#1161

EZH2 Inhibitor Tazemetostat Demonstrates Activity in Preclinical Models of Bruton's Tyrosine Kinase Inhibitor-Resistant Relapsed/Refractory Mantle Cell Lymphoma

Jeffrey Keats, Arleide Lee, Jeremy C. Cunniff, Weiqing Chen, Revonda Mehovic, Vania Estanek, Crag Markwood, Cuyue Tang, Daniel T. Dransfield, Veronica Gibaja, Alejandra Raimondi

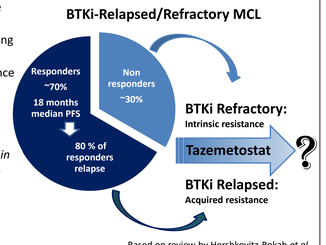
Epizyme
400 Technology Square
Cambridge, MA 02139

INTRODUCTION

Tazemetostat (EZM6438) is a potent, orally bioavailable small molecule inhibitor of EZH2, the enzymatic subunit of the polycomb repressive complex 2, which has been approved for treatment of epithelioid sarcoma and relapsed/refractory follicular lymphoma.

EZH2 plays a key role in B-cell maturation and multiple B-cell malignancies are dependent on EZH2 activity for survival. Mantle cell lymphoma (MCL) is a rare subtype of mature B cell non-Hodgkin lymphoma characterized by the t(11;14)(q13;q32) translocation leading to overexpression of cyclin D1, which plays a significant role in increased tumor cell proliferation via cell cycle dysregulation, chromosomal instability, and epigenetic regulation.

MCL often presents at an advanced stage and while initial responses occur, most cases relapse to frontline therapy, including Bruton's Tyrosine Kinase (BTK) inhibitors. The rate of intrinsic and acquired resistance to these treatments represents a high unmet medical need (as reviewed in Hershkovitz-Rokah *et al*). Treatment with EZH2 inhibitors, as monotherapy or in combination with BTK inhibitors, elicited *in vitro* anti-proliferative activity and *in vivo* tumor growth inhibition in MCL models, demonstrating that EZH2 may be a promising therapeutic target in this indication.



Based on review by Hershkovitz-Rokah *et al.*

We sought to investigate if tazemetostat, as monotherapy or in combination with standard of care (SOC) or emerging therapies, could offer therapeutic benefit to the BTK inhibitor-resistant MCL population.

MATERIALS AND METHODS

Western Blots: Cell lines were treated with 1uM tazemetostat for 4 days. 10ug of total cell lysate was run for WB analysis. Primary antibodies: EZH2, H3K27me3, and H3 (CST), Vinculin (Sigma); Secondary antibodies: Goat-anti-mouse, Goat-anti-rabbit, and Donkey anti-rabbit (Licor).

Generation of Mino IR and ZR Cell Lines: Mino was purchased from ATCC (ATCC CRL-3000) and was cultured in the presence of BTK inhibitors or DMSO for 60 days. Cells were made resistant to 300nM Ibrutinib (Mino IR) or 600nM Zanubrutinib (Mino ZR). Control cells cultured in the presence of 0.1% DMSO for 60 days (Mino D).

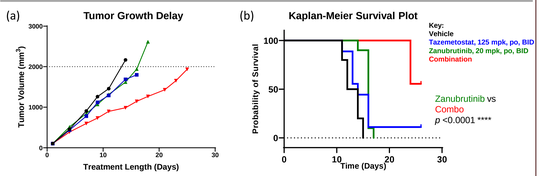
14-Day Long Term Proliferation Assays (LTP): Cell lines were treated in 96-well plates for 7 or 14 days followed by CellTiter Glo viability assay test.

10-Day Proliferation Assays of *ex-vivo* MCL Samples: Samples were treated with CD40L, anti-CD40L mAb, and compounds of interest in 12-well plates for 10 days. Tumoral B-cell viability determined by flow cytometry.

Bioinformatics: RNA-seq 100bp PE assay and primary analysis was done by Q2 solutions. Approximately 30M reads per sample were aligned to H19 using STAR and gene expression was quantified by RSEM. Further RNA-seq analyses were conducted using custom R scripts. WES assay and primary analysis were executed by GeneWiz using the Dragen variant caller against B37. All variants were validated manually in IGV.

7-day Pretreatment + 7-day Cotreatment Combination Platform: Combination potential of tazemetostat was evaluated across MCL cell lines on a 7-day Pretreatment Plus 7-Day Cotreatment Assay Model schedule as follows. Cell lines were pretreated in flasks with a dose range of tazemetostat for 7 days. On day 7, pretreated cultures were harvested and reseeded into 384-well plates with secondary agents for 7 additional days. On Day 14, cell viability was measured by CellTiter Glo. Initial concentration responses were calculated in excel before additional analyses in GraphPad Prism and CHALICE software. CHALICE software was used to calculate the Loewe volume by Loewe Additivity Model, where a positive Loewe volume denoted synergy, a negative Loewe volume antagonism, and a Loewe volume between -1 and 1 denoted additivity.

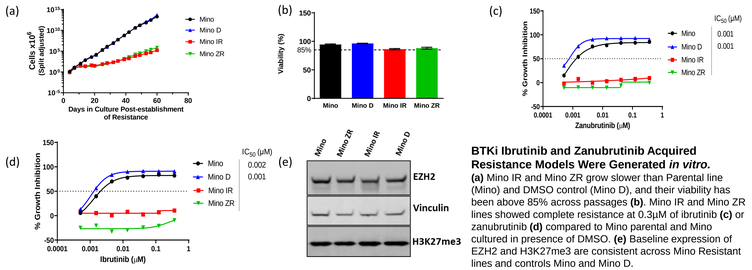
1. TAZEMETOSTAT ENHANCES ANTITUMOR ACTIVITY OF BTKi ZANUBRUTINIB IN MCL CELL LINE MOUSE XENOGRAFT



Mino Cell Line Mouse Xenograft Summary. EZH2 inhibitor EPZ011989 and BTKi ibrutinib have previously shown to have tumor growth inhibitory effects as single agents in the Mino xenograft model (Hood *et al.*). Here we confirm that **(a)** Tazemetostat and zanubrutinib both demonstrated antitumor activity as single agents in this model as shown by tumor growth delay (TGD). **(b)** Tazemetostat demonstrates significant tumor growth delay when administered in combination with BTKi zanubrutinib in this model. Statistical significance was analyzed for the combination using tumor growth delay rates observed by treatment with the standard of care alone (zanubrutinib) vs the respective combination with tazemetostat using the Log-rank (Mantel-Cox) test method.

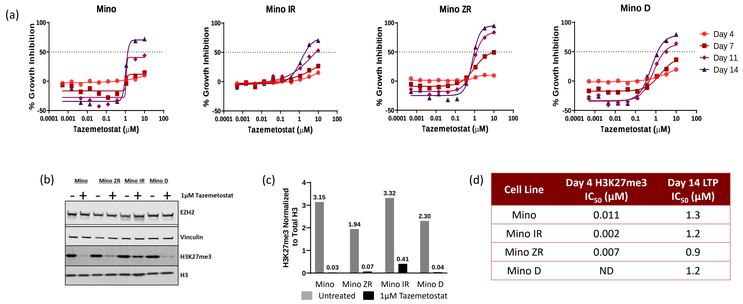
RESULTS

2. GENERATION OF MCL CELL LINES WITH ACQUIRED RESISTANCE TO BTKi



BTKi Ibrutinib and Zanubrutinib Acquired Resistance Models Were Generated *in vitro*. **(a)** Mino IR and Mino ZR grow slower than Parental line (Mino) and DMSO control (Mino D), and their viability has been above 85% across passages **(b)**. Mino IR and Mino ZR lines showed complete resistance at 0.3μM of ibrutinib **(c)** or zanubrutinib **(d)** compared to Mino parental and Mino cultured in presence of DMSO. **(e)** Baseline expression of EZH2 and H3K27me3 are consistent across Mino Resistant lines and controls Mino and Mino D.

3. BTKi-RESISTANT CELL LINES RETAIN SENSITIVITY TO TAZEMETOSTAT

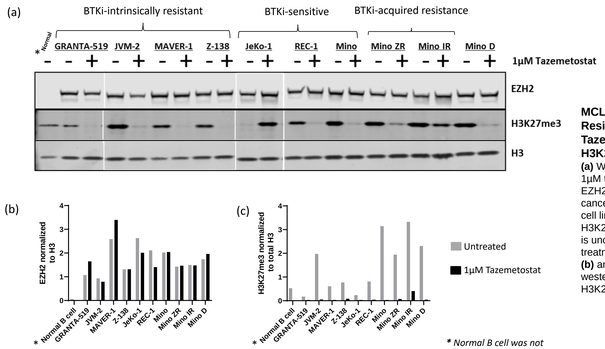


(d)

Cell Line	Day 4 H3K27me3 IC_{50} (μM)	Day 14 LTP IC_{50} (μM)
Mino	0.011	1.3
Mino IR	0.002	1.2
Mino ZR	0.007	0.9
Mino D	ND	1.2

Mino BTKi Resistant Cell Lines Are Dose and Time Sensitive to Tazemetostat. (a) Mino parental, Mino IR, Mino ZR, and Mino D respond to tazemetostat treatment *in vitro*. **(b)** Western blots from 4-day 1uM tazemetostat treatment showed inhibition of H3K27me3 in all lines **(c)** Quantification of blot shown in (b). **(d)** Western blot analysis of tazemetostat dose response in Mino lines confirms expected low IC_{50} for H3K27me3 and consistent proliferation IC_{50} across BTKi sensitive and BTKi resistant lines as seen in (a).

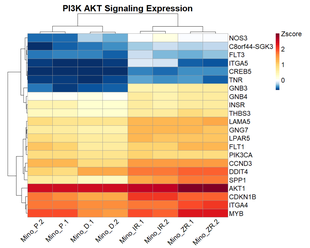
4. TAZEMETOSTAT TREATMENT DECREASES H3K27ME3 CONFIRMING ON TARGET ACTIVITY IN A PANEL OF MCL CELL LINES



MCL Models, Including BTKi Resistant Lines, Show Tazemetostat Effect on H3K27me3.
(a) Western blot analysis from 4-day 1μM tazemetostat treatment showed EZH2 and H3K27me3 levels in 10 cancer cell lines and a normal naïve B cell line. While continuing to affect H3K27me3 levels, EZH2 expression is unchanged under tazemetostat treatment of MCL cell lines. Graphs **(b)** and **(c)** show quantification of the western blot in (a) for EZH2 and H3K27me3 respectively.

5. PI3K/AKT SIGNALING PATHWAY IS UPREGULATED IN THE BTKi-ACQUIRED RESISTANCE SETTING AT THE mRNA AND PROTEIN LEVELS

(a) Upregulation of PI3K/AKT Signaling Pathway at mRNA level



(b) Activation of PI3K/AKT Signaling Pathway at Protein Level

Cell Line	Fold increase p-AKT
Mino	1.0
Mino IR	5.9
Mino ZR	2.1

(a) Gene Cluster Analysis of the PI3K/AKT Signaling Pathway: The heatmap shows that Mino cell lines with acquired BTKi-resistance cluster separately from the BTK sensitive parental and long term DMSO treated Mino cell lines based on genes in the PI3K/AKT pathway. Multiple sources have reported that besides mutations in the BTKi binding region on BTK (Chiron *et al.*), which was not found in these BTK-resistant cell lines (data not shown), upregulation of the PI3K/AKT pathway can lead to BTKi resistance in MCL. (Chiron *et al.*, Ma *et al.)* **(b)** Activation of basal levels of phospho-AKT protein was confirmed in the MINO IR and MINO ZR lines when compared to the Mino Parental cell line by western blot analysis. Fold increase over parental line shown in Table.

6. TAZEMETOSTAT SHOWS DIFFERENTIAL COMBINATORIAL ACTIVITY WITH BTK INHIBITORS BETWEEN BTKi-INTRINSIC AND ACQUIRED RESISTANCE MODELS

Drugs	Z-138	MAVER-1	GRANTA-519	JVM-2	JeKo-1	Mino	REC-1	Mino ZR	Mino IR
Taz/Acalabrutinib									
Taz/Ibrutinib									
Taz/Zanubrutinib									
Ibrutinib 7-Day IC_{50} (μM)	>1	>1	>1	1.0	0.019	0.006	0.0005	>1	>1
Taz 14-day LTP IC_{50} (μM)	1.0	0.4	0.2	>10	0.7	1.0	1.0	1.2	0.9

BTKi-intrinsically resistant | BTKi-sensitive | BTKi-acquired resistant

Combination Score	No Enhancement	Additive	Moderate Synergy	Synergy

***In Vitro* Combination Platform.** The seven standard MCL lines, both intrinsically resistant or sensitive to BTKi, show combination benefit when pretreated with tazemetostat before being cotreated with BTKis. Pretreatment with tazemetostat did not resensitize BTKi-acquired resistant cell lines to BTK inhibition.
No Enhancement (only one single agent elicits greater the 50% inhibition) or Additivity: Loewe volume -1 to 1, Moderate Synergy: Loewe volume 1 to 2 Synergy: Loewe volume >2

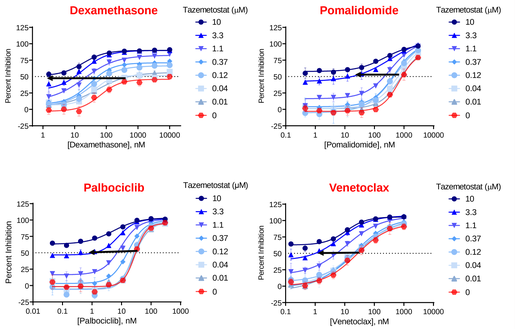
7. TAZEMETOSTAT SYNERGIZES WITH SOCs AND EMERGING THERAPIES IN BTKi-ACQUIRED RESISTANT CELL LINES

(a)

Drug Class	Drug Name	MINO Parental	Mino IR	MINO ZR
Glucocorticoid receptor agonists	Prednisolone			
	Dexamethasone			
IMiD	Pomalidomide			
	Lenalidomide			
Proteasome inhibitor	Bortezomib			
BCL2 inhibitor	Venetoclax			
CDK4/6 inhibitor	Abemaciclib			
	Palbociclib			
Anti-CD20 antibody	Rituximab			
Chemotherapies	Mafosfamide			
	Doxorubicin			
	Vincristine			
	Cytarabine			

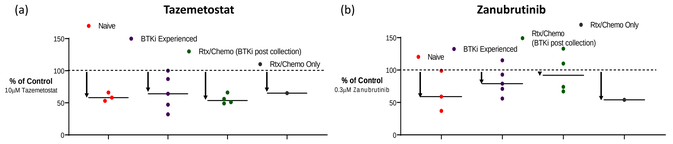
Combination Score	No Enhancement	Additive	Moderate Synergy	Synergy

(b) **COMBINATORIAL ACTIVITY IN MINO IBRUTINIB-RESISTANT MODEL**



Tazemetostat Shows Combination Benefit in Mino Parental and BTKi Acquired Resistance Cell Lines in the 7-Day Pretreatment Plus 7-Day Cotreatment Model. (a) Table showing combinatorial results when tazemetostat was combined with MCL standards of care, or emerging therapies in the 7-Day Pretreatment plus 7-Day Cotreatment combination assay. **(b)** Dose response plots depicting the IC_{50} shifts (leftward arrows) upon addition of tazemetostat to the second agents in the MINO ibrutinib-resistant cell line

8. TAZEMETOSTAT DECREASES IN VITRO PROLIFERATION OF TUMOR B-CELLS DERIVED FROM BTKi-EXPERIENCED PATIENT SAMPLES



MCL *ex vivo* Patient Samples Show Moderate Inhibition When Treated With Tazemetostat *in vitro* in a 10-Day Proliferation Assay. *In vitro* data suggest that samples from patients previously treated with BTKi or Rituximab/Chemotherapy (purple or green data points) show slightly greater sensitivity upon treatment with tazemetostat **(a)** than with BTKi zanubrutinib **(b)**.

SUMMARY AND CONCLUSIONS

- We have previously reported (Hood, *et al*) that tazemetostat resensitized a subset of MCL cell lines intrinsically resistant to BTKis, ibrutinib and acalabrutinib, and further enhanced these BTKis activity in sensitive cell lines. In the present study we show similar results when tazemetostat was combined with the recently approved BTKi zanubrutinib. MINO xenograft in vivo study shows significant tumor growth delay with the combination of tazemetostat and zanubrubinib compared to the single agents alone.
- *In vitro* acquired resistance was generated in the Mino MCL cell line to the BTK inhibitors ibrutinib and zanubrutinib and demonstrated retained sensitivity to tazemetostat.
- Preliminary analysis indicate that acquired resistance in these cell lines is not driven by BTKi binding site mutations (no mutations observed by WES, including BTK C481S mutations[3]). Data suggest that the PI3K-AKT pathway is activated as upregulation of the pathway at the mRNA level and increased pAKT and pERK proteins were observed in the BTKi acquired resistant lines compared to the parental line, consistent with the literature[3,4].
- Synergy was observed in *in vitro* combination studies with tazemetostat and IMiDs lenalidomide and pomalidomide, glucocorticoid receptor agonists prednisolone and dexamethasone, BCL2 inhibitor venetoclax, and CDK4/6 inhibitor palbociclib in the BTKi-acquired resistant models.
- Lastly, tazemetostat induced anti-proliferative effects *ex vivo* in samples derived from MCL patients that were relapsed or refractory to one or more current SOCs including ibrutinib.
- In summary, the data suggest that tazemetostat treatment (alone or in combination with a BTK inhibitor, SOCs, or emerging therapies) could be a therapeutic option in the treatment of the MCL patient population that is primary refractory/relapsed to BTK inhibitor therapy.

References:
[1] Hershkovitz-Rokah *et al.* British Journal of Haematology, 2018
[2] Hood *et al.* AACR: Cancer Res 2018;78(13 Suppl):Abstract nr808
[3] Chiron *et al. Cancer Discovery* 2014
[4] Ma *et al.* British J Haematology 2014

Acknowledgements We would like to thank the employees of Epizyme, Inc. for helpful discussions in regard to this publication. **Funding**: Epizyme, Inc.