Novel Multikinase CLK Inhibitor BH-30236 Targets Hematological Malignancies through Alternative Splicing Regulation

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Nancy Ling[1], Gregory Sims[1], Ping Jiang[1], Wei Deng[1], Dayong Zhai[1], Danan Li[1], Zhenping Wang[1], Yue Hu[1], Joshua Choi[1], Eric Wang[2], Armin Graber[1], Jean Cui[1]
[1]BlossomHill Therapeutics, Inc., San Diego, California
[2]The Jackson Laboratory for Genomic Medicine, Farmington, Connecticut

Abstract

RNA splicing dysregulation plays a critical role in tumorigenesis, cancer progression, and therapeutic resistance. Components of the spliceosome machinery are frequently mutated in myeloid malignancies, affecting 40-85% of patients with Myelodysplastic Syndrome (MDS), and 10–25% of Acute Myeloid Leukemia (AML) cases. Recurrent aberrant alternative splicing (AS) patterns have also been detected in AML patients even in the absence of somatic spliceosome gene mutations. Furthermore, genetic screens have shown that the loss of splicing factors sensitizes AML to BCL2 inhibitor venetoclax. As such, splicing modulators are now acknowledged as a promising therapeutic target for hematologic malignancies. The CDC2-like kinases (CLKs) play a pivotal role in regulating mRNA splicing *via* the phosphorylation of serine/arginine-rich splicing factor (SRSF) proteins which facilitate pre-mRNA splice site recognition. Consequently, inhibiting CLK may offer a viable strategy for targeting cancers dependent on aberrant splicing.

BH-30236 is a novel, orally bioavailable, ATP-competitive, macrocyclic inhibitor of CLKs, proviral integration for the Moloney murine leukemia virus 3 kinase (PIM3), FMS-like tyrosine kinase 3 (FLT3), and dual-specificity tyrosine-regulated kinase (DYRK) 1/2. BH-30236 potently suppressed SRSF phosphorylation and demonstrated *in vitro* efficacy at nanomolar range across immortalized and patient-derived AML cells, including cells insensitive to venetoclax. To evaluate how BH-30236 impacts mRNA expression and alternative splicing, RNA sequencing was performed on 6 patient-derived AML cells before and after BH-30236 treatment. BH-30236 modulated AS in genes associated with DNA damage response, mRNA splicing, and chromosome remodeling. Gene ontology enrichment analysis of genes downregulated by BH-30236 included terms related to mitochondrial translation, respiratory chain complex assembly, and regulation of cytochrome c release. Specifically, BH-30236 decreased the expression of anti-apoptotic BCL2 family genes MCL1 and BCL2A1, whose expression correlates with venetoclax resistance. Consistent with this, BH-30236 enhanced the potency of venetoclax in AML cell lines, including those with TP53 mutations. The combination of BH-30236 and venetoclax also synergistically suppressed tumor growth or induced deep tumor regression in venetoclax-resistant AML cell-derived xenograft models (Kasumi1, MOLM13). These observations support further evaluation of BH-30236 in combination with venetoclax for hematologic malignancies. A Phase 1 study to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary anti-leukemic activity of BH-30236 in adults with relapsed/refractory AML and higher-risk MDS is currently ongoing (NCT06501196).

BH-30236 is a Dual CLK and FLT3 Inhibitor

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

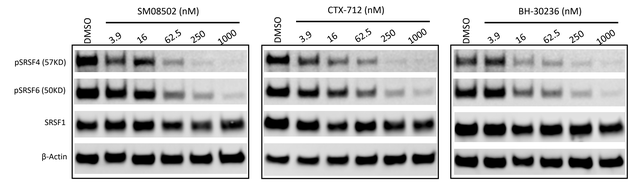
[a] CLK enzymatic activities were measured at Nanosyn and DYRK, FLT3-ITD were determined at Reaction Biology.

Cell Proliferation IC_{50} (nM)[a]					
	MV-4-11	MOLM13	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

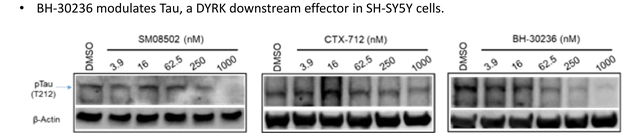
[a] CLK NanoBRET™ activities were measured at Reaction Biology

Macrocyclic small Molecule: MW 431.49

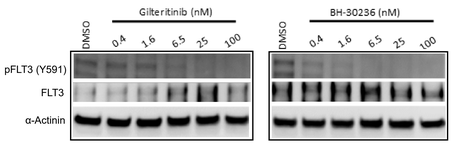
- BH-30236 inhibits phosphorylation of CLK substrate SRSFs in Kasumi1 cells.



- BH-30236 modulates Tau, a DYRK downstream effector in SH-SY5Y cells.

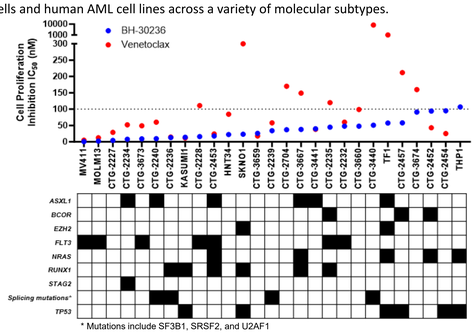


- BH-30236 inhibits FLT3 phosphorylation in MV411 cells with *FLT3-ITD* mutation.



BH-30236 Demonstrates Potent *in vitro* Efficacy in Patient-Derived AML Cells and Human AML Cell Lines

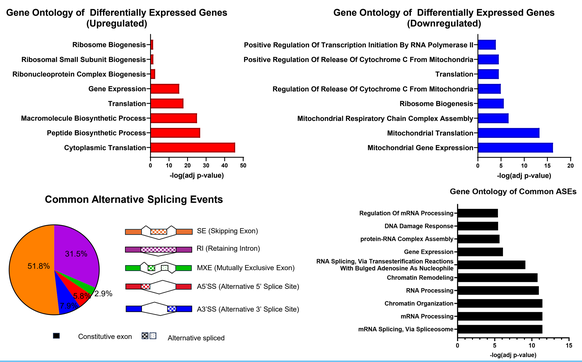
- BH-30236 exhibits stronger anti-leukemia activity than venetoclax in a majority of patient-derived AML cells and human AML cell lines across a variety of molecular subtypes.



* Mutations include SF3B1, SRSF2, and U2AF1

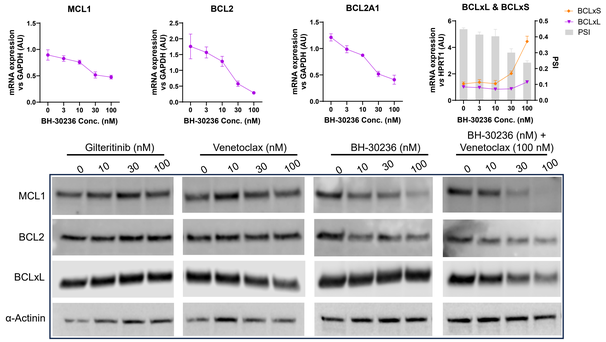
BH-30236 Regulates mRNA Splicing, DNA Damage, and Mitochondrial Function Through Regulation of Gene Expression and Alternative Splicing

- RNAseq was performed on 6 patient-derived AML cells after treatment with BH-30236.
- Differential gene expression analysis suggests BH-30236 down regulates genes involved in mitochondrial translation, respiratory chain complex assembly, and regulation of cytochrome c release.
- Alternative splicing analysis suggests BH-30236 alters splicing of genes associated with mRNA splicing, DNA damage, and chromosome remodeling



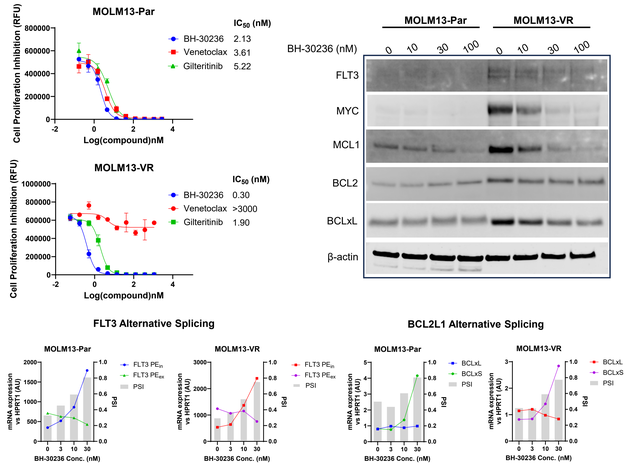
BH-30236 Modulates the Expression of Anti-Apoptotic BCL2 Family Proteins

- BH-30236 reduces anti-apoptotic proteins MCL1, BCL2, and BCL2A1, while increasing pro-apoptotic BCLxS in Kasumi1 cells.
- BCL2 inhibitor venetoclax synergizes with BH-30236 to down-regulate anti-apoptotic proteins
- Modulation of BCL2 family proteins by BH-30236 is independent of FLT3 inhibition.



BH-30236 Overcomes Venetoclax-Induced Resistance

- BH-30236 inhibits cell proliferation in *FLT3-ITD* mutant MOLM13 parental (Par) and MOLM13 venetoclax resistant (VR) cells.
- BH-30236 down-regulates FLT3, MYC, MCL1, and BCLxL proteins known to be upregulated in AML and confer resistance to venetoclax.
- BH-30236 promotes the inclusion of a poison exon (PE; exon with an in-frame stop codon whose inclusion renders the transcript NMD sensitive) in FLT3.
- BH-30236 induces splicing BCL2L1 mRNA to favor the pro-apoptotic BCLxS transcript *vs.* the anti-apoptotic BCLxL transcript.

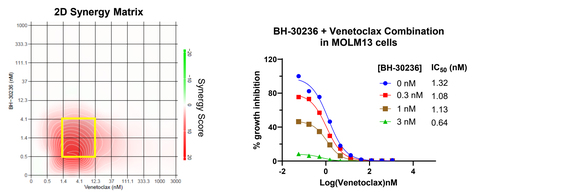


BH-30236 Synergizes With Venetoclax in Hematological Cancer Cell Lines

- BH-30236 synergizes with venetoclax in a wide spectrum of hematological cell models, include those harboring *FLT3*, *TP53*, and *NRAS* mutations.

Cell Line	Lineage	Cell Growth Inhibition IC_{50} (nM)		*c*Bliss Synergy Score*	Mutation Status
		BH-30236	Venetoclax		
MOLM13	AML	0.62	1.36	10.98	FLT3-ITD, KMT2A fusion
MV411	AML	0.7	8.88	3.93	FLT3-ITD, KMT2A, AFF1
KASUMI1	AML	9.97	3.09	10.99	RUNX1 fusion, KIT, RAD21, TP53
HNT34	AML	14.64	17.43	19.79	BCR-ABL1
TOLEDO	DLBCL	19.27	21.45	20.47	KRAS, TP53
JeKo1	MCL	39.46	>3000	26.41	CDKN2A, TP53
THP1	AML	55.63	826.3	18.75	CSNK2A1-DDX39B, KMT2A-MLLT3, NRAS, TP53
MOLM1	CML	157.5	162.7	16.38	BCR-ABL1
Jurkat	TLL	126.8	>3000	7.67	BAX, FBXW7, INPP5D, MSH2, MSH6, SYK, TP53
KG1	AML	278.9	96.38	25.38	FGFR1 fusion, NRAS, TP53
SKNO1	AML	120	149.4	21.61	RUNX1-fusion, KIT, TP53

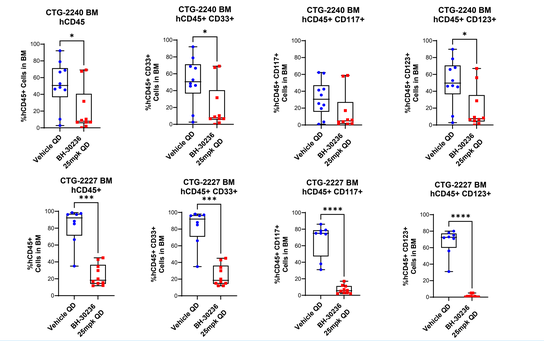
* cBliss synergy score was calculated as highest average Bliss synergy score of a 3x3 field matrix. Scores <-10 indicates antagonistic effects, scores from -10 to 10 indicates additive effects, scores > 10 indicates synergistic effects



BH-30236 Downregulates Leukemia Blasts in AML PDX Models

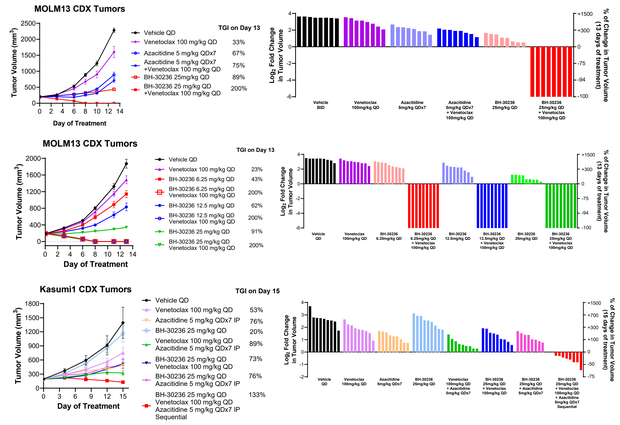
- PDX models were generated from naïve or relapsed AML patient cells and treated with BH-30236 (25mg/kg QD) for 3 weeks. The study was performed at Champion Oncology.
- Significant reduction in human (h) CD45⁺ cells in bone marrow (BM) of PDXs was observed following BH-30236 treatment in comparison to vehicle control.

PDX Model ID	Prior Treatment	Genetic Alterations
CTG-2240	Naive	U2AF1 Q157P, ZRSR2 HetLoss, SRSF4 missense var, KMT2A-MLLT3
CTG-2227	Relapsed	FLT3-ITD, IDH1 R132H, NPM, SF3A1 CNA gain, ABL2 and KMT2A intron var, CLK3 stop_gained+splice region var; U2AF1 3'UTR var



BH-30236 in Combination with Venetoclax Suppresses Tumor Growth, Showcasing Marked Synergistic Effect *in vivo*

- BH-30236 combined with venetoclax lead to robust anti-tumor efficacy in venetoclax-resistant AML MOLM13 tumor model in a dose dependent manner.
- Complete tumor regression was observed as low as 6.25 mg/kg of BH-30236 when combined with 100 mg/kg venetoclax, administered daily.
- BH-30236 in combination with SOC venetoclax+azacitidine lead to deep tumor regression in venetoclax-resistant AML model, Kasumi1.
- No significant decrease in body weight was observed during treatments.



Conclusion

- BH-30236 demonstrates *in vitro* efficacy at nanomolar range across patient-derived AML cells and human AML cell lines, including venetoclax-insensitive cells.
- BH-30236 can overcome venetoclax-resistance through FLT3-dependent and independent mechanisms.
- BH-30236 exhibits strong synergistic potential with venetoclax *in vitro* and *in vivo.*
- BH-30236 combinations with SOC demonstrates better *in vivo* efficacy than SOC venetoclax + azacitidine in venetoclax-resistant CDX AML models.
- The strong synergy between BH-30236 and venetoclax supports the clinical evaluation of BH-30236 in combination with venetoclax in hematological malignancy.
- BH-30236 is currently being evaluated as a monotherapy in an ongoing phase 1 study in relapsed/ refractory AML and HR-MDS (NCT06501196).

Corresponding Author: jean.cui@bhtherapeutics.com