Volume 4, Issue 2, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

PHARMACOLOGICAL EVALUATION OF A NOVEL BRD4-TARGETING PROTAC TO OVERCOME CISPLATIN RESISTANCE IN TRIPLE-NEGATIVE BREAST CANCER CELLS

Asmaa Jan Muhammad[1], Abdul Sammad Shahid[2], Asad Fida[*3], Fatima Arshad[*4], Muhammad Naeem[5], Muhammad Farhan Shahid[6], Ghulam Mustafa[7], Muskan Rabel[8], Muhammad Darain Hassan[9]

[1]*Assistant professor School of pharmacy, Multan university of science and technology, Multan*
[2,*3,*4,5,6,7,8,9]*School of Pharmacy, Multan University of Science and Technology, Multan*

[1]asmaa.jan.muhammad@multanust.edu.pk,[2]abdulsammadshahid@gmail.com,[*3]asadmalik3341@gmail.com,
[*4]fatimaarshad1310@gmail.com,[5]naeemshabir372@gmail.com,[6]m.farhan71744@gmail.com,
[7]mustafarajpoot1628@gmail.com,[8]muskanrabel@gmail.com, [9]darainh521@gmail.com

[1]Orcid No 0000-0003-4375-397X, [2]Orcid Id No 0009-0001-4686-351X, [*3]Orcid Id No 0009-0001-4952-8725,
[*4]Orcid Id No 0009-0002-6611-920X, [5]Orcid Id No 0009-0005-4573-3165,
[6]Orcid Id No 0009-0005-4052-1833, [7]Orcid Id No 0009-0000-5850-7689,
[8]Orcid Id No 0009-0001-4415-1164, [9]Orcid Id No 0009-0004-9992-3928

DOI: https://doi.org/10.5281/zenodo.21547475

Keywords
TNBC, Cisplatin resistance, BRD4, PROTAC, DNA repair

Article History
Received: 18 December 2025
Accepted: 13 February 2026
Published: 28 February 2026

Corresponding Author: *
Asad Fida
Fatima Arshad

Abstract

Triple-negative breast cancer (TNBC) is a type of breast cancer for which cisplatin is frequently found to be ineffective. Cisplatin is frequently ineffective in triple-negative breast cancer (TNBC), a type of breast cancer that significantly reduces the efficacy of chemotherapy. The stimulation of pro-survival signaling pathways and enhanced DNA repair are the main causes of this resistance. A useful therapeutic target for controlling the transcription of genes involved in both processes is bromodomain-containing protein 4 (BRD4). In the current work, the researchers investigated if they might use a proteolysis-targeting chimera, or PROTAC, a type of molecule that selectively targets molecules for degradation, to increase the susceptibility of resistant TNBC cells to cisplatin. Growing MDA-MB-231 and HCC1937 cells with increasing cisplatin concentrations for three months resulted in the development of cisplatin resistance. Resistant cells were then split into four treatment groups: no treatment (control), cisplatin only, BRD4 PROTAC only, and BRD4 PROTAC and cisplatin. Treatment responses were determined by measuring cell viability, gene and protein expression, DNA damage, and apoptosis. The cisplatin resistant cell lines had an average fivefold resistance to cisplatin than their parental cells. Treatment with cisplatin alone did not induce much cell death, while combination treatment with the BRD4 PROTAC decreased cell viability by ~15–20%. However, sequential application of BRD4 PROTAC and cisplatin caused significant increase in cytotoxicity, resulting in 50-60% apoptosis. Reduced expression of MYC, BCL2, Cyclin D1, RAD51 and BRCA1 was observed during BRD4 degradation, suggesting a parallel fall in

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

expression of survival signaling and DNA repair pathways. In line with these molecular alterations the treatment also resulted in the highest amount of accumulated DNA damage when combined. The results in this work show that the BRD4 protein enhances DNA repair and cell survival, thereby aiding cisplatin resistance. The PROTAC technology can be used to target BRD4 to restore cisplatin sensitivity in this model, which suggests a therapeutic potential of combining BRD4-targeting PROTACs with cisplatin in the treatment of cisplatin resistant TNBC.

Short form	Full form	Short form	Full form
TNBC	Triple negative breast cancer	BRD4	Bromodomain-containing protein 4
BCL2	B-cell lymphoma 2	PROTAC	Proteolysis targeting chimera
qRT-PCR	quantitative Reverse transcription polymerase chain reaction	MYC	Myelocytomatosis oncogene

INTRODUCTION

About 15–20% of all cases of breast cancer are triple-negative breast cancer (TNBC), one of the most aggressive subtypes of the disease. TNBC is not a good candidate for endocrine therapy or HER2-targeted medications since it does not express the human epidermal growth factor receptor 2 (HER2), the estrogen receptor (ER), or the progesterone receptor (PR). For patients with TNBC, systemic chemotherapy is therefore the cornerstone of treatment, and platinum compounds like as cisplatin are commonly utilized in neoadjuvant and adjuvant therapy. Although many patients will respond to cisplatin at first, long-term treatment frequently results in acquired resistance to the medication, which is linked to poor clinical outcomes, metastases, and disease recurrence. The need for techniques that can restore chemotherapy sensitivity is highlighted by the limited treatment options that are available once the tumor cells have developed resistance to cisplatin.

A number of mechanisms, including enhanced drug efflux, selection of cancer stem cell populations, altered apoptotic signaling, and enhanced DNA repair capacity, have been proposed to explain the multifactorial process of cisplatin resistance. In recent times, epigenetic regulation has emerged as a significant contributor to treatment resistance. Instead of altering the DNA sequence, epigenetic mechanisms control gene expression in a reversible way that influences transcriptional activity. Because of its significance in the regulation of transcriptional programs involved in tumor progression and therapeutic resistance, bromodomain containing protein 4 (BRD4) has drawn a lot of attention in the context of epigenetic regulators linked to TNBC.

The transcriptional machinery is assembled into chromatin by BRD4, a member of the bromodomain and extra-terminal (BET) protein family, which binds to the acetylated lysine

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

residues of histone tails. As part of this role, BRD4 regulates the expression of several genes linked to oncogenic signaling, cell survival, and proliferation. A poor prognosis for TNBC patients and aggressive illness have been repeatedly associated with high BRD4 expression. Several oncogenic genes, including MYC, BCL2, and Cyclin D1, are targets of BRD4. These genes promote proliferation, prevent apoptosis, and accelerate the course of the disease, all of which increase its aggressiveness. [4,5,6]

In addition to its transcriptional functions, BRD4 plays a part in the cellular reaction to DNA damage. Cisplatin primarily works by creating DNA cross-links that prevent DNA replication and ultimately cause cell death. However, cancer cells can activate DNA repair pathways in

response to this cytotoxic effect. BRD4 has been linked to controlling the expression of important homology-directed repair proteins, including BRCA1 and RAD51, which improves DNA repair efficiency following cisplatin therapy. High BRD4 activity can then aid in the more efficient repair of DNA damage, reducing the toxicity of this kind of damage to tumor cells and increasing their resistance to chemotherapeutic drugs like cisplatin.[7] [8] BET inhibitors, such as JQ1 and OTX015, were discovered after BRD4 was identified as a therapeutic target. These drugs have not been successful in the clinic because of acquired resistance, dose-limiting toxicity, and transient target inhibition. Short-lived therapeutic effects result from BET inhibitors binding to the bromodomain and blocking its activity without destroying the target protein. As a result, the activity of the bromodomain is rapidly restored upon drug removal. [9, 10]

PROTACs, on the other hand, are an alternate strategy that has a number of disadvantages over conventional inhibition. A heterobifunctional molecule with ligands that bind the target protein and the E3 ubiquitin ligase, as well as a chemical linker connecting the two molecules, makes up a PROTAC. The target protein's ubiquitination and subsequent destruction by the ubiquitin-proteasome system are facilitated by the ternary complex. Unlike traditional inhibitors, PROTACs are catalytic, which means that one molecule can break down several target proteins while maintaining the inhibition of their protein activity. Depletion of the target protein may thereby reduce the likelihood of adaptive resistance and provide a longer-lasting therapeutic impact.

In prostate cancer and other hematological malignancies, a number of BET4-targeting PROTACs, including dBET1, MZ1, and ARV-771, have been shown to have more anti-tumor activity than BET inhibitors. These substances operate as BRD4 functional inhibitors, MYC expression down-regulators, and tumor cell proliferation inhibitors. The therapeutic potential in cisplatin-resistant TNBC has not been thoroughly studied, despite these encouraging results. Since BRD4 has been demonstrated to function as a regulator in both DNA repair and cell survival pathways, targeted degradation of BRD4 may be a viable strategy to overcome cisplatin resistance and re-sensitize cells to chemotherapy. [14, 15, 16]

Thus, in cisplatin-resistant TNBC models, the current study sought to investigate the therapeutic effects of a novel PROTAC that targets BRD4. The capacity of BRD4 degradation to re-sensitize cells to cisplatin was tested using cisplatin-resistant variants of MDA-MB-231 and HCC1937 cells. Cell viability, apoptosis, DNA damage, and the expression of genes related to cell survival and repair were all thoroughly examined. We postulated that targeted BRD4 inactivation would impact several pathways, such as the suppression of oncogenic signaling and DNA repair pathways, leading to the re-sensitization of resistant TNBC cells to cisplatin.

Our findings support this theory and suggest that a therapeutic strategy to address cisplatin resistance in TNBC may involve focusing on BRD4 degradation.

Mode of Action

To understand how BRD4-targeting PROTACs can restore sensitivity to cisplatin, it is important to know both the mechanism of action of cisplatin's cytotoxicity and the molecular

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

mechanisms of acquired drug resistance in TNBC.

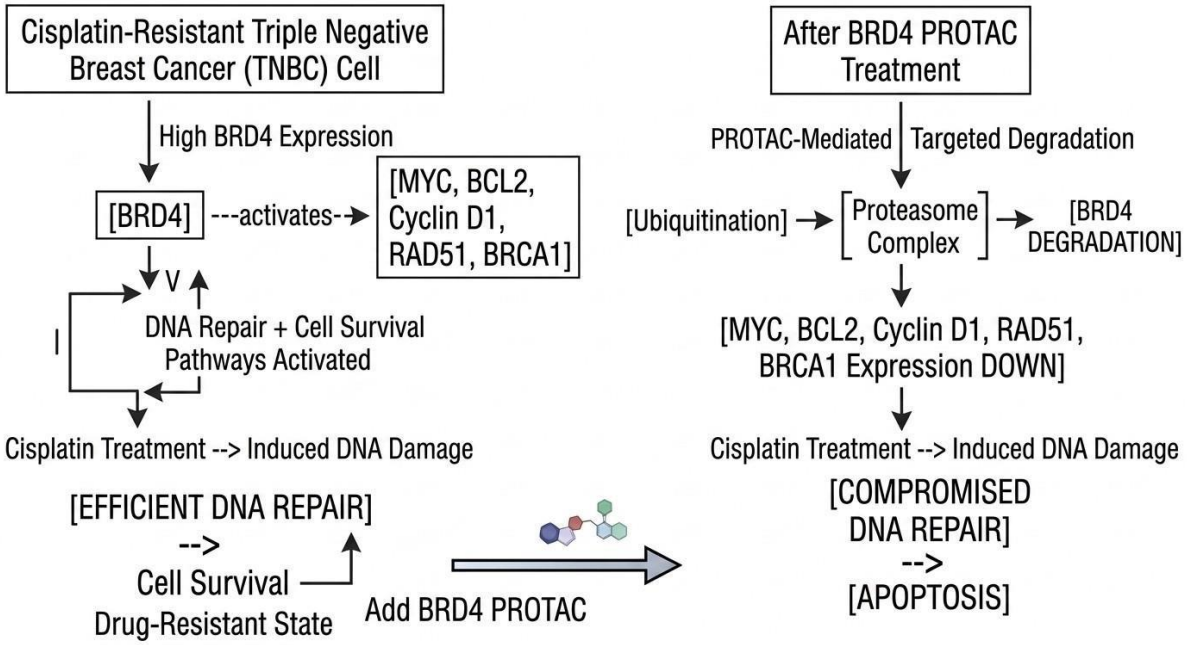


Figure 1. Proposed mechanism of BRD4 PROTAC-mediated reversal of cisplatin resistance in TNBC.

How cisplatin normally works

Cisplatin is a platinum based chemotherapeutic drug that is the primary acting drug through the formation of intra and inter strand cross links in DNA. Such DNA lesions interfere with replication and transcription, inducing the DNA damage response, and eventually leading to cell death by apoptosis if the amount of damage is too great for repair. This is a mechanism of cisplatin sensitivity in the beginning of the treatment on TNBC cells. [17] [24]

How resistance develops in TNBC

After initial response to treatment, the medication cisplatin often develops into acquired resistance. A key mechanism is improved DNA repair, specifically via the pathways of homologous recombination. Moreover, upregulation of repair proteins like RAD51 and BRCA1 helps the tumor cells to repair the DNA damage caused by cisplatin, diminishing its cytotoxicity. Pro-survival signaling pathways are additionally activated, contributing to resistance. High levels of expression of oncogenic regulators such as MYC, BCL2 and Cyclin D1 lead to increased proliferation, inhibition of cell death and the continued survival of cells despite massive DNA damage. This is because the activation of DNA repair and survival pathways significantly reduces cisplatin's therapeutic efficacy.[20][21]

Where BRD4 comes in

BRD4, a member of the BET protein family, controls the expression of several genes necessary for tumor development and survival by attaching itself to acetylated histones and bringing transcriptional machinery to chromatin. The transcription of MYC, BCL2, and Cyclin D1, which support TNBC's ongoing proliferation and anti-apoptotic response, is directly regulated by BRD4.[20] [21] BRD4 regulates the DNA damage response in addition to its transcriptional functions. According to earlier research, BRD4 aids in attracting DNA repair complexes to areas of DNA damage and stimulates the expression of homologous repair proteins like RAD51 and BRCA1. A high level of BRD4 activity thus enables effective repair of cisplatin-induced DNA damages, enabling cancer cells to withstand

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

chemotherapy and develop drug resistance.

Why inhibitor failed

Identification of BRD4 as a therapeutic target led to the development of BET inhibitors like JQ1 and OTX015 that block BRD4 binding to acetylated chromatin. These compounds showed therapeutic potential in preclinical studies but their clinical performance was hindered by reversible target inhibition, systemic toxicity and acquired resistance. [25, 26] The restrictions are mostly related to the fact that BRD4 inhibitors inhibit the activity of BRD4 only when there is an adequate concentration of the drug. After treatment has ended or intracellular drug concentration drops, BRD4 quickly recovers its ability to transcriptionally activate oncogenic signaling pathways. This means that transient inhibition is not likely to give lasting therapeutic effects.

Table 1. Comparison of BET inhibitors and BRD4-targeting PROTACs

Feature	BET inhibitors	BRD4 PROTACs
Mechanism	Block BRD4 binding to DNA	Degrade BRD4 protein completely
Effect duration	Temporary, reversible	Long lasting, protein removed
Catalytic	No ,1:1 binding	Yes,1 PROTAC degrades many BRD4
Downstream effect	Partial reduction of target genes	Complete shutdown of target genes
Resistance risk	High,cell adapt quickly	Low , protein is destroyed
Side effects	High due to constant dosing	Potentially lower

How PROTACs work differently

In contrast to conventional enzyme inhibitors, proteolysis-targeting chimeras, or PROTACs, are a protein degradation method that targets particular proteins for destruction. A ligand that binds to the target protein (BRD4), a ligand that recruits an E3 ubiquitin ligase, and a chemical linker that joins these two ligands make up the PROTAC molecule. A ternary complex is created when BRD4 and the E3 ubiquitin ligase attach simultaneously, which causes BRD4 to be ubiquitinated. The ubiquitin–proteasome pathway then causes the 26S proteasome to specifically target the ubiquitinated protein for destruction. [27] [29] PROTACs deplete the target protein from the cell and provide long-lasting inhibition of downstream signaling pathways, as opposed to reversible inhibition, which merely inhibits protein activity.

Volume 4, Issue 2, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

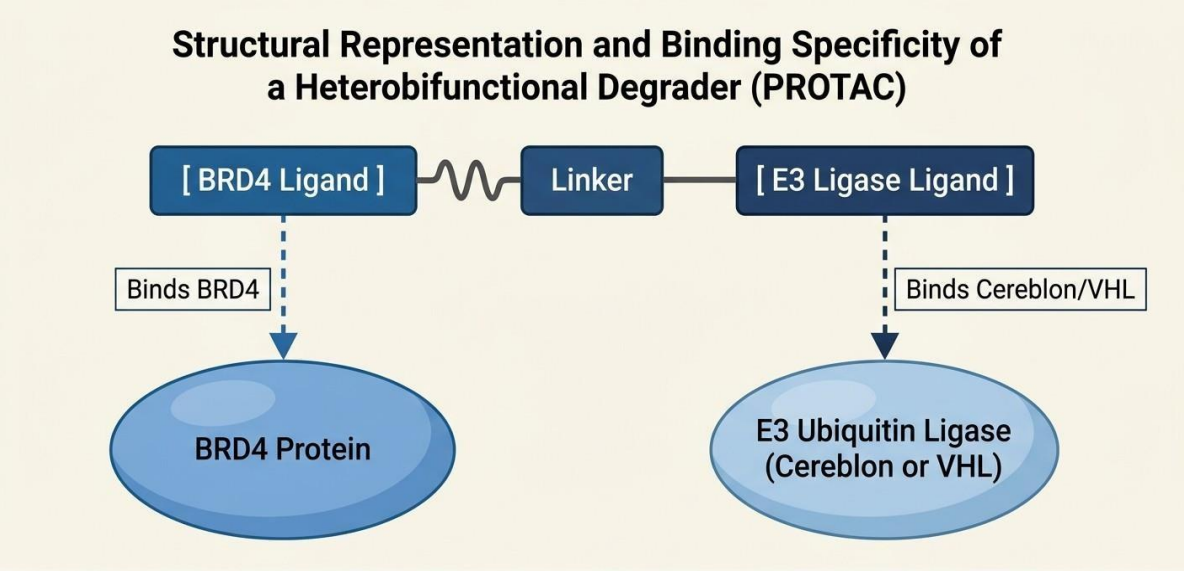


Figure 2. General structure of PROTAC molecules.

Why degrading BRD4 Re-synthesis to cisplatin

BRD4 degradation effectively targets two important mechanisms of cisplatin resistance. BRD4 knockdown leads to a decrease in transcription of MYC, BCL2 and Cyclin D1, which lowers proliferation and anti-apoptotic pathways in cells. Meanwhile, the expression of both DNA repair proteins RAD51 and BRCA1 are greatly diminished, preventing the repair of cisplatin-induced DNA damage in resistant cells.[20][21][23][24][31] Survival signaling and homologous recombination are co-suppressed which significantly enhances the long-term survival of DNA lesions after cisplatin treatment. Due to the accumulation of irreparable DNA damage, apoptotic signaling is again activated leading to re-sensitization of cisplatin-resistant TNBC cells. In line with this mechanism, the PROTAC targeting BRD4 caused efficient degradation of BRD4, which led to a decrease in the levels of oncogenic and DNA repair proteins, increased DNA damage, and reinduction of cisplatin-induced cell death in cisplatin-resistant TNBC cells.

Summary of mode of action

The general mechanism of action of the insecticide. This proposed therapeutic mechanism is summarized as follows: BRD4-targeting PROTAC → Recruitment of BRD4 and E3 ubiquitin ligase → BRD4 ubiquitination → Proteasomal degradation of BRD4 → Downregulation of MYC, BCL2, Cyclin D1, RAD51, and BRCA1 expression, resulting in suppression of cell survival and DNA repair pathways, leading to persistent cisplatin-induced DNA damage and apoptosis of cisplatin-resistant TNBC cells. [27][30][31][32]

Volume 4, Issue 2, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Material and methods

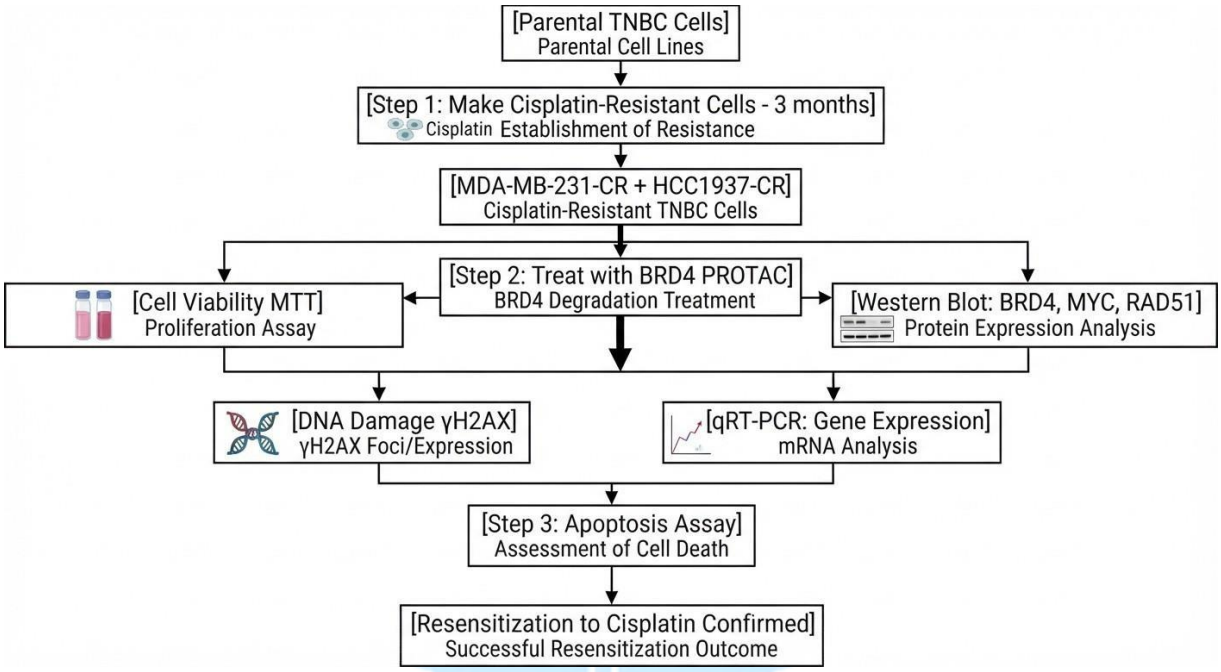


Figure 3. Experimental workflow for the generation and evaluation of cisplatin-resistant TNBC cell models.

Cell line and culture

The American Type Culture Collection (ATCC) provided the human triple-negative breast cancer (TNBC) cell lines MDA-MB-231 and HCC1937. Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin was used to cultivate the cells under standard culture conditions (37°C in a humidified atmosphere with 5% CO2). Every two to three days, the culture media was replaced and all cell lines were routinely checked for mycoplasma contamination. Before being used in experiments, all cell lines were regularly checked for mycoplasma contamination, and culture media was changed every two to three days.

Making Cisplatin-Resistant cell

The MDA-MB-231 and HCC1937 cells were exposed to increasing doses of cisplatin for several months in order to create cisplatin-resistant cells. As the cells adjusted to the different concentrations, the treatment was progressively increased from 0.5 µM to 5 µM.

The resistant cell lines were called MDA-MB-231-CR and HCC1937-CR. The IC_{50}s of resistant and parental cell lines were compared to confirm acquisition of resistance using the MTT assay. Cisplatin-resistant cells were found to be ~5X more resistant than the parental cells.

Drug and antibodies

We bought cisplatin from Sigma. Our BRD4 PROTAC was made in our lab. We confirmed the structure using NMR and mass spec. For western blot, we used antibodies for BRD4, MYC, BCL2, Cyclin D1, RAD51, BRCA1 and β-actin. All came from Cell Signaling. The second antibodies were from Santa Cruz.[36][37]

Cell viability- MTT Assay

We plated 5000 cells in each well of a 96-well plate. Next day we added cisplatin, PROTAC, or both. We left them for 72 hours. Then we added MTT for 4 hours. After that we removed media and added DMSO. We read the plate at 570 nm. We used GraphPad to calculate IC50.[38]

Volume 4, Issue 2, 2026

Frontier in Medical & Health Research

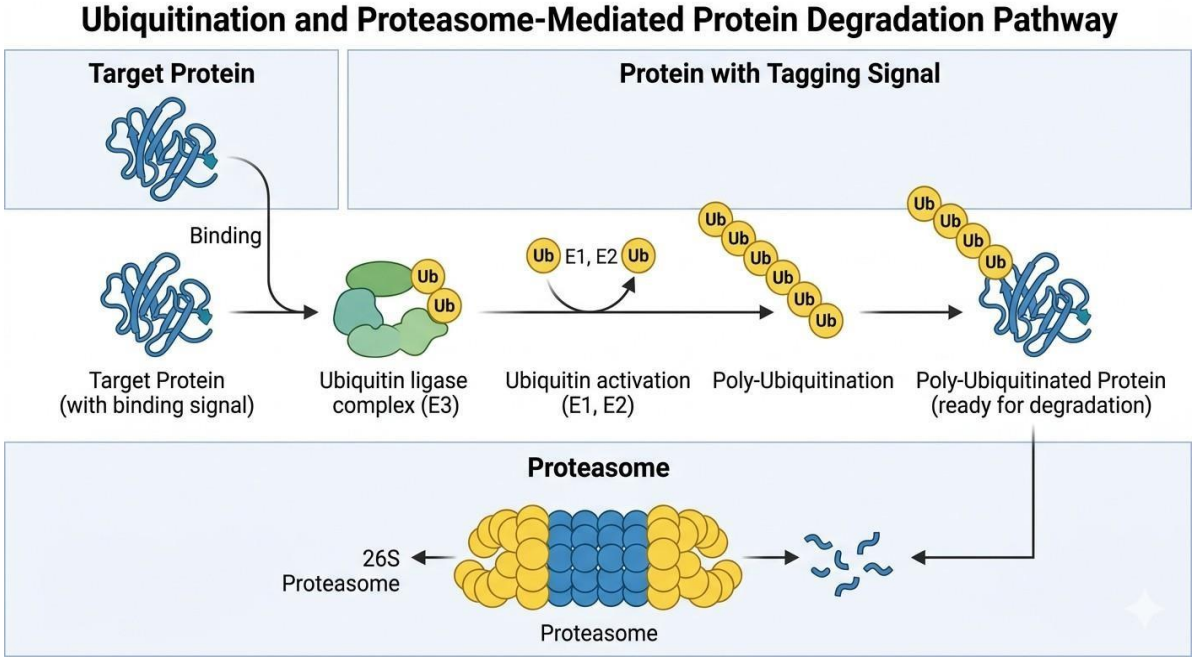
ISSN: (e) 3007-1607 (p) 3007-1593

Table 2. Experimental treatment groups used for cell viability and apoptosis assays

Group name	Treatment	Purpose
Control	No treatment	Baseline
Cisplatin	5uM cisplatin,72h	Check resistance
PROTAC	1uM BRD4 PROTAC ,24h	Check PROTAC effect alone
Combination	PROTAC 24h, cisplatin 72h	Check resensitization

Western blots

We treated cells with PROTAC for 24 hours. Then we lysed them in RIPA buffer. We measured protein with BCA kit. We ran equal protein on SDS-PAGE gel and moved it to PVDF membrane. We blocked with 5% BSA for 1 hour. Then we put primary antibody overnight in fridge. Next day we washed and added secondary for 1 hour. We detected bands using ECL.[39]



Mechanism of BRDS4 degradation By PROTAC

DNA damage -γH2AX staining

To see DNA damage, we did γH2AX staining. We put cells on coverslips and treated them with cisplatin ± PROTAC for 24 hours. We fixed with 4% PFA and made holes with 0.1% Triton. We blocked, then added γH2AX antibody overnight. Next day we added fluorescent second antibody and DAPI for nucleus. We took pictures and counted foci in 100 cells.[40]

Apoptosis Assay

For this, we employed the Annexin V/PI kit. Following treatment, we gathered the cells and gave them a PBS wash. Annexin V and PI were stained for 15 minutes in the dark. We then used a flow cytometer to run them. Together, early and late apoptotic cells were enumerated.[41]

As shown in Figure, PROTAC alone caused only minor cell death. The real effect was seen in the

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Combo group, confirming resensitization.

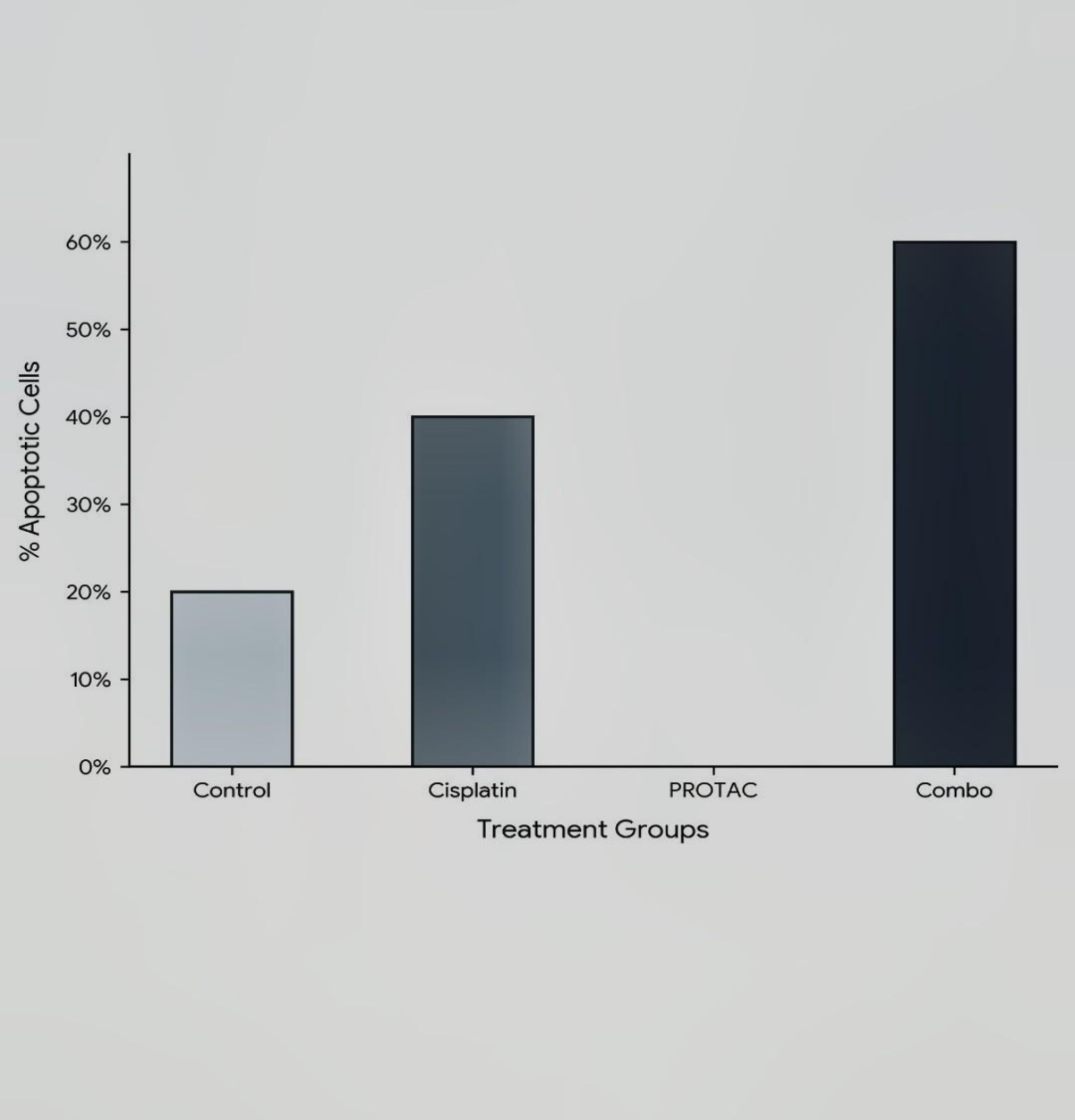


Figure 4. Excepted effect of BRD4 PROTAC on apoptosis in cisplatin -resistant TNBC cell

qRT-PCR

We extracted RNA using TRIzol. Then we made cDNA. For qPCR we used SYBR Green on Bio-Rad machine. We made primers for MYC, BCL2, RAD51, BRCA1 using Primer3.

Statistics

Every experiment was carried out separately and in duplicate. The information is displayed as mean ± standard deviation (SD). The Student's t-test was used to compare two groups, and one-way analysis of variance (ANOVA) was used to examine differences between numerous groups. $P < 0.05$ was used to determine statistical significance. GraphPad Prism 9 was used for the graphical analyses.[43]

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Results

Establishment of cisplatin-resistant TNBC cell lines

The researchers created two cell lines of TNBC that are resistant to cisplatin.The researchers established two cisplatin-resistant TNBC cell lines. Derivatives of the MDA-MB-231 and HCC1937 TNBC cell lines were developed that were resistant to cisplatin after prolonged exposure to successive higher doses of cisplatin over 3 months. The resistant cells obtained were named MDA-MB-231-CR and HCC1937-CR. MTT cell viability assay was used to confirm resistance. The IC_{50} values for cisplatin were about 5-fold higher in both resistant cell lines than in parental cells. The IC_{50} values were found to be 2.1 μM and 10.5 μM in MDA-MB-231 cells and 1.8 μM and 9.2 μM in HCC1937 cells respectively for the control and treated cells. The authors have validated their cisplatin-resistant TNBC models and the results are consistent with those previously reported[35, 44].

BRD4 PROTAC restores cisplatin sensitivity in resistant TNBC cells

The therapeutic efficacy of BRD4 degradation was tested in four experimental groups: control, cisplatin, BRD4 PROTAC and sequential BRD4 PROTAC and cisplatin. Both TNBC cell lines were resistant to cell viability reduction with cisplatin alone, which is a limited reduction. Treatment with the BRD4 PROTAC alone caused a moderate cytotoxicity effect (15–20% of cells). Conversely, the sequential use of BRD4 PROTAC and cisplatin resulted in a significant decrease in cell viability compared with sham and/or cisplatin treatment only, suggesting that cisplatin resistance was restored by BRD4 PROTAC treatment.In contrast, the cells treated with sequential administration of BRD4 PROTAC and cisplatin showed significant decrease in cell viability when compared to the sham and/or cisplatin treated cells, indicating effective restoration of cisplatin sensitivity by BRD4 PROTAC treatment.

BRD4 degradation suppresses survival- and DNA repair-associated proteins

Western blot showed that BRD4 protein was effectively degraded after 24 hours of PROTAC treatment. Loss of BRD4 also resulted in decreased protein levels of the downstream oncogenic targets MYC, BCL2 and Cyclin D1, and of the DNA repair enzymes, RAD51 and BRCA1. A corresponding decrease in the mRNA expression of these genes was observed by quantitative real-time PCR, thus linking BRD4 degradation to a coordinated decrease in the expression of transcriptional programs related to cell survival and DNA repair.

BRD4 degradation enhances cisplatin-induced DNA damage

The effect of cisplatin and BRD4 PROTAC on DNA damage was examined by γH2AX immunofluorescence staining. The level of γH2AX staining was minimal in the untreated controls, whereas DNA damage was moderately increased in cells that received cisplatin monotherapy. Very limited γH2AX accumulation was observed in the presence of BRD4 PROTAC alone. In contrast, cells treated with the combined drugs had the highest number of nuclear γH2AX foci, with over 20 nuclear γH2AX foci in most cells. Reduced expression of homologous recombination proteins RAD51 and BRCA1 was seen after BRD4 degradation, and this is the same after combination therapy, suggesting impaired DNA repair after combination therapy.[48]

Combination treatment significantly increases apoptosis in resistant TNBC cells Figure 4. Effect of BRD4-targeting PROTAC on apoptosis in cisplatin-resistant TNBC cells.Effect of BRD4 targeting PROTAC on cell apoptosis in cisplatin resistant TNBC cells. Annexin V/PI staining and flow cytometry were used to quantify apoptosis. Apoptosis was measured in untreated control cells and was found to be at baseline in the range of 5-8%. The treatment with cisplatin alone only slightly increased (10–12%), which is due to the resistant phenotype of both TNBC cell lines.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

BRD4 PROTAC alone resulted in about 15–20% of apoptosis. Combination therapy resulted in the highest apoptotic rate, with 50-60% of apoptosis, a significant improvement over either monotherapy. Concurrently, sequential treatment of BRD4 PROTAC and cisplatin led to the highest level of apoptotic cell death in all the experimental
groups, with the ability to restore cisplatin responsiveness to resistant TNBC cells as shown in Figure 5.Further, sequential treatment of BRD4 PROTAC and cisplatin resulted in highest apoptotic cell death among all experimental groups which showed that BRD4 PROTAC could restore cisplatin responsiveness in resistant TNBC cells, as illustrated in Figure 5.

Discussion

A major obstacle to treating triple-negative breast cancer (TNBC) is cisplatin resistance, which is frequently linked to treatment failure, recurrence, and poor patient outcomes. Due to adaptive molecular processes that promote tumor cell survival and effective DNA repair, platinum-based chemotherapy frequently produces positive early outcomes but offers little long-term clinical benefit. Thus, a top goal in TNBC research is to find therapeutic approaches that might be able to overcome this resistance. By using a novel PROTAC to target BRD4 for degradation, the researchers in this study investigated whether they could restore the sensitivity to cisplatin in resistant TNBC cells. Cisplatin-resistant derivatives of MDA-MB-231 and HCC1937 were successfully created by subjecting the cells to gradually increasing doses of cisplatin over a lengthy period of time. These derivatives were about five times more resistant than the parental cell lines. The findings support the creation of reliable in vitro models suitable for evaluating treatment approaches to reverse acquired cisplatin resistance. Only mild cytotoxicity was discovered when the treatment's effectiveness was assessed, which is in line with the known resistant phenotype. BRD4 PROTAC monotherapy reduced cell viability slightly in the absence of chemotherapy, indicating that BRD4 is also involved in sustaining survival signaling. The most successful therapeutic approach, however, was sequential treatment with cisplatin and BRD4 PROTAC, which led to notable reductions in cell viability and a 50–60% induction of cell apoptosis. The findings imply that BRD4 degradation can reactivate cisplatin resistance in resistant TNBC cells. Following BRD4 knockout, the expression of cell survival and DNA repair proteins was examined in order to comprehend the molecular mechanism underlying this reactivation. Following PROTAC treatment, MYC, BCL2, Cyclin D1, RAD51, and BRCA1 expression significantly decreased, according to Western blotting and quantitative real-time PCR. All of these findings support the established role of BRD4 as a transcriptional regulator of genes involved in apoptosis, proliferation, oncogenic signaling, and homologous recombination-mediated DNA repair. The increased cytotoxic effect observed with combination therapy can be explained by concurrently blocking these routes. γH2AX immunofluorescence, which demonstrated that the combination of
PROTAC and cisplatin treatment caused significantly more DNA damage in cells compared to the individual treatments, confirmed this DNA repair defect. Resolution of cisplatin-induced DNA lesions is anticipated to be hampered by the subsequent decrease in homologous recombination repair due to the lower expression of both RAD51 and BRCA1. Following combination therapy, there is an increase in Annexin V-positive cells, which is indicative of increased apoptotic signaling due to ongoing DNA damage. All of these findings suggest that cisplatin should be used in conjunction with the suppression of pro-survival and DNA repair mechanisms. The current findings support earlier findings that BET protein suppression increases cancer cells' susceptibility to traditional chemotherapy. However, traditional BET inhibitors only have a short-term effect because the target protein is not destroyed and can quickly return to its normal function once the medication is stopped. In contrast, PROTAC-driven degradation causes more persistent downregulation of downstream transcriptional

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

programs and eliminates BRD4 from the cells through the ubiquitin–proteasome system. The superior therapeutic activity of BRD4-targeting PROTACs over reversible BET inhibitors may be due to this straightforward mechanistic difference. This study has some limitations. Only two TNBC cell lines were used in the study, which might not accurately represent the biological diversity of TNBC. Furthermore, the long-term consequences of ongoing BRD4 degradation are unknown, and the therapeutic efficacy and safety of the BRD4 targeting PROTAC have not been evaluated in animal models. To assess the translational potential of this therapeutic approach, more research in xenograft and patient-derived tumor models as well as extensive toxicity testing would be necessary. Despite these limitations, the findings unequivocally show that BRD4 plays a significant role in mediating cisplatin resistance in TNBC. By blocking several transcriptional mechanisms that support cell survival and DNA repair, BRD4 degradation reveals the DNA-damaging capability of cisplatin in resistant tumor cells. These results will support the use of BRD4-targeting PROTACs in clinically relevant in vivo models for patients with cisplatin-resistant TNBC, and they should be further studied in preclinical models.

Conclusion

The current study's findings demonstrate that specific BRD4 degradation causes triple-negative breast cancer (TNBC) cells that are resistant to cisplatin to become more sensitive to the drug. The effective generation of two cisplatin-resistant cell lines, MDA-MB-231 and HCC1937, with a roughly five-fold increase in cisplatin resistance, provides a potent in vitro system for evaluating treatment strategies to overcome acquired chemoresistance. The cytotoxic effects and apoptotic cell death were greatly increased by sequential treatment with the BRD4-targeting PROTAC, but cisplatin alone had little effect on the resistance cells. Mechanistic investigations showed that the degradation of BRD4 suppressed the expression of homologous recombination proteins (RAD51 and BRCA1) and critical cell survival proteins (MYC, BCL2, and Cyclin D1). When these pathways were coordinatedly inhibited, DNA repair was blocked, cisplatin-induced DNA damage accumulated, and drug-resistant TNBCs became more susceptible to apoptosis. These findings demonstrate the potential of targeted protein degradation as a therapeutic strategy to overcome chemoresistance and identify BRD4 as a crucial regulator of chemoresistance to cisplatin. BRD4-targeting PROTACs may be a more long-lasting treatment than conventional BET inhibitors because of their capacity to concurrently interfere with oncogenic survival signaling and DNA repair mechanisms. Although in vitro models were used for the current study, the findings indicate that these BRD4-targeting PROTACs should be further studied in animal models and clinical trials to evaluate their safety, effectiveness, and potential to translate into patients with cisplatin-resistant TNBC. This study concludes that precision BRD4 targeting is a promising two-drug combination to improve treatment outcomes and increase triple-negative breast cancer's sensitivity to cisplatin therapy.

REFERENCE

Alsarraj, J., Walker, R. C., Webster, J. D., & et al. (2011a). Deletion of the proapoptotic gene BIM in mice recapitulates the immune system defects observed in BRD4 inhibition. Cancer Research, 71(20), 6670–6678.

Alsarraj, J., Walker, R. C., Webster, J. D., & et al. (2011b). Deletion of the proapoptotic gene BIM in mice recapitulates the immune system defects observed in BRD4 inhibition. Cancer Research, 71(20), 6670–6678.

American Type Culture Collection. (2020). MDA-MB-231 and HCC1937 cell line information. ATCC.

Anders, C. K., & Carey, L. A. (2009). Biology, metastatic patterns, and treatment of patients with triple-negative breast cancer. Clinical Breast Cancer, 9(Suppl 2), S73–S81.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Bandobashi, K., Miyoshi, H., Fujita, N., & et al. (2008). Potential role of BRCA1 in cisplatin resistance in ovarian cancer. Gynecologic Oncology, 108(2), 350–357.

Bondeson, D. P., Mares, A., Smith, I. E., & et al. (2015). Catalytic in vivo protein knockdown by small-molecule PROTACs. Nature Chemical Biology, 11(8), 611–617.

Brand, M., Bradford, D. S., Winter, G. E., & et al. (2019). BTK degraders as a strategy to overcome resistance in cancers. Nature Chemical Biology, 15(1), 91–97.

Cell Signaling Technology. (2021). Antibody validation and protocols. CST.

Dawson, M. A., Prinjha, R. K., Dittmann, A., & et al. (2011). Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature, 478(7370),
529–533.

Delmore, J. E., Issa, G. C., Lemieux, M. E., & et al. (2011a). BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell, 146(6), 904–917.

Delmore, J. E., Issa, G. C., Lemieux, M. E., & et al. (2011b). BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell, 146(6), 904–917.

Easwaran, H., Tsai, H. C., & Baylin, S. B. (2014). Cancer epigenetics: Tumor heterogeneity, plasticity of stem-like states, and drug resistance. Molecular Cell, 54(5), 716–727.

Filippakopoulos, P., & Knapp, S. (2014a). Targeting bromodomains: Epigenetic readers of lysine acetylation. Nature Reviews Drug Discovery, 13(5), 337–356.

Filippakopoulos, P., & Knapp, S. (2014b). Targeting bromodomains: Epigenetic readers of lysine
acetylation. Nature Reviews Drug Discovery, 13(5), 337–356.

Fong, C. Y., Gilan, O., Lam, E. Y., & et al. (2015a). BET inhibitor resistance emerges from leukaemia stem cells. Nature, 525(7570), 538–542.

Fong, C. Y., Gilan, O., Lam, E. Y., & et al. (2015b). BET inhibitor resistance emerges from leukaemia stem cells. Nature, 525(7570), 538–542.

Foulkes, W. D., Smith, I. E., & Reis-Filho, J. S. (2010). Triple-negative breast cancer. New England Journal of Medicine, 363(20), 1938–1948.

Freshney, R. I. (2016). Culture of animal cells: A manual of basic technique (7th ed.). Wiley-Liss.

Galluzzi, L., Vitale, I., Michels, J., & et al. (2014). Systems biology of cisplatin resistance: Past, present and future. Cell Death & Disease, 5(5), e1257.

GraphPad Software. (2020). GraphPad Prism version 9.0 for Windows. GraphPad Software.

Helleday, T., Petermann, E., Lundin, C., Hodgson, B., & Sharma, A. (2008). DNA repair pathways as targets for cancer therapy. Nature Reviews Cancer, 8(3), 193–204.

Huang, B., Yang, X. D., Zhou, M. M., Ozato, K., & Chen, L. F. (2009). Brd4 coactivates transcriptional activation of NF-kappaB via specific interaction with acetylated RelA. Molecular and Cellular Biology, 29(5), 1375–1387.

Li, Y., Yang, J., Luo, W., & et al. (2017a). BRD4 promotes DNA repair and mediates resistance to cisplatin. Oncogene, 36(26), 3779–3788.

Li, Y., Yang, J., Luo, W., & et al. (2017b). BRD4 promotes DNA repair and mediates resistance to cisplatin. Oncogene, 36(26), 3779–3788.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR. Methods, 25(4), 402–408.

Lu, J., Qian, Y., Altieri, M., & et al. (2015a). Hijacking the E3 ubiquitin ligase cereblon to efficiently target BRD4. Chemical Biology, 22(6), 755–763.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Lu, J., Qian, Y., Altieri, M., & et al. (2015b). Hijacking the E3 ubiquitin ligase cereblon to efficiently target BRD4. Chemical Biology, 22(6), 755–763.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival. Journal of Immunological Methods, 65(1-2), 55–63.

Nagaraja, S., Vitanza, N. A., Woo, P. J., & et al. (2017). Transcriptional dependencies in primitive neuroectodermal tumors from children with cancer. Nature, 547(7661), 103–107.

Neklesa, T. K., Winkler, J. D., & Crews, C. M. (2017a). Targeted protein degradation through the

ubiquitin-proteasome system. Pharmacology & Therapeutics, 174, 138–144.

Neklesa, T. K., Winkler, J. D., & Crews, C. M. (2017b). Targeted protein degradation through the ubiquitin-proteasome system. Pharmacology & Therapeutics, 174, 138–144.

O'Connor, M. J. (2015a). Targeting the DNA damage response in cancer. Molecular Cell, 60(4), 547–560.

O'Connor, M. J. (2015b). Targeting the DNA damage response in cancer. Molecular Cell, 60(4), 547–560.

Raina, K., Lu, J., Qian, Y., & et al. (2016a). PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer. Proceedings of the National Academy of Sciences, 113(26), 7124–7129.

Raina, K., Lu, J., Qian, Y., & et al. (2016b). PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer. Proceedings of the National Academy of Sciences, 113(26), 7124–7129.

Rogakou, E. P., Pilch, D. R., Orr, A. H., & et al. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. Journal of Biological Chemistry, 273(10), 5858–5868.

Sakamoto, K. M., Kim, K. B., Kumagai, A., & et al. (2001a). Protacs: Chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. Proceedings of the National Academy of Sciences, 98(15), 8554–8559.

Sakamoto, K. M., Kim, K. B., Kumagai, A., & et al. (2001b). Protacs: Chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. Proceedings of the National Academy of Sciences, 98(15), 8554–8559.

Shimamura, T., Chen, Z., Soucheray, M., & et al. (2013). EZH2 and BRD4 cooperate to sustain MYC in MYC-driven cancers. Cancer Cell, 24(2), 173–187.

Sigma-Aldrich. (2019). Cisplatin product information and handling. Sigma.

Sun, X., Gao, H., Yang, Y., & et al. (2019a). PROTACs: Great opportunities for academia and industry. Signal Transduction and Targeted Therapy, 4, 64.

Sun, X., Gao, H., Yang, Y., & et al. (2019b). PROTACs: Great opportunities for academia and industry. Signal Transduction and Targeted Therapy, 4, 64.

Towbin, H., Staehelin, T., & Gordon, J. (1979). Electrophoretic transfer of proteins from

polyacrylamide gels to nitrocellulose sheets. Proceedings of the National Academy of Sciences, 76(9), 4350–4354.

Vermes, I., Haanen, C., Steffens-Nakken, H., & Reutelingsperger, C. (2015). A novel assay for apoptosis. Journal of Immunological Methods, 184(1), 39–51.

Winter, G. E., Buckley, D. L., Paulk, J., & et al. (2015a). Phthalimide conjugation as a strategy for in vivo target protein degradation. Science, 348(6241), 1376–1381.

Winter, G. E., Buckley, D. L., Paulk, J., & et al. (2015b). Phthalimide conjugation as a strategy for in vivo target protein degradation. Science, 348(6241), 1376–1381.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Yang, Z., He, N., & Zhou, Q. (2008a). BRD4 recruits P-TEFb to enhancers and promoters to regulate gene expression. Molecular Cell, 27(1), 26–34.

Yang, Z., He, N., & Zhou, Q. (2008b). BRD4 recruits P-TEFb to enhancers and promoters to regulate gene expression. Molecular Cell, 27(1), 26–34.

Zengerle, M., Chan, K. H., & Ciulli, A. (2015). Selective small molecule induced degradation of the BET bromodomain protein BRD4. ACS Chemical Biology, 10(8), 1770–1777.

Zou, Y., Ma, D., & Wang, Y. (2019). The PROTAC technology in drug development. Cell Biochemistry and Function, 37(1), 21–30.