Preclinical evaluation of EZH2 inhibitor tazemetostat-based combination therapies to treat lymphoma and solid tumors

Huijing Yu, Jia Hu, Shiming Fan, Juntao Yu, Qi Ding, Ying Yu, Tingwen Li, Xiaoya Wang, Longxian Jiao, Dongxia Shi, Min Cheng, Linfang Wang, Yongxin Ren, Weiguo Su

HUTCHMED. Building 4, 720 Cai Lun Road, Z.J. Hi-Tech Park, Shanghai, China, 201203

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INTRODUCTION

- **The histone methyltransferase EZH2, as the catalytic subunit of the Polycomb Repressive Complex 2 (PRC2), mediates transcriptional repression by depositing the H3K27me3 chromatin mark [1], silencing tumor suppressor genes and driving oncogenesis. Gain-of-function mutations in EZH2 are prevalent in lymphoid malignancies, while its overexpression is a hallmark of solid tumors [2].**
- **Tazemetostat, the first-in-class selective EZH2 inhibitor, has received FDA approval for epithelioid sarcoma and follicular lymphoma. Recognizing the ubiquitous and fundamental role of epigenetic mechanisms across cancer types, tazemetostat is currently being developed for additional indications and combinatorial strategies.**
- **Amdizalisib (HMPL-689), a selective PI3Kδ inhibitor, has demonstrated favorable safety in B-cell lymphomas [3]. Surufatinib (HMPL-012), a VEGFR/FGFR1/CSF-1R inhibitor with anti-angiogenic and immuno-modulatory properties, has been approved in China for neuroendocrine tumors.**
- **Herein we report preclinical explorations of tazemetostat in combination with amdizalisib in hematologic malignancies and with surufatinib in solid tumors.**

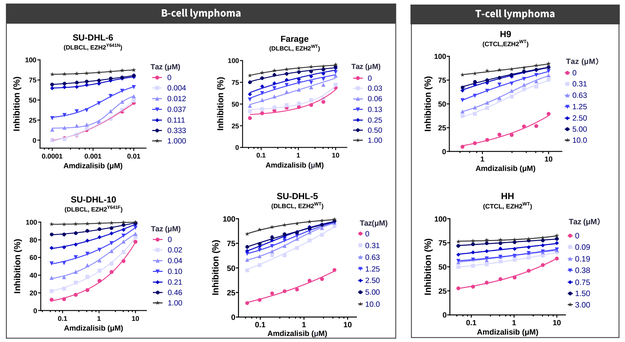
METHODS

- **Cell Viability:** SU-DHL-10 was co-treated with tazemetostat and amdizalisib for consecutive 4 days; SU-DHL-5 and SU-DHL-6 were pre-treated with tazemetostat for 4 days, Farage, H9 and HH were pre-treated for 7 days, then co-treated with amdizalisib for 3 days. Cell viability was determined using the CellTiter-Glo® 2.0 Assay (Promega).
- **Apoptosis Detection:** SU-DHL-10 was co-treated with tazemetostat and amdizalisib for consecutive 4 days; Farage and SU-DHL-5 were pre-treated with tazemetostat for 4 days, H9 was pre-treated for 7 days, then co-treated with amdizalisib for 3 days. Apoptosis was assessed via Annexin V/PI dual staining using flow cytometry, with data analyzed in FlowJo software.
- ***In vivo* Anti-tumor Efficacy:** Human cancer cell line derived xenograft models were established by subcutaneously inoculating tumor cells to immuno-deficient mice. Animals were randomized into different treatment groups , tumor volume and body weight were measured 2-3 times weekly. All compounds were administered by oral gavage.
- **Immunohistochemistry (IHC):** Tumor samples were fixed in 10% neutral buffered formalin (NBF) , embedded in paraffin and sectioned at 4 μm thickness. Sections were immunohistochemical stained with CD31(CST 77699#), images were captured from 3-5 random fields per sample and microvessel density was quantified using ImageJ software.
- **RNA-seq:** Total RNA from NCI-H69 tumor tissues (n=3/group) was extracted using RNeasy Plus Kits (QIAGEN) and subjected to paired-end sequencing on an Illumina NovaSeq platform. Sequencing reads were mapped to the human reference genome (GRCh37).

RESULTS

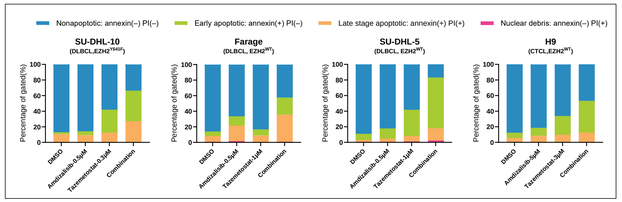
Combination of tazemetostat and amdizalisib (PI3Kδi)

➢ **Tazemetostat synergized with amdizalisib to inhibit B-cell and T-cell lymphoma proliferation *in vitro*.**



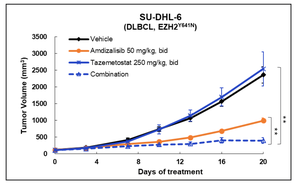
Four DLBCL cell lines (SU-DHL-6, SU-DHL-10, Farage and SU-DHL-5) and two CTCL cell lines (H9 and HH) were treated with the indicated concentrations of amdizalisib, tazemetostat and their combinations. Taz: tazemetostat; DLBCL: Diffuse large B-cell lymphoma; CTCL: Cutaneous T-cell lymphoma.

➢ **Tazemetostat in combination with amdizalisib potentiated lymphoma cells apoptosis.**



Histograms showing percentage of cells in viable, early apoptotic, late apoptotic and debris stage in DLBCL (SU-DHL-10, Farage and SU-DHL-5) and CTCL (H9) cell lines following treatments of tazemetostat, amdizalisib, or their combinations at indicated concentrations.

➢ **Tazemetostat enhanced the anti-tumor activity of amdizalisib in SU-DHL-6 xenograft model.**

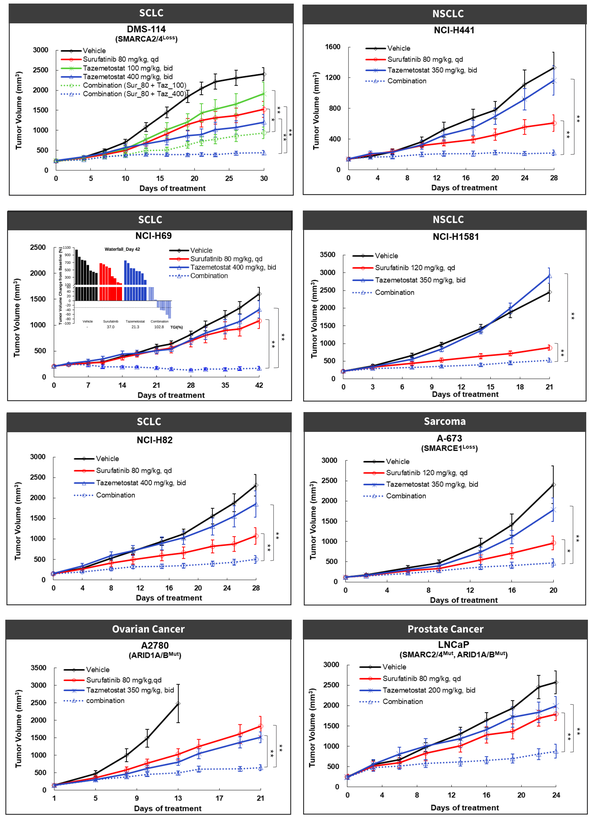


Treatment	TGI % (Day20)
Amdizalisib	60.9
Tazemetostat	-7.6
Combination	87.3

In vivo anti-tumor activity in SU-DHL-6 subcutaneous xenograft model established on CB17-SCID mice. Tumor growth curve during treatment and tumor growth inhibition rate (TGI) at day 20 were shown. Compounds were administered orally. Data was shown as Mean ± SEM. **:P<0.01, unpaired student's t test. N=8 each group.

Combination of tazemetostat and surufatinib (VEGFR/FGFR1/CSF-1Ri)

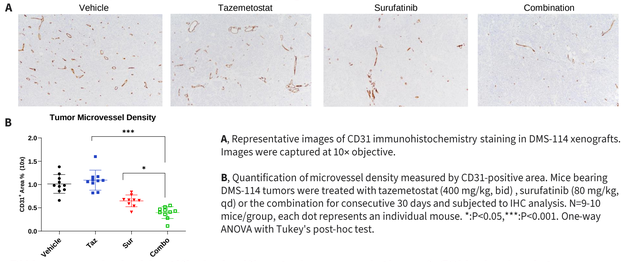
➢ **Combination of tazemetostat and surufatinib displayed stronger anti-tumor activity than single-agent across multiple solid tumor xenograft models *in vivo*.**



In vivo anti-tumor activity in different solid tumor models with or without SWI/SNF deficiency, including small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), Ewings sarcoma, ovarian cancer and prostate cancer model. Mice bearing human tumor cell line derived xenografts were administered orally with tazemetostat (bid), surufatinib (qd) or their combinations at indicated doses.

Data was shown as Mean ± SEM, *:P<0.05,**:P<0.01, unpaired student's t test. N=6-10/group in each study, no obvious body weight loss or signs of toxicity observed in all treatment conditions. qd: once daily, bid: twice daily. TGI: tumor growth inhibition.

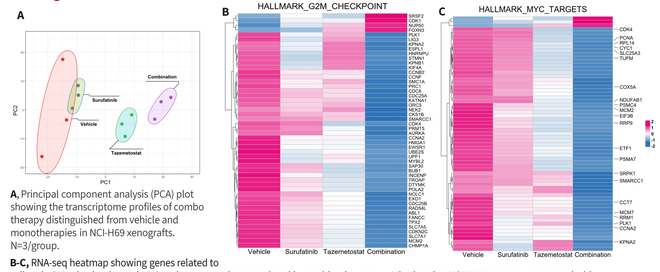
➢ **Tazemetostat potentiated the anti-angiogenic effect of surufatinib in SCLC DMS-114 tumor.**



A, Representative images of CD31 immunohistochemistry staining in DMS-114 xenografts. Images were captured at 10× objective.

B, Quantification of microvessel density measured by CD31-positive area. Mice bearing DMS-114 tumors were treated with tazemetostat (400 mg/kg, bid) , surufatinib (80 mg/kg, qd) or the combination for consecutive 30 days and subjected to IHC analysis. N=9-10 mice/group, each dot represents an individual mouse. *:P<0.05,***:P<0.001. One-way ANOVA with Tukey's post-hoc test.

➢ **RNA-seq analysis identified significant down-regulation of G2M checkpoint genes and MYC-pathway genes following the combination of tazemetostat and surufatinib in NCI-H69 xenograft model.**



A, Principal component analysis (PCA) plot showing the transcriptome profiles of combo therapy distinguished from vehicle and monotherapies in NCI-H69 xenografts. N=3/group.

B-C, RNA-seq heatmap showing genes related to cell cycle G2M checkpoint and MYC pathway were downregulated in combination group. Mice bearing NCI-H69 tumors were treated with tazemetostat (400 mg/kg, bid), surufatinib (80 mg/kg, qd) or the combination for consecutive 14 days. Hierarchical algorithm was used for clustering. Hallmark gene set enrichment analysis was performed using the gene sets from Molecular Signatures Database (MSigDB). N=3/group.

SUMMARY

- **Tazemetostat synergistically enhanced the anti-tumor efficacy of PI3Kδ inhibitor amdizalisib in B-cell and T-cell lymphoma models.**
- **Tazemetostat significantly augmented the anti-tumor efficacy of surufatinib, which was mechanistically achieved through coordinated suppression of angiogenic activity, downregulation of cell cycle regulatory genes, and attenuated oncogenic MYC signaling cascade.**
- **The novel combinations demonstrate the potential of dual-targeting strategies, with tazemetostat-amdizalisib in hematologic malignancies and tazemetostat-surufatinib in solid tumors.**

References

1. Margueron R, Reinberg D. Nature. 2011;469(7330):343-349.
2. Kim KH et al. Nat Med. 2016;22(2):128-134.
3. Cao J et al. Blood. 2020; 136 (Supplement 1): 17–18.

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