Improving Patient Outcomes through Discovery: Waldenström's Macroglobulinemia

VE RI TAS

HARVARD MEDICAL SCHOOL

Steve Treon MD, PhD, FACP, FRCP
Professor of Medicine
Bing Center for Waldenstrom's Macroglobulinemia
Dana Farber Cancer Institute
Harvard Medical School

Disclosures – Steven Treon

Research Support/P.I.	Janssen, Pharmacyclics, BMS, Eli Lilly
Consultant	Janssen, Pharmacyclics, Beigene, BMS

This presentation may contain unregistered products or indications of investigational drugs, please check the drug compendium or consult the company

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.

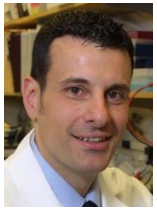
The real nature of myelomatosis.

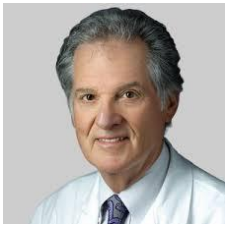
The title of this paper may at first seem somewhat surprising. The myeloma has of old had a reputation as a well defined clinical entity. With the aid of the typical changes on the X-ray film and guided by the examination of the cells from a sternal puncture the diagnosis should therefore be easy and 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.









JW describes WM

Excess Mast Cells in WM

Familial WM

Chlorambucil in WM

IGM MGUS Predisposition

Fludarabine in WM

2CDA in WM

French Coop Study Fludarabine

Rituximab in WM

No diagnostic criteria
No criteria to treat
No treatment guidelines
No response criteria

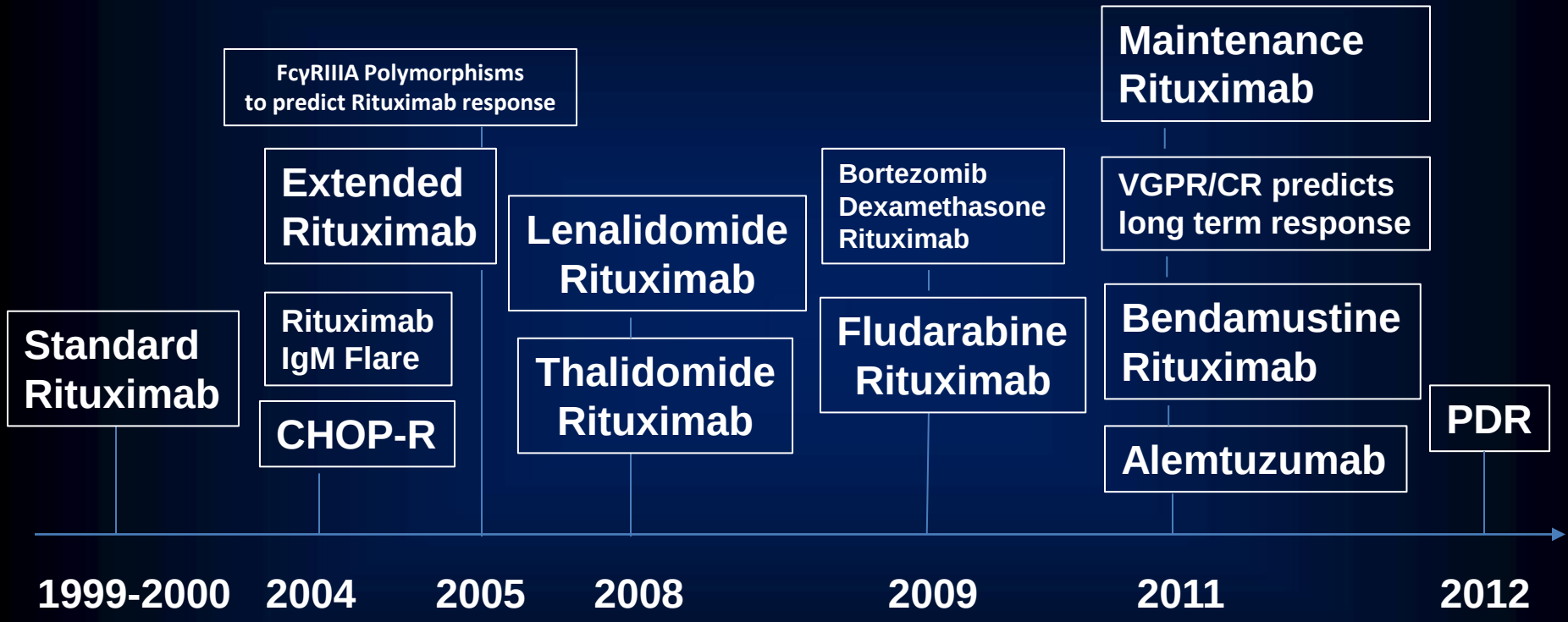
1943 1950 1962 1961 1978 1990 1993 1998 1999 2000

Arnie Smokler

IWMF GRANT TO STUDY MONOCLONAL ANTIBODY THERAPY IN WM-JANUARY 2000

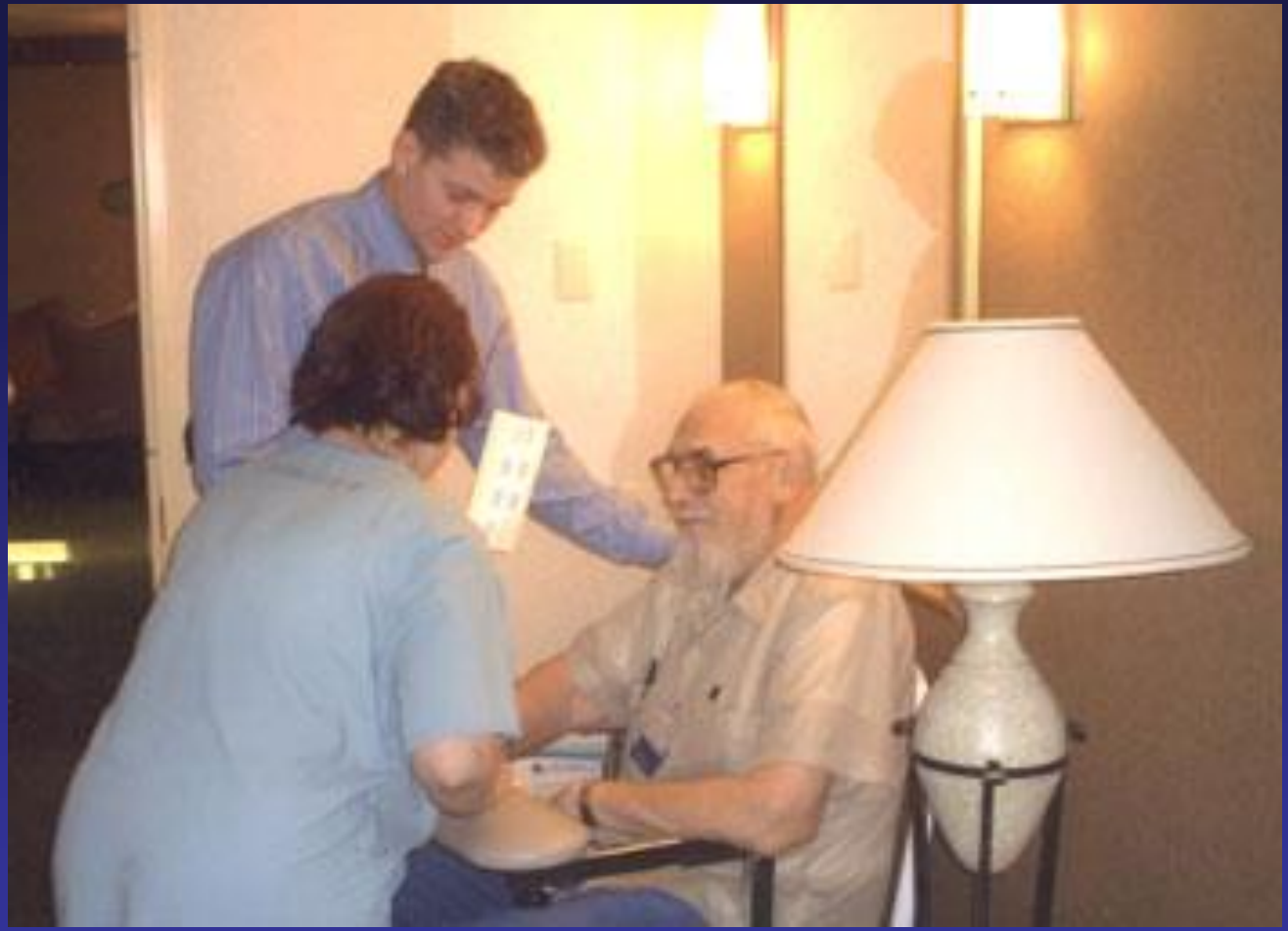
Treon, Steven P., MD, MA, PhD - Dana-Farber Cancer Institute, Boston, MA (January 2000)
TITLE: **TREATMENT OF WALDENSTROM'S MACROGLOBULINEMIA BY ANTIBODY-MEDIATED IMMUNOTHERAPY AND INDUCTION OF TUMOR SELECTIVE ANTIGENS**
[This study was to develop an antibody-mediated immunotherapy for treating WM by identifying novel tumor selective antigens to target WM plasma cells, as well as identifying agents which could be used clinically to induce such plasma cell selective antigens. The study sought to (1) identify how Rituxan, a monoclonal antibody (MoAb) works in WM patients; (2) to develop strategies to overcome the body's resistance to Rituxan; (3) to identify how protein markers on the surface of WM tumor cells block the immune system activity; and (4) to identify new therapies for use in WM. Resistant proteins were located on the WM cells, but they did not interfere with the Rituxan reaction. Other immune mechanisms appeared to be more important in determining the response to Rituxan. During the project another suitable antigen target labeled CD52 was identified on WM cells and an FDA approved monoclonal antibody (Campath-1H) has been shown to have activity against that site].

Monoclonal Antibody Clinical and Translational Studies enabled by IWMF

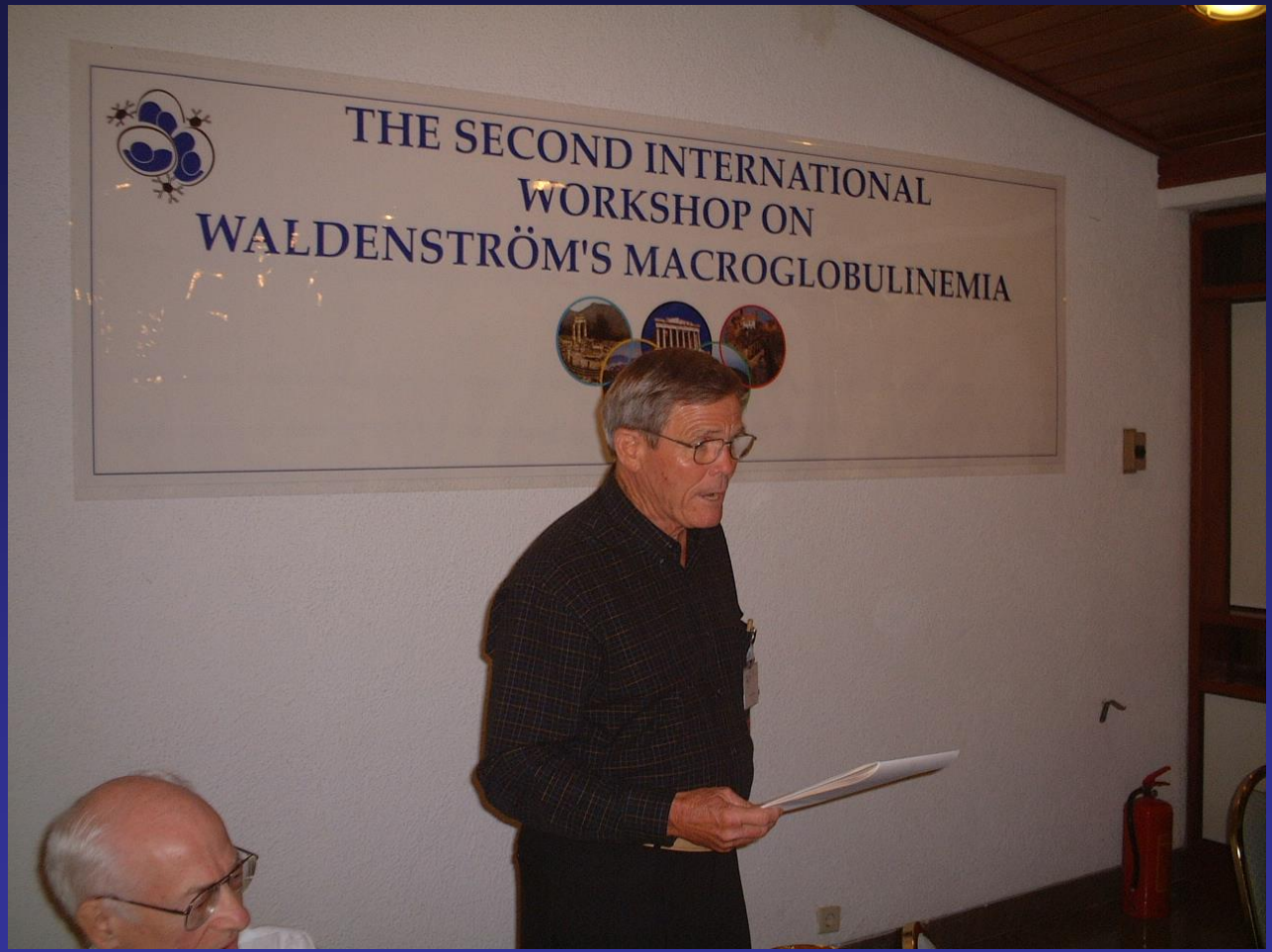


Steve Treon, Principal Investigator

Drs. Touroutoglou and Treon, joined by IWMF President Ben Rude and researchers from the Dana Farber Cancer Institute and Nevada Cancer Center at the WM Research Clinic at Las Vegas IWMF Meeting-2002. Patients attending the annual IWMF conference participated in research at this clinic which enabled identification of a genetic polymorphism that is now used to predict responses to the monoclonal antibody rituximab in patients with WM.



DFCI Clinic at Las Vegas IWMF Meeting-2002



Second International Workshop on WM-Athens, Greece 2002

Closing Ceremony at the Acropolis, Athens, Greece 2002

Second International Workshop on WM

September 2002 Consensus Panels

- Diagnostic Criteria for WM
- Criteria to Initiate Treatment
- Treatment Guidelines
- Response Criteria for WM

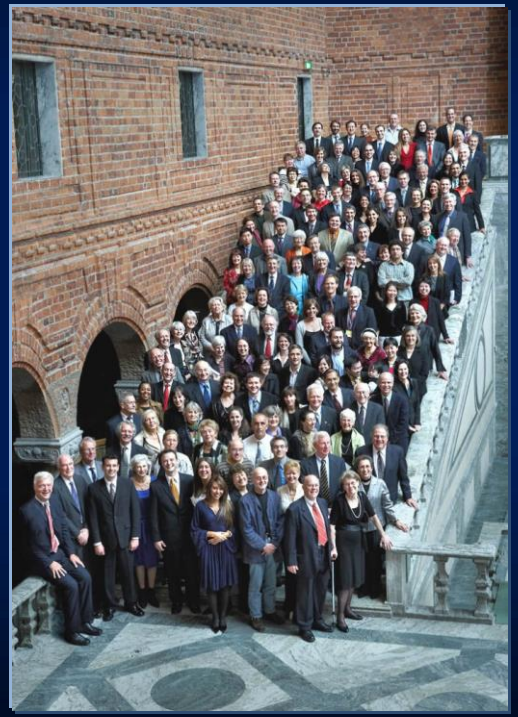
Supported by the IWMF

IWMF
International Waldenstrom's Macroglobulinemia Foundation

Second International Workshop on Waldenstrom's Macroglobulinemia
Paris 2004

Steve Treon with co-chairs Pierre Morel, Jean-Paul Fermand and Veronique LeBlond
at the Opening Ceremonies of the Third International Workshop on WM-Paris, France 2004.

5th International Workshop on Waldenstrom's Macroglobulinemia



Stockholm, Sweden • Nobel Hall, October 2008

Long-term Follow-up of WM Patients Treated With Nucleoside Analogues

- *N = 463 patients with WM*
- *176 pts with WM received either fludarabine or cladribine (2CDA) and were compared with pts treated without a nucleoside analogue or who remained on watch and wait*
- *Incidence of transformation to aggressive lymphoma increased by 7-fold and MDS/AML by 3-fold in pts treated with a nucleoside analogue.*
- *Overall survival for pts who transformed to aggressive lymphoma does not appear to be different and may reflect improvements in therapy (CHOP-R) offered to pts with transformed lymphomas.*

Leleu et al, JCO 2008

CHOP-R vs CVP-R vs. CPR in WM

- Study of outcomes for primarily untreated patients at DFCI.
- Less grade ¾ neutropenia, hospitalizations, neuropathy due to vincristine with CPR.

Response	CHOP-R (n=23)	CVP-R (n=17)	CPR (n=10)
ORR	91%	80%	90%
Major RR (>50%decrease IgM)	70%	53%	80%
CR	4%	0%	0%

Ioakimides et al, CLM 2009

UCLA Summit on WM, Los Angeles 2003

Dr. Treon joined by Mrs. Harriet Fulbright (President, Fulbright Foundation)
and DFCI President Edward Benz at the dedication of Bing Center for WM at DFCI-2005.

Dr. Peter Bing (Trustee and Chair, Stanford University) cuts the ribbon with Dr. Treon at the Dedication of Bing Center for WM at DFCI-2005.

The Bing Center for WM at DFCI- 2006

Discovery of the MYD88 Mutation

Peter Bing MD

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

MYD88 L265P Somatic Mutation in Waldenström's Macroglobulinemia

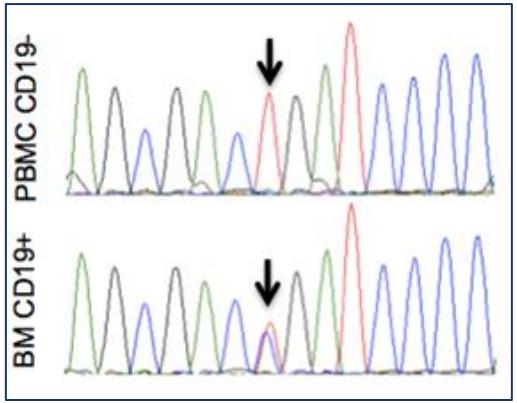
Steven P. Treon, M.D., Ph.D., Lian Xu, M.S., Guang Yang, Ph.D., Yangsheng Zhou, M.D., Ph.D., Xia Liu, M.D., Yang Cao, M.D., Patricia Sheehy, N.P., Robert J. Manning, B.S., Christopher J. Patterson, M.A., Christina Tripsas, M.A., Luca Arcaini, M.D., Geraldine S. Pinkus, M.D., Scott J. Rodig, M.D., Ph.D., 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.

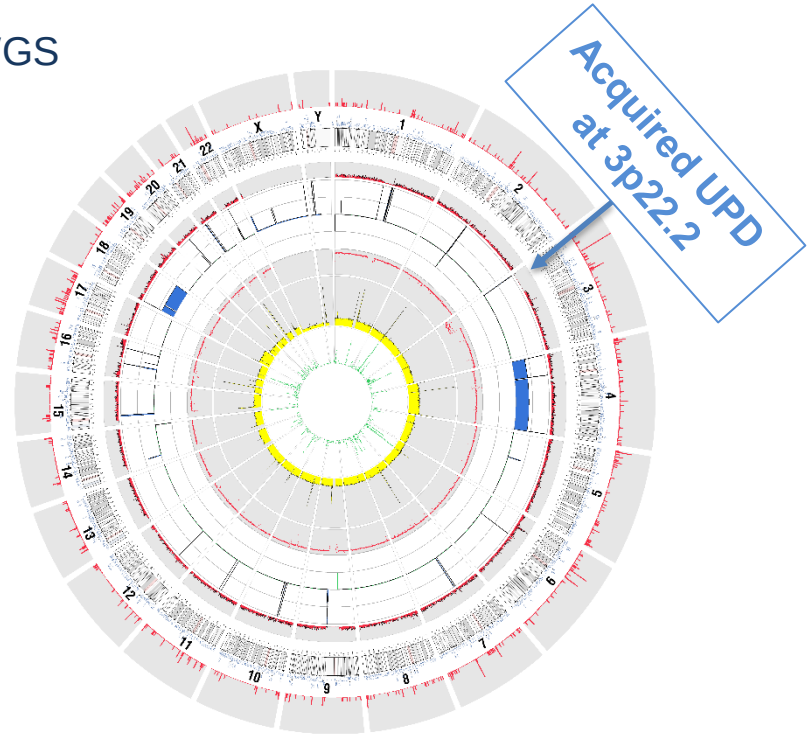
93-97% of WM patients

Treon et al, ASH 2011; NEJM 2012

MYD88 L265P Somatic Mutation in WM

C > G at position 38186241
at 3p22.2 in 91% of WM Patients by WGS

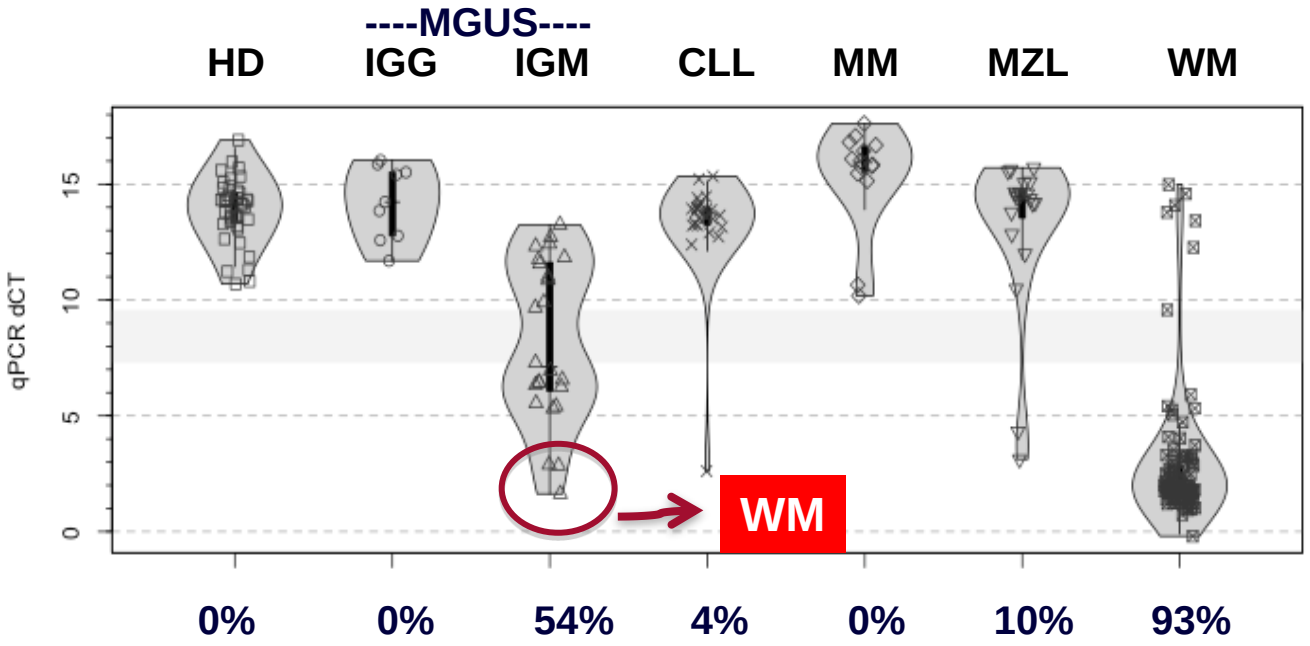




- **MYD88^{L265P} confirmed by AS-PCR in 93-97% WM pts;**
- Usually heterozygous;
- 10% WM patients homozygous due to acquired UPD.
- MYD88 homozygosity increases with time.

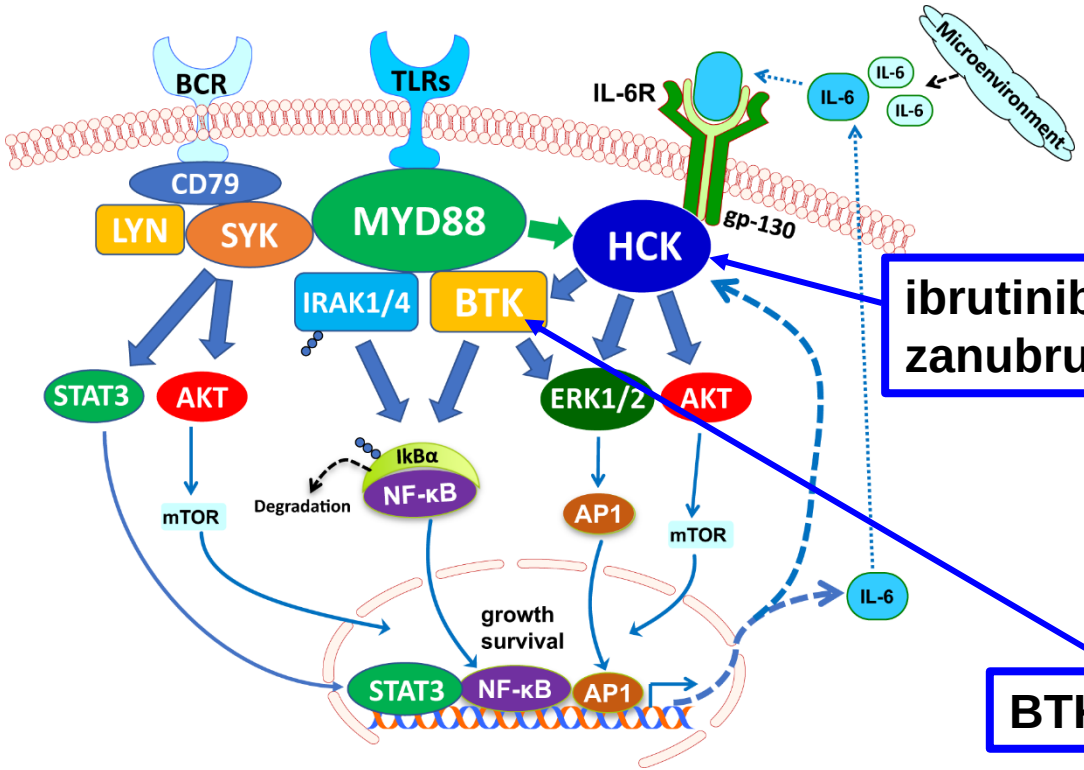
Treon et al, NEJM 367:826, 2012

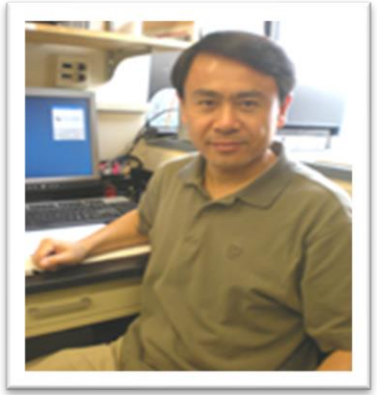
MYD88 L265P by AS-PCR can help distinguish WM from overlapping entities



Xu et al, Blood 2013

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





Yang et al, Blood 2013 122(7):1222-32;
Yang et al, Blood 2016 127(25):3237-52
Munshi and Yang et al, BCJ 2020

95-97% of WM patients have mutations in MYD88

Discovery of CXCR4 mutations in WM -2013-

Plenary Paper

LYMPHOID NEOPLASIA

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

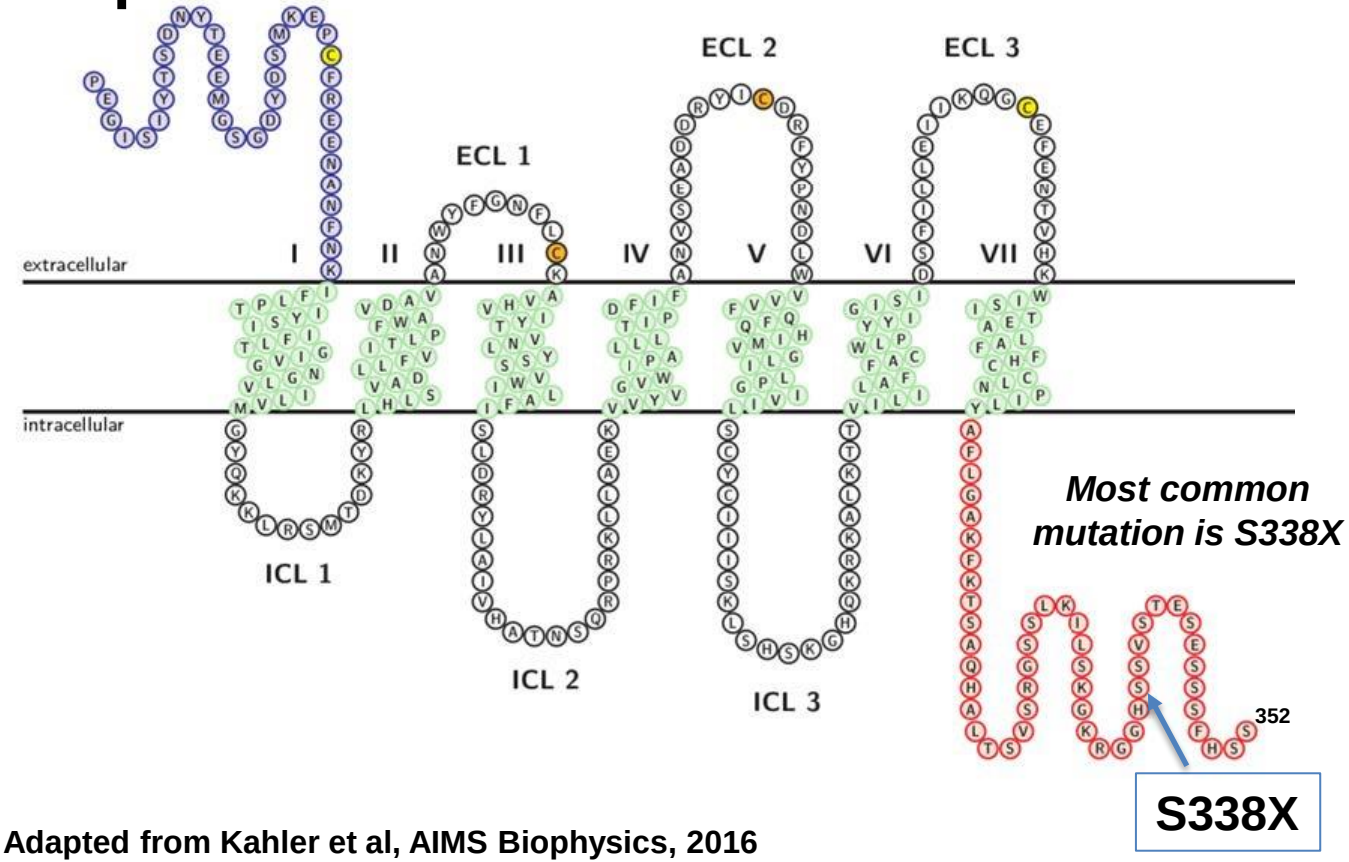
Key Points

- 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)

30-40% of WM patients

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

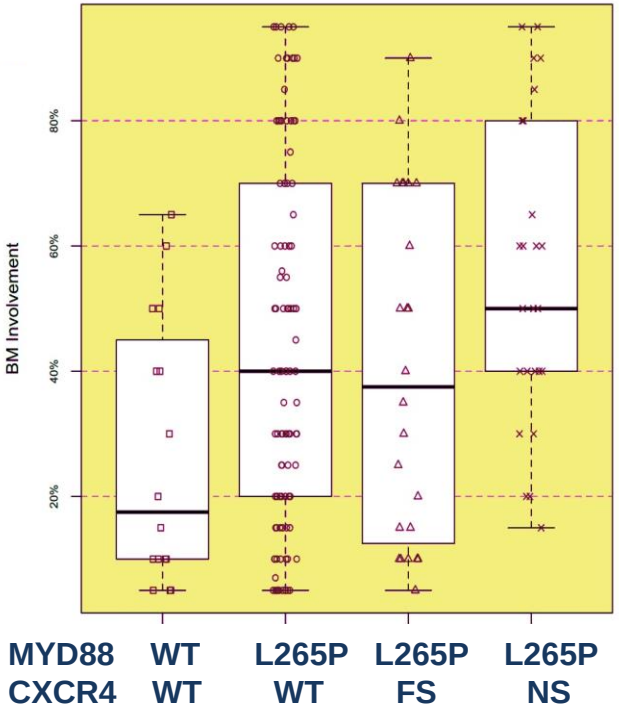
including multiple CXCR4 mutations within individual patients

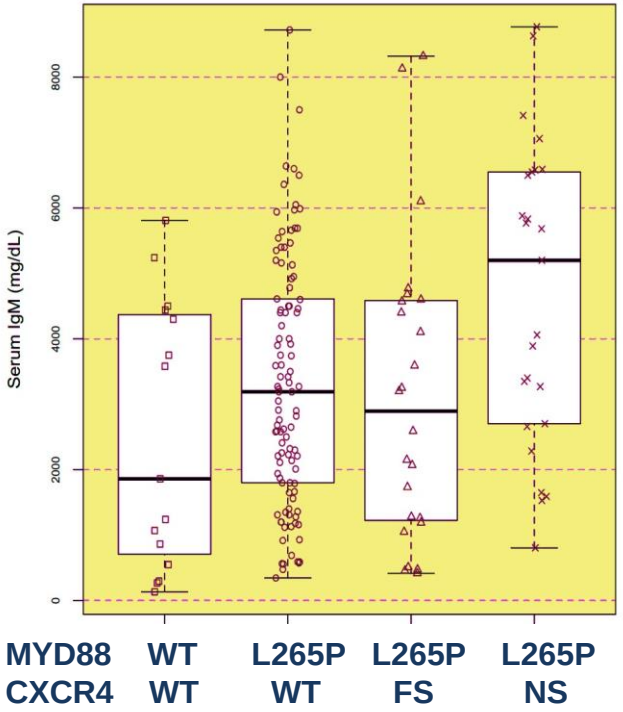
N=	MYD88 Status	CXCR4 Mutation	Nucleotide change	Amino acid change
1	L265P	Nonsense	r.997 A>T[1]	
3	L265P	Nonsense	r.1000C>T	R334X
7	L265P	Nonsense	r.1013C>A	S338X
15	L265P	Nonsense	r.1013C>G[2]	S338X[2]
1	WT	Frameshift	r.931_933insT	
3	L265P	Frameshift	r.952_954insA	T318fs
2	L265P	Frameshift	r.951_953delACCTC	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 Mutation Status Impacts Clinical Presentation of WM Patients

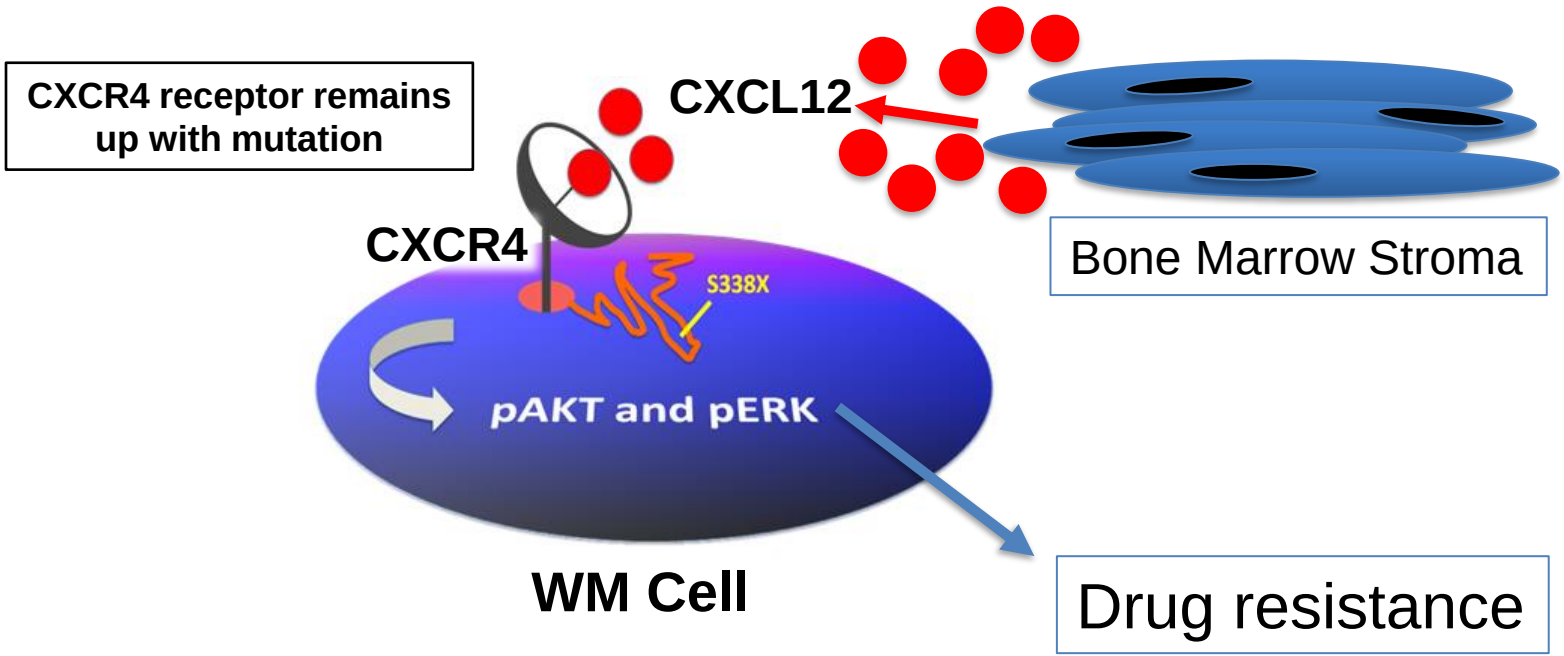




Treon et al, Blood 2014; 123(18):2791-6.

Mutated CXCR4 permits ongoing pro-survival signaling by CXCL12

30-40% of WM patients have mutations in CXCR4



Cao et al, Br J Haematol. 2015 Mar;168(5):701-7; Roccarro et al, Blood. 2014 Jun 26;123(26):4120-31

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

S Treon PI

R Advani

L Palomba

Screening

Registration

420 mg po qD Ibrutinib

Progressive Disease (PD) or Unacceptable Toxicity

Stop Ibrutinib

Event Monitoring

Stable Disease or Response Continue

Event Monitoring

✔ **MYD88, CXCR4 Mutation Status**

ClinicalTrials.gov Identifier: NCT01614821

FIRST BREAKTHROUGH
EVER GRANTED
IN ONCOLOGY-IBRUTINIB IN WM
NOVEMBER 2012

FDA MEETING JUNE 2014
IBRUTINIB
FIRST EVER APPROVAL
OF A DRUG FOR WM

Ibrutinib Activity in Previously Treated WM: Update of the Pivotal Trial (median f/u 59 mos)

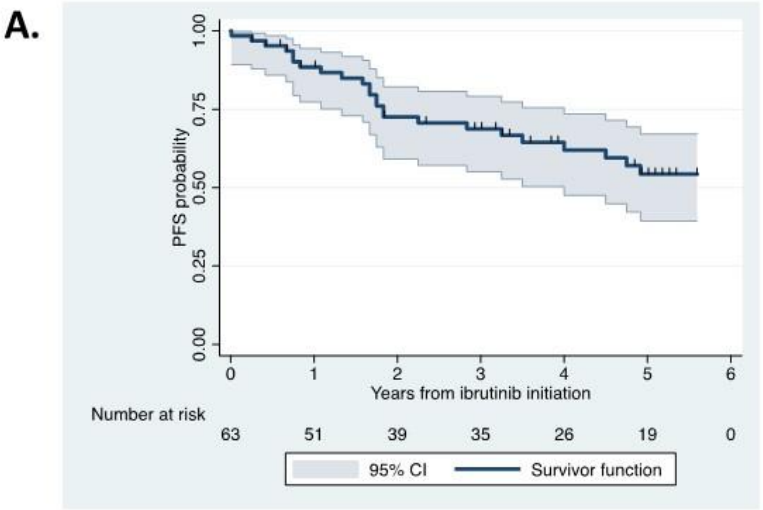
	All Patients	MYD88^{MUT} CXCR4^{WT}	MYD88^{MUT} CXCR4^{MUT}	MYD88^{WT} CXCR4^{WT}	P-value
N=	63	36	22	4	N/A
Overall Response Rate-no. (%)	90.5%	100%	86.4%	50%	<0.01
Major Response Rate-no. (%)	**79.4%**	**97.2%**	**68.2%**	**0%**	**<0.0001**
Categorical responses					
Minor responses-no. (%)	11.1%	2.8%	18.2%	50%	<0.01
Partial responses-no. (%)	49.2%	50%	59.1%	0%	0.03
Very good partial responses-no. (%)	**30.2%**	**47.2%**	**9.1%**	**0%**	**<0.01**
Median time to response (months)					
Minor response (≥Minor response)	0.9	0.9	0.9	0.9	0.38
Major response (≥Partial response)	**1.8**	**1.8**	**4.7**	**N/A**	**0.02**

***One patient had MYD88 mutation, but no CXCR4 determination and had SD.**

Updated from Treon et al, NEJM 2015

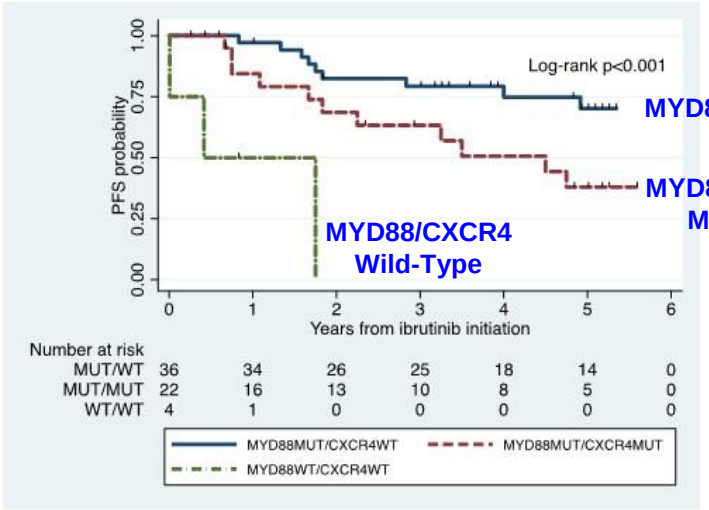
Ibrutinib in Previously Treated WM: Updated PFS

All patients



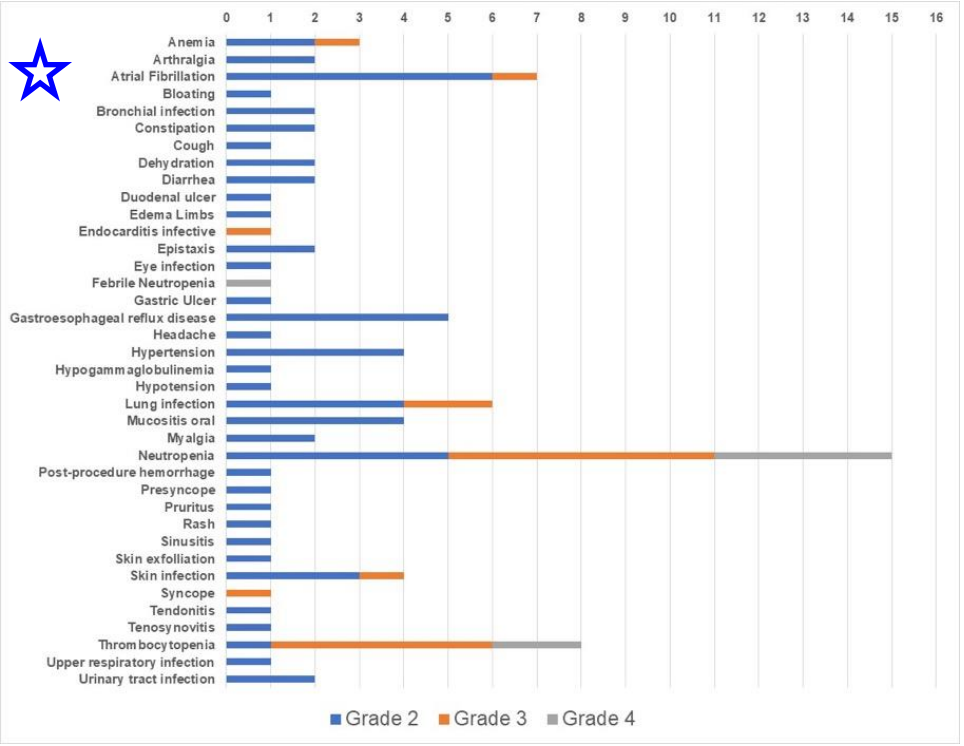
5 year PFS: 54%
5 year OS: 87%

MYD88 and CXCR4 Mutation Status



Updated from Treon et al, NEJM 2015

Long Term Toxicity Findings (grade ≥2)

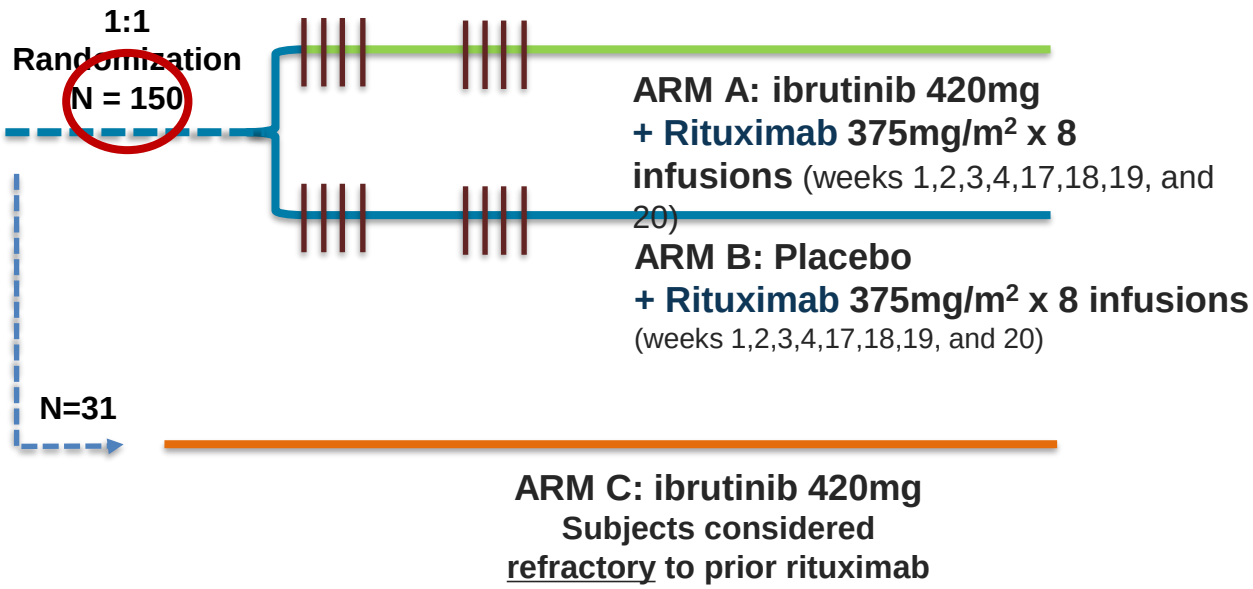


☆ **Increased since original report. 8 patients (12.7%) with Afib, including grade 1. 7 continued ibrutinib with medical management.**

iNNOVATE Study in WM

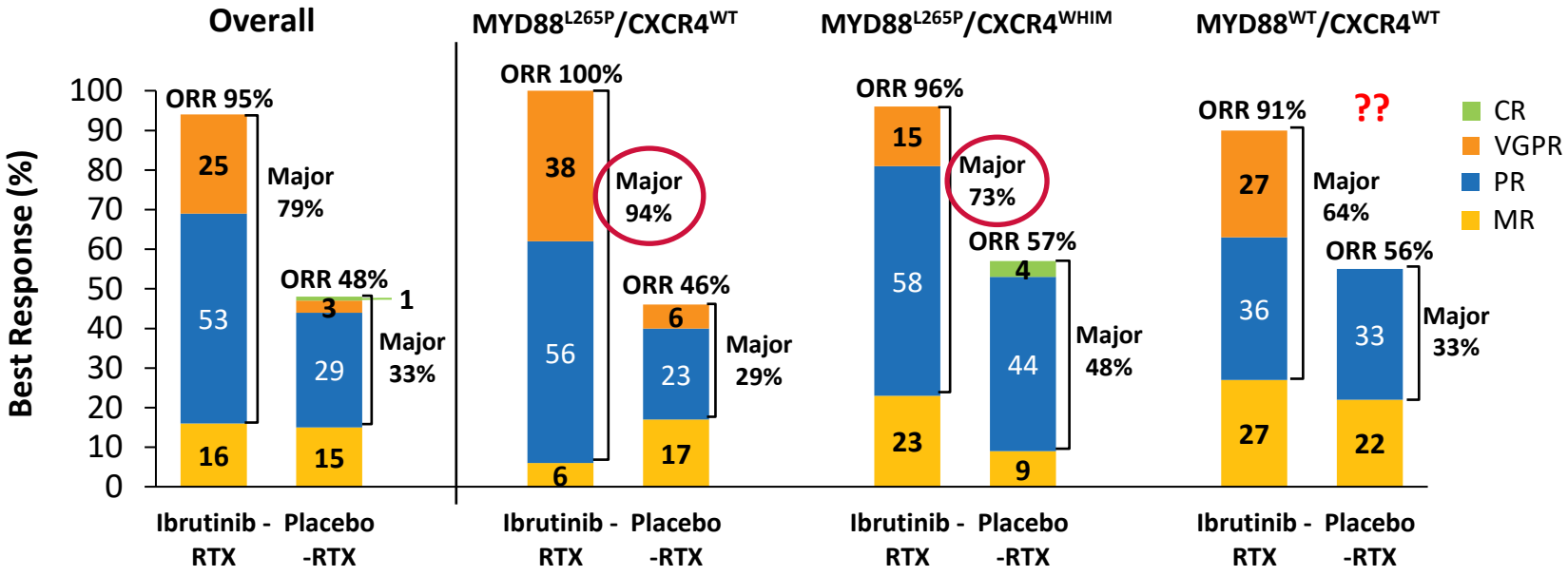
Treatment Naïve + Previously Treated

45 centers in 9 countries



ABC patients genotyped for MYD88 and CXCR4

Responses in Innovate AB Study: Update

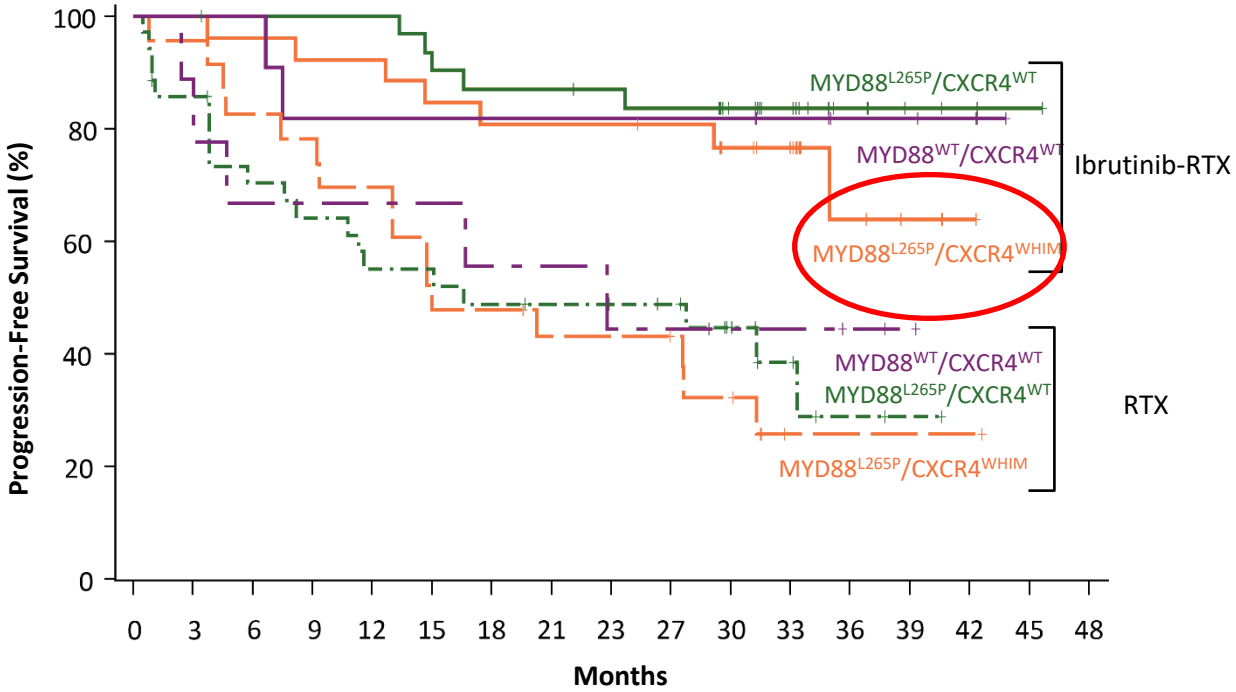


[a]Following modified 6th IWWM Response Criteria (NCCN 2014); required two consecutive assessments.

	Overall		MYD88L265P/CXCR4WT		MYD88L265P/CXCR4WHIM		MYD88WT/CXCR4WT	
	Ibrutinib - RTX	Placebo -RTX	Ibrutinib - RTX	Placebo -RTX	Ibrutinib - RTX	Placebo -RTX	Ibrutinib - RTX	Placebo -RTX
Median time to ≥PR, months (range)	2 (1–28)	6 (2–26)	2 (1–28)	5 (2–17)	3 (1–19)	11 (4–18)	6 (1–17)	6 (5–26)
Median time to ≥MR, months (range)	1 (1–18)	3 (1–24)	1 (1–18)	3 (1–24)	1 (1–11)	3 (1–8)	2 (1-17)	3 (2–17)

Buske et al., ASH 2018; abstract 149 (oral presentation)

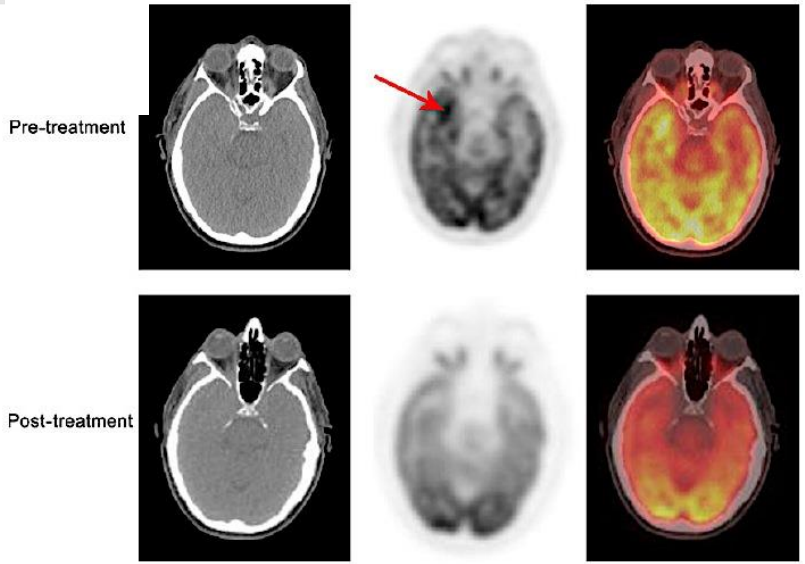
Progression-Free Survival Benefit: Impact of MYD88/CXCR4 Genotype



- **Improved PFS with ibrutinib**
- **36-month PFS rates**
  - **MYD88^{L265P}/CXCR4^{WT}: 84% vs 29%**
  - **MYD88^{L265P}/CXCR4^{WHIM}: 64% vs 26%**
  - **MYD88^{WT}/CXCR4^{WT}: 82% vs 44%**

Buske et al., ASH 2018; abstract 149 (oral presentation)

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



		Ibrutinib (nM)		
Study Day	Time post-dose (h)	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

Acalabrutinib in Treatment Naïve and Previously Treated WM

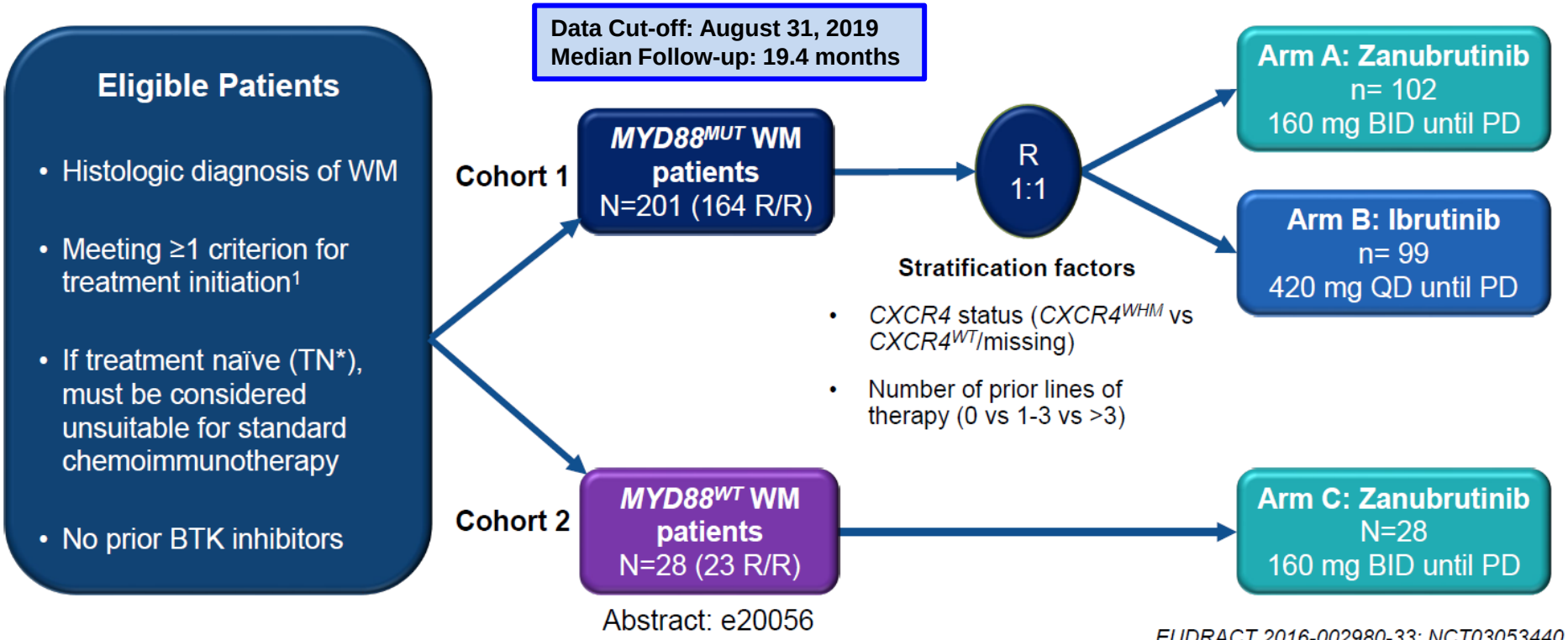
	Grade 1–2	Grade 3	Grade 4
Headache	41 (39%)	0	0
Diarrhoea	33 (31%)	2 (2%)	0
Contusion	31 (29%)	0	0
Dizziness	27 (25%)	0	0
Fatigue	22 (21%)	2 (2%)	0
Nausea	22 (21%)	2 (2%)	0
Upper respiratory tract infection	23 (22%)	0	0
Constipation	22 (21%)	0	0
Arthralgia	20 (19%)	1 (1%)	0
Back pain	18 (17%)	1 (1%)	0
Cough	18 (17%)	0	0
Lower respiratory tract infection	13 (12%)	5 (5%)	0
Neutropenia	1 (1%)	6 (6%)	11 (10%)
Pyrexia	17 (16%)	1 (1%)	0
Vomiting	17 (16%)	1 (1%)	0
Decreased appetite	14 (13%)	2 (2%)	0
Rash	16 (15%)	0	0
Pain in extremity	12 (11%)	1 (1%)	0
Epistaxis	11 (10%)	1 (1%)	0
Sinusitis	12 (11%)	0	0
Skin lesion	12 (11%)	0	0
Dyspepsia	11 (10%)	0	0
Dyspnoea	10 (9%)	1 (1%)	0
Erythema	11 (10%)	0	0
Increased tendency to bruise	11 (10%)	0	0

Afib: 5%

No atrial fibrillation event led to acalabrutinib withholding or discontinuation.

Median follow-up: 27.4 months

ASPEN Study Design: Zanubrutinib vs Ibrutinib in *MYD88*^{MUT} WM



BID, twice daily; BTK, Bruton tyrosine kinase; CXCR4, C-X-C Motif Chemokine Receptor 4; MYD88^{MUT}, myeloid differentiation primary response gene 88 mutant; PD, progressive disease; QD, daily; R, randomization; R/R, relapsed/refractory; TN, treatment naïve; WM, Waldenström Macroglobulinemia; WT, wild-type.
*Up to 20% of the overall population.

1. Dimopoulos MA, et al. *Blood*. 2014;124:1404-1411.

Tam et al, ASCO 2020

ASPEN: AE Categories of Interest (BTKi Class AEs)

AE *Categories*, n (%) (pooled terms)	All Grades		Grade ≥ 3	
	Ibrutinib (n = 98)	Zanubrutinib (n = 101)	Ibrutinib (n = 98)	Zanubrutinib (n = 101)
Atrial fibrillation/ flutter†	**15 (15.3)**	2 (2.0)	4 (4.1)	0 (0.0)
Diarrhea (PT)	**31 (31.6)**	21 (20.8)	1 (1.0)	3 (3.0)
Hemorrhage	**58 (59.2)**	49 (48.5)	8 (8.2)	6 (5.9)
Major hemorrhage[a]	9 (9.2)	6 (5.9)	8 (8.2)	6 (5.9)
Hypertension	17 (17.3)	11 (10.9)	**12 (12.2)**	6 (5.9)
Neutropenia[b]†	13 (13.3)	**30 (29.7)**	8 (8.2)	**20 (19.8)**
Infection	66 (67.3)	67 (66.3)	19 (19.4)	18 (17.8)
Second Malignancy	11 (11.2)	12 (11.9)	1 (1.0)	2 (2.0)

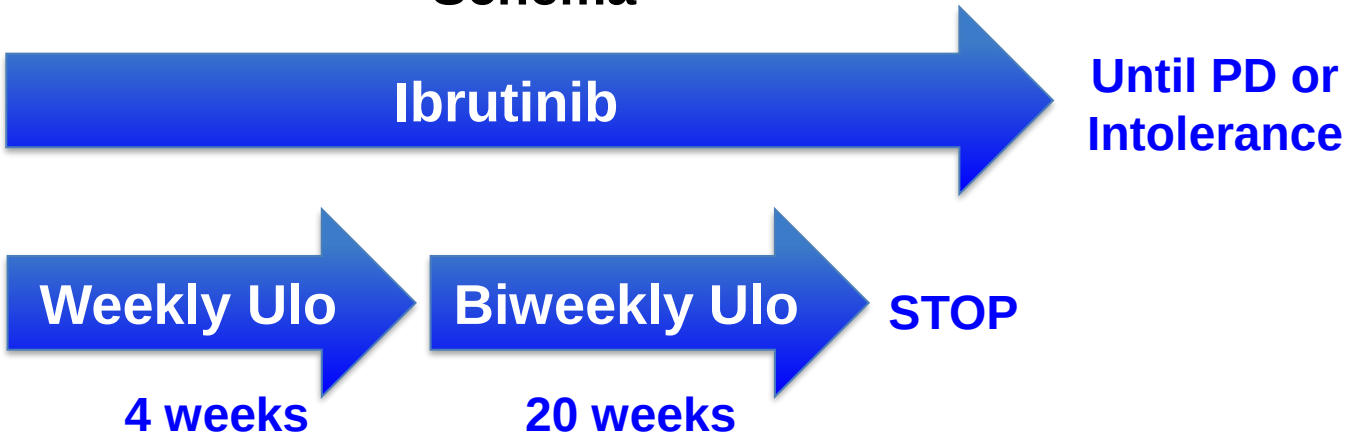
Higher AE rate in bold blue with ≥ 10% difference in any grade or ≥ 5% difference in grade 3 or above.
No tumor lysis syndrome was reported. Opportunistic infection ibrutinib (n=2), zanubrutinib (n=1).
AE, adverse event; BTKi, Bruton tyrosine kinase inhibitor; PT, preferred term.
[a]Defined as any grade ≥ 3 hemorrhage or any grade central nervous system hemorrhage.
[b]Including PT terms of neutropenia, neutrophil count decreased, febrile neutropenia, agranulocytosis, neutropenic infection and neutropenic sepsis.
† Descriptive two-sided *P*-value < 0.05.

Tam et al, ASCO 2020

Strategies to Enhance BTK Inhibitors

Phase I/II Trial of Ulocuplumab and Ibrutinib in CXCR4 mutated patients with symptomatic WM

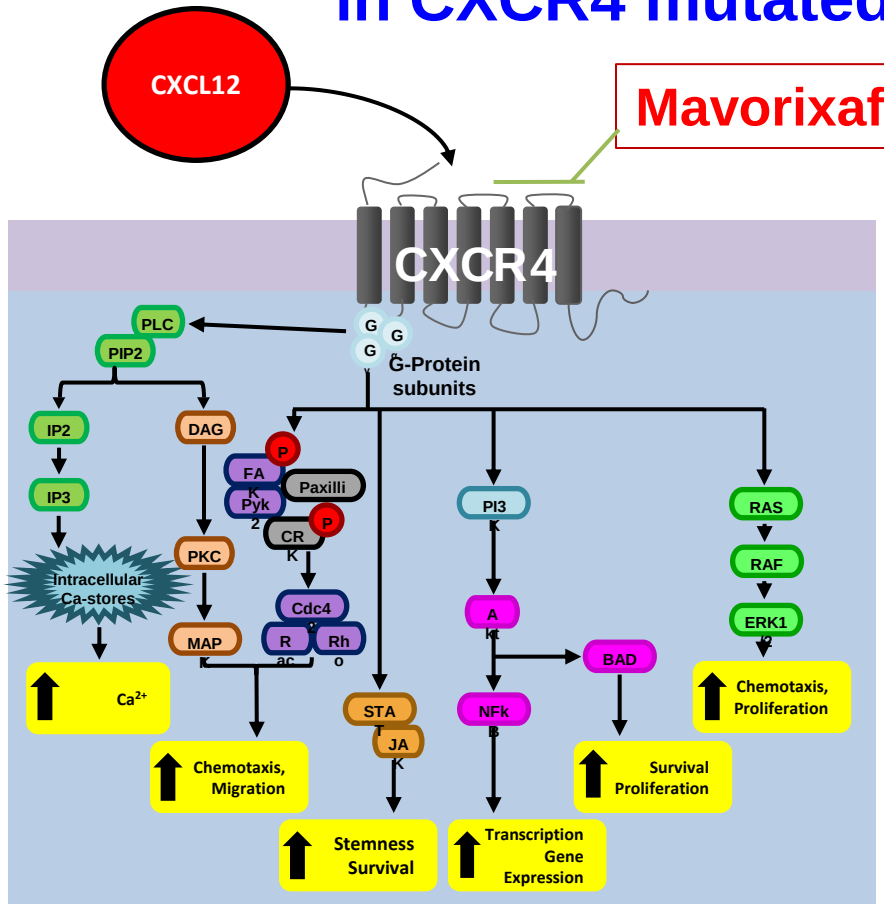
Schema

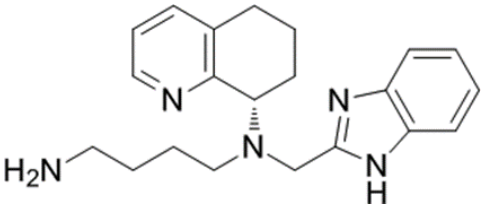


Dose Level	Ibrutinib	Ulocuplumab Cycle 1	Ulocuplumab Cycles 2-6
Level 1 –Starting dose	420mg PO DQ	400 mg weekly	800 mg every other week
Level 2	420mg PO DQ	800 mg weekly	1200 mg every other week
Level 3	420mg PO DQ	800 mg weekly	1600 mg every other week

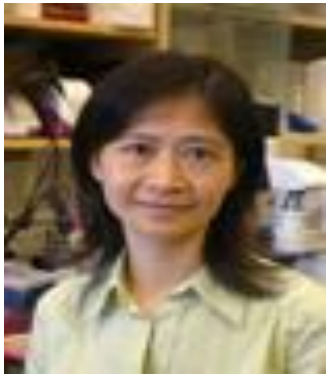
ClinicalTrials.gov Identifier: NCT03225716

Mavorixafor in combination with ibrutinib in CXCR4 mutated WM



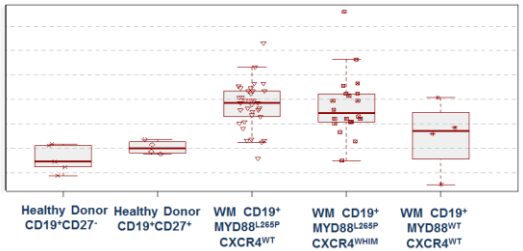


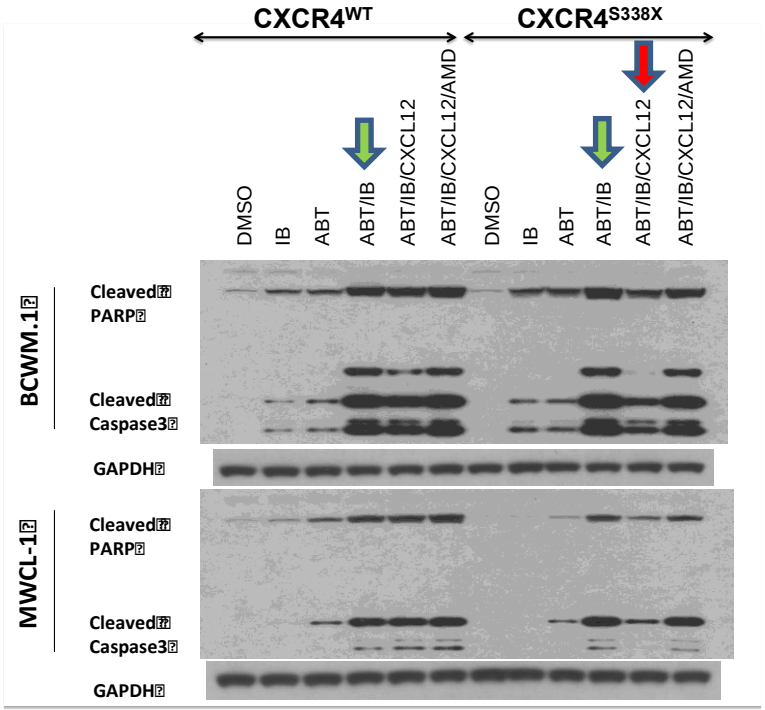
- **Non-competitive, allosteric, small molecule antagonist of CXCR4**
- **Orally Bioavailable; mean $t_{1/2}$ of ~23 hours**
- **High volume of distribution**



Venetoclax (ABT-199) augments ibrutinib induced apoptosis

Higher BCL2 levels in MYD88 mutated WM



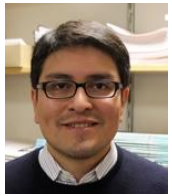


Cao et al, BJH 2015

Ibrutinib and Venetoclax in Treatment Naïve WM

24 months

Ibrutinib 420 mg/day x 4 weeks	Ibrutinib 420 mg/day Add Venetoclax 100 mg/day week 5 200 mg/day week 6 400 mg/day weeks 7,8	Ibrutinib 420 mg/day And Venetoclax 400 mg/day	Observation
4 weeks	**4 weeks**	**22 months**	**Follow to PD or off study**



Jorge Castillo, PI (DFCI)

Personal communication

Resistance Mechanisms to Ibrutinib

Regular Article

blood

LYMPHOID NEOPLASIA

Acquired mutations associated with ibrutinib resistance in Waldenström macroglobulinemia

Lian Xu,[1] Nicholas Tsakmaklis,[1] Guang Yang,[1,2] Jiaji G. Chen,[1] Xia Liu,[1] Maria Demos,[1] Amanda Kofides,[1] Christopher J. Patterson,[1] Kirsten Meid,[1] Joshua Gustine,[1] Toni Dubeau,[1] M. Lia Palomba,[3] Ranjana Advani,[4] Jorge J. Castillo,[1,2] Richard R. Furman,[5] Zachary R. Hunter,[1,2] and Steven P. Treon[1,2]

[1]Bing Center for Waldenström's Macroglobulinemia, Dana-Farber Cancer Institute, and [2]Department of Medicine, Harvard Medical School, Boston, MA; [3]Lymphoma Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY; [4]Division of Oncology, Stanford University Medical Center, Stanford, CA; and [5]Division of Hematology and Oncology, Weill Cornell Medical School, New York, NY

Key Points

- BTK^{Cys481} mutations, including multiple mutated variants within individual patients are common in ibrutinib-progressing WM patients.
- BTK^{Cys481} mutations were associated with mutated CXCR4 in WM patients progressing on ibrutinib.

Ibrutinib produces high response rates and durable remissions in Waldenström macroglobulinemia (WM) that are impacted by MYD88 and CXCR4^{WHIM} mutations. Disease progression can develop on ibrutinib, although the molecular basis remains to be clarified. We sequenced sorted CD19$^+$ lymphoplasmacytic cells from 6 WM patients who progressed after achieving major responses on ibrutinib using Sanger, TA cloning and sequencing, and highly sensitive and allele-specific polymerase chain reaction (AS-PCR) assays that we developed for Bruton tyrosine kinase (*BTK*) mutations. AS-PCR assays were used to screen patients with and without progressive disease on ibrutinib, and ibrutinib-naïve disease. Targeted next-generation sequencing was used to validate AS-PCR findings, assess for other *BTK* mutations, and other targets in B-cell receptor and MYD88 signaling. Among the 6 progressing patients, 3 had *BTK*^{Cys481} variants that included *BTK*$^{Cys481Ser(c.1635G>C\ and\ c.1634T>A)}$ and *BTK*$^{Cys481Arg(c.1634T>C)}$. Two of these patients had multiple *BTK* mutations. Screening of 38 additional patients on ibrutinib without clinical progression identified *BTK*^{Cys481} mutations in 2 (5.1%) individuals, both of whom subsequently progressed. *BTK*^{Cys481} mutations were not detected in baseline samples or in 100 ibrutinib-naive WM patients. Using mutated *MYD88* as a tumor marker, *BTK*^{Cys481} mutations were subclonal, with a highly variable clonal distribution. Targeted deep-sequencing confirmed AS-PCR findings, and identified an additional *BTK*$^{Cys481Tyr(c.1634G>A)}$ mutation in the 2 patients with multiple other *BTK*^{Cys481} mutations, as well as *CARD11*$^{L\text{-}eu878Phe(c.2632C>T)}$ and *PLC*γ*2*$^{Tyr495His(c.1483T>C)}$ mutations. Four of the 5 patients with *BTK*^{C481} variants were CXCR4 mutated. *BTK*^{Cys481} mutations are common in WM patients with clinical progression on ibrutinib, and are associated with mutated *CXCR4*. (*Blood*. 2017;129(18):2519-2525)

Introduction

Regular Article

blood

LYMPHOID NEOPLASIA

BTK^{Cys481Ser} drives ibrutinib resistance via ERK1/2 and protects BTK$^{wild\text{-}type}$ MYD88-mutated cells by a paracrine mechanism

Jiaji G. Chen,[1] Xia Liu,[1] Manit Munshi,[1] Lian Xu,[1] Nicholas Tsakmaklis,[1] Maria G. Demos,[1] Amanda Kofides,[1] Maria Luisa Guerrera,[1] Gloria G. Chan,[1] Christopher J. Patterson,[1] Kirsten Meid,[1] Joshua Gustine,[1] Toni Dubeau,[1] Patricia Severns,[1] Jorge J. Castillo,[1,2] Zachary R. Hunter,[1,2] Jinhua Wang,[3] Sara J. Buhrlage,[3] Nathanael S. Gray,[3] Steven P. Treon,[1,2] and Guang Yang[1,2]

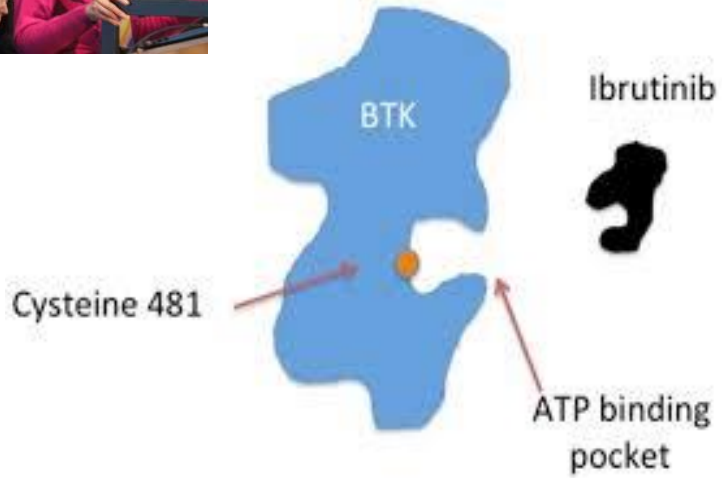
[1]Bing Center for Waldenstrom's Macroglobulinemia, [2]Department of Medical Oncology, Dana Farber Cancer Institute, and [3]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA

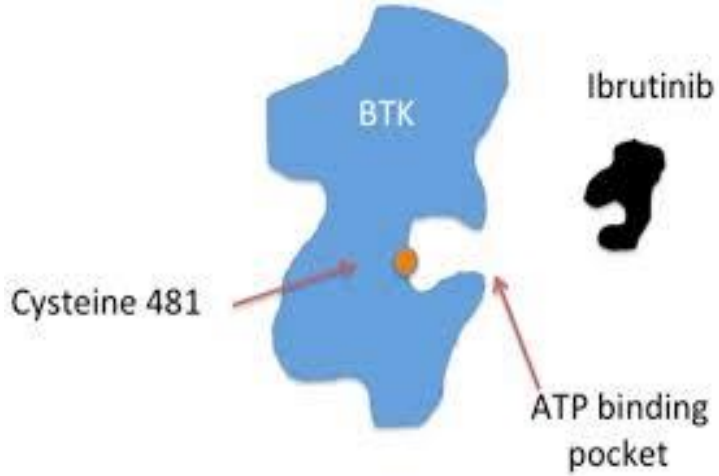
KEY POINTS

- BTK^{Cys481} mutation results in ERK1/2 mediated survival signaling and ibrutinib resistance in MYD88-mutated cells.
- BTK^{Cys481} mutation confers a protective effect against ibrutinib on neighboring BTK wild-type cells through a paracrine mechanism.

Acquired ibrutinib resistance due to BTK^{Cys481} mutations occurs in B-cell malignancies, including those with MYD88 mutations. BTK^{Cys481} mutations are usually subclonal, and their relevance to clinical progression remains unclear. Moreover, the signaling pathways that promote ibrutinib resistance remain to be clarified. We therefore engineered BTK^{Cys481Ser} and BTK^{WT} expressing MYD88-mutated Waldenström macroglobulinemia (WM) and activated B-cell (ABC) diffuse large B-cell lymphoma (DLBCL) cells and observed reactivation of BTK-PLCγ2-ERK1/2 signaling in the presence of ibrutinib in only the former. Use of ERK1/2 inhibitors triggered apoptosis in BTK^{Cys481Ser}-expressing cells and showed synergistic cytotoxicity with ibrutinib. ERK1/2 reactivation in ibrutinib-treated BTK^{Cys481Ser} cells was accompanied by release of many prosurvival and inflammatory cytokines, including interleukin-6 (IL-6) and IL-10 that were also blocked by ERK1/2 inhibition. To clarify if cytokine release by ibrutinib-treated BTK^{Cys481Ser} cells could protect BTK^{WT} MYD88-mutated malignant cells, we used a Transwell coculture system and showed that nontransduced BTK^{WT} MYD88-mutated WM or ABC DLBCL cells were rescued from ibrutinib-induced killing when cocultured with BTK^{Cys481Ser} but not their BTK^{WT}-expressing counterparts. Use of IL-6 and/or IL-10 blocking antibodies abolished the protective effect conferred on nontransduced BTK^{WT} by coculture with BTK^{Cys481Ser} expressing WM or ABC DLBCL cell counterparts. Rebound of IL-6 and IL-10 serum levels also accompanied disease progression in WM patients with acquired BTK^{Cys481} mutations. Our findings show that the BTK^{Cys481Ser} mutation drives ibrutinib resistance in MYD88-mutated WM and ABC DLBCL cells through reactivation of ERK1/2 and can confer a protective effect on BTK^{WT} cells through a paracrine mechanism. (*Blood*. 2018;131(18):2047-2059)

Resistance to Ibrutinib

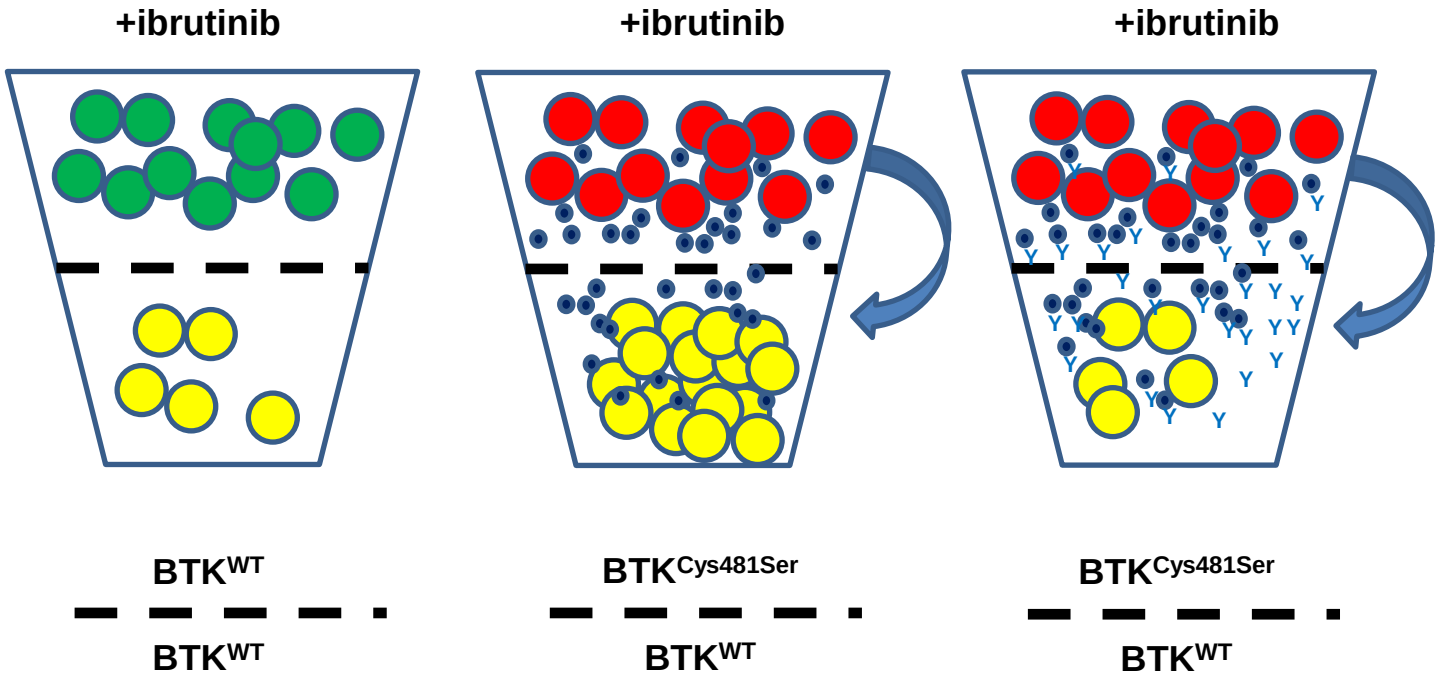




Resistance to Ibrutinib is commonly mediated by acquired mutations at BTK Cys481 in WM

BTK Cys481 is also the covalent binding site for zanubrutinib, acalabrutinib and tirabrutinib.

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



Chen et al, Blood 2018

Non-covalent BTK inhibitors in WM

- **Vecabrutinib**

  Targets BTK (T474). HCK (276 nM). Phase I included 3 WM patients (1 BTK^{Cys481}; 1 PLCg2 mutation; Allan et al, ASH 2019)

- **ARQ-531**

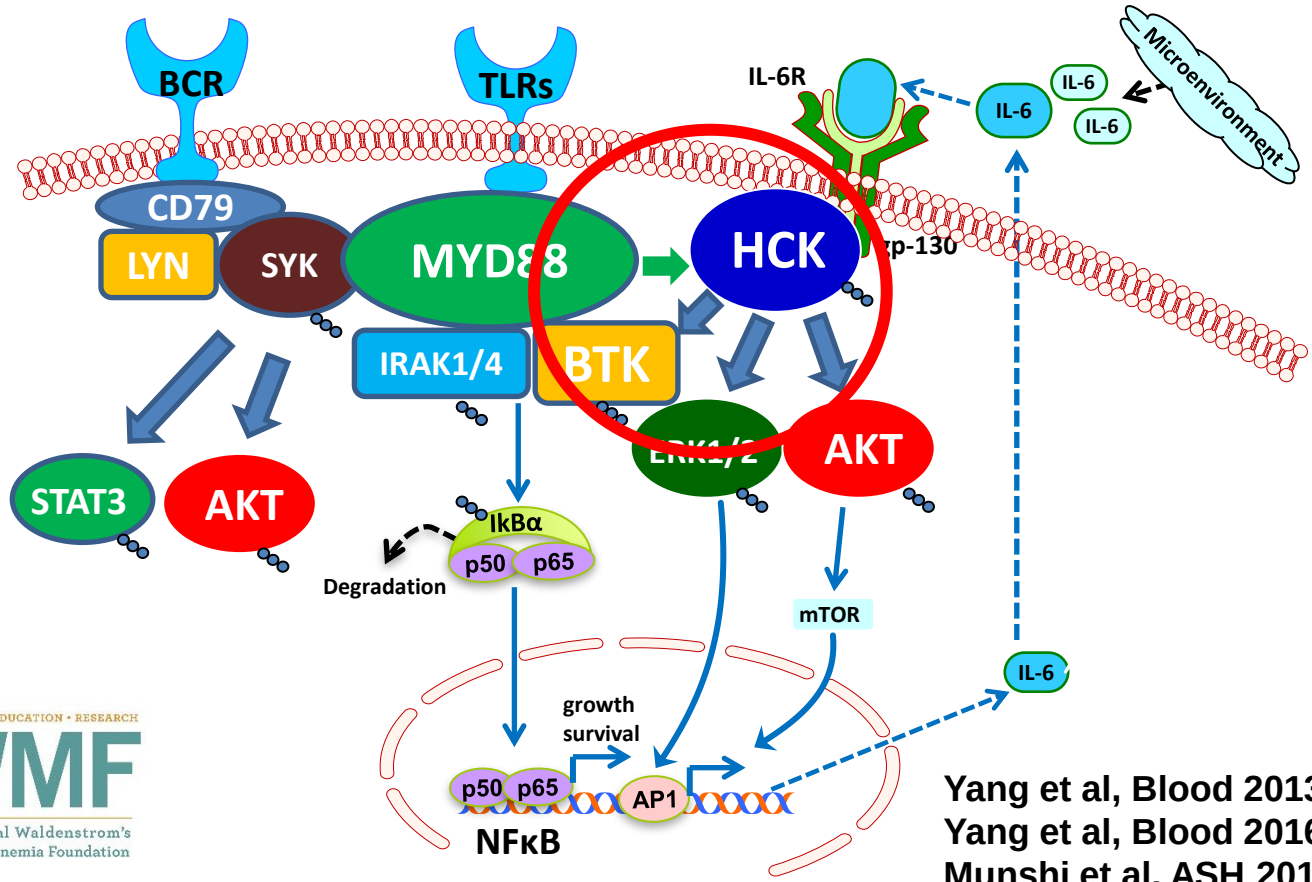
  Targets BTK (E475/Y476) and HCK (18 nM). Phase I study completed. (Reiff et al, Cancer Discovery 2018; Woyach et al, ASH 2019).

- **LOXO-305**

  Targets BTK (G473-K483) Ongoing Phase I study included 2 WM patients. (Brandhuber et al, SOHO 2018; Mato et al, ASH 2019)

  Pre-clinical and Clinical Studies in WM initiated at DFCI.

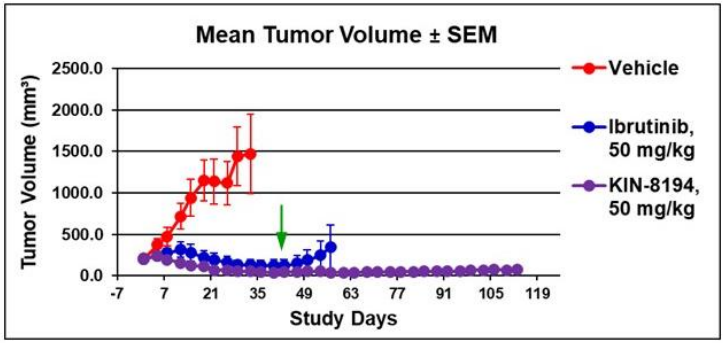
Targeting BTK Cys481 Mutation signaling in MYD88 mutated lymphomas by inhibition of upstream HCK activation of BTK

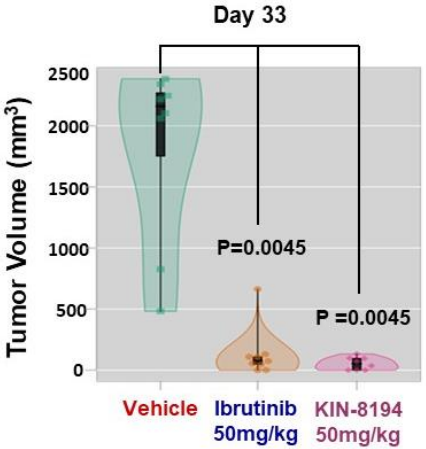


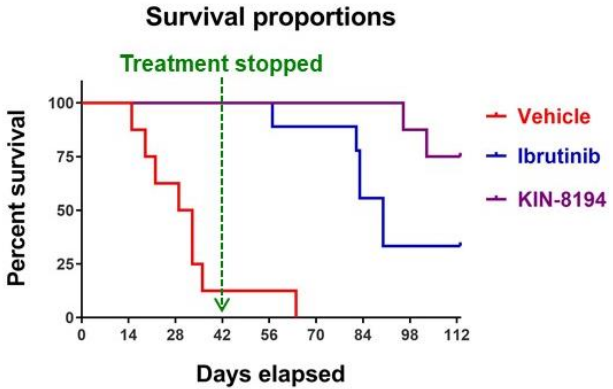
SUPPORT • EDUCATION • RESEARCH
IWMF
International Waldenstrom's Macroglobulinemia Foundation

Yang et al, Blood 2013
Yang et al, Blood 2016
Munshi et al, ASH 2018

Development of a dual HCK/BTK inhibitor: KIN-8194 Ibrutinib sensitive model



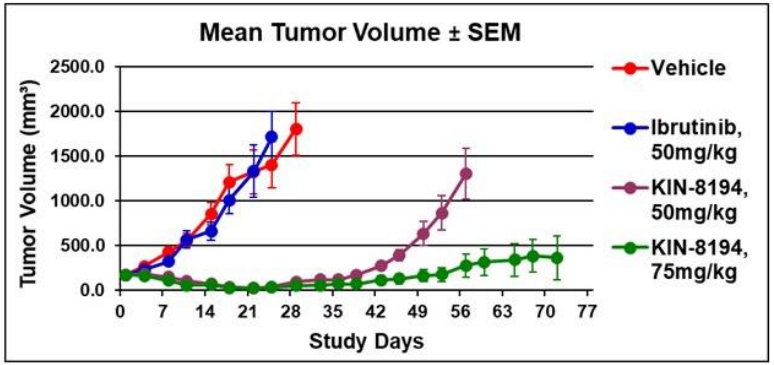


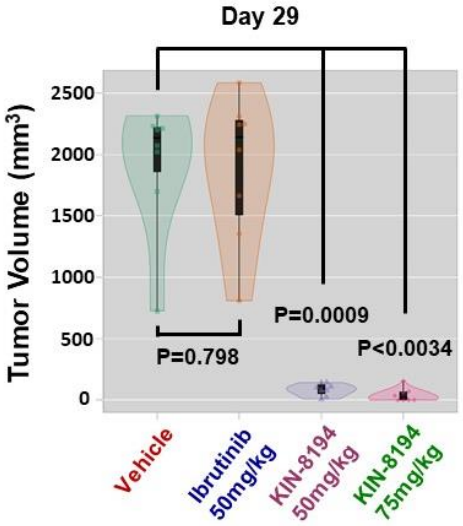


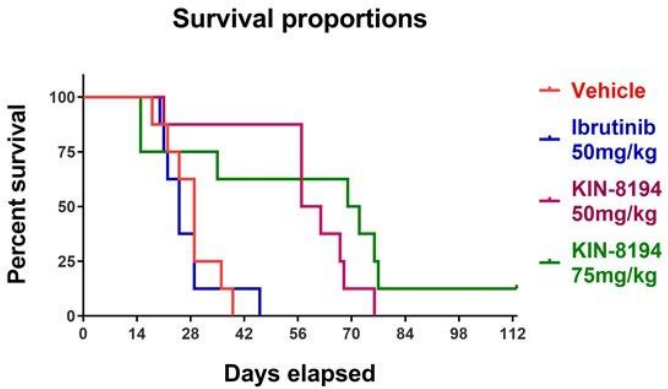
	Vehicle	Ibrutinib (50mg/kg)	KIN-8194 (50mg/kg)
Median Survival (days)	31	90	Undefined

Yang et al, ASH 2019

KIN-8194 can overcome mutated BTKCys481 resistance to ibrutinib



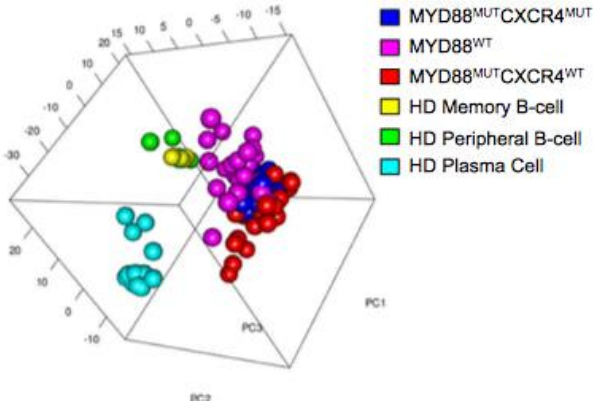




	Vehicle	Ibrutinib 50mg/kg	KIN-8194 50mg/kg	KIN-8194 75mg/kg
Median Survival (days)	29	25	57.5	70.5

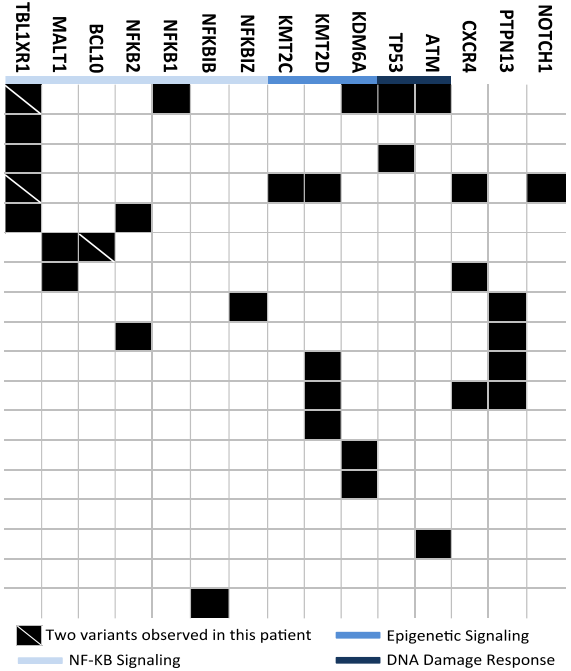
Yang et al, ASH 2019

New Driver Mutations Identified in WM Patients without MYD88 mutation

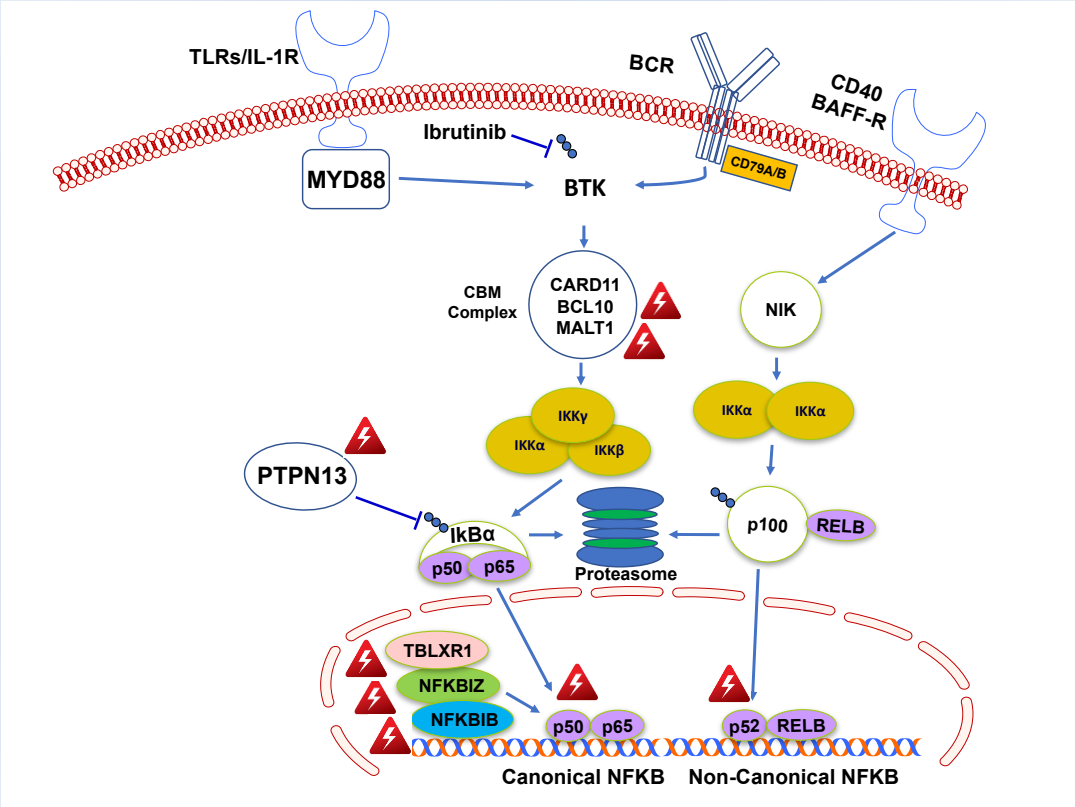


Principal component analysis of top 500 high variance genes.

Hunter et al, Blood. Adv 2018



Genomic Landscape of MYD88 Wild-Type WM



Hunter et al, Blood Adv. 2018

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

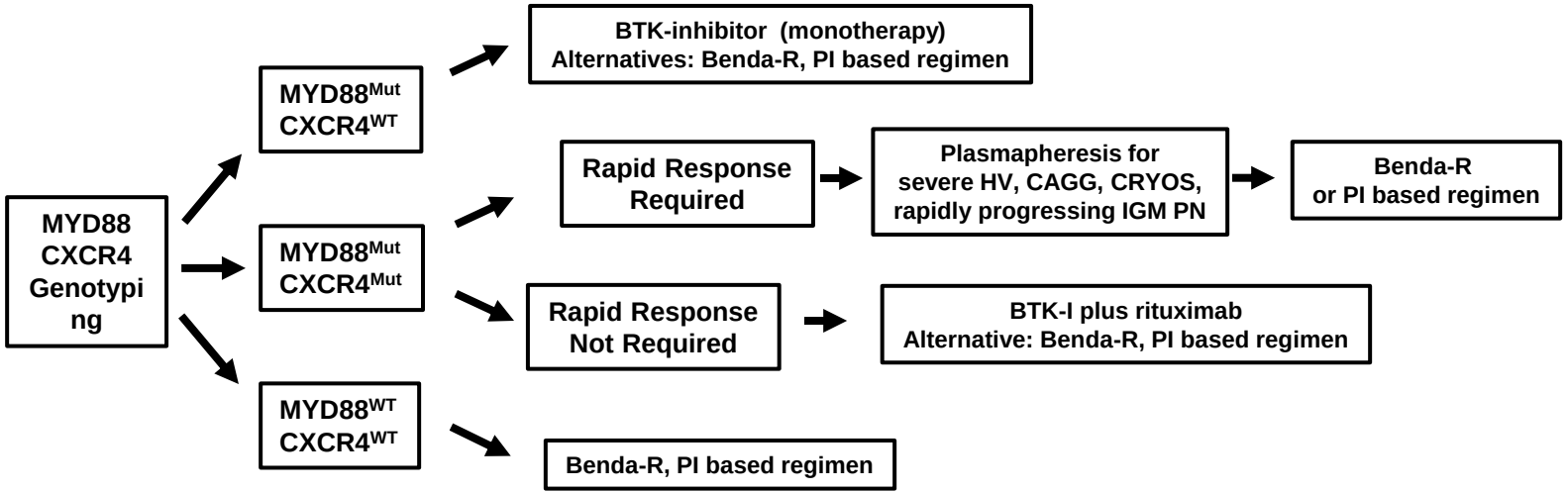
WMFC
Waldenstrom`s Macroglobulinemia Foundation of Canada

300 PROJECT

Sequence and Track 300 Symptomatic Untreated WM Patients

-Determine mutations in the DNA by Whole Exome Seq.
-Determine transcriptional (RNA) changes, incl. aberrant splicing
-Map epigenome and its regulatory changes
-Understand impact on disease presentation, course, survival
-Develop targeted therapies based on mutation profile for individual patients

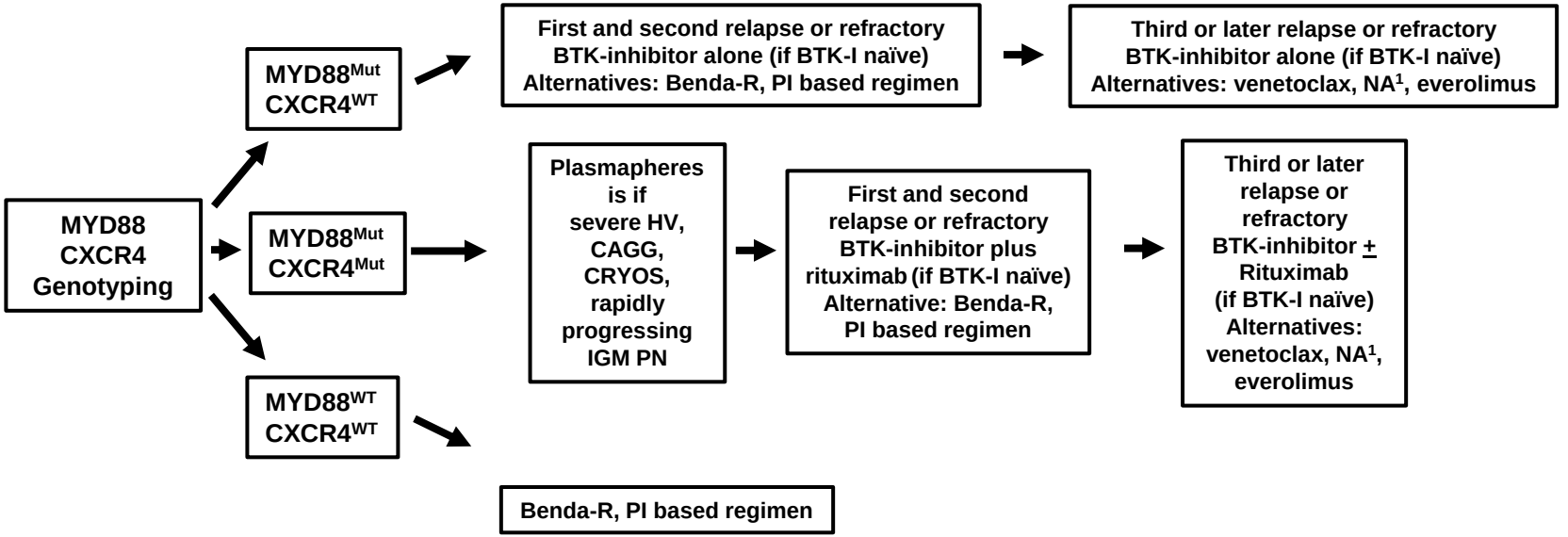
Genomic Based Treatment Approach to Symptomatic Treatment Naïve WM



- Rituximab should be held for serum IgM ≥4,000 mg/dL
- Benda-R for bulky adenopathy or extramedullary disease.
- PI based regimen for symptomatic amyloidosis, and possible ASCT as consolidation.
- Rituximab alone, or with ibrutinib if MYD88^{Mut} or bendamustine for IgM PN depending on severity and pace of progression.
- Maintenance rituximab may be considered in patients responding to rituximab based regimens.

Treon et al, JCO 2020

Genomic Based Treatment Approach to Symptomatic Relapsed or Refractory WM



- **Nucleoside analogues (NA) should be avoided in younger patients, and candidates for ASCT.[1]**
- **ASCT may be considered in patients with multiple relapses, and chemosensitive disease.**

Treon et al, JCO 2020