Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Targeted BTK degradation via PROTACs enhances therapeutic efficacy against lymphoma through synergy with METTL3 inhibitors

Eunji Na
Inje University
HwaJeong Kang
Inje University
Yurim Jeong
Inje University
Hyewon Jang
Inje University
Cheol Won Jeong
Inje University
Wan-Su Choi
Inje University
Jung-Yeon Lim
limjy@inje.ac.kr

Inje University

Article

Keywords: Burkitt's lymphoma, PROTAC, BTK, METTL3, Apoptosis

Posted Date: April 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9330895/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Bruton's tyrosine kinase (BTK) plays a pivotal role in the development and survival of B cells, rendering it a well-established therapeutic target in various hematologic malignancies. Although small-molecule BTK inhibitors (iBTKs) have been widely investigated and successfully implemented in clinical practice, their therapeutic efficacy is frequently compromised by the emergence of resistance mechanisms, including compensatory signaling pathways. To overcome these limitations, proteolysis-targeting chimeras (PROTACs) have gained attention as an alternative strategy that facilitates selective degradation of BTK rather than mere enzymatic inhibition. In this study, we systematically compared the antitumor activities of conventional iBTKs and PROTAC-based BTK degraders (dBTKs) to assess their relative effectiveness. Notably, dBTKs exhibited superior suppression of cell proliferation and more robust induction of apoptosis in Burkitt's lymphoma cell lines. Simultaneously, we explored the therapeutic potential of targeting METTL3, a methyltransferase involved in N6-methyladenosine (m^6A) RNA modification, which has been increasingly implicated in tumor progression. Inhibition of METTL3 has shown promise in disrupting oncogenic RNA regulatory networks, suggesting its utility as a combinatorial partner in targeted cancer therapies. Building upon these insights, we evaluated the combinatorial efficacy of dBTKs with a METTL3 inhibitor (iMETTL3). Co-treatment with dBTKs and iMETTL3 resulted in significantly enhanced antitumor effects, as evidenced by greater reductions in cell viability and a synergistic increase in apoptosis relative to monotherapies. These findings highlight the therapeutic advantage of simultaneously targeting BTK for degradation and inhibiting METTL3, providing a compelling rationale for dual-targeted treatment strategies in lymphoma and potentially other hematologic cancers.

Introduction

Bruton's tyrosine kinase (BTK) is a non-receptor tyrosine kinase that plays a pivotal role in the B cell receptor (BCR) signaling pathway, where it governs critical processes such as B cell development, proliferation, and differentiation [1,2]. Aberrant BTK activation has been implicated in the pathogenesis of several B cell malignancies, including Burkitt's lymphoma. While BTK inhibition has emerged as a promising therapeutic approach, clinical outcomes remain suboptimal—particularly in pediatric, elderly, or treatment-refractory patient populations [3,4].

Covalent BTK inhibitors, such as Ibrutinib, Zanubrutinib, and Acalabrutinib, have shown significant efficacy in the treatment of B cell lymphomas [5,6]. Among them, Ibrutinib irreversibly binds to BTK and effectively disrupts downstream signaling, leading to impaired survival and proliferation of malignant B cells. However, the emergence of resistance—often driven by mutations that hinder drug binding—has limited the long-term efficacy of these agents [7]. Consequently, alternative strategies that go beyond enzymatic inhibition are being actively explored.

Proteolysis-targeting chimeras (PROTACs) represent a novel modality for targeted protein degradation. By recruiting E3 ubiquitin ligases to the target protein, PROTACs facilitate its ubiquitination and

subsequent degradation via the proteasome [8,9]. BTK-targeting PROTACs, such as MT-802, have demonstrated potent anti-proliferative activity in B cell malignancies, offering a promising solution to overcome resistance associated with conventional inhibitors [10].

In parallel, recent studies have highlighted the role of epitranscriptomic regulation in cancer, particularly the function of METTL3, an RNA methyltransferase responsible for N6-methyladenosine (m^6A) modification. METTL3 has been shown to promote leukemogenesis and support the survival of malignant hematopoietic cells [11]. STM2457, a selective METTL3 inhibitor, has demonstrated antitumor activity by reducing m^6A modification levels and impairing cancer cell growth and differentiation. Although primarily studied in acute myeloid leukemia, its relevance in lymphoid malignancies is increasingly being recognized [12].

Given the distinct yet complementary mechanisms of BTK degradation and METTL3 inhibition, this study investigates the potential synergistic effects of combining MT-802 (a BTK degrader) and STM2457 (a METTL3 inhibitor) in Burkitt's lymphoma. By targeting both protein signaling and RNA modification pathways, we aim to identify therapeutic vulnerabilities and assess whether this dual-targeted approach can enhance antitumor efficacy and overcome drug resistance. Our findings may inform future strategies for treating aggressive lymphomas with poor prognosis.

Results

Comparative analysis of iBTK and dBTK in Burkitt's lymphoma cells

To evaluate the relative efficacy of covalent BTK inhibition (iBTK) versus targeted BTK degradation (dBTK), we performed CCK-8 assays using Burkitt's lymphoma cell lines Ramos and Daudi. Cells were treated with increasing concentrations (2.5, 5, and 10 μM) of each compound, and viability was assessed at 24, 48, and 72 hours (Fig. 1a–c). In both cell lines, dBTK treatment led to a stronger reduction in cell viability compared to iBTK in a dose-dependent manner. Notably, Ramos cells exhibited markedly enhanced sensitivity to dBTK at all assessed time points, with consistently lower cell viability compared to iBTK treatment (Fig. 1a). Time-course analysis further supported these findings. dBTK treatment resulted in a more pronounced and sustained decrease in cell viability over 72 hours compared to iBTK in both Ramos (Fig. 1d) and Daudi cells (Fig. 1e). These results suggest that BTK degradation by dBTK is more effective in suppressing Burkitt's lymphoma cell proliferation than conventional BTK inhibition.

Comparison of the efficacy of iBTK and dBTK in inducing G2/M phase arrest in Burkitt's lymphoma cells

To determine whether the observed reduction in cell viability was associated with alterations in cell cycle progression, we performed flow cytometric analysis to evaluate the distribution of cell cycle phases following treatment with iBTK or dBTK. After 72 hours of treatment, both compounds significantly

increased the proportion of Ramos cells in the G2/M phase, accompanied by a reduction in the G0/G1 population, with a greater effect observed in dBTK-treated cells. (Fig. 2a). Compared with Ramos cells, Daudi cells showed no significant difference in cell cycle distribution between iBTK- and dBTK-treated groups (Fig. 2b). These results suggest that while both agents affect cell cycle progression, with a more evident response observed in Ramos cells than in Daudi cells.

Evaluation of the apoptosis-inducing effects and mechanisms of iBTK and dBTK

To evaluate the pro-apoptotic effects of iBTK and dBTK, Annexin V/PI staining was performed to quantify early (Annexin V^+/PI^-) and late (Annexin V^+/PI^+) apoptotic populations. Both compounds significantly increased the proportions of apoptotic cells compared to the control group, indicating their capacity to induce apoptosis in Burkitt's lymphoma cells (Fig. 3a, b). Notably, dBTK treatment led to a markedly higher accumulation of early and late apoptotic populations in Ramos cells than iBTK, suggesting superior apoptotic potency (Fig. 3a). To elucidate the molecular mechanisms underlying this apoptosis induction, we evaluated the expression of apoptosis-related proteins, including PARP and caspase-3, via Western blotting. PARP, a substrate of caspase-3, undergoes cleavage during apoptosis and is widely used as a marker of apoptotic activation [13]. Drug-treated cells exhibited increased levels of cleaved PARP and activated caspase-3 relative to the control group (Fig. 3c, d). Consistent with the Annexin V/PI staining results, Ramos cells treated with dBTK showed the highest levels of cleaved PARP and caspase-3 activation (Fig. 3c). Together, these results demonstrate that both iBTK and dBTK effectively induce apoptosis, with dBTK showing enhanced efficacy—particularly in Ramos cells—through activation of key apoptotic signaling pathways.

Enhanced inhibitory effects on Burkitt's lymphoma cell proliferation by the combination of dBTK and iMETTL3

To assess the combined effects of BTK degradation and METTL3 inhibition on Burkitt's lymphoma cell proliferation, CCK-8 assays were conducted using Ramos and Daudi cell lines. Cells were treated with dBTK, iMETTL3, or a combination of both agents, and cell viability was measured at 24, 48, and 72 hours (Fig. 4a–c). Additionally, time-course analysis was performed to examine treatment effects over time in each cell line (Fig. 4d, e). The combination of dBTK and iMETTL3 resulted in significantly greater suppression of cell proliferation compared with either treatment alone at all time points examined. This synergistic effect was particularly evident in Ramos cells, which exhibited the greatest reduction in viability following combination treatment. Collectively, these findings demonstrate that dual targeting of BTK and METTL3 enhances anti-proliferative effects in Burkitt's lymphoma cells, suggesting potential advantages of this combination strategy.

Combination of dBTK and iMETTL3 enhances G2/M phase arrest in Burkitt's lymphoma cells

To further explore the mechanism underlying the antiproliferative effects of dBTK and iMETTL3, we performed cell cycle analysis using flow cytometry. Ramos and Daudi cells were treated with dBTK, iMETTL3, or a combination of both, and the distribution of cells across G0/G1, S, and G2/M phases was assessed (Fig. 5a, b). In Ramos cells, dBTK or iMETTL3 treatment alone induced accumulation of cells in the G2/M phase, whereas combination treatment further enhanced G2/M phase arrest, accompanied by a reduction in the G0/G1 population (Fig. 5a). These findings suggest a synergistic effect of the dual treatment on disrupting cell cycle progression. Compared with Ramos cells, Daudi cells showed less pronounced but noticeable changes in cell cycle distribution across all treatment conditions, with an increase in the G2/M phase population upon combination treatment (Fig. 5b). These results indicate that the combination of dBTK and iMETTL3 synergistically enhances G2/M phase arrest in Ramos cells, while also affecting cell cycle progression in Daudi cells, albeit less prominently. This differential response may suggest intrinsic cell line–specific vulnerabilities to the dual-targeted approach.

Enhanced apoptosis induction by the combination of dBTK and iMETTL3 in Burkitt's lymphoma cells

To assess the pro-apoptotic effects of dBTK and iMETTL3, alone or in combination, we performed Annexin V/PI staining to quantify early and late apoptotic cell populations. Both dBTK and iMETTL3 monotherapies significantly increased the proportion of apoptotic cells compared to the control group. Notably, the combination treatment resulted in a further increase in both early and late apoptosis, indicating a synergistic effect in promoting cell death (Fig. 6a, b). To further validate these findings, Western blot analysis was conducted to examine the expression of key apoptotic markers, including cleaved PARP and activated caspase-3. While each agent alone enhanced cleavage of PARP and activation of caspase-3 relative to the control, combination treatment led to the most pronounced increase in apoptotic protein expression (Fig. 6c, d). Together, these results demonstrate that co-targeting BTK and METTL3 significantly enhances apoptosis in Burkitt's lymphoma cells compared with single-agent treatment.

Discussion

Burkitt's lymphoma is an aggressive subtype of B-cell non-Hodgkin's lymphoma characterized by high proliferation rates and poor outcomes in relapsed or refractory cases, especially among older patients [14]. While frontline chemoimmunotherapy is often effective in younger individuals, resistance to standard regimens underscores the need for novel therapeutic approaches [15,16].

The B-cell receptor (BCR) signaling pathway plays a pivotal role in B-cell survival and is frequently deregulated in B-cell malignancies [1]. BTK, a key component of this pathway, has emerged as a

therapeutic target, with inhibitors such as Ibrutinib showing clinical efficacy in certain lymphoma subtypes [17]. However, in Burkitt's lymphoma, which often exhibits tonic BCR signaling independent of antigen stimulation, BTK inhibitors have shown limited effectiveness [18].

In this study, we demonstrated that the PROTAC-based BTK degrader, MT-802 (dBTK), exhibits superior cytotoxicity compared to the covalent BTK inhibitor Ibrutinib (iBTK) in Burkitt's lymphoma cell lines (Fig. 1). dBTK induced stronger antiproliferative effects, G2/M cell cycle arrest (Fig. 2), and apoptotic activity, as evidenced by Annexin V/PI staining and elevated levels of cleaved PARP and caspase-3 (Fig. 3). These results suggest that targeted degradation of BTK may overcome the limitations of conventional inhibition by eliminating both enzymatic and scaffolding functions of the protein.

We further investigated the therapeutic impact of combining dBTK with STM2457 (iMETTL3), a selective inhibitor of METTL3, an m⁶A methyltransferase implicated in oncogenic RNA regulation and therapy resistance. METTL3 promotes tumor progression through various mechanisms, including modulation of mRNA stability and DNA damage response pathways [19,20]. Co-treatment with dBTK and iMETTL3 led to significantly greater inhibition of cell viability than either agent alone. This was consistently observed across multiple time points and confirmed by increased G2/M phase arrest (Fig. 5). and apoptosis markers (Fig. 6).

Interestingly, the combinatorial effect was more pronounced in the EBV-negative Ramos cell line than in the EBV-positive Daudi cells. This discrepancy may be attributed to the influence of Epstein–Barr virus (EBV) infection, which is known to promote lymphomagenesis through multiple pathways [21]. EBV has been reported to upregulate AID, activate Wnt/β-catenin signaling, and suppress apoptosis by downregulating caspase-3 via viral microRNAs [22,23]. Moreover, EBV can modulate m⁶A RNA methylation, thereby contributing to immune evasion and altered cell cycle regulation [24]. Given that BTK negatively regulates Wnt/β-catenin signaling, its loss in the context of EBV-driven oncogenesis may yield complex and less predictable outcomes.

Taken together, these findings highlight the therapeutic potential of co-targeting BTK and METTL3 in Burkitt's lymphoma, particularly in EBV-negative contexts. However, the distinct molecular features associated with EBV infection may modulate drug sensitivity and require further exploration.

In conclusion, our study proposes a promising dual-targeted approach combining BTK degradation and METTL3 inhibition for the treatment of Burkitt's lymphoma. Future studies involving additional cell models and in vivo validation are warranted to further elucidate the mechanistic basis and clinical relevance of this combination strategy.

Methods

Cell Lines and Cell Culture

Burkitt's lymphoma cell lines including, Ramos and Daudi cells were used. These cell lines were obtained from Korean Cell Line Bank. These cell lines were grown in RPMI 1640 medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco), 2 mM L-glutamine (Gibco), 1% antibiotics (10 U/mL penicillin and 10 g/mL streptomycin; Gibco). All cell lines were incubated at 37℃ and 5% CO_2.

Drug Preparations

Ibrutinib (BTK inhibitor), MT802 (BTK degrader) and STM2457 (METTL3 inhibitor) were purchased from MedChemExpress. Both were dissolved in dimethyl sulfoxide as recommended by the manufacturer and stored at −80℃. All these drugs were diluted with the RPMI 1640 medium with 10% heat-inactivated FBS, 2 mM L-glutamine, and 1% antibiotics before being used for the treatment of cell lines.

Cell Proliferation Assay

Cell growth was assessed using the CCK-8 (Dojindo Laboratories Co., Ltd.) assay according to the manufacturer's protocol. Ramos and Daudi cell lines were seeded at an initial cell density of 2×10^4 cells/100 μL culture medium in 96-well plates. These cells were treated with various doses of Ibrutinib, MT802, and STM2457 drugs without any drugs, 2.5 μM, 5 μM, and 10 μM for each drug. The synergistic effects were assessed using 5 μM for both drugs. Cultures were maintained at 37℃ in a 5% CO_2 atmosphere. CCK-8 solution was added in 10 μL to each well after 24, 48, and 72 hours. The plates were incubated for 1∼4 hours in a CO_2 incubator and the optical density were measured at 450 nm using a microplate reader.

Western Blotting

One million Ramos or Daudi cells were lysed in 2× laemmli sample buffer (Bio-Rad) with β-mercaptoethanol and boiled at 95℃ for 10 minutes. After removal of the insoluble fraction by centrifugation at 10,000×g for 10 min, protein samples were separated by SDS gel electrophoresis and transferred to a polyvinylidene difluoride membrane. The membranes were stained with cleaved poly (ADP-ribose) polymerase (PARP), PARP, cleaved caspase-3 antibodies (Cell Signaling Technology) at a dilution of 1:1,000 or GAPDH antibody (Cell Signaling Technology) at a dilution of 1:5,000 at 4℃ overnight. After overnight incubation at 4℃, HRP-conjugated secondary antibodies were added. After washing with Tris-buffered saline and Tween 20, the hybridized bands were detected using an enhanced chemiluminescence (ECL) detection kit (Amersham Pharmacia Biotech).

Cell Cycle Analysis

The 5×10^5 cells were treated with the drugs described. After incubating for 72 hours in CO_2 incubator, the cells were harvested and centrifuged 2,000 rpm 5 minutes with DPBS (Gibco). These cells were performed twice for wash and discarded the supernatant. For cell cycle analysis, the Annexin V-propidium iodide (PI) Apoptosis Kit (BioVision) was used following the manufacturer's protocol. The cell cycle was detected using the Novocyte Advanteon (Agilent).

Statistical Analysis

Each experiment was repeated at least three times to ensure the reproducibility of the results. All data were statistically evaluated by Student's t-test using GraphPad Prism 8 (GraphPad Software Inc.). The values are depicted using the mean and SEM. Differences between the means of the groups were considered to be statistically significant if $P < 0.05$, $P < 0.01$, and $P < 0.001$.

Declarations

Conflicts of Interest: The authors declare no competing interests.

Data Availability

The datasets generated during the current study available from the corresponding author on reasonable request.

Acknowledgements

The authors would like to thank the Clinical Medicine Research Institute, Yeouido St. Mary's Hospital, Catholic University of Korea, for kindly providing the compounds used in this study.

Funding Declaration

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. NRF-2022R1F1A1061005, RS-2026-25473344).

Author contributions

J.-Y.L. conceived the study; H.K, E.N, Y.J, H.J, C.J, W.-S.C and J-Y.L. conducted experiments and analyzed data; H.K, E.N and J-Y.L. wrote the manuscript with input from all authors.

References

1. Pal Singh, S., Dammeijer, F. & Hendriks, R. W. Role of Bruton's tyrosine kinase in B cells and malignancies. *Mol Cancer***17**, 57 (2018). https://doi.org:10.1186/s12943-018-0779-z
2. Mouhssine, S., Maher, N., Matti, B. F., Alwan, A. F. & Gaidano, G. Targeting BTK in B Cell Malignancies: From Mode of Action to Resistance Mechanisms. *Int J Mol Sci***25** (2024). https://doi.org:10.3390/ijms25063234
3. Anoop, P. *et al.* Outcome of childhood relapsed or refractory mature B-cell non-Hodgkin lymphoma and acute lymphoblastic leukemia. *Leuk Lymphoma***53**, 1882-1888 (2012). https://doi.org:10.3109/10428194.2012.677534
4. Lopez, C. *et al.* Burkitt lymphoma. *Nat Rev Dis Primers***8**, 78 (2022). https://doi.org:10.1038/s41572-022-00404-3

5. Alsuhebany, N., Pan, C., Holovac, E., Do, B. & McBride, A. Zanubrutinib in Mantle Cell Lymphoma Management: A Comprehensive Review. *Blood Lymphat Cancer***13**, 67-76 (2023). https://doi.org:10.2147/BLCTT.S426588
6. Shirley, M. Correction to: Bruton Tyrosine Kinase Inhibitors in B-Cell Malignancies: Their Use and Differential Features. *Target Oncol***17**, 93 (2022). https://doi.org:10.1007/s11523-021-00864-9
7. Dobrovolsky, D. *et al.* Bruton tyrosine kinase degradation as a therapeutic strategy for cancer. *Blood***133**, 952-961 (2019). https://doi.org:10.1182/blood-2018-07-862953
8. Lai, A. C. & Crews, C. M. Induced protein degradation: an emerging drug discovery paradigm. *Nat Rev Drug Discov***16**, 101-114 (2017). https://doi.org:10.1038/nrd.2016.211
9. Bekes, M., Langley, D. R. & Crews, C. M. PROTAC targeted protein degraders: the past is prologue. *Nat Rev Drug Discov***21**, 181-200 (2022). https://doi.org:10.1038/s41573-021-00371-6
10. Li, X. & Song, Y. Proteolysis-targeting chimera (PROTAC) for targeted protein degradation and cancer therapy. *J Hematol Oncol***13**, 50 (2020). https://doi.org:10.1186/s13045-020-00885-3
11. Wang, X. *et al.* Structural basis of N(6)-adenosine methylation by the METTL3-METTL14 complex. *Nature***534**, 575-578 (2016). https://doi.org:10.1038/nature18298
12. Yankova, E. *et al.* Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia. *Nature***593**, 597-601 (2021). https://doi.org:10.1038/s41586-021-03536-w
13. Herceg, Z. & Wang, Z. Q. Failure of poly(ADP-ribose) polymerase cleavage by caspases leads to induction of necrosis and enhanced apoptosis. *Mol Cell Biol***19**, 5124-5133 (1999). https://doi.org:10.1128/MCB.19.7.5124
14. Cowling, V. H., Turner, S. A. & Cole, M. D. Burkitt's lymphoma-associated c-Myc mutations converge on a dramatically altered target gene response and implicate Nol5a/Nop56 in oncogenesis. *Oncogene***33**, 3519-3527 (2014). https://doi.org:10.1038/onc.2013.338
15. Blum, K. A., Lozanski, G. & Byrd, J. C. Adult Burkitt leukemia and lymphoma. *Blood***104**, 3009-3020 (2004). https://doi.org:10.1182/blood-2004-02-0405
16. Dunleavy, K. *et al.* Low-intensity therapy in adults with Burkitt's lymphoma. *N Engl J Med***369**, 1915-1925 (2013). https://doi.org:10.1056/NEJMoa1308392
17. Chu, Y. *et al.* Ibrutinib significantly inhibited Bruton's tyrosine kinase (BTK) phosphorylation,in-vitro proliferation and enhanced overall survival in a preclinical Burkitt lymphoma (BL) model. *Oncoimmunology***8**, e1512455 (2019). https://doi.org:10.1080/2162402X.2018.1512455
18. Schmitz, R. *et al.* Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. *Nature***490**, 116-120 (2012). https://doi.org:10.1038/nature11378
19. Zeng, C., Huang, W., Li, Y. & Weng, H. Roles of METTL3 in cancer: mechanisms and therapeutic targeting. *J Hematol Oncol***13**, 117 (2020). https://doi.org:10.1186/s13045-020-00951-w
20. Esteva-Socias, M. & Aguilo, F. METTL3 as a master regulator of translation in cancer: mechanisms and implications. *NAR Cancer***6**, zcae009 (2024). https://doi.org:10.1093/narcan/zcae009

21. Baran-Marszak, F. *et al.* Gene array identification of Epstein Barr virus-regulated cellular genes in EBV-converted Burkitt lymphoma cell lines. *Lab Invest***82**, 1463-1479 (2002). https://doi.org:10.1097/01.lab.0000035025.51772.2b
22. Shackelford, J. & Pagano, J. S. Tumor viruses and cell signaling pathways: deubiquitination versus ubiquitination. *Mol Cell Biol***24**, 5089-5093 (2004). https://doi.org:10.1128/MCB.24.12.5089-5093.2004
23. Yu, S., Han, R. & Gan, R. The Wnt/beta-catenin signalling pathway in Haematological Neoplasms. *Biomark Res***10**, 74 (2022). https://doi.org:10.1186/s40364-022-00418-9
24. Yanagi, Y. *et al.* EBV Exploits RNA m(6)A Modification to Promote Cell Survival and Progeny Virus Production During Lytic Cycle. *Front Microbiol***13**, 870816 (2022). https://doi.org:10.3389/fmicb.2022.870816

Figures

Figure 1

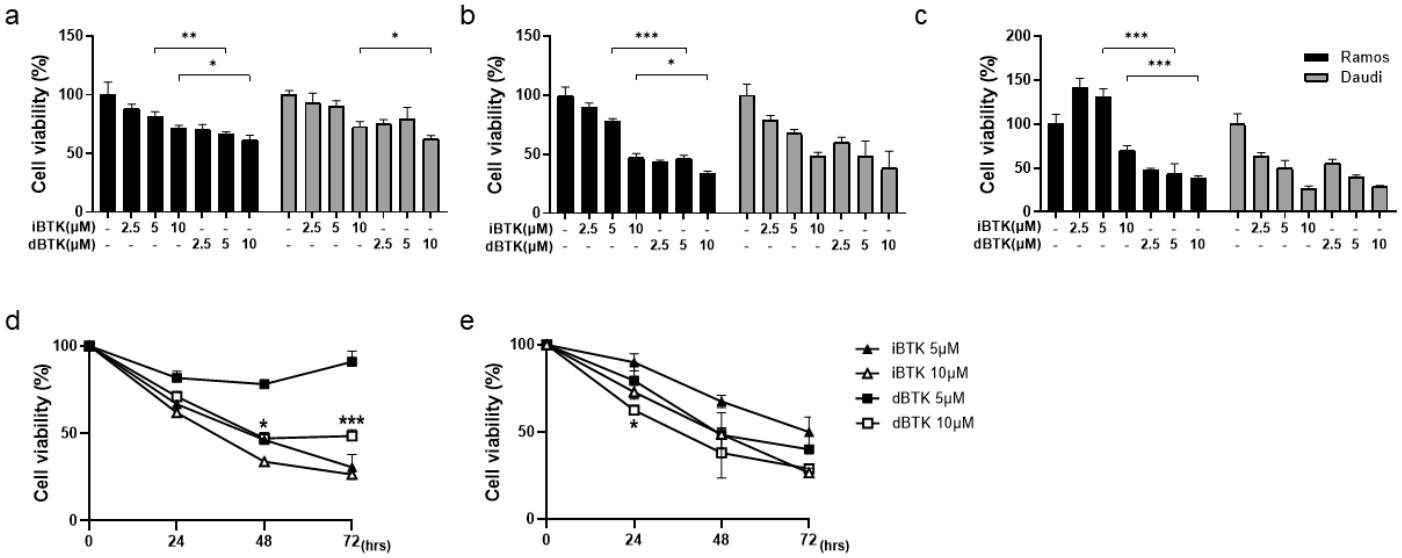


Figure 1

Dose- and time-dependent effects of iBTK and dBTK on the survival of Ramos and Daudi cells. Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay. Each cell line was seeded at a density of 5×10³ cells per well, and the cells were treated with iBTK or dBTK at concentrations of 1.25 μM, 2.5 μM, 5 μM, and 10 μM for (a) 24 hours, (b) 48 hours, and (c) 72 hours. Untreated cells served as the control. iBTK and dBTK were treated at concentrations of 5μM and 10μM each, and the cell viability of (d) Ramos cells and (e) Daudi cells was evaluated after 24, 48, and 72 hours. Statistical analysis used Prism's t-test and was expressed as *P<0.05; **P<0.01; ***P<0.001. Error bars indicate ± standard deviation (SD).

Figure 2

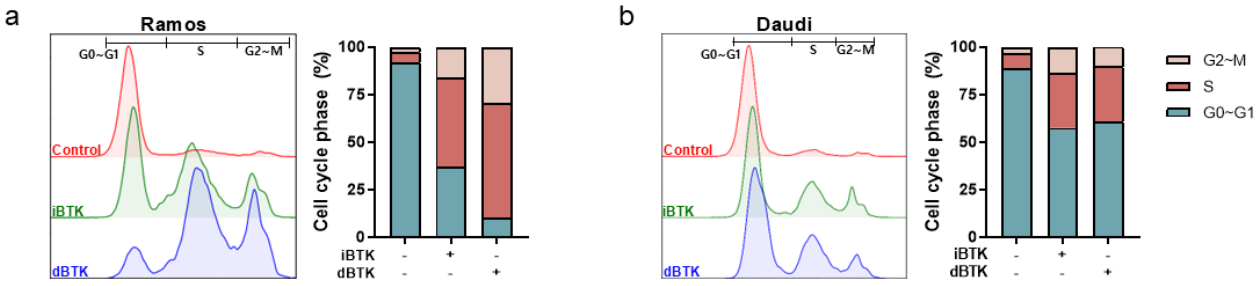


Figure 2

Flow cytometry analysis revealed an increase in cells arrested in the G2/M phases when treated with iBTK or dBTK individually. The percentage of cells at each stage of the cell cycle was determined using flow cytometry. (a) Ramos and (b) Daudi cell lines were treated with 10 μM of iBTK or dBTK for 72 hours. The cell cycle distribution was analyzed as a percentage, excluding cells outside the measurement range.

Figure 3

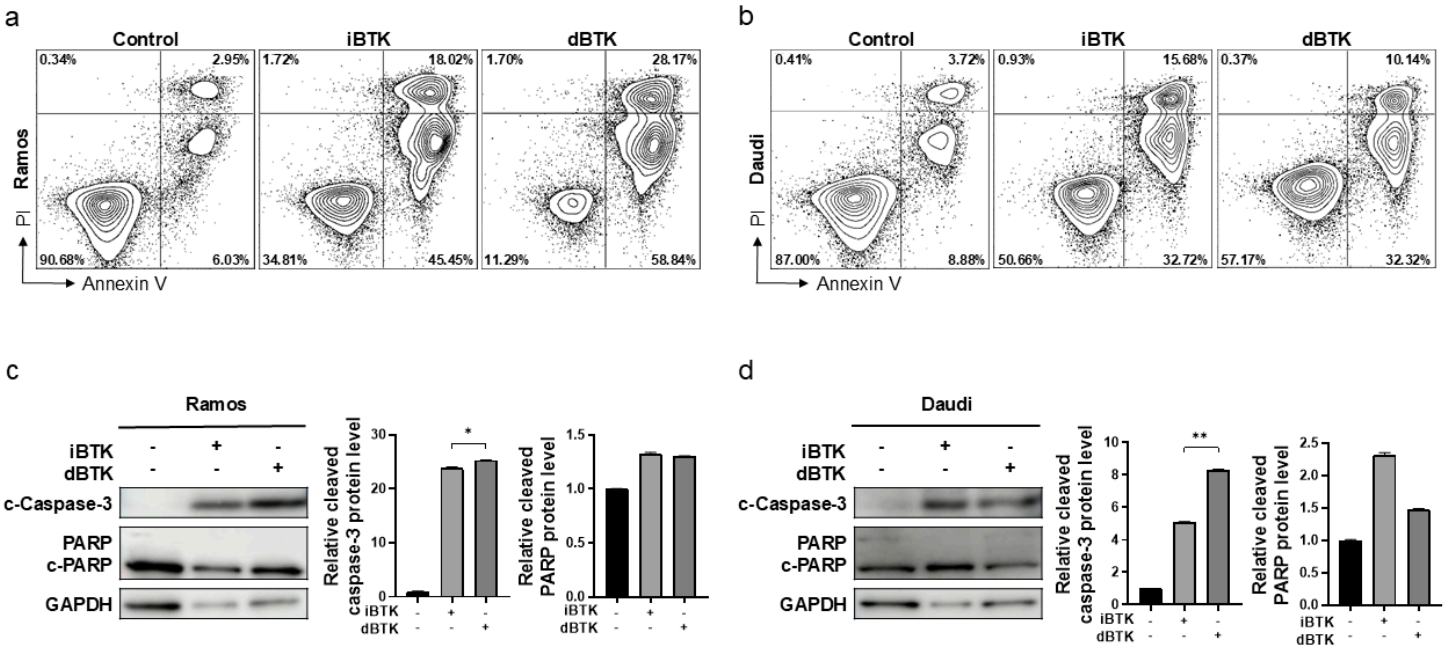


Figure 3

Apoptosis-inducing effects of iBTK and dBTK in Daudi and Ramos cells. Cells were treated with 10 μM iBTK or dBTK, and untreated cells served as the control. After 72 hours of treatment, apoptosis was evaluated using Annexin V-FITC/PI staining by flow cytometry. (a) Apoptosis analysis in Ramos cells. (b) Apoptosis analysis in Daudi cells. The expression levels of apoptosis-related proteins, including cleaved

Caspase-3 (c-Caspase-3), PARP, and cleaved PARP (c-PARP), were analyzed in Ramos and Daudi cells treated with 10 μM iBTK or dBTK using Western blot. (c) Protein expression levels of c-Caspase-3, PARP, and c-PARP in Ramos cells normalized to GAPDH. (d) Protein expression levels of c-Caspase-3, PARP, and c-PARP in Daudi cells normalized to GAPDH. Statistical analysis used t-test, and error bars represent ± standard deviation (SD).

Figure 4

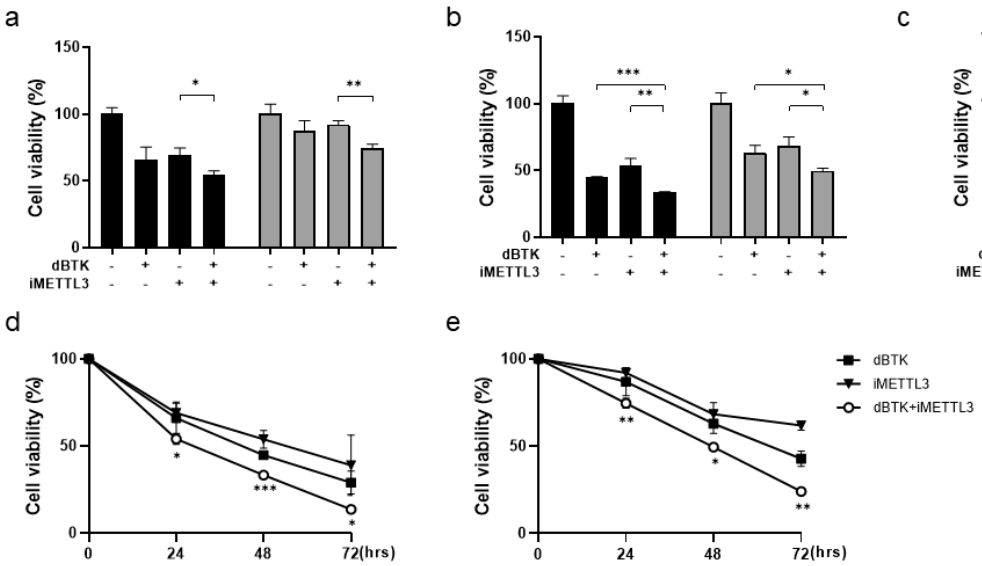


Figure 4

Effects of dose and time on the viability of Ramos and Daudi cells treated with dBTK and iMETTL3, either alone or in combination. Cell viability was assessed using the CCK-8 assay. Each cell line was plated at a density of 5 × 10^3 cells per well. Cells were treated with 5 μM or 10 μM of each drug individually, or with a combination of 5 μM of dBTK and 5 μM of iMETTL3 (total 10 μM). Viability was measured at (a) 24 hours, (b) 48 hours, and (c) 72 hours. The cell viability of (d) Ramos cells and (e) Daudi cells was evaluated after 24, 48, and 72 hours following treatment with dBTK and iMETTL3 at 5μM each, both individually and in combination. Statistical analysis used Prism's t-test and was expressed as *P<0.05; **P<0.01; ***P<0.001. Error bars denote ± standard deviation (SD).

Figure 5

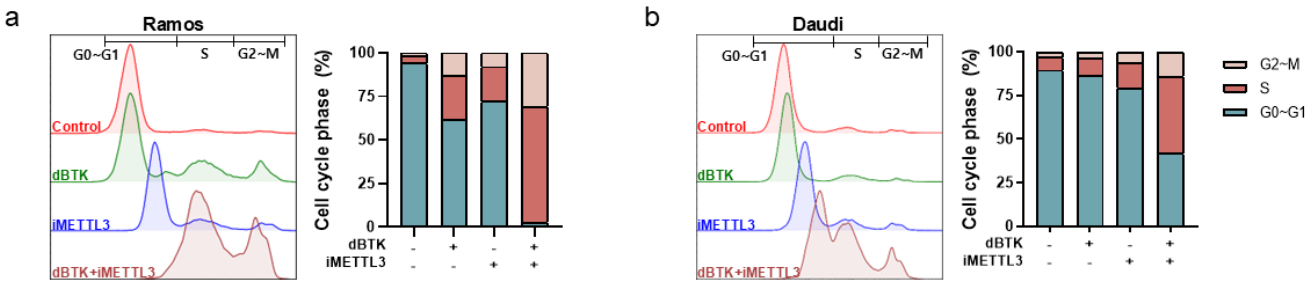


Figure 5

Flow cytometric analysis of cell cycle stages revealed that the combination of dBTK and iMETTL3 resulted in an increased proportion of cells arrested in the G2/M phase compared to individual treatments. The percentage of cells in each cell cycle stage was determined using flow cytometry. (a) Ramos and (b) Daudi cell lines were treated with 5 μM of each drug individually or in combination for 72 hours. Cell cycle stages were analyzed as percentages, excluding cells outside the measurement range. Statistical tests were performed with t-tests. Error bars indicate ±SD.

Figure 6

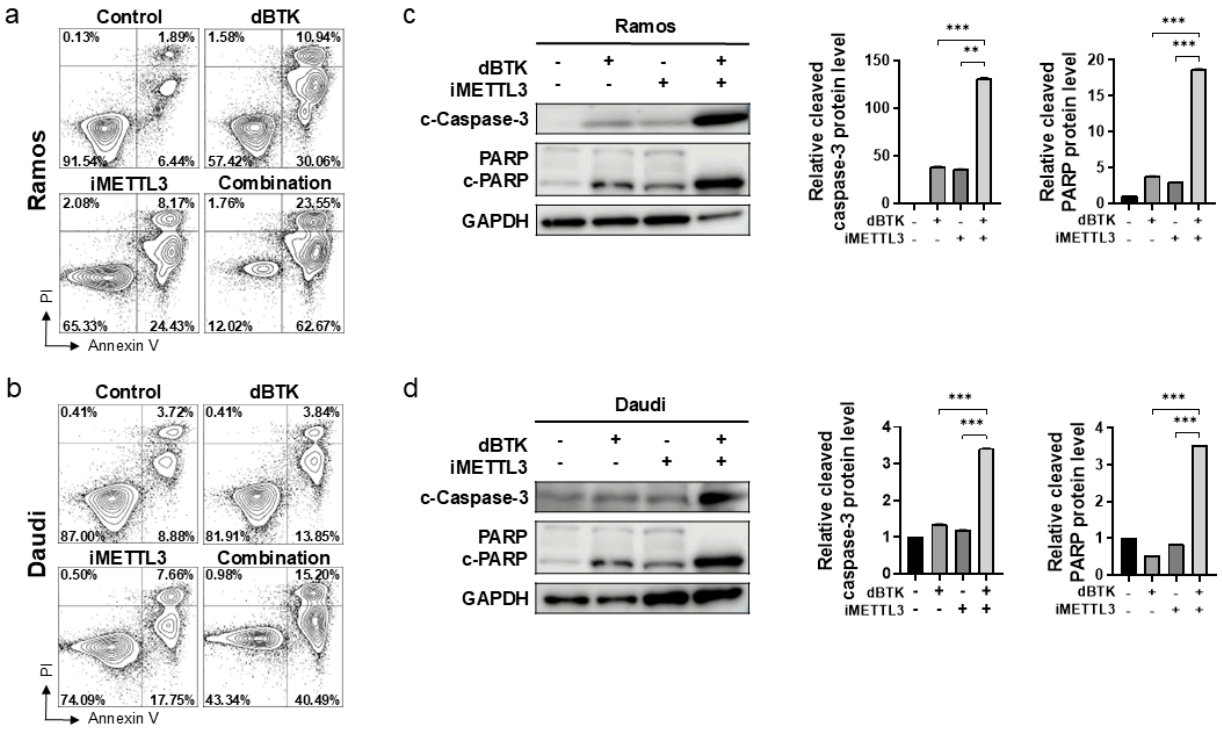


Figure 6

Effects of individual and combination treatments of dBTK and iMETTL3 on apoptosis in Ramos and Daudi cells. Cells were treated with 5 μM of each drug individually or in combination, with untreated cells serving as the control. After 72 hours of treatment, apoptosis was evaluated using flow cytometry. (a) Analysis of apoptosis in Ramos cells. (b) Analysis of apoptosis in Daudi cells. Protein expression levels of cleaved Caspase-3 (c-Caspase-3), PARP, and cleaved PARP (c-PARP) were analyzed in Ramos and Daudi cell lines treated with dBTK and iMETTL3, both individually (5 μM each) and in combination, using Western blotting. (c) Protein expression levels of c-Caspase-3, PARP, and c-PARP in Ramos cells, normalized to GAPDH. (d) Protein expression levels of c-Caspase-3, PARP, and c-PARP in Daudi cells, normalized to GAPDH. Statistical analysis used t-tests, and error bars represent ± standard deviation (SD).