CLK Inhibitor BH-30236 Synergizes with Venetoclax in Anti-Leukemia Activity via Splicing Modulation in Preclinical AML and CLL Models

AACR 2026
Abstract # 5872

Wei Deng, Ping Jiang, Zhenping Wang, Yue Hu, Nancy Ling, Danan Li, Joshua Choi, Eugene Rui, Zachary Zimmerman, Geoffrey Oxnard and Jean Cui
BlossomHill Therapeutics, Inc., San Diego, California

Abstract

Alterations of splicing patterns are increasingly recognized as a hallmark of cancer by promoting proliferation, evading apoptosis, changing cell signaling, and driving drug resistance through aberrantly spliced isoforms. In hematologic malignancies of both myeloid and lymphoid lineages, including myelodysplastic syndromes (MDS), acute myeloid leukemia (AML), and chronic lymphocytic leukemia (CLL), frequent mutations or overexpression of RNA splicing factors are associated with aberrant alternative splicing. CDC-like kinases (CLKs) regulate alternative splicing by phosphorylating serine/arginine-rich splicing factors (SRSFs) which are essential for splice site recognition and spliceosome assembly. Thus, targeting CLKs represents a promising therapeutic strategy, especially in hematologic malignancies with splicing dysregulation.

BH-30236 is a novel, potent, orally bioavailable, ATP-competitive, small molecule kinase inhibitor of CLK1/2/4. BH-30236 inhibited the phosphorylation of SRSFs in cancer cells, leading to modulation of splicing patterns and RNA/protein expression of genes related to apoptosis and DNA damage response pathways, thereby promoting apoptosis. In addition, the expression of stem cell markers was downregulated by BH-30236 in AML cell lines. BH-30236 effectively inhibited the proliferation of hematologic cancer cell lines or leukemia cells from AML or CLL patients. In addition, effective anti-tumor activity of BH-30236 was observed in tumor models derived from AML cell lines or leukemia patients. A synergistic anti-cell proliferation effect between BH-30236 and venetoclax, a BCL2 inhibitor, was consistently observed across multiple hematologic cancer cell lines of diverse lineages. The synergy is likely driven by BH-30236 mediated suppression of anti-apoptotic and pro-survival proteins including MCL1, a pro-survival factor whose upregulation is associated with resistance to venetoclax. Moreover, the combination of BH-30236 and venetoclax synergistically induced complete tumor regression in the highly resistant MOLM13 cell-derived xenograft tumor model. This in vivo synergistic effect was observed even when BH-30236 or venetoclax was administered at low dose levels. Notably, modification of combinatory treatment regimen after achieving complete tumor regression to single agent BH-30236 treatment sustained tumor-free survival in mice and maintained tumor free for more than 80 days, even after treatment discontinuation, that is consistent with BH-30236 modulation of leukemia stem cells via regulation of alternative splicing.

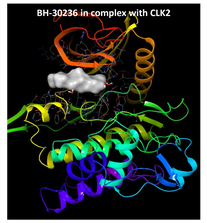
BH-30236 is currently under clinical investigation, either as a single agent or in combination with venetoclax, in adults with relapsed or refractory AML or higher risk MDS in a Phase 1/1b clinical trial (NCT06501196).

BH-30236 is a Multi-Kinase CLK Inhibitor

Recombinant Enzymatic Assay IC_{50} (nM) [a]								
	CLK1	CLK2	CLK3	CLK4	DYRK1A	DYRK1B	PIM3	FLT3_ITD
BH-30236	0.13	0.17	5.87	0.45	0.11	0.15	0.12	0.54
CTX712	0.21	0.06	1.66	0.52	0.15	NA	NA	NA
Cirtuvivint (SM08502)	0.10	0.26	5.86	0.41	0.08	NA	623	10.2

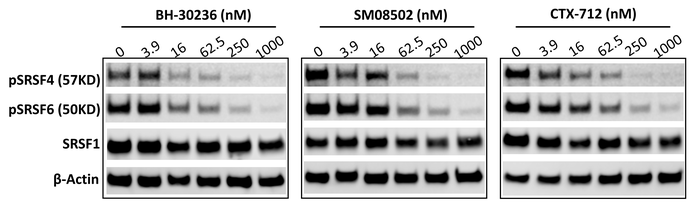
[a] CLK enzymatic activities were measured at Nanosyn and DYRK, FLT3-ITD, PIMs were determined at Reaction Biology.

Cell Proliferation Inhibition in FLT3-Mutated Cells: IC_{50} (nM)					
	MV-4-11 (FLT3-ITD)	MOLM13 (FLT3-ITD)	BaF3 FLT3-ITD	BaF3 FLT3-ITD/F691L	BaF3 FLT3-ITD/D835V
BH-30236	0.98	2.06	0.70	10.2	0.78
Gilteritinib	2.45	8.9	1.53	15.1	2.05

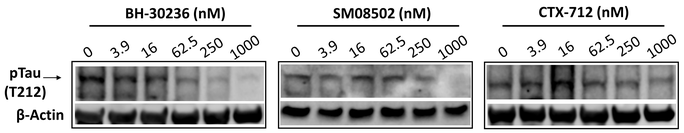


Macrocyclic small molecule: MW 431

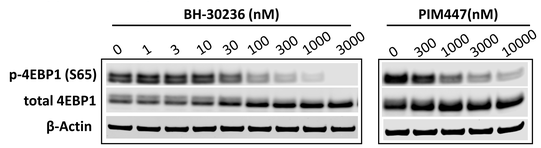
- BH-30236 effectively inhibited the phosphorylation of CLK substrate SRSFs in Kasumi-1 Cells



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



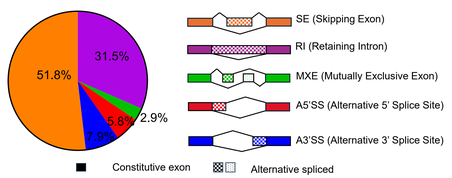
- BH-30236 suppressed the phosphorylation of PIM downstream effector 4EBP1 in MM1S Cells



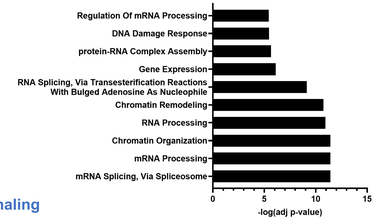
Note: All test articles used in the poster except BH-30236 were proxy chemical compounds purchased from a commercial source.

The Effect of BH-30236 on RNA Processing, Apoptosis and DNA Damage Response Signaling

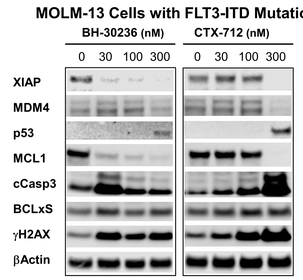
Common Alternative Splicing Events (ASE) Affected by BH-30236 in 6 Primary AML Samples

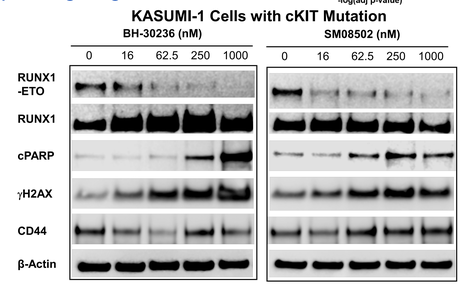


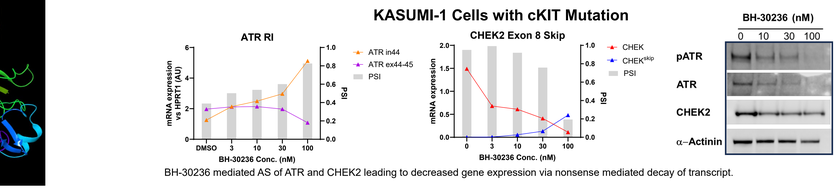
Gene Ontology of Common ASE Affected by BH-30236 in 6 Primary AML Samples



- BH-30236 Promoted Apoptosis and DNA Damage Response Signaling

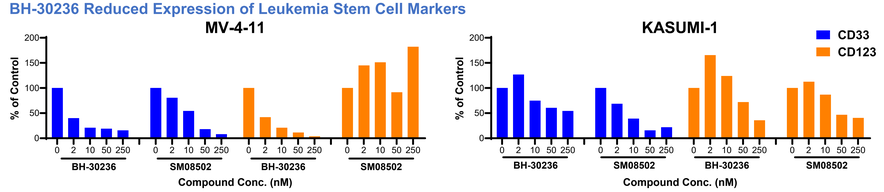




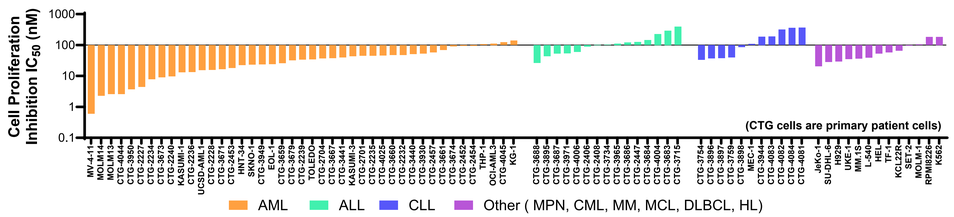


BH-30236 mediated AS of ATR and CHEK2 leading to decreased gene expression via nonsense mediated decay of transcript.

- BH-30236 Reduced Expression of Leukemia Stem Cell Markers



- BH-30236 Inhibited Proliferation of a Broad Panel of Hematologic Malignancies in Cell Proliferation Assays



Conclusion

- BH-30236 is a CLK inhibitor that demonstrates *in vitro* efficacy at nanomolar range across multiple types of blood malignancies, including cancer cell lines and patient-derived AML, ALL and CLL cells.
- BH-30236 can overcome venetoclax-resistance through FLT3-dependent and independent mechanisms.
- BH-30236 exhibits synergistic potential with venetoclax *in vitro* in multiple types of blood cancers, including AML, CLL and MCL..
- Combination of BH-30236 with venetoclax or venetoclax and azacitidine induced tumor regression in venetoclax-resistance CDX AML models.
- BH-30236 is currently being evaluated as a monotherapy or in combination with venetoclax in an ongoing phase 1 study in relapsed/ refractory AML and HR-MDS (NCT06501196).

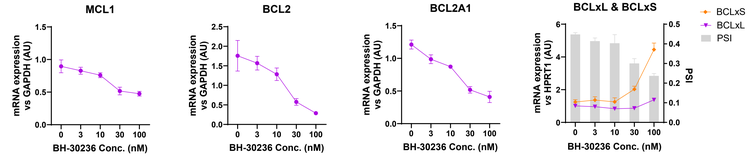
BH-30236 Synergizes With Venetoclax in Hematological Cancer Cell Lines

- Synergy between BH-30236 and Venetoclax Across Multiple Types of Hematological Cancer Cell Lines in Matrix Assays

Cell Line Name	Cancer Type	Proliferation Inhibition IC_{50} (nM)		Synergy Score [1]	Mutation Status
		BH-30236	Venetoclax		
MOLM-13	AML	0.62	1.36	11.0	FLT3-ITD, KMT2A fusion
MV-4-11	AML	0.70	8.88	3.93	FLT3-ITD, KMT2A, AFF1
HNT34	AML	14.6	17.4	19.8	BCR-ABL1
KASUMI-1	AML	26.4	12.4	12.1	RUNX1 fusion, KIT, RAD21, TP53
THP1	AML	55.6	826	18.8	CSNK2A1-DDX39B, KMT2A-MLLT3, NRAS, TP53
SKNO1	AML	120	149	21.6	RUNX1-fusion, KIT, TP53
HL60	AML	244	10.0	17.5	NRAS Q61L, TP53, CDKN2A, cMyc-Ampl.
KG1	AML	279	96.4	25.4	FGFR1 fusion, NRAS, TP53
HG3	CLL	86.1	>1000	17.6	NA
MEC1	CLL	164	>1000	12.9	R3HCC1L-HTRA1, TP53
JeKo1	MCL	39.5	>3000	26.4	CDKN2A, TP53
MINO	MCL	65.9	10.0	11.1	CDKN2A, NRAS, TP53
MOLM-1	CML	158	163	16.4	BCR-ABL1
SUPB15	B-ALL	53.3	1.20	10.1	BCR-ABL1
Jurkat E6.1	TLL	127	>3000	7.67	BAX, FBXW7, INPP5D, MSH2, MSH6, SYK, TP53
TOLEDO	DLBCL	19.3	21.5	20.5	KRAS, TP53

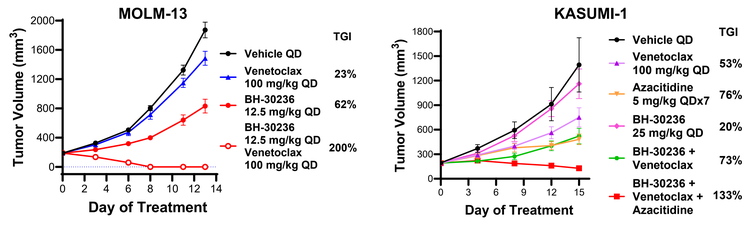
[1] Synergy score was calculated as highest average Bliss or HSA score of a 3x3 field matrix. Scores <-10 indicates antagonistic effects, scores from -10 to 10 indicates additive effects, scores > 10 indicates synergistic effects.

- BH-30236 Reduced Expression of MCL1 and Other Apoptotic Factors in KASUMI-1 cells

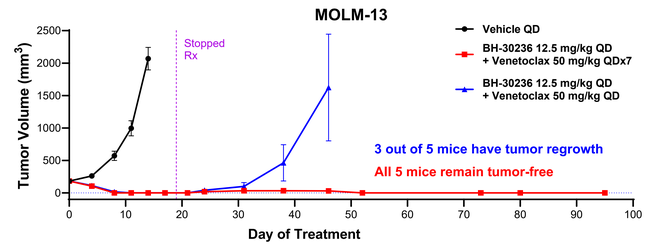


Combination Effect of BH-30236 and Venetoclax in AML CDX Tumor Models

- BH-30236 in Combination with Venetoclax or Venetoclax+Azacitidine Induced Tumor Regression in AML CDX Tumor Models



- BH-30236 in Combination with Venetoclax Induced Complete Tumor Regression and Maintained Tumor Free Status After Withdrawal of Treatment in MOLM-13 CDX Tumor Model



Corresponding Author: jean.cui@bhtherapeutics.com