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# The CDK9 inhibitor enitociclib overcomes resistance to BTK inhibition and CAR-T therapy in mantle cell lymphoma

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## Abstract

Inhibitors of Bruton's tyrosine kinase (BTKi) and chimeric antigen receptor T-cell (CAR-T) therapy targeting CD19 are paradigm-shifting advances in treating patients with aggressive mantle cell lymphoma (MCL). However, clinical relapses following BTKi and CD19-directed CAR-T treatments are a fast-growing medical challenge. Development of novel therapies to overcome BTKi resistance (BTKi-R) and BTKi-CAR-T dual resistance (Dual-R) are urgently needed. Our single-cell RNA sequencing data revealed major transcriptomic reprogramming, with great enrichment of MYC-targets evolving as resistance to these therapies developed. Interestingly, cyclin-dependent kinase 9 (CDK9), a critical component of the positive transcription elongation factor-b complex, was among the top upregulated genes in Dual-R vs. BTKi-R samples. We therefore hypothesized that targeting CDK9 may turn off MYC-driven tumor survival and drug resistance. Enitociclib (formerly VIP152) is a selective CDK9 inhibitor whose potency against MCL has not been assessed. In this study, we found that enitociclib was highly potent in targeting lymphoma cells, with the half-maximal inhibitory concentration ( $IC_{50}$ ) ranging from 32 to 172 nM in MCL and diffuse large B-cell lymphoma cell lines. It inhibited CDK9 phosphorylation and downstream events including *de novo* synthesis of the short-lived proteins c-MYC, MCL-1, and cyclin D1, and induced apoptosis in a caspase-3-dependent manner. Enitociclib potently inhibited *in vivo* tumor growth of cell line-derived and patient-derived xenografts having therapeutic resistance. Our data demonstrate the potency of enitociclib in overcoming therapeutic resistance in MCL models and provide evidence in favor of its clinical investigation.

**Keywords** CDK9, Mantle cell lymphoma, Enitociclib, VIP152, MYC, MCL-1, Relapse, Therapeutic resistance, BTK inhibitors, CAR-T therapy.

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## To the editor

Mantle cell lymphoma [1] is a very aggressive subtype of non-Hodgkin lymphoma. There have been paradigm-shifting therapeutic advances in the last decade, including BTKi therapies (ibrutinib, acalabrutinib, zanubrutinib, and pirtobrutinib) and anti-CD19 CAR-T therapy [2–6]. However, therapeutic relapse frequently occurs, and there is a rising need to prevent or overcome resistance in patients who relapse. Our single-cell RNA sequencing data showed that MYC targets were progressively enriched with BTKi resistance (Fig. 1A). MYC mRNA expression was upregulated in BTKi-R compared to BTKi-sensitive (BTKi-S) cells, and its high expression correlated with poor patient survival in our patient cohort ( $p=0.037$ ) (Fig. 1B) and two independent cohorts [7, 8] ( $p=0.0032$  and  $0.0027$ , respectively) (Supplementary Figure S1 A-B). Moreover, cyclin-dependent kinase 9 (CDK9) was among the top upregulated genes in Dual-R samples vs. solely BTKi-R samples [9]. Therefore, we targeted the transcription gatekeeper CDK9 to see if that approach could overcome therapeutic resistance. CDK9 inhibition by small molecules such as AZD4573 induces acute loss of short-lived mRNA and proteins, including c-MYC and MCL-1 [10]. Enitociclib is a selective and potent CDK9 inhibitor with a better safety profile than AZD4573 [11]; however, its potency in treating MCL and whether it overcomes therapeutic resistance is not known.

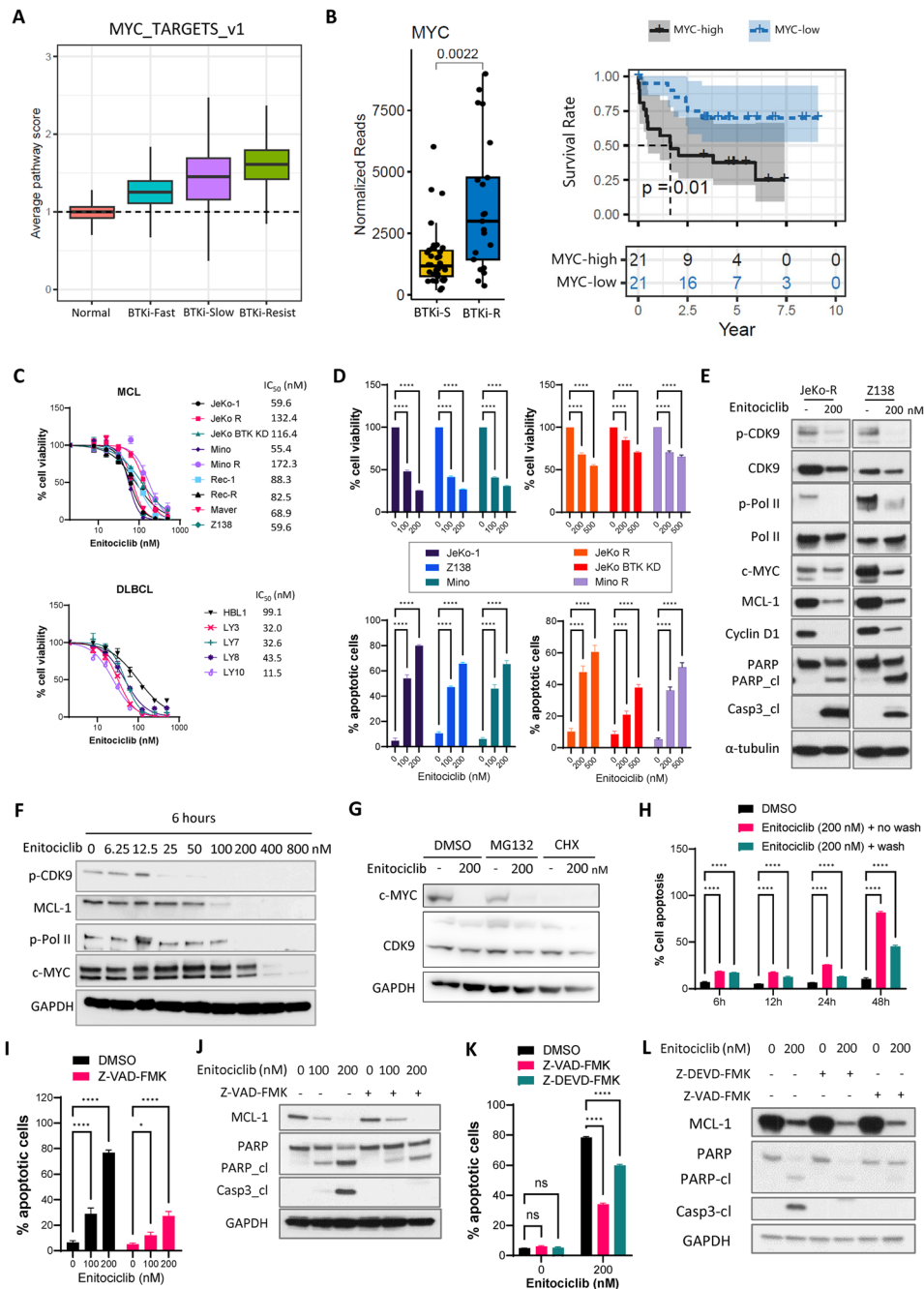
Enitociclib was highly potent in primary MCL cells, MCL cell lines, and diffuse large B-cell lymphoma (DLBCL) cell lines, with an  $IC_{50}$  of 32–172 nM (Fig. 1C and Supplementary Figure S2 A). Enitociclib inhibited cell viability in a dose- and time-dependent manner by robustly inducing apoptosis (Fig. 1D and Supplementary Figure S2 B-C and S3A-B). Upon treatment with enitociclib for 6 h, CDK9 phosphorylation was markedly inhibited in both JeKo-R cells with *acquired* BTKi-resistance and Z138 cells with *primary* BTKi-resistance (Fig. 1E). Correspondingly, CDK9 downstream signaling events, including phosphorylation of RNA polymerase II (Pol II) at Ser 2, were also downregulated, along with reduced expression of the short-lived proteins c-MYC, MCL-1, and cyclin D1 (Fig. 1E). These changes were dose-dependent in JeKo-R cells (Fig. 1F). Similarly, cycloheximide (CHX), a translation elongation inhibitor, diminished c-MYC expression, while the proteasome inhibitor MG132 failed to rescue enitociclib-induced c-MYC downregulation (Fig. 1G and Supplementary Figure S4). These indicate that enitociclib blocks *de novo* gene expression of short-lived proteins but not protein degradation.

Additionally, the two apoptosis indicators, cleaved PARP (poly (ADP-ribose) polymerase) and cleaved caspase-3, were markedly upregulated upon CDK9

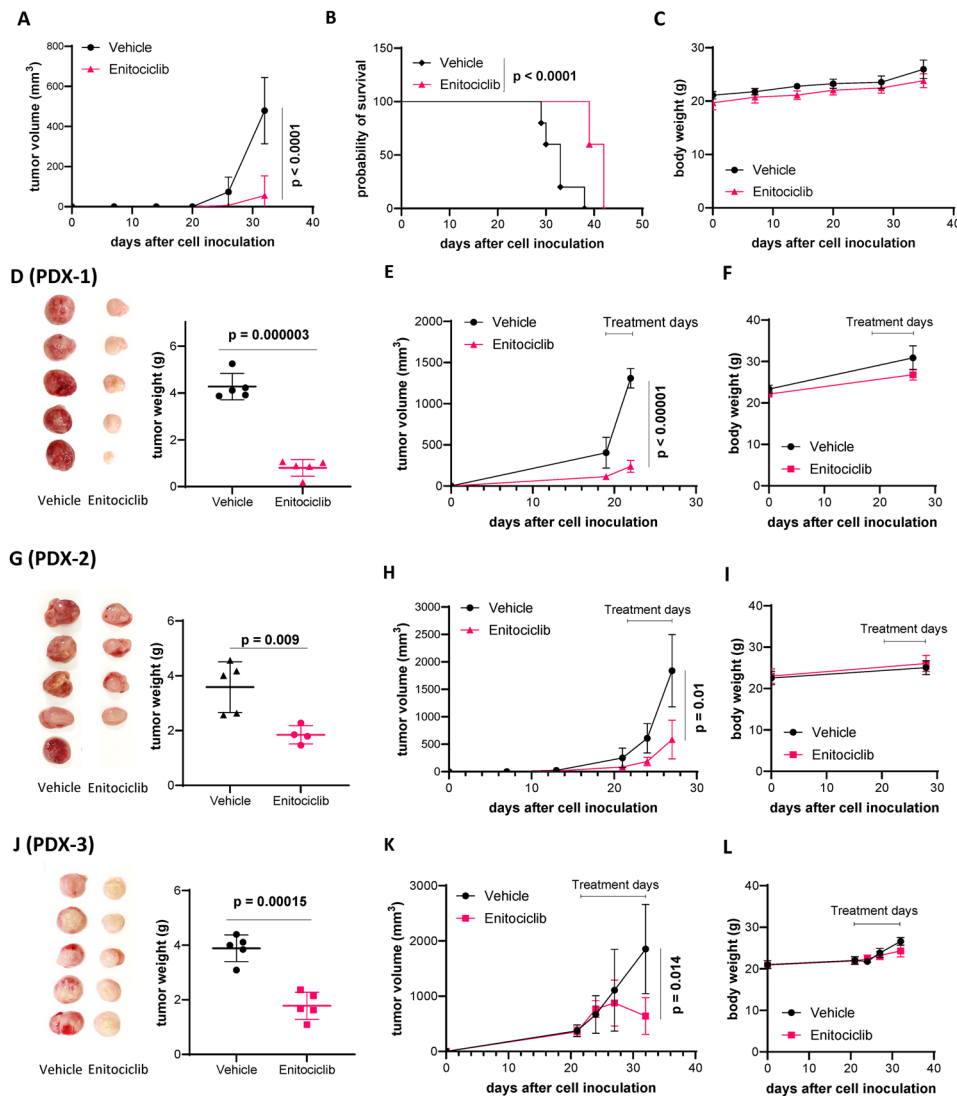
inhibition (Fig. 1E). Enitociclib treatment triggered apoptosis as early as 6 h and further augmented it at 24 and 48 h in JeKo-R cells (Fig. 1H). Enitociclib-triggered apoptosis was blocked by the pan-caspase inhibitor Z-VAD-FMK and by the specific caspase-3 inhibitor Z-VEAD-FMK in JeKo-R and JeKo-1 cells (Fig. 1I-L and Supplementary Figure S5 A-B). Together, these data demonstrate that targeting CDK9 with enitociclib triggered apoptosis in a caspase-3-dependent manner.

To determine whether targeting CDK9 with enitociclib can effectively and safely overcome therapeutic resistance in MCL, we first tested its *in vivo* efficacy using JeKo-1 cell line-derived xenografts (CDXs). Enitociclib at 10 mg/kg (IV, twice a week) markedly inhibited the tumor growth of JeKo-1 CDXs in immunodeficient NSG (NOD.Cg-Prkdc<sup>scid</sup>Il2rg<sup>tm1Wjl</sup>/SzJ) mice ( $p<0.0001$ ) and correspondingly prolonged mouse survival ( $p<0.0001$ ) (Fig. 2A-B) without significant body weight loss (Fig. 2C) or other apparent adverse effect. To further address this, we established patient-derived xenograft (PDX) models from three patients with different types of therapeutic resistance: PDX-1 having BTKi resistance (Fig. 2D-F), PDX-2 having dual resistance to the BTKi ibrutinib and the Bcl-2 inhibitor venetoclax (Fig. 2G-I), and PDX-3 having dual BTKi-CAR-T resistance (Fig. 2J-L). Enitociclib efficaciously inhibited *in vivo* PDX growth of PDX-1 ( $p=0.00015$ ), PDX-2 ( $p=0.009$ ), and PDX-3 ( $p=0.000003$ ) without causing significant body weight loss (Fig. 2D-L).

Our findings showed that targeting CDK9 with its specific inhibitor enitociclib led to potent anti-lymphoma activity *in vitro* and *in vivo*. Enitociclib induced rapid CDK9 inhibition and a rapid decline in c-MYC, MCL-1, and cyclin D1 to robustly induce apoptosis, which is predominantly dependent on caspase-3 activation. Enitociclib also significantly impeded tumor growth in mouse CDX and PDX models. These data demonstrate that CDK9 is a promising target in MCL and may be utilized to overcome therapeutic resistance to BTKi and CART therapy in MCL. In a phase I dose-escalation trial, enitociclib was reported to be safe and effective in treating double-hit DLBCL patients [12]. Altogether, it highlights the targeting of CDK9 as a potentially effective regimen for treatment of advanced disease. Translational and mechanistic studies are ongoing to understand how targeting CDK9 can overcome therapeutic resistance in lymphoma.



**Fig. 1** Targeting CDK9 with the specific inhibitor entitociclib potently inhibited lymphoma cell growth by suppressing *de novo* expression of short-lived proteins and inducing apoptosis. **(A)** MYC-TARGETS-v1 was progressively enriched in BTKi-fast responders (-Fast), -slow responders (-Slow) and non-responders (-Resist) based on GSEA analysis of single-cell RNA-seq data from MCL patient samples. **(B)** MYC mRNA expression was higher in BTKi-resistant (BTKi-R) than BTKi-sensitive (BTKi-S) MCL cells (left panel), and its high expression correlated with poor patient survival (right panel). **(C)** Cell viability assay assessing the in vitro efficacy of entitociclib in 9 MCL cell lines (top panel) and 5 DLBCL cell lines (bottom panel). The IC50 is presented to the right of each cell line. **(D)** Entitociclib at the indicated concentrations inhibited cell viability and induced apoptosis in a dose-dependent manner in MCL cells after 24 hr of treatment. **(E)** Western blots show that entitociclib inhibited CDK9 phosphorylation and Pol II phosphorylation at Ser 2, reduced expression of c-MYC, MCL-1, and cyclin D1, and induced cleavage of PARP and caspase-3 in JeKo-R and Z138 cells. **(F)** Entitociclib dose-dependently suppressed CDK9 phosphorylation, Pol II phosphorylation, and expression of c-MYC and MCL-1 by 6 hr after treatment in JeKo-R cells. **(G)** Pretreatment of cycloheximide (CHX, 50?g/ml) for 1 hour diminished c-MYC expression, while pretreatment of MG132 (10?M) failed to rescue entitociclib (200 nM)-induced c-MYC downregulation in JeKo-R cells. **(H)** Entitociclib (200 nM) induced apoptosis at 24 and 48 hr even when the cells were treated with entitociclib for only the first 6 hr in JeKo-R cells. **(I-J)** Pan-caspase inhibitor Z-VAD-FMK (10?M) blocked entitociclib (200 nM)-induced apoptosis by cell apoptosis assay **(I)** and western blot **(J)** in JeKo-R cells. **(K-L)** Caspase-3-specific inhibitor Z-DEAD-FMK (20?M) rescued entitociclib (200 nM)-induced apoptosis by cell apoptosis assay **(K)** and western blot **(L)** in JeKo-R cells. \*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001; \*\*\*\*, p < 0.0001



**Fig. 2** Enitociclib potently inhibited MCL cell growth in MCL cell line-derived xenografts (CDX) and patient-derived xenografts (PDX) models in mice. (A–C) Enitociclib (10 mg/kg, IV, twice a week) markedly inhibited tumor growth in JeKo-1 CDXs. Tumor volume (A), mouse survival (B), and mouse body weight (C) are plotted. Statistical significance is indicated in the graphs. (D–F, PDX-1) Enitociclib (10 mg/kg, IV, twice a week) effectively inhibited tumor growth in PDX models with ibrutinib resistance (D–F, PDX-1), ibrutinib-venetoclax dual resistance (G–I, PDX-2), or dual resistance to ibrutinib and CAR-T therapy (J–L, PDX-3). Mouse tumors were dissected, imaged, and weighed (D, G, and J). Tumor size (E, H, and K), and mouse body weight (F, I, and L) are plotted

**Abbreviations**

BTK	Bruton's tyrosine kinase
BTKi	BTK inhibitor
CAR-T	chimeric antigen receptor T cell
CDK9	cyclin-dependent kinase 9
CDX	cell line-derived xenograft
DLBCL	diffuse large B-cell lymphoma
BTKi-Fast	BTKi fast responder
BTKi-R	BTKi-resistant
BTKi-Resist	BTKi non-responders
BTKi-S	BTKi-sensitive
BTKi-Slow	BTKi slow responder
Dual-R	BTKi-CAR-T dual resistant
IC <sub>50</sub>	Half-maximal inhibitory concentration
MCL	mantle cell lymphoma
PDX	patient-derived xenograft
Pol II	RNA polymerase II

**Supplementary Information**

The online version contains supplementary material available at <https://doi.org/10.1186/s40364-024-00589-7>.

- Supplementary Material 1
- Supplementary Material 2
- Supplementary Material 3
- Supplementary Material 4
- Supplementary Material 5
- Supplementary Material 6

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#### Author contributions

MW and VJ conceived the idea and designed the experiments; WL, VJ, TZ, AJ, QC, FY, JC, YL, and JM performed the experiments; WL, TZ, QC, FY, and MW performed data analysis; WL and VJ drafted the manuscript; VJ, WL, and MW revised the manuscript. All authors read and approved the final manuscript.

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#### Data availability

The single-cell RNA sequencing dataset and bulk RNA sequencing dataset have been deposited in the European Genome-Phenome Archive (EGA) database under the accession codes EGAS00001005019 and EGAS00001003418. All other data generated or analyzed during this study are included in this published article and its supplementary information files.

#### Declarations

##### Ethics approval and consent to participate

The patient samples were collected from peripheral blood, bone marrow, or apheresis after obtaining informed consent and approval from the Institutional Review Board at The University of Texas MD Anderson Cancer Center. All animal experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center. According to this protocol, the maximal tumor size should be limited to 20 mm, which was not exceeded in this study.

##### Consent for publication

Not applicable.

##### Competing interests

MW has the following potential competing interests: Consultancy: AbbVie, Acerta Pharma, ADC Therapeutics America, Amphista Therapeutics Limited, AstraZeneca, Be Biopharma, BeiGene, BioInvent, Bristol Myers Squibb, Deciphera, DTRM Biopharma (Cayman) Limited, Genentech, InnoCare, Janssen, Kite Pharma, Leukemia & Lymphoma Society, Lilly, Merck, Miltenyi Biomedicine, Milken Institute, Oncternal, Parexel, Pepromene Bio, Pharmacyclics, VelosBio. Research: Acerta Pharma, AstraZeneca, BeiGene, BioInvent, Celgene, Genmab, Genentech, Innocare, Janssen, Juno Therapeutics, Kite Pharma, Lilly, Loxo Oncology, Molecular Templates, Oncternal, Pharmacyclics, VelosBio, Vincerox. Honoraria: AbbVie, Acerta Pharma, AstraZeneca, Bantam Pharmaceutical, BeiGene, BioInvent, Bristol Myers Squibb, CAHON, Dava Oncology, Eastern Virginia Medical School, Genmab, i3Health, ICML, IDEOlogy Health, Janssen, Kite Pharma, Leukemia & Lymphoma Society, Medscape, Meeting Minds Experts, MD Education, MJH Life Sciences, Merck, Moffit Cancer Center, NIH, Nurix, Oncology Specialty Group, OncLive, Pharmacyclics, Physicians Education Resources (PER), Practice Point Communications (PPC), Scripps, Studio ER Congressi, WebMD. No other authors have potential competing interests.

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