Novel Approaches for Targeting MYD88 in Waldenstrom's Macroglobulinemia

Steven P. Treon MD, PhD, FRCP, FACP

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Novel Approaches for targeting MYD88 in Waldenstrom's Macroglobulinemia

Steven P. Treon MD, PhD, FRCP, FACP
Harvard Medical School
Bing Center for Waldenstrom's Macroglobulinemia
Dana Farber Cancer Center, Boston MA

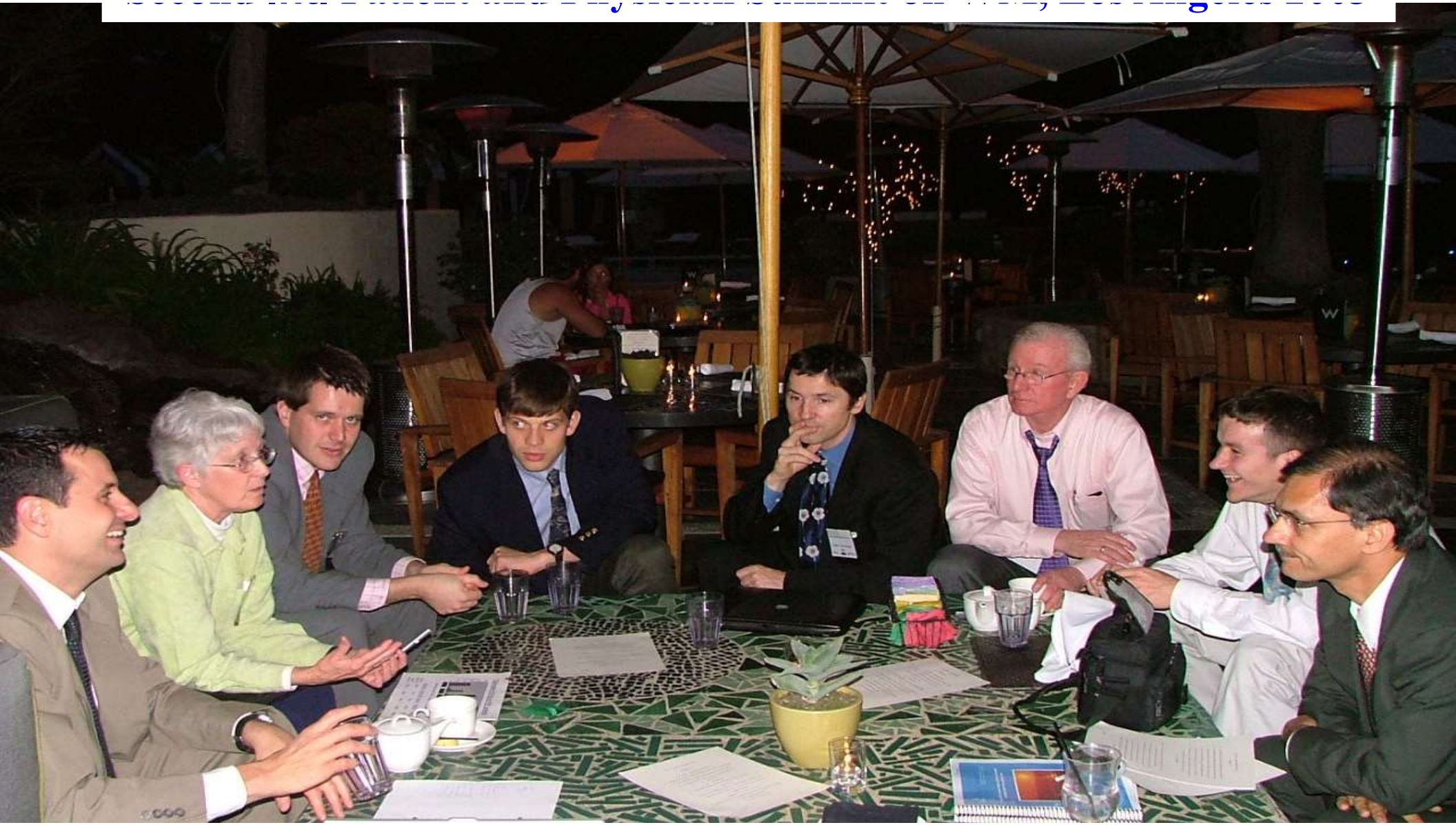
In Memoriam of Glenn Cantor, D.V.M., Ph.D.

Pre-MYD88

WM Treatment Approach

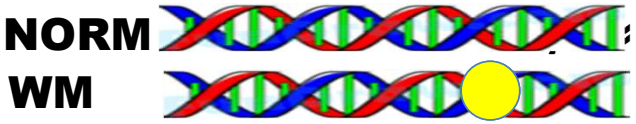
"Hand me down medicines"

Athens, Greece 2002

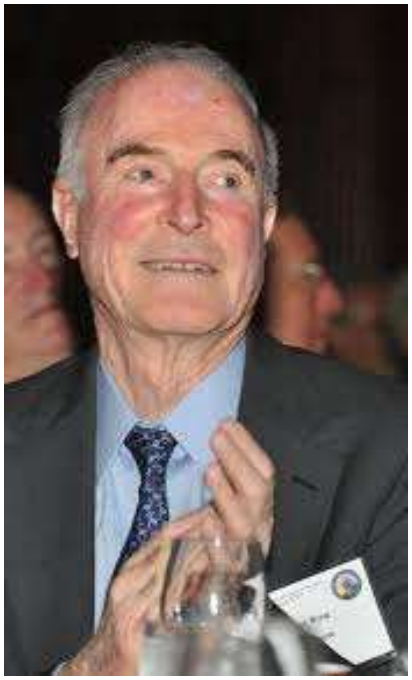


Dedication of Bing Center for WM at DFCI 2005

WHOLE GENOME SEQUENCING: DISCOVERY OF MYD88 MUTATION IN WM-2011



Discovery of the MYD88 Mutation in WM
Best of ASH 2011; NEJM 2012



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.

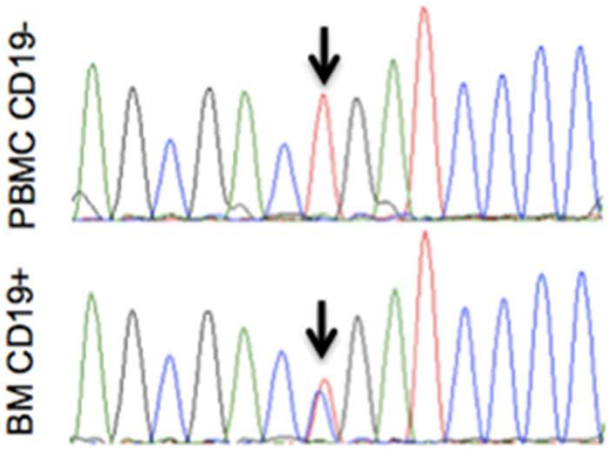
IWMF
International Waldenstrom's Macroglobulinemia Foundation

90% of WM patients had MYD88 mutation

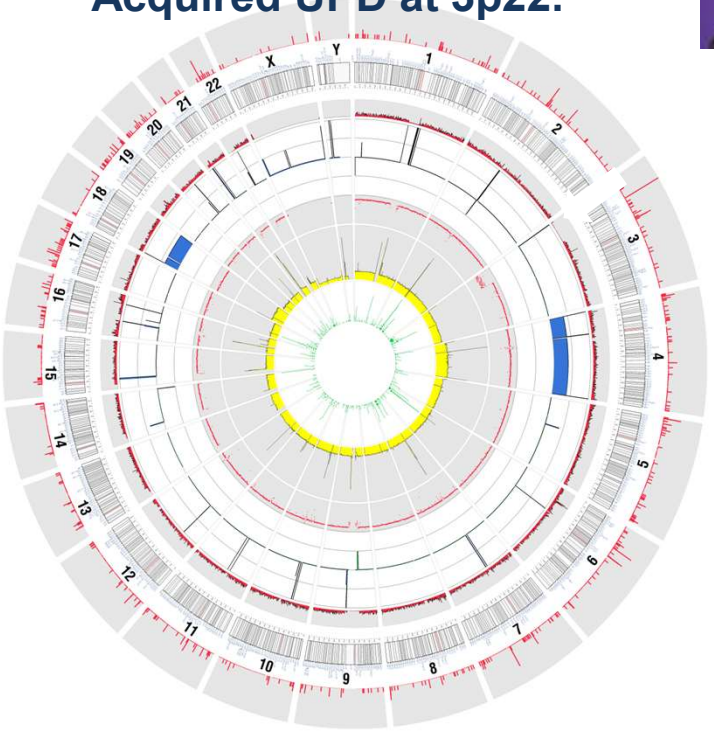
MYD88 L265P Somatic Mutation in WM

Zach Hunter

C to G at position 38186241 at 3p22.2



Acquired UPD at 3p22.



- **MYD88^{L265P} confirmed by AS-PCR in 95-97% WM pts.**
- **50-80% IGM MGUS pts.**

Treon et al, NEJM 367:826, 2012

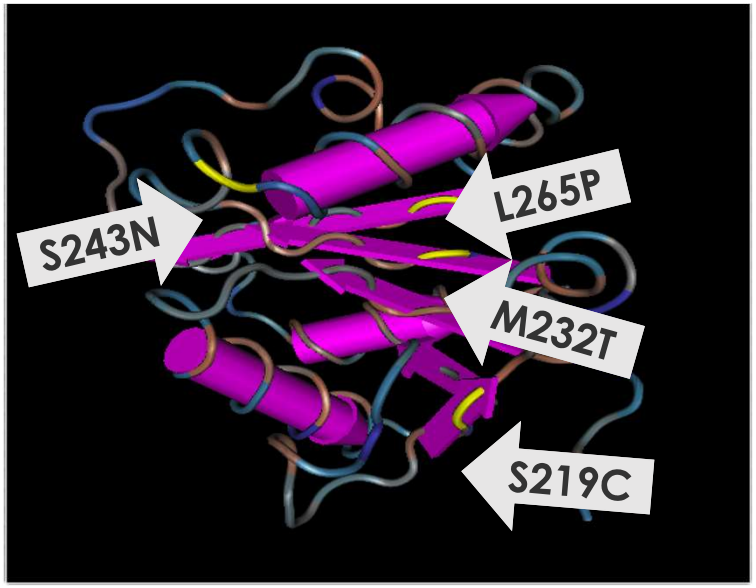
MYD88 L265P in WM/IGM MGUS

	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

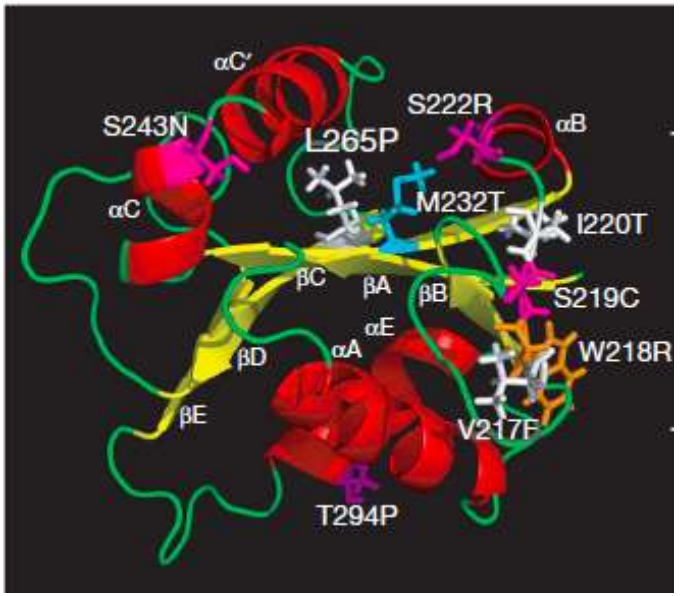
MYD88 Mutations in B-cell LPD

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.

Comment on Poulain et al, page ■■■

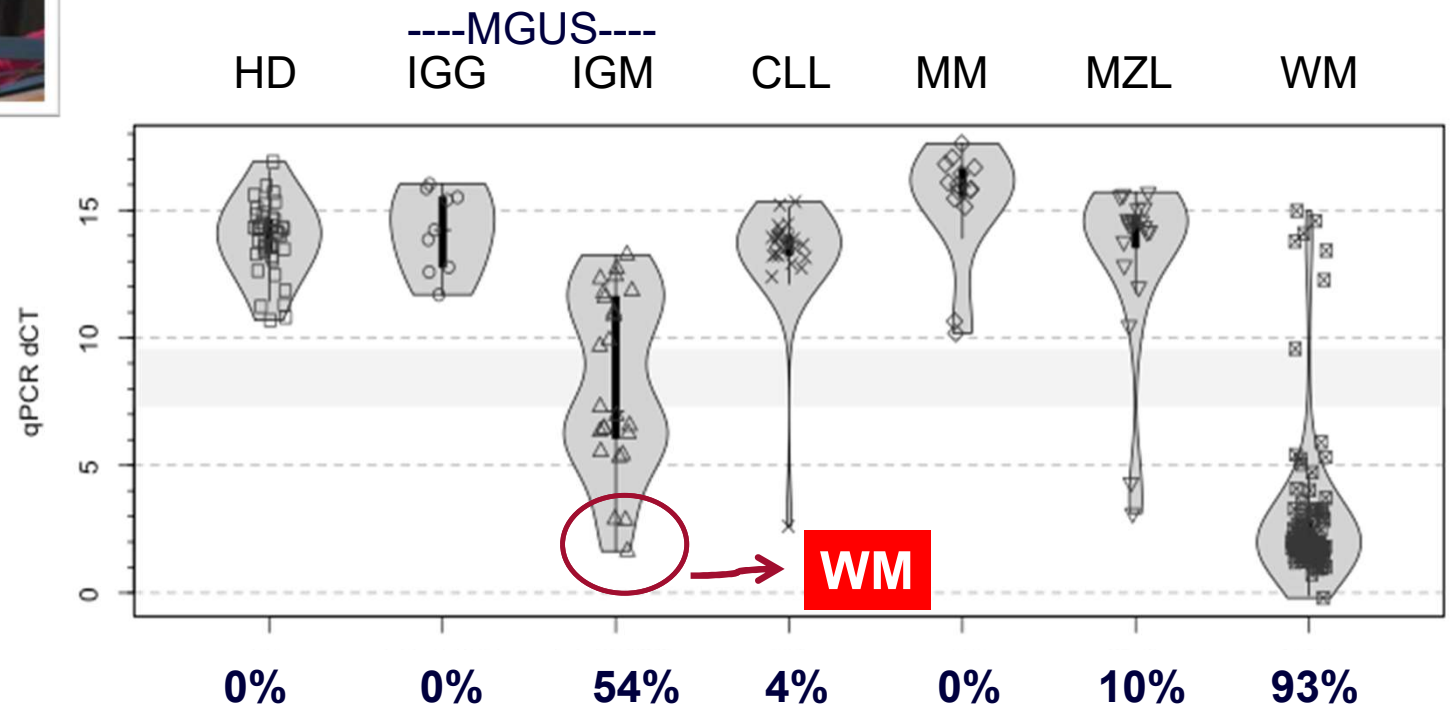
A new era for Waldenstrom macroglobulinemia: MYD88 L265P

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]

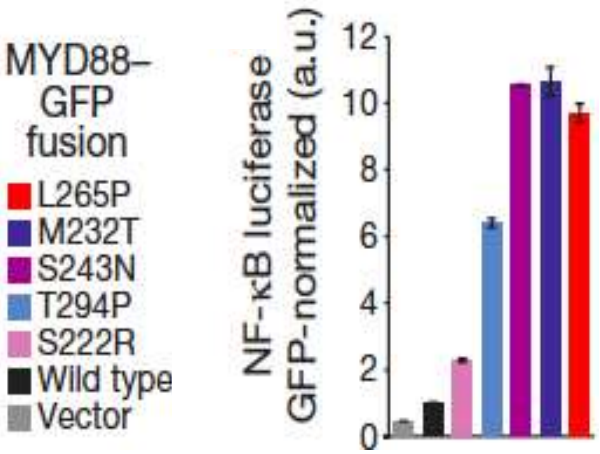
Lian Xu MS

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

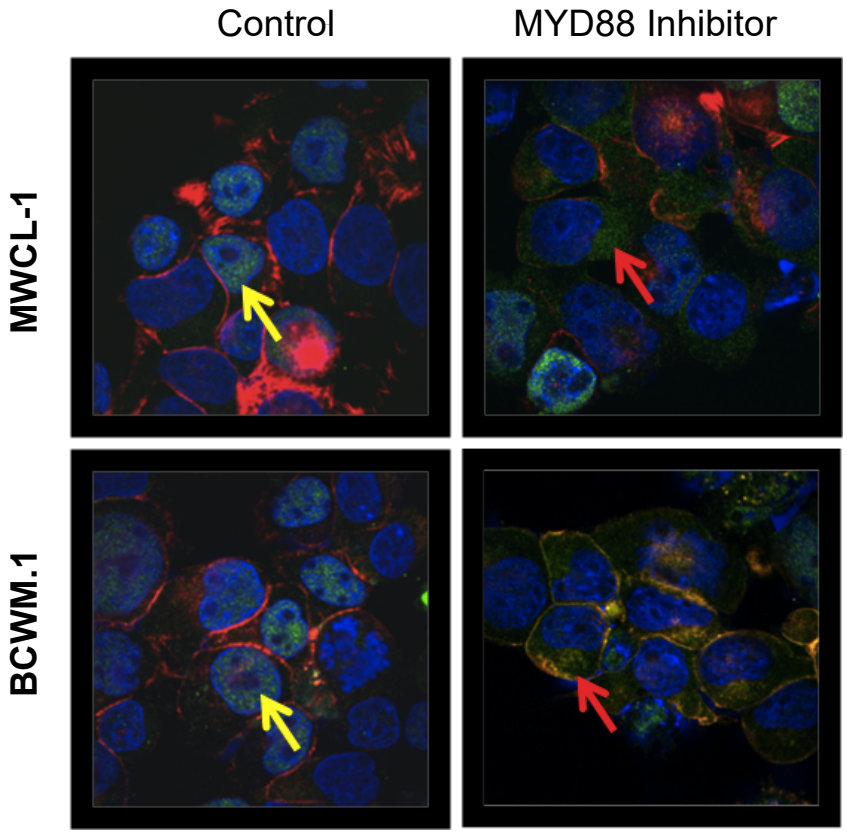


Xu et al, Blood 2013

MYD88 mutations transactivate NFKB

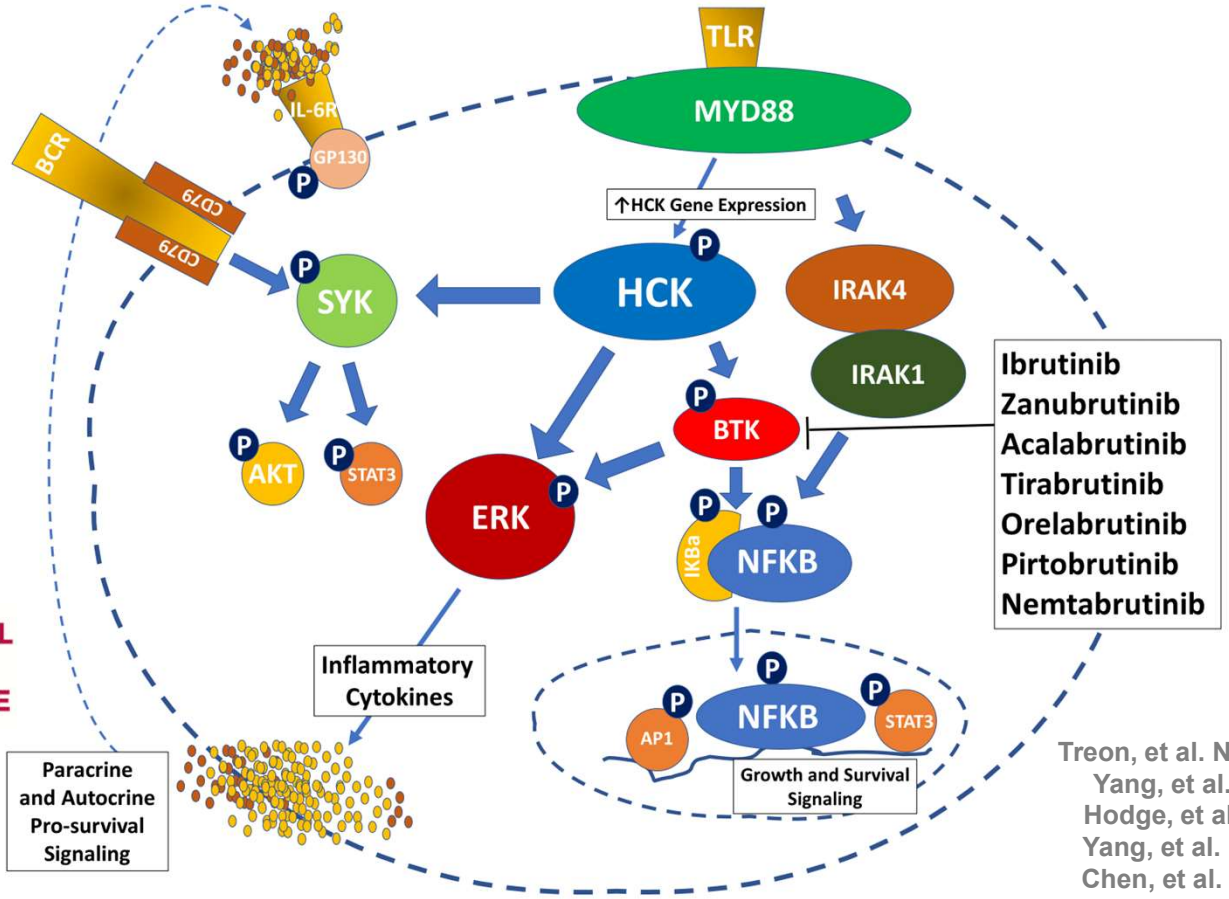


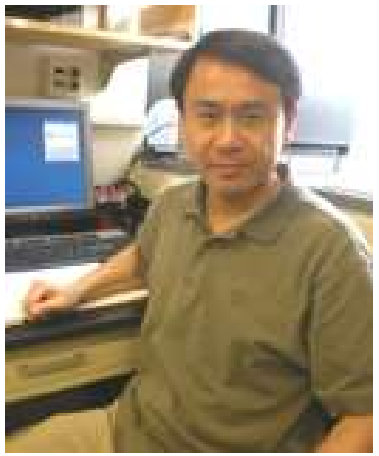
Ngo et al, Nature 2011
Treon et al, NEJM 2012



MYD88 L265P mutated WM cells

MYD88 Directed Pro-survival Signaling in WM





Guang Yang Ph.D.

Treon, et al. N Engl J Med. 2012;367(9):826-833.
Yang, et al. Blood. 2013;122(7):1222-1232.
Hodge, et al. Blood. 2014;123(7):1055-1058.
Yang, et al. Blood. 2016;127(25):3237-3252.
Chen, et al. Blood. 2018;131(18):2047-2059.
Liu, et al. Blood Adv. 2020;4(1):141-153.
Munshi, et al. Blood Cancer J. 2020;10:12.
Munshi, et al. Blood Adv. 2022.

New directions in WM

First Breakthrough Pathway for any Oncology Indication
First Drug Approval for Waldenstrom's Macroglobulinemia

BTK-Inhibitor Trials in WM

Study	Cohort	Agent (s)	N=	Time to Major Resp.	ORR/Major RR	≥VGPR	PFS
Pivotal Study	R/R	Ibrutinib	63	2 mo.	91% / 79%	30%	54% @ 60 mo.
INNOVATE Arm C	R/R	Ibrutinib	31	2 mo.	87% / 77%	29%	40% @ 60 mo.

Median ORR: 93%; Major RR: 81%; ≥VGPR: 30%;
PFS 76% @ 4 yrs

Very Few or no complete responses!

Phase 2	TN, R/R	Acalabrutinib	106	N/A	94% / 81%	39%	84% TN / 52% R/R (@ 66 mo.)
Phase 2	TN, R/R	Tirabrutinib	27	1.9 TN 2.1 R/R	96% / 93%	33%	93% @ 24 mo.
Phase 2	R/R	Pirtobrutinib	80	N/A	81% / 67% (prior cBTKi) 88% / 88% (cBTKi naïve)	24% (prior cBTKi) 29% (cBTKi naïve)	57% @ 18 mo. (for prior cBTKi) N/A for cBTKi naïve.

Mutated CXCR4 permits ongoing pro-survival signaling by CXCL12

Plenary Paper

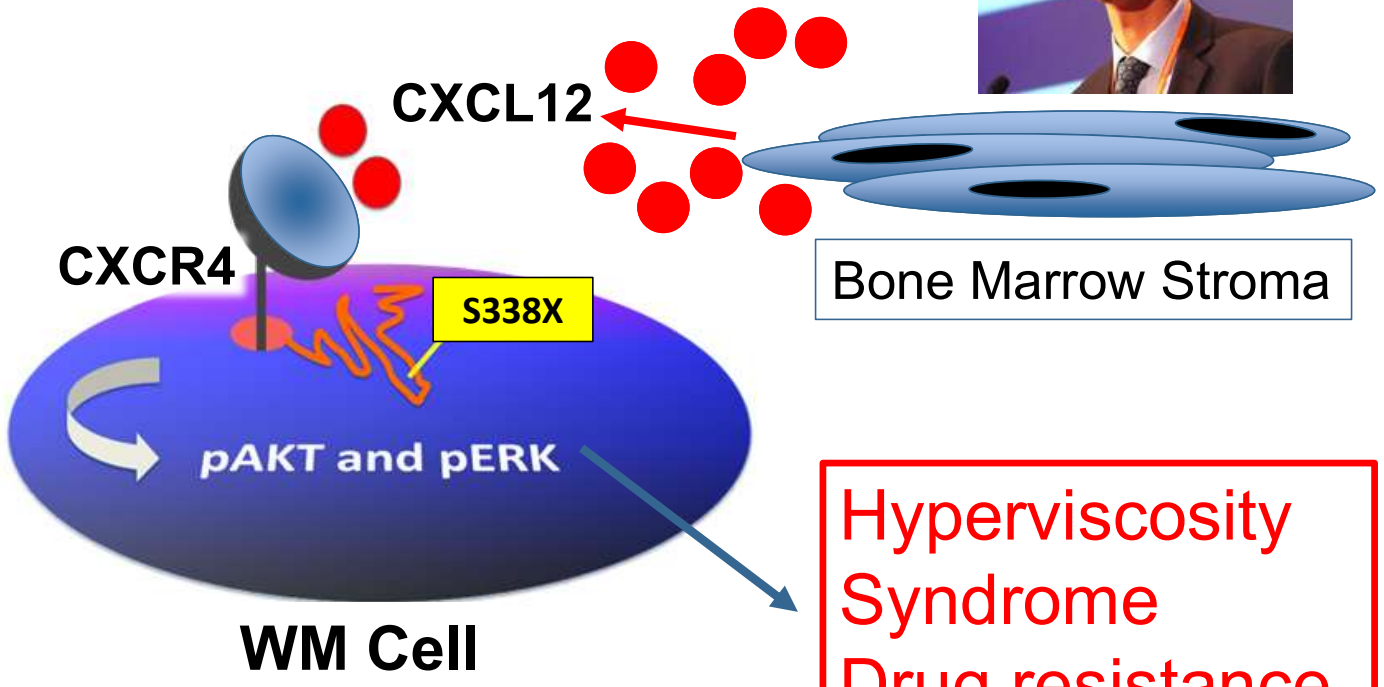
LYMPHOID NEOPLASIA

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

- **30-40% of WM patients have mutated CXCR4.**
- **>40 different CXCR4 mutations, most common is S338X.**



Hunter et al, Blood 2013; Treon et al, Blood 2014; Roccarro et al, Blood 2014; Cao et al, Leukemia 2014.

Impact of CXCR4 Mutation Status in BTK-Inhibitor Studies in WM

Study	Patient Population	Agent (s)	Time to Major Response ($CXCR^{Mut\ vs.\ WT}$)	Major Response Rate ($CXCR^{Mut\ vs.\ WT}$)	≥VGPR ($CXCR^{Mut\ vs.\ WT}$)	PFS ($CXCR^{Mut\ vs.\ WT}$)
Pivotal Study	R/R	Ibrutinib	4.7 vs.1.8 mo.	68% vs. 97%	9% vs. 47%	38% vs. 70% (@ 60 mo.)
ASPEN Cohort 1	TN, R/R	Ibrutinib	6.6 vs. 2.8 mos.	65% vs. 82%	10% vs. 24%	49% vs. 75% (@ 42 mo.)
	TN, R/R	Zanubrutinib	3.4 vs. 2.8 mos.	70% vs. 82%	18% vs. 34%	73% vs. 81% (@ 42 mo.)

$CXCR4^{Mut}$ vs $CXCR4^{WT}$

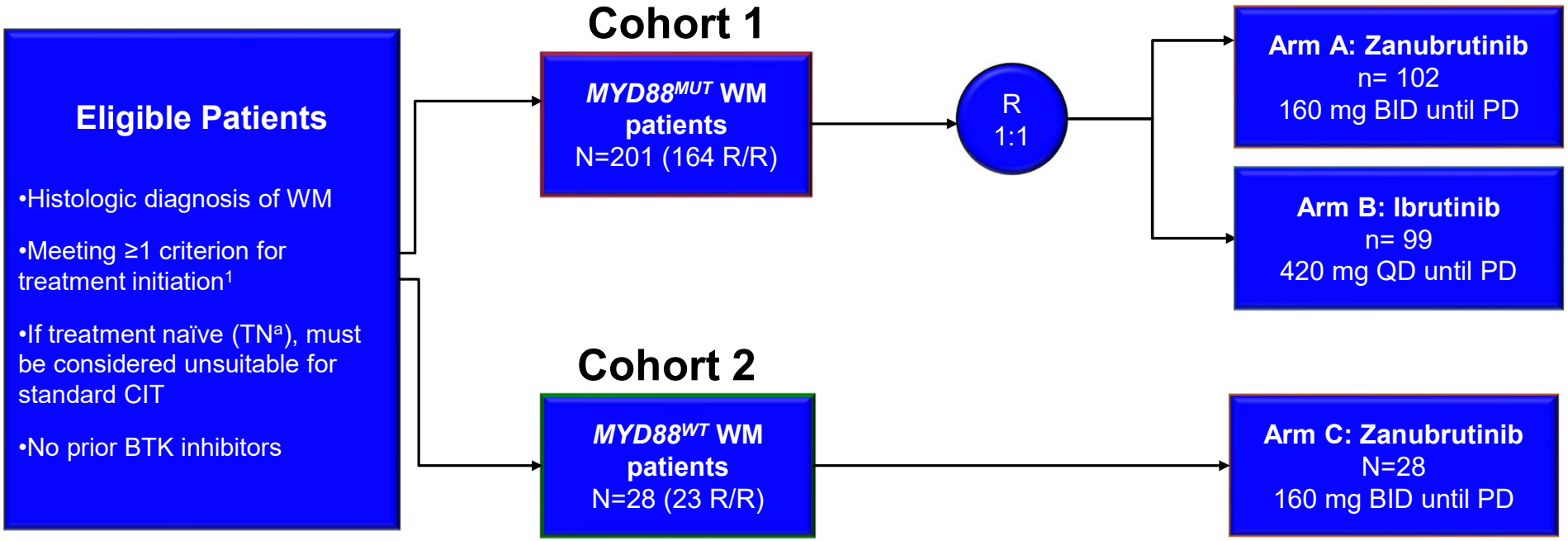
Median Time to Major Response: (4.2 vs. 1.9 mos)

Median Major RR: 71% vs. 87%

Median ≥VGPR: 14% vs. 41%

PFS: 59% vs. 75% @4 years

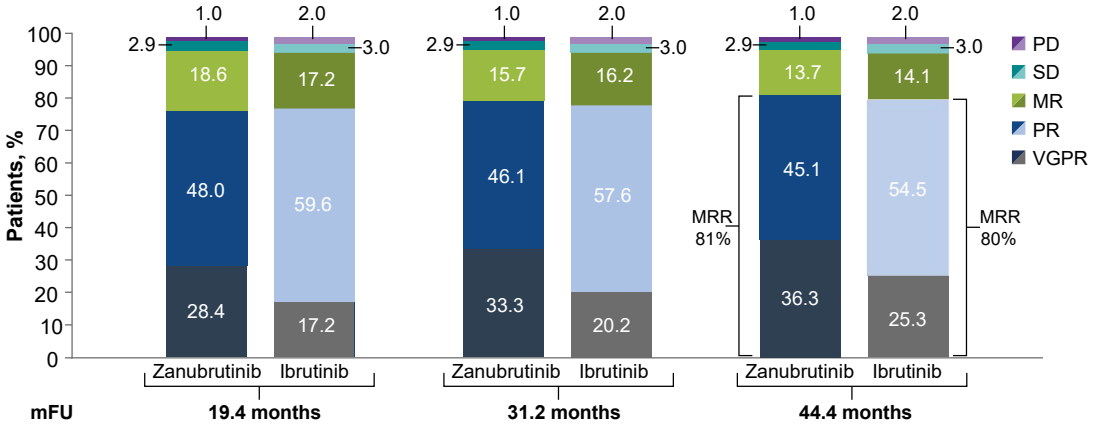
Phase 3 ASPEN Study
Zanubrutinib vs. Ibrutinib in WM



NCT03053440

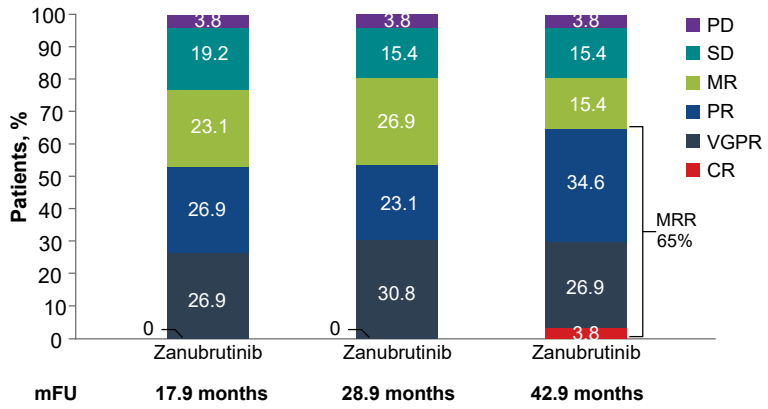
ASPEN: Best Overall Response and PFS by Investigator Assessment

$MYD88^{MUT}$



- *At 44.4 months event free rates for PFS were 78.3% and 69.7% for zanubrutinib and ibrutinib, respectively. For OS, 87.5% and 85.2%, respectively.*

$MYD88^{WT}$



- *At 42.9 months event-free rates for PFS and OS were 53.8% and 83.9%, respectively.*

Dimopoulos MA et al, JCO 2023

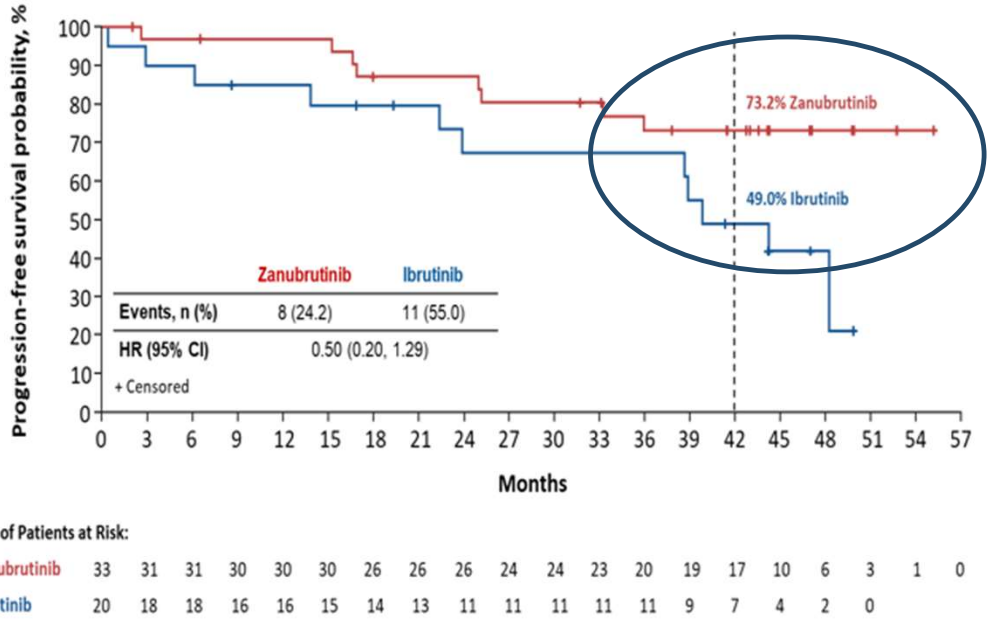
Response by CXCR4 Mutation Status for Cohort 1 WM Patients on Zanubrutinib

Response Assessment by *CXCR4* Status[a]

Response	*CXCR4*^{MUT} Ibrutinib (n=20)	*CXCR4*^{MUT} Zanubrutinib (n=33)	*CXCR4*^{WT} Ibrutinib (n=72)	*CXCR4*^{WT} Zanubrutinib (n=65)
VGPR or better, n (%)	2 (10.0)	**7 (21.2)**	22 (30.6)	**29 (44.6)**
Major response, n (%)	13 (65.0)	**26 (78.8)**	61 (84.7)	54 (83.1)
Overall response, n (%)	19 (95.0)	30 (90.9)	68 (94.4)	63 (96.9)
Time to MR, median (months)	6.6	3.4	2.8	2.8
Time to VGPR, median (months)	31.3	11.1	11.3	6.5

Bold blue text indicates >10% difference between arms.

PFS in Patients With *MYD88*^{MUT}*CXCR4*^{MUT}



Tam et al, Blood Adv. 2024; Dimopoulos et al JCO 2023

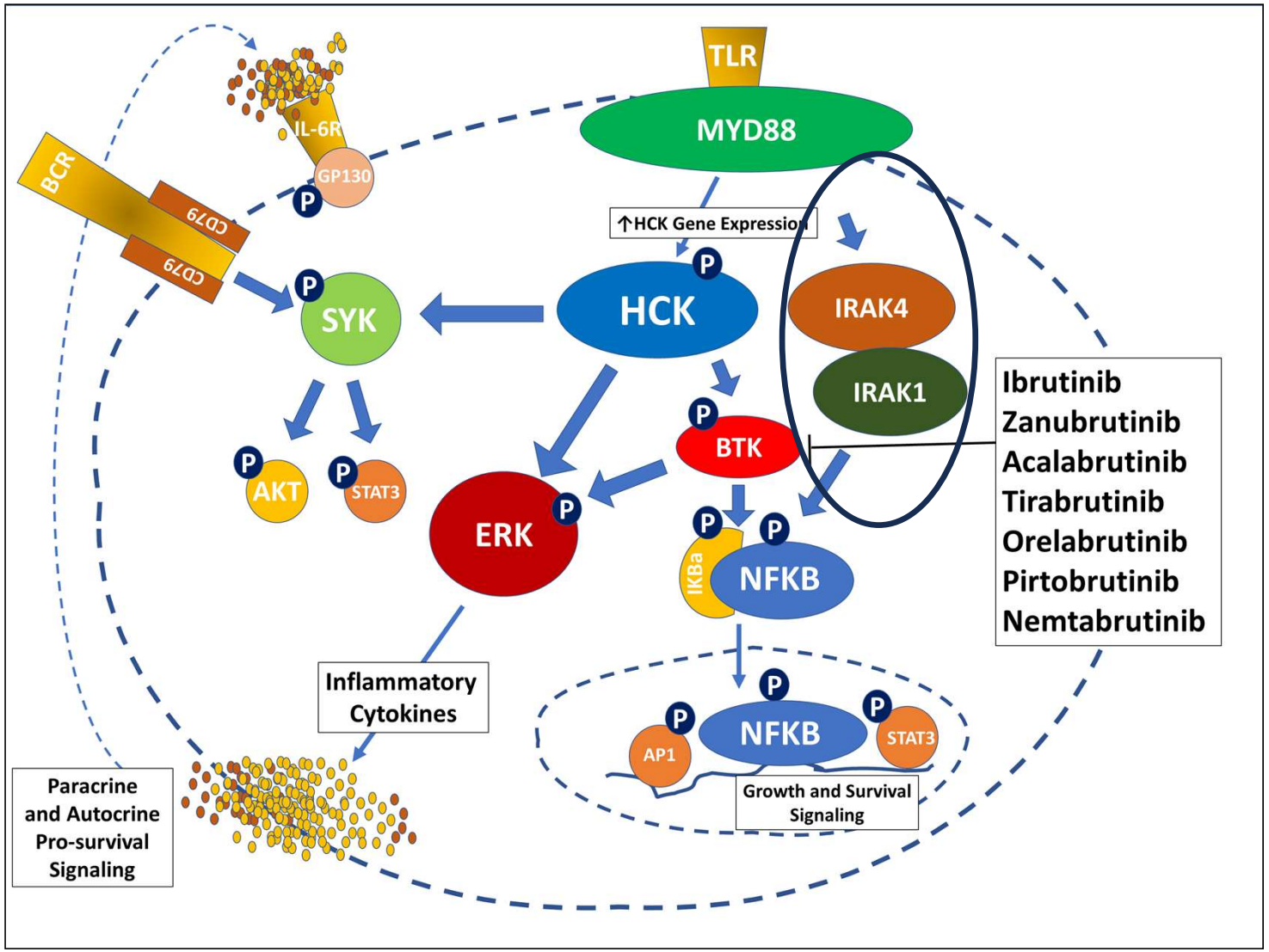
ASPEN STUDY Adverse Events of Interest (Cohort 1)

AEs,[a] n (%)	Any grade		Grade ≥3	
	Ibrutinib (n=98)	Zanubrutinib (n=101)	Ibrutinib (n=98)	Zanubrutinib (n=101)
Infection	78 (79.6)	80 (79.2)	**27 (27.6)**	22 (21.8)
Bleeding	61 (62.2)	56 (55.4)	10 (10.2)	9 (8.9)
Diarrhea	**34 (34.7)**	23 (22.8)	2 (2.0)	3 (3.0)
Hypertension*	**25 (25.5)**	15 (14.9)	**20 (20.4)***	10 (9.9)
Atrial fibrillation/ flutter*	**23 (23.5)***	8 (7.9)	**8 (8.2)***	2 (2.0)
Anemia	22 (22.4)	18 (17.8)	6 (6.1)	**12 (11.9)**
Neutropenia*[b]	20 (20.4)	**35 (34.7)***	10 (10.2)	**24 (23.8)***
Thrombocytopenia	17 (17.3)	17 (16.8)	6 (6.1)	11 (10.9)
Second primary malignancy/ nonskin cancers	17 (17.3)/ 6 (6.1)	17 (16.8)/ 6 (5.9)	3 (3.1)/ 3 (3.1)	6 (5.9)/ 4 (4.0)

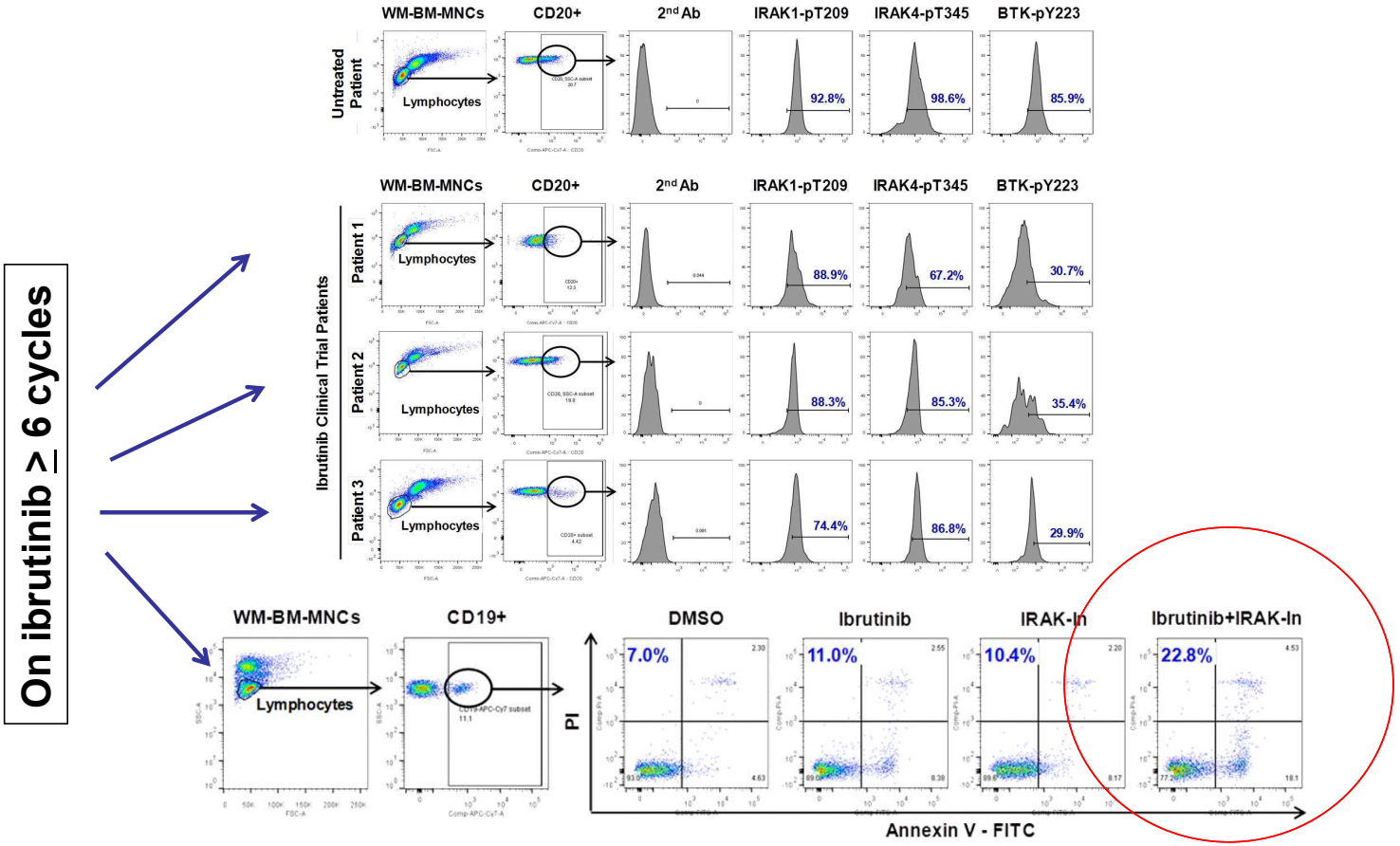
Dimopoulos et al, JCO 2023

Intrinsic Resistance to BTK-Inhibitors

Targeting Intrinsic Resistance: IRAK Inhibitors

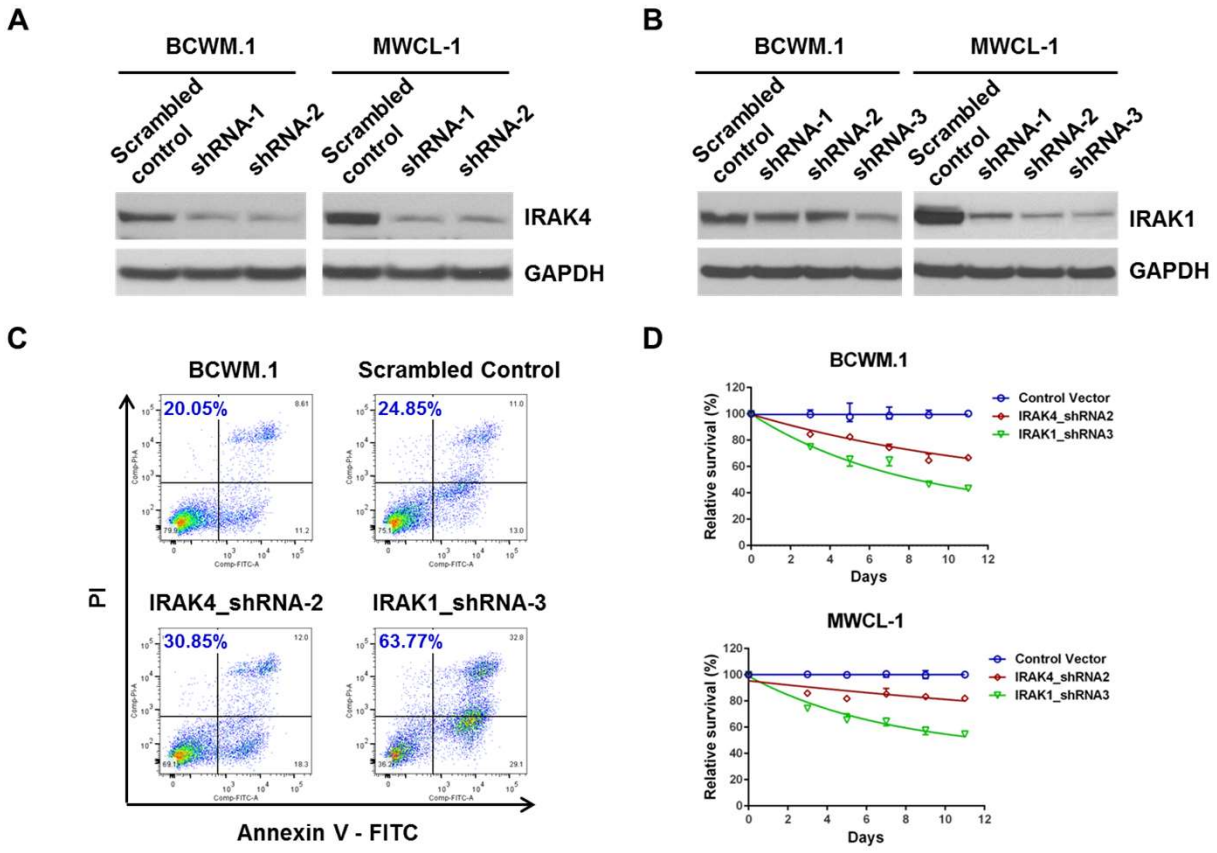


IRAK1/4 kinase survival signaling remains intact in WM cells from ibrutinib treated patients.



Yang G, Liu X, Chen J, et al. Targeting IRAK1/IRAK4 signaling in Waldenstrom's Macroglobulinemia. Blood 2015; 126(23):4004.

Cell survival depends on IRAK1>IRAK4 in MYD88 Mutated WM Cells



Yang et al, ASH 2015

Medicinal Chemistry Team for Novel WM Drug Development

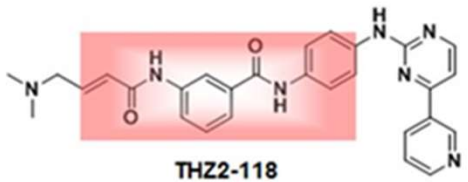
Nathanael Gray
Ph.D.

Sara Buhrlage
Ph.D.

John Hatcher
Ph.D.

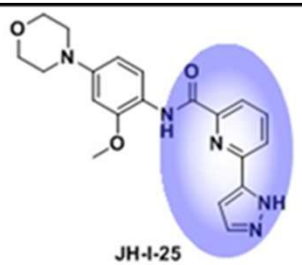
Jinhua Wang
Ph.D.

Development of IRAK1 Inhibitor JH-X-119-01



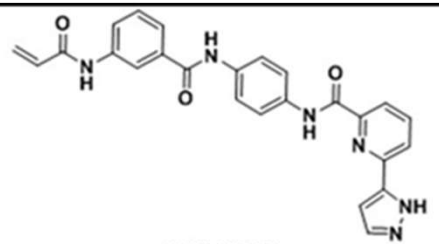
THZ2-118

IRAK1 IC50 = 14.2 nM
IRAK4 IC50 > 10,000 nM
JNK1 IC50 = 1.54 nM
JNK2 IC50 = 1.99 nM
JNK3 IC50 = 0.75 nM



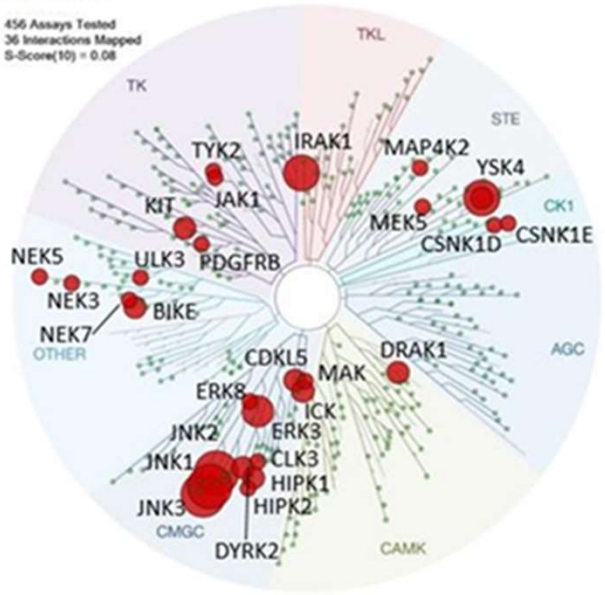
JH-I-25
IRAK1 IC50 = 9 nM
IRAK4 IC50 = 17nM

Merge scaffolds

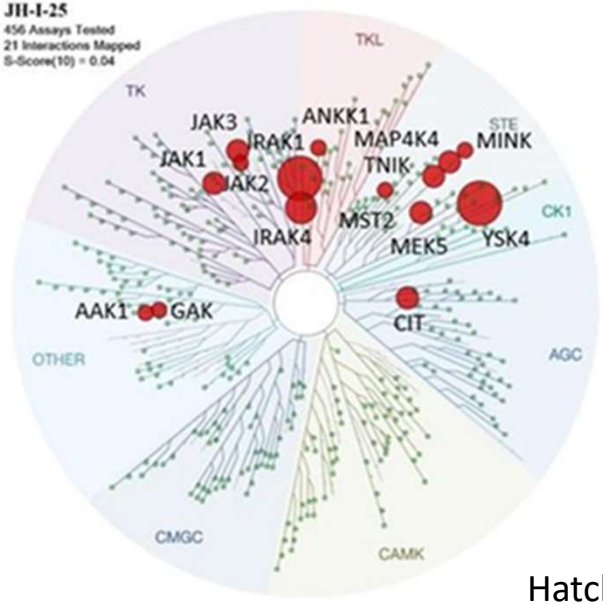


JH-X-119-01
IRAK1 IC50 = 9 nM
IRAK4 IC50 > 10,000 nM

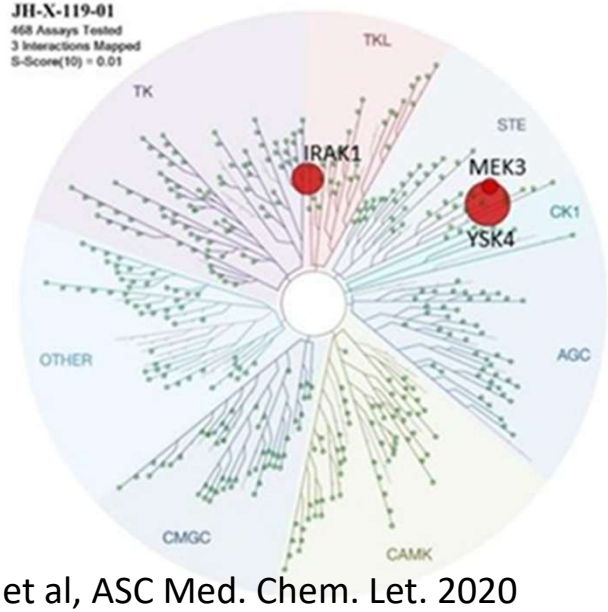
THZ2-118



JH-I-25



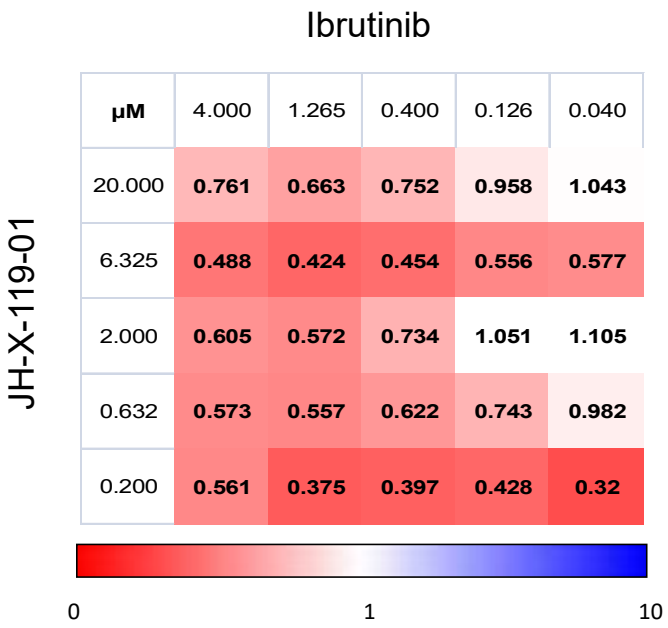
JH-X-119-01



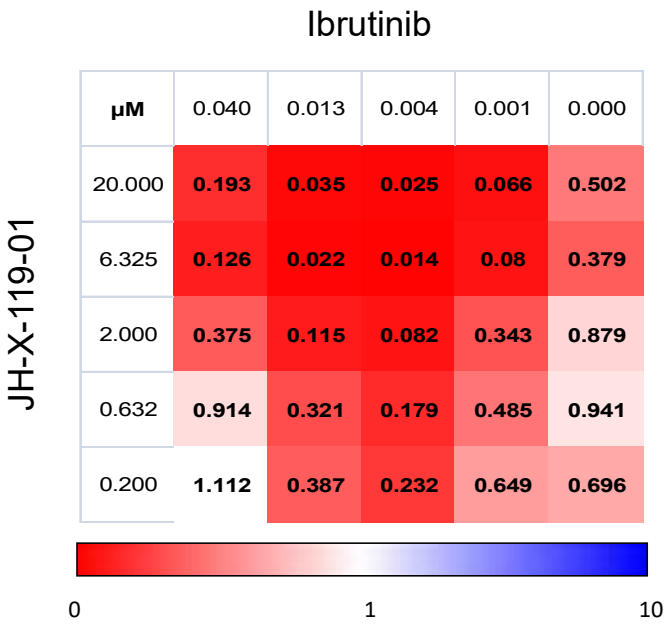
Hatcher et al, ASC Med. Chem. Let. 2020

Combining JH-X-119-01 with Ibrutinib Showed Synergistic Killing of MYD88 Mutated Cells

BCWM.1



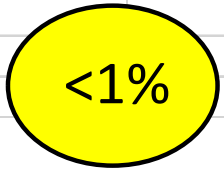
TMD-8



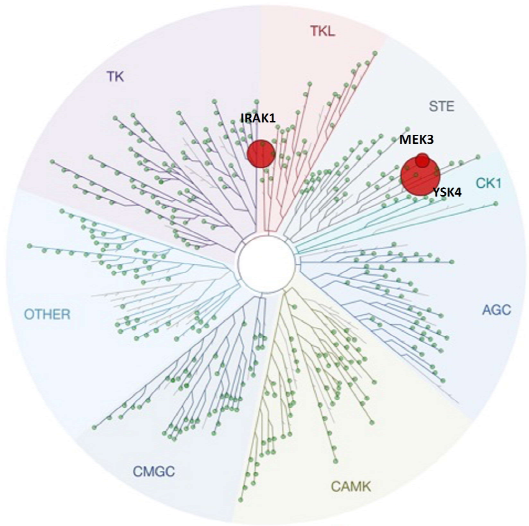
Hatcher et al, ASC Med. Chem. Let. 2020

JH-X-119-01 Pharmacokinetics

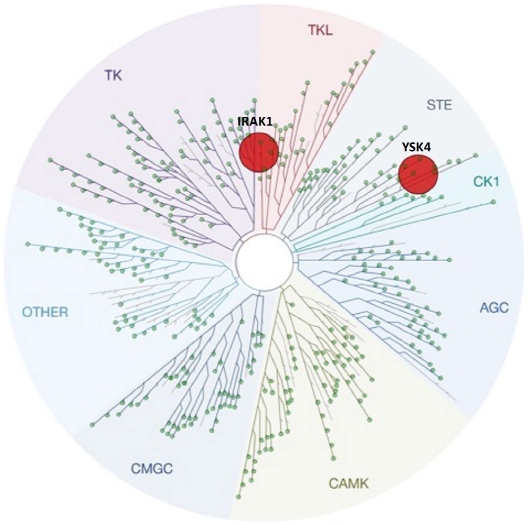
JH-X-119-01Mouse PK											
Dose: 2mg/Kg IV and 10mg/Kg PO											
Formulation: 0.4mg/mL solution in 5/95 DSMSO/30%Captisol											
Subject	**$T_{1/2}$**	**T_{max}**	**C_{max}**	**C_{max}**	**AUC_{last}**	**AUC_{last}**	**AUC_{INF_obs}**	**AUC**	**Cl_obs**	**MRT_{INF_obs}**	**Vss_obs**
	hr	***hr***	***ng/mL***	***µM***	***min*ng/mL***	***µM.hr***	***min*ng/mL***	***%Extrap***	***mL/min/kg***	***hr***	***L/kg***
IV Mouse-1	1.68	0.08	4880	10.78	129905	4.78	130457	0.42	15.33	0.51	0.47
IV Mouse-2	1.41	0.08	4140	9.15	75923	2.80	76300	0.49	26.21	0.26	0.41
IV Mouse-3	1.74	0.08	4490	9.92	132660	4.89	133429	0.58	14.99	0.57	0.52
Avg.	**1.61**	**0.08**	**4503**	**9.95**	**112829**	**4.16**	**113395**	**0.50**	**18.84**	**0.45**	**0.46**
Subject	**$T_{1/2}$**	**T_{max}**	**C_{max}**	**C_{max}**	**AUClast**	**AUC_{last}**	**AUC_{INF_obs}**	**$AUC_{\%Extrap}$**	**Cl_obs**	**F%**	
	hr	***hr***	***ng/mL***	***µM***	***min*ng/mL***	***µM.hr***	***min*ng/mL***		***mL/min/kg***		
PO Mouse-4	2.91	0.08	46	0.10	4726	0.17	5405	12.56	1850.15		
PO Mouse-5	2.06	0.25	28	0.06	6093	0.22	6611	7.83	1512.66		
PO Mouse-6	1.75	0.50	39	0.09	4562	0.17	4773	4.43	2095.04		
Avg.	**2.24**	**0.28**	**38**	**0.08**	**5127**	**0.19**	**5596**	**8.28**	**1819.28**		



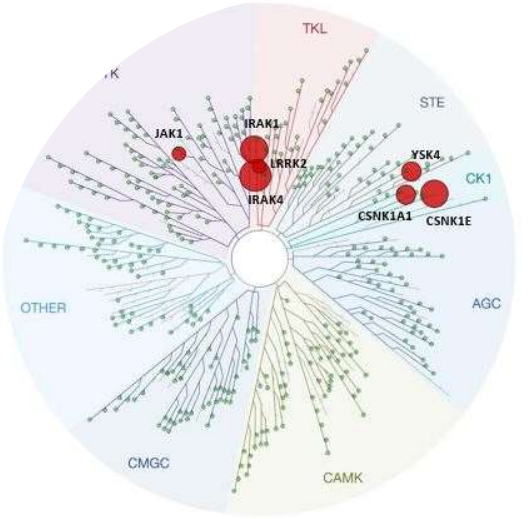
Development of Bioavailable Dual IRAK 1,4 Inhibitors



JHX-119-01



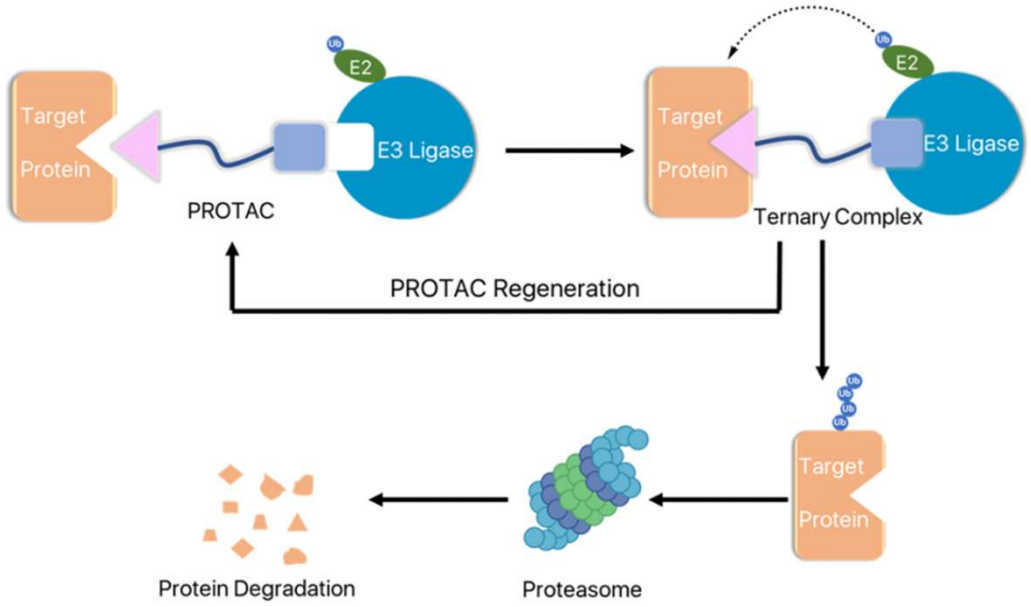
PS-IV-91



JH-XIII-82-01

F=14%

PROTACs-PROteolysis TArgeting Chimeras



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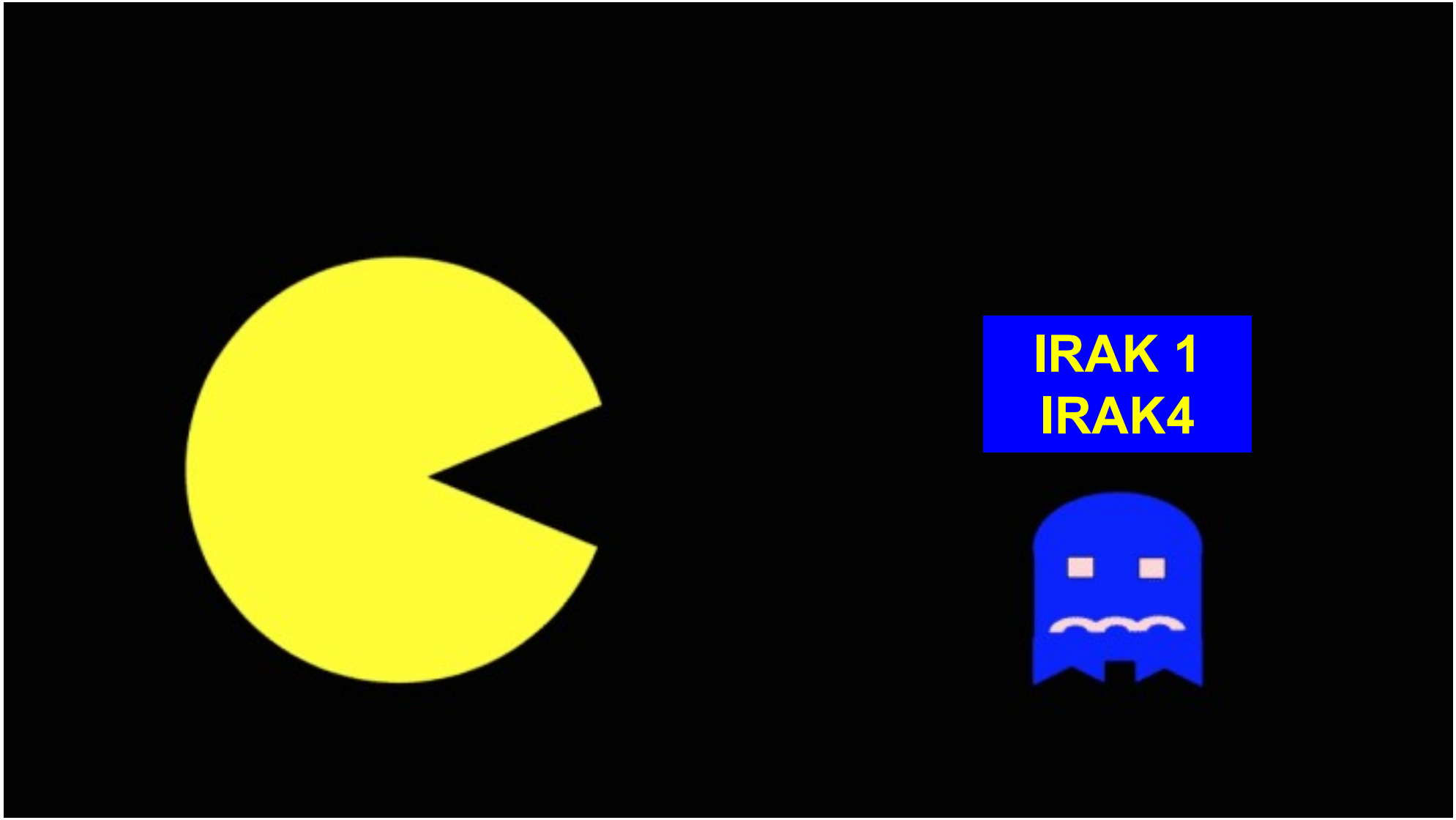
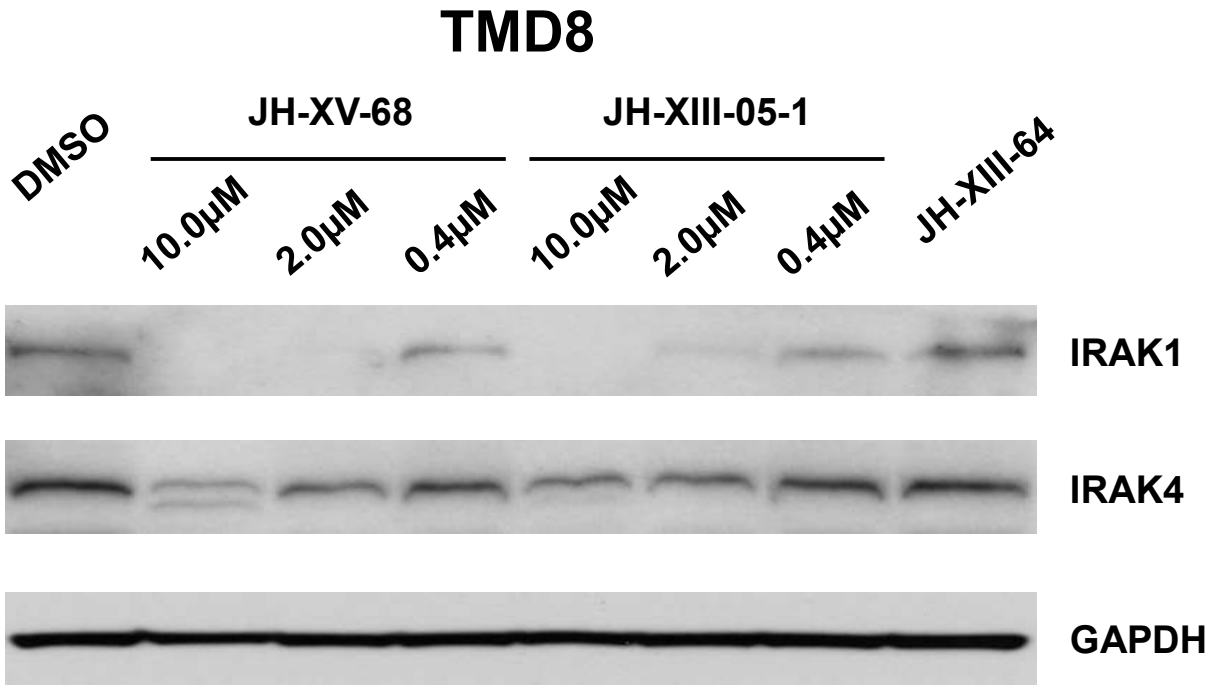
IWMF
International Waldenstrom's Macroglobulinemia Foundation

- **Hijack the cell's garbage disposal system to promote destruction of disease-causing proteins.**
- **Efficacy dependent on protein resynthesis instead of only on-off rate of inhibitor.**
- **Powerful strategy for traditionally undruggable targets.**
- **Quickly growing approach with 18 PROTACs undergoing clinical evaluation.**


IRAK 1
IRAK4

Dual IRAK1/4 degrader JH-XIII-05-1 in TMD8 cells

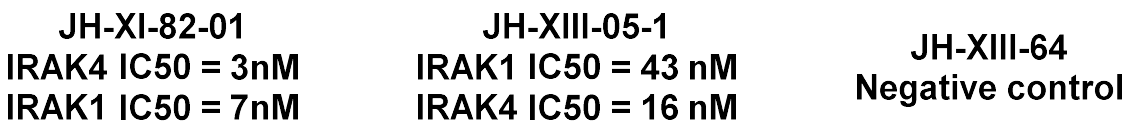


- JH-XIII-05-1 utilizes cereblon-targeting warhead.
- JH-XV-68 utilizes VHL-targeting warhead.
- JH-XIII-64 has no warhead (negative control).

Preliminary Data, DFCI.

Dual IRAK1/4 PROTAC JH-XIII-05-1 shows increased killing compared to parental dual IRAK1/4 inhibitor

ED50	JH-VIII-64	JH-XI-82-01	JH-XIII-05-1
BCWM.1	1.67E-05	1.19E-06	6.96E-09
MWCL-1	1.91E-05	2.19E-06	1.57E-05
TMD8	1.23E-05	1.05E-06	9.55E-09
HBL-1	6.89E-06	6.01E-07	3.04E-08
OCI-Ly7	8.41E-06	1.43E-06	2.30E-08
OCI-Ly19	1.12E-05	2.91E-07	2.93E-09
Ramos	5.64E-06	2.74E-07	9.27E-08

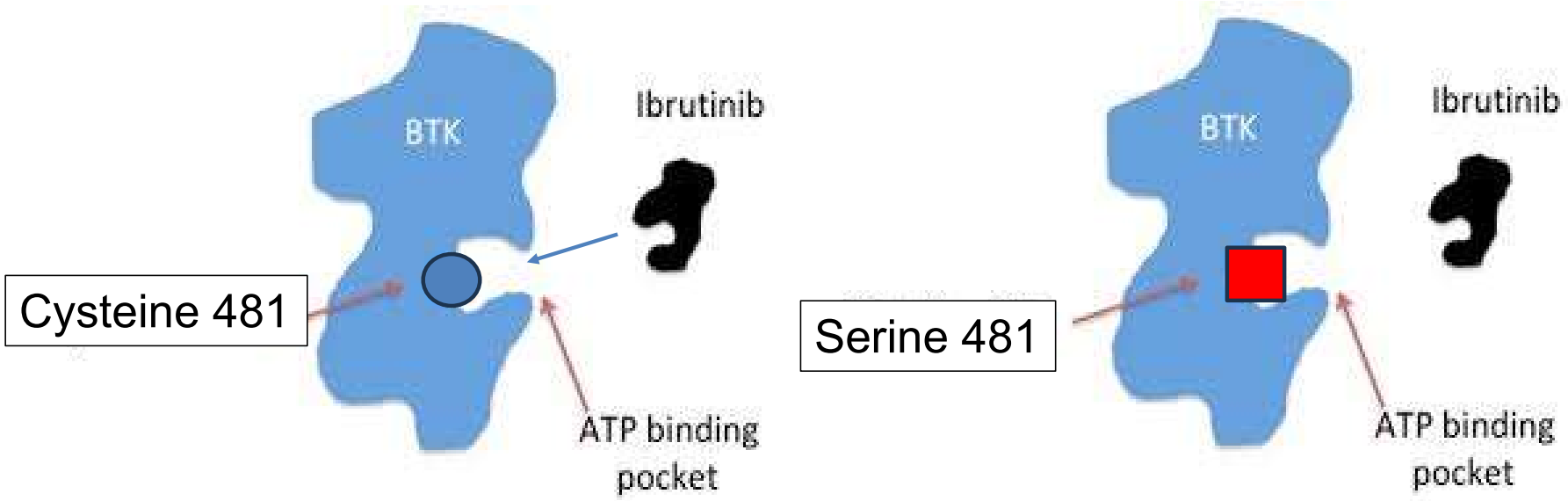


- JH-XI-82-01 is the parental IRAK1/4 inhibitor from which JH-XIII-05-1 was designed.
- JH-XIII-64 is the negative control of JH-XIII-05-1 that cannot cause degradation.
- JH-XIII-05-1 shows a drastic increase in cell killing compared to the parental IRAK1/4 inhibitor as well as the negative control.

Preliminary Data, DFCI.

Acquired Resistance to BTK-Inhibitors

Acquired Resistance to Ibrutinib



-Acquired ibrutinib resistance is commonly due to mutations at amino acid position 481 on BTK which is the site for drug binding.

-BTK Cys481 is also where zanubrutinib, acalabrutinib and tirabrutinib bind.

Lian Xu MS

BTK Cys481 Mutations are common in WM Patients who progress on Ibrutinib.

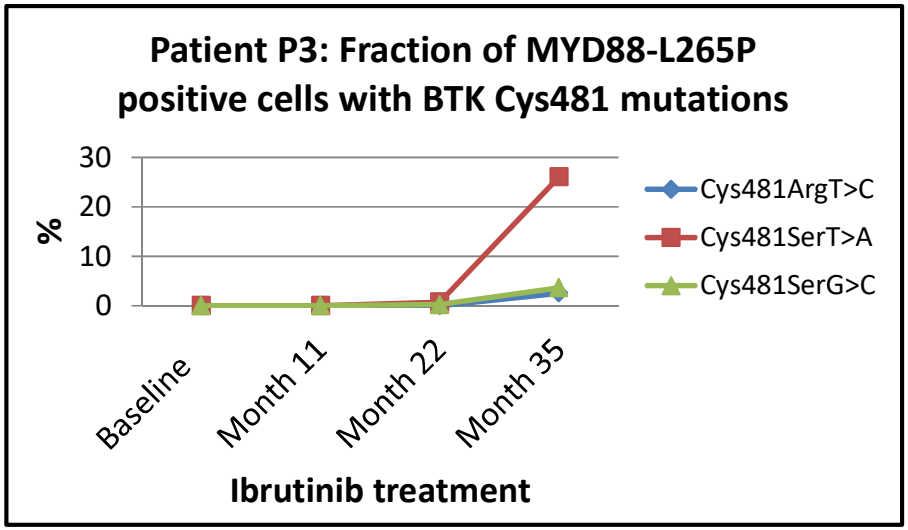
Patient*	L265P positive cells with BTK C481R$^{T>C}$	L265P positive cells with BTK C481S$^{T>A}$	L265P positive cells with BTK C481S$^{G>C}$	L265P positive cells with BTK C481Y$^{G>A}$	L265P positive cells with PLCG2 Y495H$^{T>C}$	L265P positive cells with CARD11 L878F$^{C>T}$
P1	None	None	None	None	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 with multiple BTK Cys^{481} mutations



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%

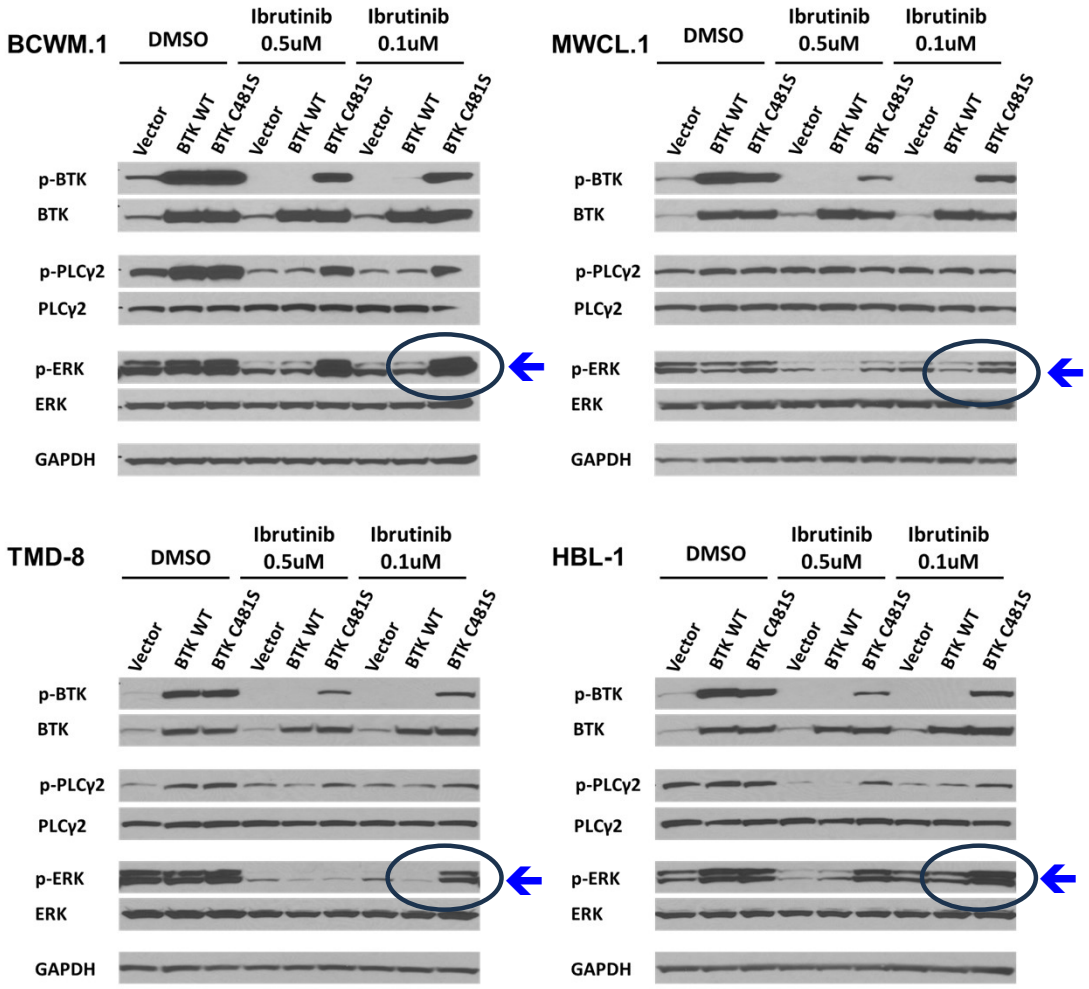
Xu et al, Blood 2017

BTK Cys481 mutant expressing cells show ERK 1/2 activation in the presence of ibrutinib.

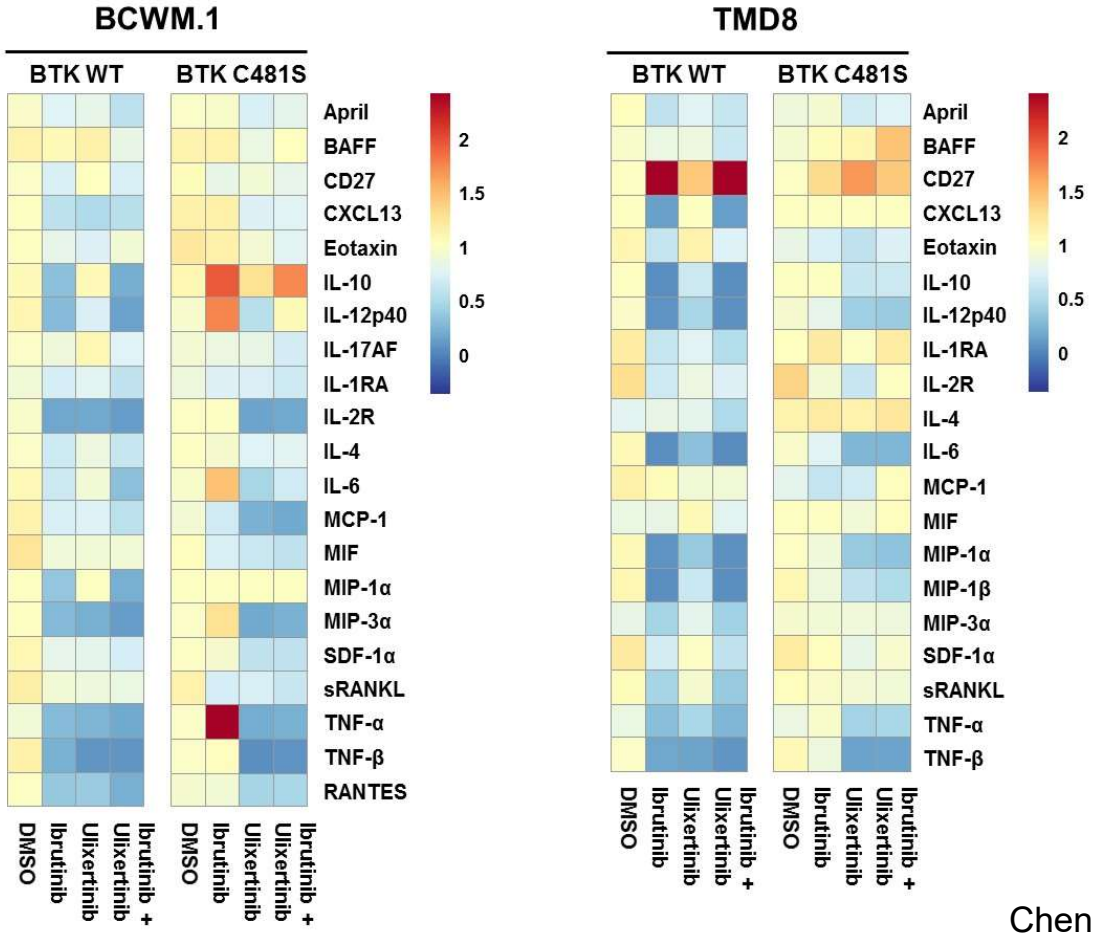
Jiaji Chen

Xia Liu MD

Chen et al, Blood 2018

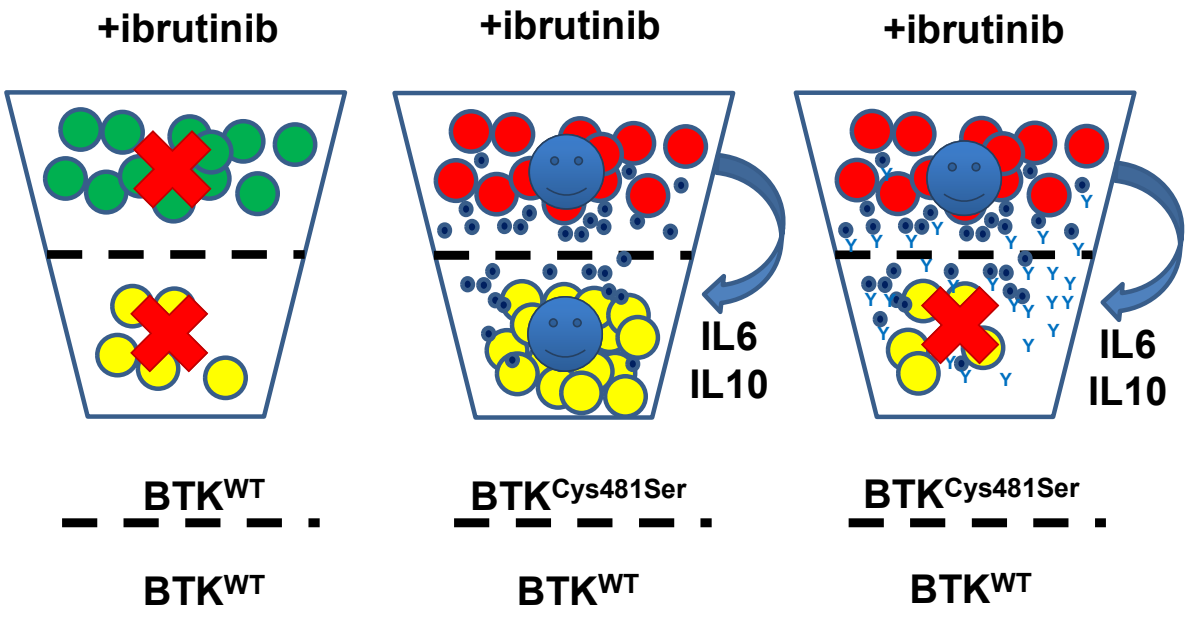


BTK mutated cells release inflammatory cytokines in the presence of ibrutinib that can be blocked by the ERK-inhibitor ulixertinib



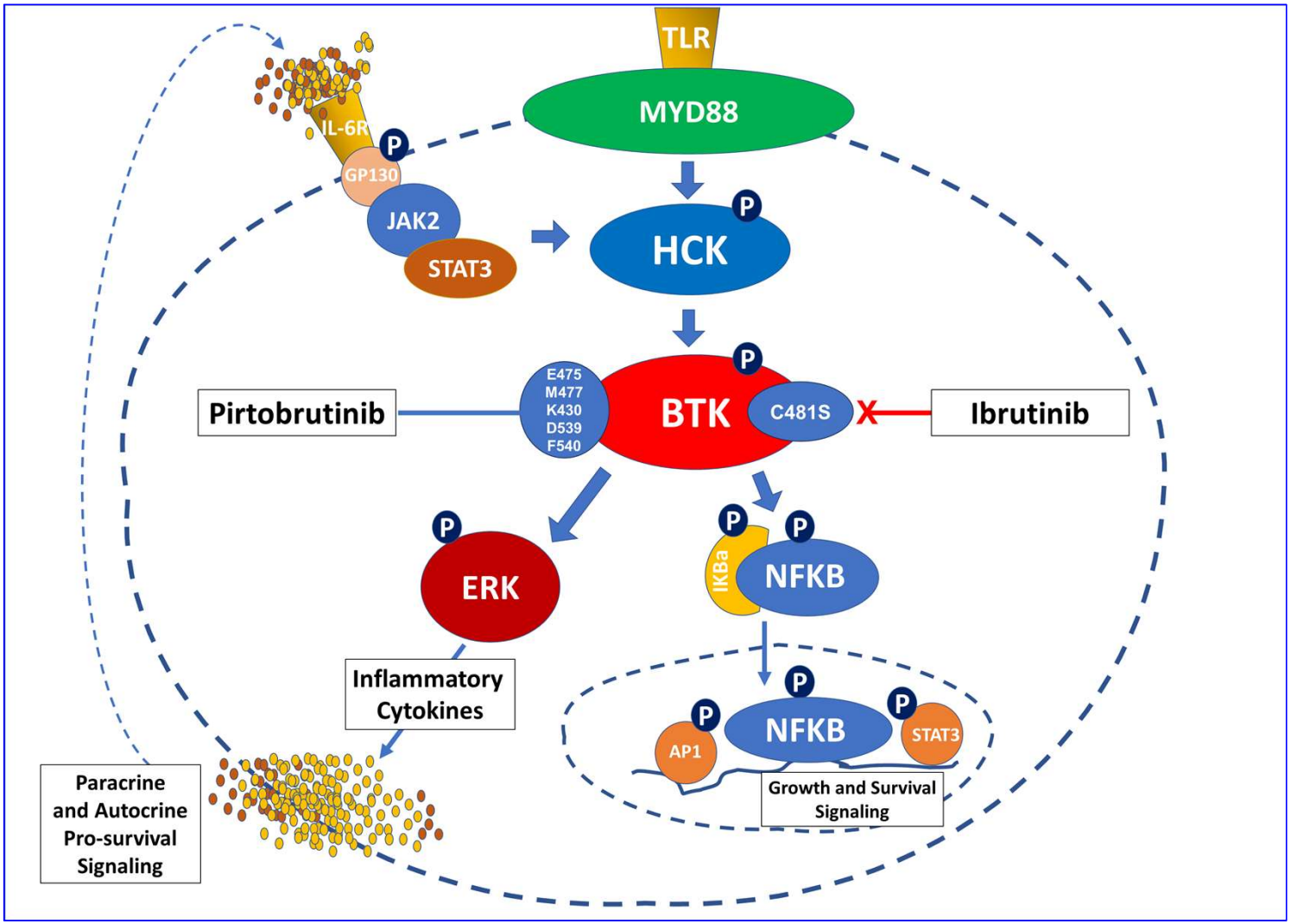
Chen et al, BLOOD 2018

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

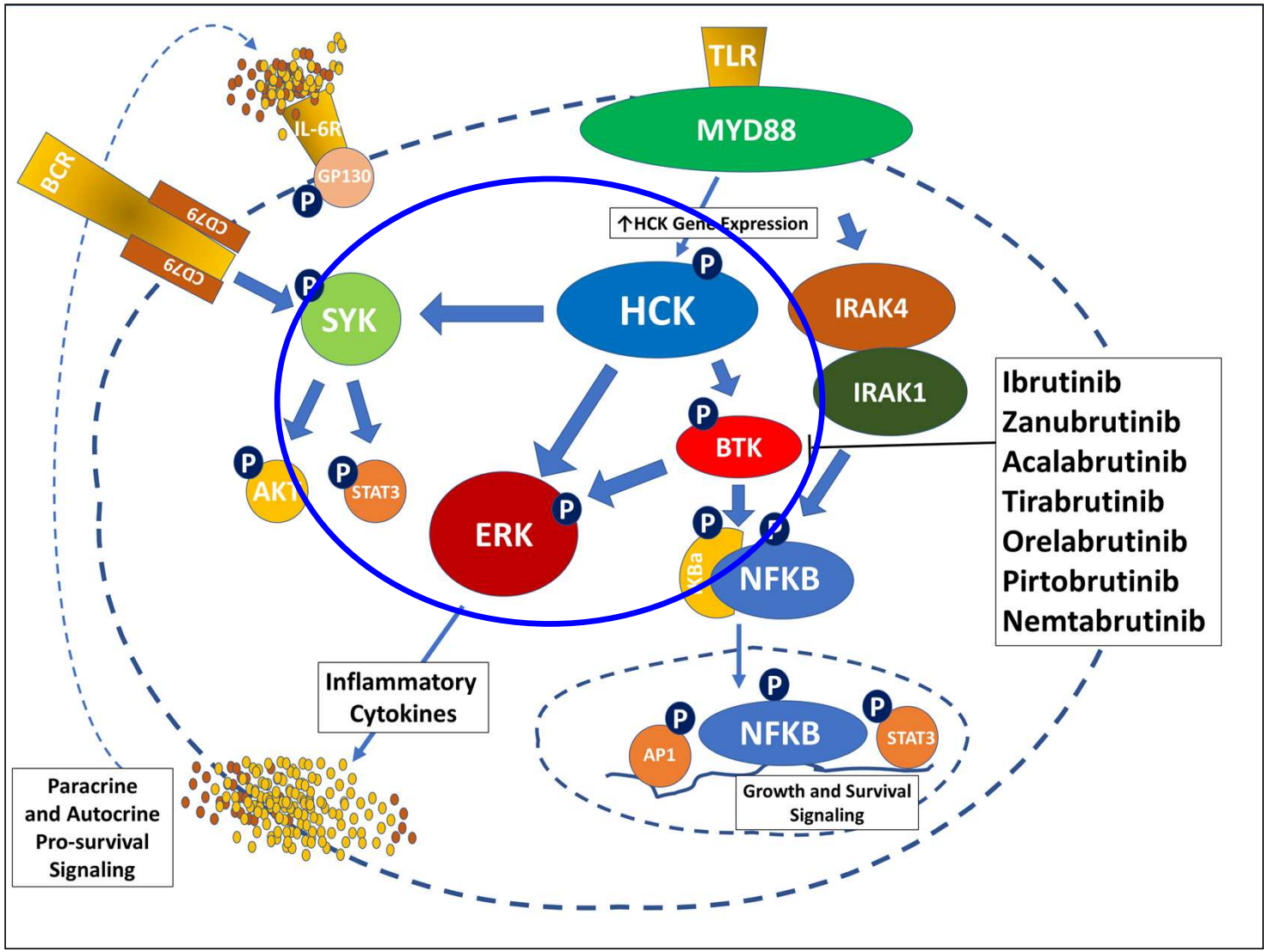


Chen et al, Blood 2018

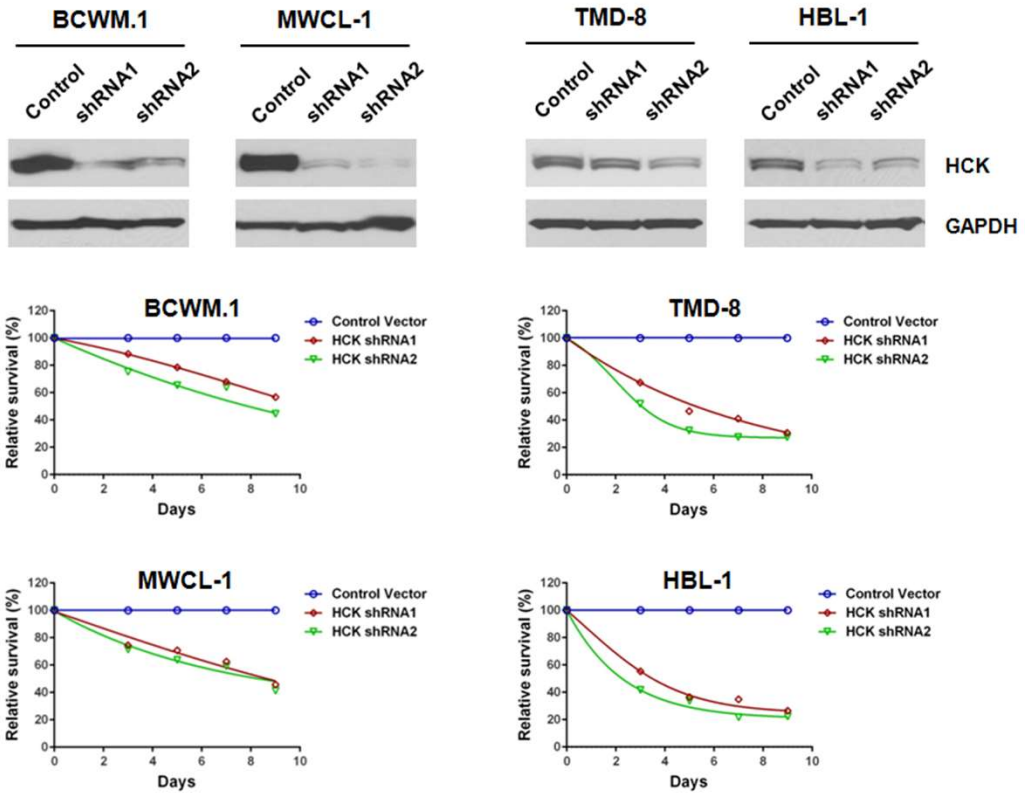
Non-Covalent BTK Inhibitors to Overcome Acquired Resistance to Ibrutinib



Targeting Acquired Resistance: HCK Inhibitors

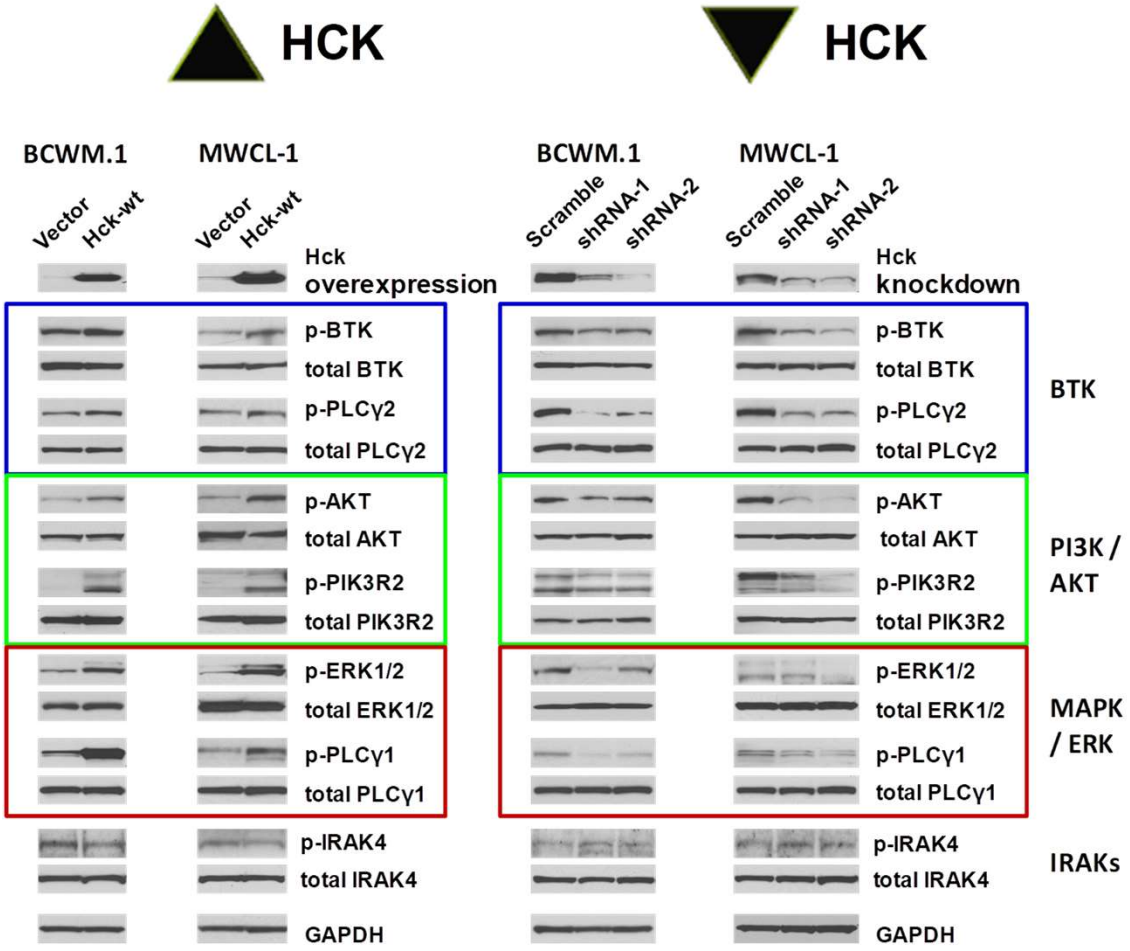


HCK is an important survival determinant in MYD88 mutated WM and ABC DLBCL cells.



Yang et al, Blood 2016.

HCK promotes pro-survival signaling in MYD88 mutated WM cells

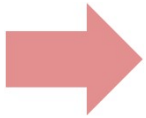


Yang et al, Blood 2016.

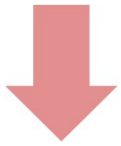
Development of HCK Inhibitors

Collaboration between Bing Center for WM and Harvard Medicinal Chemistry Labs to develop novel, potent HCK inhibitors. Supported by NIH, IWMF, LLS.

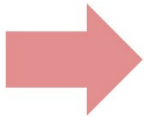
Chemical leads
identified in a screen of 220 clinical and preclinical kinase inhibitors for antiproliferative effect on WM cell lines



Lead optimization
> 400 analogs synthesized and triaged according to assay flowchart

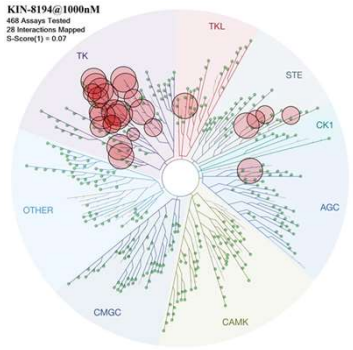


Target deconvolution
HCK, BTK, LYN



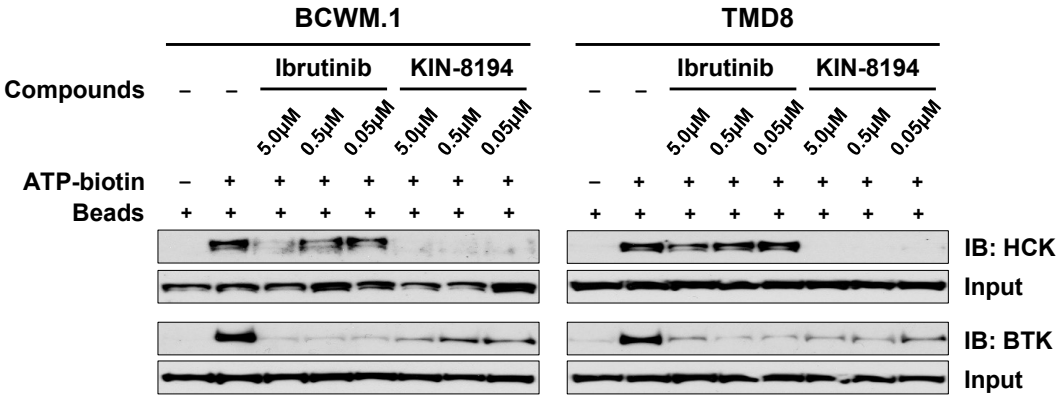
KIN-8194

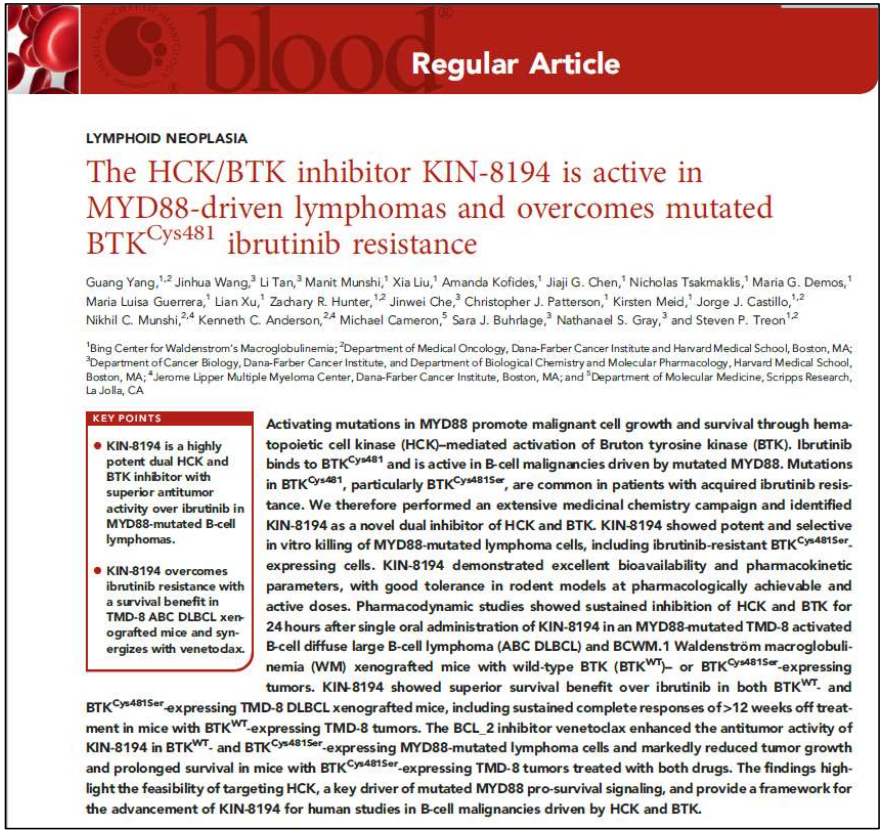
KIN-8194 is a highly potent, dual HCK/BTK Inhibitor



KINOMEscan® against a panel of 468 kinases. KIN-8194 at 1.0 uM showed good selectivity (S10=0.07)

Kinases	Enzymatic IC50 (nM)	Kinase group	Kinase family
HCK	<0.495	TK	SRC
BLK	<0.495	TK	SRC
BTK	0.915	TK	TEC
LYN	1.150	TK	SRC
FRK	1.400	TK	SRC
ACK (TNK2)	7.780	TK	ACK
CSK	16.100	TK	CSK
ErbB2	52.600	TK	EPH
ABL	98.600	TK	ABL




blood
Regular Article

LYMPHOID NEOPLASIA

The HCK/BTK inhibitor KIN-8194 is active in MYD88-driven lymphomas and overcomes mutated BTK^{Cys481} ibrutinib resistance

Guang Yang,[1,2] Jinhua Wang,[3] Li Tan,[3] Manit Munshi,[1] Xia Liu,[1] Amanda Kofides,[1] Jiaji G. Chen,[1] Nicholas Tsakmaklis,[1] Maria G. Demos,[1] Maria Luisa Guerrera,[1] Lian Xu,[1] Zachary R. Hunter,[1,2] Jinwei Che,[3] Christopher J. Patterson,[1] Kirsten Meid,[1] Jorge J. Castillo,[1,2] Nikhil C. Munshi,[2,4] Kenneth C. Anderson,[2,4] Michael Cameron,[5] Sara J. Buhrlage,[3] Nathanael S. Gray,[3] and Steven P. Treon[1,2]

[1]Bing Center for Waldenstrom's Macroglobulinemia; [2]Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA; [3]Department of Cancer Biology, Dana-Farber Cancer Institute, and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA; [4]Jerome Lipper Multiple Myeloma Center, Dana-Farber Cancer Institute, Boston, MA; and [5]Department of Molecular Medicine, Scripps Research, La Jolla, CA

KEY POINTS

- KIN-8194 is a highly potent dual HCK and BTK inhibitor with superior antitumor activity over ibrutinib in MYD88-mutated B-cell lymphomas.
- KIN-8194 overcomes ibrutinib resistance with a survival benefit in TMD-8 ABC DLBCL xenografted mice and synergizes with venetoclax.

Activating mutations in MYD88 promote malignant cell growth and survival through hematopoietic cell kinase (HCK)–mediated activation of Bruton tyrosine kinase (BTK). Ibrutinib binds to BTK^{Cys481} and is active in B-cell malignancies driven by mutated MYD88. Mutations in BTK^{Cys481}, particularly $BTK^{Cys481Ser}$, are common in patients with acquired ibrutinib resistance. We therefore performed an extensive medicinal chemistry campaign and identified KIN-8194 as a novel dual inhibitor of HCK and BTK. KIN-8194 showed potent and selective in vitro killing of MYD88-mutated lymphoma cells, including ibrutinib-resistant $BTK^{Cys481Ser}$–expressing cells. KIN-8194 demonstrated excellent bioavailability and pharmacokinetic parameters, with good tolerance in rodent models at pharmacologically achievable and active doses. Pharmacodynamic studies showed sustained inhibition of HCK and BTK for 24 hours after single oral administration of KIN-8194 in an MYD88-mutated TMD-8 activated B-cell diffuse large B-cell lymphoma (ABC DLBCL) and BCWM.1 Waldenström macroglobulinemia (WM) xenografted mice with wild-type BTK (BTK^{WT})– or $BTK^{Cys481Ser}$–expressing tumors. KIN-8194 showed superior survival benefit over ibrutinib in both BTK^{WT}- and $BTK^{Cys481Ser}$–expressing TMD-8 DLBCL xenografted mice, including sustained complete responses of >12 weeks off treatment in mice with BTK^{WT}–expressing TMD-8 tumors. The BCL_2 inhibitor venetoclax enhanced the antitumor activity of KIN-8194 in BTK^{WT}- and $BTK^{Cys481Ser}$–expressing MYD88-mutated lymphoma cells and markedly reduced tumor growth and prolonged survival in mice with $BTK^{Cys481Ser}$–expressing TMD-8 tumors treated with both drugs. The findings highlight the feasibility of targeting HCK, a key driver of mutated MYD88 pro-survival signaling, and provide a framework for the advancement of KIN-8194 for human studies in B-cell malignancies driven by HCK and BTK.

IWMF International Waldenstrom's Macroglobulinemia Foundation

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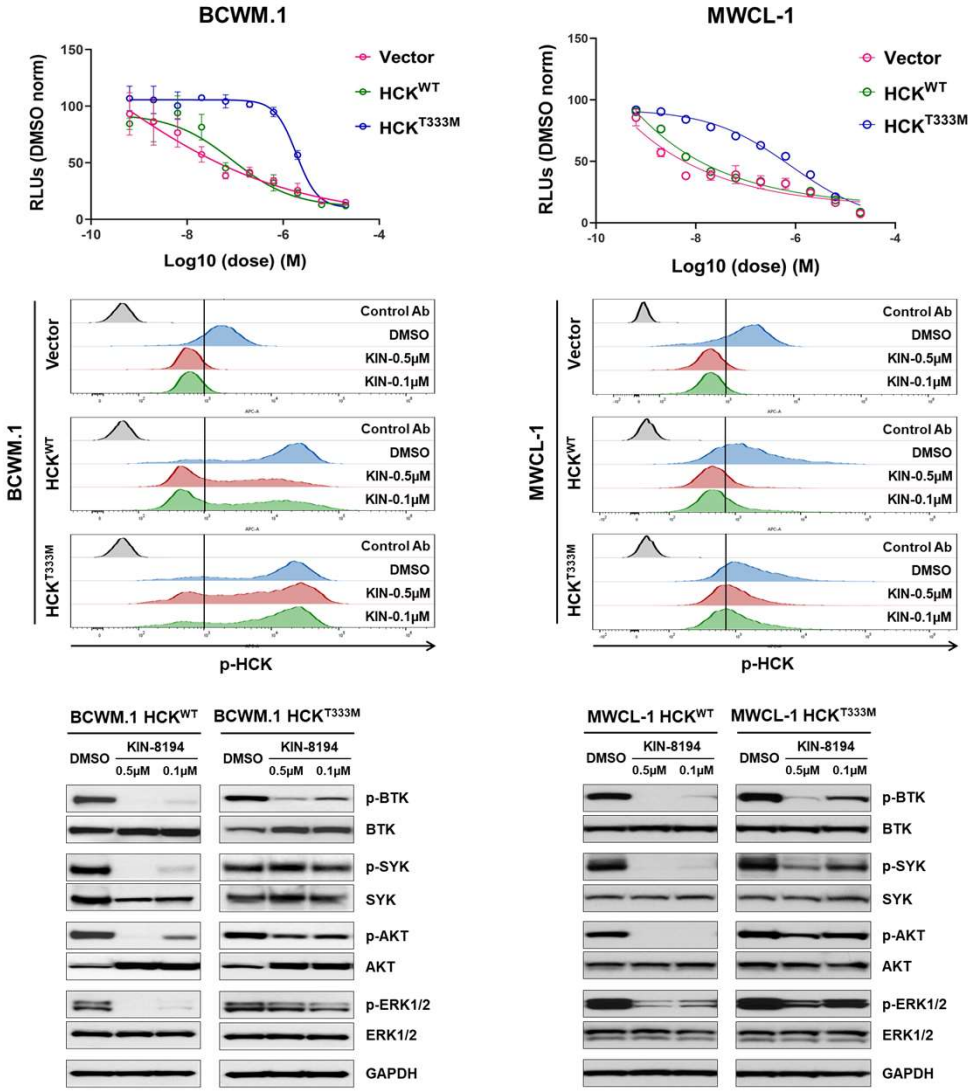
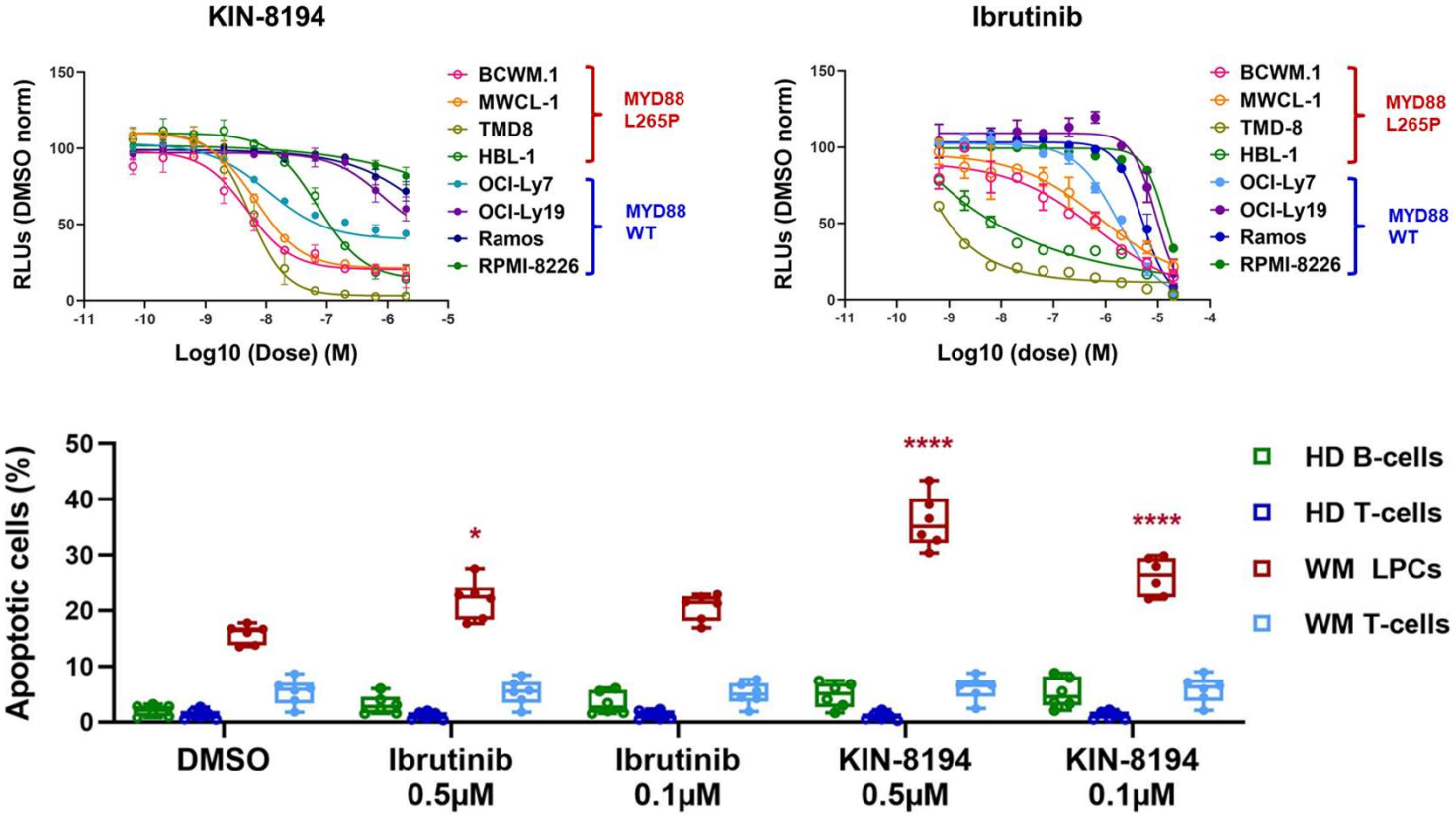
HCK and BTK are key targets of KIN-8194 activity in MYD88 mutated WM cells.



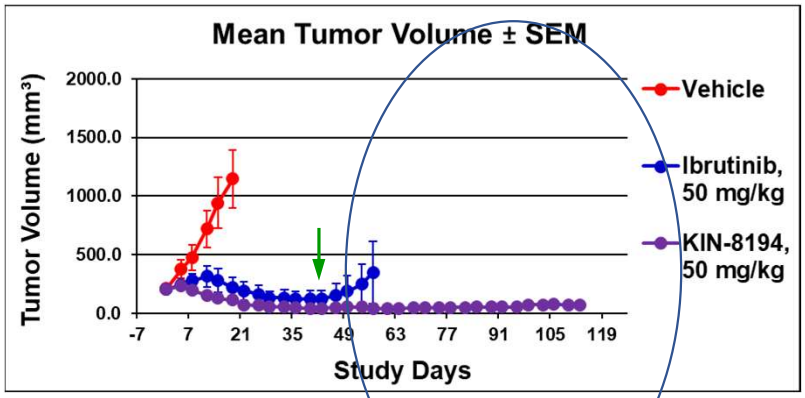
Yang et al, Blood. 2021;138(20):1966-1979

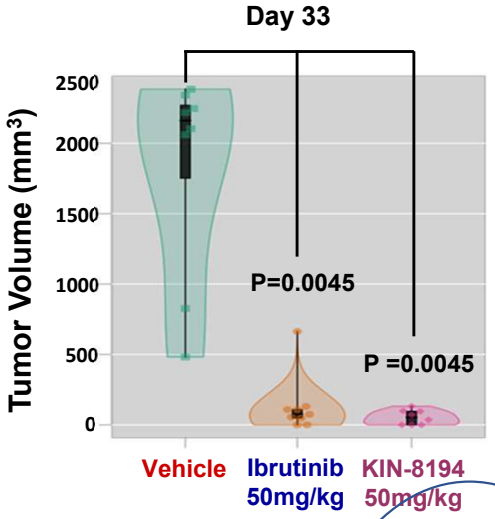
KIN-8194 shows selective killing of MYD88 tumor cells

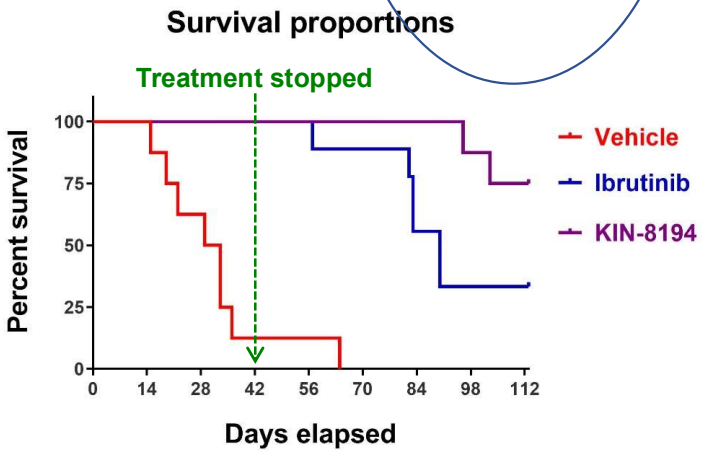


Yang et al, Blood. 2021;138(20):1966-1979

KIN-8194 in BTK unmutated TMD8 xenografted mice





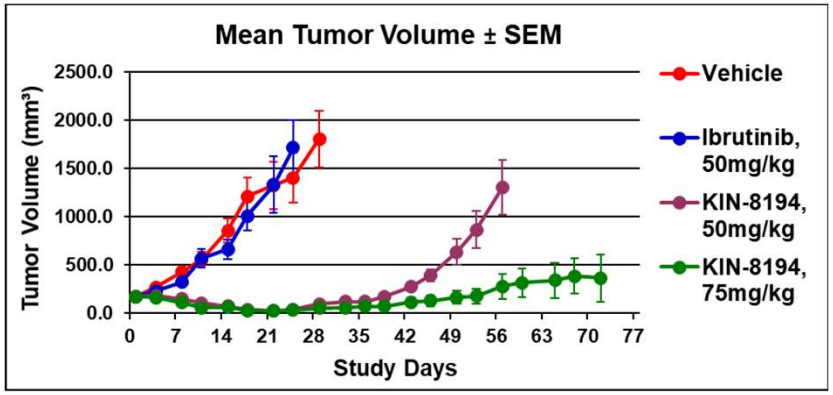


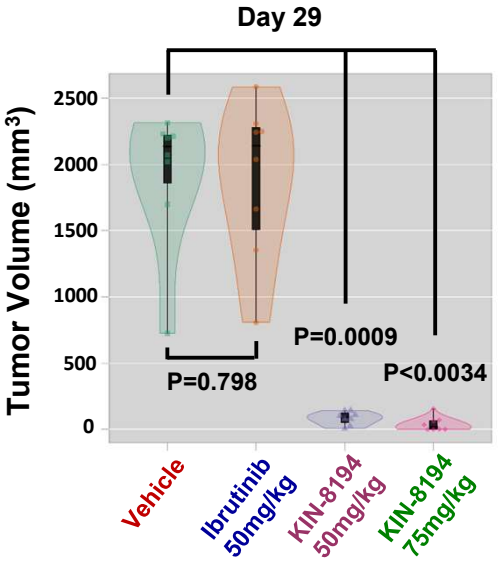
Median Survival	Vehicle	Ibrutinib (50mg/kg)	KIN-8194 (50mg/kg)
(days)	31	90	Undefined

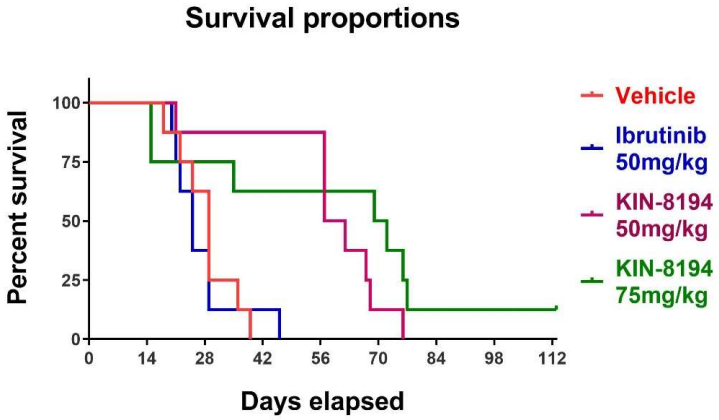
Log-rank (Mantel-Cox) test, P<0.0001

Yang et al, Blood 2021

KIN-8194 in BTK Cys481 mutated TMD8 xenografted mice







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

Log-rank (Mantel-Cox) test, P=0.0007

Yang et al, Blood 2021

Activity of KIN-8194 in Mantle Cell Lymphoma

ARTICLE OPEN

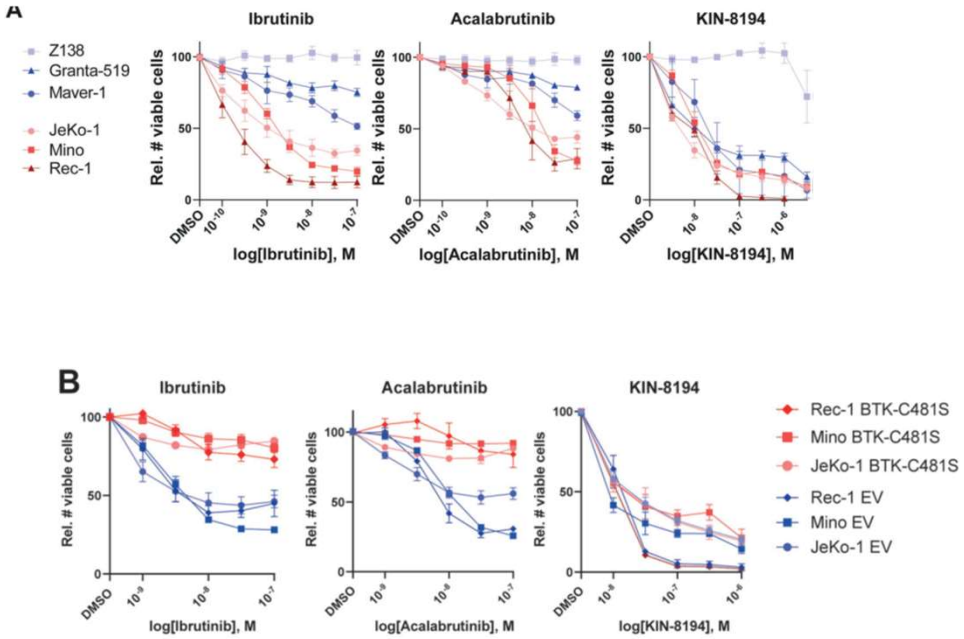
LYMPHOMA

The dual HCK/BTK inhibitor KIN-8194 impairs growth and integrin-mediated adhesion of BTKi-resistant mantle cell lymphoma

Hildo C. Lantermans[1,2,3], Fangxue Ma[1,2,3], Annemieke Kuil[1,2,3], Sanne van Kesteren[1,2,3], Sevtap Yasinoglu[1,2,3], Guang Yang[4,5,9], Sara J. Buhrlage[6], Jinhua Wang[6], Nathanael S. Gray[7], Marie José Kersten[2,8], Steven P. Treon[4,5], Steven T. Pals[1,2,3,10] and Marcel Spaargaren[1,2,3,10]

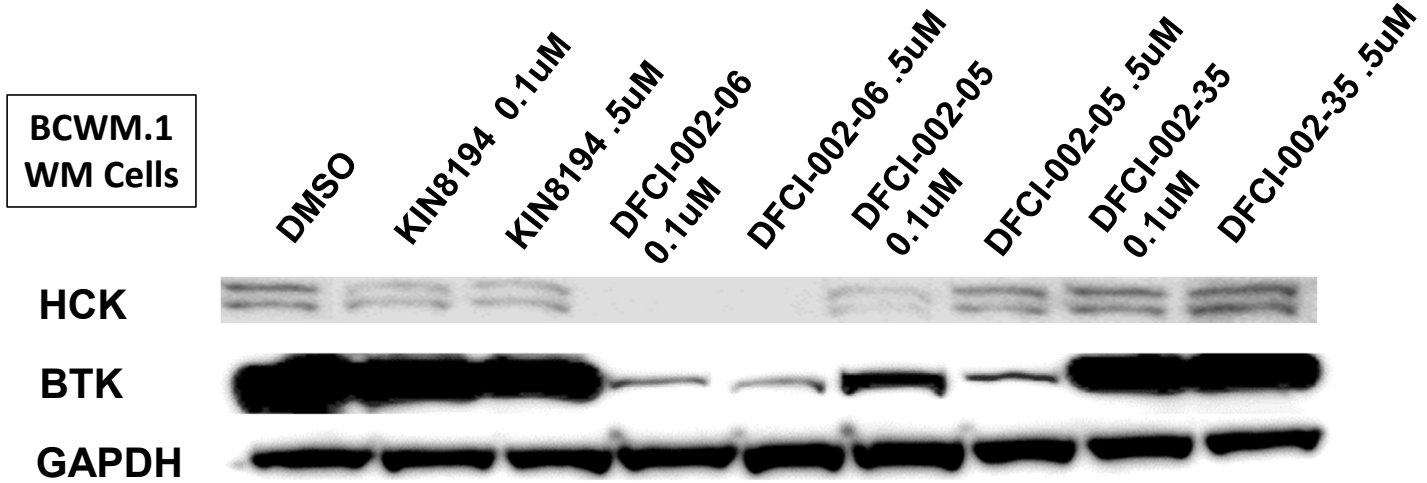
Although Bruton's tyrosine kinase (BTK) inhibitors (BTKi) have significantly improved patient prognosis, mantle cell lymphoma (MCL) is still considered incurable due to primary and acquired resistance. We have recently shown that aberrant expression of the Src-family tyrosine kinase hematopoietic cell kinase (HCK) in MCL correlates with poor prognosis, and that genetic HCK perturbation impairs growth and integrin-mediated adhesion of MCL cells. Here, we show that KIN-8194, a dual inhibitor of BTK and HCK with in vivo activity against Myd88-L265P-driven diffuse large B-cell lymphoma and Waldenström Macroglobulinemia, has a potent growth inhibitory effect in MCL cell lines and primary MCL cells, irrespective of their sensitivity to BTKi (ibrutinib and acalabrutinib). In BTKi-resistant cells this is mediated by inhibition of HCK, which results in repression of AKT-S6 signaling. In addition, KIN-8194 inhibits integrin-mediated adhesion of BTKi-sensitive and insensitive MCL cells to fibronectin and stromal cells in an HCK-dependent manner. Finally, we show that MCL cells with acquired BTKi resistance retain their sensitivity to KIN-8194. Taken together, our data demonstrate that KIN-8194 inhibits growth and integrin-mediated adhesion of BTKi-sensitive MCL cells, as well as MCL cells with primary or acquired BTKi resistance. This renders KIN-8194 a promising novel treatment for MCL patients.

Leukemia; https://doi.org/10.1038/s41375-024-02207-9



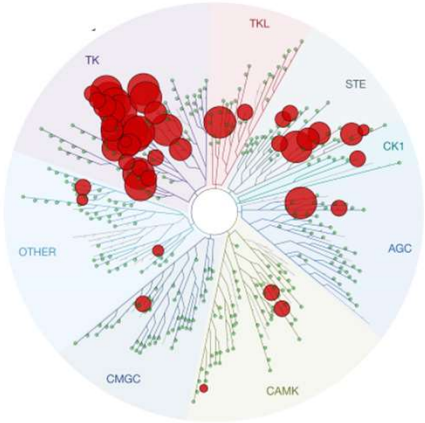
Lantermans et al, Leukemia. 2024 Mar 7. doi: 10.1038/s41375-024-02207-9.

Characterization of Novel bifunctional BTK/HCK PROTACS

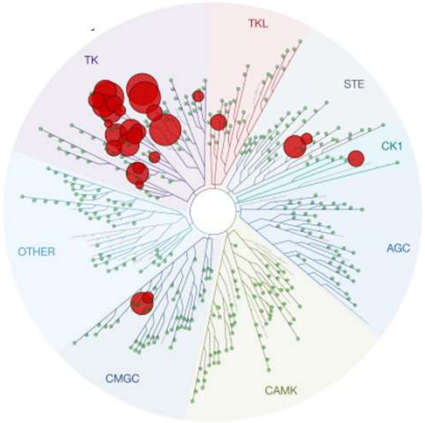


John Hatcher Ph.D.

KIN-8194



DFCI-002-05



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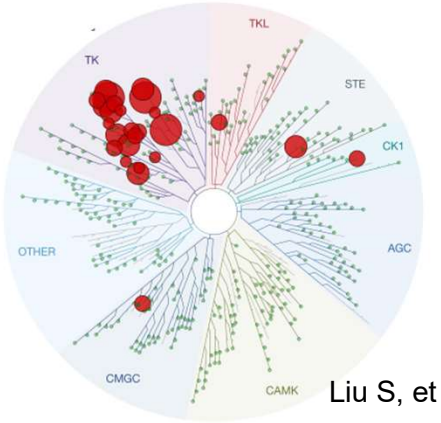
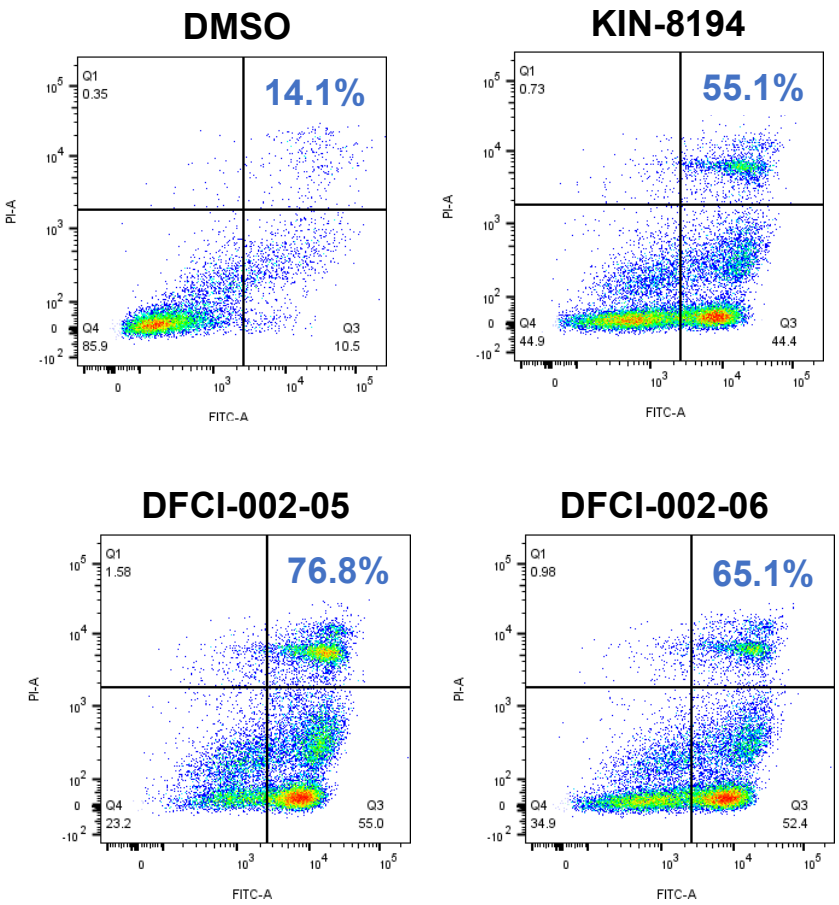
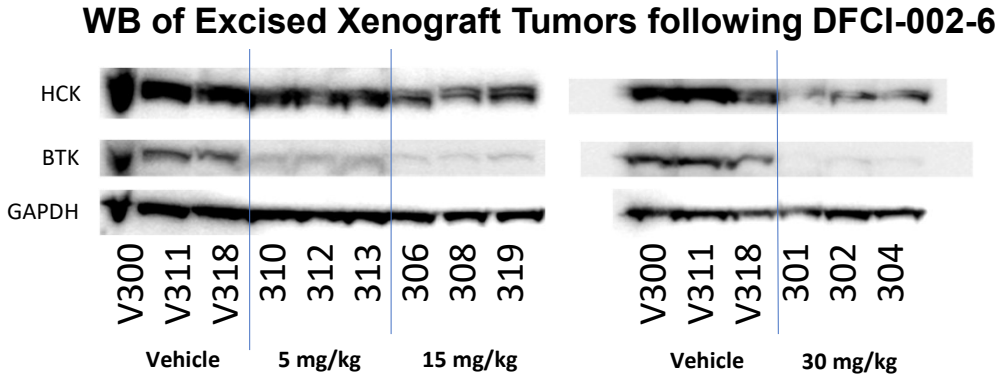


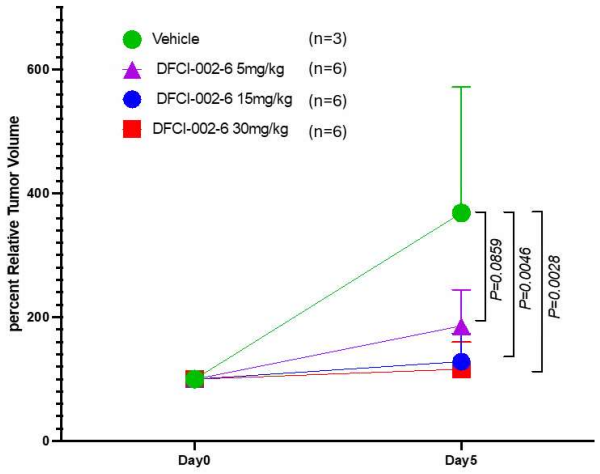
Kinome Studies performed at 1 uM

Liu S, et al. Blood (2023) 142 (Supplt 1): 3298.

Development of Highly Potent and Bioavailable dual Bifunctional BTK/HCK PROTACS







Liu S, et al. Blood (2023) 142 (Supplt 1): 3298.

Preliminary Data, DFCI

DFCI-002-05 Pharmacokinetics

Individual and mean plasma concentration-time data of DFCI-002-5-B2 after an IV dose at 2 mg/kg in male C57BL/6 mice

Dose (mg/kg)	Dose route	Sampling time (hr)	Concentration (ng/mL) Individual			Mean (ng/mL)	SD	CV(%)
2	IV	Pre-dose	BQL	BQL	BQL	**BQL**	NA	NA
		0.083	595	329	519	**481**	137	28.5
		0.25	601	196	386	**394**	203	51.4
		0.5	435	342	350	**375**	51.4	13.7
		1	419	248	303	**323**	87.4	27.0
		2	336	176	345	**286**	95.4	33.4
		4	267	276	276	**273**	4.82	1.77
		8	219	104	136	**153**	59.5	38.9
		24	69.7	39.0	54.9	**54.6**	15.3	28.1

PK parameters	Unit	Estimated Value
Rsq_adjusted		0.975
CL	L/hr/kg	0.448
V_{ss}	L/kg	5.34
$T_{1/2}$	hr	8.96
Regression Points	hr	5
AUC_{last}	hr*ng/mL	3761
AUC_{INF}	hr*ng/mL	4466
MRT_{last}	hr	7.23
MRT_{INF}	hr	11.9
C_0	ng/mL	531

Individual and mean plasma concentration-time data of DFCI-002-5-B2 after a PO dose at 10 mg/kg in male C57BL/6 mice

Dose (mg/kg)	Dose route	Sampling time (hr)	Concentration (ng/mL) Individual			Mean (ng/mL)	SD	CV(%)
10	PO	Pre-dose	BQL	BQL	BQL	**BQL**	NA	NA
		0.083	5.73	19.5	40.0	**21.7**	17.3	79.4
		0.25	68.6	84.3	40.1	**64.3**	22.4	34.9
		0.5	173	129	175	**159**	26.4	16.6
		1	168	243	163	**191**	44.7	23.4
		2	356	445	155	**318**	148	46.6
		4	533	518	604	**552**	45.8	8.31
		8	423	540	346	**436**	97.8	22.4
		24	136	194			46.3	32.2

PK parameters	Unit	Estimated Value
Rsq_adjusted		
T_{max}	hr	
C_{max}	ng/mL	
$T_{1/2}$	hr	
Regression Points	hr	
AUC_{last}	hr*ng/mL	7868
AUC_{INF}	hr*ng/mL	
MRT_{last}	hr	
MRT_{INF}	hr	
F	%	

552 nM

42%

Individual and mean plasma concentration-time data of DFCI-002-5-B2 after an PO dose at 30 mg/kg in male C57BL/6 mice

Dose (mg/kg)	Dose route	Sampling time (hr)	Concentration (ng/mL) Individual			Mean (ng/mL)	SD	CV(%)
30	PO	Pre-dose	BQL	BQL	BQL	**BQL**	NA	NA
		0.083	12.0	24.1	24.9	**20.3**	7.22	35.5
		0.25	133	67.8	94.1	**98.4**	33.0	33.5
		0.5	215	308	240	**254**	48.3	19.0
		1	292	325	382	**333**	45.3	13.6
		2	963	516	718	**732**	224	30.6
		4	958	1250	1060	**1089**	148	13.6
		8	970	700	1143	**938**	223	23.8
		24	584	510	324	**473**	134	28.3

PK parameters	Unit	Estimated Value
Rsq_adjusted		0.999
T_{max}	hr	4.00
C_{max}	ng/mL	1089.4 (1.37 uM)
$T_{1/2}$	hr	16.5
Regression Points	hr	3
AUC_{last}	hr*ng/mL	17894
AUC_{INF}	hr*ng/mL	29133
MRT_{last}	hr	10.1
MRT_{INF}	hr	24.7
F	%	31.7

Individual and mean plasma concentration-time data of DFCI-002-5-B2 after a PO dose at 60 mg/kg in male C57BL/6 mice

Dose (mg/kg)	Dose route	Sampling time (hr)	Concentration (ng/mL) Individual			Mean (ng/mL)	SD	CV(%)
60	PO	Pre-dose	BQL	BQL	BQL	**BQL**	NA	NA
		0.083	13.8	10.8	24.8	**16.5**	7.37	44.8
		0.25	106	84.9	93.3	**94.8**	10.7	11.3
		0.5	247	184	184	**205**	36.3	17.7
		1	367	320	362	**350**	25.6	7.32
		2	813	640	638	**697**	100	14.4
		4	1183	723	505	**804**	346	43.1
		8	760	803	1188	**917**	236	25.7
		24	470	398	561	**477**	81.5	17.1

PK parameters	Unit	Estimated Value
Rsq_adjusted		NA
T_{max}	hr	8.00
C_{max}	ng/mL	916.83333 (1.15 uM)
$T_{1/2}$	hr	NA
Regression Points	hr	NA
AUC_{last}	hr*ng/mL	16798
AUC_{INF}	hr*ng/mL	NA
MRT_{last}	hr	10.5
MRT_{INF}	hr	NA
F	%	14.9

DFCI-002-06 Pharmacokinetics

Individual and mean plasma concentration-time data of DFCI-002-6-B2 after an IV dose at 2.61 mg/kg in male C57BL/6 mice

Dose (mg/kg)	Dose route	Sampling time (hr)	Concentration (ng/mL) Individual			Mean (ng/mL)	SD	CV(%)
2.61	IV	Pre-dose	BQL	BQL	BQL	**BQL**	NA	NA
		0.083	1380	1380	761	**1174**	357	30.4
		0.25	1410	1520	1300	**1410**	110	7.80
		0.5	1420	1170	1250	**1280**	128	9.97
		1	1250	1120	709	**1026**	282	27.5
		2	1160	1360	1260	**1260**	100	7.94
		4	1170	920	961	**1017**	134	13.2
		8	826	717	189	**577**	341	59.0
		24	242	258	238	**246**	10.6	4.30

PK parameters	Unit	Estimated Value
Rsq_adjusted		0.943
CL	L/hr/kg	0.146
V_{ss}	L/kg	1.97
$T_{1/2}$	hr	9.70
Regression Points	hr	7
AUC_{last}	hr*ng/mL	14421
AUC_{INF}	hr*ng/mL	17866
MRT_{last}	hr	7.66
MRT_{INF}	hr	13.5
C0	ng/mL	1174

Individual and mean plasma concentration-time data of DFCI-002-6-B2 after a PO dose at 10 mg/kg in male C57BL/6 mice

Dose (mg/kg)	Dose route	Sampling time (hr)	Concentration (ng/mL) Individual			Mean (ng/mL)	SD	CV(%)
10	PO	Pre-dose	BQL	BQL	BQL	**BQL**	NA	NA
		0.083	34.1	48.0	78.9	**53.7**	22.9	42.7
		0.25	129	343	205	**226**	108	48.1
		0.5	326	158	466	**317**	154	48.7
		1	645	470	790	**635**	160	25.2
		2	347	1500	900	**916**	577	63.0
		4	1180	325	1300	**935**	532	56.9
		8	1450	978	1270		238	19.3
		24	210	962				68.6

PK parameters	Unit	Estimated Value
Rsq_adjusted		
T_{max}	hr	
C_{max}	ng/mL	
$T_{1/2}$	hr	
Regression Points	hr	
AUC_{last}	hr*ng/mL	
AUC_{INF}	hr*ng/mL	
MRT_{last}	hr	
MRT_{INF}	hr	
F	%	

1232 nM

39%

Individual and mean plasma concentration-time data of DFCI-002-6-B2 after an PO dose at 30 mg/kg in male C57BL/6 mice

Dose (mg/kg)	Dose route	Sampling time (hr)	Concentration (ng/mL) Individual			Mean (ng/mL)	SD	CV(%)
30	PO	Pre-dose	BQL	BQL	BQL	**BQL**	NA	NA
		0.083	44.6	95.9	32.6	**57.7**	33.6	58.3
		0.25	285	219	296	**267**	41.6	15.6
		0.5	427	479	251	**386**	119	31.0
		1	975	1020	971	**989**	27.2	2.75
		2	1900	1570	1470	**1647**	225	13.7
		4	1510	1500	1050	**1353**	263	19.4
		8	2390	1610	2360	**2120**	442	20.8
		24	1430	1980	1290	**1567**	365	23.3

PK parameters	Unit	Estimated Value
Rsq_adjusted		NA
Tmax	hr	8.00
Cmax	ng/mL	2120 (2.66 uM)
T1/2	hr	NA
Regression Points	hr	NA
AUClast	hr*ng/mL	41212
AUCINF	hr*ng/mL	NA
MRTlast	hr	11.9
MRTINF	hr	NA
F	%	24.9

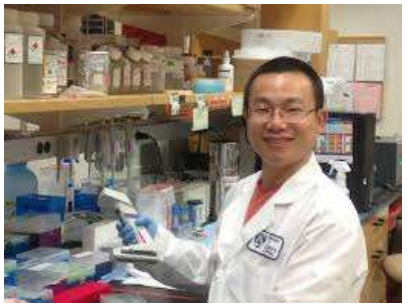
Individual and mean plasma concentration-time data of DFCI-002-6-B2 after a PO dose at 60 mg/kg in male C57BL/6 mice

Dose (mg/kg)	Dose route	Sampling time (hr)	Concentration (ng/mL) Individual			Mean (ng/mL)	SD	CV(%)
60	PO	Pre-dose	BQL	BQL	BQL	**BQL**	NA	NA
		0.083	37.3	45.7	55.8	**46.3**	9.26	20.0
		0.25	239	227	118	**195**	66.7	34.2
		0.5	304	494	533	**444**	123	27.6
		1	941	1080	1030	**1017**	70.4	6.92
		2	1530	1450	543	**1174**	548	46.7
		4	2000	2000	1540	**1847**	266	14.4
		8	1900	2300	2190	**2130**	207	9.70
		24	1900	2000	917	**1606**	598	37.3

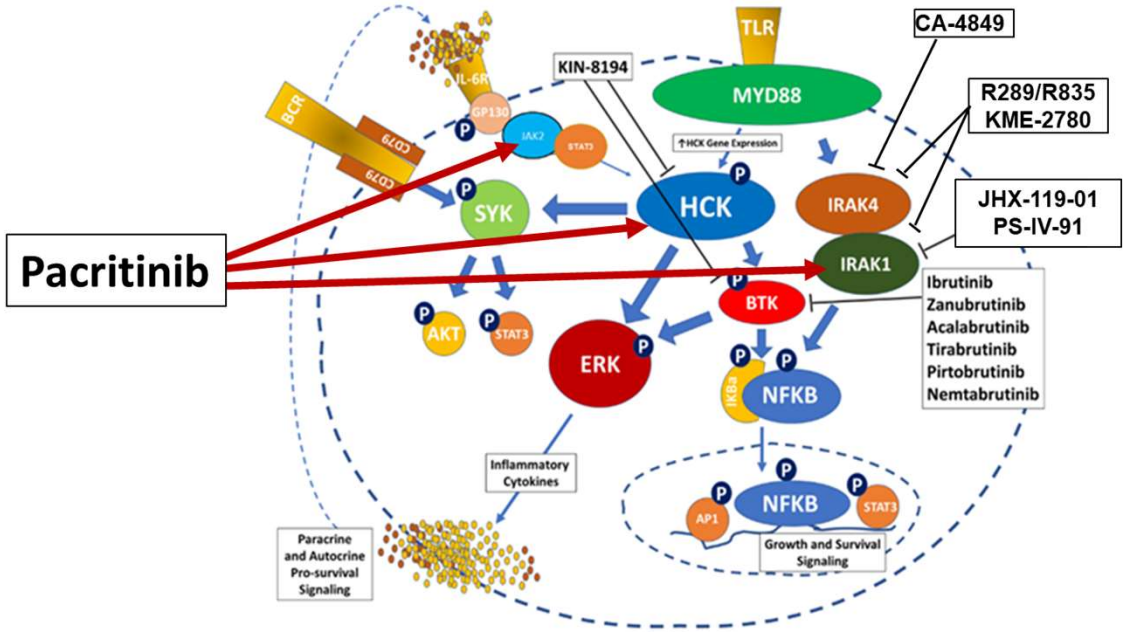
PK parameters	Unit	Estimated Value
Rsq_adjusted		NA
T_{max}	hr	8.00
C_{max}	ng/mL	2130 (2.68 uM)
$T_{1/2}$	hr	NA
Regression Points	hr	NA
AUC_{last}	hr*ng/mL	42422
AUC_{INF}	hr*ng/mL	NA
MRT_{last}	hr	11.9
MRT_{INF}	hr	NA
F	%	12.8

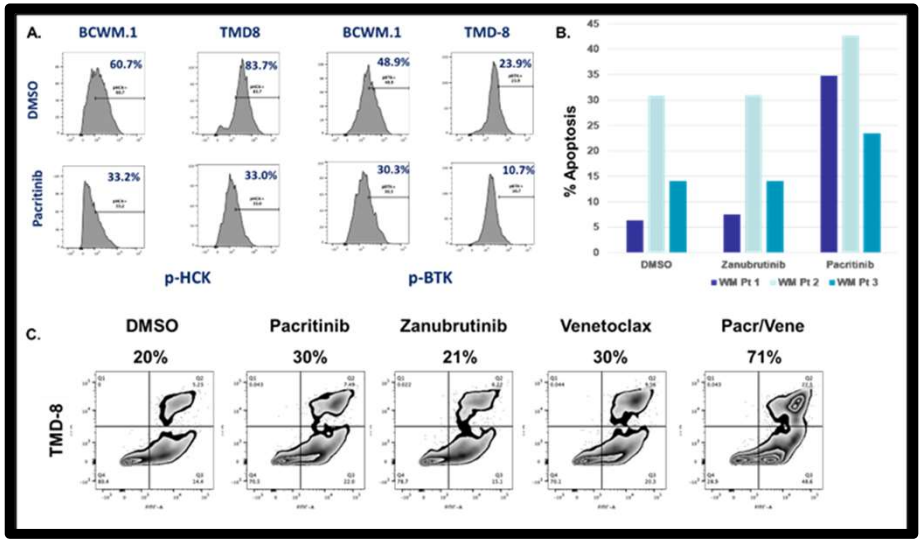
Liu S, et al. Blood (2023) 142 (Supplt 1): 3298.

Novel Treatment Approaches: Pacritinib



Shirong Liu MD, PhD Shayna Sarosiek, MD





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