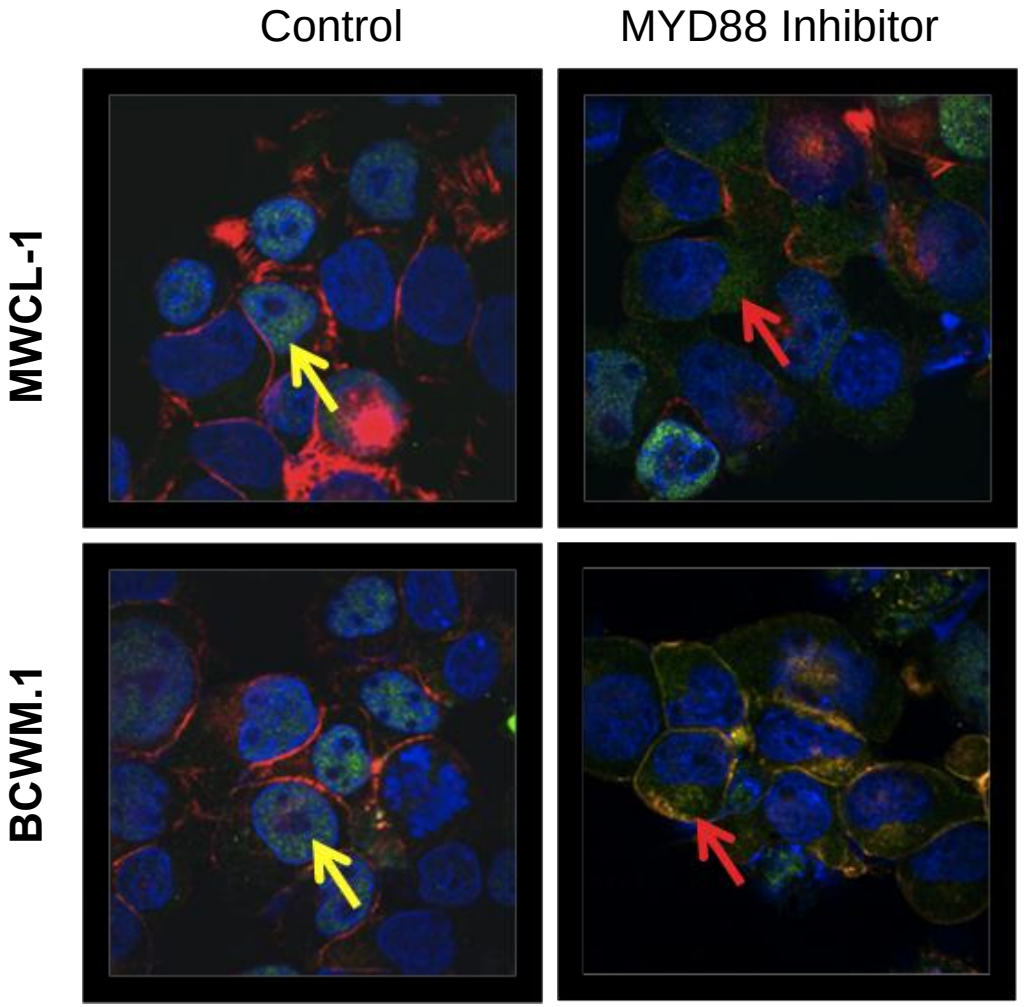
93-95% MYD88 L265P
2% Non-L265P MYD88

Ngo et al, Nature 2011; Treon et al, NEJM 2012; Treon et al, NEJM 2015

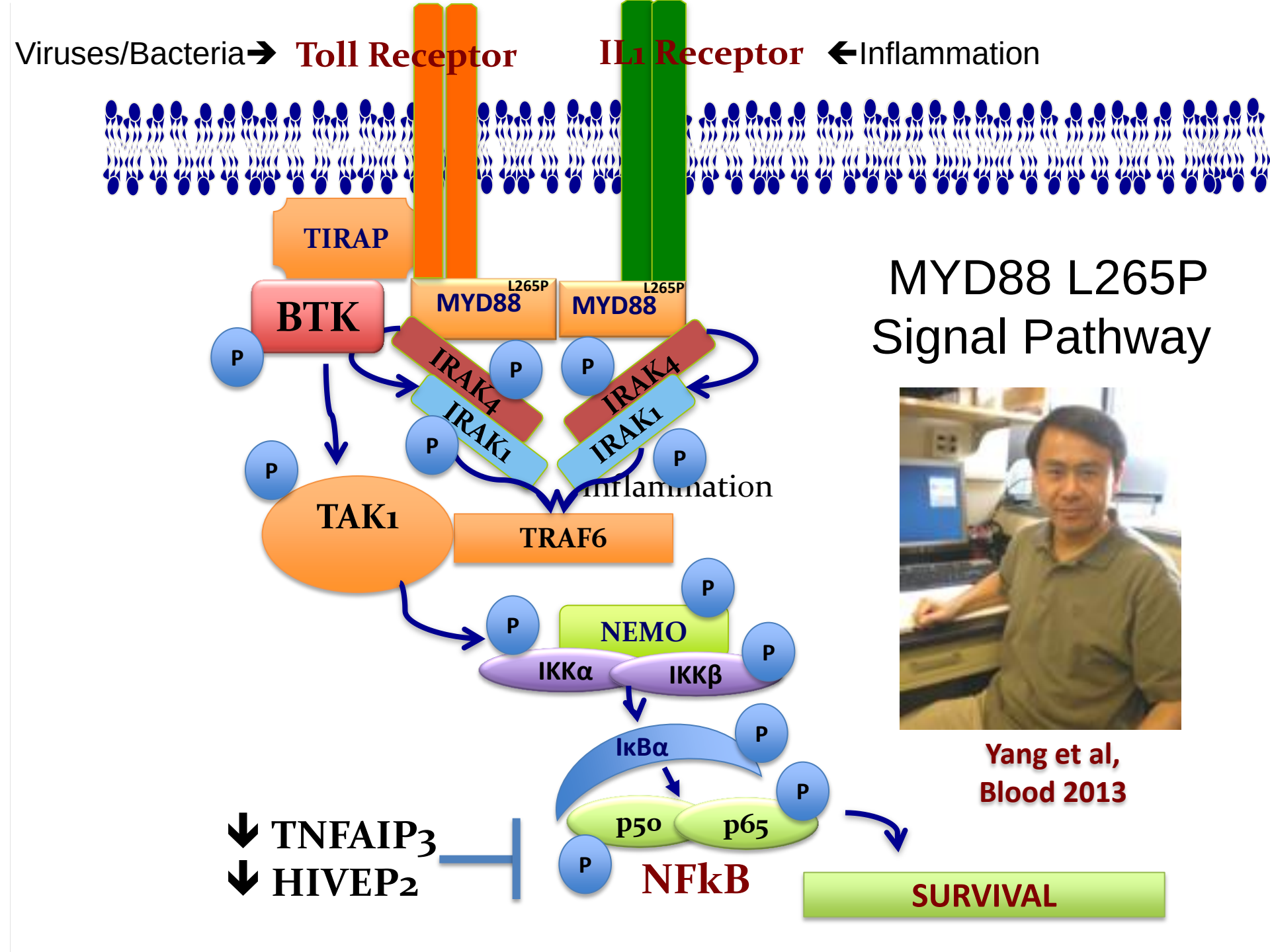
MYD88 mutations transactivate NFKB



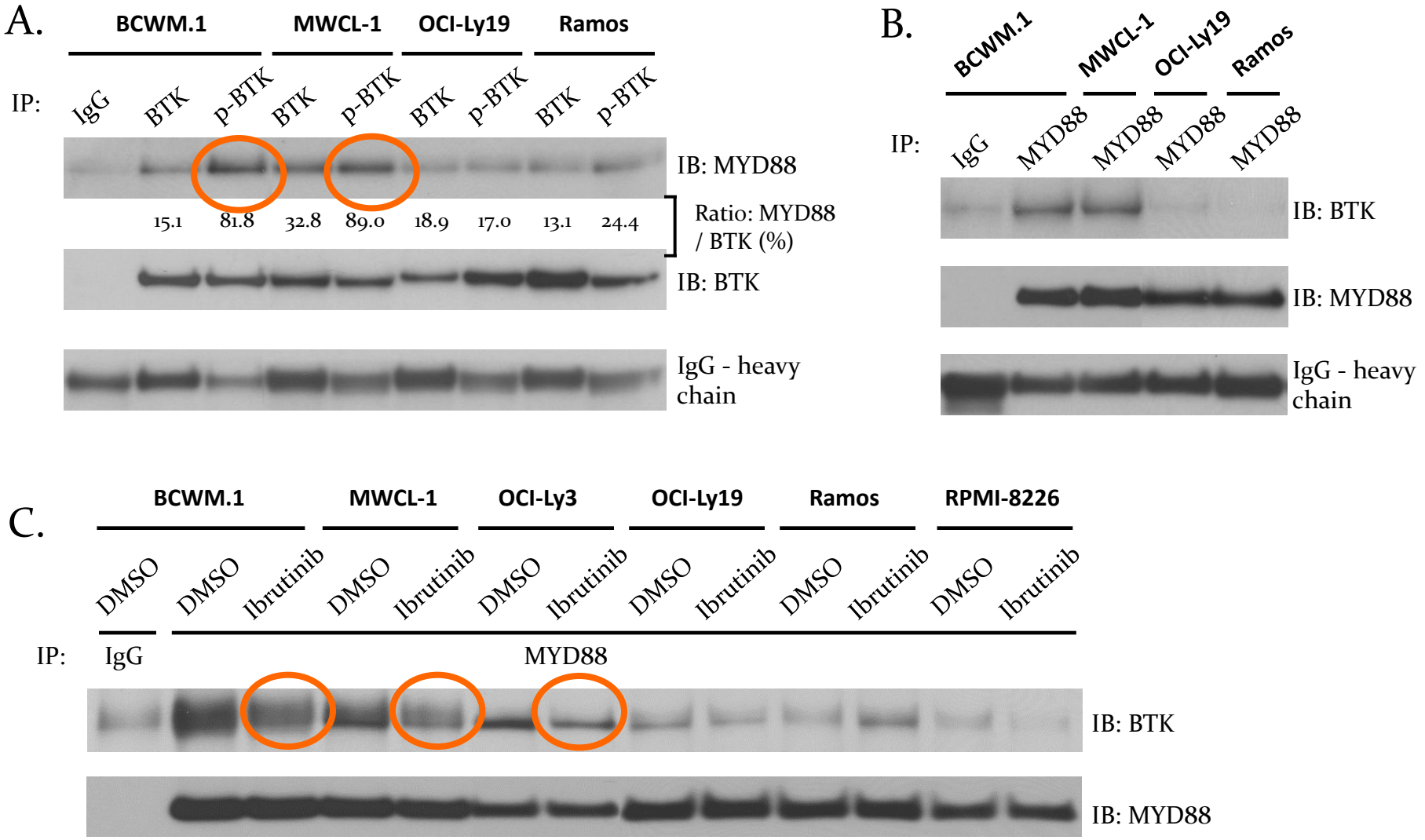
Ngo et al, Nature 2011
Treon et al, NEJM 2012



MYD88 L265P mutated WM cells

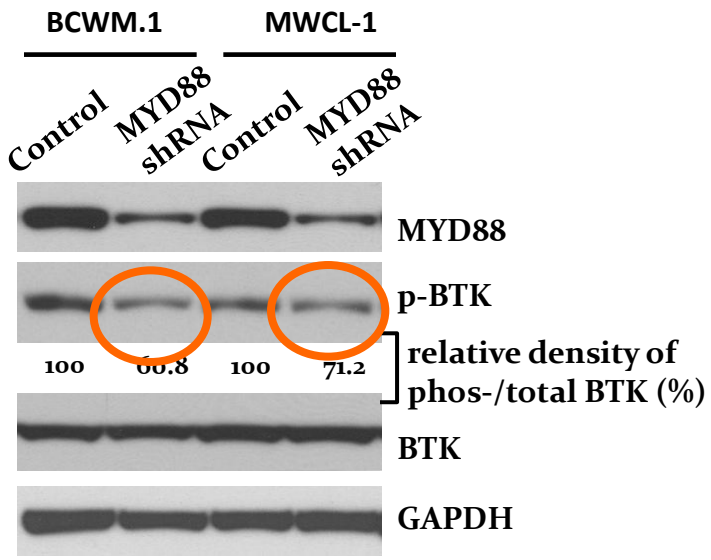

Viruses/Bacteria
Toll Receptor
IL1 Receptor
Inflammation
TIRAP
BTK
MYD88 L265P
MYD88 L265P
IRAK4
IRAK1
IRAK4
IRAK1
P
TAK1
TRAF6
Inflammation
NEMO
IKKα
IKKβ
IκBα
p50
p65
NFkB
↓ TNFAIP3
↓ HIVEP2
SURVIVAL
MYD88 L265P Signal Pathway
Yang et al, Blood 2013

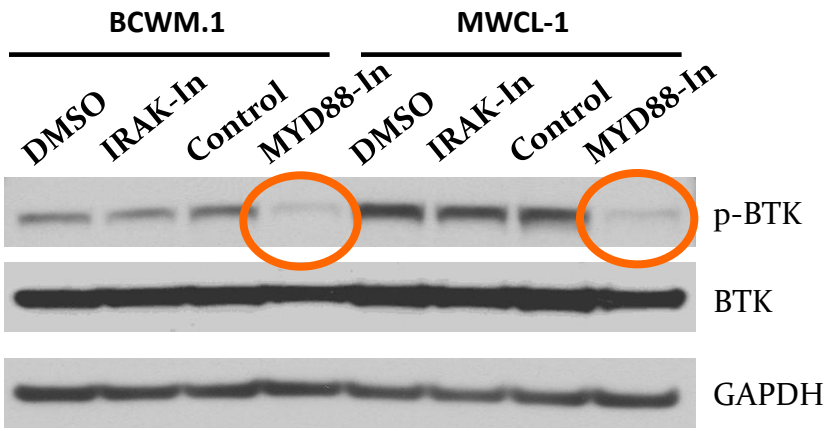
MYD88 binds to the active form of Bruton's Tyrosine Kinase (BTK) in L265P expressing WM cells

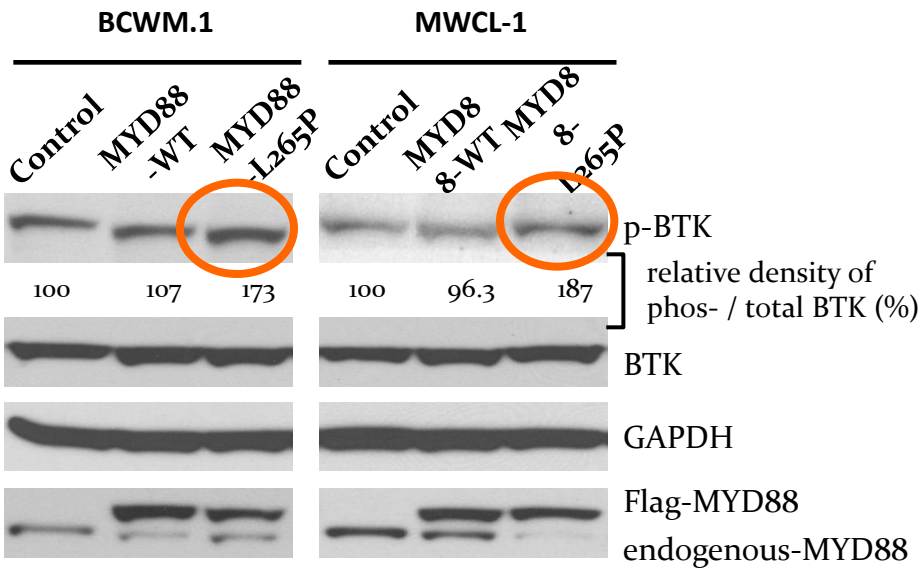


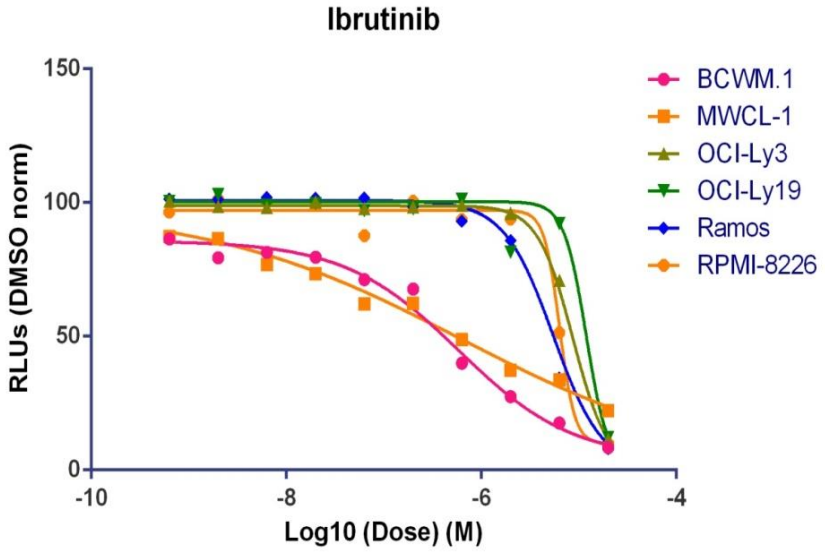
Yang et al, Blood 2013; 122:1222-32.

MYD88 L265P induces BTK in WM cells





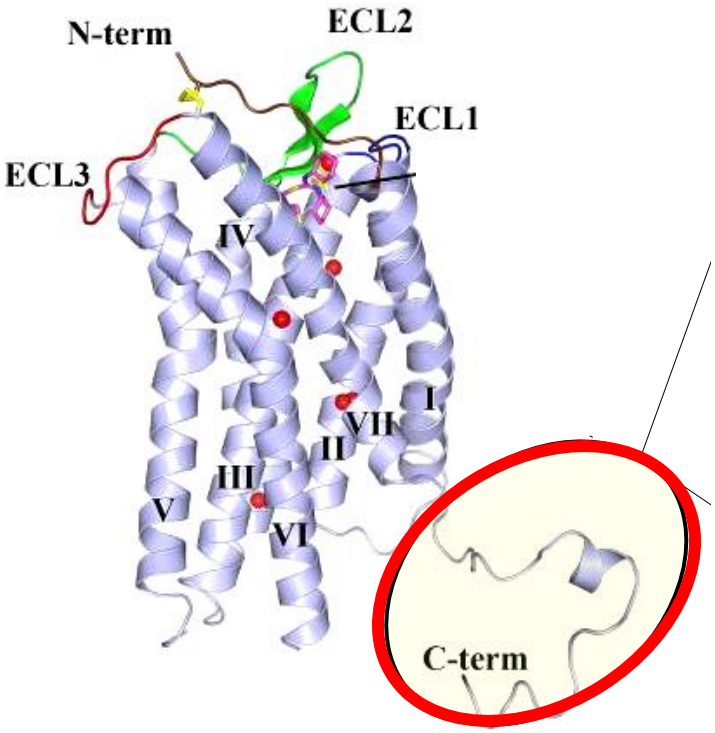




Yang et al, Blood 2013; 122:1222-32.

WHIM-like CXCR4 C-tail mutations in WM

Warts, Hypogammaglobulinemia, Infection, and Myelokathexis.



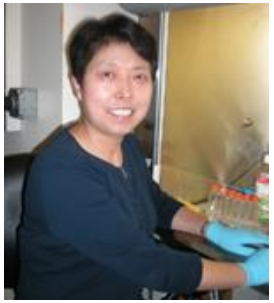
- 30-40% of WM patients
- > 30 Nonsense and Frameshift Mutations
- Almost always occur with $MYD88^{L265P}$

Hunter et al, Blood 2013; Rocarro et al, Blood 2014; Poulain et al, ASH 2014; Schmidt et al, BJH 2014.

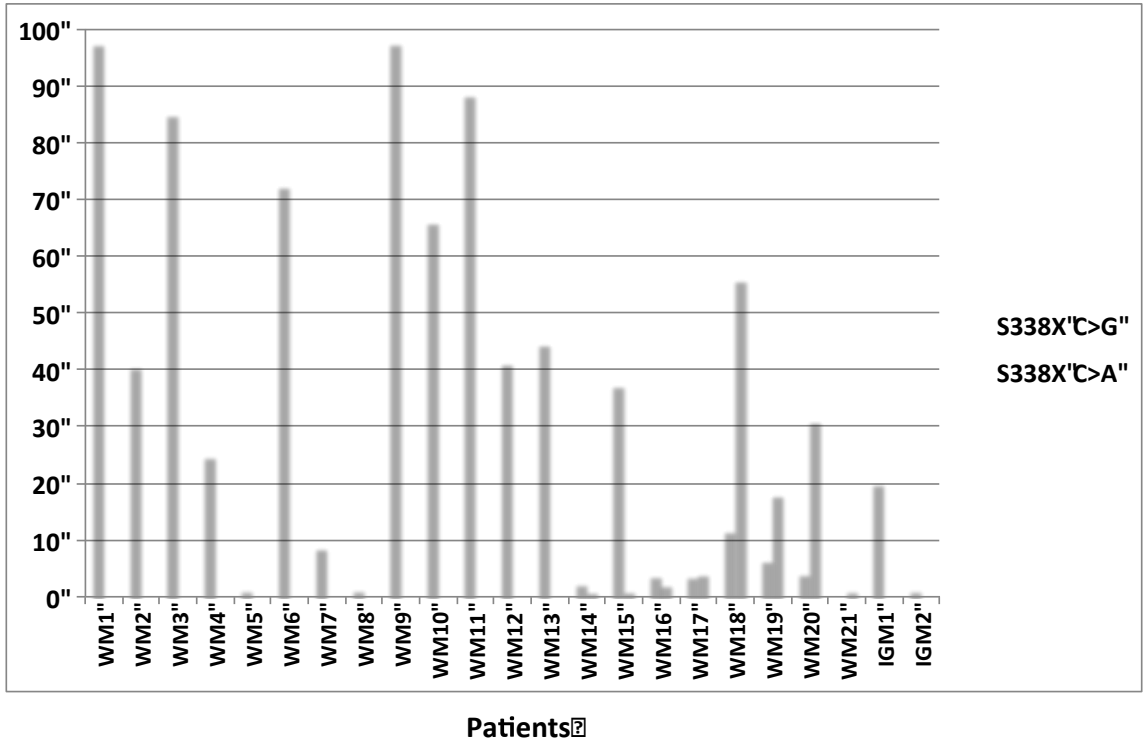
MYD88 and CXCR4 MUTATIONS in B-CELL MALIGNANCIES

	(N=)	MYD88 ^{L265P}	CXCR4^{WHIM}
Healthy Donors	32	0 (0%)	0 (0%)
IgM MGUS	12	6 (50%)	2 (17%)
Non-IgM MGUS	7	0 (0%)	0 (0%)
Untreated WM	102	97 (95%)	44 (43%)
Treated WM	62	57 (92%)	21 (34%)
MZL	20	2 (10%)	1 (5%)
CLL	32	1 (3%)	0 (0%)
Multiple Myeloma	14	0 (0%)	0 (0%)

Xu et al, British J. Hematol. 2015



Not all WM cells carry the CXCR4 mutation and some patients even carry multiple CXCR4 mutations!

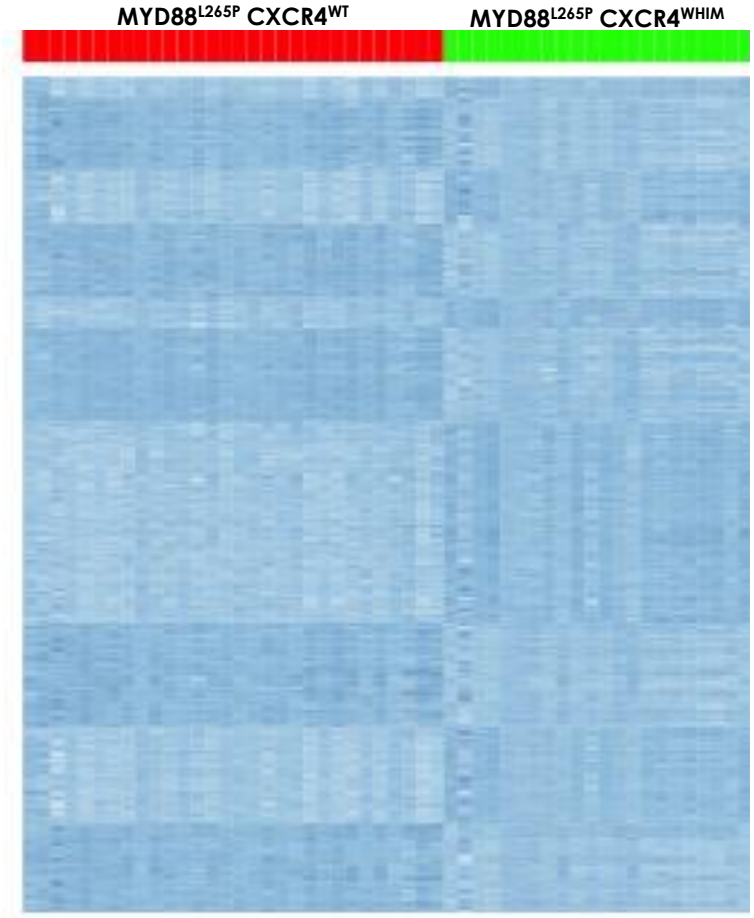


Cancer cell fraction analysis of CXCR4^{S338X} showed that mutations were primarily subclonal, with highly variable clonal distribution (median 45.1%, range 1.2%-97.5%)

Multiple CXCR4 mutations can be found in some individual WM patients!!

Xu et al, British J. Hematol. 2015

MYD88 and CXCR4 Transcriptome



Supervised Clustering of 3,103 genes found to be significantly differentially expressed between $MYD88^{L265P}CXCR4^{WT}$ (N=29) and $MYD88^{L265P}CXCR4^{WHIM}$ (N=23) WM patients

Hunter et al, BLOOD (In press)

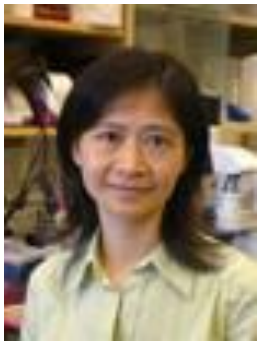
CXCR4 Signaling in WM Patients with WHIM mutations

Plerixafor
Ulucuplomab

Busillo et al, JBC 2010
Mueller et al, PLOS ONE 2013
Cao et al, Leukemia 2014
Rocarro et al, Blood 2014
Cao et al, BJH 2015

SDF-1

CXCR4



Yang Cao

B-arrestins

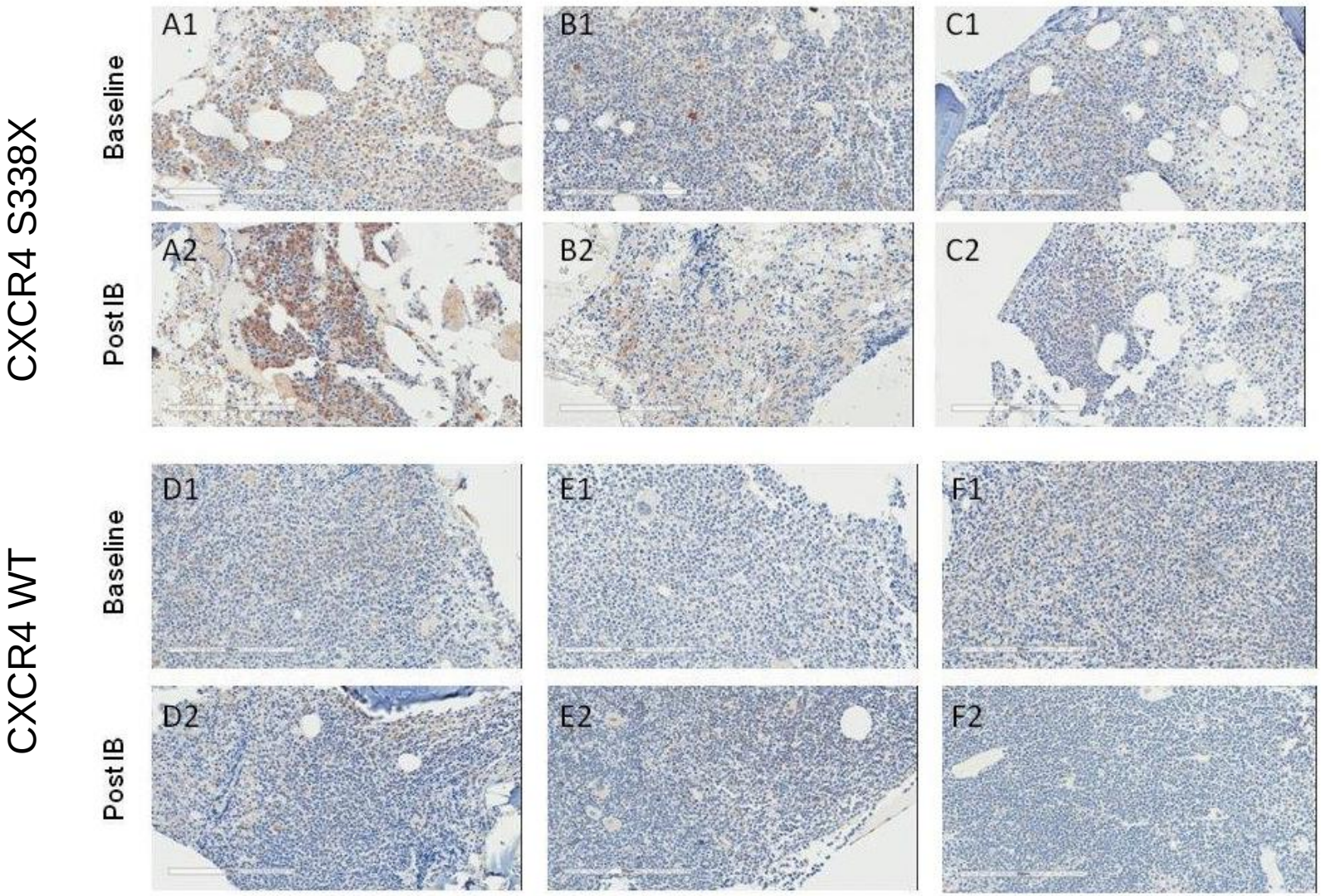
Ser346/7

ERK
AKT

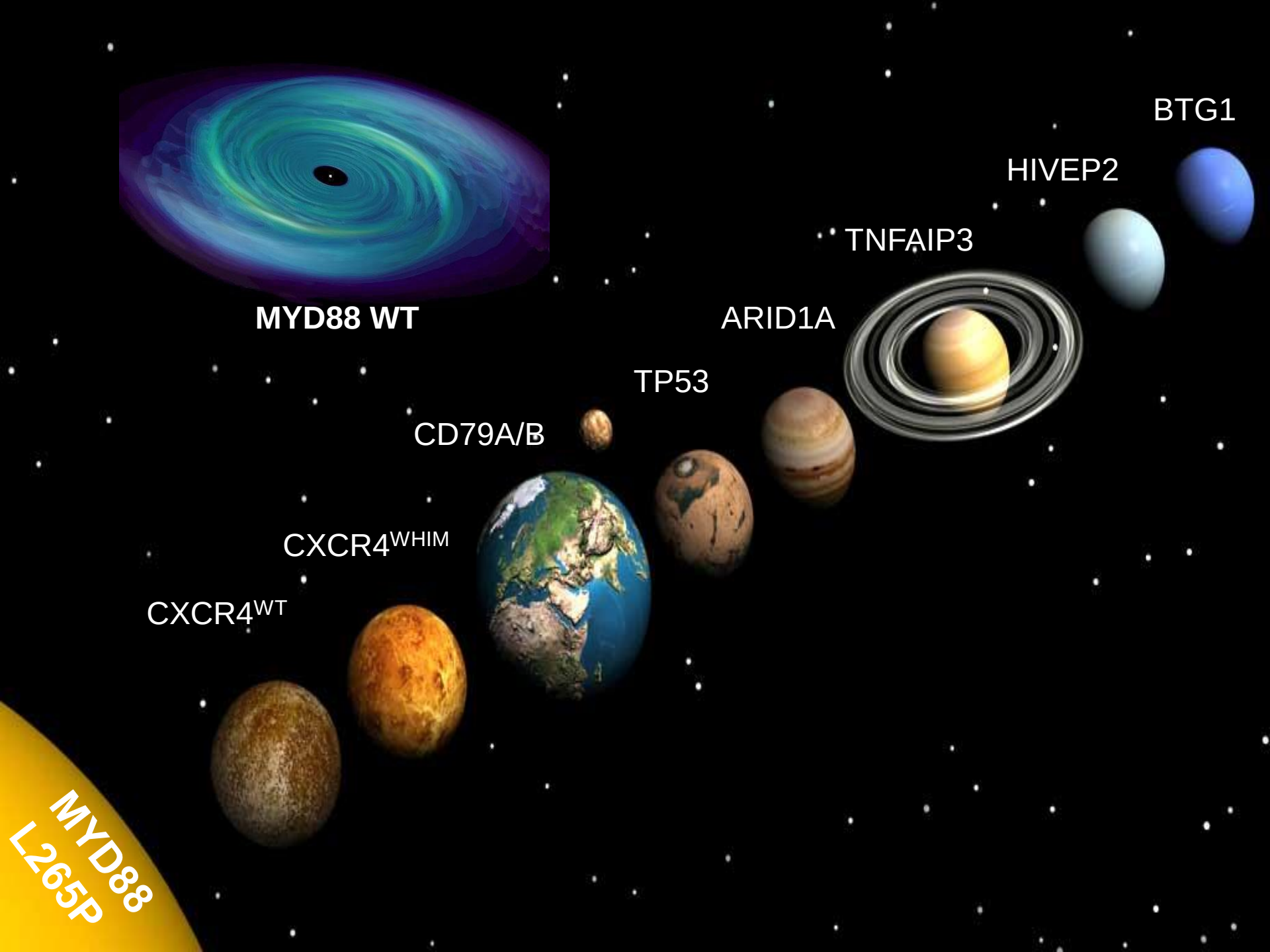
SURVIVAL
DRUG RESISTANCE

GRK 2/3

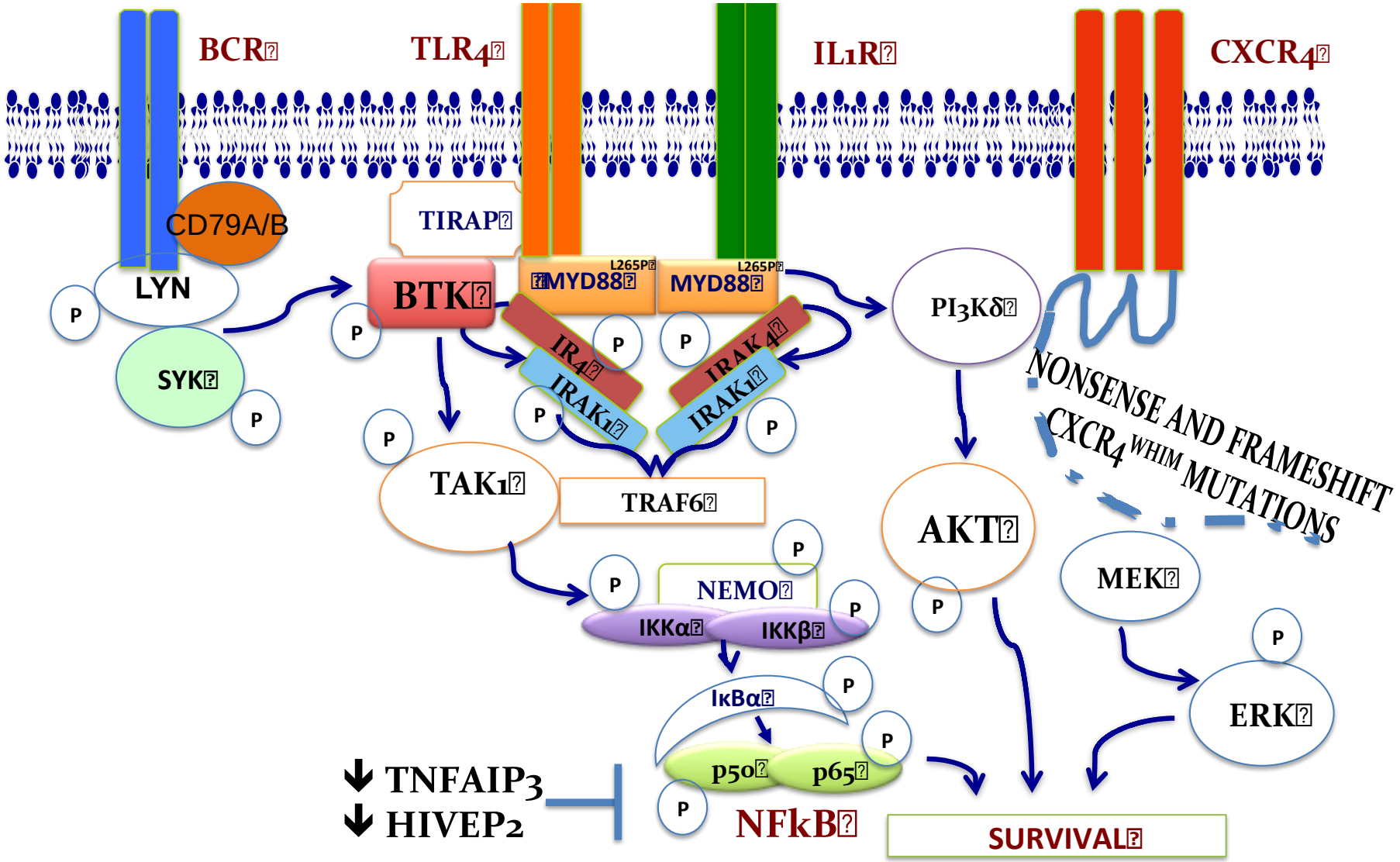
Constitutive pAKT expression in CXCR4 S338X WM patients on Ibrutinib



Cao et al, Leukemia 2014

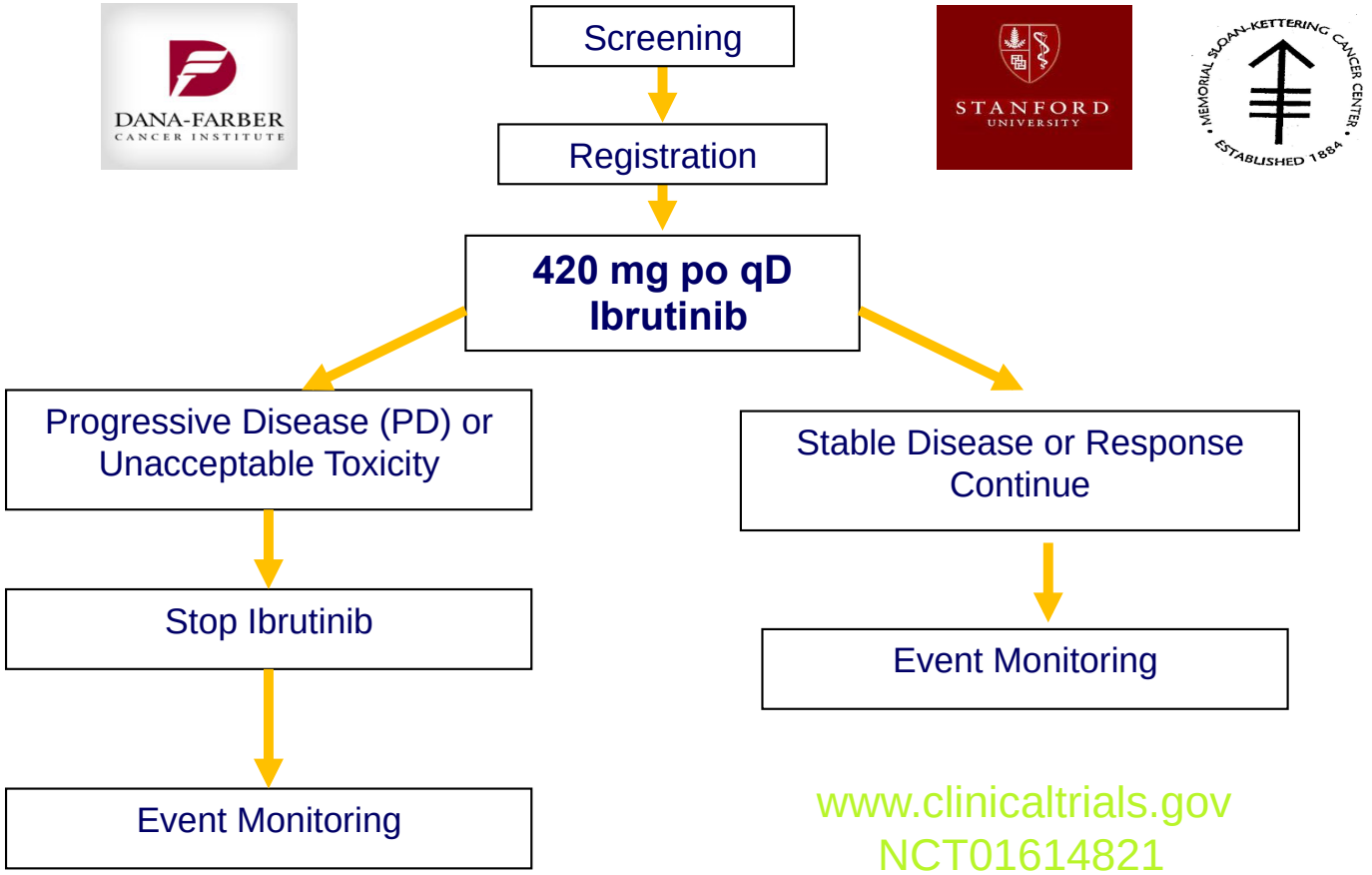

MYD88 WT
BTG1
HIVEP2
TNFAIP3
ARID1A
TP53
CD79A/B
CXCR4WHIM
CXCR4WT
MYD88 L265P

Activating mutations trigger survival signaling in Waldenstrom's Macroglobulinemia




Targeting
Actionable
Mutations
MYD88

Multicenter study of Ibrutinib in Relapsed/Refractory WM (≥1 prior therapy)



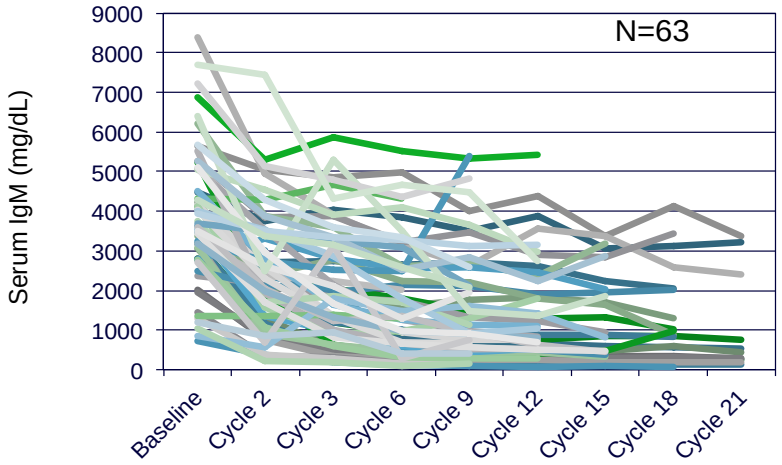
Baseline Characteristics for Study Participants (n=63)

	Median	Range
Age (yrs)	63	44-86
Prior therapies	2	1-9
Hemoglobin (mg/dL)	10.5	8.2-13.8
Serum IgM (mg/dL)	3,520	724-8,390
B_2M (mg/dL)	3.9	1.3-14.2
BM Involvement (%)	60	3-95
Adenopathy >1.5 cm	37 (59%)	N/A
Splenomegaly >15 cm	7 (11%)	N/A

Treon et al, NEJM 372: 1430, 2015

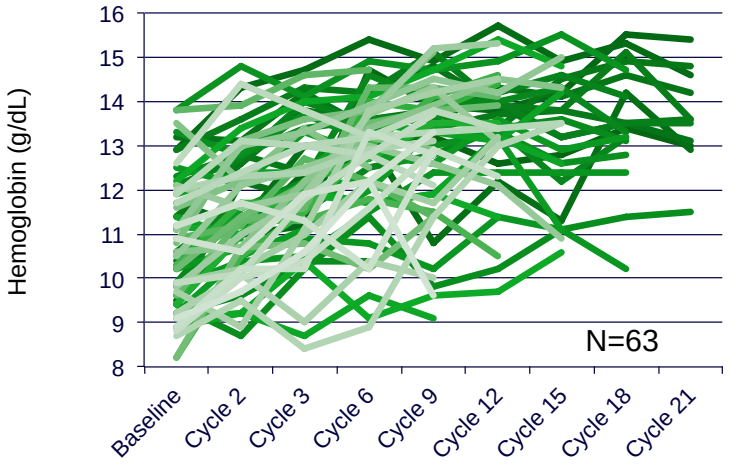
Serum IgM and Hb Levels Following Ibrutinib

Serum IgM



Best IgM Response:
3,520 to 880 mg/dL; p<0.001

Hb



Best Hemoglobin Response:
10.5 to 13.8; p<0.001

Best Clinical Responses to Ibrutinib

Median duration of treatment: 19.1 (range 0.5-29.7) months

ORR: 91% Major RR (≥ PR): 73%

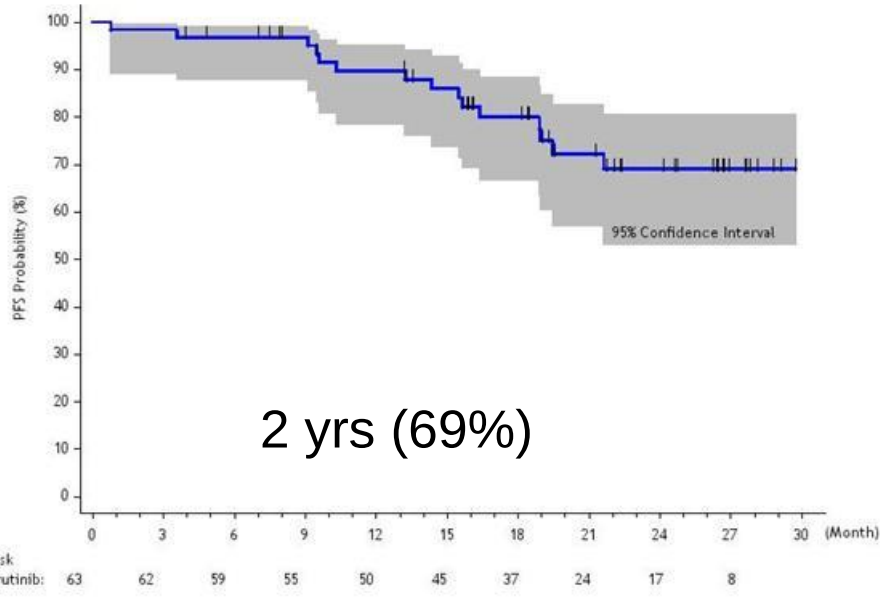
	(N=)	(%)
VGPR	10	16
PR	36	57
MR	11	17

Median time to ≥ MR: 4 weeks
Median time to ≥ PR or better: 8 weeks

Treon et al, N Engl J Med. 2015; 372(15):1430-40.

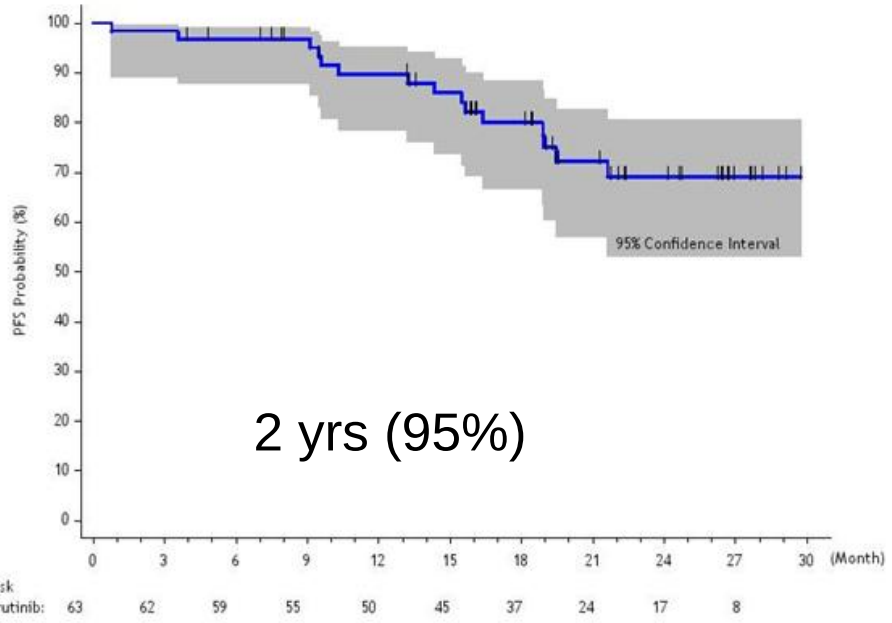
Progression-free and overall survival for 63 previously WM patients treated with ibrutinib.

PFS



2 yrs (69%)

OS

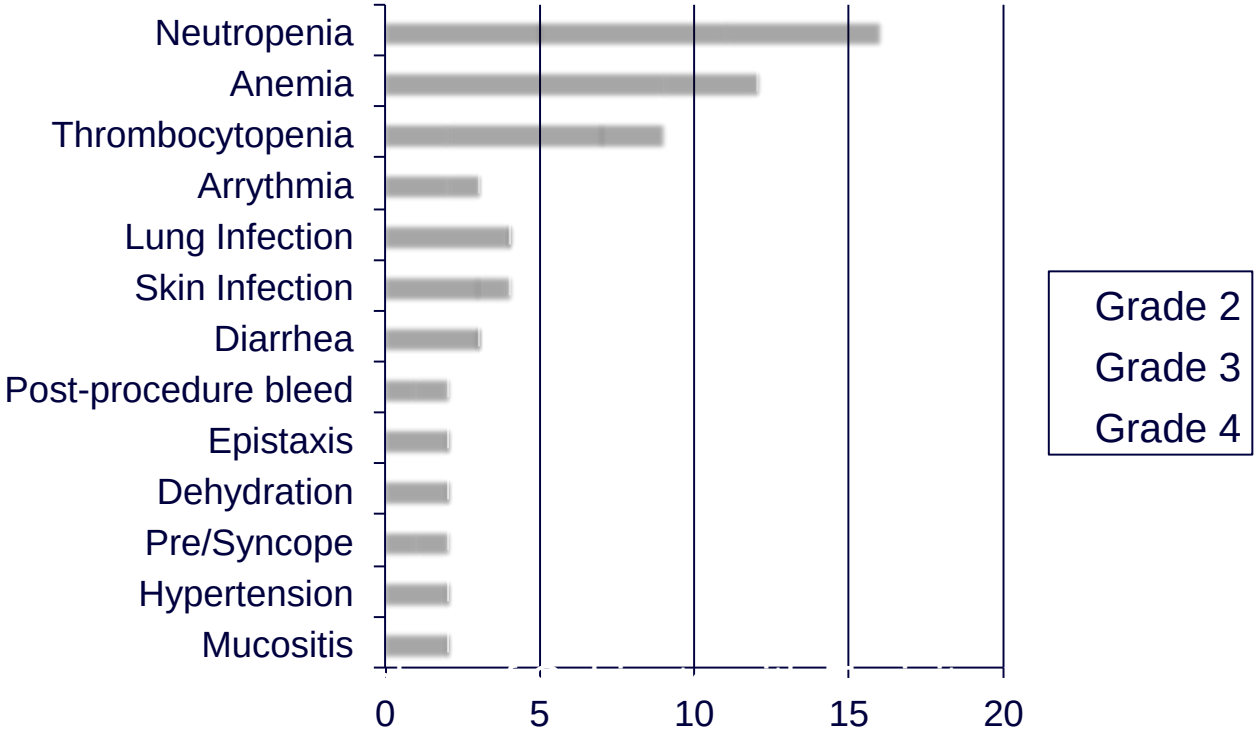


2 yrs (95%)

Treon et al, N Engl J Med. 2015; 372(15):1430-40.

Ibrutinib Related Adverse Events in previously treated WM patients

Toxicities >1 patient; N=63



Treon et al, N Engl J Med. 2015; 372(15):1430-40.

FDA News Release
FDA expands approved use of Imbruvica for rare form of non-Hodgkin lymphoma
First drug approved to treat Waldenstrom's
January 29, 2015

EMA Approval for symptomatic previously treated and chemoimmunotherapy unsuitable frontline WM
First ever for Waldenstrom's

July 8, 2015

Health Canada Santé Canada

April 5, 2016

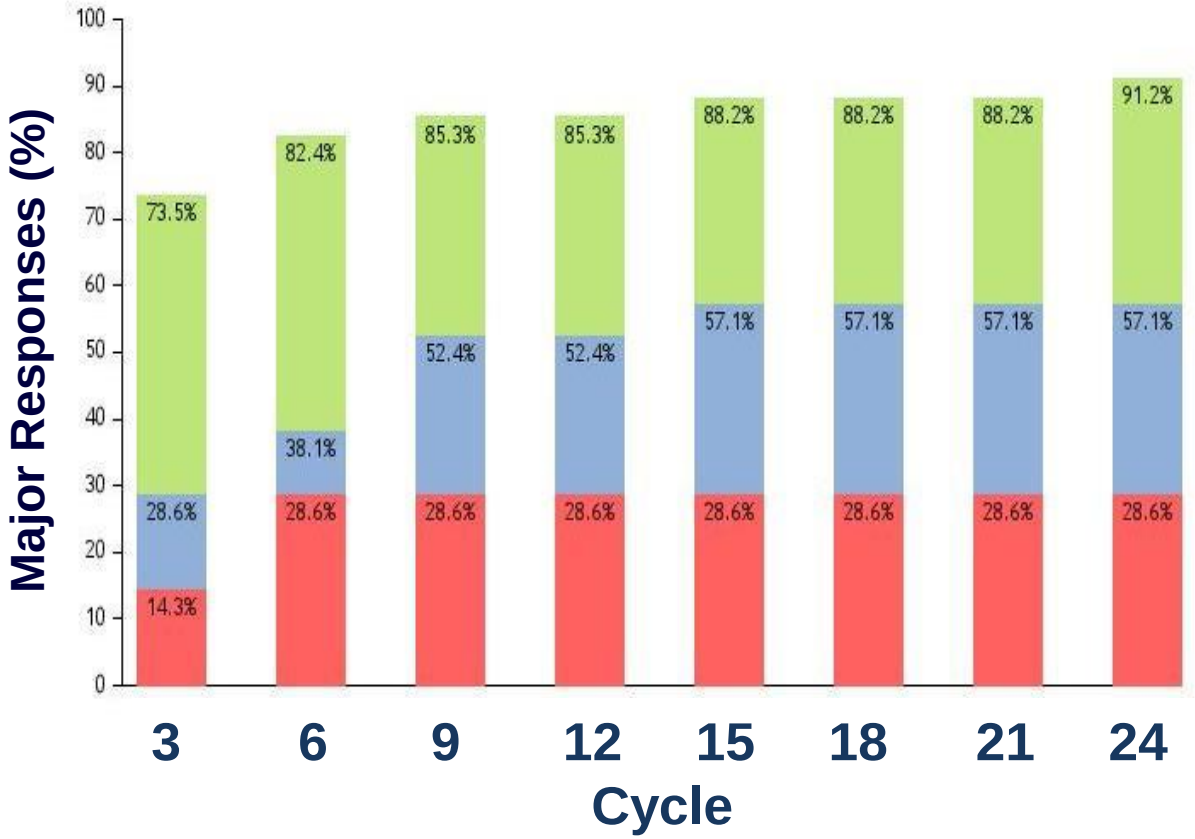
Responses to ibrutinib are impacted by MYD88 (L265P and non-L265P) and CXCR4 mutations.

	$MYD88^{MUT}$ $CXCR4^{WT}$	$MYD88^{MUT}$ $CXCR4^{WHIM}$	$MYD88^{WT}$ $CXCR4^{WT}$	p-value
N=	36	21	5	
Overall RR	100%	85.7%	60%	<0.01
Major RR	91.7%	61.9%	0%	<0.01

2 patients subsequently found to have other MYD88 mutations not picked up by AS-PCR

Treon et al, N Engl J Med. 2015; 372(15):1430-40; NEJM 2015; Letter, August 6, 2015.

Kinetics of major responses following ibrutinib therapy in genotyped WM patients.



MYD88^{L265P}
CXCR4^{WT}

MYD88^{L265P}
CXCR4^{WHIM}

MYD88^{WT}
CXCR4^{WT}

Treon et al, NEJM 372: 1430, 2015


Targeting
Actionable
Mutations
CXCR4

Phase I Study of Ulocuplumab in Relapsed/Refractory CXCR4^{WHIM} WM Patients

LEUKEMIA & LYMPHOMA SOCIETY®
fighting blood cancers

Screening,Informed Consent, Registration

Ulucuplomab Weekly for 4 weeks, then every other week for 24 weeks.

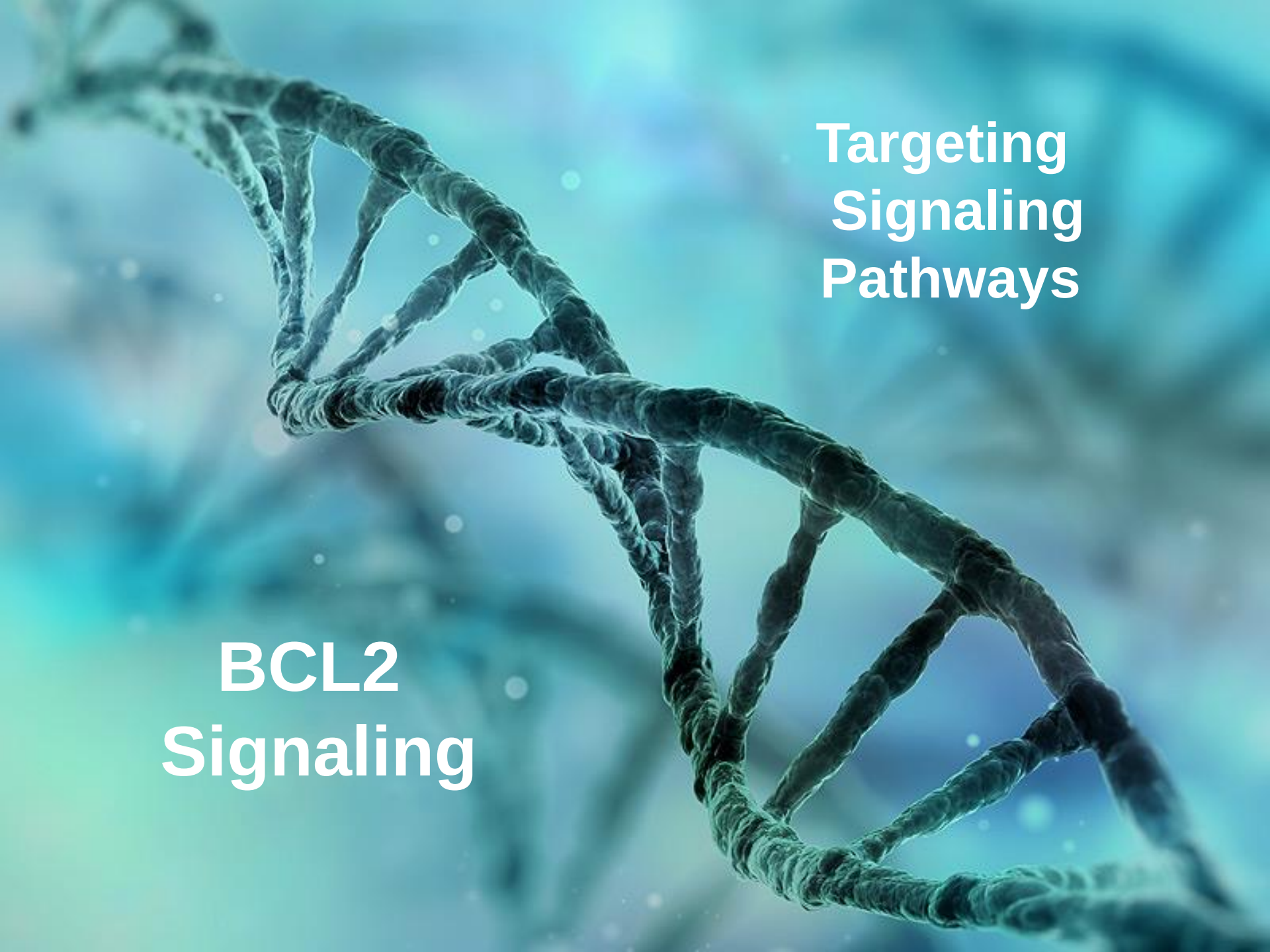
Progressive Disease or Unacceptable Toxicity

Stop Ulocuplumab

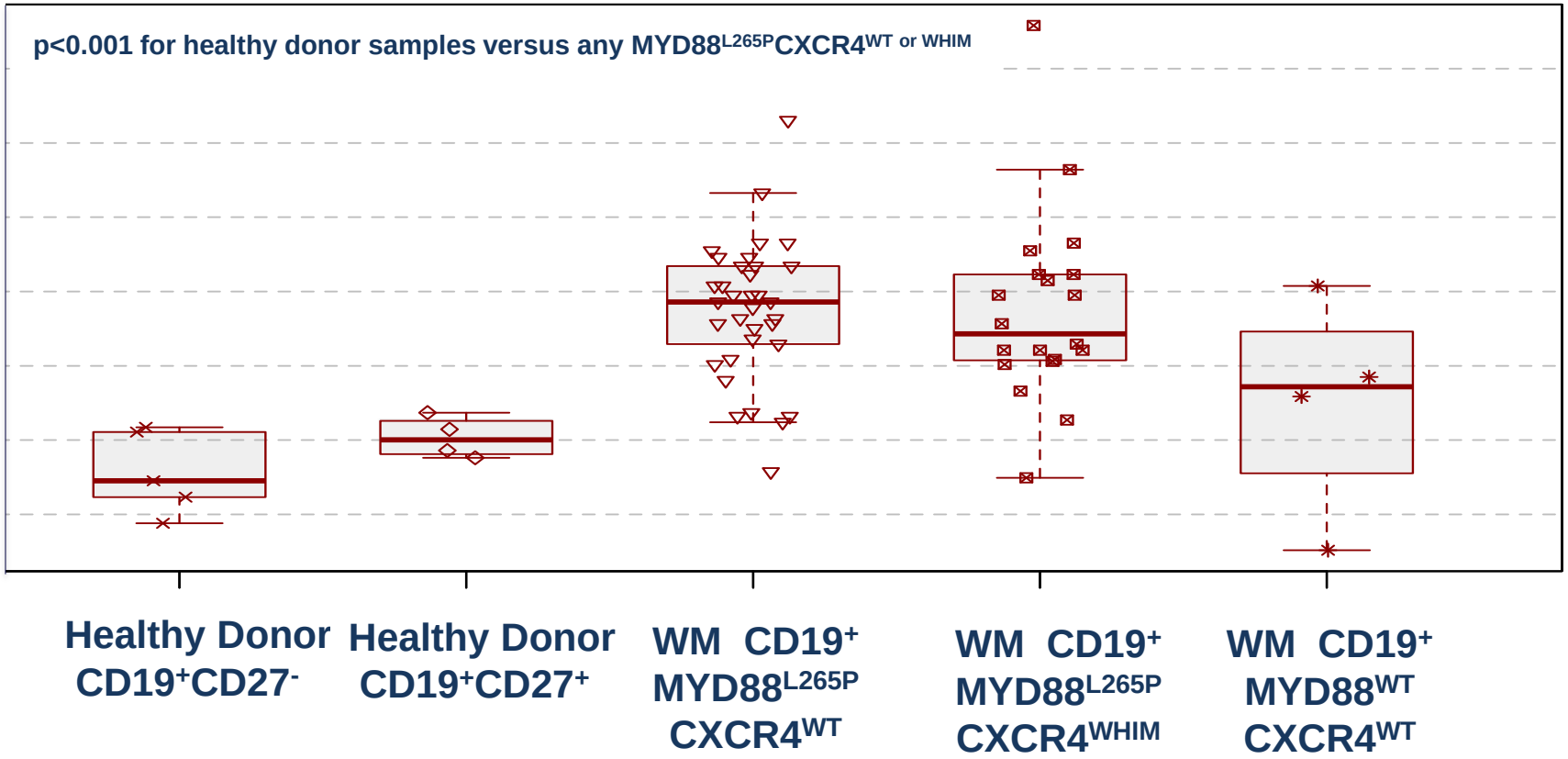
Event Monitoring

SD or Response Continue

Event Monitoring


Targeting
Signaling
Pathways
BCL2
Signaling

BCL-2 is overexpressed in primary WM patient cells by next generation sequencing (RNAseq) in MYD88 and CXCR4 mutated and unmutated patients.



Hunter et al, ASH 2015; Abstract 128

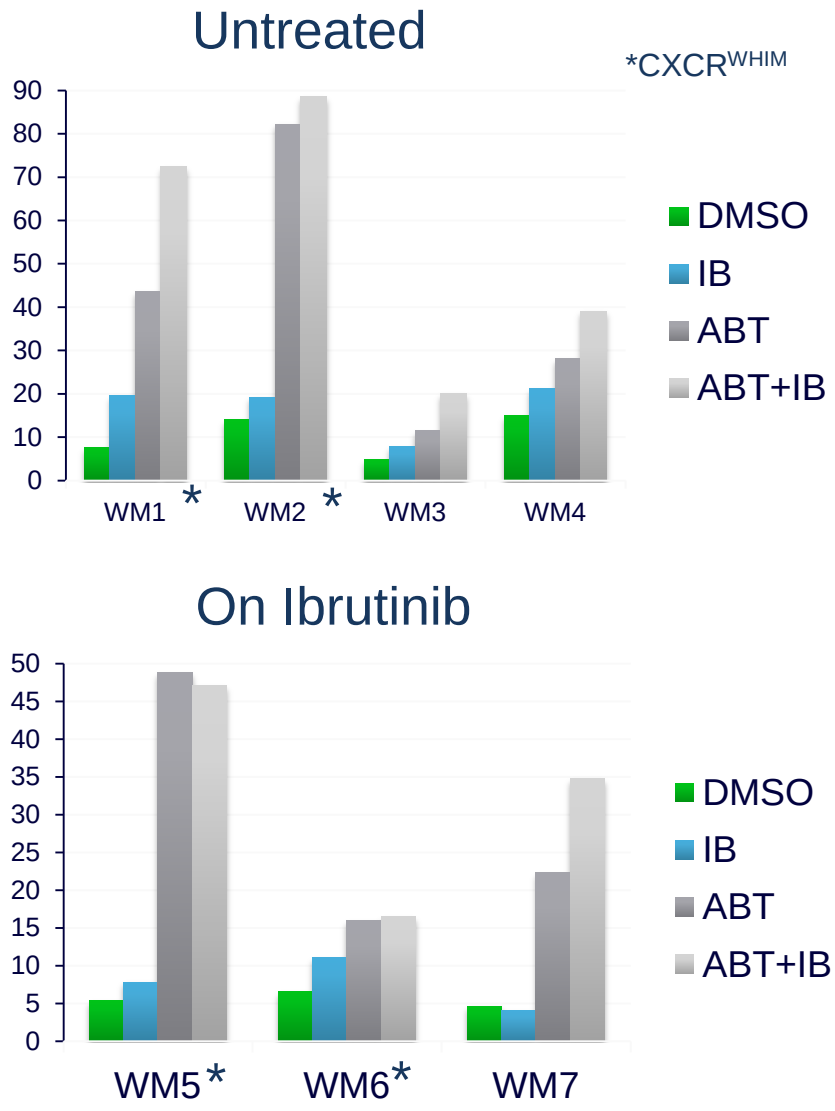

BCL2
giaccherini
13


BCL2

Venetoclax (ABT-199)

- Highly selective BCL2 inhibitor
- Approved for the treatment of 17p-deleted CLL
- Under investigation in CLL (other indications) and other B-cell malignancies.
- Tumor lysis syndrome, neutropenia.

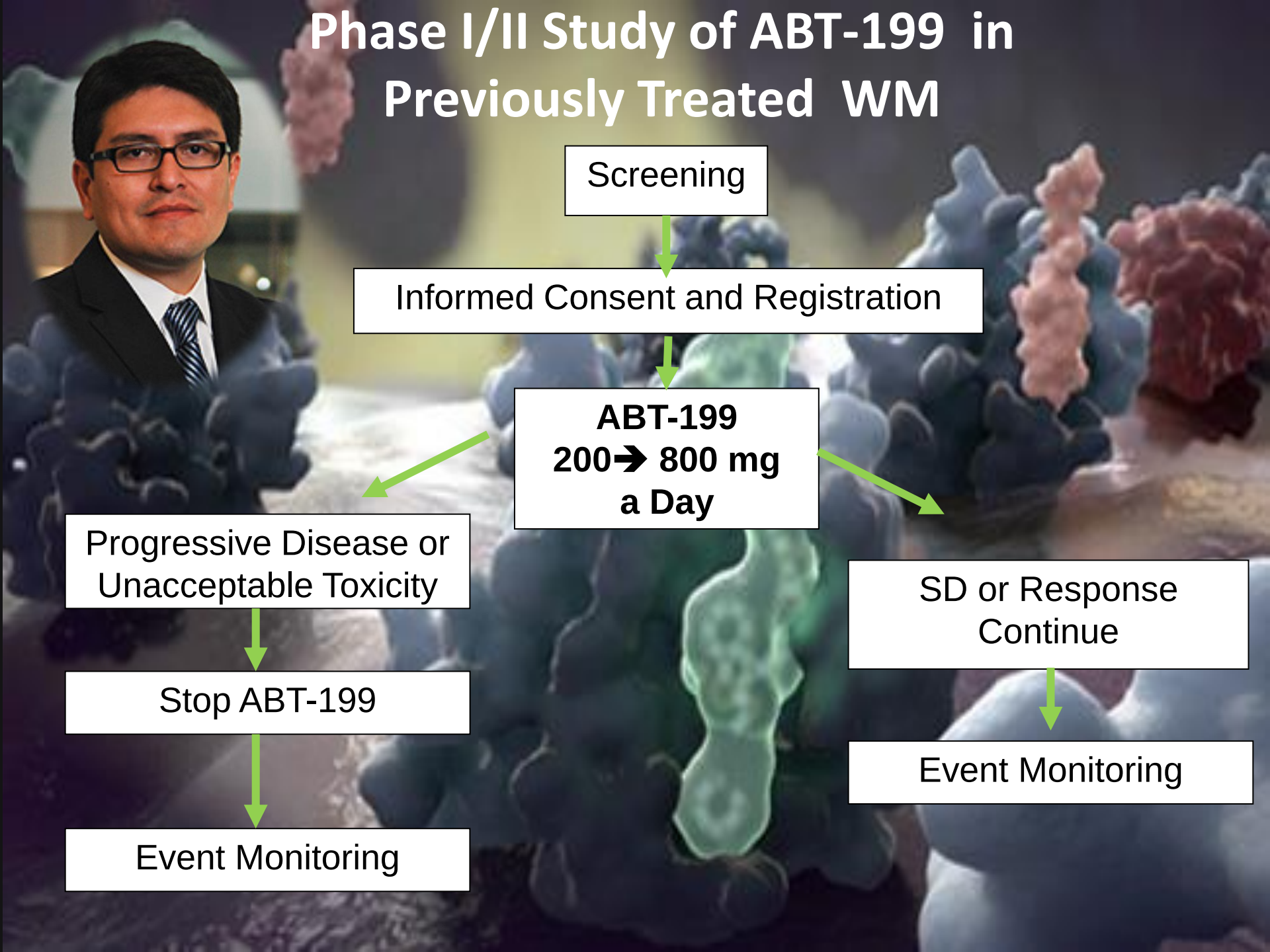
The anti-BCL2 agent Venetoclax (ABT-199) shows pre-clinical and clinical activity in WM.

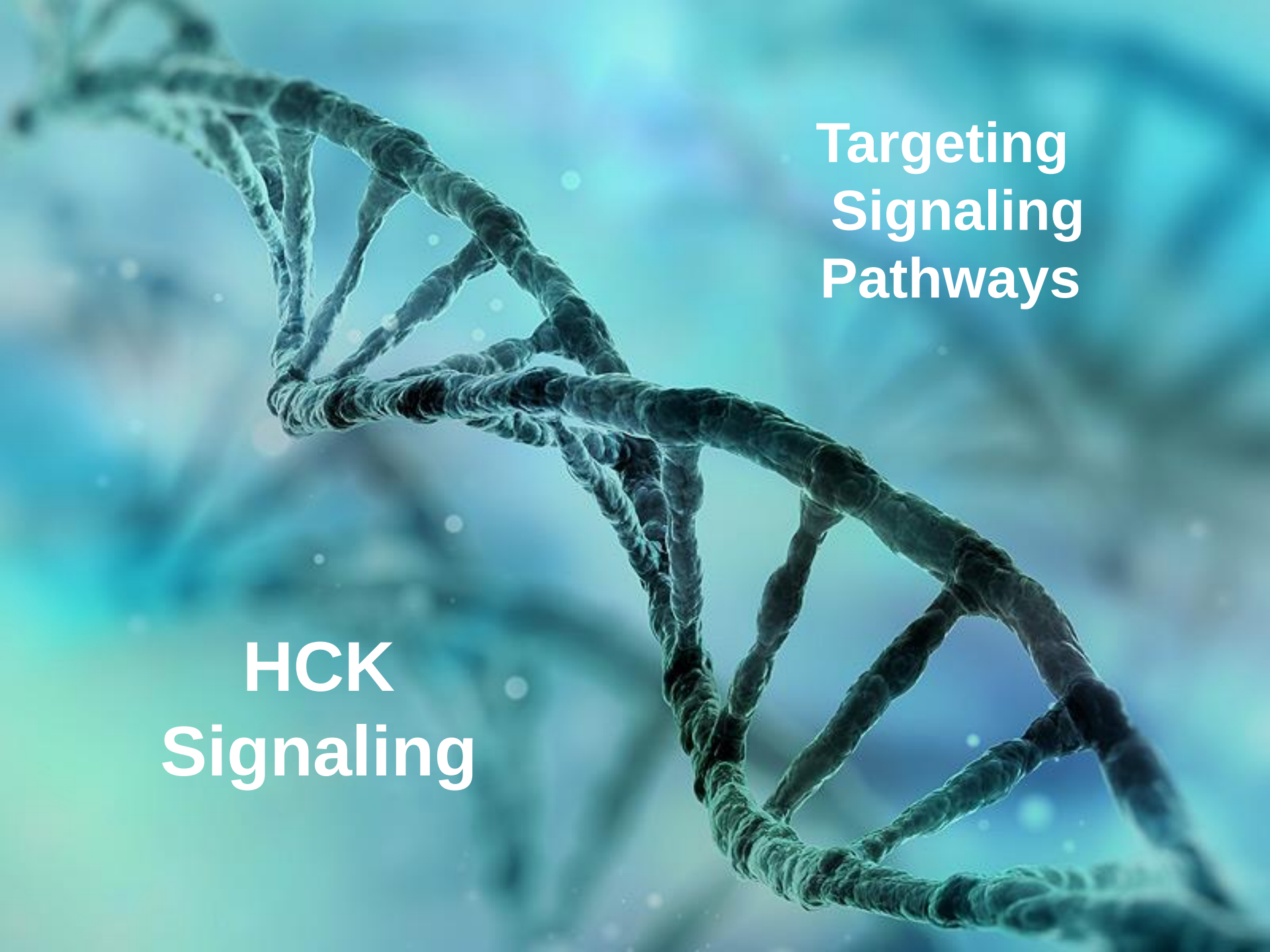


N=4
ORR=100%; all major responders
PFS: 18, 25, 38+, 40+ months.

Cao et al, BJH 2015;
Gericitano et al, ASH 2015. Abstract 254.

Phase I/II Study of ABT-199 in Previously Treated WM



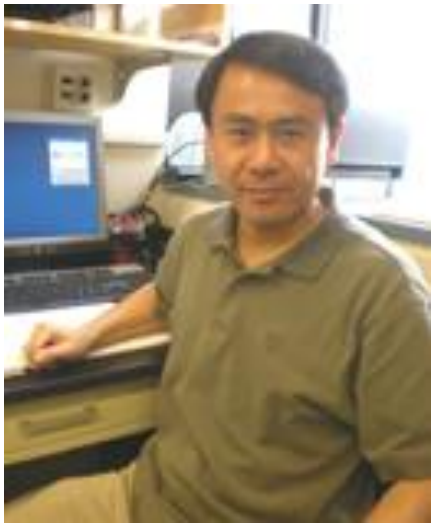

Targeting
Signaling
Pathways
HCK
Signaling

Hematopoeitic Cell Kinase (HCK)

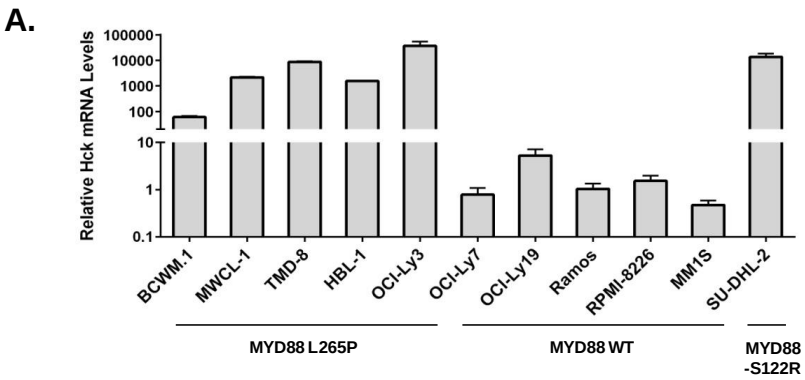
- SRC family kinase
- Expressed at early stages of B-cell development
- GEP in WM shows HCK among the most aberrantly over-transcribed genes.
  (2 highest HCK, IL6)
- In MM cells, HCK transactivated by IL6 through IL6ST.
- HCK among 285 genes down-regulated in MYD88 knockdown HBL-1 MYD88 mutated cells.

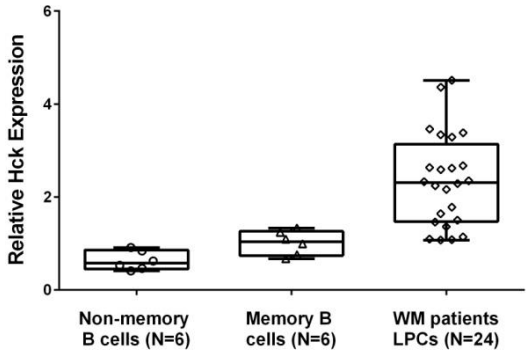
Hallek et al, Exp Hematol 1997; Guttierez et al, Leukemia 2007;
Ngo et al, Oncogene 2011

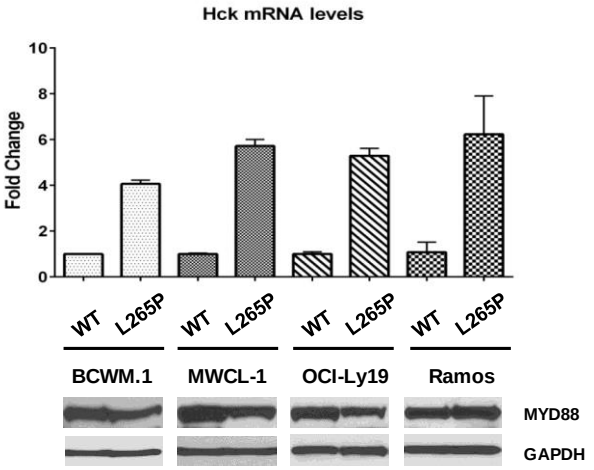
Hematopoeitic Cell Kinase (HCK) is induced by Mutated MYD88 in WM and ABC DLBCL cells.



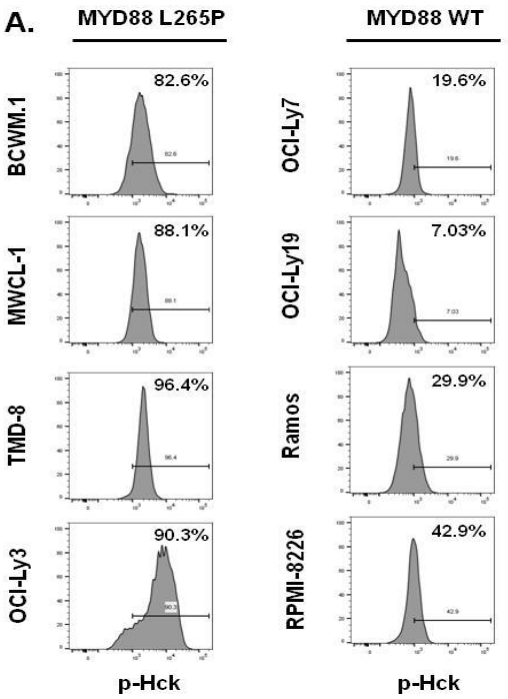
Yang et al, ASH 2015

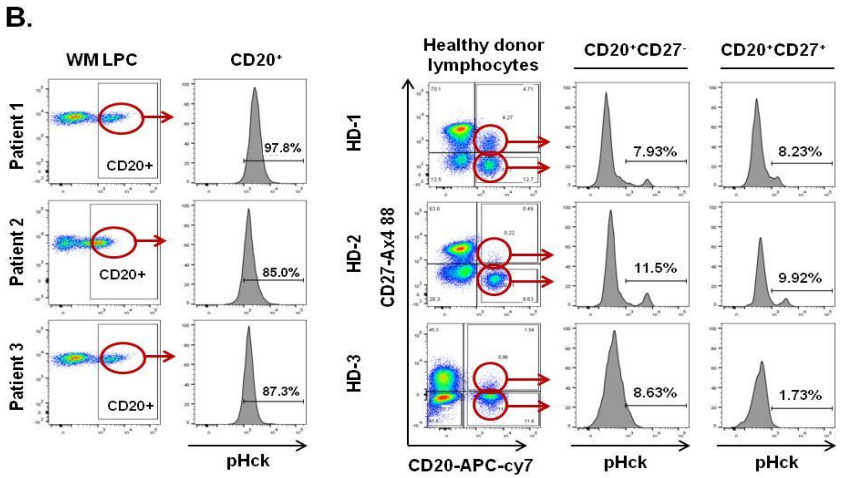


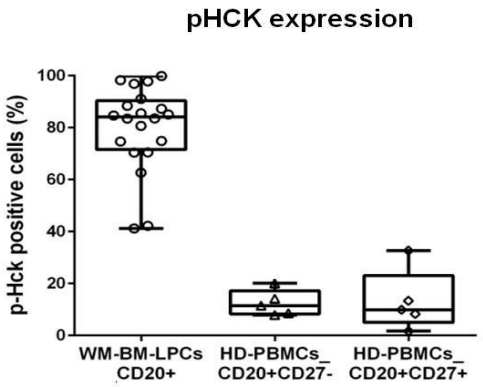




HCK is activated in MYD88 Mutated Cell Lines and Primary WM Patient Cells.

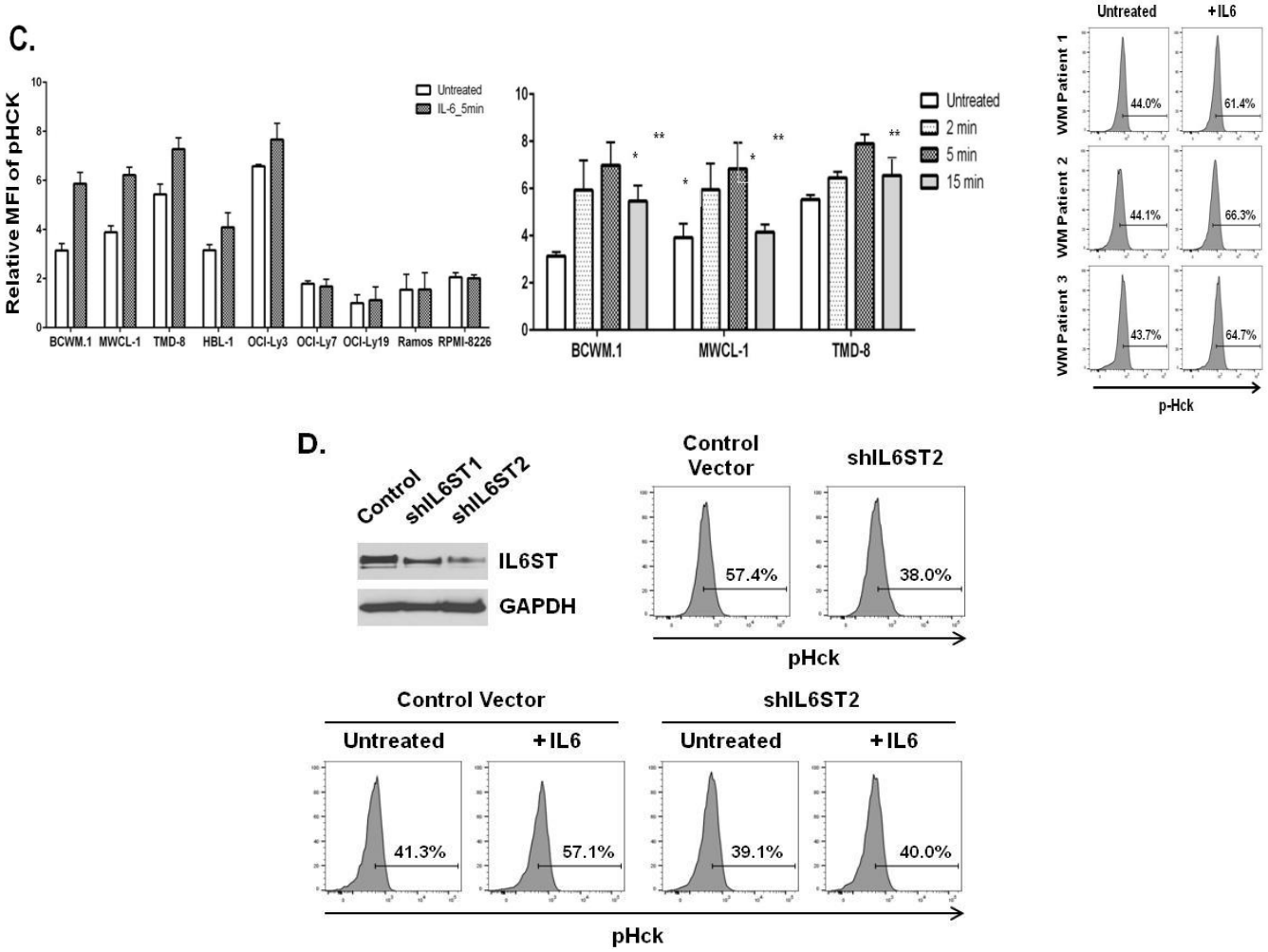






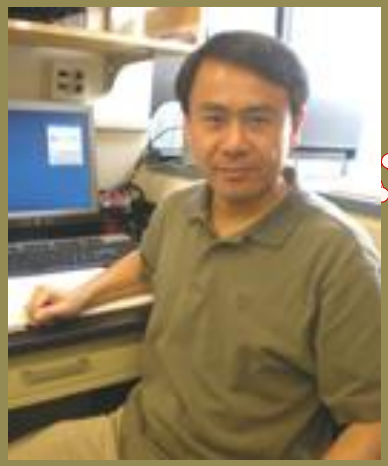
Yang et al, ASH 2015

IL-6 transactivates HCK in MYD88 Mutated Cell Lines and Primary WM Cells

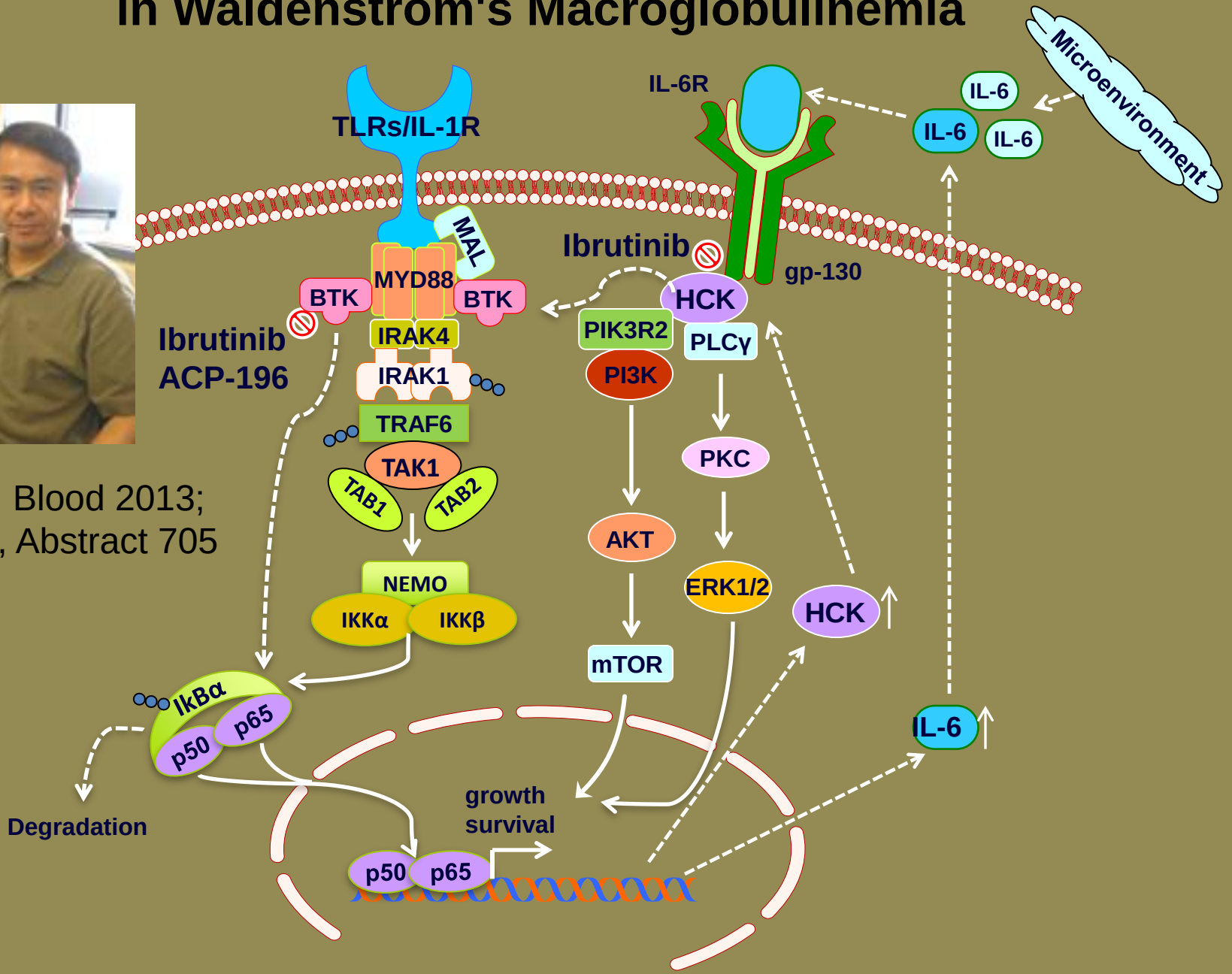


Yang et al, ASH 2015

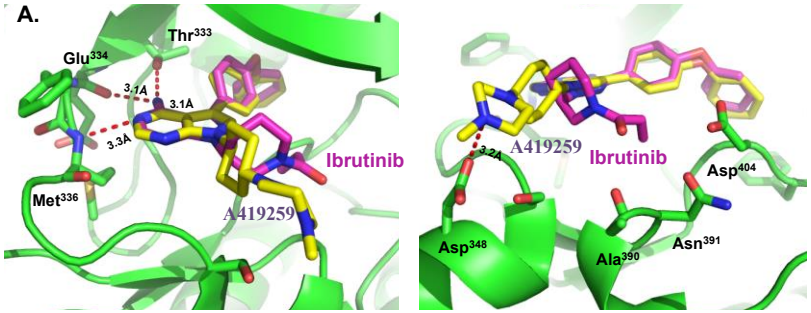
Signaling Pathways Driven by Mutated MYD88 in Waldenström's Macroglobulinemia

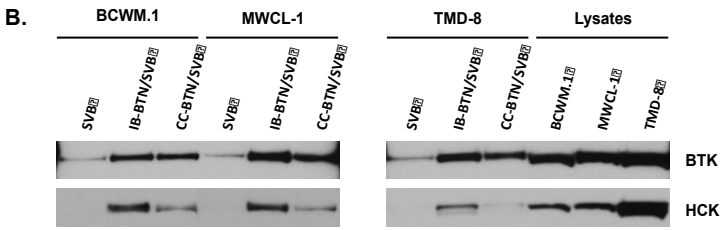


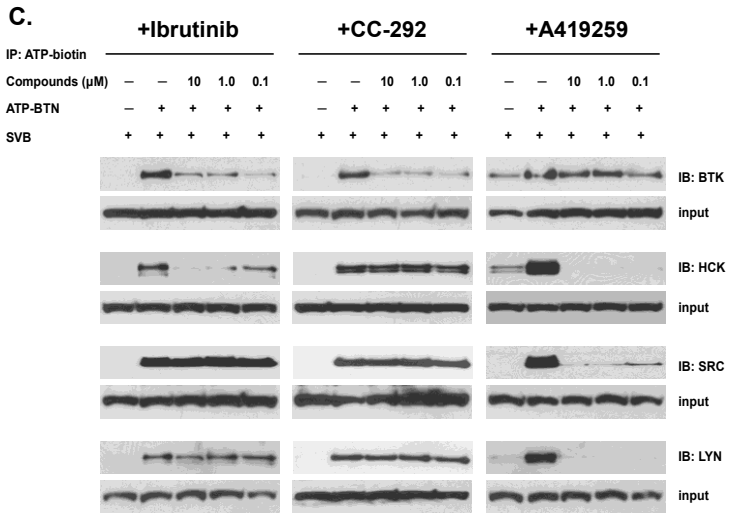
Yang et al, Blood 2013;
ASH 2015, Abstract 705

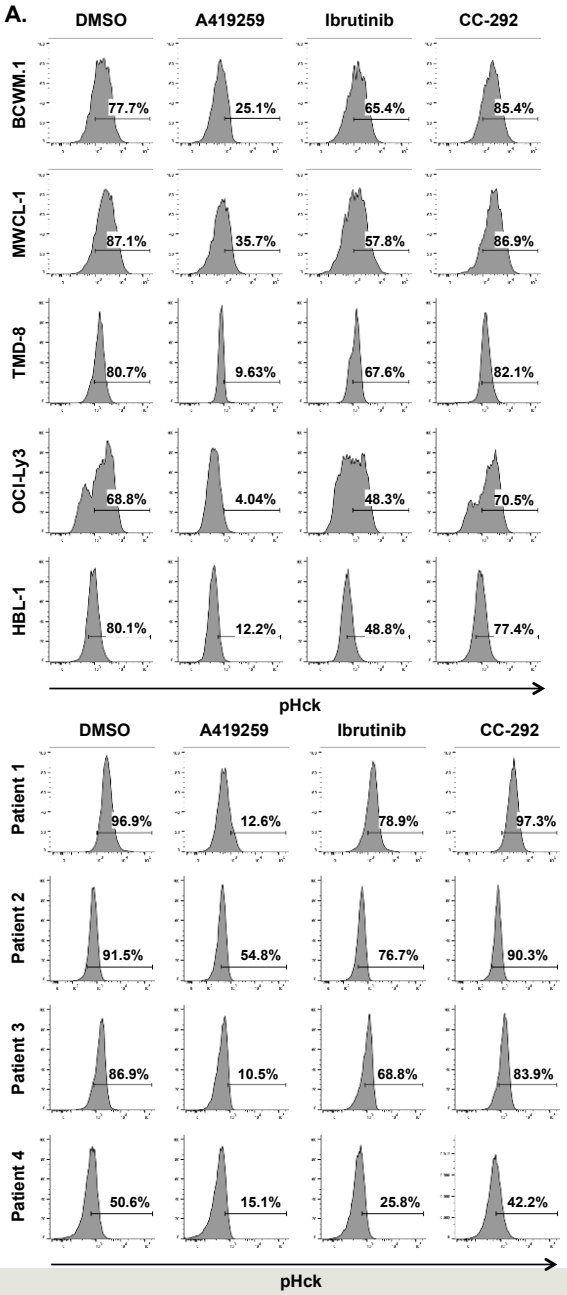


Ibrutinib and A419259 bind to and block HCK kinase activity

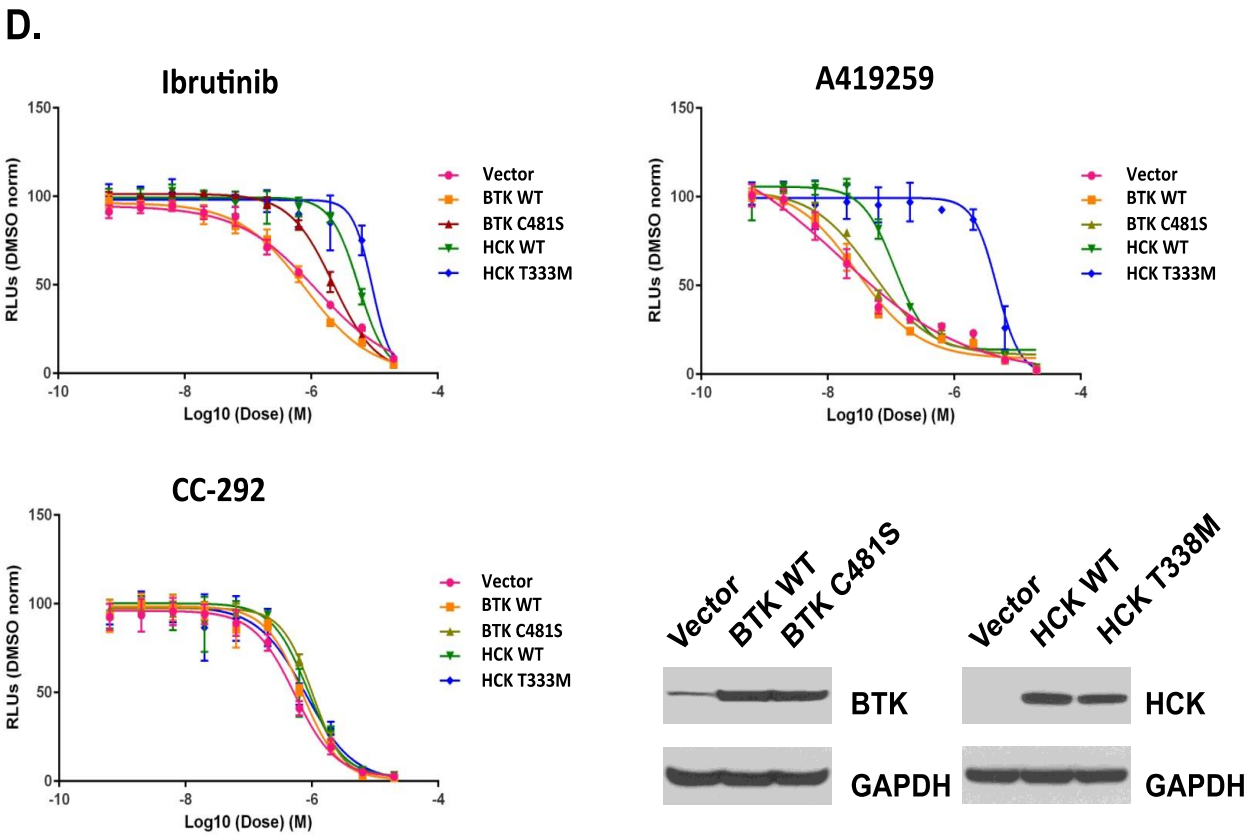




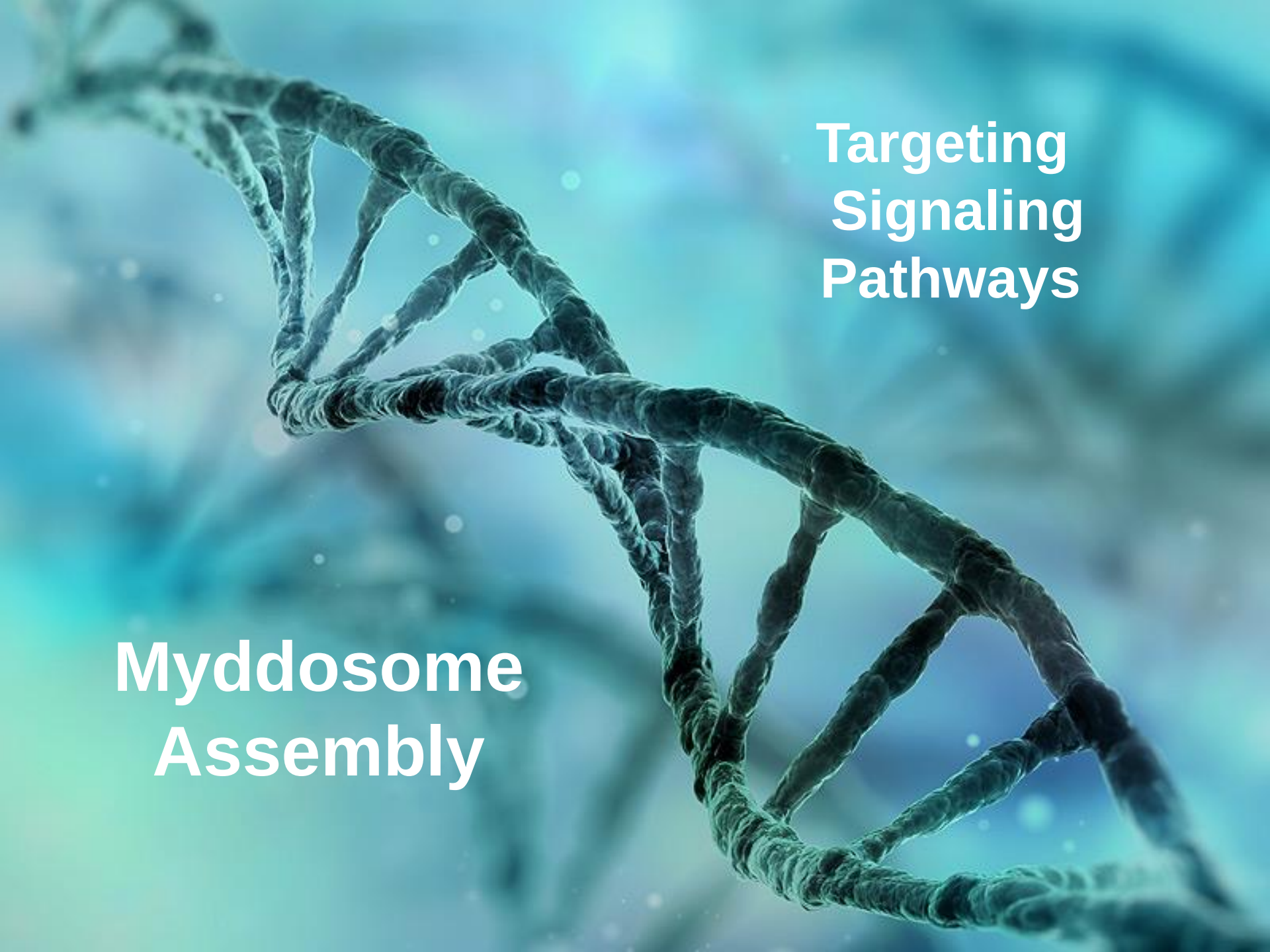




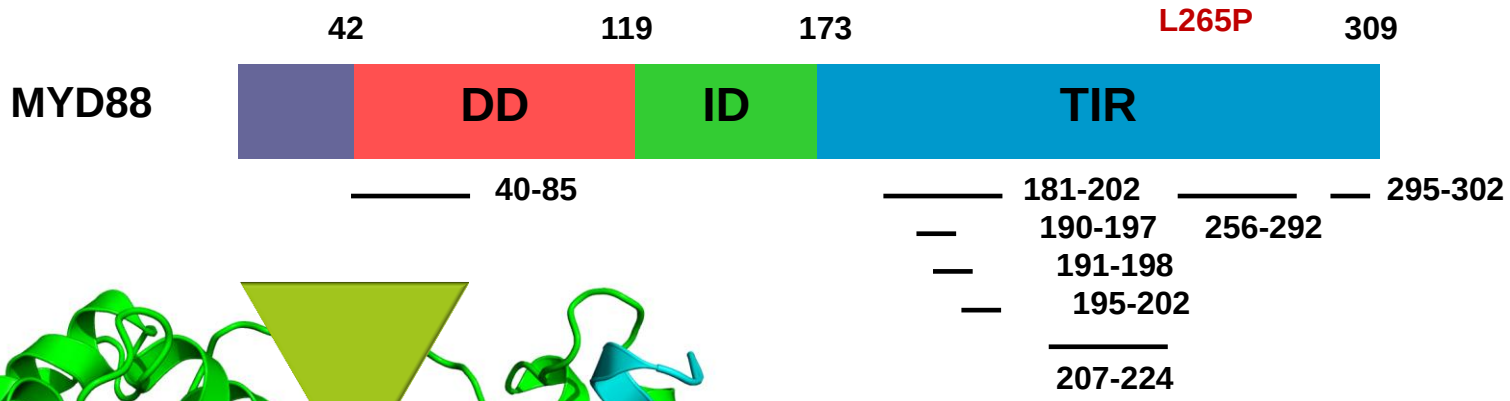
Transduction with HCK gatekeeper mutant (T333M) promotes resistance in MYD88 mutated WM cells treated with Ibrutinib or A419259

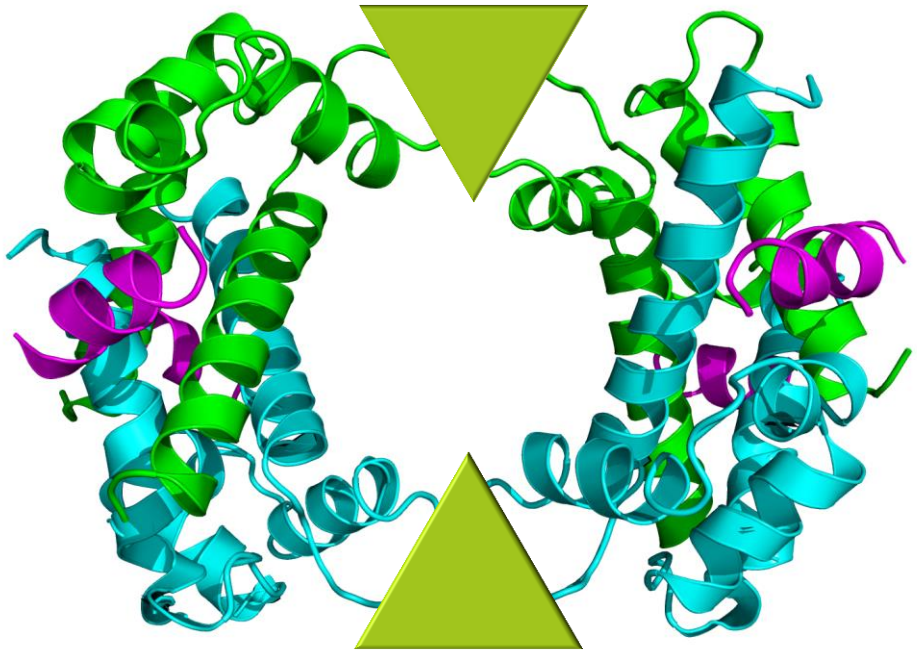


Yang et al, ASH 2015


Targeting
Signaling
Pathways
Myddosome
Assembly

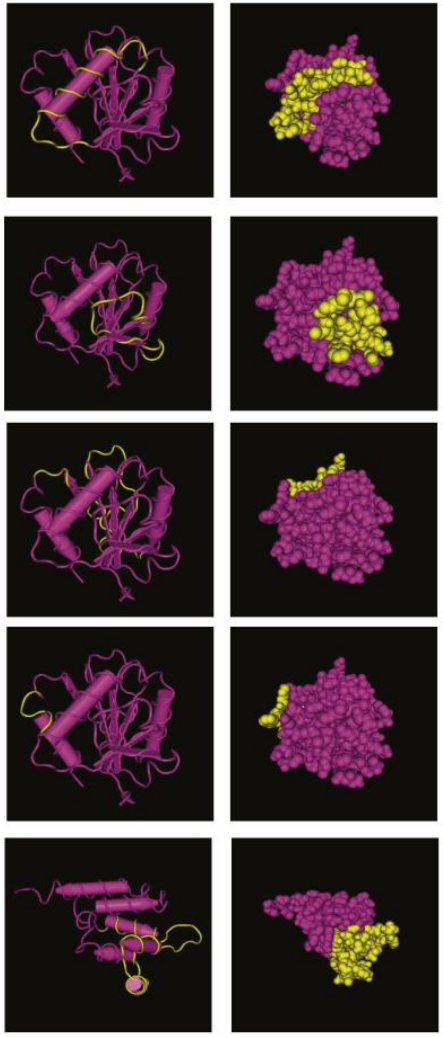
MYD88 Signaling is dependent on TIR domain homodimerization.





DD: death domain
ID: intermediate domain
TIR: TIR domain

MYDDOSOME ASSEMBLY in MYD88 Mutated WM



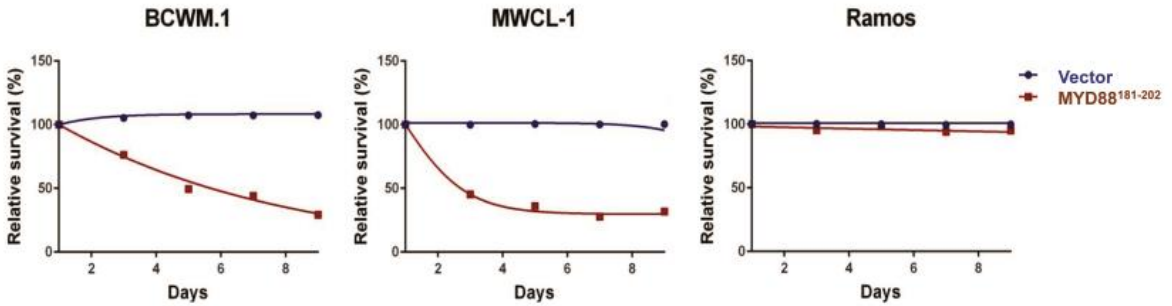
MYD88 TIR 181-202

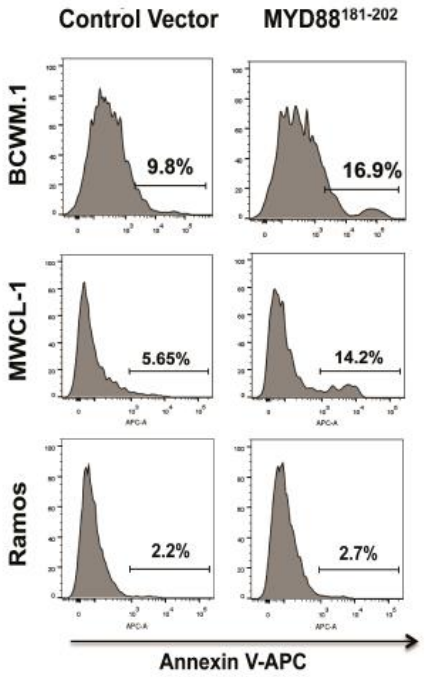
MYD88 TIR 207-224

MYD88 TIR 256-292

MYD88 TIR 295-302

MYD88 DD 40-85



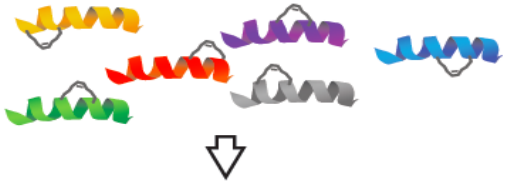


Liu et al, BJH 2016

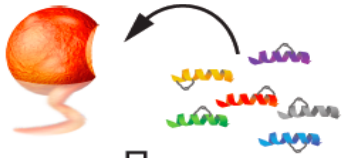
Targeting Myddosome Assembly

Collaboration
TREON/WALENSKY LABS

Synthesize library of stapled peptides

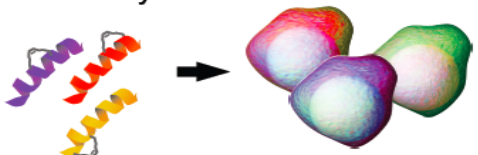


Screen library of stapled peptides to identify optimal binders



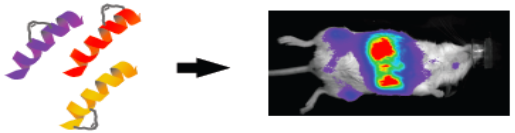
MYD88

Apply cell-permeable stapled peptides in cellular analyses



WM Cells

In vivo efficacy and mechanism of action studies in cancer models



Summary

- MYD88 and CXCR4 mutations are the most common mutations present in WM patients, respectively. Other mutations exist in WM at lower frequencies, and remain to be investigated.
- MYD88 triggers BTK, and the BTK inhibitor ibrutinib is highly active in patients with MYD88 mutations. Lower response activity is observed in patients with CXCR4 mutations.
- Ulocuplumab is a novel CXCR4 inhbitor that will be entering Phase I testing in WM patients.
- BCL2 is overexpressed in WM, the BCL-2 inhibitor venetoclax has entered a dedicated Phase I/II Study in WM.
- New therapeutics under development include HCK and Myddosome assembly inhibitors.

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The Leukemia & Lymphoma Society®
Fighting Blood Cancers

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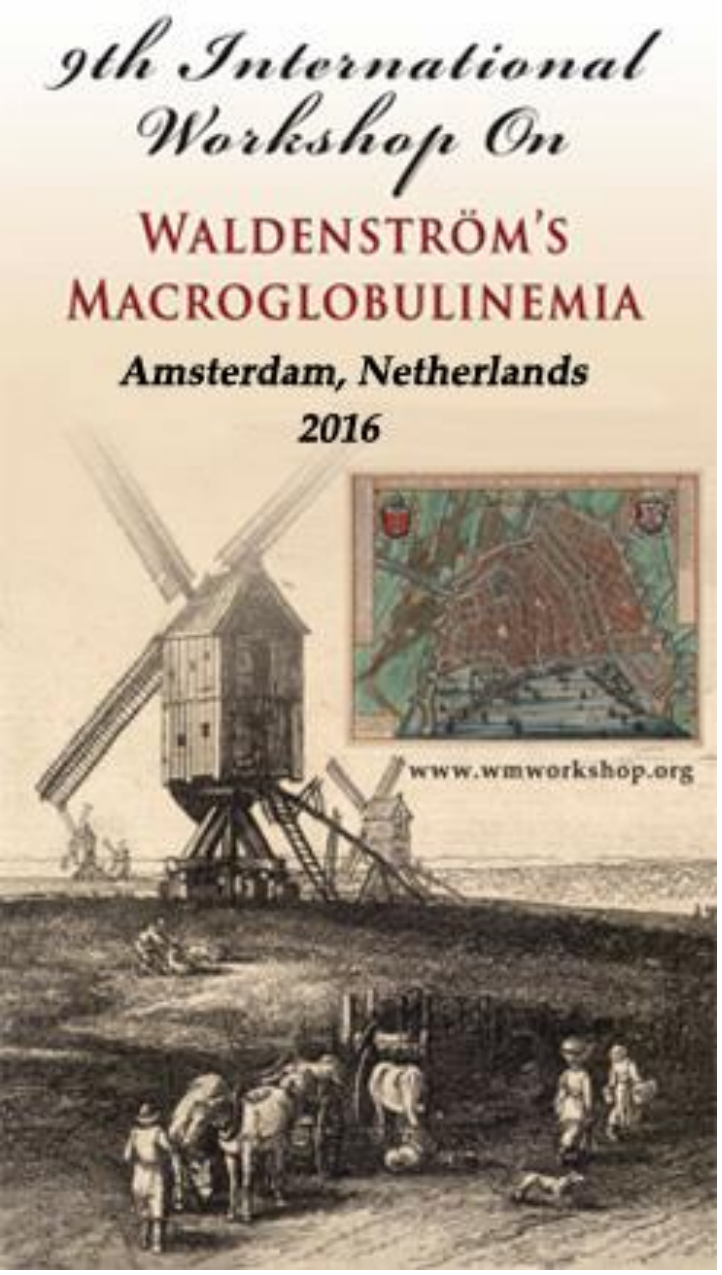

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