Waldenström's Macroglobulinemia: Genomic and Treatment Advances

HARVARD
MEDICAL SCHOOL

Zachary Hunter PhD, Guang Yang PhD,
and Steve Treon MD, PhD
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Dana Farber Cancer Institute
Harvard Medical School

Acta Medica Scandinavica. Vol. CXVII, fasc. III—IV, 1944.

Incipient myelomatosis or «essential« hyperglobulinemia with fibrinogenopenia — a new syndrome?

By

JAN WALDENSTRÖM.

Submitted for publication September 2, 1943.

The real nature of myelomatosis.

The title of this paper may at first seem somewhat surprising. The myeloma has of old had a reputation as a well defined clinical entity. With the aid of the typical changes on the X-ray film and guided by the examination of the cells from a sternal puncture the diagnosis should therefore be easy and there ought not to be found any serious diagnostical troubles. In the following I am going to give a description of two cases, who have several symptoms suggesting myelomatosis but also show decided differences. They are very much alike even as regards details in the chemistry of the blood proteins and it seems probable according to my opinion, that they suffer from the same malady. A third case very much resembles these two patients but also shows other signs, that do not fit in so well with the picture.

Waldenström's Macroglobulinemia – first described by Jan Gosta Waldenström in 1944.

Second International Workshop on WM
Athens, Greece 2002

2nd Intl Patient and Physician Summit on WM, Los Angeles 2003

Dedication of Bing Center for WM at DFCI 2005

Discovery of the MYD88 Mutation in WM -2012-

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

MYD88 L265P Somatic Mutation in Waldenström's Macroglobulinemia

Steven P. Treon, M.D., Ph.D., Lian Xu, M.S., Guang Yang, Ph.D., Yangsheng Zhou, M.D., Ph.D., Xia Liu, M.D., Yang Cao, M.D., Patricia Sheehy, N.P., Robert J. Manning, B.S., Christopher J. Patterson, M.A., Christina Tripsas, M.A., Luca Arcaini, M.D., Geraldine S. Pinkus, M.D., Scott J. Rodig, M.D., Ph.D., Aliyah R. Sohani, M.D., Nancy Lee Harris, M.D., Jason M. Laramie, Ph.D., Donald A. Skifter, Ph.D., Stephen E. Lincoln, Ph.D., and Zachary R. Hunter, M.A.

WHOLE GENOME SEQUENCING IN WM

3,000,000,000 nucleotides

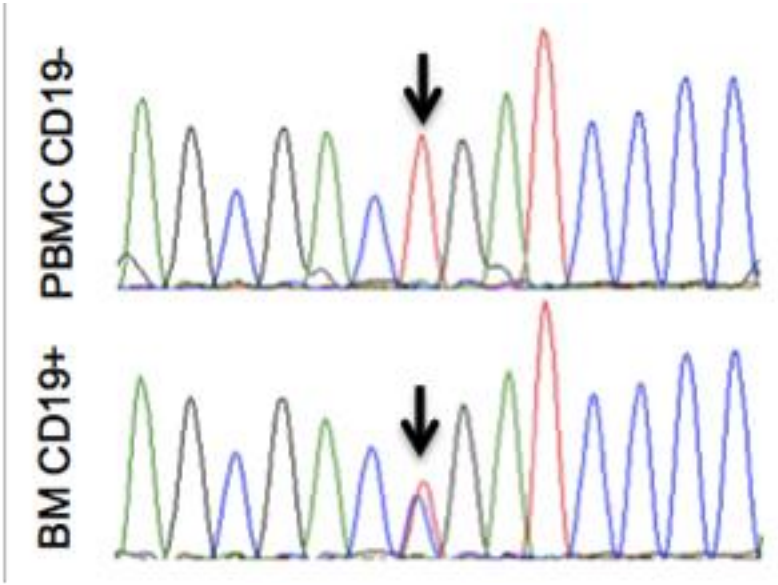
Paired Sequencing from same individuals

NORM

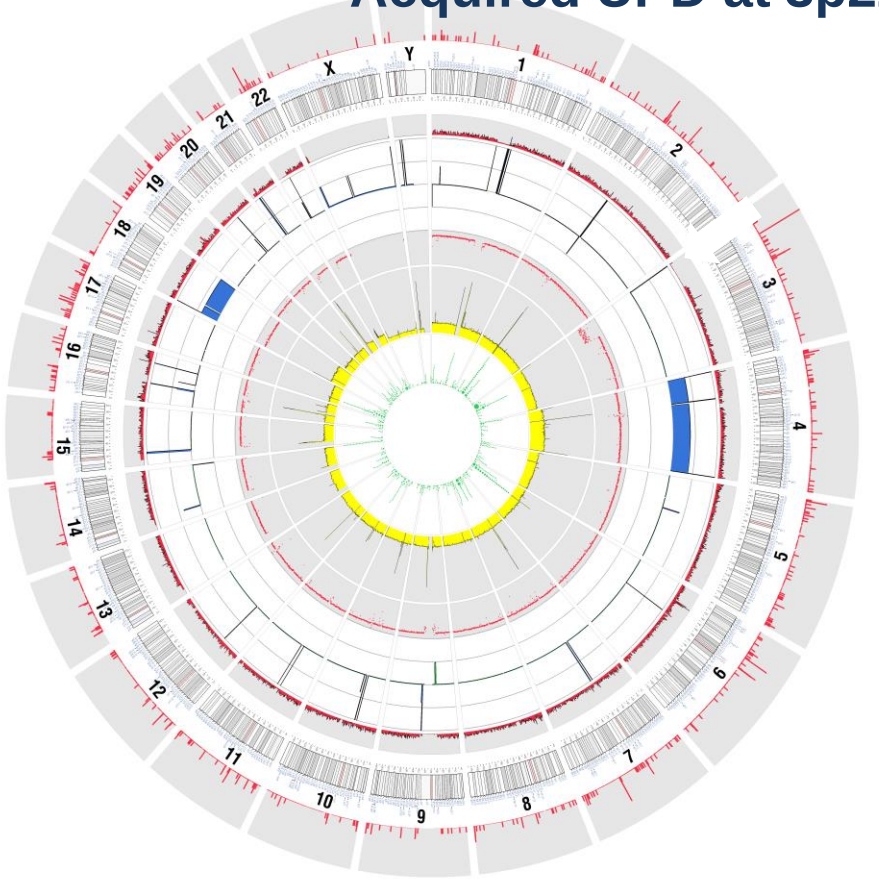
WM

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 93-97% WM pts;
- 50-80% IGM MGUS pts;
- Usually heterozygous

Treon et al, NEJM 367:826, 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 CD19+	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 CD19+	97%	
Patkar	AS-PCR	BM	85%	

>50 CONFIRMATIONAL STUDIES PUBLISHED

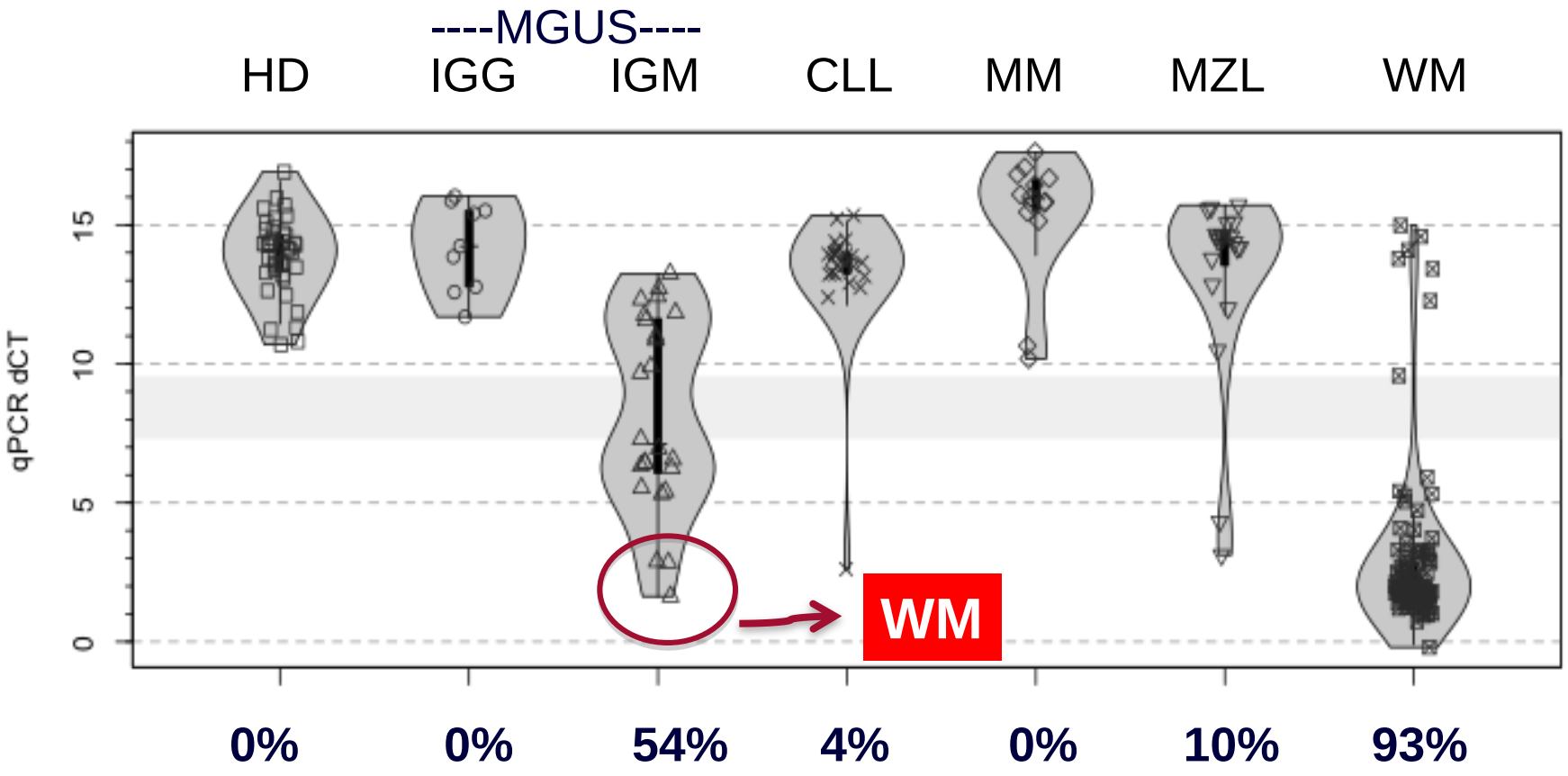
Comment on Poulain et al, page ■■■

A new era for Waldenstrom macroglobulinemia: MYD88 L265P

Steven P. Treon[1] and Zachary R. Hunter[1] [1]BING CENTER FOR WALDENSTROM'S MACROGLOBULINEMIA, DANA FARBER CANCER INSTITUTE AND HARVARD MEDICAL SCHOOL

In this edition of *Blood*, Poulain et al demonstrate the high prevalence of the MYD88 L265P somatic mutation in patients with Waldenstrom macroglobulinemia (WM) and provide insight into its biological relevance in the growth and survival of WM.[1]

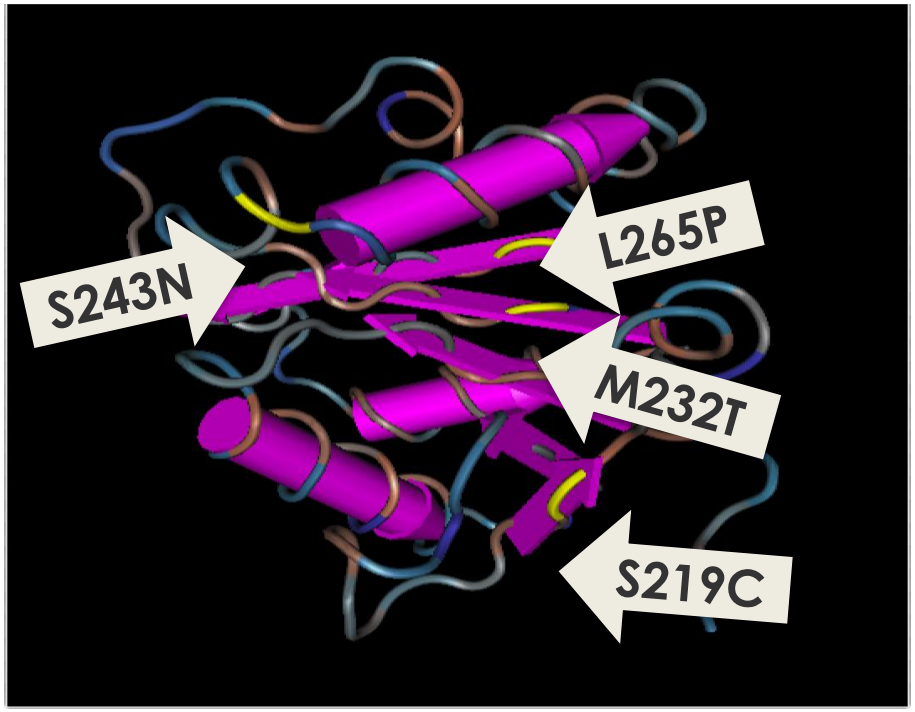
MYD88 L265P by AS-PCR can help distinguish WM from overlapping entities



Xu et al, Blood 2013

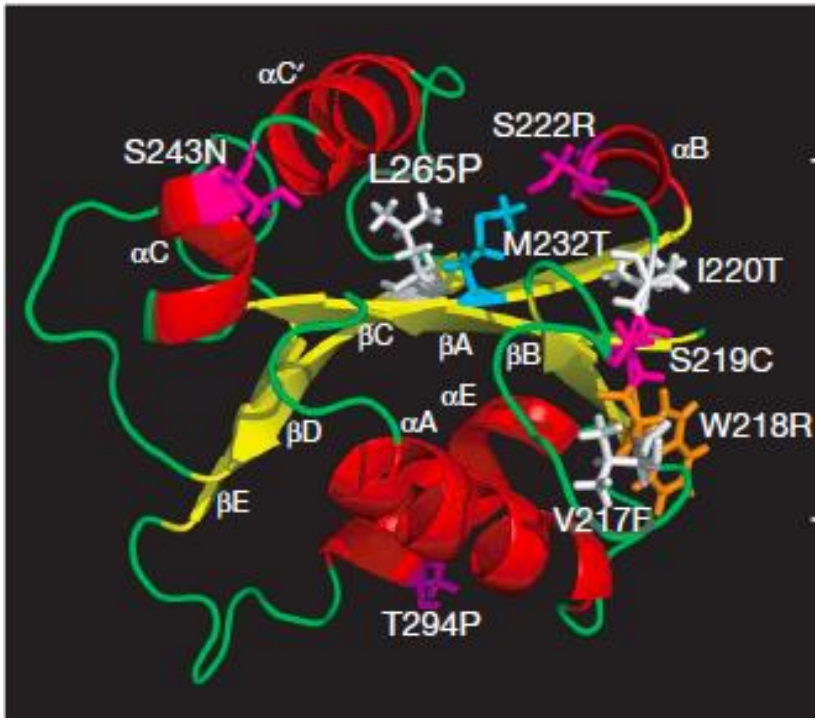
MYD88 Mutations in B-cell LPD

WM



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

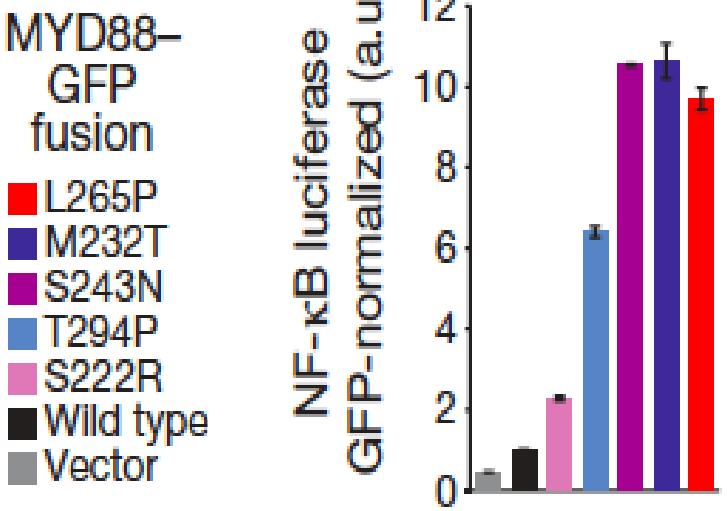
ABC DLBCL



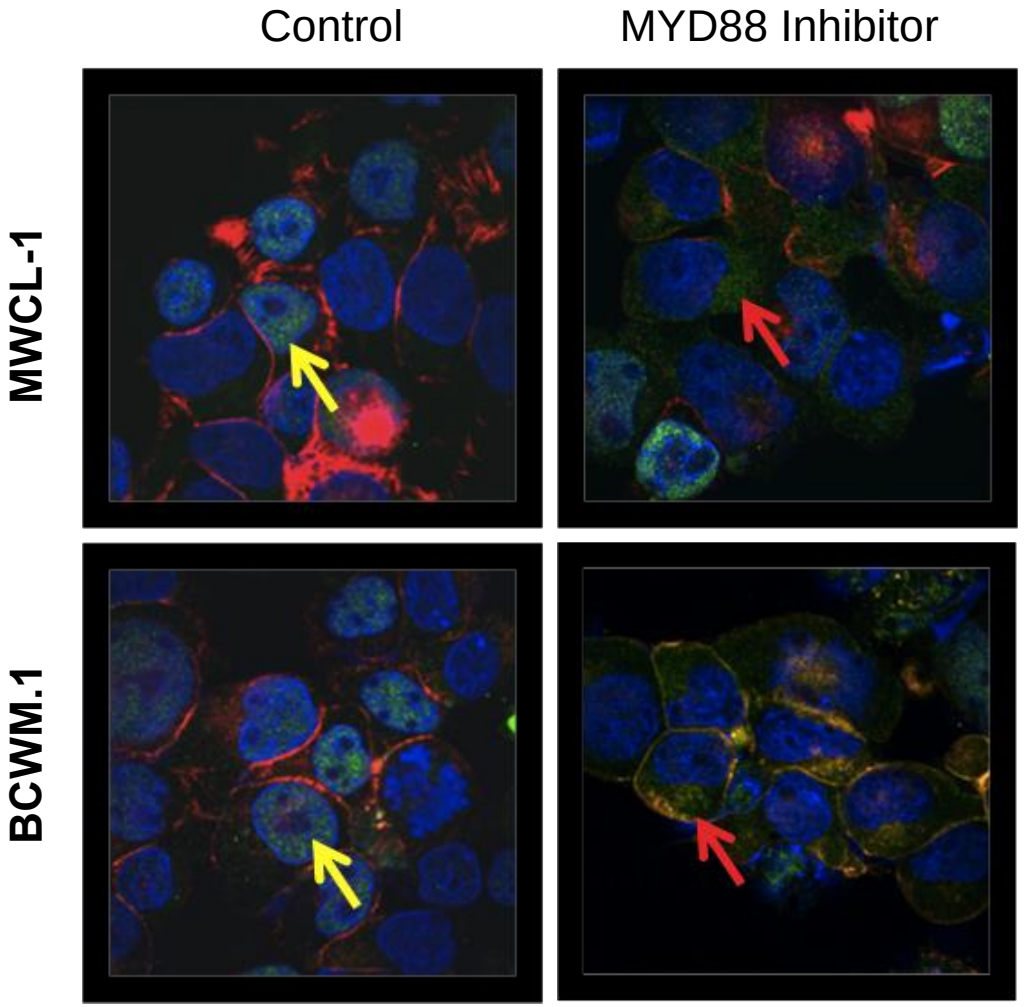
29% MYD88 L265P
10% Non-L265P MYD88

Treon et al, NEJM 2012; Treon et al, NEJM 2015; Jiménez et al, 2013; Varettoni et al 2013; Poulain et al, 2013, Xu et al, 2013.

MYD88 mutations transactivate NFKB

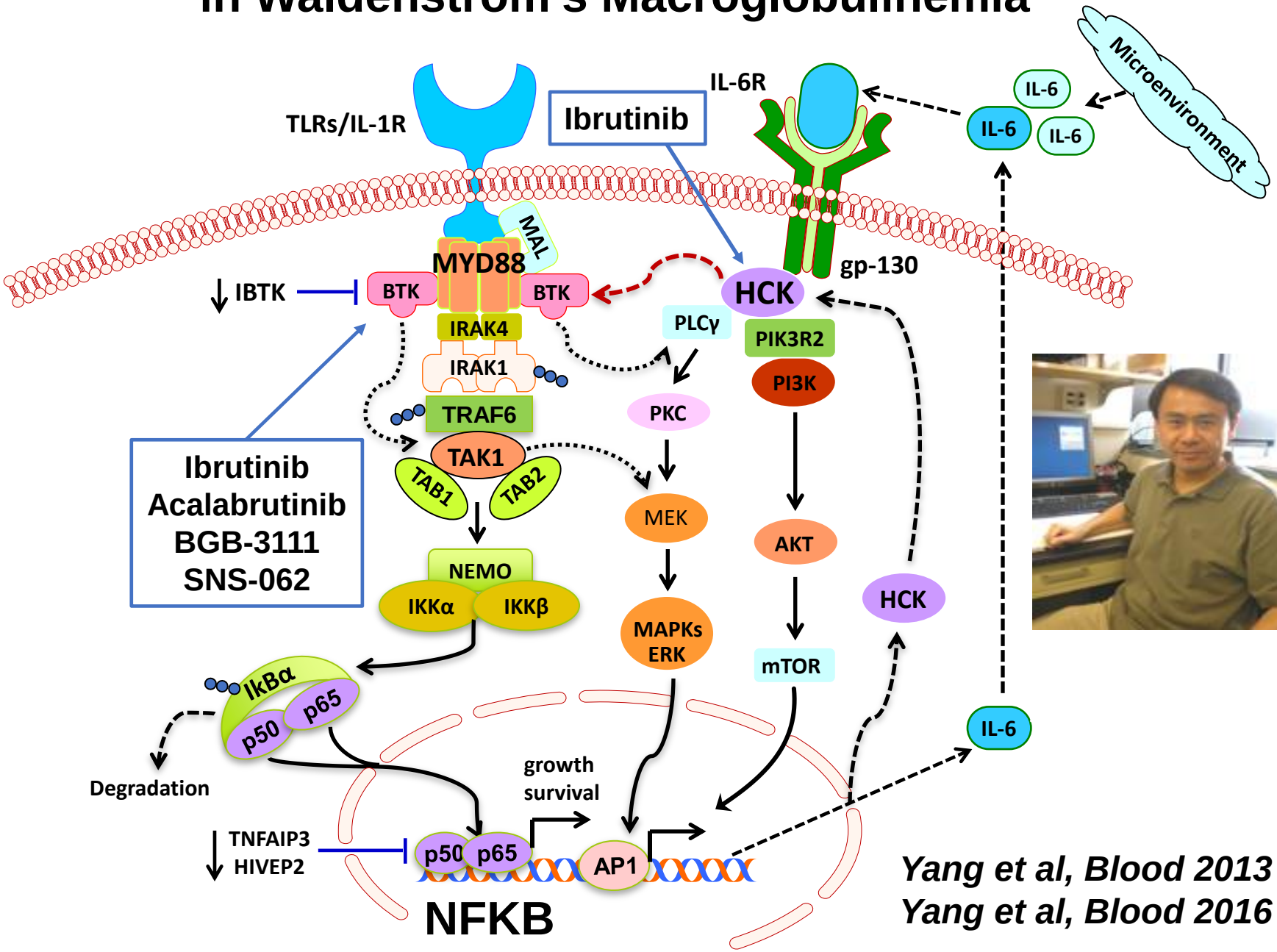


Ngo et al, Nature 2011
Treon et al, NEJM 2012



MYD88 L265P mutated WM cells

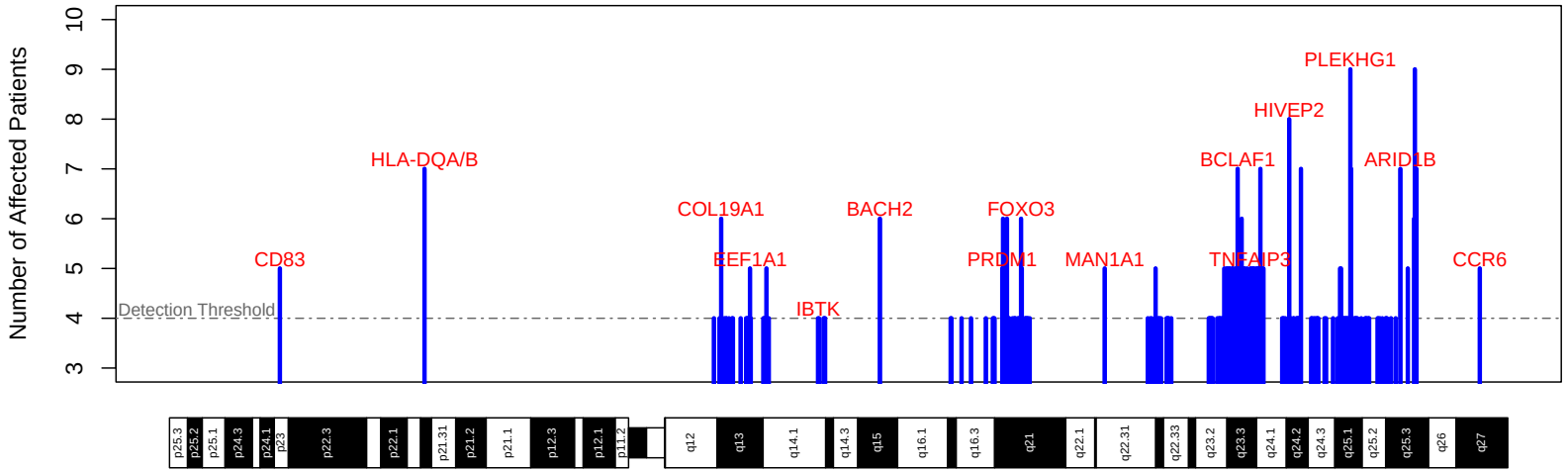
Pro-Survival Signaling Driven by Mutated MYD88 in Waldenström's Macroglobulinemia



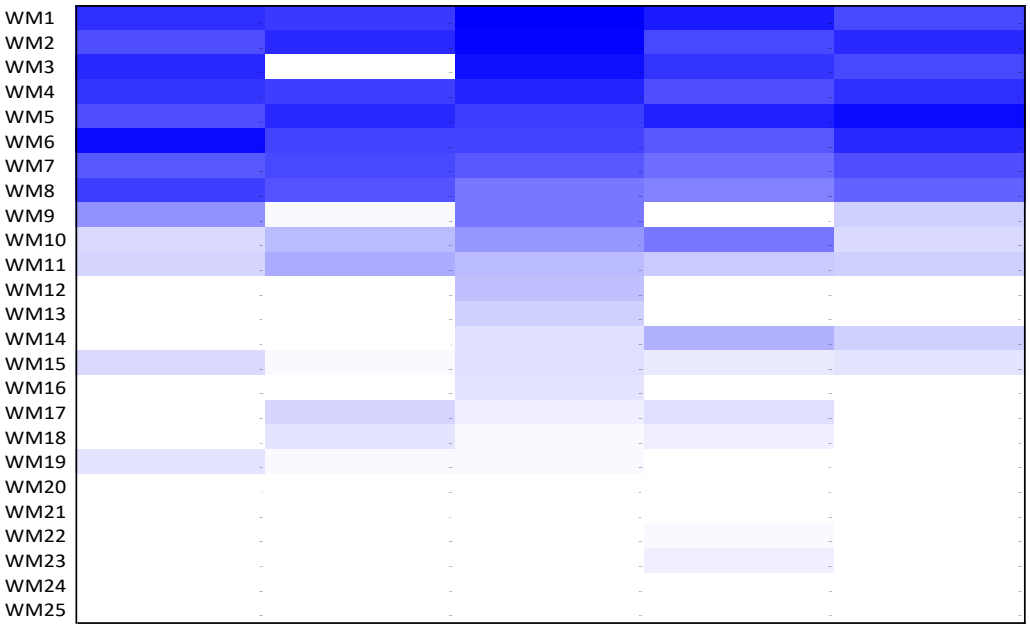
Yang et al, Blood 2013
Yang et al, Blood 2016


Loss of “Brakes” permits
MYD88 to signal unimpeded

Chr. 6q clonal loss is common in WM and impacts BTK, BCL2, and NFKB regulatory genes



Hunter, et al. Blood 2014



Guerrera et al, Haematologica 2018

Discovery of CXCR4 mutations in WM -2013-

Plenary Paper

LYMPHOID NEOPLASIA

SUPPORT • EDUCATION • RESEARCH
IWMF
International Waldenstrom's Macroglobulinemia Foundation

The genomic landscape of Waldenström macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis

Zachary R. Hunter,[1,2] Lian Xu,[1] Guang Yang,[1] Yangsheng Zhou,[1] Xia Liu,[1] Yang Cao,[1] Robert J. Manning,[1] Christina Tripsas,[1] Christopher J. Patterson,[1] Patricia Sheehy,[1] and Steven P. Treon[1,3]

[1]Bing Center for Waldenström's Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA; [2]Department of Pathology and Laboratory Medicine, Boston University School of Graduate Medical Sciences, Boston, MA; and [3]Harvard Medical School, Boston, MA

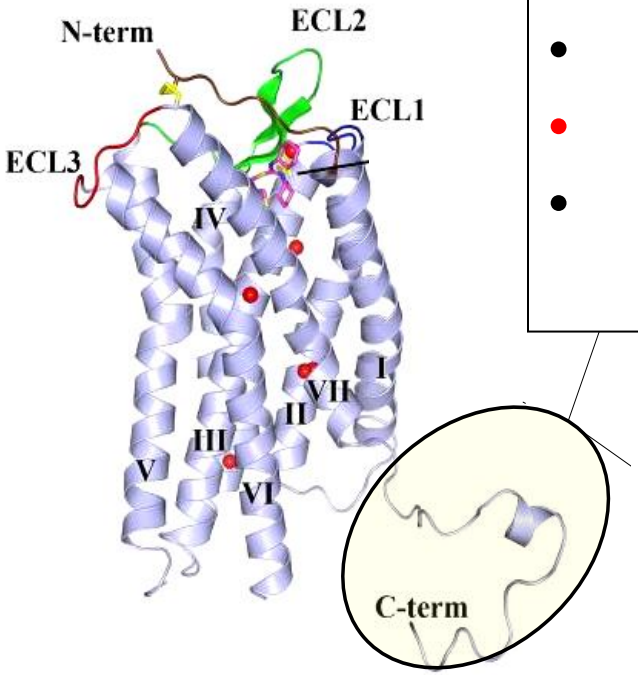
Key Points

- Highly recurring mutations are present in WM, including MYD88 L265P, warts, hypogammaglobulinemia, infection, and myelokathexis-syndrome–like mutations in CXCR4, and ARID1A.
- Small, previously undetected CNAs affecting B-cell regulatory genes are highly prevalent in WM.

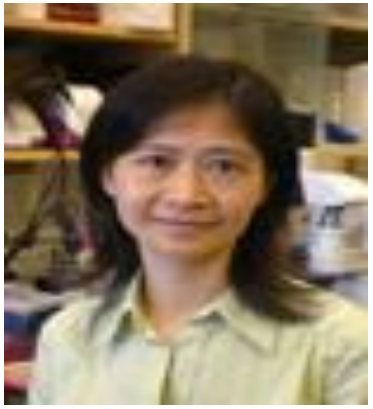
The genetic basis for Waldenström macroglobulinemia (WM) remains to be clarified. Although 6q losses are commonly present, recurring gene losses in this region remain to be defined. We therefore performed whole genome sequencing (WGS) in 30 WM patients, which included germline/tumor sequencing for 10 patients. Validated somatic mutations occurring in >10% of patients included *MYD88*, *CXCR4*, and *ARID1A* that were present in 90%, 27%, and 17% of patients, respectively, and included the activating mutation L265P in MYD88 and warts, hypogammaglobulinemia, infection, and myelokathexis-syndrome–like mutations in CXCR4 that previously have only been described in the germline. WGS also delineated copy number alterations (CNAs) and structural variants in the 10 paired patients. The CXCR4 and CNA findings were validated in independent expansion cohorts of 147 and 30 WM patients, respectively. Validated gene losses due to CNAs involved *PRDM2* (93%), *BTG1* (87%), *HIVEP2* (77%), *MKLN1* (77%), *PLEKHG1* (70%), *LYN* (60%), *ARID1B* (50%), and *FOXP1* (37%). Losses in *PLEKHG1*, *HIVEP2*, *ARID1B*, and *BCLAF1* constituted the most common deletions within chromosome 6. Although no recurrent translocations were observed, in 2 patients deletions in 6q corresponded with translocation events. These studies evidence highly recurring somatic events, and provide a genomic basis for understanding the pathogenesis of WM. (*Blood*. 2014;123(11):1637-1646)

CXCR4 C-tail mutations in WM

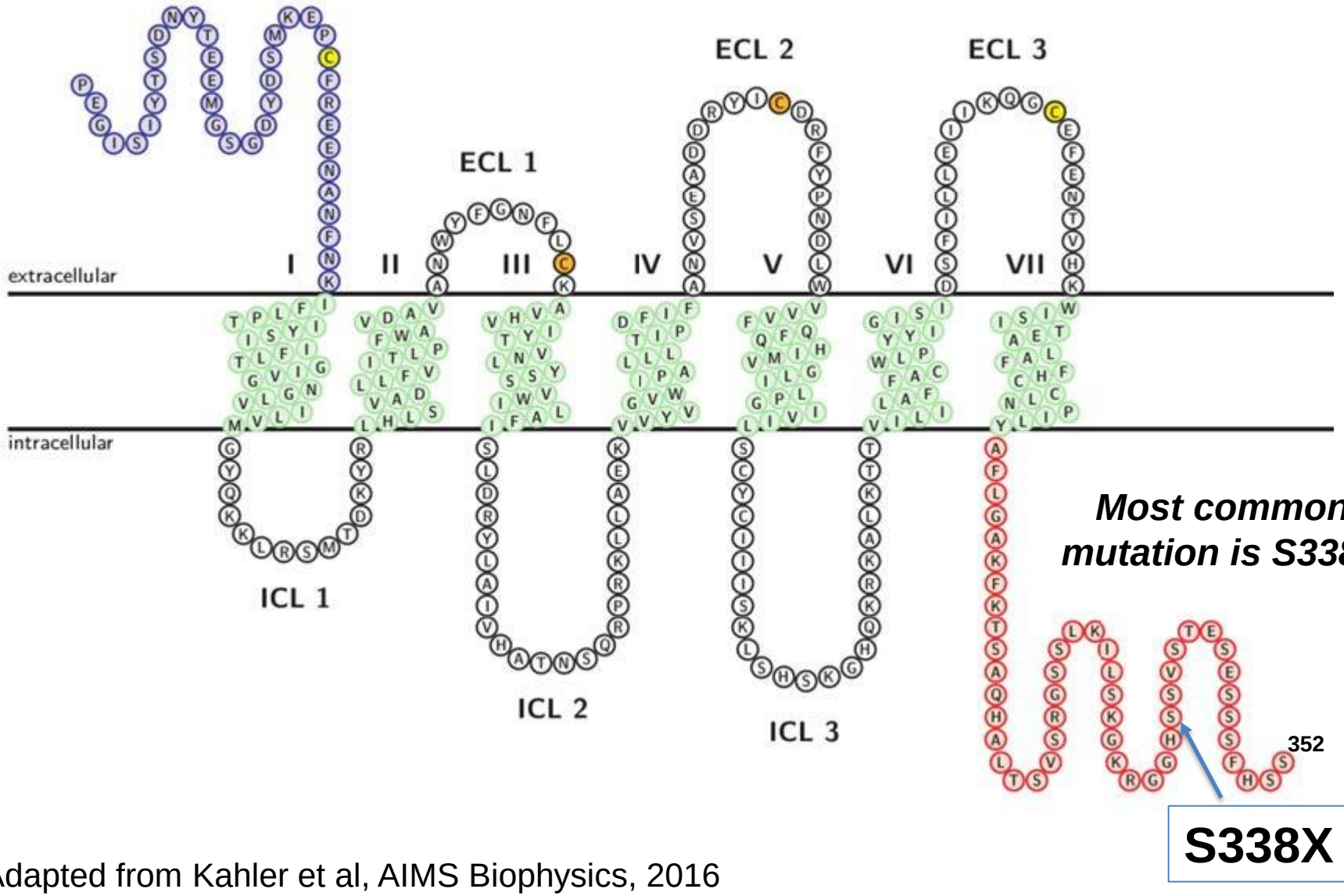
- 30-40% of WM patients; rare in other LPD
- Accompany MYD88 mutations
- Exclusive of del 6q
- Usually subclonal (median 45%)
- **High serum IgM levels/hyperviscosity**
- Promote **ibrutinib resistance** through enhanced AKT/ERK signaling.



Hunter et al, Blood 2013;
Rocarro et al, Blood 2014:
Poulain et al, Blood 2016;
Cao et al, Leukemia 2014;
Cao et al, BJH 2015



Mutations impact the “tail” of the CXCR4 receptor



Adapted from Kahler et al, AIMS Biophysics, 2016

>40 types of CXCR4 C-terminal somatic mutations in WM

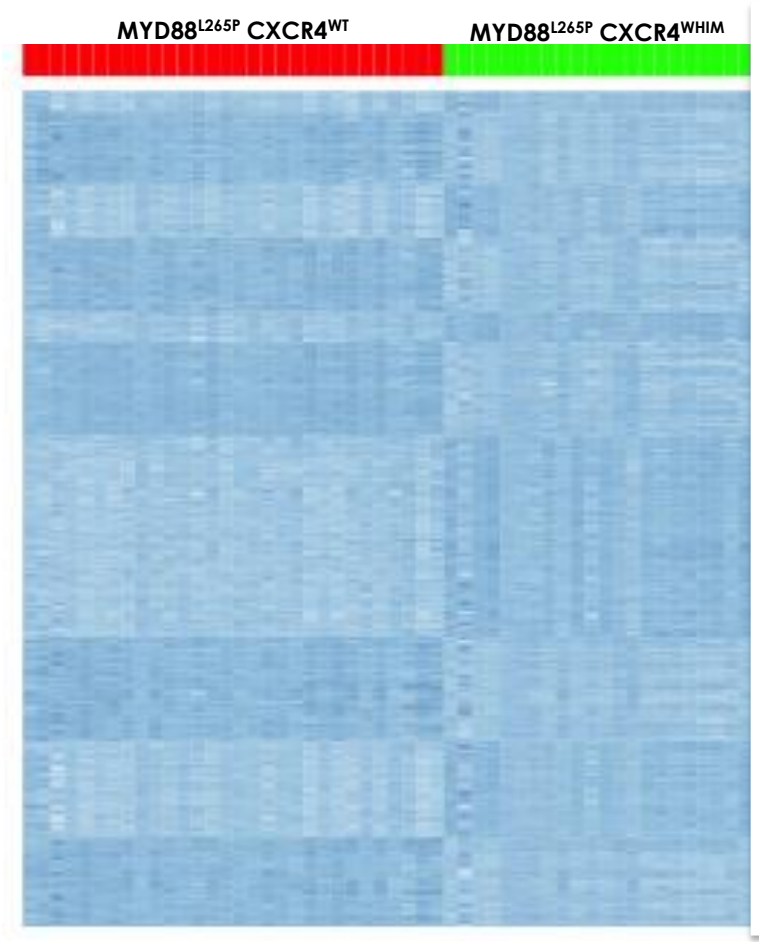
including multiple CXCR4 mutations within individual patients

N=	MYD88 Status	CXCR4 Mutation	Nucleotide change	Amino acid change
1	L265P	Nonsense	r.997 A>T[1]	[illegible]
3	L265P	Nonsense	r.1000C>T	R334X
7	L265P	Nonsense	r.1013C>A	S338X
15	L265P	Nonsense	r.1013C>G[2]	S338X[2]
1	WT	Frameshift	r.931_933insT	
3	L265P	Frameshift	r.952_954insA	T318fs
2	L265P	Frameshift	r.951_953delACCTC	T318fs
1	L265P	Frameshift	r.954_956insC	S319fs
1	L265P	Frameshift	r.958_960delTG	V320fs
1	L265P	Frameshift	r.963_965insC	R322fs
1	L265P	Frameshift	r.969_971insG	S324fs
1	L265P	Frameshift	r.978_980insT	K327fs
1	L265P	Frameshift	r.984_986insT	L329fs
1	L265P	Frameshift	r.993_995insA	G332fs
1	L265P	Frameshift	r.1005_1007insT	G336fs
2	L265P	Frameshift	r.1013_1015delATCT	S338fs
1	L265P	Frameshift	r.1013_1015delATCTGTTTCCACTGAGT	S338fs
3	L265P	Frameshift	r.1012_1014insT	S338fs
1	L265P	Frameshift	r.1015_1017delCT	S339fs
1	L265P	Frameshift	r.1020_1022delT	S341fs
1	L265P	Frameshift	r.1024_1026delCT	S342fs
1	L265P	Frameshift	r.1030_1041CTGAGTCTTC>GT	S344fs
1	L265P	Frameshift	r.1033_1035delAG	E345fs

@50%

Treon et al, Blood 2014; Poulain et al, CCR 2016; Baer et al, Leukemia 2017

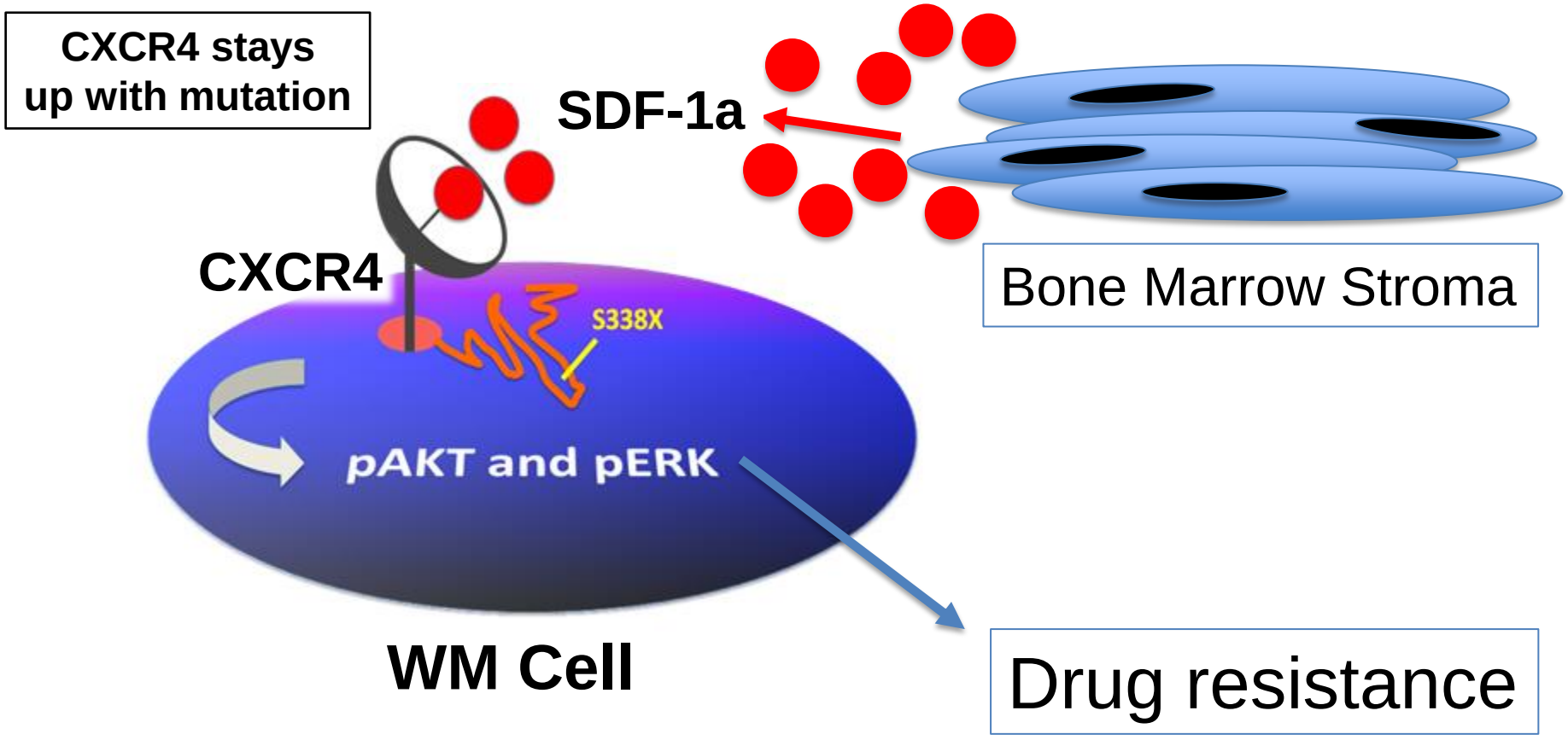
MYD88 and CXCR4 Transcriptomic Profiling

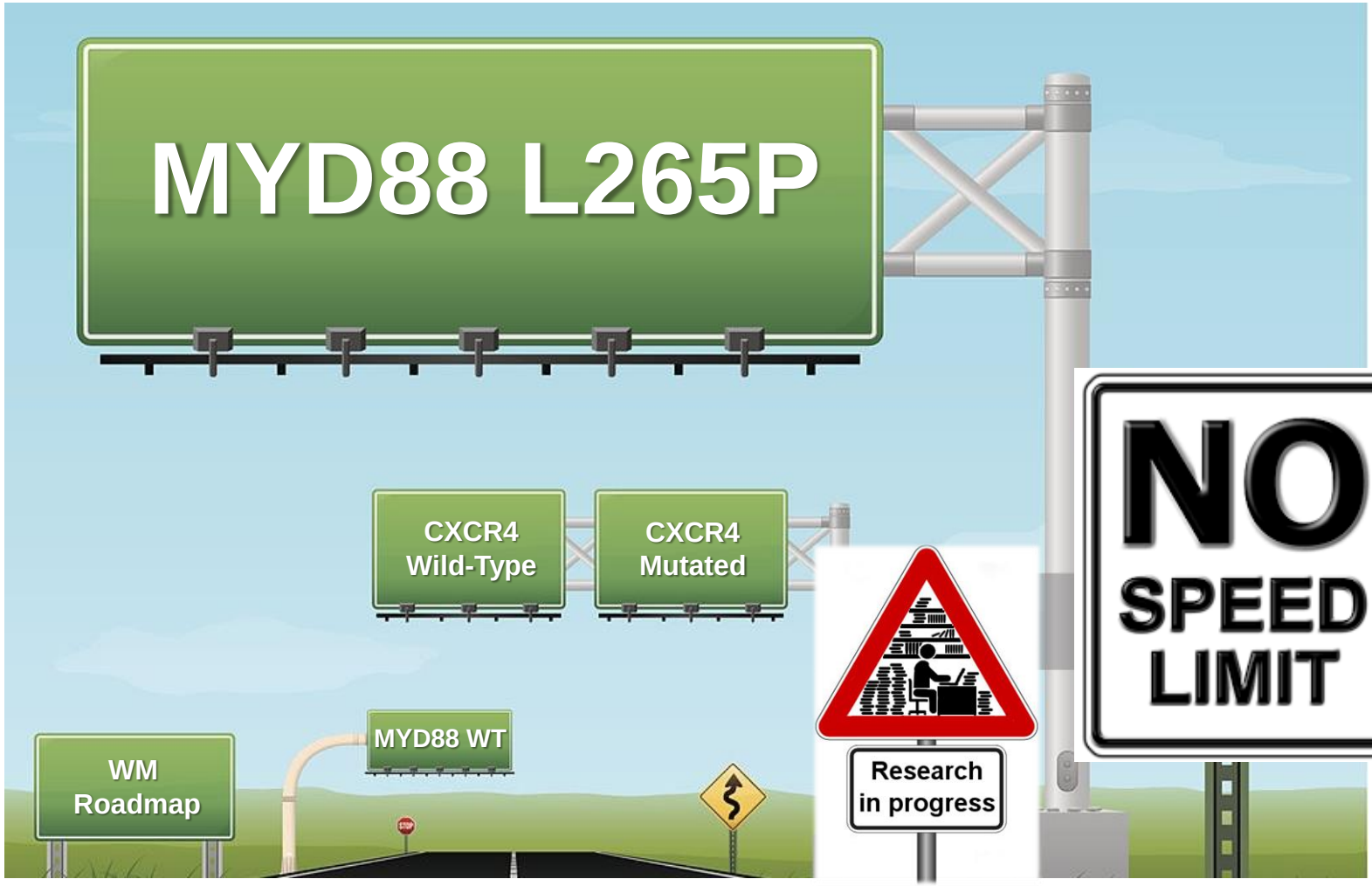


Supervised Clustering of 3,103 genes found to be significantly differentially expressed between $MYD88^{L265P}CXCR4^{WT}$ (N=29) and $MYD88^{L265P}CXCR4^{WHIM}$ (N=23) WM patients

Hunter et al, BLOOD 2016

Mutations in CXCR4 permit ongoing pro-survival signaling by SDF-1a, the ligand for CXCR4 that is manufactured in the bone marrow stroma.




MYD88 L265P
CXCR4
Wild-Type
CXCR4
Mutated
NO
SPEED
LIMIT
MYD88 WT
WM
Roadmap
Research
in progress

Differential Diagnosis of MYD88 WT WM

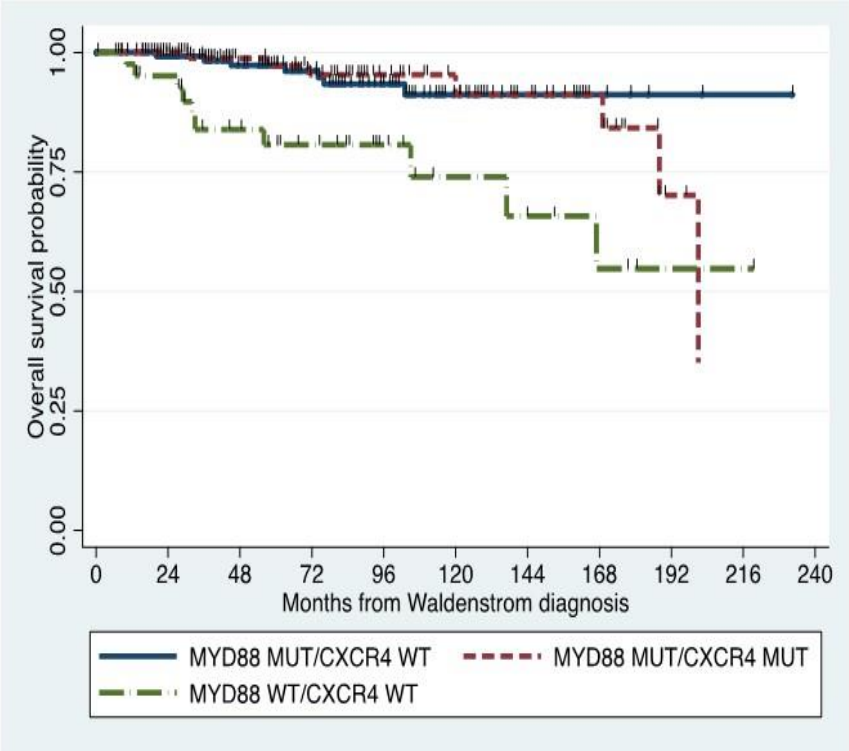
Diagnosis	N=	Age (yrs)	Gender (% male)	BM (%)	sIgM (mg/dL)	Hb (g/dL)	Adenopathy (%)	Splenomegaly (%)
WM	46	58.5	48	35	2,980	11.0	35	28
IgM MM	7	59	71	60	8,375	9.0	14	14
MZL	6	64.5	0	10	1,642	11.3	67	33
IgM PC MGUS	3	62	33	5	1,846	13.9	0	0
CLL	1	83	0	5	1,822	13.2	0	0
DLBCL	1	76	0	5	355	9.5	0	100

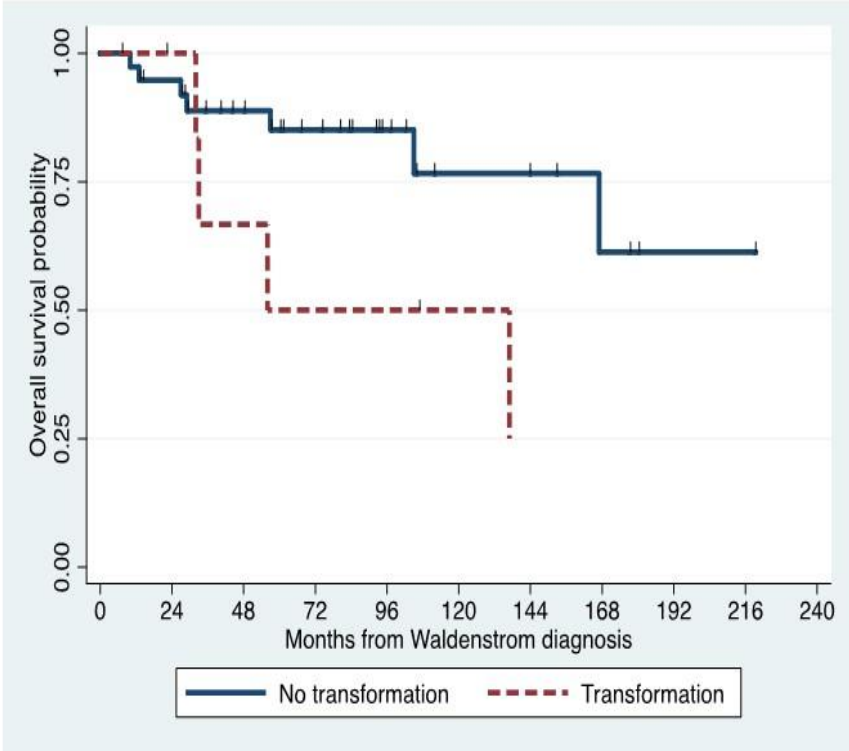
t(11;14)

N=64

Treon et al, BJH 2017

High risk of transformation and poorer survival accompany MYD88^{WT} WM

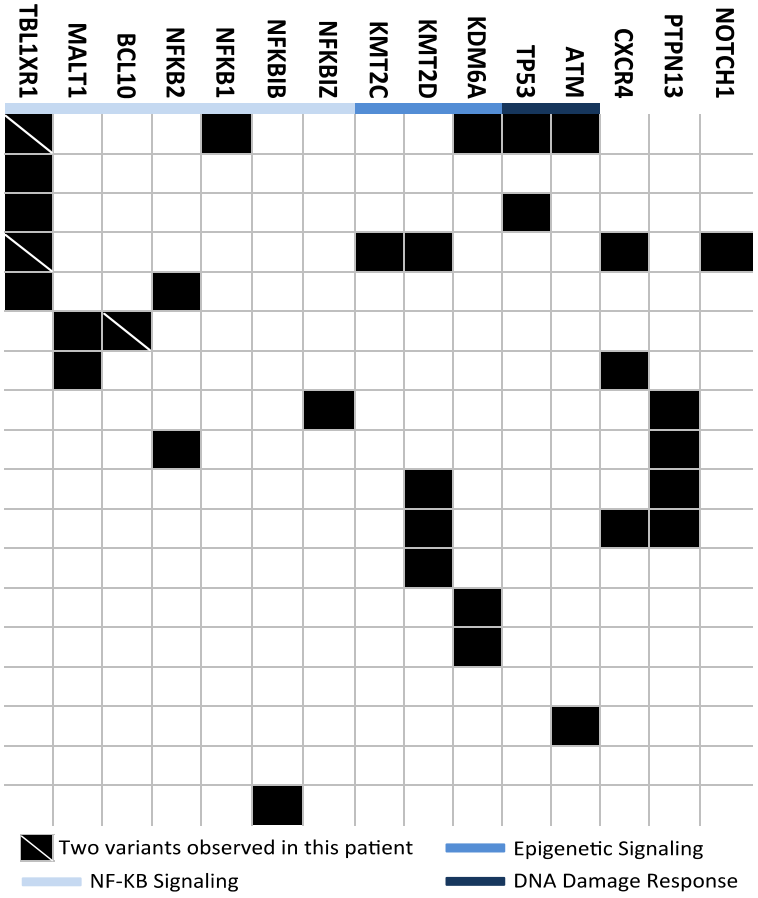


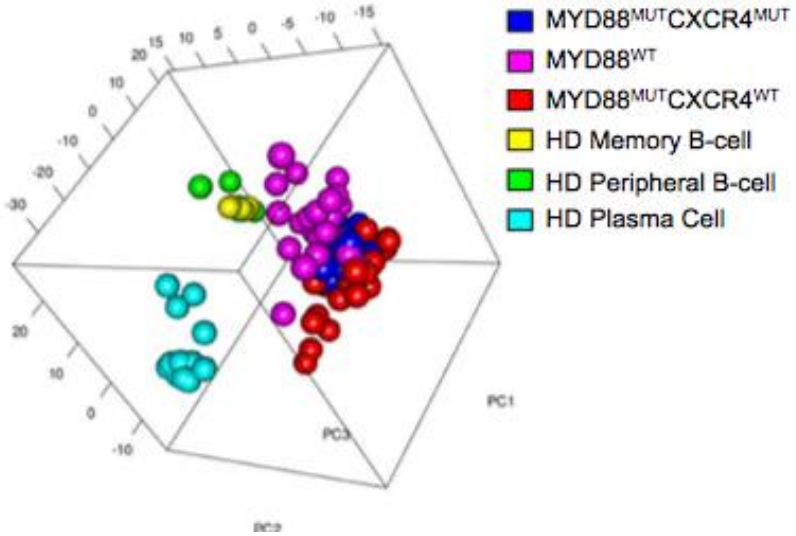


Transformation risk for MYD88 WT
(Odds ratio 23·3; 95% CI 4·2-233·8; p<0·001).

Treon et al, BJH 2017

New Driver Mutations Identified in MYD88 WT WM

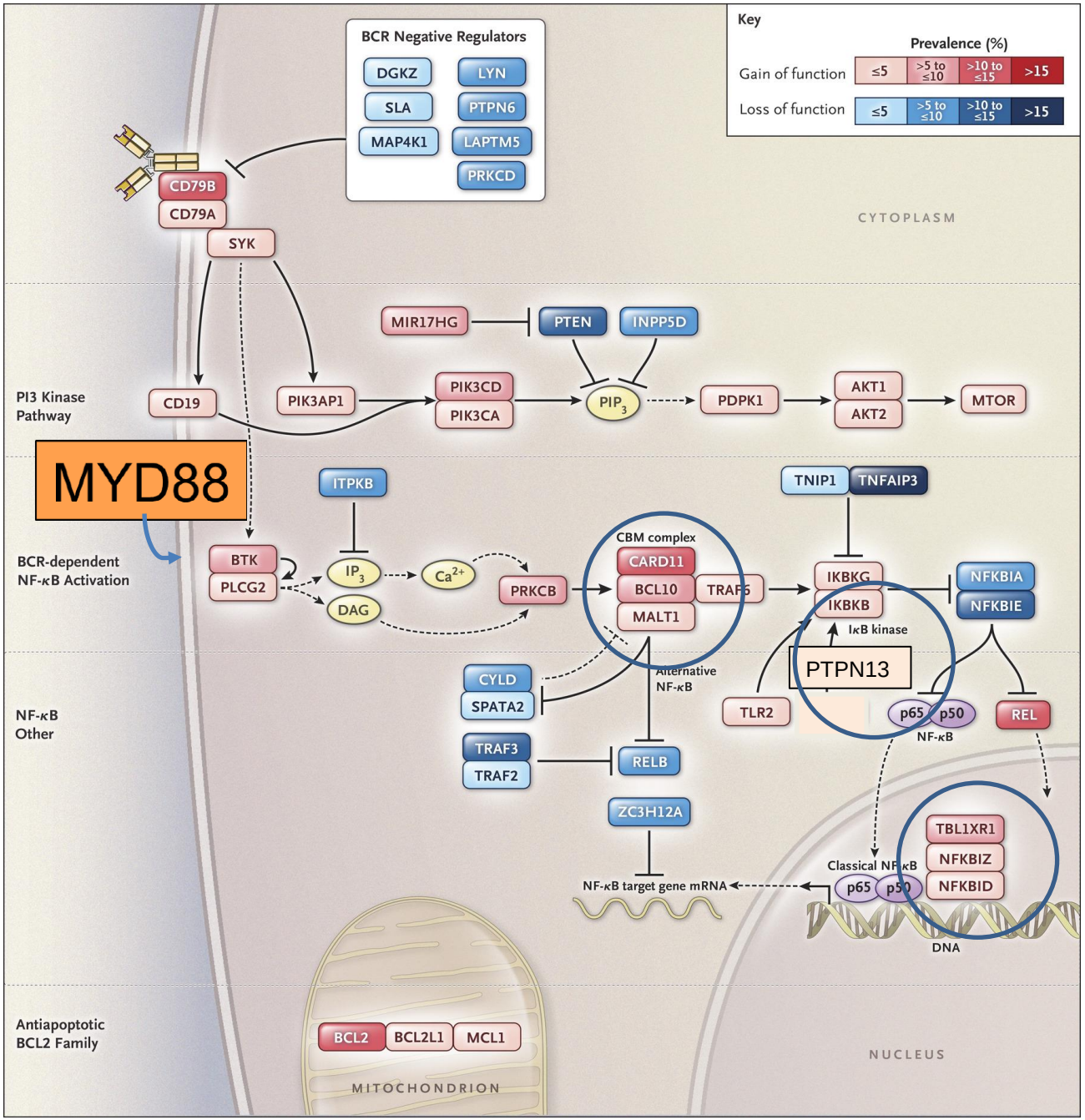




Principal component analysis of top 500 high variance genes.

Hunter et al, ASH 2017

Mutations in MYD88 WT WM are downstream of BTK



Adapted from Schmitz et al, NEJM 2018

What about other mutations in WM?

300 PROJECT

- **Sequence 300 Untreated Symptomatic Patients**
- **Understand impact of other mutations on disease course, transformation, survival**
- **Develop individualized targeted treatments**

Bench to Bedside

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

R. Advani L. Palomba

DANA-FARBER CANCER INSTITUTE

Screening

Registration

420 mg po qD Ibrutinib

Progressive Disease (PD) or Unacceptable Toxicity

Stop Ibrutinib

Event Monitoring

Stable Disease or Response Continue

Event Monitoring

www.clinicaltrials.gov
NCT01614821

✔ **MYD88, CXCR4 Mutation Status**

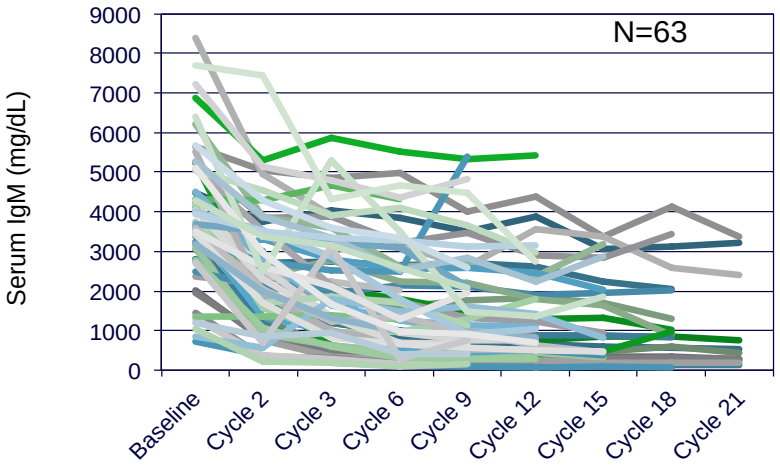
Baseline Characteristics for Study Participants (n=63)

	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 2015; 372:1430

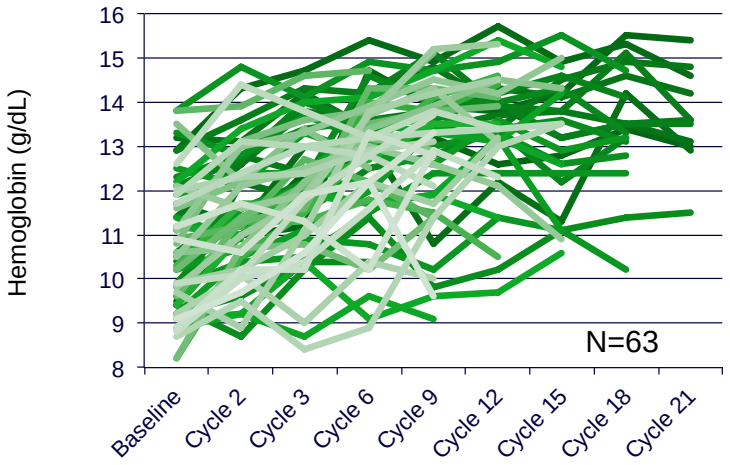
Rapid Changes in Serum IgM and Hemoglobin Levels Following Ibrutinib

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

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

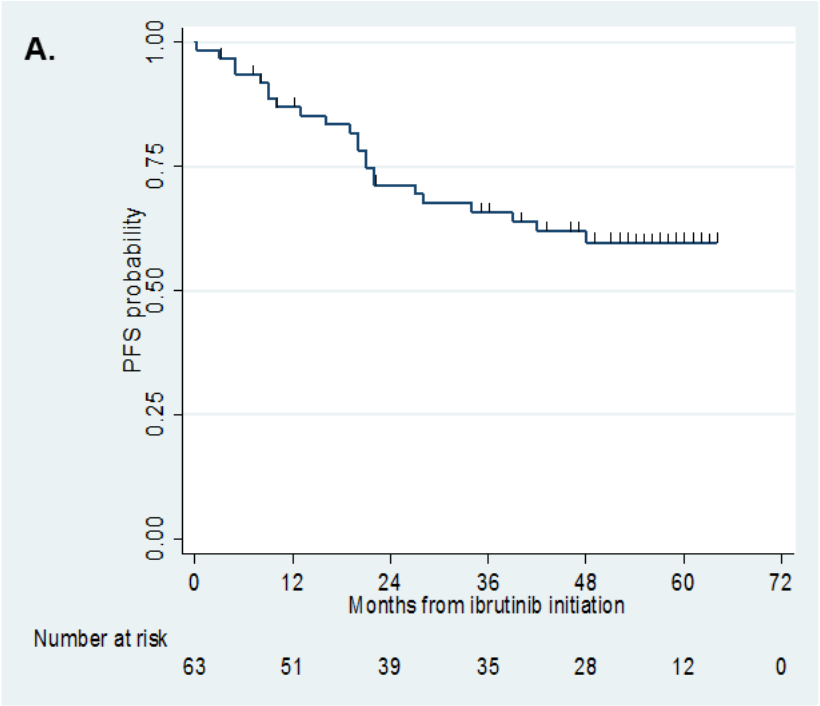
Responses to ibrutinib are impacted by MYD88 (L265P and non-L265P) and CXCR4 mutations.

	ALL	$MYD88^{Mut}$ $CXCR4^{WT}$	$MYD88^{Mut}$ $CXCR4^{Mut}$	$MYD88^{WT}$ $CXCR4^{WT}$	P-value
N=	**63**	36	21	5	
ORR	**90.4%**	100%	85.7%	60%	0.005
Major (>PR)	**77.7%**	97.2%	66.6%	0%	<0.001
VGPR	**27.0%**	44.4%	9.5%	0%	0.007
Time to Minor Response (mos.)	**1.0**	1.0	1.0	1.0	0.10
Time to Major response (mos.)	**2.0**	2.0	6.0	N/A	0.05

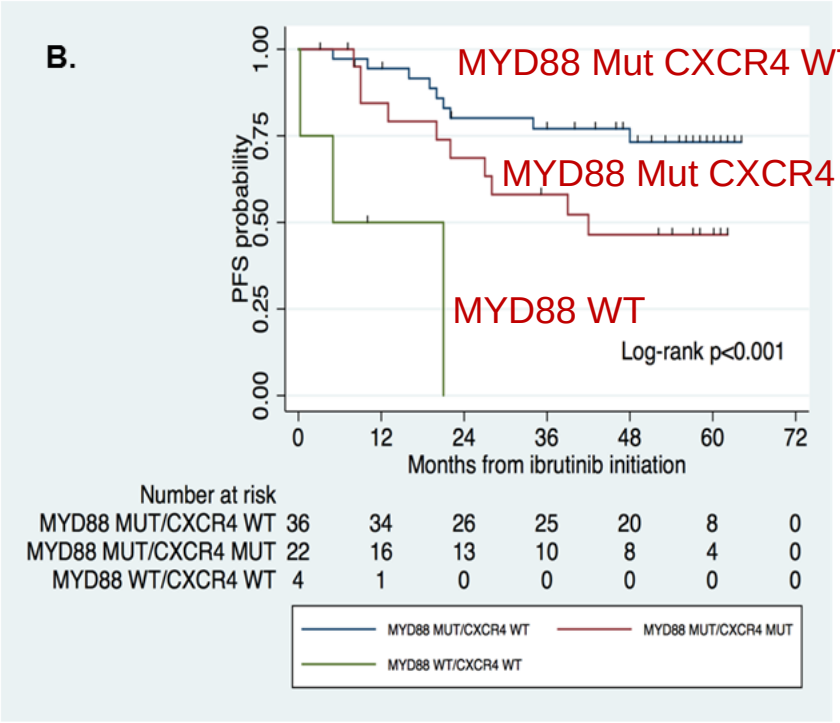
Treon et al, NEJM 2015; Treon et al, ASH 2017

Ibrutinib in Previously Treated WM: PFS

All patients



MYD88 and CXCR4 Status

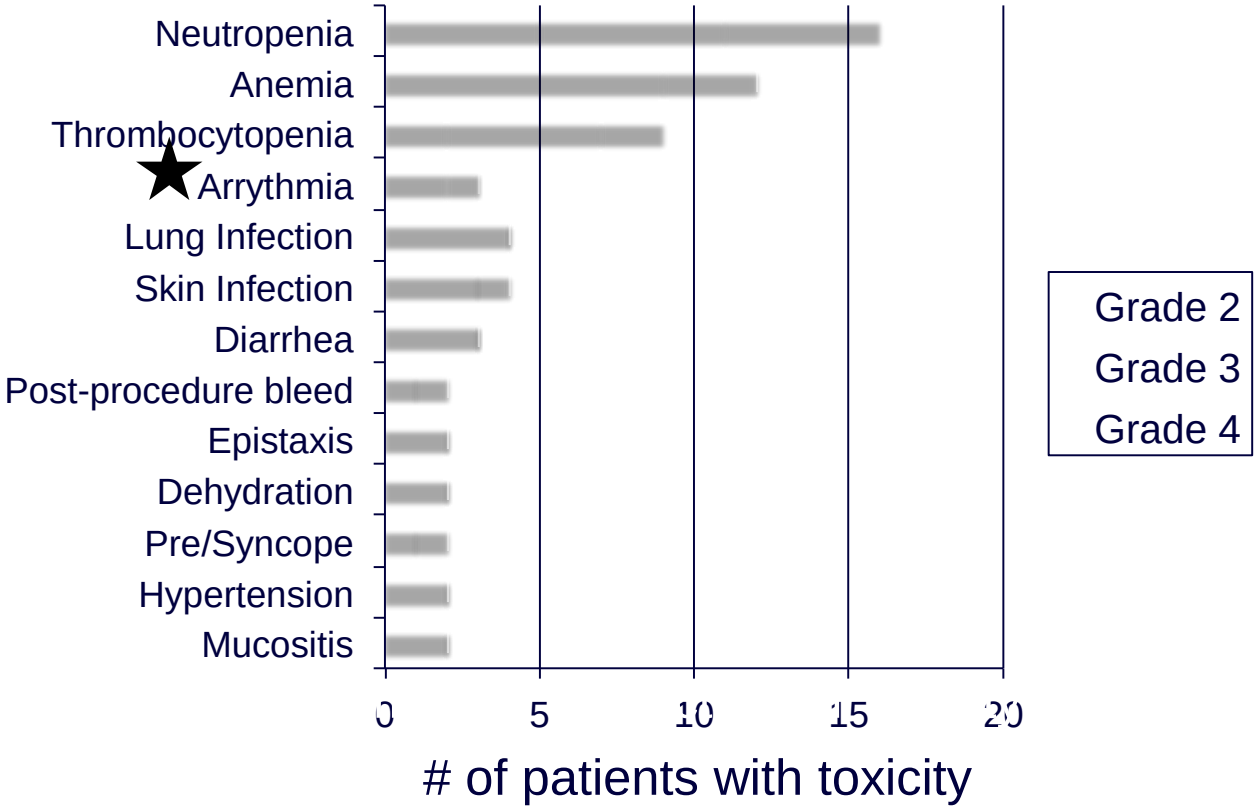


Median PFS > 5 years

Treon et al, NEJM 2015; EHA 2018

Ibrutinib Related Adverse Events in previously treated WM patients

Toxicities >1 patient; N=63



- **No impact on IGA and IGG immunoglobulins**

★10% incidence with larger WM Experience; earlier presentation for those patients with prior Afib history.

Treon et al, NEJM 2015; Gustine et al, AJH 2016

Supported FDA Approval of Ibrutinib for WM -2015-

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Ibrutinib in Previously Treated Waldenström's Macroglobulinemia

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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

>80 countries

EMA Approval for symptomatic previously treated and chemoimmunotherapy unsuitable frontline WM
First ever for Waldenstrom's
July 8, 2015

משרד הבריאות
לחיים בריאים יותר

September, 2015

Health Canada Santé Canada

April 5, 2016

Ibrutinib in Rituximab-Refractory WM Patients: Multicenter, Open-Label Phase 3 Substudy (iNNOVATE™)

Median Prior Therapies: 4 (range 1-7)
Median follow-up: 18.1 (range 6.3-21.1 months)

ORR: 90% Major RR (≥ PR): 71%

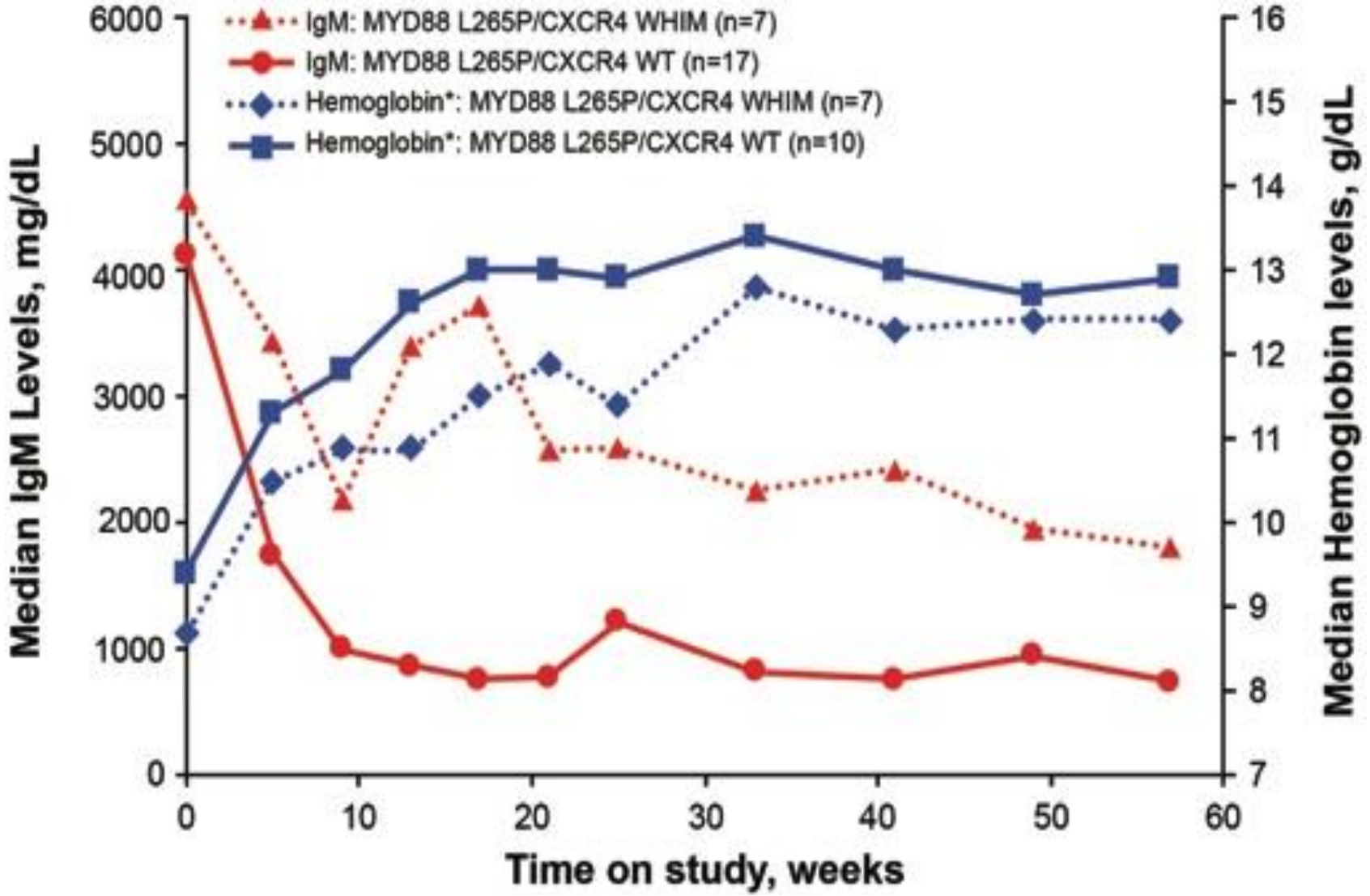
	(N=)	(%)
VGPR	4	13
PR	18	58
MR	6	19

Median time to ≥ MR: 4 weeks
Median time to best response: 8 weeks

18 mo PFS: 86%
18 mo OS: 97%

Dimopoulos et al, IWWM9 2016; Lancet Oncol 2017.

Impact of CXCR4 Mutation Status on IgM and HgB Response



Dimopoulos et al, IWWM9; Lancet Oncology, 2017

Primary Therapy of WM with Ibrutinib

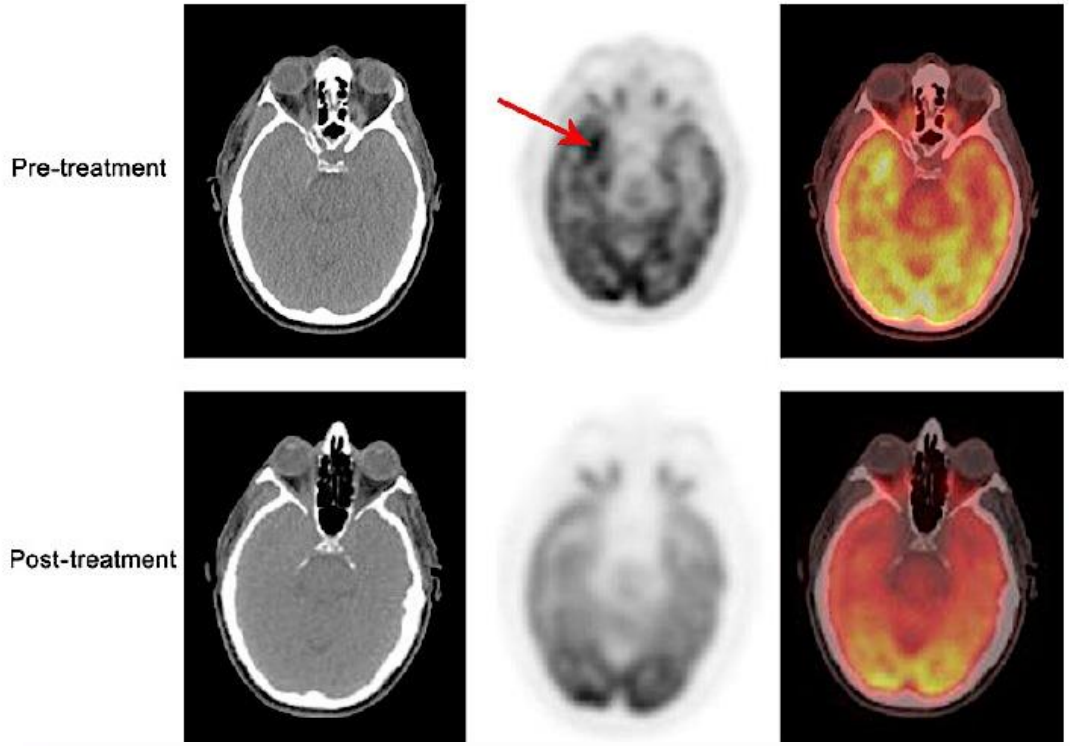
	All Patients	MYD88^{MUT} CXCR4^{WT}	MYD88^{MUT} CXCR4^{MUT}	P-value
N=	30	16	14	N/A
Overall Response Rate-no. (%)	30 (100%)	16 (100%)	14 (100%)	1.00
Major Response Rate-no. (%)	25 (83%)	15 (94%)	10 (71%)	0.16
Categorical responses				
Minor responses-no. (%)	5 (17%)	1 (6%)	4 (29%)	0.16
Partial responses-no. (%)	19 (63%)	10 (63%)	9 (64%)	1.00
Very good partial responses-no. (%)	6 (20%)	5 (31%)	1 (7%)	0.18
Median time to response (months)				
Minor response (≥Minor response)	1.0	0.9	1.7	0.07
Major response (≥Partial response)	1.9	1.8	7.3	0.01

Median on treatment duration of 13.4 (range 1.8-21.1 months)

18 mo: PFS 92%; OS 100%. PD patients were CXCR4 mutated.

Treon et al, ASH 2017; EHA 2018

Ibrutinib (560 mg/day) induced response in a WM patient with Bing Neel Syndrome



Study Day	Time post-dose (h)	Ibrutinib (nM)		
		CSF	Plasma	%CSF/Plasma
Day 1	0	BLQ	BLQ	NA
	2	34	1133	3·0
1 Month	3	16	463	3·5
4 Months	2·5	7	318	2·2

Mason et al, BJH 2016

Other BTK Inhibitors

- **Acalabrutinib** (Phase II Study Completed, Awaiting Results)
- **BGB-3111** (Phase II Study Completed, Phase III randomized study for newly diagnosed and previously treated patients is ongoing)
- **SNS-062** (Non-covalent inhibitor that binds to a different site from other BTK inhibitors; use in resistant disease due to BTK mutations)

Strategies to Enhance BTK Inhibitors in WM

iNNOVATE Study in WM

Treatment Naïve + Previously Treated

45 centers in 9 countries

1:1 Randomization N = 150

ARM A: ibrutinib 420mg
+ Rituximab 375mg/m² x 8 infusions
(weeks 1,2,3,4,17,18,19, and 20)

ARM B: Placebo
+ Rituximab 375mg/m² x 8 infusions
(weeks 1,2,3,4,17,18,19, and 20)

N=31

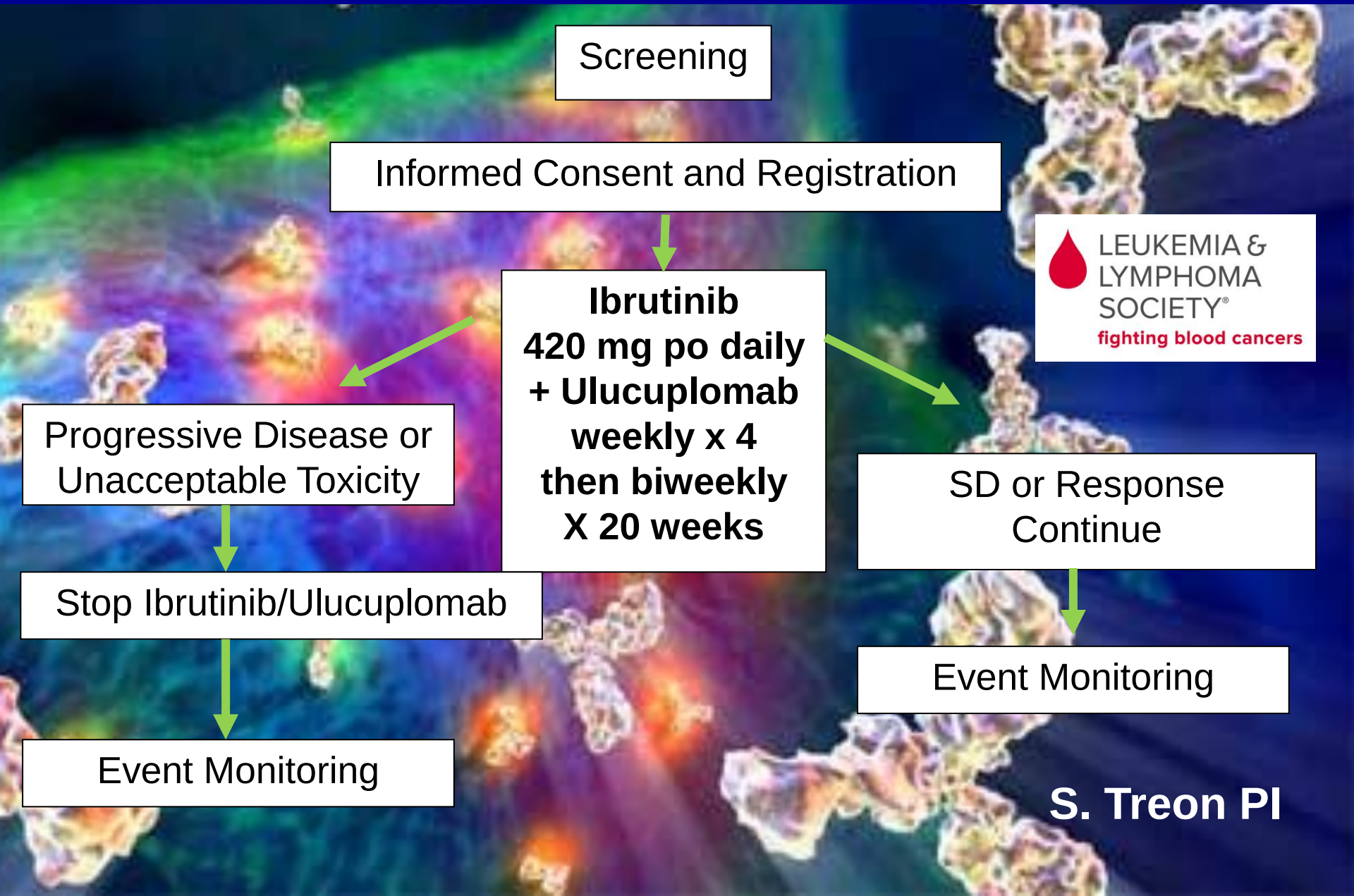
ARM C: ibrutinib 420mg
Subjects considered
refractory to prior rituximab

ABC patients genotyped for MYD88 and CXCR4

What is still unknown after iNNOVATE?

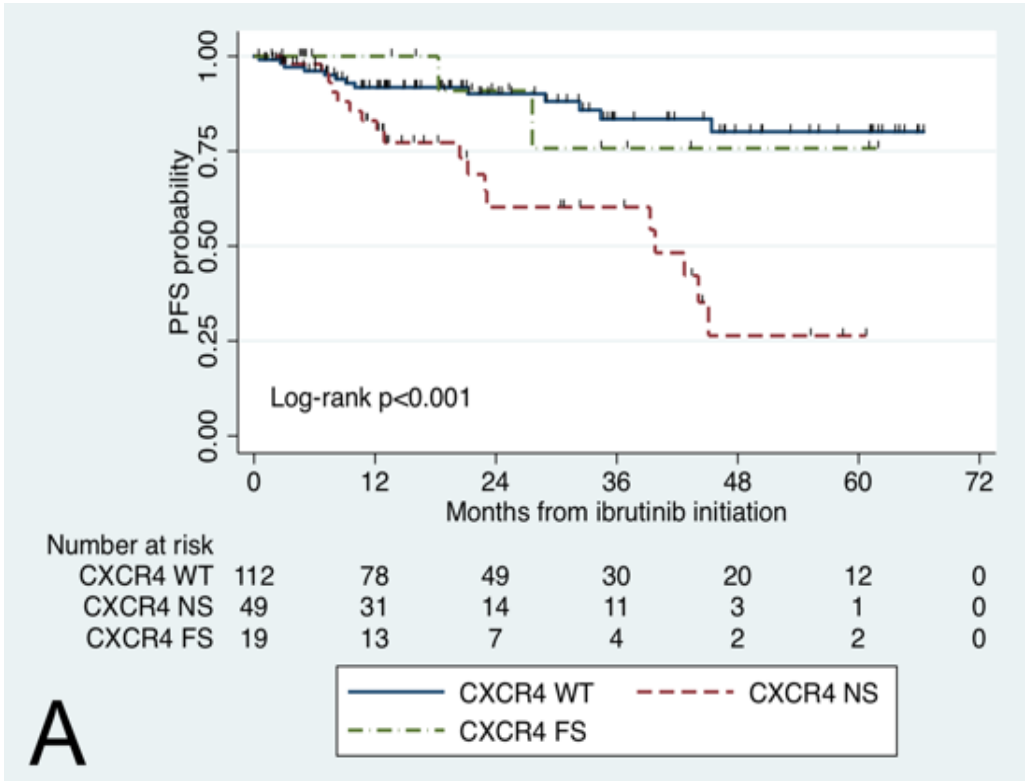
I think we all agree…we need another study

Phase II Study of Ibrutinib plus Ulucuplomab in CXCR4^{WHIM} WM Patients

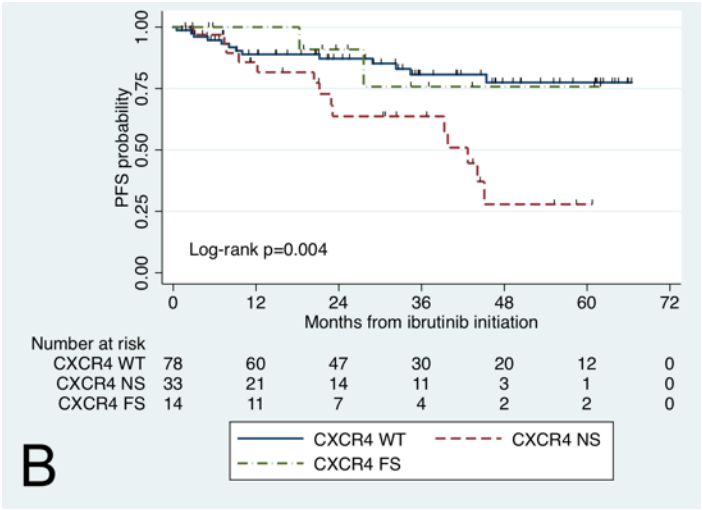


Role of Nonsense vs. Frameshift CXCR4 mutations in Ibrutinib Response

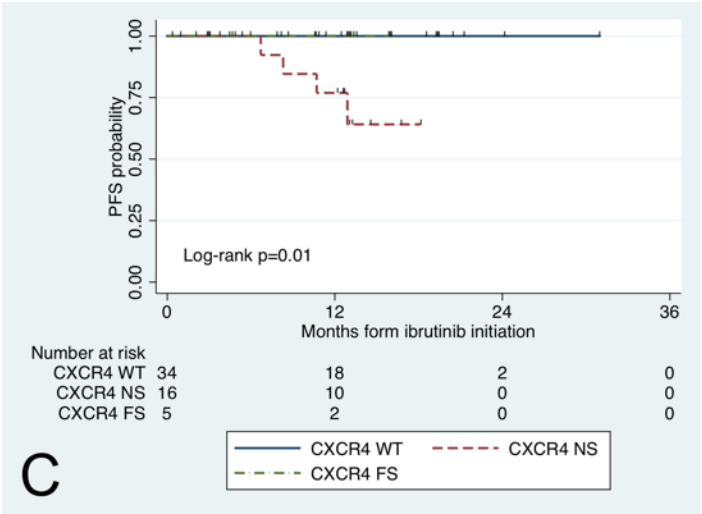
All patients



Previously Treated

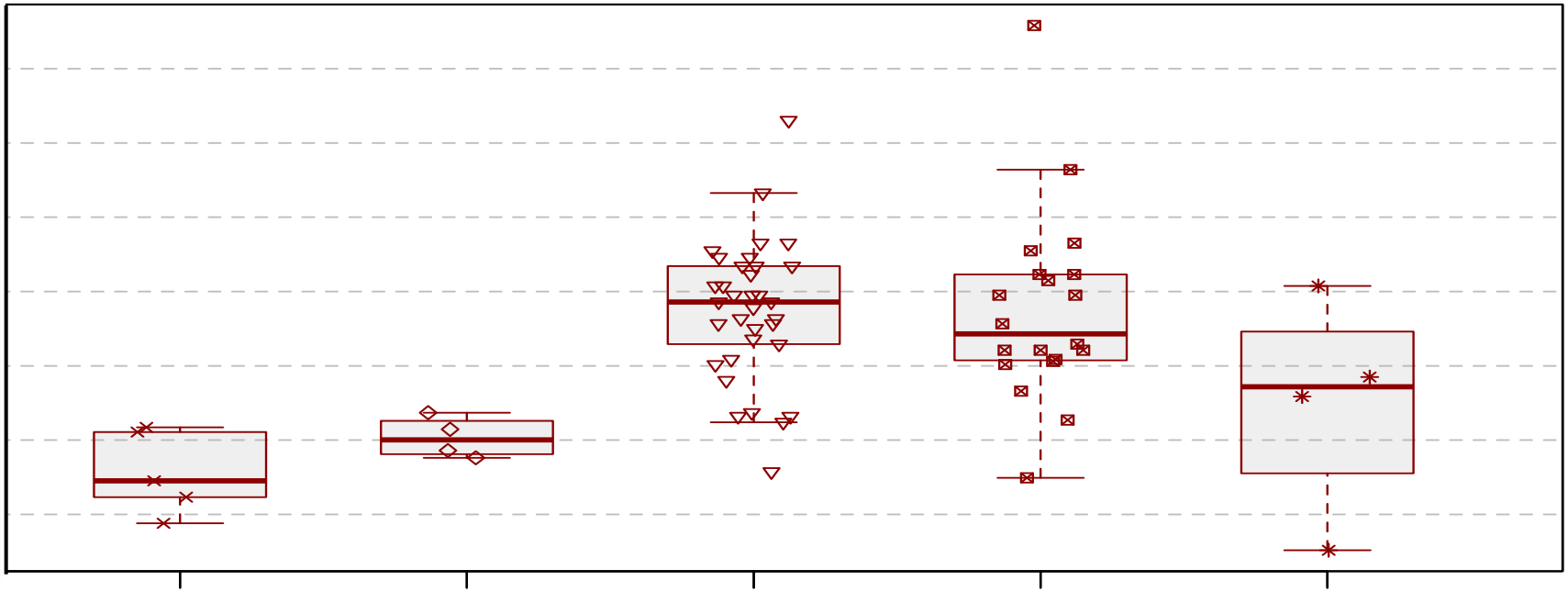


Untreated



Castillo et al, EHA 2018

BCL-2 is overexpressed in primary WM patient cells by transciptome analysis in MYD88 mutated patients regardless of CXCR4 mutation status.



Healthy Donor $CD19^{+}CD27^{-}$

Healthy Donor $CD19^{+}CD27^{+}$

WM $CD19^{+}$ $MYD88^{L265P}$ $CXCR4^{WT}$

WM $CD19^{+}$ $MYD88^{L265P}$ $CXCR4^{WHIM}$

WM $CD19^{+}$ $MYD88^{WT}$ $CXCR4^{WT}$

$p<0.001$ for healthy donor samples versus any $MYD88^{L265P}CXCR4^{WT\ or\ WHIM}$

Hunter et al, BLOOD 2016


Brazil
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TIME
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Argentina
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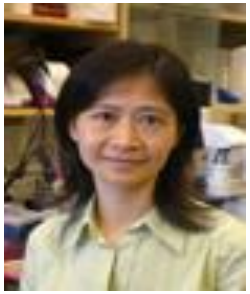
Ibrutinib
BCL2

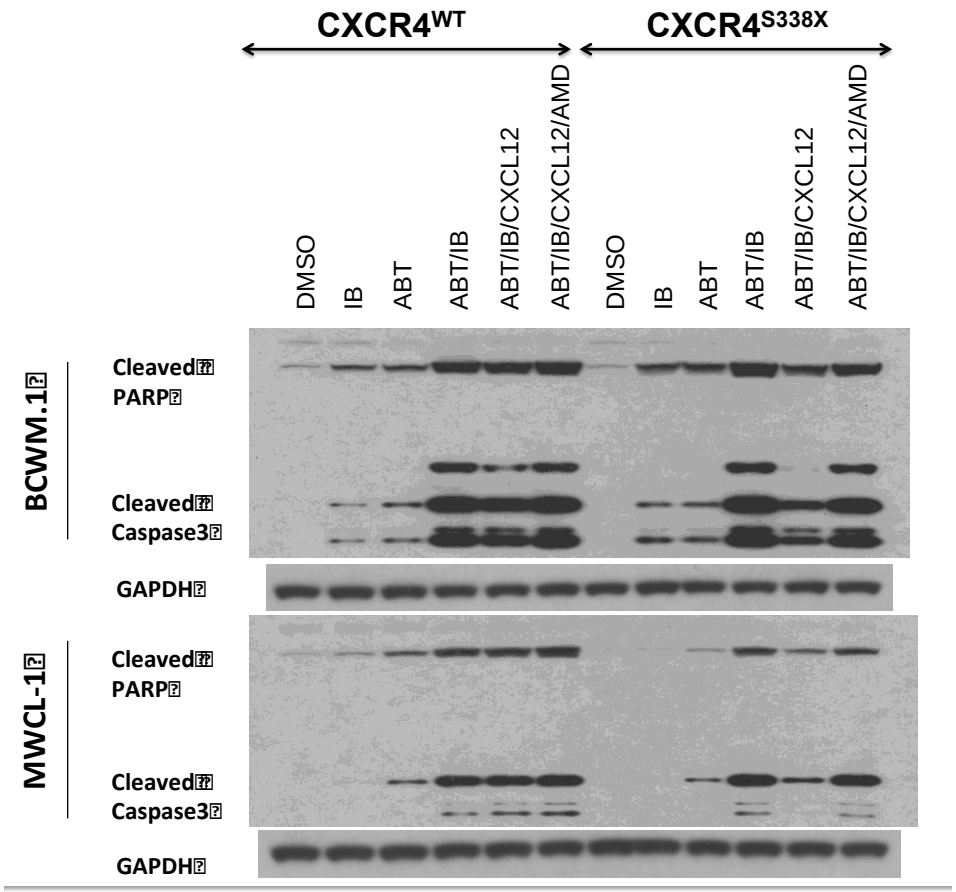
Ibrutinib

BCL2


Ibrutinib
BCL2

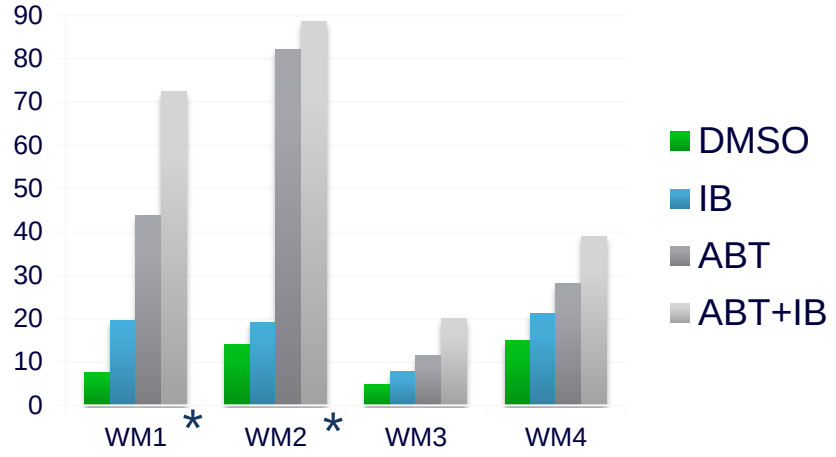


Venetoclax (ABT-199) enhances Ibrutinib killing in MYD88 mutated WM Cells.

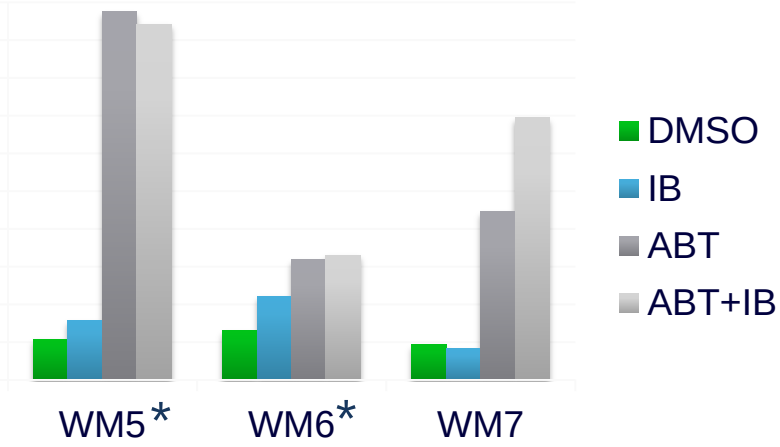


Cao et al, BJH 2015

Untreated

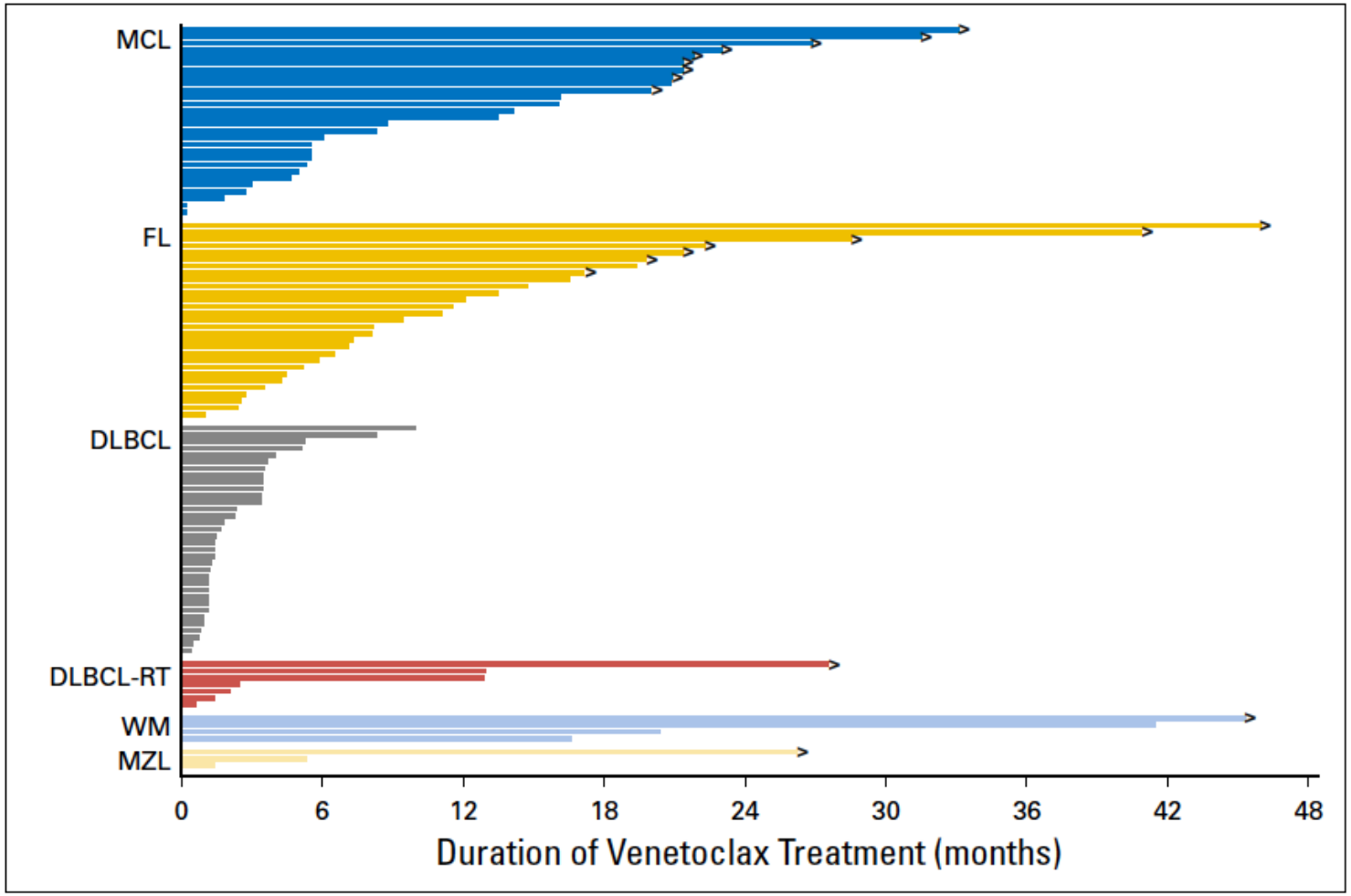


Ibrutinib >6 mo.



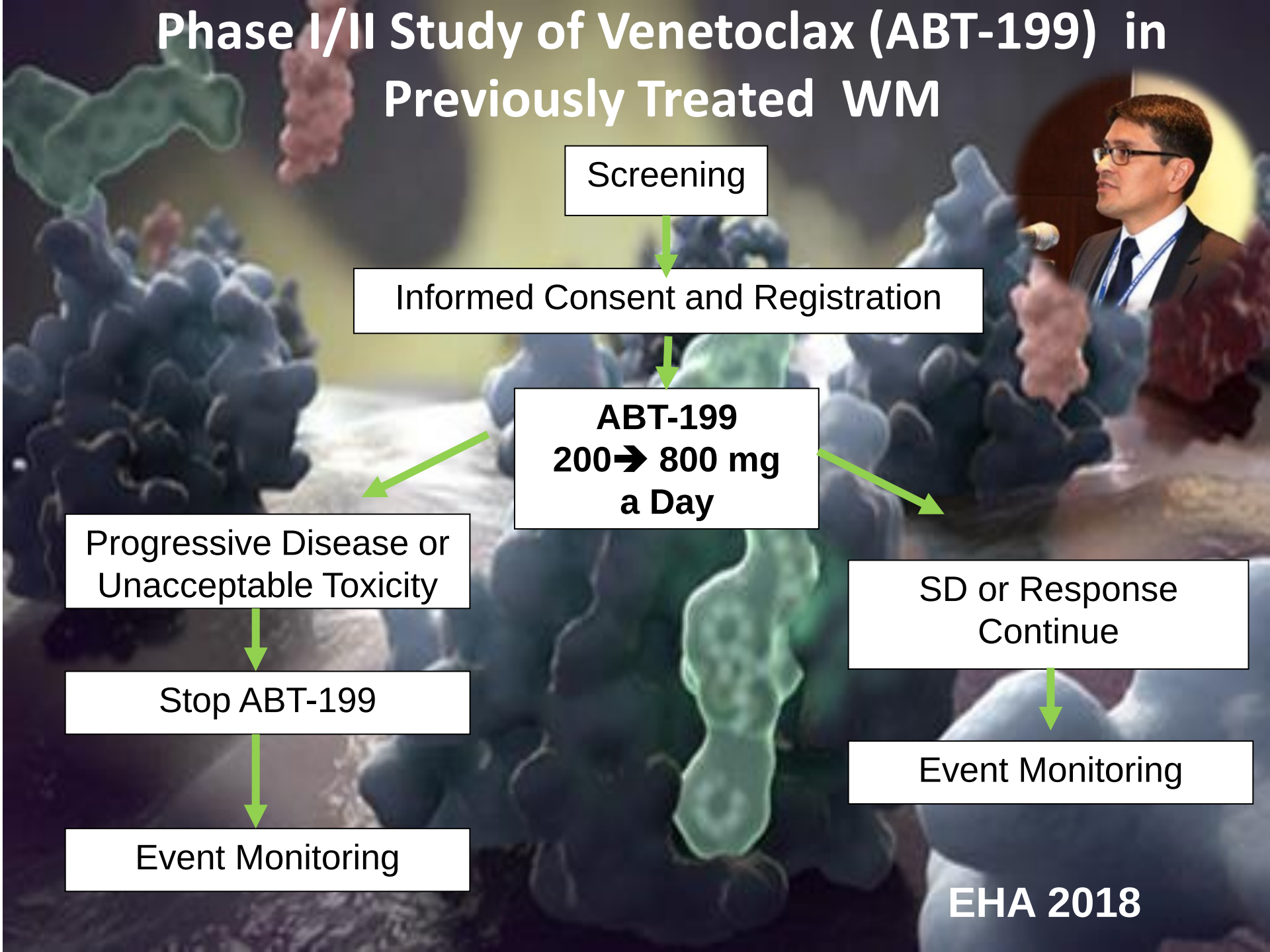
*CXCRWHIM

Activity of the anti-BCL2 agent Venetoclax (ABT-199) in previously treated NHL Patients



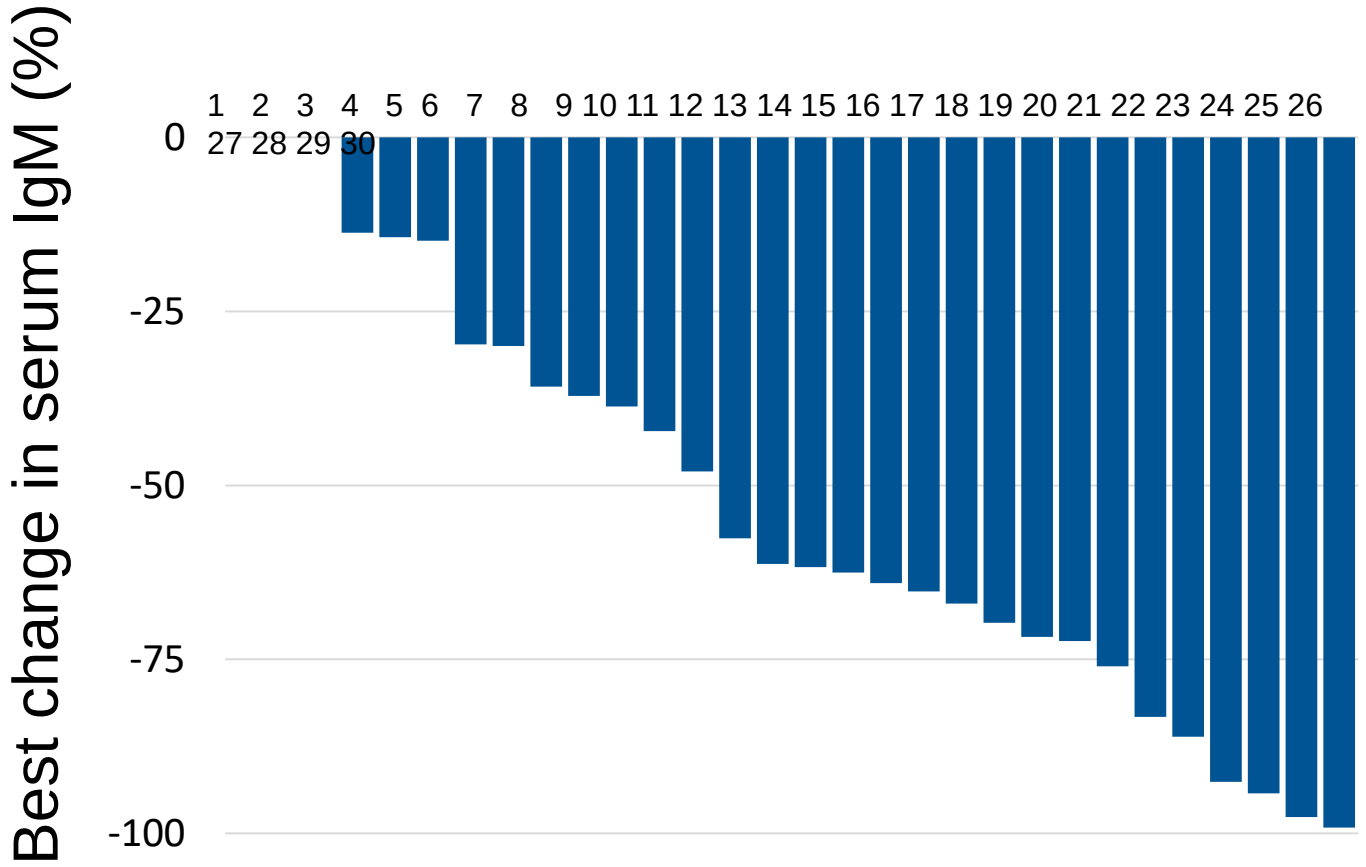
Davids et al, JCO 2017

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



EHA 2018

Venetoclax responses in previously treated WM



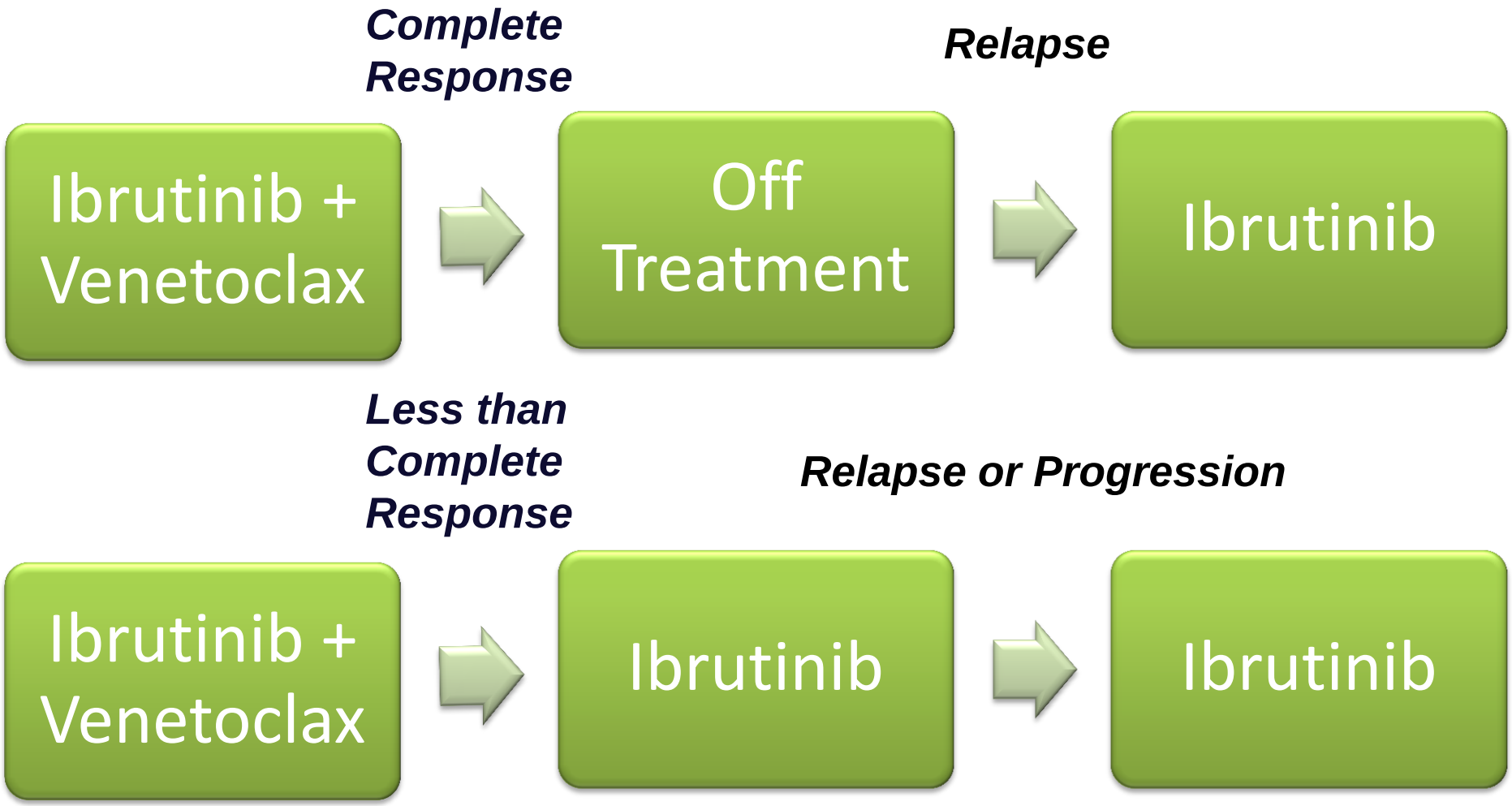
ORR: 80% **Major Responses: 56%** **VGPR: 13%**

Median Prior therapies: 2 (1-10)

Median f/u: 8 months

Castillo et al, EHA 2018

Ibrutinib and Venetoclax in Treatment Naïve WM

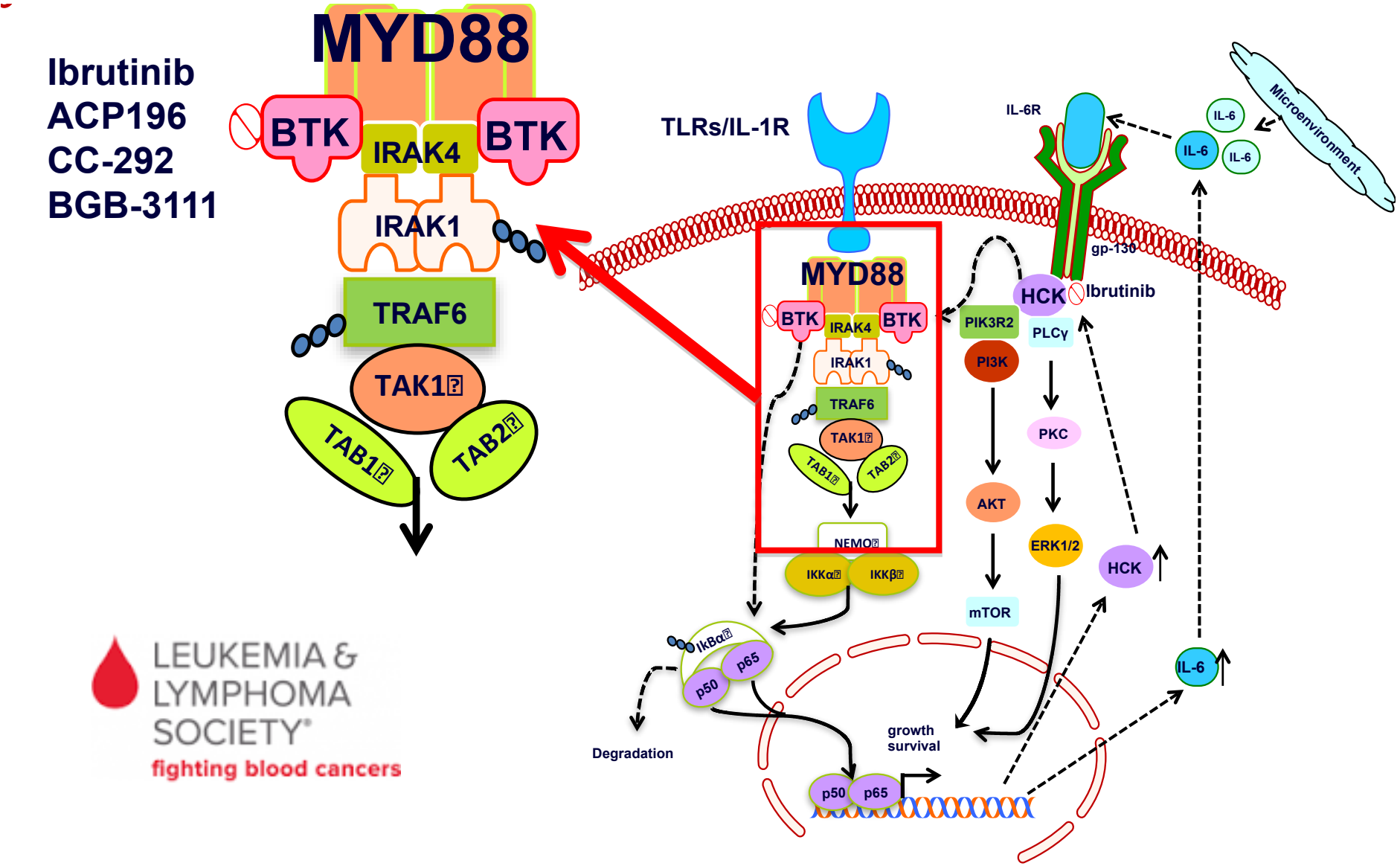


Bedside to Bench

Why are there no Complete Responses to Ibrutinib?

Primary Resistance to Ibrutinib: Role of IRAKs

Signaling Pathways Driven by Mutated MYD88 in Waldenström's Macroglobulinemia



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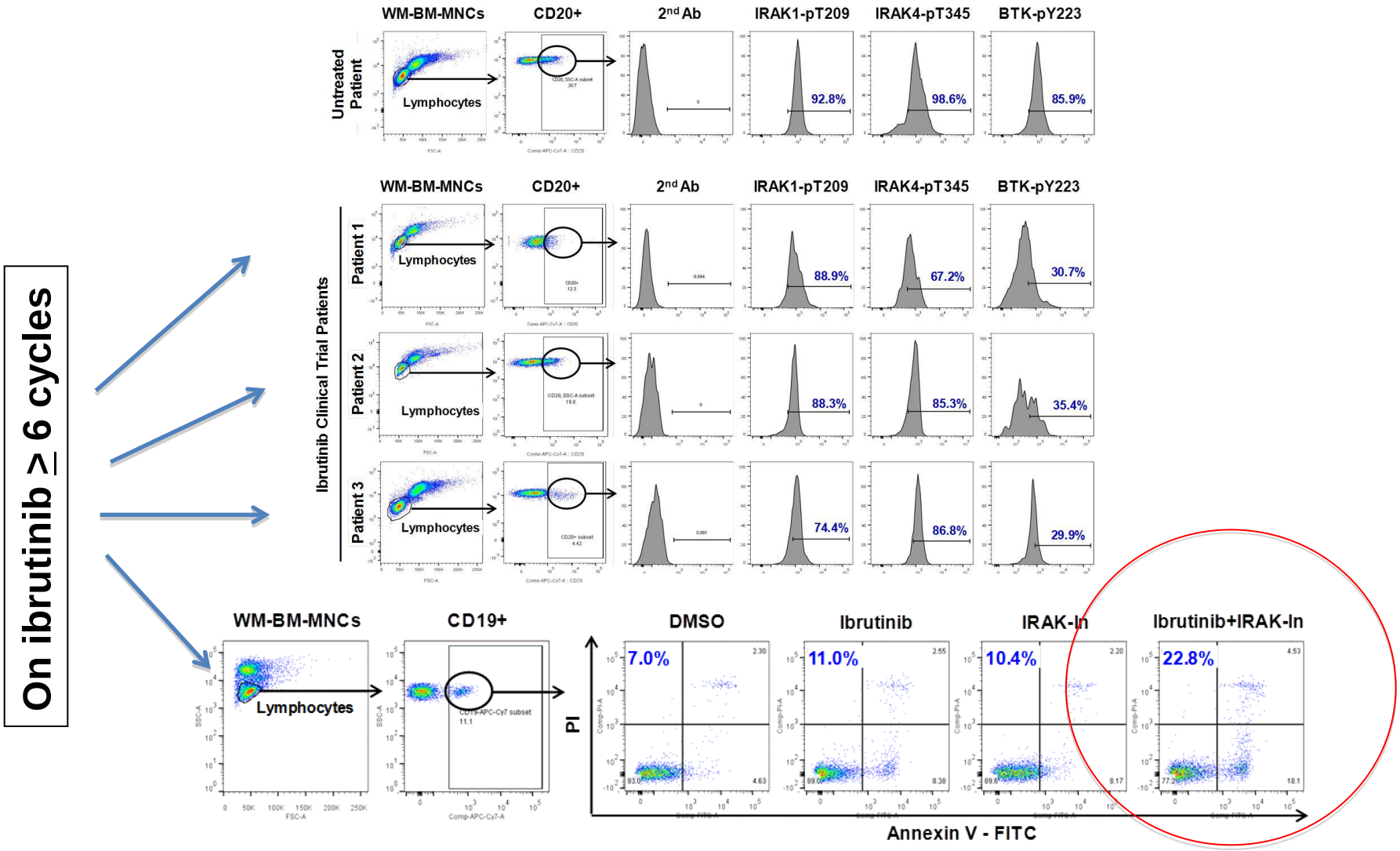
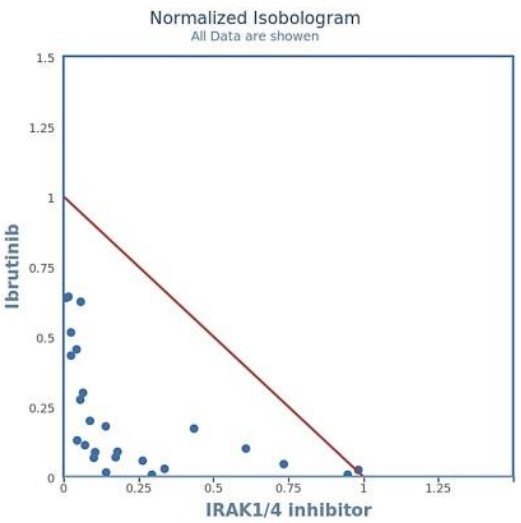
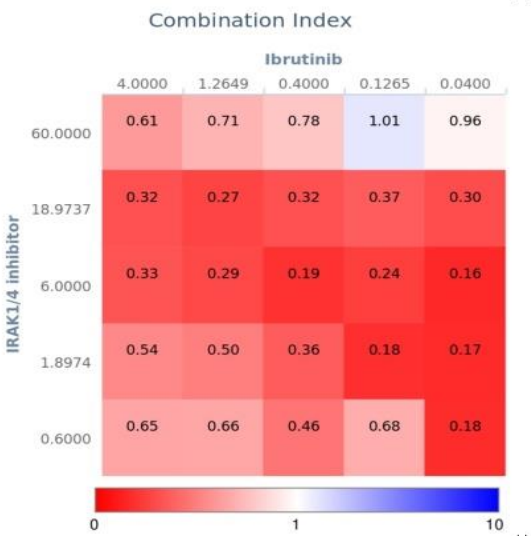
IRAK1/4 kinase survival signaling remains intact in WM cells from ibrutinib treated patients.



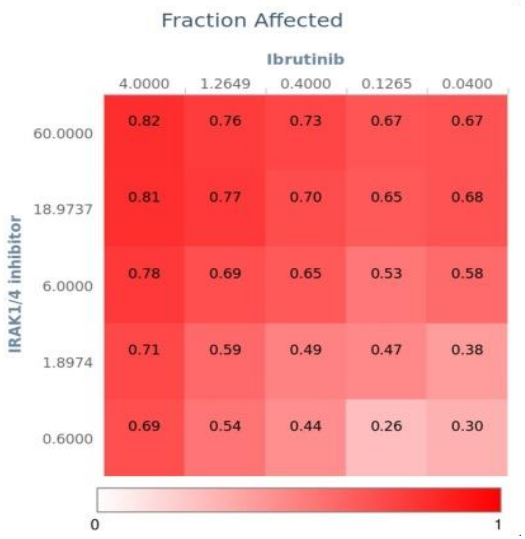
Combination of Ibrutinib and IRAK inhibitors show synergistic NFkB inhibition and WM cell killing (Yang et al, Blood 2013).



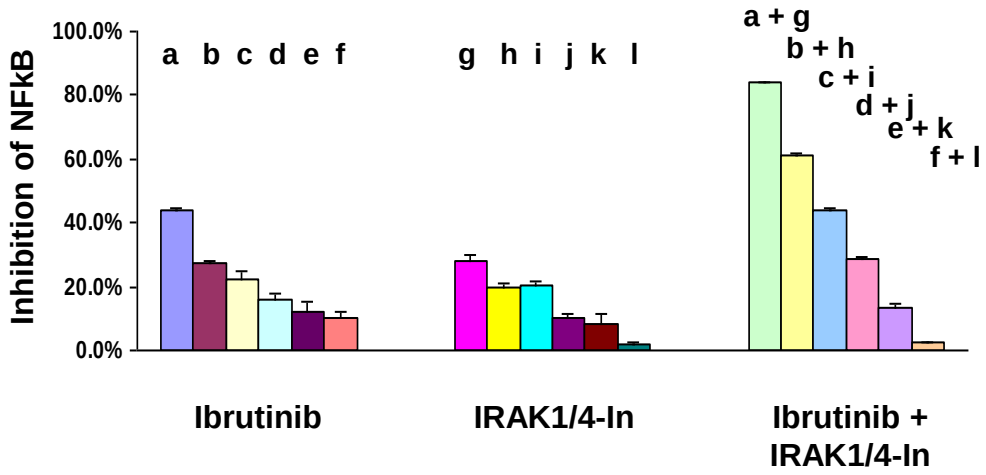
Normalized Isobologram

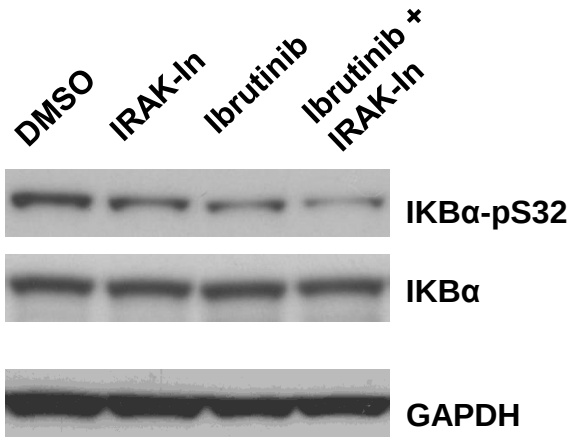


Combination Index

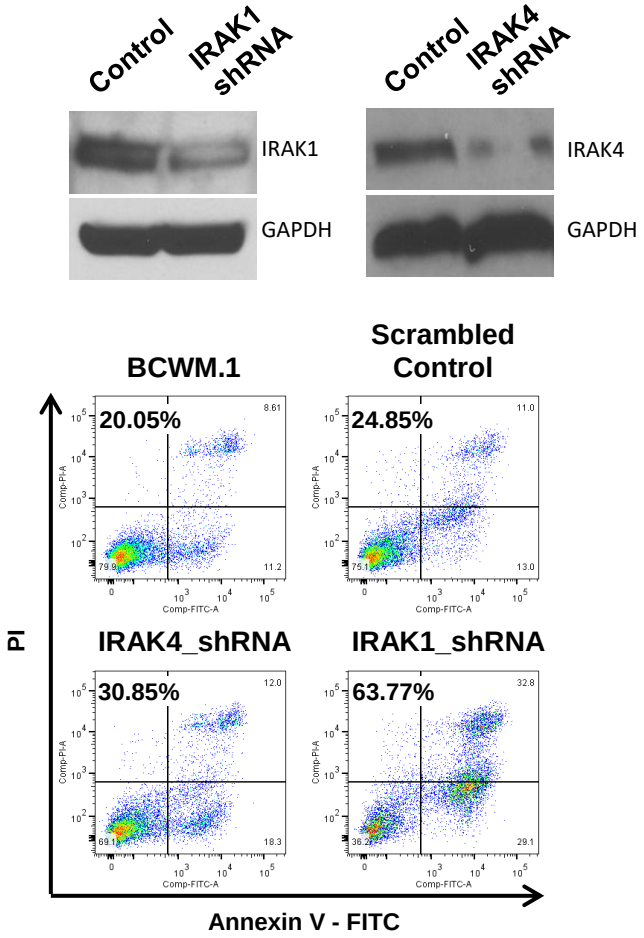


Fraction Affected



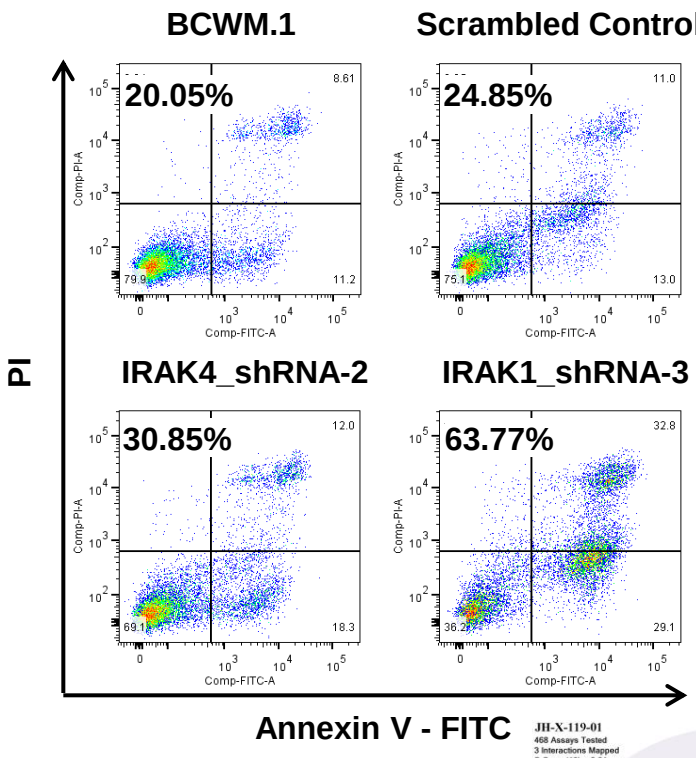


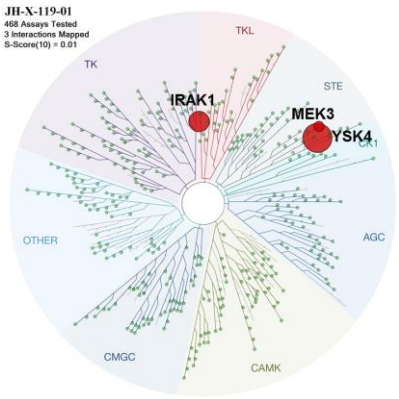
Survival studies following constitutive knockdown of IRAK1 and IRAK4 in MYD88 mutated BCWM.1 WM cells.



Yang et al, ASH 2017

IRAK1 inhibitor JH-X-119 with Ibrutinib Shows Synergistic Killing in MYD88 Mutated Cells





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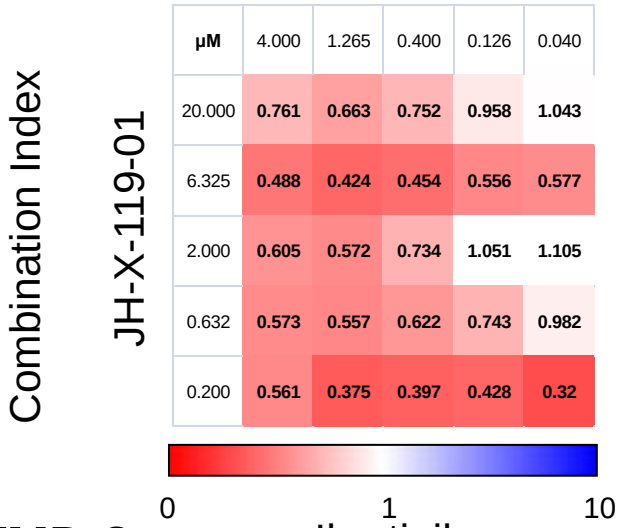
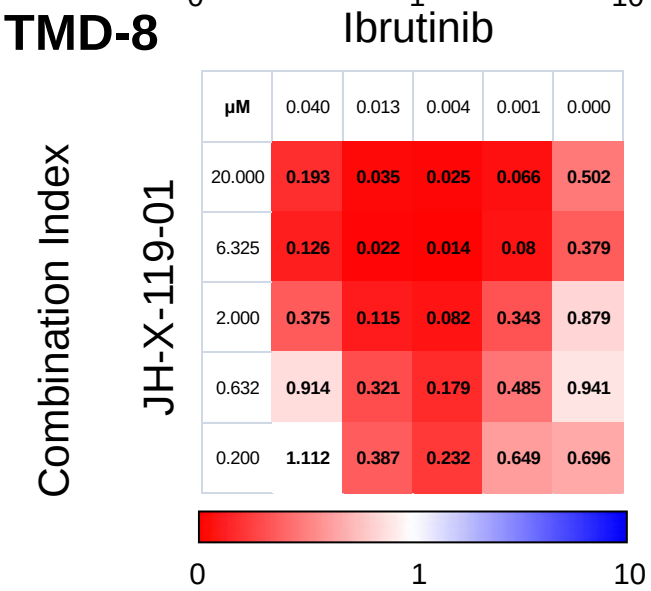
BCWM.1



TMD-8



Novel Approaches
Secondary Resistance to Ibrutinib: Role of BTK

Acquired Resistance in WM Patients on Ibrutinib.

Patient*	L265P positive cells with BTK C481R$^{T>C}$	L265P positive cells with BTK C481S$^{T>A}$	L265P positive cells with BTK C481S$^{G>C}$	L265P positive cells with BTK C481Y$^{G>A}$	L265P positive cells with PLCG2 Y495H$^{T>C}$	L265P positive cells with CARD11 L878F$^{C>T}$
P1	None	None	None	None	None	None
P2	32.4%	6.6%	5.8%	1.0%	None	None
P3	0.3%	34.4%	6.5%	0.3%	None	0.2%
P4	None	None	None	None	None	None
P5	None	None	None	None	None	None
P6	None	None	10.3%	None	11.9%	None

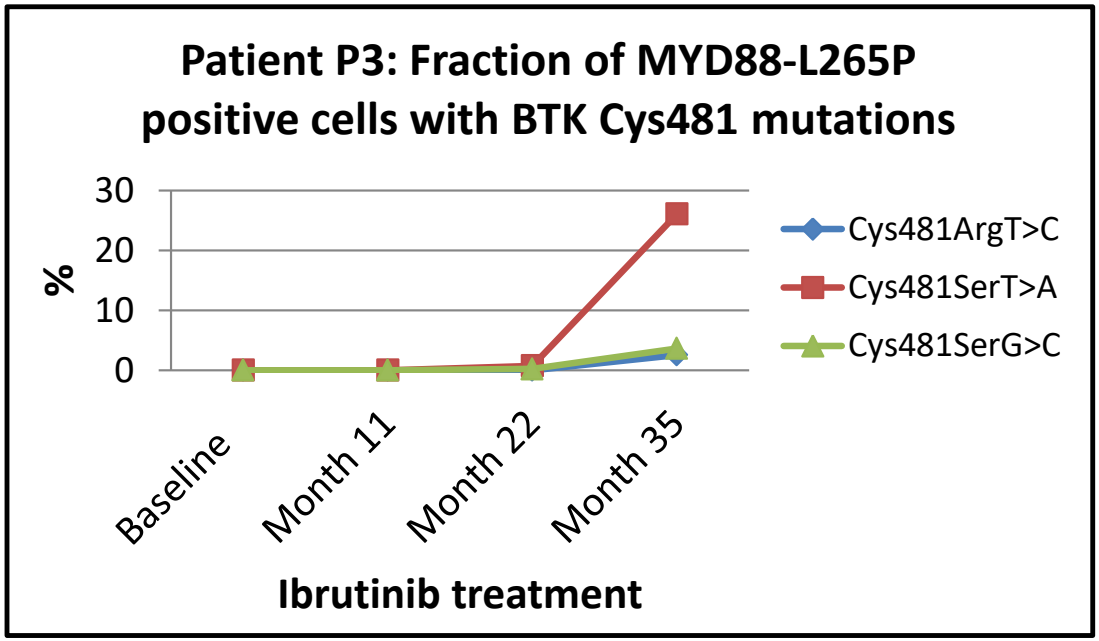
Targeted next-generation sequencing for MYD88, CXCR4, BTK, PLCG2, CARD11, LYN. All patients are MYD88 Mutated.

P2, P3, P6 are CXCR4 WHIM Mutated.

Xu et al, BLOOD 2017

Serial samples from WM Patient P3 with multiple Cys^{481} mutations

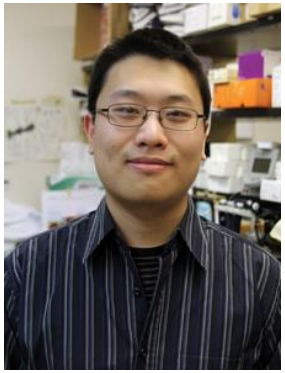
BTK



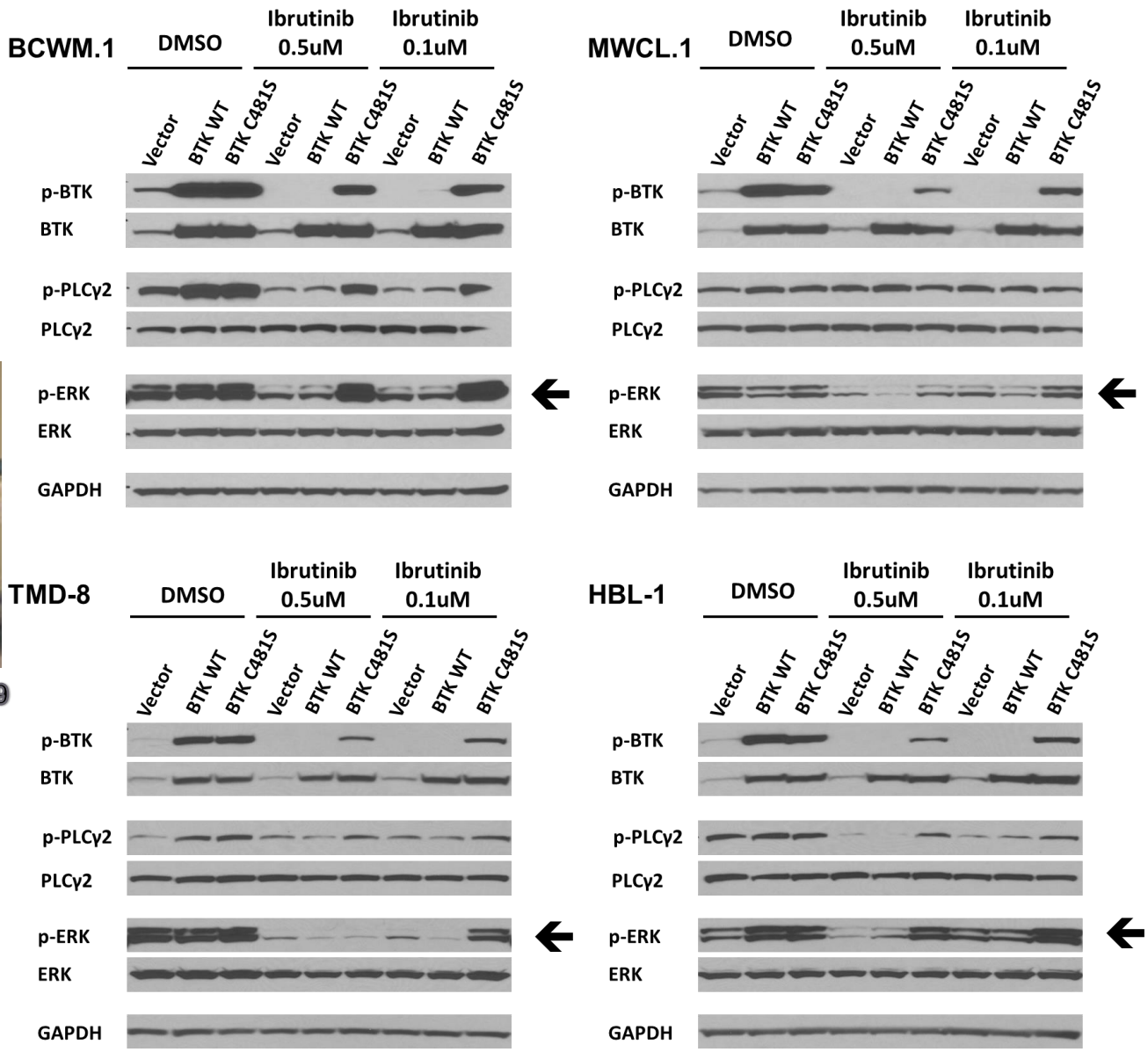
Sampling date	Cys481ArgT>C	Cys481SerT>A	Cys481SerG>C
Baseline	0.00	0.00	0.00
Month 11	0.00	0.00	0.00
Month 22	0.00	0.71%	0.19%
Month 35	2.54%	26.08%	3.62%

Xu et al, BLOOD 2017

BTK C481S expressing cells displayed persistent activation of BTK and ERK1/2 following Ibrutinib treatment.

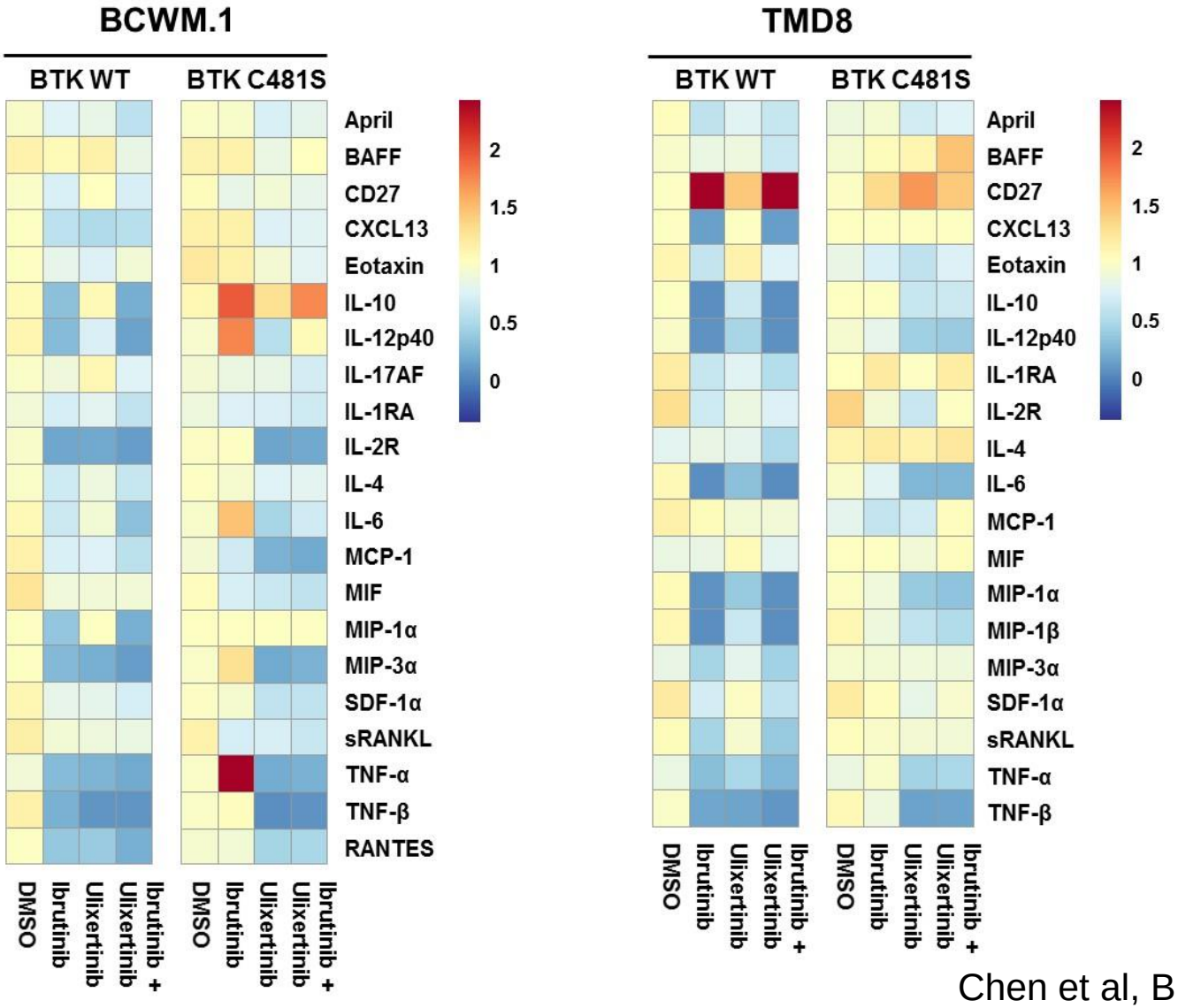


Chen et al, IWWM9



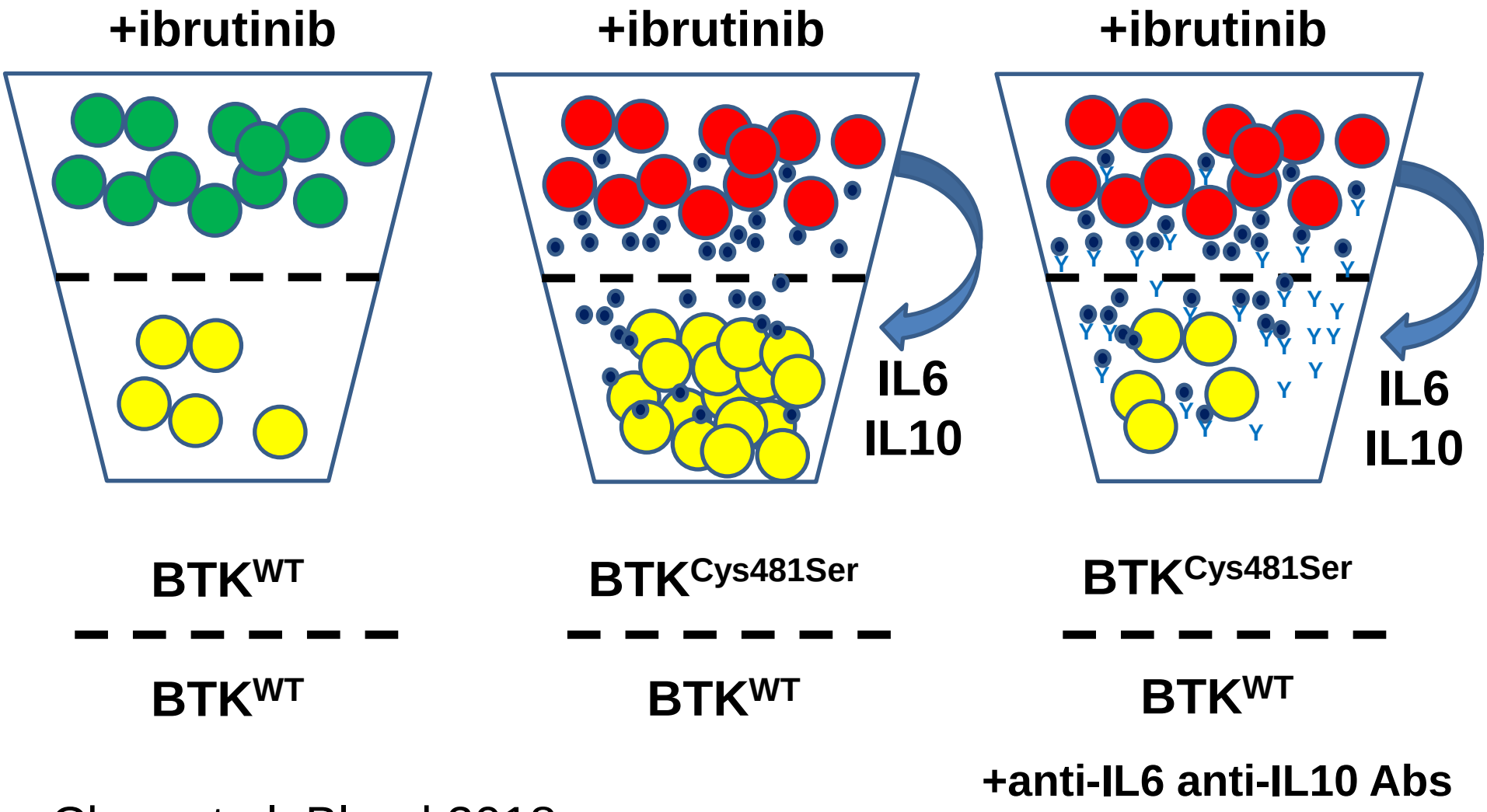
ASH 2016

BTK mutated cells release inflammatory cytokines in the presence of ibrutinib that can be blocked by the ERK-inhibitor ulixertinib



Chen et al, BLOOD 2018

$BTK^{Cys481Ser}$ mutated clones release cytokines that protect BTK^{WT} clones from ibrutinib triggered cytotoxicity



Chen et al, Blood 2018


'Roman Formation' © Jason Juta / Karwansaray Publishers - www.jasonjuta.com
Product: Ancient Warfare Magazine VII.6

blood® commentary

LYMPHOID NEOPLASIA

Comment on Chen et al, page 2047

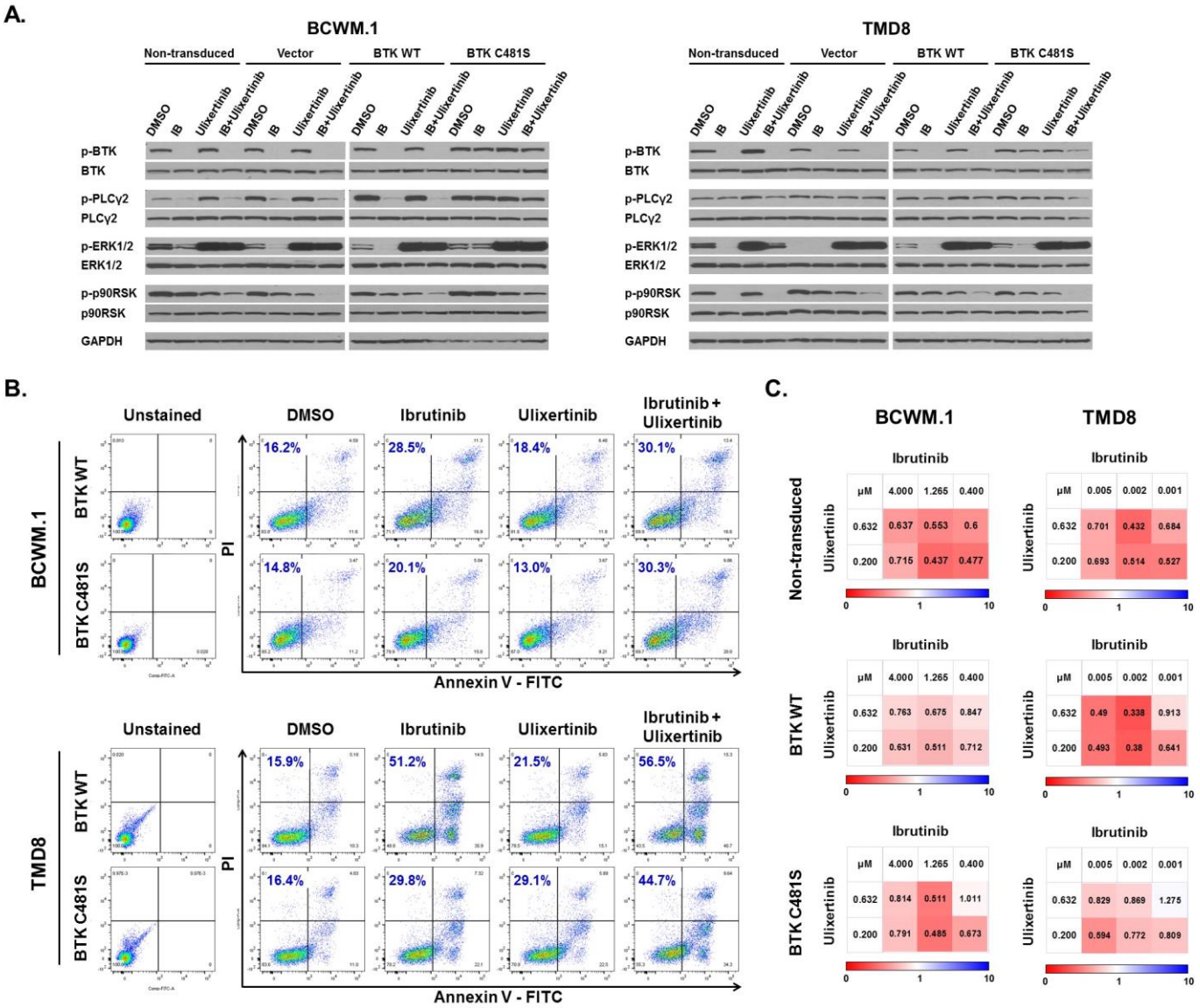
New pieces in the BTKi resistance puzzle

Jan A. Burger | University of Texas MD Anderson Cancer Center

In this issue of *Blood*, Chen et al[1] report about novel mechanisms of ibrutinib resistance related to BTK Cys481 point mutations in Waldenström macroglobulinemia (WM). They transfected WM and diffuse large B-cell lymphoma (DLBCL) cells with vectors containing wild-type (BTK^{WT}) or Cys481Ser mutated ($BTK^{Cys481Ser}$) BTK, and examined effects of the transfected genes on ibrutinib sensitivity and signaling pathways, especially on ERK activation. The authors report that $BTK^{Cys481Ser}$ promotes ibrutinib resistance via reactivation of ERK1/2 signaling (see figure). Next, they examined how WM and DLBCL cells carrying $BTK^{Cys481Ser}$ can confer survival benefit to BTK^{WT} cells, an important question because BTK resistance mutations often only affect a subpopulation of the malignant B cells. A prosurvival effect on WT cells was seen when mixing mutated and WT cells, which apparently did not depend on cell-cell contact, as demonstrated in micropore filter experiments to separate BTK^{WT} from $BTK^{Cys481Ser}$ cells. In this setting, $BTK^{Cys481Ser}$ cells still conferred protection of BTK^{WT} cells in a paracrine fashion, via secretion of cytokines, especially interleukin-6 (IL-6) and IL-10, which were found to be elevated in supernatants from $BTK^{Cys481Se}$ but not from BTK^{WT} cells.

pathway activation that is independent from BTK. In addition, ibrutinib therapy can also promote the expansion of CLL subclones carrying del(8p), with additional driver mutations.[6] Based on a highly sensitive droplet method for detection of single cells with somatic gene mutations,[6] it is apparent that miniscule populations of resistant cells already can be present before therapy initiation, which then become selected and expand, as an example of clonal evolution under therapeutic pressure. Patients with WM generally have durable remissions while on ibrutinib therapy, and, as in CLL, *BTK* C481S mutations emerge in those patients developing resistance. In contrast, development of resistance during therapy is more common in patients with mantle cell lymphoma (MCL), where C481S *BTK* mutations can be associated with resistance, along with additional PI3K-AKT and CDK4 resistance pathway activity.[7] Besides infrequent C481S *BTK* mutations, resistance to ibrutinib in MCL has been shown to arise from adaptive changes in the kinome usage in tumor cells, in particular, enhanced PI3K-AKT signaling.[7,8] In part, adaptive changes appear to be facilitated by integrin β1 signaling and tumor microenvironment interactions.[8]

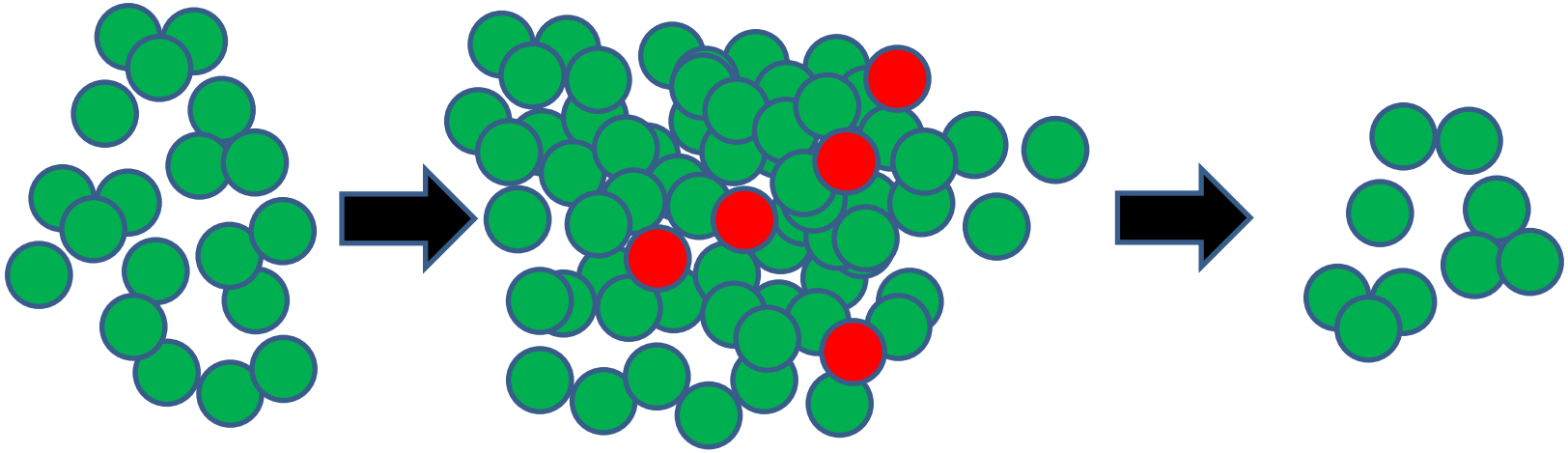
Ulixertinib blocks ERK-downstream signaling and overcomes mutated BTK Cys 481 resistance to ibrutinib.



Chen et al, BLOOD 2018

Novel approach to treating BTK Cys 481 mutated WM

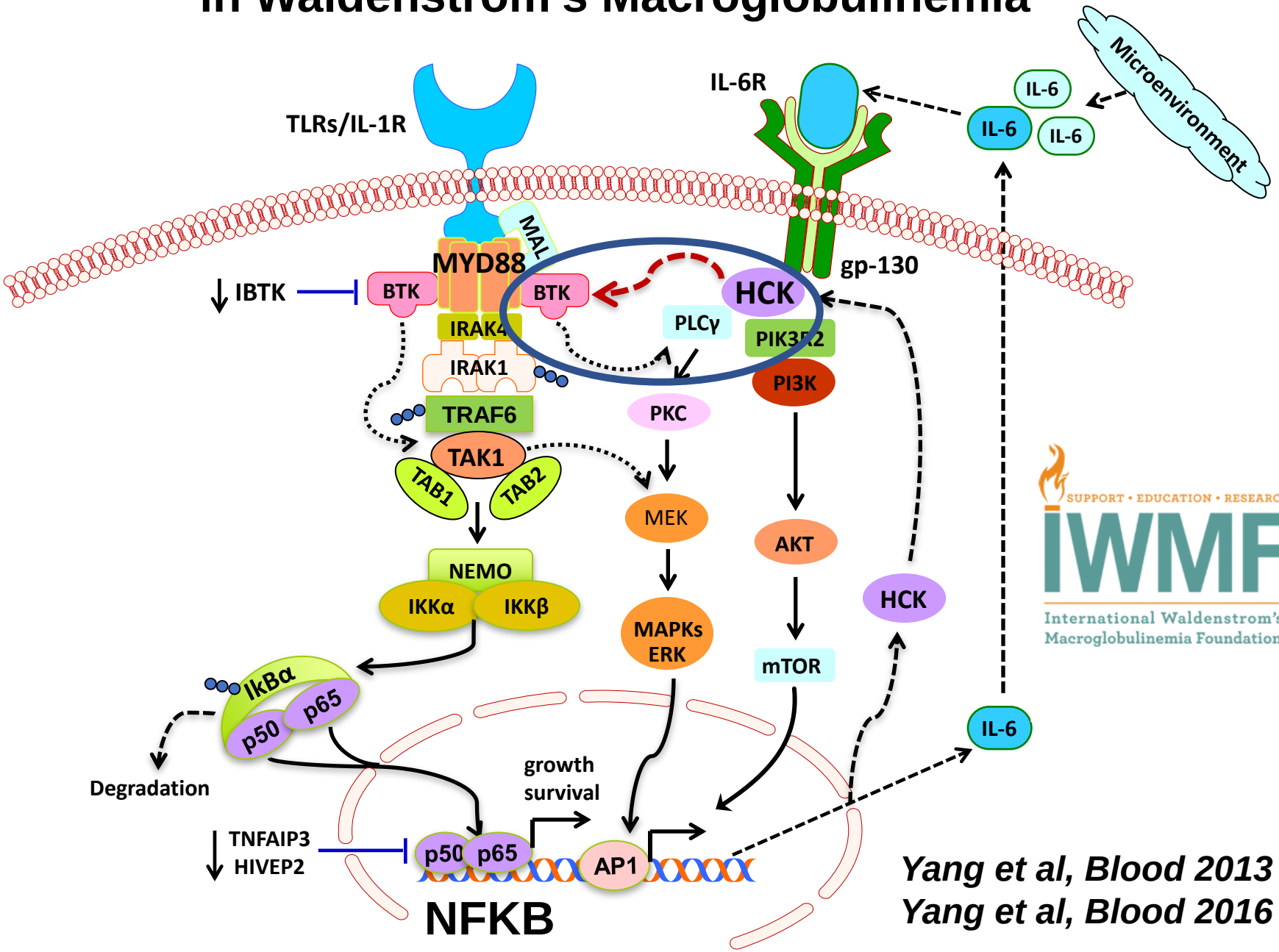
Monitor for BTK Cys481 mutation, when positive add ERK-inhibitor



Ibrutinib

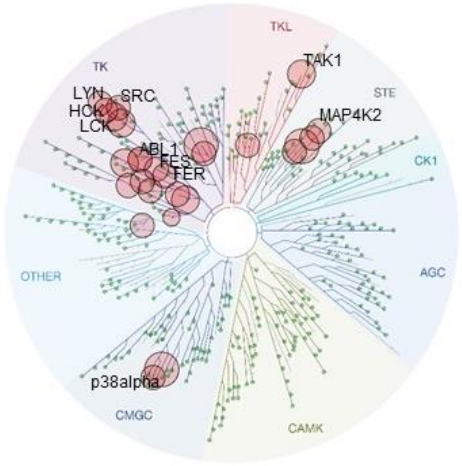
+ ERK-inhibitor

Pro-Survival Signaling Driven by Mutated MYD88 in Waldenström's Macroglobulinemia



Development of a highly potent HCK inhibitor: SB1-G-33

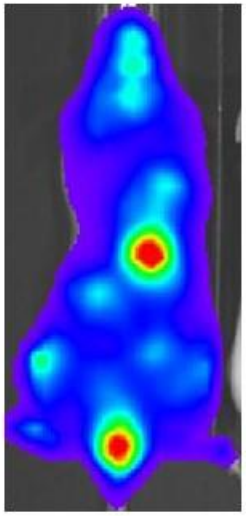
A.



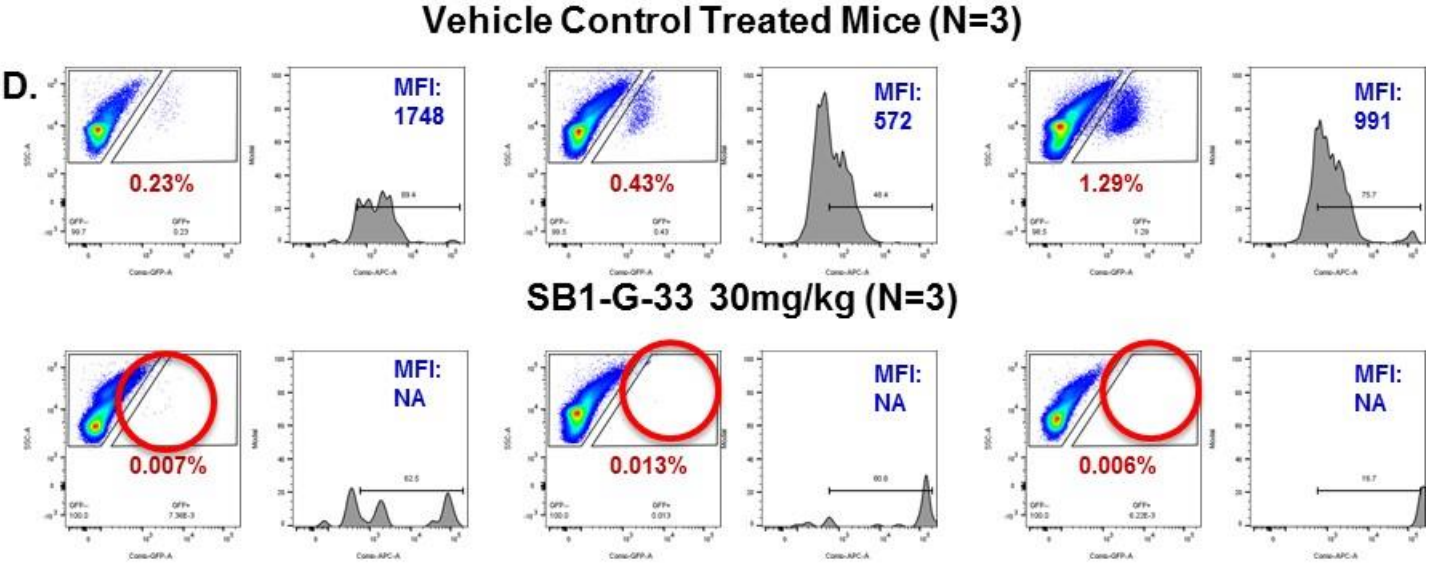
B.

PK Parameters	Average	SD
Cmax (ng/mL)	1054.75	91.96
Tmax (h)	4.56	5.19
AUC (0-48) ng.h/mL	25382.32	2525.20
AUC (0-∞) ng.h/mL	29024.75	2313.63
K (h^{-1})	0.03	0.01
Half-life (h)	22.38	10.38
F (% bioavailability)	78.61	7.82
Cl (mL/h/kg)	678.18	57.42
Vd (mL/kg)	21249.12	7456.06

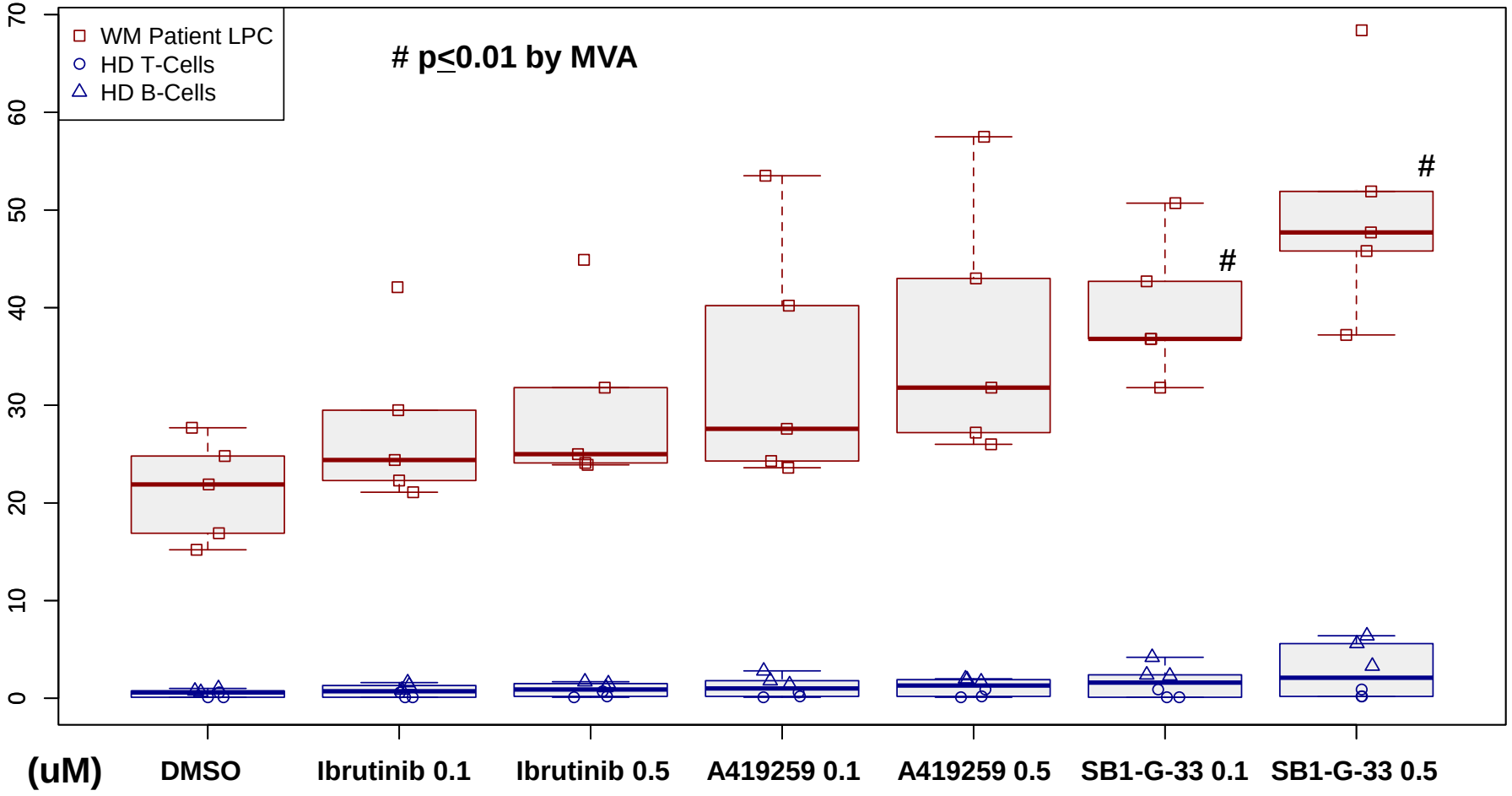
C.



D.



Apoptotic activity for SB1-G-33 in primary symptomatic WM patient and healthy donor cells



WM cells incubated in whole BM microenvironment

The HCK inhibitor SB1-G-33 blocks BTK and overcomes BTK^{C481S} mutated ibrutinib resistance.

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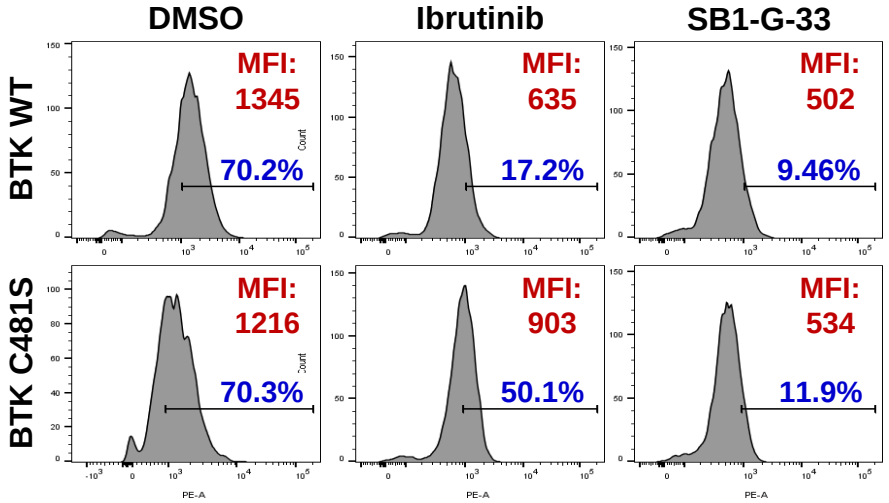

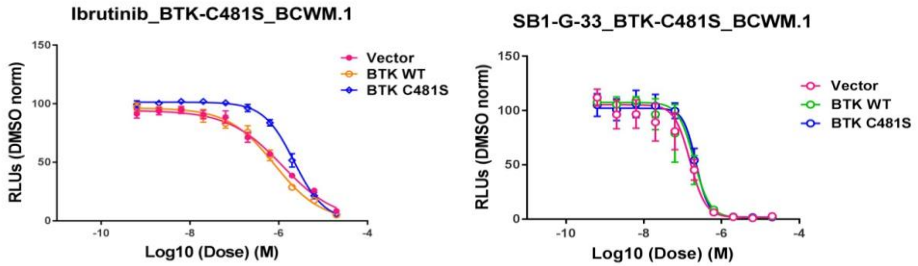

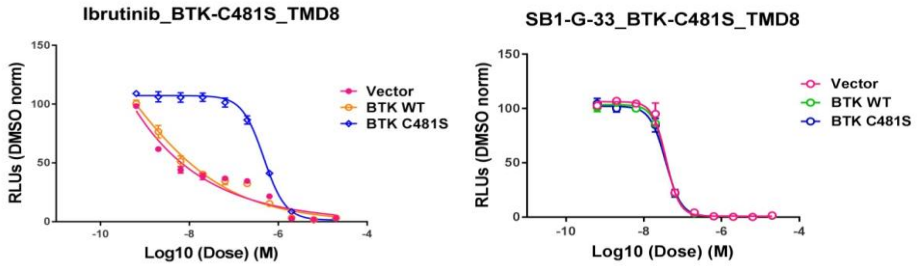

Summary

- MYD88 and CXCR4 mutations are common in WM. MYD88 activates BTK and HCK in WM cells, both targets of ibrutinib.
- Ibrutinib produces high response rates and durable responses in R/R WM. No Complete Responses.
- Mutated CXCR4, aberrant IRAK and BCL2 signaling contribute to ibrutinib resistance.
- BTK mutations are common with acquired ibrutinib resistance, and trigger ERK1/2 survival signaling.
- Novel strategies to overcome intrinsic and acquired resistance include targeting CXCR4, ERK, IRAK and BCL2 signaling.

Bing Center for WM

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WM Clinic Appointments
Jorge Castillo, MD
Toni Dubeau, NP
Patricia Sheehy, NP

WM Clinical Trials

- **Venetoclax**
- **Ibrutinib/Ulucuplomab**
- **Daratumumab (CD38)**
- **Ibrutinib vs. BGB-3111**

WM Workshop/Patient Symposium

5th International Patient and Physician Summit on Waldenstrom's Macroglobulinemia

October 13-14, 2018
NY Marriott Downtown
New York, NY

www.waldenstromsummit.org