

Carfilzomib: A Promising Proteasome Inhibitor For the Treatment of Cervical Cancer

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Abstract

Cervical cancer is still a major health problem around the world. It is the fourth most common cancer in women. Even though screening and vaccination have gotten better, there are still not many good treatments for advanced and recurrent cervical cancer. Platinum-based chemotherapy works only a little and causes a lot of resistance to build up. Proteasome inhibitors are a new type of drug that has changed the way we treat blood cancers, especially multiple myeloma. Carfilzomib, a second-generation irreversible proteasome inhibitor, has exhibited encouraging preclinical efficacy across multiple solid tumors. This thorough review brings together the latest research on carfilzomib and other proteasome inhibitors that could be used to treat cervical cancer. It looks at how these drugs work at the molecular level, how well they work in preclinical studies, how they could be used in combination with other drugs, how they might become resistant, and what this means for real-world use. Although direct clinical evidence for carfilzomib in cervical cancer is scarce, preclinical studies involving related proteasome inhibitors exhibit substantial anti-tumor activity via various mechanisms, including the induction of apoptosis, cell cycle arrest, and increased sensitivity to conventional chemotherapy. The ubiquitin-proteasome system has been confirmed as a viable therapeutic target in cervical cancer, with combination strategies demonstrating significant potential for addressing drug resistance and enhancing therapeutic efficacy.

Keywords: Carfilzomib, Cervical Cancer, Proteasome Inhibitor, Conventional Chemotherapy, Multiple Myeloma.

1. Introduction

Cervical cancer is a big health problem around the world. In 2020, there were about 604,000 new cases and 342,000 deaths from it. Even though there has been a lot of progress in prevention thanks to human papillomavirus (HPV) vaccination and screening programs, advanced and recurrent cervical cancer is still very hard to treat [19]. Current treatment paradigms heavily depend on platinum-based chemotherapy, especially cisplatin, which shows limited efficacy and is often linked to the emergence of drug resistance [6], [16], [19].

The ubiquitin-proteasome system (UPS) has become a vital regulator of cellular homeostasis and a recognized therapeutic target in cancer [7], [8], [9]. Proteasome inhibitors have demonstrated significant clinical efficacy in hematological malignancies, with bortezomib, carfilzomib, and ixazomib obtaining FDA approval for the treatment of multiple myeloma [4, 7, 9]. However, their effectiveness in solid tumors, such as cervical cancer, has not been completely established, creating both challenges and opportunities for therapeutic advancement [20].

Carfilzomib, a second-generation epoxyketone-based proteasome inhibitor, presents several theoretical advantages over first-generation agents, including irreversible binding to the proteasome catalytic subunit and potentially enhanced selectivity [2], [3], [9]. Although direct clinical evidence for carfilzomib in cervical cancer is still scarce, recent preclinical studies involving various proteasome inhibitors indicate substantial anti-tumor efficacy in cervical cancer models [6, 13, 15, 16]. This review thoroughly analyzes the existing knowledge on

proteasome inhibition, particularly focusing on carfilzomib, as a therapeutic approach for cervical cancer treatment.

2. Background and Theoretical Foundataion

2.1. The Ubiquitin-Proteasome System in Cancer

The ubiquitin-proteasome system is the main way that eukaryotic cells break down proteins in a controlled way. It controls about 80–90% of the turnover of proteins inside cells [7], [8], [28]. This system is very important for controlling the cell cycle, apoptosis, the response to DNA damage, immune surveillance, and signal transduction [7, 9, 17]. Cancer cells are more reliant on proteasome activity to keep protein homeostasis when their metabolism is working harder, they are growing quickly, and they are getting more misfolded proteins [17].

The UPS has three main parts: E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes, and E3 ubiquitin ligases. These enzymes attach ubiquitin molecules to target proteins in order [5], [10]. The 26S proteasome, a big multi-catalytic protease complex, then recognizes and breaks down polyubiquitinated proteins [7, 16]. Dysregulation of the UPS has been associated with the pathogenesis of cervical cancer, particularly via HPV-mediated degradation of the tumor suppressors p53 and pRb [10, 16].

Recent evidence underscores the complexity of proteasome composition, revealing that constitutive proteasomes, immunoproteasomes, and intermediate proteasome subtypes possess unique catalytic properties and tissue distributions [2, 3]. This diversity has significant ramifications for proteasome inhibitor selectivity and resistance mechanisms [2, 3, 4].



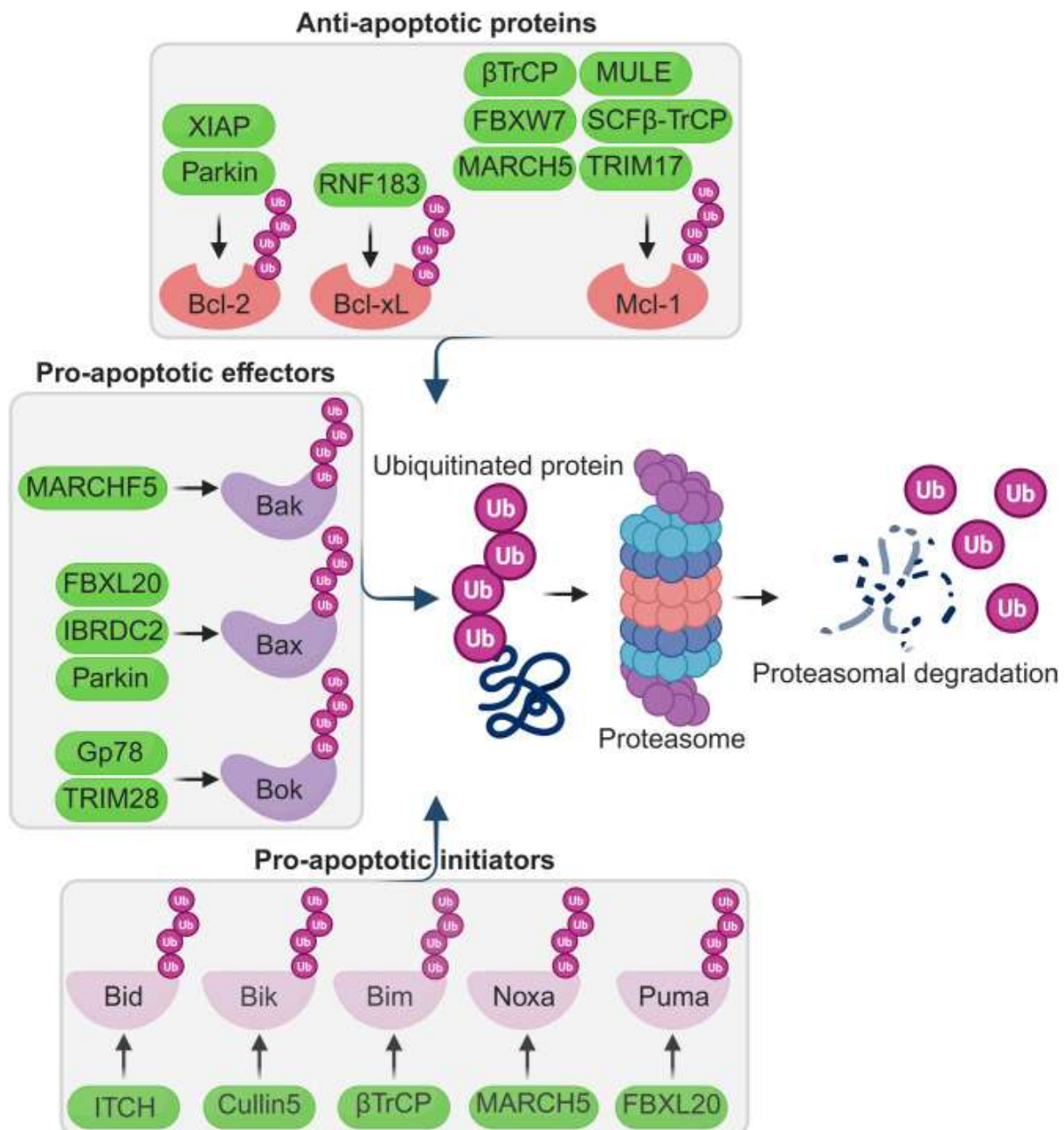


Figure 1: Ubiquitin-proteasome system (UPS)-mediated regulation of Bcl-2 family proteins [8]. This figure illustrates the role of E3 ubiquitin ligases in targeting Bcl-2 family proteins for ubiquitination and subsequent proteasomal degradation. Central to the figure is the ubiquitin-proteasome system (UPS), where polyubiquitinated proteins are recognized and degraded by the 26 S proteasome. Proteins marked with multiple “Ub” (ubiquitin) icons are designated for degradation. **Top panel:** Anti-apoptotic Bcl-2 family proteins (Bcl-2, Bcl-xL, and Mcl-1) are regulated by specific E3 ligases (green ovals) which promote their ubiquitination. **Left panel:** Pro-apoptotic effector proteins (Bak, Bax, and Bok) are similarly targeted by specific E3 ligases (green ovals) to modulate apoptosis. **Bottom panel:** BH3-only pro-apoptotic initiator proteins (Bid, Bik, Bim, Noxa, Puma) are shown to be ubiquitinated by specific E3 ligases (green ovals). The central flow in the figure depicts the general UPS process, E3 ligases catalyze the attachment of ubiquitin to substrates, leading to recognition and degradation by the proteasome. Ubiquitinated proteins (tagged with purple Ub chains) enter the proteasome, are degraded, and free ubiquitin molecules are recycled. Green ovals indicate E3 ubiquitin ligases; arrows denote direction of ubiquitination or degradation. Shapes and colors of Bcl-2 family proteins represent their functional class: red (anti-apoptotic), purple (pro-apoptotic effectors), and pink (BH3-only proteins). Ub = Ubiquitin

2.2. Proteasome Inhibitors: Development and Clinical Success

The advancement of proteasome inhibitors as anticancer agents signifies a significant breakthrough in targeted cancer therapy [7], [9], [17]. Bortezomib, the first proteasome inhibitor to be approved in 2003, showed amazing

results in treating multiple myeloma, changing the way this disease is treated forever [4, 7, 20]. The clinical efficacy of bortezomib confirmed the proteasome as a viable therapeutic target and catalyzed the advancement of next-generation inhibitors with enhanced pharmacological characteristics [9].

The FDA has approved three proteasome inhibitors for blood cancers: bortezomib (a reversible boronic acid-based drug), carfilzomib (an irreversible epoxyketone-based drug), and ixazomib (an oral agent that reversibly binds to boronic acid) [4], [7], [9]. These substances have different ways of binding, selectivity profiles, and pharmacokinetic properties [2], [3], and [9]. Proteasome inhibitors have been very successful in treating multiple myeloma, but they haven't worked as well on their own in solid tumors. This has led to research into new combinations and formulations [1, 20].

Proteasome inhibitors derived from natural products have attracted considerable attention, with compounds like marizomib (salinosporamide A) exhibiting distinctive inhibitory characteristics and promising benefits in solid tumor contexts [6, 17]. Marizomib irreversibly inhibits all three catalytic activities of the proteasome (chymotrypsin-like, trypsin-like, and caspase-like), potentially providing more extensive anti-tumor effects [6].

2.3. Carfilzomib: Mechanism and Pharmacology

Carfilzomib (PR-171) is a tetrapeptide epoxyketone proteasome inhibitor that binds selectively and irreversibly to the $\beta 5$ (chymotrypsin-like) catalytic subunit of the 20S proteasome [2], [3], [9]. The epoxyketone pharmacophore creates a stable morpholino adduct with the N-terminal threonine residue of the $\beta 5$ subunit. This leads to longer proteasome inhibition than reversible inhibitors like bortezomib [2, 3].

Carfilzomib also stops the immunoproteasome subunits $\beta 1i$ (LMP2) and $\beta 2i$ (MECL1) at higher doses, which helps it fight tumors [2, 3]. The fact that carfilzomib binds irreversibly means that proteasome activity can't be restored. This could make it more effective against cancer and help it get around some of the resistance mechanisms that bortezomib has [2, 3, 4].

Carfilzomib exhibits various pharmacological benefits, such as diminished off-target effects and potentially reduced neurotoxicity in comparison to bortezomib [9]. But carfilzomib has been linked to cardiovascular toxicity, which means that patients need to be watched closely [9]. The drug has a fast plasma clearance and needs to be given through an IV, which may make it less useful in some situations [9].



Figure 2: FDA-approved proteasome inhibitors [3]. Bortezomib and ixazomib contain a boronic acid pharmacophore, while a tetrapeptide carfilzomib has an epoxyketone pharmacophore.

Carfilzomib-induced proteasome inhibition results in the accumulation of polyubiquitinated proteins, which instigates proteotoxic stress, endoplasmic reticulum (ER) stress, the activation of the unfolded protein response (UPR), and ultimately leads to apoptosis [2], [8], [9]. Other mechanisms involve blocking NF- κ B signaling, stopping the cell cycle, making the DNA damage response less effective, and changing the tumor microenvironment [2, 9, 18].

3. Cervical Cancer: Current Treatment Landscape and Unmet Needs

3.1. Epidemiology and Clinical Challenges

Cervical cancer is still the fourth most common cancer among women worldwide, and it is most prevalent in low- and middle-income nations with limited access to screening and immunization programs [19]. Treatment options for advanced, recurrent, or metastatic disease are still insufficient, despite the fact that HPV vaccination and improved screening have decreased incidence in developed countries [19].

With 5-year survival rates for metastatic disease less than 20%, advanced cervical cancer has a dismal prognosis [19]. About 30–40% of patients with locally advanced disease experience recurrence even after receiving the best

possible primary treatment, which includes concurrent chemoradiotherapy [19]. These alarming figures highlight how urgently new therapeutic strategies are needed.

3.2. Current Treatment Modalities and Limitation

The standard treatment for advanced cervical cancer includes platinum-based chemotherapy, usually cisplatin alone or in conjunction with paclitaxel [6], [16], [19], [20]. Cisplatin shows initial response rates of 15-25% as a single agent, but the emergence of chemoresistance poses a significant clinical challenge [6, 16, 20]. Cisplatin resistance mechanisms encompass augmented DNA repair, modified drug uptake and efflux, heightened detoxification, and the dysregulation of apoptotic pathways [6], [20].

Recent progress has integrated targeted therapies and immunotherapies into the treatment paradigm for cervical cancer. Bevacizumab, an anti-VEGF antibody, showed a survival benefit when added to chemotherapy in the GOG 240 trial [19]. Immune checkpoint inhibitors, especially pembrolizumab, have demonstrated efficacy in PD-L1-positive recurrent or metastatic cervical cancer [19]. But response rates are still low, and most patients eventually get worse, which shows that new treatment options are still needed.

3.3. Molecular Targets in Cervical Cancer

Cervical cancer pathogenesis is closely associated with high-risk HPV infections, specifically HPV-16 and HPV-18, which represent about 70% of cases [10], [16], [19]. Oncoproteins E6 and E7 from HPV cause cancer by breaking down the tumor suppressors p53 and pRb, respectively, using the ubiquitin-proteasome system [10], [16]. This HPV-mediated takeover of the UPS gives a strong biological reason to use proteasome inhibition as a treatment for cervical cancer.

In addition to HPV-related mechanisms, cervical cancer demonstrates dysregulation of various signaling pathways suitable for therapeutic intervention, including PI3K/AKT/mTOR, MAPK/ERK, NF- κ B, and apoptotic pathways [13], [16], [18], [19]. The p53 pathway is often turned off by HPV E6, but proteasome inhibition can help bring it back to life by stabilizing p53 and its transcriptional targets [13, 16]. Likewise, the alteration of Bcl-2 family proteins constitutes a significant mechanism through which proteasome inhibitors facilitate apoptosis [8, 13, 16].

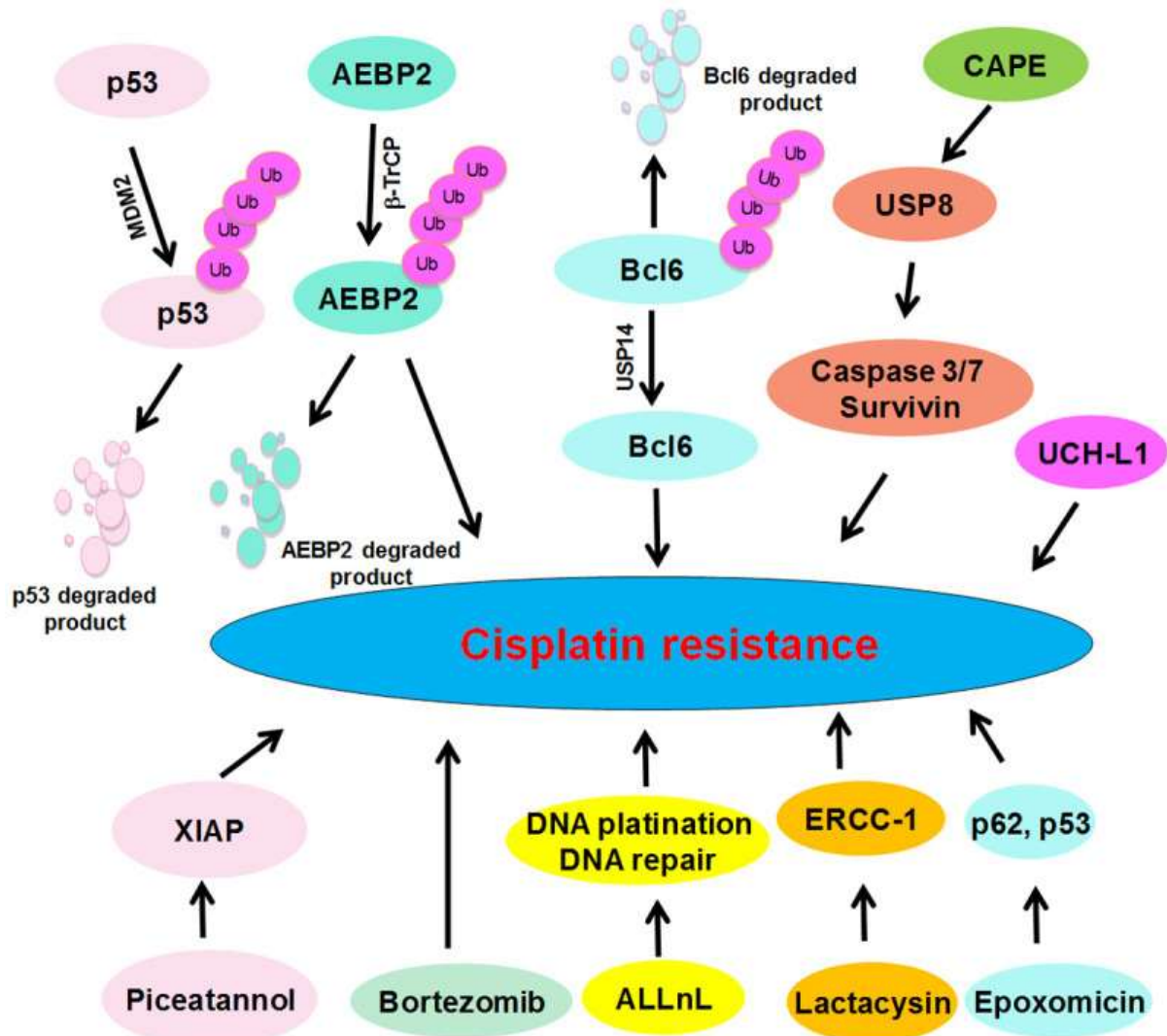


Figure 3: Ubiquitination plays a critical role in cisplatin resistance in ovarian cancer cells [10]. Multiple compounds target ubiquitination to overcome the drug resistance of ovarian cancer cells.

4. Preclinical Evidence for Proteasome Inhibition in Cervical Cancer

4.1. Direct Studies in Cervical Cancer Model

Numerous preclinical studies have directly examined proteasome inhibitors in cervical cancer models, yielding substantial evidence for their therapeutic potential. Guo et al. showed that delanzomib (CEP-18770), a new proteasome inhibitor, had strong pro-apoptotic and cytotoxic effects on five cervical cancer cell lines (HeLa, CaSki, C33A, SiHa, and ME-180) that represent the main molecular subtypes [16]. Delanzomib inhibited proteasomal activity and markedly enhanced the sensitivity of cervical cancer cells to doxorubicin, with combination therapy demonstrating synergistic effects [16].

Zhang et al. examined marizomib, a second-generation proteasome inhibitor, in cervical cancer cell lines (HeLa, CaSki, C33A) and xenograft models [6]. Marizomib alone exhibited significant cytotoxic effects, and its combination with cisplatin led to markedly increased cytotoxicity and apoptosis both in vitro and in vivo [6]. Significantly, the combination was well-tolerated in mice, indicating clinical viability [6].

Meng et al. found that double-stranded RNA poly(I:C) works with proteasome inhibitors to kill cervical cancer cells by increasing the levels of interferon- β (IFN β) and genes linked to apoptosis. This activates both intrinsic and extrinsic apoptotic pathways [15]. This discovery indicates the possibility of immunomodulatory combination strategies.

Sun et al. recently showed that the MDM2 inhibitor APG-115 works with bortezomib to kill cervical cancer cells and xenografts [13]. The combination increased the levels of p53 and p21, lowered the levels of Ki67 and Bcl-2, and greatly slowed the growth of tumors [13]. This study concentrated on bortezomib instead of carfilzomib, yet it demonstrates proof-of-concept for proteasome inhibitor combinations in cervical cancer.

4.2. Molecular Mechanisms of Action

Proteasome inhibitors have anti-tumor effects in cervical cancer through a number of interconnected ways. The main way it works is by stabilizing tumor suppressor proteins, especially p53 and its transcriptional targets p21, PUMA, and Noxa [13], [16]. Guo et al. showed that treating cervical cancer cells with delanzomib caused p53, p21, PUMA, and Noxa to build up in a dose-dependent way [16]. This activation of the p53 pathway is especially important in cervical cancer, where HPV E6 usually targets p53 for proteasomal degradation [10], [16].

Another important process is the activation of stress-activated protein kinases. Delanzomib caused the phosphorylation of p38 MAPK and JNK, which are stress response kinases that help cells die [16]. When used with doxorubicin, delanzomib made p38 phosphorylation even stronger, which added to the cytotoxicity of the two drugs working together [16].

The modulation of Bcl-2 family proteins is pivotal in apoptosis induced by proteasome inhibitors. Sun et al. demonstrated that the combination of APG-115 and bortezomib decreased the levels of anti-apoptotic proteins Bcl-2, Bcl-xL, and Mcl-1, while enhancing the levels of pro-apoptotic proteins BAK, BAX, and BIM [13]. This change in the balance of Bcl-2 family members toward a pro-apoptotic state makes it easier for the outer membrane of the mitochondria to become permeable and for caspases to be activated [8, 13].

Caspase activation constitutes the terminal common pathway of proteasome inhibitor-induced apoptosis. Numerous studies have reported the activation of caspases-3, -8, and -9, signifying the involvement of both intrinsic (mitochondrial) and extrinsic (death receptor) apoptotic pathways [6], [15], [16]. Zhang et al. showed that marizomib caused PARP cleavage and caspase-3 activation, and these effects were stronger when marizomib was used with cisplatin [6].

Blocking the proteasome also has an effect on pathways that lead to angiogenesis and metastasis. Zhang et al. discovered that marizomib altered the expression of angiogenesis-related factors, specifically increasing angiopoietin-1 (Ang-1) levels and decreasing those of Flt-3L, SCF, and Tie-2 [6]. These modifications may facilitate anti-angiogenic effects and diminish metastatic potential.

4.3. Efficacy Data from In Vitro and In Vivo Studies

In vitro studies have consistently shown that proteasome inhibitors have strong anti-proliferative and pro-apoptotic effects on cervical cancer cell lines. Guo et al. documented IC₅₀ values for delanzomib between 10-50 nM across five cervical cancer cell lines, indicating more advantageous cytotoxicity profiles relative to bortezomib [16]. Marizomib exhibited dose-dependent cytotoxicity in HeLa, CaSki, and C33A cells [6].

Multiple xenograft models have shown that it works in living organisms. Zhang et al. demonstrated that marizomib alone diminished tumor volume in cervical cancer xenografts, while combination therapy (marizomib plus cisplatin) resulted in markedly enhanced tumor growth inhibition relative to either agent administered singularly [6]. It is important to note that the combination therapy was well-tolerated, with no significant weight loss or obvious toxicity seen in the mice that were treated [6].

Sun et al. showed that APG-115 and bortezomib together greatly slowed the growth of tumors in cervical cancer xenografts. The combination worked better than either drug alone [13]. Immunohistochemical analysis demonstrated reduced Ki67 (proliferation marker) and Bcl-2 expression, alongside elevated p21 expression in tumors subjected to combination treatment [13].

These preclinical findings collectively substantiate the therapeutic efficacy of proteasome inhibitors in cervical cancer, especially when administered in conjunction with standard chemotherapy or targeted therapies. Nonetheless, it is crucial to acknowledge that the majority of direct cervical cancer studies have employed proteasome inhibitors other than carfilzomib, specifically delanzomib, marizomib, and bortezomib. This underscores a deficiency in the literature concerning carfilzomib-specific efficacy in cervical cancer models.

5. Combination Strategies and Synergistic Effects

5.1. Proteasome Inhibitors with Platinum-Based Chemotherapy

Combining proteasome inhibitors with platinum-based chemotherapy is a very promising way to treat cervical cancer. Chao et al. examined the molecular mechanisms that facilitate the synergistic effects of proteasome inhibitors and platinum agents in solid tumors, pinpointing apoptosis and autophagy modulation as critical mediators of increased efficacy [20]. Proteasome inhibition can counteract cisplatin resistance by stabilizing pro-apoptotic proteins and inhibiting the activation of survival pathways [20].

Zhang et al. showed that marizomib makes cervical cancer cells more sensitive to cisplatin in several ways [6]. The combination increased PARP cleavage and caspase-3 activation caused by cisplatin, which showed that apoptotic signaling was stronger [6]. Mechanistically, marizomib increased Ang-1 expression and decreased Flt-3L, SCF, and Tie-2, which may have stopped angiogenic support for tumor growth [6]. These results indicate that proteasome inhibitors can counteract cisplatin resistance by altering both apoptotic and angiogenic pathways.

Guo et al. demonstrated that delanzomib markedly enhanced the sensitivity of cervical cancer cells to doxorubicin,

a widely utilized chemotherapeutic agent [16]. The combination synergistically augmented p53 stabilization and p38 MAPK phosphorylation, resulting in heightened apoptosis [16]. This synergy was evident in various cervical cancer cell lines exhibiting distinct molecular characteristics, indicating widespread applicability [16].

Chemosensitization by proteasome inhibitors is based on a number of molecular mechanisms. Proteasome inhibition first stops the breakdown of pro-apoptotic proteins caused by chemotherapy. This lets these proteins build up to levels that are needed for apoptosis to happen [16], [20]. Second, proteasome inhibitors can stop NF- κ B from being activated. This is a key survival pathway that is often turned on when chemotherapy causes stress [2, 9, 20]. Third, blocking the proteasome may make it harder for cells to fix DNA damage, which makes DNA-damaging drugs like cisplatin more toxic [20].

5.2. Combination with Targeted Therapies

The combination of proteasome inhibitors and targeted therapies presents opportunities to leverage synthetic lethality and surmount adaptive resistance mechanisms. Sun et al. showed that the MDM2 inhibitor APG-115 and bortezomib worked together to treat cervical cancer [13]. This combination fully activated p53 and blocked Bcl-2 at the same time, which was a strong pro-apoptotic signal [13]. The synergy was apparent both in vitro and in vivo, as xenografts treated with the combination exhibited markedly enhanced tumor growth inhibition compared to either single agent [13].

Bi et al. examined the combined inhibition of HDAC and proteasome in gynecologic cancers, discovering that ixazomib, in conjunction with romidepsin (an HDAC inhibitor), synergistically induced apoptosis in ovarian and endometrial cancer models [12]. Although this study did not specifically investigate cervical cancer, the findings may be applicable due to the common gynecologic cancer biology [12]. Autophagy was identified as a resistance mechanism, and its inhibition reversed this resistance to the combination [12].

The idea of targeting compensatory survival pathways is a key way to make proteasome inhibitors work better. Thompson et al. demonstrated that glutaminase inhibition synergizes with carfilzomib in multiple myeloma by targeting metabolic adaptation [26]. Cervical cancer may exhibit analogous metabolic vulnerabilities that necessitate further investigation.

Lee et al. showed that carfilzomib stops the growth of and kills HPV-negative head and neck cancer cells by stopping PI3K/AKT/mTOR signaling and turning on p21 [18]. Although this study concentrated on head and neck cancer instead of cervical cancer, the mechanistic insights into PI3K/AKT/mTOR pathway inhibition are pertinent, given that this pathway is often dysregulated in cervical cancer [18], [19].

5.3. Immunomodulatory Combinations

The convergence of proteasome inhibition and immunotherapy signifies a burgeoning field of interest. Meng et al. showed that poly(I:C), which activates TLR3 and boosts the immune system, works with proteasome inhibitors to kill cervical cancer cells by raising the levels of IFN β and genes linked to apoptosis [15]. This discovery indicates that proteasome inhibitors might augment immunogenic cell death and anti-tumor immune responses.

Shi et al. created a cyclic peptide-based PROTAC that breaks down palmitoyltransferase DHHC3 and lowers the levels of PD-L1 in cervical cancer cells [11]. This study concentrated on targeted protein degradation instead of direct proteasome inhibition, yet it underscores the possibility of altering immune checkpoint expression via UPS-related mechanisms [11]. The cyclic peptide improved the effectiveness of cisplatin and increased the release of IFN- γ and TNF- α in T cell co-culture systems, which suggests that it boosted anti-tumor immunity [11].

Proteasome inhibitors may improve the effectiveness of immunotherapy by various means, such as boosting antigen presentation, changing the expression of immune checkpoints, and boosting immunogenic cell death [9], [11]. These immunomodulatory effects necessitate further exploration in cervical cancer, especially in light of the recent efficacy of immune checkpoint inhibitors in this malignancy [19].

6. Mechanisms of Resistance and Strategies to Overcome Them

6.1. Proteasomal Adaptations and Mutations

Resistance to proteasome inhibitors constitutes a significant clinical challenge, especially in multiple myeloma, where acquired resistance is nearly ubiquitous [3], [4]. Kim et al. examined proteasomal adaptations to FDA-approved proteasome inhibitors, pinpointing point mutations in the β 5 catalytic subunit as a principal resistance mechanism [3]. Mutations like Ala49Thr and Thr21Ala make it harder for inhibitors to bind while keeping catalytic activity [3]. Overexpression of the β 5 subunit can also make cells resistant by raising the total amount of proteasome that can be inhibited [3].

Bennett et al. conducted a thorough review of resistance mechanisms to bortezomib, carfilzomib, and ixazomib in multiple myeloma, highlighting that resistance develops through various intricate mechanisms [4]. These encompass modifications in proteasome subunit composition, characterized by transitions towards immunoproteasome or intermediate proteasome subtypes that may display varying inhibitor sensitivities [2], [3],

[4].

Carmony et al. examined the influence of proteasome catalytic subunit composition on resistance, illustrating that cells can adjust to proteasome inhibition by modifying the relative abundance of constitutive proteasome and immunoproteasome subunits [2]. This variability in proteasome composition poses a challenge for single-agent proteasome inhibitor therapy and indicates the potential efficacy of inhibitors aimed at multiple proteasome subtypes [2].

P-glycoprotein (Pgp)-mediated drug efflux has been associated with carfilzomib resistance in certain contexts [3]. Nonetheless, carfilzomib is typically regarded as less vulnerable to Pgp-mediated efflux than bortezomib, potentially providing benefits in resistant contexts [3].

6.2. Autophagy-Mediated Resistance

Autophagy has become an essential adaptive resistance mechanism against proteasome inhibition. Bi et al. showed that autophagy helps gynecologic cancers resist both HDAC and proteasome inhibition at the same time [12]. Sensitive cells displayed diminished autophagy following treatment with ixazomib and romidepsin, whereas resistant cells demonstrated enhanced autophagy that facilitated cell survival [12]. Pharmacological or genetic suppression of autophagy counteracted this resistance, augmenting anti-tumor responses both in vitro and in vivo [12].

The connection between stopping proteasomes and starting autophagy is complicated and depends on the situation. Inhibition of the proteasome can trigger compensatory autophagy as cells strive to preserve protein homeostasis via alternative degradation pathways [2], [12]. This adaptive autophagy can help cells stay alive by getting rid of damaged organelles and proteins that have built up over time, which lowers proteotoxic stress [12].

Wu et al. showed that titanium nitride (TiN) nanoshells that carry carfilzomib have their own autophagy-inhibiting properties that work together to make carfilzomib work better [1]. This discovery indicates that the concurrent inhibition of proteasomes and autophagy may constitute an effective approach to surmount resistance [1], [12].

6.3. Compensatory Survival Pathways

When proteasome activity is blocked, cancer cells turn on several backup survival pathways. Oron et al. discovered that HSP70 family chaperones (HSPA1A/B) act as universal responders to proteasome inhibition in various cancer types [29]. The induction of HSPA1A/B was transcriptionally regulated and dependent on HSF1/2, and these chaperones safeguarded the 26S proteasome from carfilzomib inhibition [29]. Blocking HSPA1A/B made cancer cells more sensitive to carfilzomib in cell lines, organoids from patients, and xenograft models [29]. The unfolded protein response (UPR) is another way that cells try to make up for proteasome inhibition. When proteins fold incorrectly, they build up in the endoplasmic reticulum (ER), which causes stress and activates the unfolded protein response (UPR). Depending on how strong and long the stress is, this can either kill cells or help them live [2], [9]. Targeting UPR components may improve the effectiveness of proteasome inhibitors by stopping adaptive survival responses.

The activation of the NF- κ B pathway is a well-known way for cancer cells to stay alive. Proteasome inhibitors can stop NF- κ B from being activated by stopping the breakdown of I κ B. However, some cells that are resistant may use other NF- κ B signaling pathways to do so [2], [9]. Combination strategies that target both proteasome and NF- κ B may be able to get around this resistance mechanism.

7. Novel Drug Delivery Systems and Formulations

Advanced drug delivery systems provide prospects to augment proteasome inhibitor effectiveness while mitigating systemic toxicity. Wu et al. created titanium nitride (TiN) nanoshells that contained carfilzomib for use in both chemotherapy and phototherapy [1]. These empty nanoshells showed that they could hold a lot of carfilzomib and convert light into heat very well in the second near-infrared (NIR-II) region [1]. The TiN nanoshells showed natural autophagy-inhibiting effects that worked together to make carfilzomib work better [1]. In vivo studies showed that TiN nanoshells easily drained into lymph nodes, which are common places for cervical cancer to spread [1]. TiN nanoshells loaded with carfilzomib, along with NIR-II photothermal therapy and surgery, greatly slowed the growth of tumors and the spread of cancer to lymph nodes in solid tumor models [1]. Although this study did not specifically investigate cervical cancer, the lymphatic drainage characteristics of TiN nanoshells are particularly pertinent due to the tendency of cervical cancer for lymphatic dissemination [1].

This "combined chemo-phototherapy assisted surgery" strategy exemplifies a novel methodology that may be applicable to cervical cancer treatment [1]. The capacity to target lymph nodes and integrate various therapeutic modalities (chemotherapy, phototherapy, surgery) tackles critical issues in cervical cancer management, such as local control and the prevention of metastatic dissemination [1].

Nanoparticle-based delivery systems provide numerous benefits for proteasome inhibitors in solid tumors, such

as increased tumor accumulation via the enhanced permeability and retention (EPR) effect, safeguarding against premature degradation, regulated release kinetics, and the possibility of combination drug loading [1]. Future advancements in carfilzomib formulations tailored for cervical cancer must take into account these innovative delivery methods.

8. Translational Considerations and Clinical Implications

8.1. Lessons from other Solid Tumors

Proteasome inhibitors have demonstrated significant success in hematological malignancies; however, their effectiveness in solid tumors has been comparatively restricted [9], [20]. There are a number of reasons why this is the case, such as differences in proteasome dependence, effects on the tumor microenvironment, barriers to drug penetration, and the activation of compensatory survival pathways [9, 20].

Lee et al. showed that carfilzomib has strong anticancer effects in HPV-negative head and neck squamous cell carcinoma (HNSCC). The IC50 values were more than four times lower in sensitive cell lines than in resistant lines [18]. Carfilzomib diminished tumor proliferation in vivo and triggered apoptosis via modulation of the PI3K/AKT/mTOR and p21 pathways [18]. Crucially, carfilzomib demonstrated the potential to surmount cisplatin resistance in HNSCC [18]. These findings in head and neck cancer, which shares squamous cell histology with cervical cancer, offer promising translational evidence.

Zulkifli et al. demonstrated that carfilzomib enhances the unfolded protein response and induces apoptosis in cetuximab-resistant colorectal cancer [22]. Chen et al. showed that carfilzomib stops LDHA-mediated metabolic reprogramming in esophageal squamous cell carcinoma [23]. These studies in other solid tumors underscore the various mechanisms through which carfilzomib can exert anti-tumor effects beyond mere proteasome inhibition.

It is clear from studies of many different types of solid tumors that proteasome inhibitors only work when they are used in combination with other drugs [1], [6], [13], [16], [20]. Single-agent proteasome inhibitor therapy seldom yields sustained responses in solid tumors; however, combinations with chemotherapy, targeted agents, or immunotherapy demonstrate improved efficacy [6, 13, 16, 20].

8.2. Safety and Toxicity Profiles

Safety and tolerability are essential factors for the clinical translation of proteasome inhibitors in cervical cancer. Carfilzomib exhibits a distinct toxicity profile relative to bortezomib, characterized by diminished peripheral neuropathy and heightened cardiovascular toxicity [9]. Cardiovascular adverse events, such as heart failure, hypertension, and arrhythmias, have been documented with carfilzomib and necessitate vigilant monitoring [9].

Preclinical studies utilizing cervical cancer models have generally indicated satisfactory tolerability of proteasome inhibitors. Zhang et al. observed that marizomib, both as a monotherapy and in conjunction with cisplatin, was well-tolerated in xenograft-bearing mice, exhibiting no significant weight loss or overt toxicity [6]. However, extrapolating from preclinical to clinical toxicity necessitates caution, as murine models may not entirely replicate human toxicity profiles.

The toxicity profile of proteasome inhibitors may be affected by their combination partners. Bi et al. determined that concurrent inhibition of HDAC and proteasome was generally well-tolerated in gynecologic cancer models, although some toxicity was noted at elevated doses [12]. For combination regimens to be developed for use in the clinic, it will be important to carefully choose the right dose and schedule.

New formulations and delivery systems might make it possible to make proteasome inhibitors more effective. Wu et al. showed that tumor-bearing mice could handle TiN nanoshell-encapsulated carfilzomib combined with photothermal therapy well [1]. Targeted delivery methods that increase tumor accumulation while lowering systemic exposure could help with toxicity issues.

8.3. Patient Selection and Biomarkers

Finding predictive biomarkers will be very important for choosing the best patients for future clinical trials of proteasome inhibitors for cervical cancer. Several prospective biomarkers merit examination based on mechanistic insights and preclinical evidence.

The levels of proteasome activity may be able to tell if someone is sensitive to proteasome inhibitors. Oron et al. discovered that elevated HSPA1A/B mRNA expression, indicative of reduced proteasome activity, was linked to unfavorable outcomes in cancer patients [29]. This indicates that tumors exhibiting elevated baseline proteasome activity may exhibit increased susceptibility to proteasome inhibition.

The status of p53 is another possible biomarker. Most cervical cancers use HPV E6 to break down p53, but blocking the proteasome can bring p53 back to life [13], [16]. Tumors possessing intact p53 pathway components downstream of p53 may exhibit a heightened response to proteasome inhibitor-induced p53 stabilization [13, 16].

The expression of Bcl-2 family proteins may serve as a predictor for sensitivity to apoptosis induced by

proteasome inhibitors. Tumors exhibiting elevated Bcl-2/Bcl-xL expression and diminished pro-apoptotic protein expression may exhibit heightened sensitivity to proteasome inhibitors that promote apoptosis [8], [13].

Autophagy markers may help find patients who would benefit from combination therapies that target both proteasome and autophagy. Bi et al. showed that starting autophagy predicts resistance to both HDAC and proteasome inhibition, which means that the level of autophagy at the start of treatment or during treatment could help choose the best combination therapy [12].

9. Future Directions and Emerging Strategies

9.1. Next-Generations Proteasome Inhibitors

The development of next-generation proteasome inhibitors with enhanced selectivity, potency, and pharmacological characteristics is ongoing. Rahimi examined the progression and diversification of proteasome inhibitors, emphasizing strategies such as isoform-selective inhibitors, inhibitors aimed at novel binding sites, and agents with enhanced solid tumor penetration [9].

Isoform-selective inhibitors that preferentially target immunoproteasome subunits ($\beta 1i$, $\beta 2i$, $\beta 5i$) instead of constitutive proteasome subunits may be beneficial in certain cancer scenarios and could mitigate toxicity to normal tissues [2], [9]. Kraus et al. showed that LU-102, a $\beta 2$ -selective inhibitor, works with bortezomib and carfilzomib to get around proteasome inhibitor resistance in myeloma cells [24]. Similar selective inhibitors might be beneficial in cervical cancer, especially for addressing resistance.

Oral proteasome inhibitors like ixazomib are easier to use and may allow for long-term dosing schedules that could be helpful for solid tumors [4], [9]. Creating oral forms of stronger inhibitors like carfilzomib could make them more useful in medicine.

9.2. PROTACs and Targeted Protein Degradation

Proteolysis-targeting chimeras (PROTACs) are a new way to break down specific proteins that uses the ubiquitin-proteasome system [5], [11], [14]. Traditional proteasome inhibitors stop the proteasome from working, but PROTACs only target certain proteins for ubiquitination and proteasomal degradation [5, 11, 14].

Movahed et al. examined PROTACs in gynecological cancers, emphasizing their capacity to address the shortcomings of existing therapies, such as drug resistance and off-target effects [5]. PROTACs can target proteins that can't be treated with traditional small molecule inhibitors because they don't have the right binding pockets [5]. The catalytic mechanism of PROTACs, which allows one PROTAC molecule to break down many target protein molecules, could make them more powerful [5].

Numerous PROTAC investigations have focused on cervical cancer. Shi et al. created a cyclic peptide-based PROTAC that breaks down palmitoyltransferase DHHC3 and lowers the amount of PD-L1 in cervical cancer cells [11]. This PROTAC improved the effectiveness of cisplatin and boosted immune responses that fight tumors [11]. Gunasekaran et al. created an N-degron-based PROTAC (NC1) that targets PLK1. This PROTAC successfully removed PLK1 protein, stopped cells from moving from G2 to M phase, and killed cancer cells in cervical cancer xenografts [14].

The combination of PROTACs and proteasome inhibitors is an interesting but possibly complicated strategy. Although PROTACs necessitate operational proteasomes for the degradation of target proteins, temporary proteasome inhibition could potentially augment PROTAC-mediated target engagement or alter compensatory pathways. This area needs to be looked into very carefully.

9.3. Personalized Medicine Approaches

The variability of cervical cancer, both among patients and within specific tumors, requires personalized medicine strategies for effective proteasome inhibitor therapy. Li et al. examined the function of ubiquitination in drug resistance within gynecological cancers, highlighting the intricate relationship between ubiquitination and chemoresistance [10]. This complexity indicates that universal solutions are improbable to succeed.

Combining multi-omic profiling (genomics, transcriptomics, proteomics, and metabolomics) could help find groups of patients who would benefit the most from proteasome inhibitor therapy. Profiles of proteasome subunit expression, changes in UPS components, and baseline proteasome activity levels are all possible biomarkers for stratification [2], [3], and [10].

Patient-derived organoids (PDOs) and xenografts (PDXs) serve as robust platforms for individualized pharmacological assessment. Bi et al. employed patient-derived organoids to elucidate autophagy-mediated resistance to concurrent HDAC and proteasome inhibition [12]. Similar methodologies may be utilized to forecast individual patient responses to carfilzomib-based regimens and enhance combination strategies.

Adaptive clinical trial designs that include real-time biomarker assessment and treatment changes based on early response indicators could speed up the search for effective proteasome inhibitor regimens for cervical cancer.

Combining imaging biomarkers, functional assays, and monitoring of circulating tumor DNA (ctDNA) could make it possible to change treatments on the fly.

10. Conclusion

Inhibiting the proteasome is a promising treatment for cervical cancer because it targets some of the disease's biggest weaknesses, such as the way HPV messes up the ubiquitin-proteasome system, the need for proteasome activity for rapid growth, and the ability to trigger apoptosis by restoring the p53 pathway. There isn't much direct clinical evidence for carfilzomib in cervical cancer yet, but preclinical studies with other proteasome inhibitors show that they can kill tumors in a number of ways, such as by causing apoptosis, stopping the cell cycle, and making cells more sensitive to chemotherapy.

Carfilzomib's irreversible binding mechanism and good selectivity profile give it theoretical advantages over first-generation proteasome inhibitors like bortezomib. Preclinical data from cervical cancer models utilizing delanzomib, marizomib, and bortezomib demonstrate proof-of-concept for the efficacy of proteasome inhibitors in this malignancy [6], [13], [15], [16]. Combination strategies, especially those involving platinum-based chemotherapy, targeted therapies, and immunomodulatory agents, exhibit increased efficacy and signify the most promising direction for future development.[6], [13], [15], [16], [20].

To fully realize the clinical potential of carfilzomib in cervical cancer, several challenges must be overcome. Resistance mechanisms, such as proteasomal adaptations, autophagy induction, and the activation of compensatory survival pathways, necessitate combinatorial strategies that target multiple vulnerabilities [2], [3], [4], [12], [29]. New ways to deliver drugs, like nanoparticle formulations, might help target tumors better and lower the risk of systemic toxicity [1]. Choosing patients based on predictive biomarkers will be important for finding the people who are most likely to benefit from proteasome inhibitor therapy.

References

- [1]. Wu, X., Wang, L., Xu, Y. N., Chen, J. L., Luo, K. Q., Yuan, M. H., ... & Jiang, R. (2022). Chemo-Phototherapy with carfilzomib-encapsulated TiN nanoshells suppressing tumor growth and lymphatic metastasis. *Small*, 18(29), 2200522.
- [2]. Carmony, K. C. (2016). *Elucidating proteasome catalytic subunit composition and its role in proteasome inhibitor resistance*. University of Kentucky.
- [3]. Kim, K. B. (2021). Proteasomal adaptations to FDA-approved proteasome inhibitors: a potential mechanism for drug resistance?. *Cancer Drug Resistance*, 4(3), 634.
- [4]. Bennett, M. K., Pitson, S. M., & Wallington-Beddoe, C. T. (2021). Mechanisms Driving Resistance to Proteasome Inhibitors Bortezomib, Carfilzomib, and Ixazomib in Multiple Myeloma. In *Resistance to Targeted Therapies in Multiple Myeloma* (pp. 39-59). Cham: Springer International Publishing.
- [5]. Movahed, F., Ourang, Z., Neshat, R., Hussein, W. S., salih Saihood, A., shallan Alarajy, M., & Zareii, D. (2024). PROTACs in gynecological cancers: Current knowledge and future potential as a treatment strategy. *Pathology-Research and Practice*, 263, 155611.
- [6]. Zhang, Z., Zhang, S., Lin, B., Wang, Q., Nie, X., & Shi, Y. (2022). Combined treatment of marizomib and cisplatin modulates cervical cancer growth and invasion and enhances antitumor potential in vitro and in vivo. *Frontiers in Oncology*, 12, 974573.
- [7]. Chen, X., Wu, X., Li, L., & Zhu, X. (2024). Development of proteasome inhibitors for cancer therapy. *International Journal of Drug Discovery and Pharmacology*, 100004-100004.
- [8]. Soni, S., Anang, V., Zhao, Y., Horowitz, J. C., Nho, R. S., & Mebratu, Y. A. (2025). A new era in cancer therapy: targeting the Proteasome-Bcl-2 axis. *Journal of Experimental & Clinical Cancer Research*, 44(1), 246.
- [9]. Rahimi, N. (2025). The Evolution and Diversification of Proteasome Inhibitors in Cancer and Beyond.
- [10]. Li, Y., Chen, Z., & Wu, Y. (2024). Unraveling role of ubiquitination in drug resistance of gynecological cancer. pmc. ncbi. nlm. nih. govL Yu. *Z Chen, Y Wu, M Xu, D Zhong, H Xu, W Zhu.American Journal of Cancer Research*, 2523-2537.
- [11]. Shi, Y. Y., Dong, D. R., Fan, G., Dai, M. Y., & Liu, M. (2023). A cyclic peptide-based PROTAC induces intracellular degradation of palmitoyltransferase and potentially decreases PD-L1 expression in human cervical cancer cells. *Frontiers in immunology*, 14, 1237964.

- [12]. Bi, J., Zhang, Y., Malmrose, P. K., Losh, H. A., Newton, A. M., Devor, E. J., ... & Leslie, K. K. (2022). Blocking autophagy overcomes resistance to dual histone deacetylase and proteasome inhibition in gynecologic cancer. *Cell death & disease*, 13(1), 59.
- [13]. Sun, C., Meng, X., Cui, X., Liang, S., Sun, J., Zhang, B., ... & Chen, Y. (2025). APG-115 synergizes with bortezomib to induce apoptosis in cervical cancer cells. *Anti-Cancer Drugs*, 10-1097.
- [14]. Gunasekaran, P., Shin, S. C., Hwang, Y. S., Lee, J., La, Y. K., Yim, M. S., ... & Bang, J. K. (2025). N-Degron-Based PROTAC Targeting PLK1: A Potential Therapeutic Strategy for Cervical Cancer. *Pharmaceutics*, 17(8), 1027.
- [15]. Meng, X., Cui, X., Shao, X., Liu, Y., Xing, Y., Smith, V., ... & Chen, Y. (2022). poly (I: C) synergizes with proteasome inhibitors to induce apoptosis in cervical cancer cells. *Translational Oncology*, 18, 101362.
- [16]. Guo, K. Y., Han, L., Li, X., Yang, A. V., Lu, J., Guan, S., ... & Zhang, H. (2017). Novel proteasome inhibitor delanzomib sensitizes cervical cancer cells to doxorubicin-induced apoptosis via stabilizing tumor suppressor proteins in the p53 pathway. *Oncotarget*, 8(69), 114123.
- [17]. Wang, H., Yang, Q., Dou, Q. P., & Yang, H. (2018). Discovery of natural proteasome inhibitors as novel anticancer therapeutics: Current status and perspectives. *Current Protein and Peptide Science*, 19(4), 358-367.
- [18]. Lee, H. Y., Kim, J. Y., Wang, Z., Amornphimoltham, P., Gutkind, J. S., & Jeong, W. J. (2025). Noncanonical Pathways of Proteasome Inhibition in HPV-Negative Head & Neck Cancer. *Molecular Carcinogenesis*, 64(11), 1838-1850.
- [19]. Nagasaka, K. (2024). Molecular Target Drug for Cervical Cancer. In *Recent Topics on Prevention, Diagnosis, and Clinical Management of Cervical Cancer* (pp. 217-230). Singapore: Springer Nature Singapore.
- [20]. Chao, A., & Wang, T. H. (2016). Molecular mechanisms for synergistic effect of proteasome inhibitors with platinum-based therapy in solid tumors. *Taiwanese Journal of Obstetrics and Gynecology*, 55(1), 3-8.
- [21]. Li, J., Pohl, L., Schüller, J., Korzeniewski, N., Reimold, P., Kaczorowski, A., ... & Duensing, S. (2021). Targeting the proteasome in advanced renal cell carcinoma: complexity and limitations of patient-individualized preclinical drug discovery. *Biomedicines*, 9(6), 627.
- [22]. Zulkifli, A., Tan, F. H., Areeb, Z., Stuart, S. F., Gomez, J., Paradiso, L., & Luwor, R. B. (2021). Carfilzomib promotes the unfolded protein response and apoptosis in cetuximab-resistant colorectal cancer. *International Journal of Molecular Sciences*, 22(13), 7114.
- [23]. Chen, L., Shi, H., Zhang, W., Zhu, Y., Chen, H., Wu, Z., ... & Li, Q. (2024). Carfilzomib suppressed LDHA-mediated metabolic reprogramming by targeting ATF3 in esophageal squamous cell carcinoma. *Biochemical Pharmacology*, 219, 115939.
- [24]. Kraus, M., Bader, J., Geurink, P. P., Weyburne, E. S., Mirabella, A. C., Silzle, T., ... & Driessen, C. (2015). The novel β 2-selective proteasome inhibitor LU-102 synergizes with bortezomib and carfilzomib to overcome proteasome inhibitor resistance of myeloma cells. *Haematologica*, 100(10), 1350.
- [25]. Rausch, J. L., Ali, A. A., Lee, D. M., Gebreyohannes, Y. K., Mehalek, K. R., Agha, A., ... & Duensing, A. (2020). Differential antitumor activity of compounds targeting the ubiquitin-proteasome machinery in gastrointestinal stromal tumor (GIST) cells. *Scientific Reports*, 10(1), 5178.
- [26]. Thompson, R. M., Dytfeld, D., Reyes, L., Robinson, R. M., Smith, B., Manevich, Y., ... & Dolloff, N. G. (2017). Glutaminase inhibitor CB-839 synergizes with carfilzomib in resistant multiple myeloma cells. *Oncotarget*, 8(22), 35863.
- [27]. Bi, J., Zhang, Y., Malmrose, P. K., Losh, H. A., Newton, A. M., Devor, E. J., ... & Leslie, K. K. (2022). Blocking autophagy overcomes resistance to dual histone deacetylase and proteasome inhibition in gynecologic cancer. *Cell death & disease*, 13(1), 59.
- [28]. Zhang, X., Linder, S., & Bazzaro, M. (2020). Drug development targeting the ubiquitin-proteasome system (UPS) for the treatment of human cancers. *Cancers*, 12(4), 902.
- [29]. Oroń, M., Grochowski, M., Jaiswar, A., Legierska, J., Jastrzębski, K., Nowak-Niezdoda, M., ... & Walerych, D. (2021). The response network of HSP70 defines vulnerabilities in cancer cells with the inhibited proteasome.
- [30]. Tanaka, Y., Okabe, S., Ohyashiki, K., & Gotoh, A. (2022). Potential of a sphingosine 1-phosphate receptor antagonist and sphingosine kinase inhibitors as targets for multiple myeloma treatment. *Oncology Letters*, 23(4), 111.