MYELOID KINOME INHIBITOR HM43239 OVERCOMES ACQUIRED RESISTANCE IN ACUTE MYELOID LEUKEMIA MODELS

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INTRODUCTION

Oral HM43239 is in development for the treatment of acute myeloid leukemia (AML) because of its capacity to potently inhibit kinases that drive myeloid malignancies, including diverse forms of the FLT3, SYK, JAK and c-KIT kinases. Wildtype FLT3 is overexpressed in most AML patients, and approximately 30% of newly diagnosed adult AML patients harbor internal tandem duplications (ITDs) or point mutations in the tyrosine kinase domain (TKD). These mutations drive aberrant activation of downstream proliferation pathways and are associated with a high risk of relapse. Likewise, the c-KIT alternative receptor kinase, as well as the SYK and JAK1/2 intracellular kinases, mediate oncogenic signaling in AML that can promote drug resistance to certain FLT3 inhibitors. HM43239 was developed to overcome shortcomings of other FLT3 inhibitors. It inhibits a broad set of mutant and wildtype forms of FLT3, while simultaneously disrupting downstream SYK, JAK/STAT5, ERK, and other rescue signaling pathways. This rationale supports the development of HM43239.

OBJECTIVE

Evaluate the activity of HM43239, an orally active drug, as a Myeloid Kinome Inhibitor in human AML models.

METHOD

Biochemical kinase assays were performed by Thermo Fisher Scientifics and DiscoverX USA. The effects of HM43239 on cell proliferation (IC_{50}), growth rate (GR_{50}) and concentration at half-maximal effect (Growth Effective Concentration; GEC_{50}) were determined using the MTS assay with vehicle controls[1]. Cell-based inhibition of target phosphorylation was assessed by Western blot and flow cytometric analyses. The AML cell lines tested included MV-4-11, MOLM-13, MOLM-14 and BAF3/ITD. *In vivo* efficacy was assessed using the MOLM-14 FLT3-Mutated xenograft model.

Inhibition of Kinases by HM43239

Assay Methodology	Kinase	Mutation Type	Activity
Binding Affinity (K_D, nM)	FLT3	WT	0.58
		ITD	0.37
		D835Y	0.29
		D835H	0.4
		ITD/D835V	0.48
		ITD/F691L	1.3
Inhibition of Kinase Enzyme Activity (IC_{50}, nM)	FLT3	WT	1.1
		ITD	1.8
		D835Y	1.0
	SYK	WT	2.9
	JAK	JAK-1	2.8
		JAK-2	6.3
		JAK -2 (V617F)	9.9
	c-KIT	WT	> 500
		D816H	3.6
		D816V	3.5

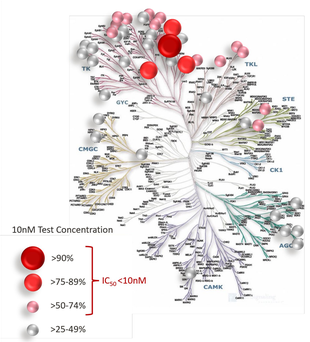
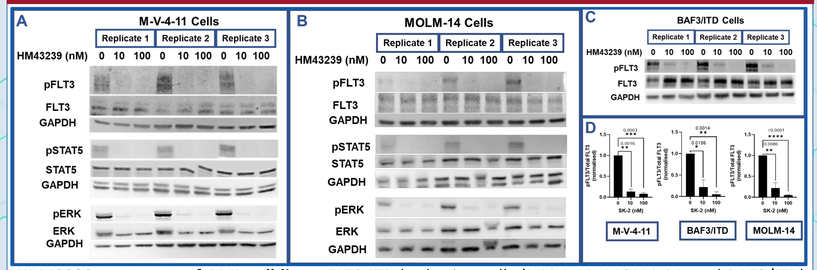
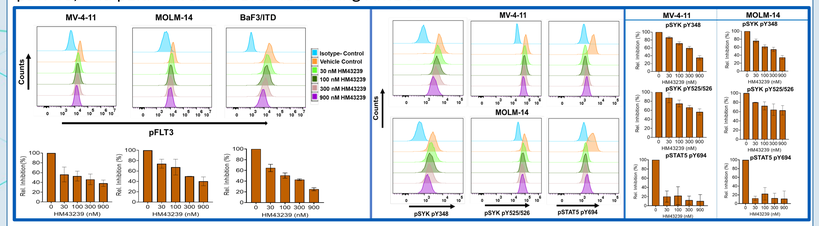


Table and Figure: Assays for Kinase inhibitory constant (IC_{50}) and binding constant (K_D) were performed by Thermo Fisher Scientifics (MA, USA) and DiscoverX (CA, USA), respectively. Consensus composite kinome scan as confirmed by both IC_{50} or K_D screening technologies.

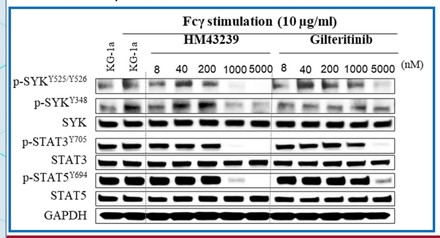
HM43239 Suppression of AML Survival Signaling



HM43239 treatment of AML cell lines. FLT3-ITD harboring cells (MV-4-11, MOLM-14, and BAF3/ITD) were exposed to different concentrations of HM43239 for 2 h and then evaluated by western blot for pFLT3, pSTAT5, and pERK relative to total level of each protein. HM43239 significantly reduced pFLT3, pSTAT5, and pERK levels in FLT3-ITD bearing AML cells.

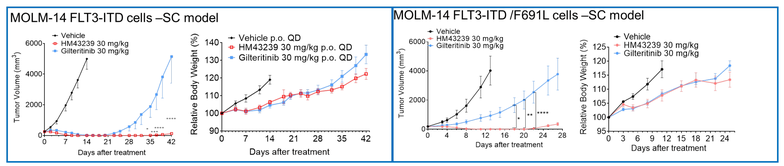


HM43239 reduced pFLT3, pSYK, and pSTAT5 levels in HM43239 treated AML cells as detected by Flow cytometry. MV-4-11, MOLM-14, BAF3/ITD cells were exposed to different concentrations of HM43239 for 2 h and then analyzed with antibodies specific for the phosphorylated form and total level of each protein.



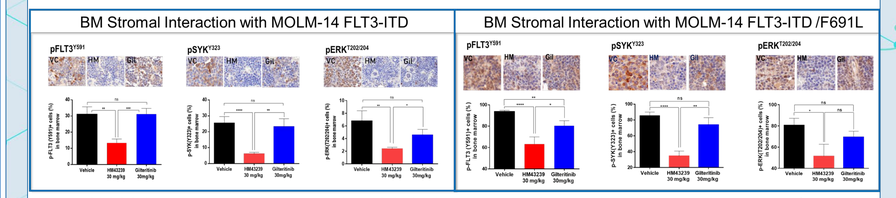
HM43239 is more potent than gilteritinib on Fcγ-induced KG-1a Cells (FLT3-WT). KG-1a cells were treated with various concentrations of HM43239 or gilteritinib, followed by treatment with 10 μg/mL humanIgG for 10 min, and then crosslinked with 1 μg/mL anti-humanIgG for 10 min. SYK and JAK/STAT5 activation were more potently inhibited by HM43239 than gilteritinib.

Effect of HM43239 and Gilteritinib on Subcutaneous Model of Human AML in Mice Model



HM43239 is more potent than gilteritinib in AML murine xenograft studies: Three million MOLM-14 FLT3-ITD or MOLM-14 FLT3-ITD/F691L cells were implanted SC in nude mice. Fifteen days later they were randomized by their tumor volume into 3 groups of 5 mice each The mice were then treated orally QD with either placebo, 30mg/kg HM43239 or 30 mg/kg gilteritinib for 28 days. Statistical analysis utilized two-way ANOVA followed by Sidak's test.

Effect of HM43239 and Gilteritinib on the Interactions of Bone Marrow Stroma with AML in Orthotopic Mice Model



HM43239 is more potent than gilteritinib in orthotopic murine model of AML. Mice were administered i.v. either MOLM-14 FLT3-ITD or MOLM-14 FLT3-ITD/F691L cells and allowed to populate the bone marrow for 7 days, after which drugs were administered orally QD for 12 to 14 days. Representative images of IHC were collected using a Dako REAL Envision Detection System (400x) and quantified with a Vectra 3 Pathology Imaging Analyzer (200x images). Positive DAB % = DAB positive area pixel / (Hematoxylin pixel + DAB positive area pixel) × 100. ▪ VC, vehicle control; HM, HM43239 30 mg/kg; Gil, Gilteritinib 30mg/kg. ▪ ns, not significant; * p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (unpaired t test using GraphPad PRISM®, GraphPad Software)

CONCLUSIONS

- HM43239 inhibits wild type and mutant forms of FLT3 at low nM concentrations.
- HM43239 inhibits phospho-FLT3, phospho-SYK, phospho-EKR1/2 and phospho-JAK/STAT5 that participate in signaling and rescue pathways.
- HM43239 has potential to kill cells and tumors resistant to other FLT3 inhibitors.
- HM43239, at doses that are well tolerated, demonstrates *in vivo* efficacy on tumors resistant to other FLT3 inhibitors.
- A Phase 1/2 trial (NCT03850574) of HM43239 in R/R AML patients is ongoing.

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REFERENCES

1. Hafner M., Niepel M., Chung M., and Sorger P.K. (2016) Growth rate inhibition metrics correct for confounders in measuring sensitivity to cancer drugs, *Nat. Method* 13 (6):521-527.

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