BH-30236, a Novel, Macrocyclic CLK Inhibitor Modulating RNA splicing, Demonstrates Potent Anti-Proliferation Activity in a Broad Panel of Cancer Cell Lines

Ping Jiang, Danan Li, Nancy Ling, Dayong Zhai, Wei Deng, Zhenping Wang, Yue Hu, Eugene Rui, J. Jean Cui
BlossomHill Therapeutics, Inc., San Diego, California

Abstract # 617

Abstract

Alternative splicing (AS) is a primary mechanism for mRNA transcript diversification and protein expression regulation. In cancer, altered mRNA splicing promotes oncogenic transformation, induces metastasis, and confers resistance to cancer treatment. Mutations or imbalanced expression/activity of splicing factors (SF), such as serine-arginine-rich SFs (SRSFs), often result in deregulation of RNA splicing and tumor progression. CDC-like kinases (CLKs) and dual-specificity tyrosine-regulated kinases (DYRKs) are key regulators of AS *via* phosphorylation of SRSFs. BH-30236 is a novel, macrocyclic, ATP-competitive inhibitor of CLK1/2/4. It also inhibits DYRK1/2, provirus integration site for Moloney murine leukemia virus 3 (PIM3) and FMS-like tyrosine kinase 3 (FLT3) at clinically relevant concentrations. The inhibitory activity of BH-30236 against CLK, DYRK, PIM3 and FLT3 in cancer cells was investigated *via* the phosphorylation inhibition of SRSF, Tau, 4EBP1 and FLT3, respectively. A comprehensive analysis of anti-proliferation activity for BH-30236 was conducted using a panel comprising 116 hematological and solid tumor cell lines. To understand the mechanism of anti-cancer cell growth, the effect of BH-30236 on AS and protein expression of key tumor related biomarkers were examined. In conclusion, BH-30236 effectively inhibited pSRSFs (IC_{50} 40-60 nM in IMR-32 cells), pTau (IC_{50} ~50 nM in SH-SY5Y cells), p4EBP1 (IC_{50} ~80 nM in MM1S cells) and pFLT3-ITD (IC_{50} 0.16 nM in MV-4-11 cells). BH-30236 demonstrated broad inhibition of cancer cell proliferation with a median IC_{50} of 85.2 nM (range 0.55-393 nM) across 12 tumor types. BH-30236 showed greater sensitivity against hematological malignancy (median IC_{50} 23.34 nM), neuroblastoma (median IC_{50} 25.73 nM), breast cancer (median IC_{50} 83.80 nM), colon cancer (median IC_{50} 85.17 nM), and lung cancer (median IC_{50} 102.65 nM). Furthermore, BH-30236 demonstrated synergy with KRAS inhibitors in KRAS mutant cell lines and BCL2 inhibitor Venetoclax in MOLM-13 cells. Mechanistically, BH-30236 modulated AS by increase of pro-apoptotic and anti-proliferative splicing variants of key factors, such as BCL2L1, S6K, and BCLAF1. Meanwhile, BH-30236 downregulated RNA expression of SRSFs and modulated apoptosis family to increase cell death. These results strongly support the clinical applications of the novel multikinase CLK inhibitor BH-30236 in hematological malignancies and solid tumors as a single agent or in combination with other therapies.

Introduction

Pre-mRNA splicing is a complex, highly regulated process involving the removal of introns and the ligation of exons to produce mature mRNAs for protein translation. More than 94% of human protein-coding genes are alternatively spliced in nearly all human organs, leading to proteome diversity and regulation of cell functions. Dysregulated pre-mRNA splicing has been identified in almost all tumor types. Cancer-associated splicing alterations arise from either recurrent mutations in splicing factors or altered expression of trans-acting factors governing splicing catalysis and regulation, leading to cancer cell proliferation, migration, and metastasis, escaping from cell death, rewiring cell metabolism or cell signaling, promoting an abetting microenvironment, altering immune response or enabling drug resistance.[1] The expression of the key splicing regulators serine/arginine-rich splicing factors (SRSFs) is tightly regulated at the post-translational level. The localization and activity of SRSFs are modulated by a dynamic cycle of phosphorylation/dephosphorylation, mostly at serine residues within the RS-domain of SRSF proteins. The phosphorylation of SRSFs is controlled mainly by three families of splicing kinases: serine-rich protein kinases (SRPKs), CDC-like kinases (CLKs), and dual-specificity tyrosine-regulated kinases (DYRKs). The CLK family, which comprises CLK1-4, collaborates with SRPKs at nuclei to adjust the degree of phosphorylation of SR proteins. SRSFs are often upregulated in many cancers. Therefore, inhibition of CLK/DYRK kinases can be an effective approach to modulate aberrant alternative splicing for cancer therapy.

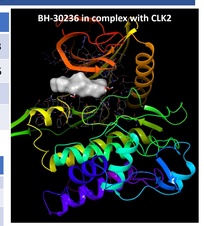
Characterization of BH-30236

Compd	Recombinant Enzymatic Assay[a] IC_{50} (nM)							
	CLK1	CLK2	CLK3	CLK4	DYRK1A	DYRK1B	FLT3	PIM3
BH-30236	0.134	0.165	5.87	0.446	0.11	0.148	0.248	0.115
CTX-712[b]	0.205	0.064	1.66	0.518	0.153	NA	NA	NA
SM08502[b]	0.097	0.262	5.86	0.407	0.078	NA	5.9	623

[a] CLK enzymatic activities were determined at Nanosyn and DYRK, FLT3-ITD and PIMs were determined at Reaction Biology
[b] Proxy chemical compound purchased from a commercial source

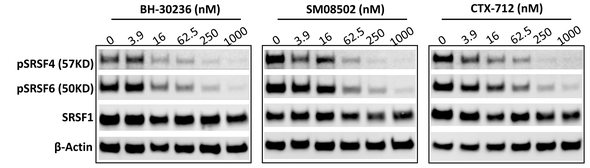
Compound ID	NanoBRET™ IC_{50} (nM)[c]		
	CLK1	CLK2	CLK4
BH-30236	0.111	1.387	< 0.058
SM08502	< 0.058	1.954	< 0.058

[c] CLK NanoBRET™ activities were determined at Reaction Biology

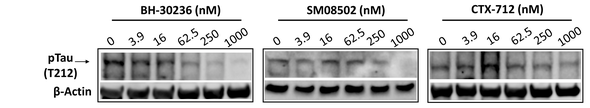


Macrocyclic small molecule: MW 431.49

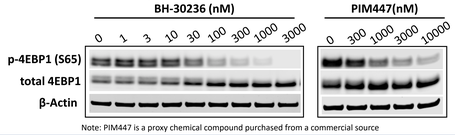
- **BH-30236 effectively inhibited the phosphorylation of CLK substrate SRSFs in Kasumi-1 Cells**



- **BH-30236 modulated the phosphorylation of DYRK downstream effector Tau in SH-SY5Y Cells**



- **BH-30236 suppressed the phosphorylation of PIM downstream effector 4EBP1 in MM1S Cells**

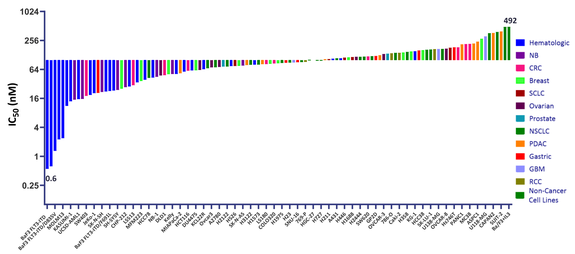


Note: PIM447 is a proxy chemical compound purchased from a commercial source

BH-30236 Potently Inhibited Cancer Cell Growth in a Broad Panel of Cancer Cells (116) and Normal Cells (3)

- **BH-30236 demonstrated a broad spectrum of anti-cancer cell proliferation efficacy in a panel of 116 cancer cell lines and 3 normal cell lines**
- **BH-30236 is most sensitive in hematological cancer cell lines**

5-Day Proliferation IC_{50} of BH-30236 in a Panel of 119 Cell Lines



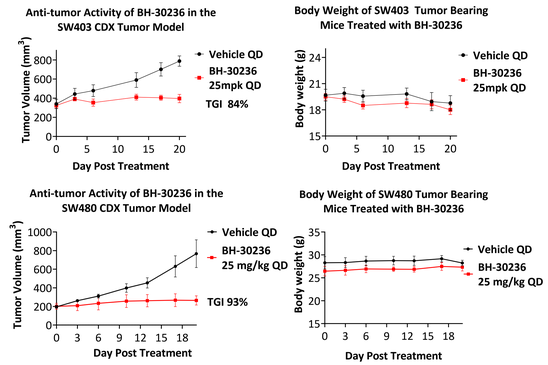
Disease Type	Mean IC_{50} (nM)	Median IC_{50} (nM)	N
Hematologic Cancer	39.43	23.34	26
Neuroblastoma	34.70	25.73	8
Breast Cancer	99.14	83.80	13
Colon Cancer	107.31	85.17	11
Skin Cancer	92.13	92.13	2
Ovarian Cancer	102.35	94.48	6
Gastric Cancer	106.87	95.29	6
Lung Cancer	118.37	102.65	27
Prostate Cancer	104.57	104.57	2
Kidney Cancer	155.45	155.45	2
Pancreatic Cancer	187.64	169.35	10
Glioblastoma	189.57	169.50	3
Non-cancer Cell Lines	455.02	489.11	3

- **The anti-proliferation IC_{50} values of BH-30236 in CRC and ovarian cancer cell lines**

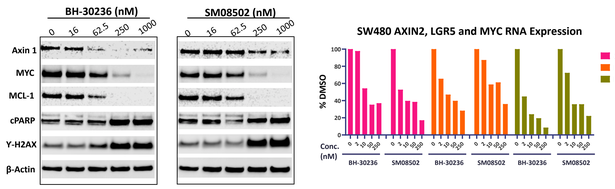
Disease	Cell Lines	Relevant Mutations	BH-30236 IC_{50} (nM)	SM08502 IC_{50} (nM)
Colorectal Cancer	SW403	APC $KRAS^{G12V}$ $TP53^{E51*}$	18.04	69.3
	LS 513	CTNNB1 $KRAS^{G12D}$	30.11	201.7
	DLD1	APC $KRAS^{G12D}$ $TP53^{S241F}$	48.7	83.65
	HCT116	CTNNB1 $KRAS^{G13D}$	60.2	77.3
	LS 180	CTNNB1 $KRAS^{G12D}$	82.71	155.1
	COLO320	$TP53^{R248W}$	85.17	68.4
	SW620	APC $KRAS^{G12V}$ $TP53^{R273H}$	118.9	78.7
	GP2D	$KRAS^{G12D}$	121.30	118.7
	SW480	APC $KRAS^{G12V}$ $TP53^{R273H}$	183.7	143.5
	CT26	$KRAS^{G12D}$	214.6	375.2
	MC38	$TP53^{R172H}$	216.9	294.7
Ovarian Cancer	PA-1	$NRAS^{G12D}$ $TP53^{N239D}$	48.0	71.8
	TOV-112D	CTNNB1 $TP53^{R175H}$	62.5	77.9
	Ovcar3	PIK3R1 $TP53^{R248W}$	70.7	79.4
	A2780	ATM PTEN	72.1	NA
	SKOV3	APC $TP53^{S90Prof*33}$ PIK3CA	116.9	88.6
	Ovcar8	CTNNB1 KRAS TP53	172.8	NA

Evaluation of BH-30236 Applications in Colon and Ovarian Cancers

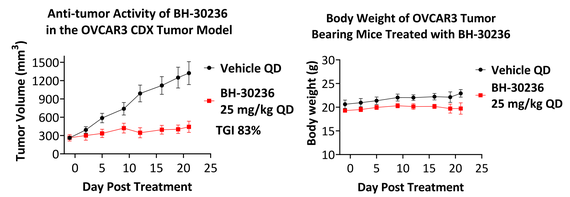
- **BH-30236 induced tumor growth inhibition in CRC models regardless of mutation status**
- **The combination of BH-30236 with standard therapies will be evaluated**



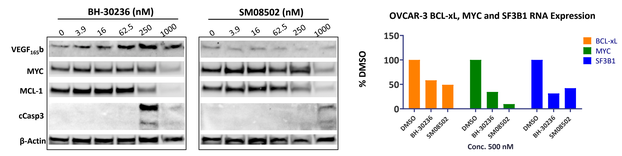
- **BH-30236 downregulated WNT signaling leading to down regulation of MYC and induced apoptosis in SW480 cells**



- **BH-30236 induced tumor growth inhibition in ovarian cancer model**



- **BH-30236 downregulated MYC, MCL-1 and induced apoptosis in OVCAR3 Cells**



- **Combination benefit observed with BH-30236 and KRAS inhibitors**

Cell Line	Cancer Type	KRAS Inhibitors	HSA Synergy Score	Most Synergistic Area Score
H358	NSCLC	sotorasib[d]	4.8	14.2
Suit-2	PDAC	MRTX1133[d]	4.1	13.6
GP2D	CRC	MRTX1133[d]	5.3	11.15
SW1463	CRC	adagrasib[d]	7.4	25.5

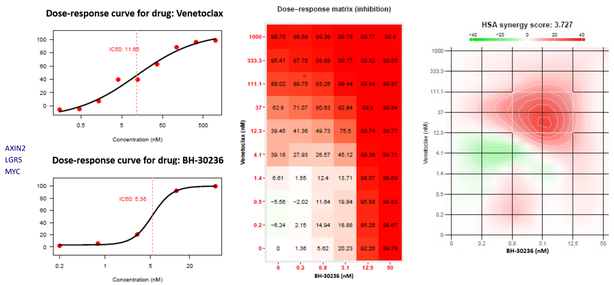
[d]Proxy chemical compound purchased from a commercial source

BH-30236 Potently Inhibited the Viability of Primary AML Cells and Demonstrated Strong Synergy with BCL2 Inhibitor in an AML Cell Line

- **BH-30236 demonstrated strong inhibitory activity in ex vivo cell viability assays in 31 patient-derived human AML models from Champions Oncology**

Model ID	IC_{50} (nM)	Model ID	IC_{50} (nM)	Model ID	IC_{50} (nM)
CTG-2227	4.5	CTG-2456	17.7	CTG-3673	9.1
CTG-2228	16	CTG-2457	58	CTG-3674	91
CTG-2232	48	CTG-2701	45	CTG-3679	32
CTG-2234	7.9	CTG-2704	37	CTG-3930	52
CTG-2235	45	CTG-3440	51	CTG-3949	24
CTG-2236	13	CTG-3441	40	CTG-3950	3.7
CTG-2239	34	CTG-3659	26	CTG-4025	46
CTG-2240	9.7	CTG-3660	48	CTG-4044	2.6
CTG-2452	94	CTG-3661	70	CTG-4045	120
CTG-2453	18	CTG-3667	38	Mean IC_{50}	39.9
CTG-2454	95	CTG-3671	17	Median IC_{50}	37.5

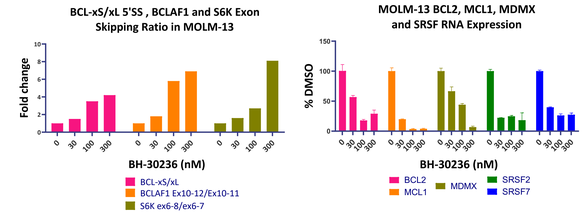
- **The combination effect of BH-30236 with Venetoclax in MOLM-13 cell line**



Venetoclax[e] (nM)	BH-30236 (nM)	Relative Inhibition (%)	HSA Synergy Score
12.4	3.1	75.5	35
37	3.1	92.8	29.2
37	0.8	80.6	17.3

[e]Proxy chemical compound purchased from a commercial source

- **BH-30236 dose-dependently modulated the alternative splicing to favor anti-tumor Isoforms and downregulated BCL2, MCL-1, MDMX and SRSFs in MOLM-13 Cells**



Conclusion

- BH-30236 is a potent CLK/PIM/FLT3 inhibitor
- BH-30236 demonstrated potent in vitro and in vivo anti-cancer activity across different tumor types with good safety profile
- BH-30236 showed potent ex vivo anti-leukemic activity in patient derived AML models
- BH-30236 inhibited tumor cell proliferation, induced apoptosis and DNA damage through modulation of alternative splicing of key factors favoring anti-proliferation and pro-apoptotic splicing variants
- Potential synergistic benefit observed when BH-30236 was used in combination with BCL2 and KRAS inhibitors
- Preclinical pharmacology evaluation in solid tumors and hematological malignancies strongly supports the evaluation of BH-30236 in human clinical trials as a single agent or in combination with standard therapies in solid tumors and hematological malignancies

Additional preclinical activities of BH-30236 will be presented on Tuesday, Apr 9, 2024, 1:30 PM - 5:00 PM at Poster Section 26, Poster Board 25, Abstract #5944

Reference

1. Bradley R, Anczuków O. RNA splicing dysregulation and the hallmarks of cancer. Nat Rev Cancer. 2023; 23(3):135-155

Contact: jean.cui@bhtherapeutics.com