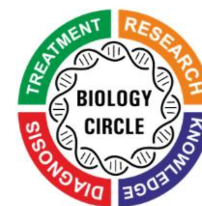
Cancer Research and Medicine
DOI: https://doi.org/10.63626/xkbx3b64
ISSN Online: 3104-4131
ISSN Print: XXXX-XXXX



Research Article

Alkyl-phospholipids and cell cycle check point inhibitor induces antineoplastic effects in breast cancer cells: Evidence from in vitro investigations

Kinzah Kanwal, Marriyam Nasim
Medical Laboratory Technology, Riphah International University, Islamabad, Pakistan

Abstract

Background: Combination of anticancer compounds often lead to better control of tumor progression. In this context, searching for novel combinations of anticancer compounds is a continuous process. Alkyl-phospholipids are synthetic lipids and have shown anticancer effects. Combining the alkyl-phospholipids with potential other drugs is an attractive filed of interest. In this study, we investigated the combined anticancer effects of an alkyl-phospholipids (erufosine and perifosine) and a cell cycle check point inhibitor (ATR) with the hypothesis that combining the two above-mentioned classes can be instrumental against breast cancer.

Methods: Human breast cancer cell lines (MDA-MB-231 and MCF-7) were exposed to the two compounds and resulting toxicity was assessed by using a mitochondrial based dye reduction assay. The effects were calculated numerically, and percentages were compared with untreated controls. To give insights at gene levels, expression profiles of cell cycle associated genes (CDKN family) were investigated by using real-time PCR methos. The effects in both assays were uncovered as single agent and combinational approaches in parallel for comparison purposes.

Results: Exposure with the compounds (erufosine, perifosine and ATR inhibitor) reduced the cell proliferation of the breast cancer cells. Overall, alkyl-phospholipids inhibited more effectively the cell proliferation as shown by the MTT assay. Combination of the two classes (alkyl-phospholipids and ATR inhibitor) showed synergistic effects while inhibiting cell proliferation. The effects were comparable in the two breast cancer cell lines. The compounds either as single agent or in combination also induced the expression of CDKN family members. However, these effects were less pronounced in ER/PR/HER2 negative cells (MDA-MB-231) as compared to ER/PR positive cells (MCF-7).

Conclusion: Alkyl-phospholipids and ATR inhibitor can be combined to achieve synergistic antineoplastic effects against breast cancer. The compounds can be applied as a combinational therapeutic approach against breast cancer.

Key Words: Breast Cancer, Alkyl-phospholipids, Cell cycle inhibitor, Combinational therapy

INTRODUCTION

Deregulation in cell cycle is a hallmark of cancer cell transformation leading to fast cell proliferation. Cell cycle regulation is governed by specific checkpoints that ensure integrity of the DNA content while going through the cell cycle stages. However, when compromised, the cell cycle machinery leads to defects in DNA duplication and damage of genome. This condition initiate DNA damage response (DDR) through multiple players including ATM and ATR serine/threonine kinases. Among these, ATR is activated by a considerably broader spectrum of genotoxic stressors and serves as a key mechanism for genome stability maintenance [1-4].

Corresponding Author: Kinzah Kanwal
Email: kinzah.kanwal@riphah.edu.pk
Received: 29.06.2025
Revised: 22.07.2025
Accepted: 23.07.2025
Published: 24.07.2025

Many chemotherapeutic medicines and radiation have an anti-tumor impact by inducing DNA damage in cancer cells; hence, stimulation DDR pathways are regarded as a significant factor influencing treatment outcomes. When DDR pathways are inactivated, the inhibition of other pathways can induce cancer cell-specific lethality. For cancer treatment, ATR is a promising therapeutic target due to its functions as facilitator in regulating responses to DNA mutilation. Many types of cancer are being addressed with chemotherapeutic medicines in combination with inhibitors of ATR [5, 6]. EPT-46464, AZ20 (AstraZeneca), AZD6738, VE-821, and VX 970 (VE-822) (Vertex) are some of the ATR inhibitors that have been identified and are currently in Phase I clinical studies [7]. VE-821 was shown to be a potent ATP-competitive inhibitor of ATR with minimal cross-reactivity against related kinases. Under replication stress, ATR and CHK1 keep cancer cells alive, and

inhibitors of both kinases are now being tested in clinical studies. Indeed, when the CHK1 inhibitor AZD7762 is combined with ATR inhibitor VE-821 in cancer cells, replication stress is induced, resulting in replication arrest and apoptosis. *In vitro* and *in vivo*, combination of ATR and CHK1 inhibitors causes replication fork arrest, ssDNA buildup, replication collapse, and synergistic cell death in cancer cells. VE-822 was the first selective ATR inhibitor, while AZD6738 was second to enter clinical development [8-10].

Synthetic chemicals known as alkyl-phospholipids (ALPs) have been known to harbor anti-cancer and anti-proliferative properties. ALPs are glycosylated lipids and glycosylated phospholipids. Due to their resemblance to cellular phospholipids, these agents disrupt lipid homeostasis, targeting membrane lipid rafts and changing lipid-linked signaling, resulting in cell death. ALPs have an advantage over traditional anti-cancer drugs in that they target cell membrane phospholipids rather than cancer cells' DNA. These ALPs produce biophysical disruption by inserting a micelle in the lipid bilayer at clinical concentrations. Destructive cellular pathways are triggered when cell membrane metabolism is disrupted [11, 12].

Perifosine is a variation of edelfosine (prototype ALP) in which choline component of alkyl phosphocholines is replaced with a heterocyclic piperidine group. This modification was an advantage over miltefosine (1st generation ALP) as stability and half-life by approximately 140 hours increased, thus halting rapid metabolic degradation [11]. Phosphocholine, that is the main metabolic product of miltefosine, is responsible for severe GI toxicity after oral therapy. Perifosine, on the other hand, is unable to produce phosphocholine and allowing for a more thorough investigation *in vitro* and *in vivo* [13, 14]. Perifosine is a 2nd generation ALP that may be given to people orally and has a low toxicity profile, as well as a high rate of tumor uptake and accumulation. In phase II clinical trials, perifosine exhibited toxicity against a variety of advanced and metastatic cancers such as breast cancer, head and neck cancer, melanoma, and hepatocellular carcinoma, but it had a toxicological profile like miltefosine and caused GI side effects (nausea, vomiting, diarrhea). It inhibits many important signal transduction pathways in human malignancies, including PI3K/AKT, one of the most important pathways for cancer cell proliferation. Perifosine has shown significant clinical effectiveness as an Akt inhibitor in combination treatments with other potent antineoplastic medicines, despite its limitations as single oral drug for treating common solid tumors [14-16].

Erufosine is a 3rd generation ALP molecule that has shown itself to be effective against leukemia, multiple myeloma, breast, prostate, colorectal and squamous carcinoma cell lines. When evaluated for anticancer potential, erufosine has demonstrated considerably reduced off target effects due to structural alteration. *In vivo*, erufosine is more metabolically stable, with lower hemolytic activity and less bone marrow damage. Because of these properties, erufosine can be administered intravenously to obtain clinically meaningful doses that are not achievable with other ALPs. In different cancer cell lines, erufosine induces anticancer effects through autophagy, apoptosis, and G2 cell cycle arrest. Erufosine has demonstrated the ability to penetrate blood-brain barrier and accumulate brain tissue, as well as having a responsive nature in brain cancers *in vitro* and *in vivo*. As far as anticancer activities are concerned, it is limited to pre-clinical studies so far and increased understanding about its anti-cancer properties will foster the evaluations in phase I trials. So far, *in vitro* investigations are successfully conducted against acute myeloid leukemia, astrocytoma, glioblastoma, multiple myeloma, breast, colorectal and oral squamous cell carcinoma [17-25].

The objectives of current research were to investigate the cytotoxic effects imposed by ATR inhibitor and erufosine/perifosine alone and in combinations in breast cancer cells. Furthermore, the aim was to determine the effects of single and combinational exposures of the test compounds on CDKN family of genes in breast cancer cells by real-time PCR methodology.

METHODS

Toxicity Assay

Two breast cancer cell lines (MDA-MB-231 and MCF-7) were cultured in the cell culture medium (21875-034) along with 10% fetal bovine serum (10270-106), 2mM L-glutamate (A2916801), penicillin (100IU/ml) and streptomycin (100µg/ml) (15140-122) in 96-well plates. To assess toxicity response towards compounds (erufosine, perifosine and ATR), the cells were cultured and incubated at 5% CO_2, 37˚C, humidified culture conditions. By using the MTT dye reduction assay methodology, a colorimetric assay, cell viability was investigated after exposure with the compounds. Cells (4000cells/well) were propagated in 96-well plates (100µl medium/well) and treated with variable concentrations (1.56-50µM) of erufosine, perifosine and ATR inhibitor (Ab219506). The treatment was carried out for three intervals i.e., 24-72 hours. The treatment was carried out as a single agent and combinations of the selected compounds as shown in Table 1 and 2. Following treatment intervals, MTT solution (20395.01) was added to dissolve formazan formed by viable cells. Optical densities were measured to calculate cell survival rates as percentage of untreated control. Inhibitory concentrations (IC) required for subsequent real-time

PCR experiments were assessed using GraphPad Prism software.

Expressional Analysis Treatments

Breast cancer cells were cultured in 6-well plates and allowed to grow overnight. Afterwards, the cells were exposed to various ICs of erufosine, perifosine and ATR alone and in combinations (Table 3). After 48 hours of incubation, cells were washed, trypsinized and the palettes were collected. Total RNA, extracted from treated and control cells by using spin column extraction kit (K0731), was quantified and used to synthesize cDNA via reverse transcriptase kit (K1622). Prepared cDNA samples were confirmed by amplifying reference gene (HPRT1) and gel electrophoresis.

Real Time PCR

Primers of cell cycle related genes (CDKN1A, CDKN1B) were designed (Table 4) and optimized by gradient PCR followed by gel electrophoresis to visualize the products. Real time PCRs were performed by using SybrGreen dye (K0221), cDNA samples and gene specific primers. The samples were amplified in triplicate. Quantstudio-3 real-time machine was used for expression profiling. Expression level of HPRT1 reference gene was used to normalize the data. Fold changes were calculated by Livak (2-ΔΔCT) method.

Table 1: Treatment of breast cancer cells against different concentrations of selected compounds for MTT assay

Cell Lines	Erufosine (µM)	Perifosine (µM)	ATR (µM)
MDA-MB-231	1.56, 3.12, 6.25, 12.5, 25, 50	1.56, 3.12, 6.25, 12.5, 25, 50	1.56, 3.12, 6.25, 12.5, 25, 50
MCF-7	1.56, 3.12, 6.25, 12.5, 25, 50	1.56, 3.12, 6.25, 12.5, 25, 50	1.56, 3.12, 6.25, 12.5, 25, 50

Table 2: Exposure with inhibitors and compounds in combinations

Cell Lines	Erufosine + ATR		Perifosine + ATR	
	Erufosine	**ATR**	**Perifosine**	**ATR**
MDA-MB-231	IC25	IC25	IC25	IC25
MCF-7	IC25	IC25	IC25	IC25

Table 3: Exposure with single agents and combinations for real-time PCR analysis

Cell Lines	Single Agent Treatment			Combinational Treatment
MDA-MB-231	Erufosine IC25	Perifosine IC25	ATR IC25	Erufosine IC25+ ATR IC25
	Erufosine IC50	Perifosine IC50	ATR IC50	Perifosine IC25+ ATR IC25
MCF-7	Erufosine IC25	Perifosine IC25	ATR IC25	Erufosine IC25+ ATR IC25
	Erufosine IC50	Perifosine IC50	ATR IC50	Perifosine IC25+ ATR IC25

Table 4: Primer sequence for amplification of selected genes

Gene	Forward Primer	Reverse Primer
CDKN1A	GCTTCATGCCAGCTACTTCC	CTGTGCTCACTTCAGGGTCA
CDKN1B	CCGGCTAACTCTGAGGACAC	TGCAGGTCGCTTCCTTATTC
HPRTI	GACCAGTCAACAGGGGACAT	CTTGCGACCTTGACCATCTT

RESULTS

Cytotoxic Effects of Compounds

The compounds (erufosine, perifosine and ATR) imposed the anti-proliferative effects in breast cancer cells. Effects of the two ALPs (erufosine and perifosine) were comparable while inhibiting the viability of breast cancer cells. At lower concentrations (≤3.13μM), there were some stimulatory effects on growth. While at further higher concentrations, the decline in viable cells were observed. At highest concentrations (50μM), a steep decline in viability was observed (≥80%) in both breast cancer cell lines. As far as ATR inhibitor is concerned, growth inhibitory effects were concentration dependent. However, these effects were less prominent in both cell lines while comparing with ALPs. With the highest concentration (50μM) of ATR inhibitor, a maximum of ~50% growth inhibition was observed (Figure 1, 2). When considered combinations of ALPs (erufosine and perifosine) with ATR inhibitor, synergistic impact on inhibition of viability was observed (Figure 3, 4). These synergistic effects were witnessed in both breast cancer cell lines. Furthermore, the effects were clearer at initial time intervals (24 hours) as compared to later time intervals (48 and 72 hours). All in all, erufosine and perifosine when combined with ATR inhibitor, showed synergism in inhibiting cell viability (Figure 3, 4).

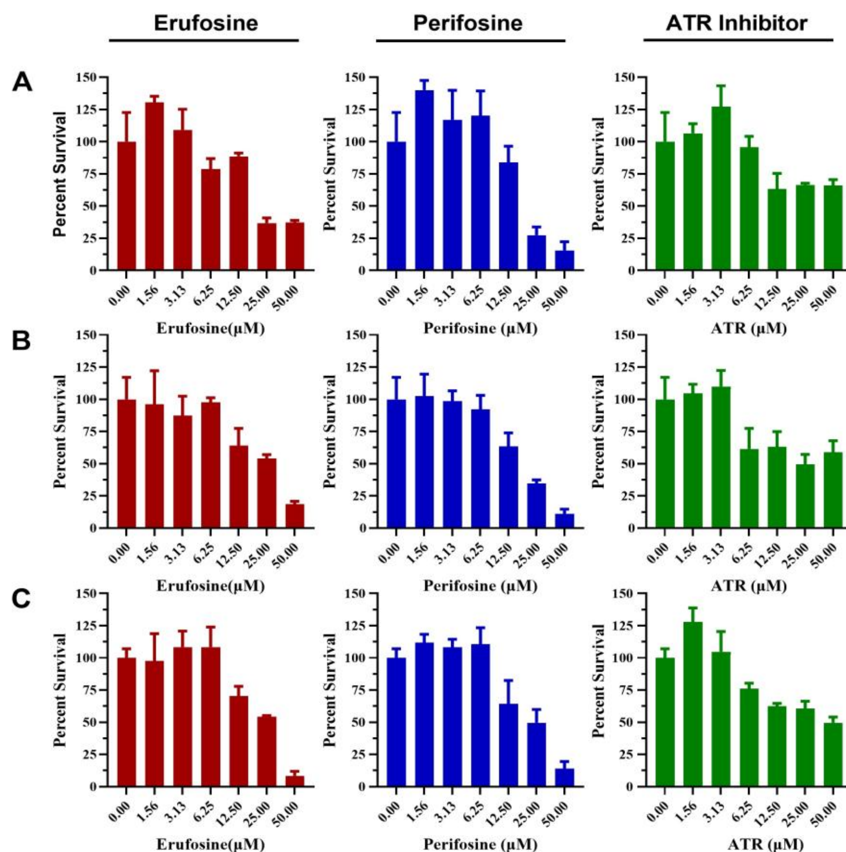


Figure 1: MTT results of breast cancer cell line (MDA-MB-231) after treatment with selected compounds (erufosine, perifosine, ATR inhibitor). The cells were cultured and exposed to compounds as single agents' treatments. Afterwards, MTT dye reduction assay was performed to evaluate the effects on cell viability.

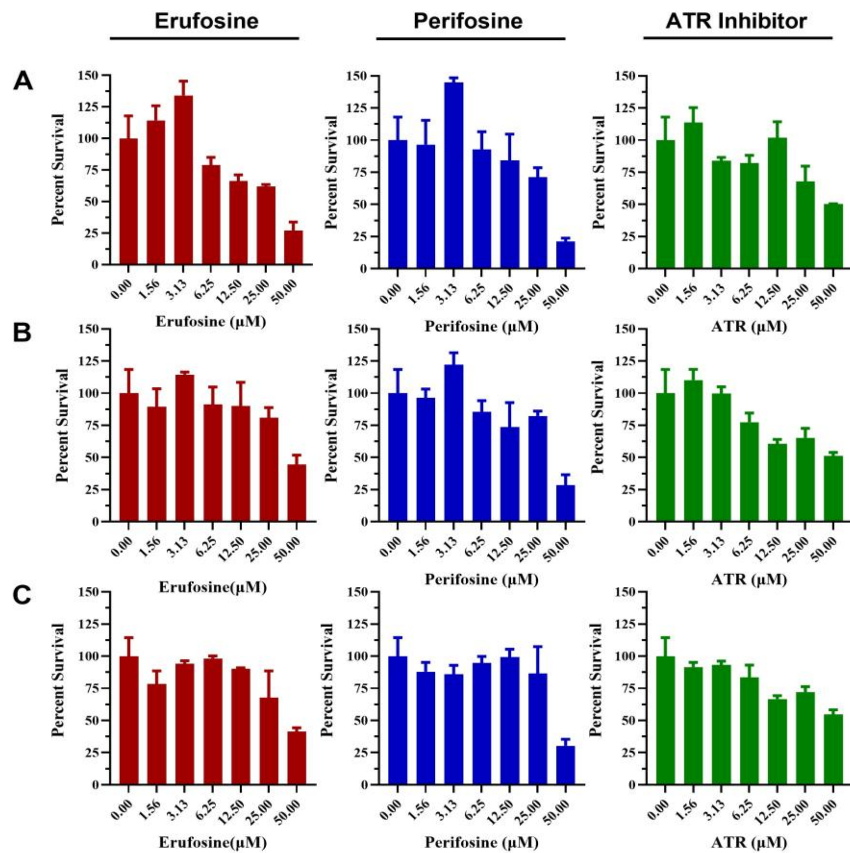


Figure 2: MTT results of breast cancer cell line (MCF-7) after treatment with selected compounds (erufosine, perifosine, ATR inhibitor). The cells were cultured and exposed to compounds as single agents' treatments. Afterwards, MTT dye reduction assay was performed to evaluate the effects on cell viability.

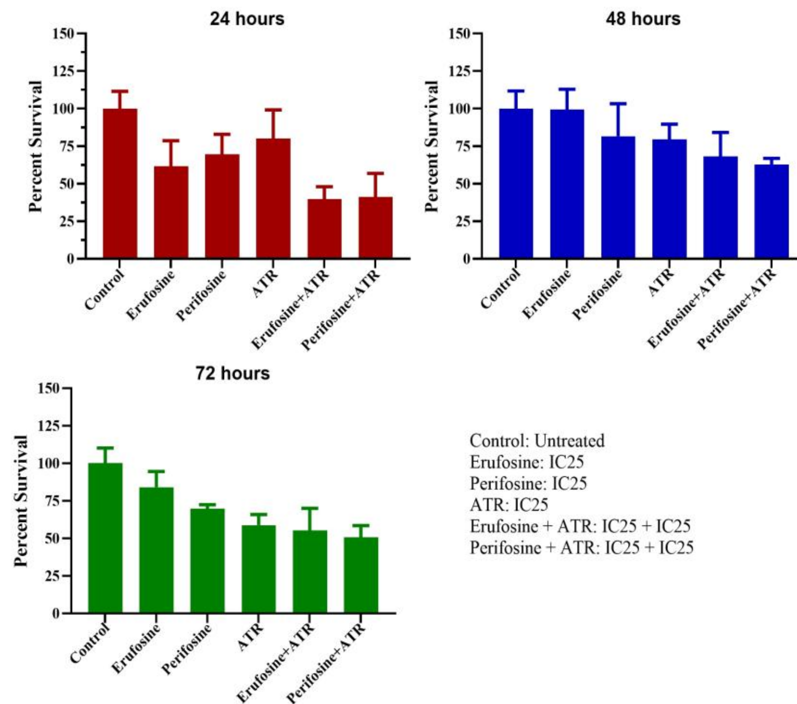


Figure 3: MTT results of breast cancer cell line (MDA-MB-231) after treatment with selected compounds (erufosine, perifosine, ATR inhibitor). The cells were cultured and exposed to compounds as combinational treatments. Afterwards, MTT dye reduction assay was performed to evaluate the effects on cell viability.

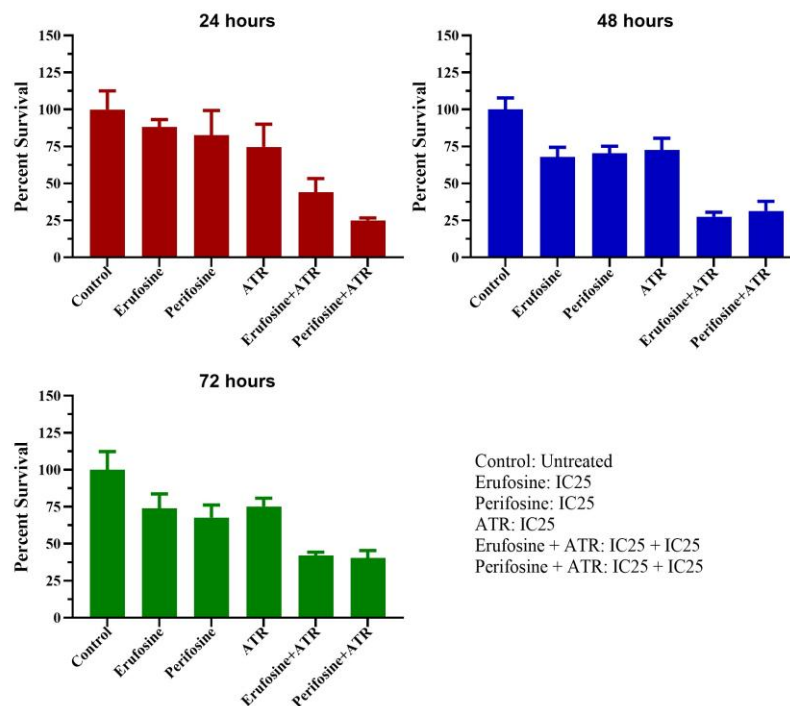


Figure 4: MTT results of breast cancer cell line (MCF-7) after treatment with selected compounds (erufosine, perifosine, ATR inhibitor). The cells were cultured and exposed to compounds as combinational treatments. Afterwards, MTT dye reduction assay was performed to evaluate the effects on cell viability.

Expressional Profiling of Genes

The cells were exposed to compounds (single and combinations) followed by evaluation of expression modifications via real-time PCR method. Single agent treatments uniformly induced the CDKN1A and CDKN1B genes in MDA-MB-231 cells. However, these effects were more intense in response to perifosine (up to 14fold) followed by erufosine (up to 11fold) and ATR inhibitor (up to 2fold). Furthermore, these inducing effects were concentration dependent as maximum up regulation of the genes was observed with IC50 concentrations of compounds. Considering the combinational effects, again there was induction of the gene in response to erufosine/ATR and perifosine/ATR combinations. Surprisingly, these agents imposed a more pronounced induction relatively as single agent when compared to combined effects of any two compounds as shown in Figure 5.

In MCF-7 cells, CDKN1A gene was highly up-regulated after erufosine exposure (up to 40fold) followed by perifosine and ATR exposures (up to 10fold). These inductions were concentration dependent as perifosine, and ATR inhibitor induced maximum up regulation with highest applied concentration (IC50). Conversely, erufosine did not follow this pattern as more pronounced effects were seen with IC25 concentration. In CDKN1B gene, there was a mild induction (up to 3fold) after exposure to erufosine and ATR inhibitor, while there were negligible effects after perifosine exposure. As far as combinations are concerned, overall induction in both genes was witnessed in MCF-7 cells. However, this induction was more pronounced in CDKN1A gene as compared to CDKN1B gene (Figure 6).

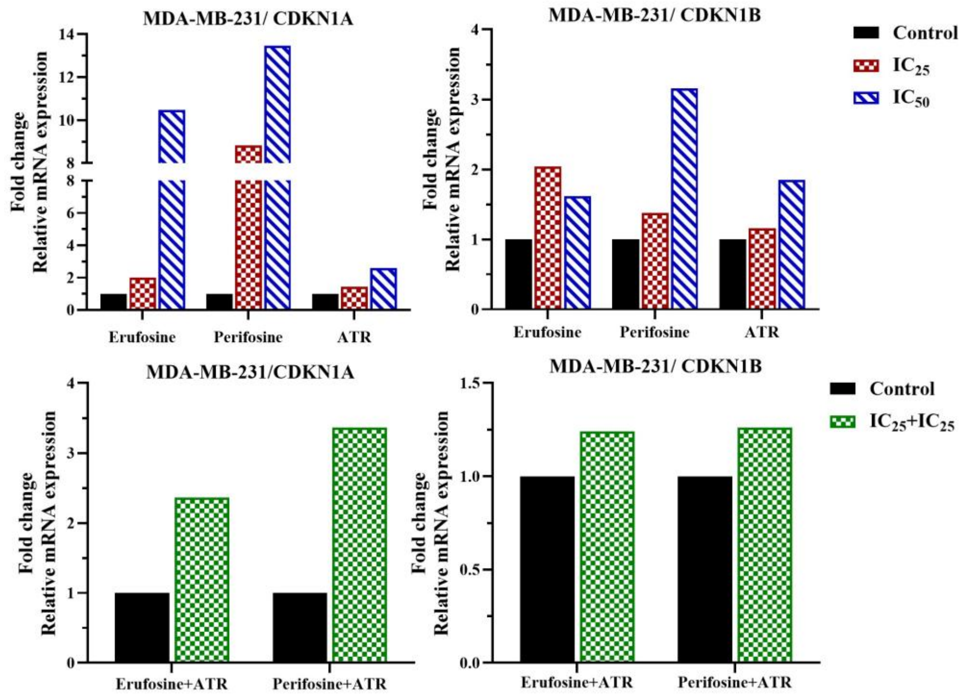


Figure 5: Expressional changes in CDKN1A and CDKN1B gene in MDA-MB-231 cells. The cells were exposed to erufosine, perifosine and ATR inhibitor as single agent or combinations followed by expression analysis by real-time PCR methodology.

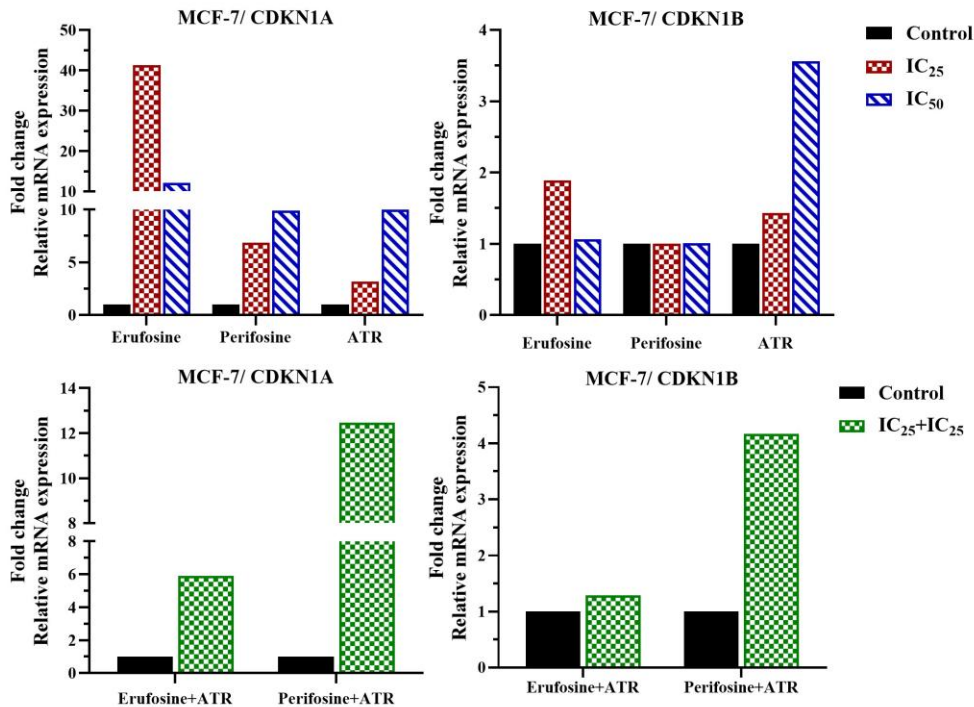


Figure 6: Expressional changes in CDKN1A and CDKN1B gene in MCF-7 cells. The cells were exposed to erufosine, perifosine and ATR inhibitor as single agent or combinations followed by expression analysis by real-time PCR methodology.

DISCUSSION

Cancer treatment is challenging with the availability of limited therapeutic options. Furthermore, treatment of cancer with available anticancer compounds imposes a huge burden of side effects in the patients. In these conditions, it is inevitable to find the novel anticancer compounds which can be used for the treatment of cancer patients with better physiological profile and safety. Considering this, the present study was designed to evaluate the effects of three compounds named erufosine, perifosine and ATR inhibitor as a single agent and in combinations. Erufosine and perifosine are ALPs and have shown substantial anticancer effects in pre-clinical settings. On the other hand, ATR inhibitor (VE-821) is a commercially available ATP competitive inhibitor. Among the selected compounds, ALPs interact with cell surface membranes, especially the lipid rafts and induce tumor cell specific lethal effects. These effects have been validated *in vitro* and *in vivo* against multiple cancers which indicate the broad-spectrum anticancer potential of the two compounds [26-29]. On the other end, ATR is a promising therapeutic target as it is involved in multiple cellular functions and facilitates responses including cell cycle progression, cell stress and DNA damages. Clinical utilization of ATR inhibitors is an open secret, and further research is being reported to highlight the utilization of these targets [30-32]. As we know, combining the anticancer compounds for clinical utilization often led to better control of tumor progression with minimal side-effects. On this ground, combining the selected ALPs and ATR inhibitor was a promising area of research. In this study, the breast cancer cell lines of different molecular subtypes (MCF-7: ER^+/PR^+, MDA-MB-231:$ER^-/PR^-/HER2^-$) were exposed to the compounds as single agent and combinational treatment followed by the determination of cytotoxic profile of the compounds. Afterwards, the cells were exposed to compounds and expression change in two important cell cycle inhibitor genes (CDKN1A, CDKN1B) were evaluated by using the real time PCR method.

For the said purpose, breast cancer cell lines were cultured in 96-well plates and treated with erufosine, perifosine and ATR inhibitor alone and in combinations. Afterwards, effects on viability of cells were assessed by MTT dye reduction assay (Table 1 and 2). The results highlighted that ALPs were more potent while inhibiting viability of breast cancer cells in comparison to ATR inhibitor (Figure 1 and 2). These effects were uniform in both cancer cell lines which were distinct from each other on molecular grounds. A similar type of trend has been observed in the breast cancer cell lines treated with ATM inhibitor in combination with the selected alkyl-phospholipid [33]. As far as combinational effects are concerned, the cells were more responsive towards the combination of alky-phospholipids with ATR inhibitor as more prominent inhibition of the cancer cell growth was observed after treatment with the combinations of compounds. This shows that combining the two classes with lower concentrations impose substantial antitumor effects, which can be instrumental while treating cancers with minimal side effects.

Deregulation of genetic expression is a common phenomenon observed during carcinogenic processes. Among these deregulations, pro-tumor genes are often upregulated while antitumor genes are downregulated. Among the important genes, cell cycle inhibitors like CDKN family are master regulators and often are inhibited during cancer cell progression [34, 35]. Considering the importance of this gene family, we selected the two important members (CDKN1A, CDKN1B) and evaluated the impact of selected compounds on expression profile in breast cancer cell lines. The cells were exposed to the compounds as single or combination treatments and resulting effects on expression profile were determined via real-time PCR method. When exposed to single agents, the two genes were upregulated in two cell lines. However, these induction levels vary among the cell lines and genes themselves. Specifically, CDKN1A was overall induced more prominently in both cell lines as compared to CDKN1B. In addition, the inductions were more intense in receptor negative (MDA-MB-231) cells as compared to counterparts. This shows the cell line and gene specific modifications by the selected compounds. Surprisingly, when treated with combinations of the compounds (alkyl-phospholipids and ATR inhibitor), the effects were less pronounced overall as compared to single agent treatments. Thus, there was no synergism among the compounds while inducing the CDKN family in breast cancer cells, which reflect the possible involvement of additional feedback loops working in different directions. Nevertheless, the compounds showed potential of inducing cell cycle inhibitor genes in breast cancer cells thus can be used as significant cytostatic agents.

To conclude, findings suggest that combining ALPs (erufosine and perifosine) with ATR inhibitor can be instrumental to impose anti-proliferative effects in breast cancer cells. Furthermore, the agents can deregulate the key master regulators of cell cycle (CDKN family) while imposing the cytostatic effects. Further research is required to understand the potential of these agents while using in combinations to treat breast cancer to obtain optimal results.

REFERENCES

1. Matthews HK, Bertoli C, de Bruin RA. Cell cycle control in cancer. Nature reviews Molecular cell biology. 2022 Jan;23(1):74-88.
2. Groelly FJ, Fawkes M, Dagg RA, Blackford AN, Tarsounas M. Targeting DNA damage response pathways in cancer. Nature Reviews Cancer. 2023 Feb;23(2):78-94.
3. Phan LM, Rezaeian AH. ATM: main features, signaling pathways, and its diverse roles in DNA damage response, tumor suppression, and cancer development. Genes. 2021 May 30;12(6):845.
4. Feng W, Dean DC, Hornicek FJ, Wang J, Jia Y, Duan Z, Shi H. ATR and p-ATR are emerging prognostic biomarkers and DNA damage response targets in ovarian cancer. Therapeutic advances in medical oncology. 2020 Dec;12:1758835920982853.
5. Cheng B, Pan W, Xing Y, Xiao Y, Chen J, Xu Z. Recent advances in DDR (DNA damage response) inhibitors for cancer therapy. European Journal of Medicinal Chemistry. 2022 Feb 15;230:114109.
6. Bradbury A, Hall S, Curtin N, Drew Y. Targeting ATR as Cancer Therapy: A new era for synthetic lethality and synergistic combinations?. Pharmacology & therapeutics. 2020 Mar 1;207:107450.
7. Ngoi NY, Pilié PG, McGrail DJ, Zimmermann M, Schlacher K, Yap TA. Targeting ATR in patients with cancer. Nature Reviews Clinical Oncology. 2024 Apr;21(4):278-93.
8. Jo U, Senatorov IS, Zimmermann A, Saha LK, Murai Y, Kim SH, Rajapakse VN, Elloumi F, Takahashi N, Schultz CW, Thomas A. Novel and highly potent ATR inhibitor M4344 kills cancer cells with replication stress, and enhances the chemotherapeutic activity of widely used DNA damaging agents. Molecular cancer therapeutics. 2021 Aug 1;20(8):1431-41.
9. Lloyd RL, Wijnhoven PW, Ramos-Montoya A, Wilson Z, Illuzzi G, Falenta K, Jones GN, James N, Chabbert CD, Stott J, Dean E. Combined PARP and ATR inhibition potentiates genome instability and cell death in ATM-deficient cancer cells. Oncogene. 2020 Jun 18;39(25):4869-83.
10. Mihanfar A, Asghari F, Majidinia M. Ataxia telangiectasia and Rad3-related (ATR) inhibition by VE-822 potently reversed 5-flourouracil resistance in colorectal cancer cells through targeting DNA damage response. Molecular Biology Reports. 2024 Dec;51(1):474.
11. van Blitterswijk WJ, Verheij M. Anticancer mechanisms and clinical application of alkylphospholipids. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2013 Mar 1;1831(3):663-74.
12. van Blitterswijk WJ, Verheij M. Anticancer alkylphospholipids: mechanisms of action, cellular sensitivity and resistance, and clinical prospects. Current pharmaceutical design. 2008 Jul 1;14(21):2061-74.
13. Richardson PG, Eng C, Kolesar J, Hideshima T, Anderson KC. Perifosine, an oral, anti-cancer agent and inhibitor of the Akt pathway: mechanistic actions, pharmacodynamics, pharmacokinetics, and clinical activity. Expert opinion on drug metabolism & toxicology. 2012 May 1;8(5):623-33.
14. Fensterle J, Aicher B, Seipelt I, Teifel M, Engel J. Current view on the mechanism of action of perifosine in cancer. Anti-Cancer Agents in Medicinal Chemistry-Anti-Cancer Agents). 2014 May 1;14(4):629-35.
15. Pinton G, Manente AG, Angeli G, Mutti L, Moro L. Perifosine as a potential novel anti-cancer agent inhibits EGFR/MET-AKT axis in malignant pleural mesothelioma. PLoS One. 2012 May 10;7(5):e36856.
16. Crul M, Rosing H, De Klerk GJ, Dubbelman R, Traiser M, Reichert S, Knebel NG, Schellens JH, Beijnen JH, ten Bokkel Huinink WW. Phase I and pharmacological study of daily oral administration of perifosine (D-21266) in patients with advanced solid tumours. European journal of cancer. 2002 Aug 1;38(12):1615-21.
17. Kapoor V, Zaharieva MM, Das SN, Berger MR. Erufosine simultaneously induces apoptosis and autophagy by modulating the Akt–mTOR signaling pathway in oral squamous cell carcinoma. Cancer letters. 2012 Jun 1;319(1):39-48.
18. Ansari SS, Akgün N, Berger MR. Erufosine increases RhoB expression in oral squamous carcinoma cells independent of its tumor suppressive mode of action-a short report. Cellular oncology. 2017 Feb;40:89-96.
19. Dineva IK, Zaharieva MM, Konstantinov SM, Eibl H, Berger MR. Erufosine suppresses breast cancer in vitro and in vivo for its activity on PI3K, c-Raf and Akt proteins. Journal of cancer research and clinical oncology. 2012 Nov;138:1909-17.
20. Kaleağasioğlu F, Berger MR. Differential effects of erufosine on proliferation, wound healing and apoptosis in colorectal cancer cell lines. Oncology Reports. 2014 Mar;31(3):1407-16.
21. Yosifov DY, Todorov PT, Zaharieva MM, Georgiev KD, Pilicheva BA, Konstantinov SM, Berger MR. Erucylphospho-N, N, N-trimethylpropylammonium (erufosine) is a potential antimyeloma drug devoid of myelotoxicity. Cancer chemotherapy and pharmacology. 2011 Jan;67:13-25.
22. Abdik EA, Kaleagasioglu F, Abdik H, Sahin F, Berger MR. ABT-737 and erufosine combination against castration-resistant prostate cancer: a promising but cell-type specific response

associated with the modulation of anti-apoptotic signaling. Anti-Cancer Drugs. 2019 Apr 1;30(4):383-93.
23. Akhtar MS, Ali SS. Erufosine alters the genes associated with G2/M Phase of cell cycle in cancers: Molecular evidence from gene expression analysis. Biomedical Current Insights. 2024;1(2).
24. Masood M, Iqbal S. Alkyl-phospholipid mediated cytotoxic and cytostatic effects in liver cancer cells. Cancer Research and Medicine. 2024;1(2).
25. Nasim M, Batool A, Kanwal K. Cytotoxic effects and expressional modifications induced by alkyl-phospholipid (erufosine) in lung cancer cells. Cancer Research and Medicine. 2024 Dec 26;1(1).
26. Pervaiz A, Mahmood S, Berger MR. Erufosine, a third-generation alkylphosphocholine with cytotoxic and cytostatic effects in breast and colorectal cancer cells. Cancer Research. 2017 Jul 1;77(13_Supplement):116-.
27. Königs SK, Pallasch CP, Lindner LH, Schwamb J, Schulz A, Brinker R, Claasen J, Veldurthy A, Eibl H, Hallek M, Wendtner CM. Erufosine, a novel alkylphosphocholine, induces apoptosis in CLL through a caspase-dependent pathway. Leukemia research. 2010 Aug 1;34(8):1064-9.
28. Shen J, Hong Y, Zhao Q, Zhang JL. Preclinical evaluation of perifosine as a potential promising anti-rhabdomyosarcoma agent. Tumor Biology. 2016 Jan;37:1025-33.
29. Weber AM, Ryan AJ. ATM and ATR as therapeutic targets in cancer. Pharmacology & therapeutics. 2015 May 1;149:124-38.
30. Peasland A, Wang LZ, Rowling E, Kyle S, Chen T, Hopkins A, Cliby WA, Sarkaria J, Beale G, Edmondson RJ, Curtin NJ. Identification and evaluation of a potent novel ATR inhibitor, NU6027, in breast and ovarian cancer cell lines. British journal of cancer. 2011 Jul;105(3):372-81.
31. Fokas E, Prevo R, Hammond EM, Brunner TB, McKenna WG, Muschel RJ. Targeting ATR in DNA damage response and cancer therapeutics. Cancer treatment reviews. 2014 Feb 1;40(1):109-17.
32. Wilson Z, Odedra R, Wallez Y, Wijnhoven PW, Hughes AM, Gerrard J, Jones GN, Bargh-Dawson H, Brown E, Young LA, O'Connor MJ. ATR inhibitor AZD6738 (ceralasertib) exerts antitumor activity as a monotherapy and in combination with chemotherapy and the PARP inhibitor olaparib. Cancer research. 2022 Mar 15;82(6):1140-52.
33. Kanwal K, Batool A, Nasim M. Cytotoxic effects of alkyl-phospholipids (Erufosine and Perifosine) in combination with cell cycle check point inhibitor (ATM) in breast cancer cells. Biomedical Current Insights. 2024 Dec 28;1(1).
34. Lee HA, Chu KB, Moon EK, Quan FS. Histone deacetylase inhibitor-induced CDKN2B and CDKN2D contribute to G2/M cell cycle arrest incurred by oxidative stress in hepatocellular carcinoma cells via forkhead box M1 suppression. Journal of Cancer. 2021 Jun 22;12(17):5086.
35. Wang Z, Ren M, Liu W, Wu J, Tang P. Role of cell division cycle-associated proteins in regulating cell cycle and promoting tumor progression. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2024 Jun 30:189147.

Ethics Approval: Not Applicable
Author Contributions: KK executed experiments and drafted manuscript. MN performed data analysis and figures preparation.
Competing Interests: No declared competing interests.
Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Open Access
This article is licensed under a Creative Commons Attribution 4.0 International License. The license permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source provide a link to the Creative Commons license, and indicate if changes were made. View the copy of this license at http://creat iveco mmons .org/licen ses/by/4.0/.