1258P HJM-353: A Potent, Selective and Orally Bioavailable EED Inhibitor with Robust Anti-tumor Activities

Xin Ma[1*], Wei Qi[2], Yong Du[1], Desheng Kong[1], Ya Geng[1], Li Zeng[1]

[1]Jing Medicine Technology (Shanghai), Ltd. 6th Floor, Y Building, 393 Middle Huaxia Road, Shanghai, China

[2]Gene Editing Center, School of Life Science and Technology, ShanghaiTech University, 393 Middle Huaxia Road, Shanghai, China

*corresponding author: xma@jingmedicine.com

Introduction

- Polycomb Repressive Complex 2 (PRC2), a multimeric complex that catalyzes the methylation of histone H3 at lysine 27 with core subunits EZH2, EED and SUZ12, functions as an epigenetic modulator in regulating cell proliferation and development. Dysregulation of the PRC2 complex is implicated in hematological and solid malignancies and shown to correlate with poor prognosis in cancers.
- EZH2 inhibitor Tazemetostat (EPZ-6438) has been approved by FDA for the treatment of advanced epithelioid sarcoma and follicular lymphoma. However, acquired mutations in EZH2 and the complementary activity of EZH1 might impair the effectiveness of this class of PRC2 inhibitors.
- Targeting the allosteric subunit EED represents a new strategy to fully inhibit PRC2 activity and address the limitations of EZH2 inhibition.
- HJM-353 is a potent, selective and orally bioavailable EED inhibitor with robust anti-tumor activities, and good DMPK properties.

In vitro Activity of HJM-353

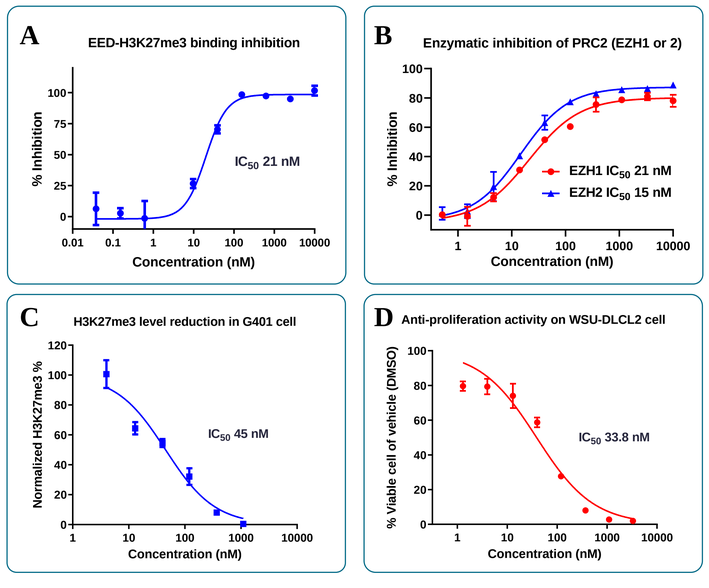


Figure 1 A: HJM-353 inhibits EED-H3K27me3 peptide binding activity, determined by AlphaScreen assay; **B**: HJM-353 inhibits enzymatic histone methyltransferase activity of PRC2 (EZH2) and PRC2 (EZH1) equally, determined by AlphaLISA assay; **C**: HJM-353 effectively inhibits PRC2 activity in G401 cell, indicated by reduction of cellular H3K27me3 level, determined by quantified analysis of Western Blot results after 3-day incubation; **D**: HJM-353 potently inhibits WSU-DLCL2 cell proliferation after 9-day incubation, viable cell numbers were counted by Vi-CELL.

Activity of HJM-353 on Tazemetostat-Resistant Cell

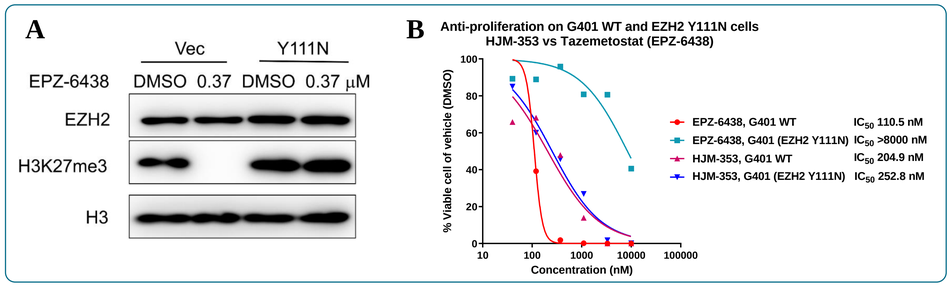
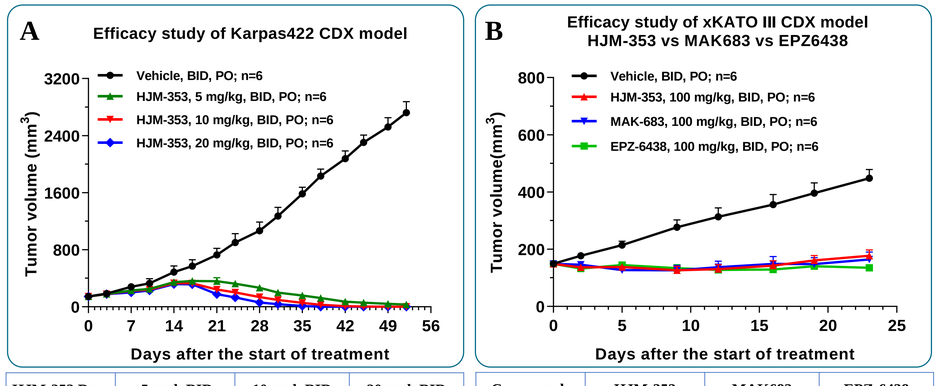


Figure 2 A: Validation of constructed Tazemetostat-resistant rhabdoid G401 cell line (EZH2-Y111N), H3K27me3 level could not be reduced after Tazemetostat (EPZ-6438) treatment at 0.37 μM; **B**: Anti-proliferation activities of HJM-353 and Tazemetostat on G401 WT and EZH2-Y111N mutated cells after 15-day incubation. HJM-353 is not affected by this resistant mutation, while IC_{50} values of Tazemetostat shifted over 80-fold.

DMPK Profiles and Pre-clinical Evaluation of HJM-353

Microsomal stability CL_{int} (m/r/d/cm/h, μL/min/mg protein)		**<7.5/7.6/<7.5/15.6/<7.5**	Plasma stability $t_{1/2}$ (min, m/r/d/h)	**>240**
CYP inhibition (7 isoforms)		**IC_{50} > 30 μM**	CYP TDI (7 isoforms)	**No**
CYP induction (1A2, 2B6, 3A4)		**No induction below 20 μM**	Fu (% free, m/r/d/cm/h @ 2 μM)	**17.1/20.2/28.3/32.2/28.8**
Mouse PK	IV 1 mg/kg	CL **6.17 mL/min/kg**		Vss **0.84 L/kg**
	PO 10 mg/kg	$t_{1/2}$ **2.14 h**	C_{max} **9358 ng/mL**	AUC_{0-24h} **35000 ng·h/mL** F% **130%**
hERG		**IC_{50} > 80 μM**	Mini-Ames/CAA/Rat MN	**All negative**
Histone Methyltransferase panel		**Clean**	SAFETY Scan 47 panel	**IC_{50} or EC_{50} > 10 μM**

In vivo Anti-tumor Activity Evaluation in CDX Models



HJM-353 Dose	5 mpk,BID	10 mpk,BID	20 mpk,BID
TGI @ Day 52	104.4%	105.6%	105.6%
Tumor regression	3/6 mice Complete regression @ D52	All Complete regression @ D49	All Complete regression @ D38

Compounds	HJM-353	MAK683	EPZ-6438
TGI @ Day 23	90.6%	94.9%	104.7%

Figure 3 A: HJM-353 showed highly anti-tumor activity in B cell NHL Karpas422 CDX model, induced tumor complete regression as low as 5 mg/kg, BID dosage; **B**: HJM-353 showed quite similar tumor growth suppression in xKATO III gastric cancer CDX model as MAK683 and Tazemetostat.

PK/PD Study in Karpas422 CDX Model

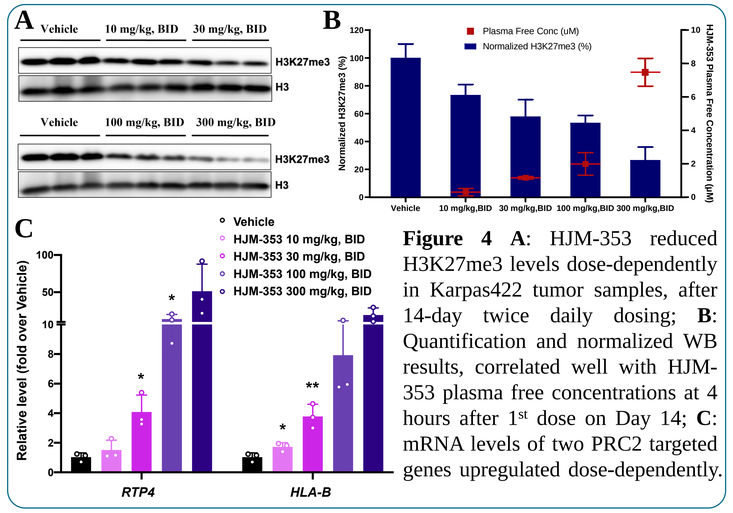


Figure 4 A: HJM-353 reduced H3K27me3 levels dose-dependently in Karpas422 tumor samples, after 14-day twice daily dosing; **B**: Quantification and normalized WB results, correlated well with HJM-353 plasma free concentrations at 4 hours after 1st dose on Day 14; **C**: mRNA levels of two PRC2 targeted genes upregulated dose-dependently.

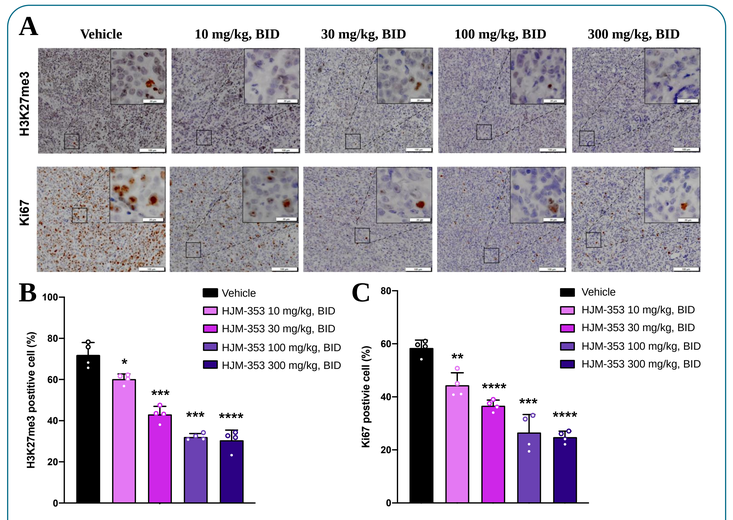


Figure 5 A: IHC staining of H3K27me3 and Ki67 signals in Karpas422 tumor sample slices, scale bars are 100 μm and 20 μm in magnified box; **B**/**C**: Quantification by counting. HJM-353 dose-dependently inhibited PRC2 activity (H3K27me3+) and tumor cell proliferation (Ki67+).

Conclusion

- In summary, HJM-353 demonstrated potent *in vitro* and *in vivo* anti-tumor activities, good drug-like properties and PK/PD correlation, efficacious on Tazemetostat-resistant tumor cells as well (*in vivo* data is not shown).
- GLP toxicity studies were completed, and IND filing is expected in 2022 Q3.

All authors declare no conflict of interests on the poster.