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Mechanisms and Applications of AZD6738 on Breast Cancer

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Abstract. Breast cancer (BC) is a cancer that is prevalent in women. However, the heterogeneity of BC in patients, especially triple-negative breast cancer (TNBC), leads to cases with poor outcomes, which requires more efficient treatment approaches. AZD6738, also known as ceralasertib, a selective inhibitor of Ataxia telangiectasia and Rad3-related (ATR) kinase, can be used for cancer treatment. In clinical practice, it is often used in combination with other drugs or chemotherapy to enhance treatment efficacy. ATR kinase is activated during DNA damage, affecting the cell cycle and responding to DNA replication stress (RS) by activating CHK1 and a series of downstream reactions. AZD6738 can block the ATR pathway by inhibiting the activation of CHK1, thereby inducing cancer cell apoptosis. Moreover, researchers conducted clinical studies on AZD6738 and explored the efficacy of its combination therapy with other drugs, such as PARP inhibitor olaparib. This review focuses on the structure and function of AZD6738, as well as its applications in TNBC clinical trials. Future studies of AZD6738 are required to further explore the mechanisms and clinical applications in the treatment of breast cancer and other cancers.

Keywords: AZD6738, breast cancer, Ataxia telangiectasia and Rad3-related (ATR) kinase, clinical trials.

1. Introduction

Breast cancer (BC) is a common kind of cancer. According to statistics, in 2020, there reported about 2.3 million new cases of female BC, making it the highest incidence category of cancer in the world, accounting for 11.7% in all cancer cases [1]. Among women, BC accounts for about a quarter of all cancer cases, posing a significant threat to women's health. At the same time, it is the fifth leading cause of cancer-related death globally, with one in six deaths from cancer being due to BC. The incidence rate and mortality rate of breast cancer are ranked first in many countries in the world [1]. Therefore, the effective treatment of breast cancer is very important for society.

Rad3-related (ATR) is a tumor suppressor that maintains the genomic stability in normal cells. Activated by DNA damage induced during DNA replication-associated stress. Critical roles in DNA repair, cell-cycle progression, and survival [2]. AZD6738 is an ATR inhibitor that competitively inhibits ATR kinase with a high selectivity. It inhibits DNA damage repair, thus promoting the apoptosis of cancer cells. It also works with drugs such as olaparib (causes replication fork stalling and collapse) and gemcitabine as combined therapies for cancer [3].

This review summarizes the general mechanism of action and partial effects of AZD6738 in tumor treatment. This review explains the molecular mechanism of ATR, the characteristics of AZD6738, and clinical studies using AZD6738 to treat breast cancer.

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2. ATR

Some errors arise during DNA replication, collectively called DNA replication stress (RS), which leads to genome instability and leading to cancer or apoptosis. Cellular response to replication stress cincludes 2 pathways. One of them is the activation of DNA damage tolerance pathway and the other is activation of the ataxia telangiectasia mutated (ATM)- and ATR pathway [2].

ATR is a kinase that can phosphorylate serine/threonine residues of proteins, which is upregulated in multiple cancer cell types and participates in the coordination of DNA damage response (DDR) and cell cycle checkpoints caused by stress related to DNA replication.

Genomic damage exposes single-stranded DNA (ssDNA). At the stopped fork, replicating protein A (RPA) binds to ssDNA, which then aggregates ATR via ATRIP protein [2]. The recruited ATR-ATRIP complex and the two ATR-activating proteins (TOPBP1 and ETAA 1) work together. DNA topoisomerase 2-binding protein 1 (TOPBP1) then activates ATR [2]. The activated ATR kinase further phosphorylates to activate CHK 1. CHK 1 inhibits the activation of cyclin-dependent kinase 2 (CDK 2) and then causes cell cycle during S-G2 phase, as a result, slower DNA replication in S phase [2]. In addition, CHK 1 is able to limit mitosis by phosphorylating cyclin-dependent kinase 1 (CDK 1). Through the above pathway, ATR regulates the G2 / M cell cycle checkpoint after DNA damage, delaying cell cycle progression to recover from DNA damage and stalled replication forks.

The mechanism of ATR at different periods of the cell cycle. When ATR is activated in s-phase cells, it hinders initiation of replication initiation and limits DNA synthesis, whereas activation in G2 cells promotes arrest of the cell cycle at the G2 / M transition [4].

In preclinical models, targeting the ATR showed antitumor activity can be used. Currently, there are several ATR inhibitors (ATRi) in clinical development and will be used as anticancer drugs in the treatment of BC in the future.

3. Structure and function of AZD6738

AZD6738 (PubChem CID 54761306), also known as Ceralasertib, is an oral ATR kinase inhibitor based on morpholin-pyrimidine. Figure 1 shows the chemical structure of AZD6738. By targeting the inhibition of ATR, it acquires potential antitumor activity. Studies showed that AZD6738 can block the phosphorylation of the protein kinase CHK 1 to selectively inhibit ATR activity. This subsequently inhibits the activation of DNA damage checkpoints and DNA damage repair and induces apoptosis in tumor cells [3]. In addition, AZD6738 can make tumor cells more sensitive to chemotherapy and radiotherapy [3].

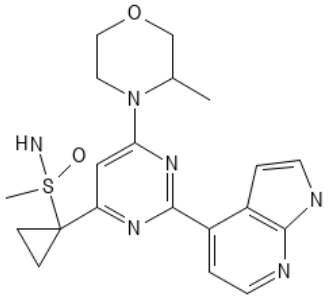

Figure 1. Chemical structure of AZD6738.

4. Application of AZD6738

Monotherapy treatment with AZD6738 has been shown to reduce the rate of tumor growth [5]. The combined efficacy of AZD6738 and some drugs was also observed. AZD6738 is commonly used in cotreatment with drugs such as carboplatin and irinotecan, which are associated with replication fork cessation and collapse, or the PARP inhibitor olaparib. For example, AZD6738 can achieve better therapeutic effects by enhancing olaparib activity. Moreover, combination therapy requires fewer drug doses than monotherapy. Table 1 summarizes the application of AZD6738 on breast cancer.

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4.1. In vitro and in vivo studies

In an *in vitro* study, human BC cell lines MDA-MB-231 (TNBC cell line) and MCF-7 (hormone receptor-positive breast cancer) were used to verify that DNA repair inhibitors were more conducive to the treatment of BC than single-dose radiotherapy [6]. All experiments were conducted with cells in the logarithmic growth phase and AZD0156 and AZD6738 were used as targets. Olaparib was used for PARP inhibition [6]. This experiment uses colony formation assays to evaluate cell survival rates after radiotherapy, DDR inhibitors, or combination therapy. The simultaneous use of a single dose or fractional ionizing radiation (IR) treatment allows for the evaluation of cell survival and sublethal injury repair [7]. Through flow cytometry, researchers analyze the cell cycle based on the distribution of DNA content in cells. Meanwhile, immunofluorescence microscopy will also be used to evaluate the kinetics of DNA double strand break repair [6]. The experimental results showed that all inhibitors exhibited significant radiosensitizing effects, and the effect after fractional IR is significantly greater than that of single-dose IR. More unrepaired DNA double strand breaks were caused by these inhibitors 24 hours after IR [6].

Cyclin E is a type of protein used to drive G1/S conversion, which binds to CDK2 and exerts its function. Low molecular weight cyclin E (LMW-E) is a post-translational modification subtype of LMW-E, which is mainly detected in tumor tissues and exists in many breast cancer [8]. In a clinical trial using immortalized human mammalian epithelial cell lines (hMECs) 76NE6 and 76NF2V, inducible 76NE6-EKO-LMWE cells were cultured in medium containing DMSO (Dox-) or 100ng/mL doxycycline (Dox+, inducing LMW-E expression) for 24 hours. Then RAD51 inhibitor B02, CHK1 inhibitor rabusertib, and ATR inhibitor AZD6738 were added to the medium [8]. After 96 hours of inhibitor exposure, cells were measured and the half maximal inhibitory concentration (IC50) of each inhibitor was calculated [8]. The data showed that compared to uninduced cells, the half maximal inhibitory concentrations (IC50) of B02 (37%), rabusertib (84%), and AZD6738 (75%) were significantly reduced in LMW-E expressing inducible 76NE6-EKO cells. At the same time, it was found that the expression of RAD51 decreased and the level of cleaved PARP (a cell apoptosis marker) increased [8]. These results indicate that LMW-E expression increases sensitivity to drugs targeting the ATR-CHK1-RAD51 pathway. This provides a potential therapeutic strategy for breast cancer cells overexpressing LMW-E [8].

4.2. Clinical trials

Clinical studies have shown that daily AZD6738 coincides with olaparib in a BRCA mutant TNBC xenograft model. As a drug therapy, AZD6738 has potential capabilities in the treatment of BC. A study suggests that if AZD6738 is administered simultaneously with carboplatin daily, tumor regression takes at least 2 days, while after irinotecan, it needs to be administered twice a day [7]. In the TNBC xenotransplantation model derived from BRCA2 mutation patients, AZD6738 and Olapanide were administered three to five days a week at the same time to make the tumor eliminated completely. Even in the TNBC xenograft model with BRCA wild-type, increasing the dose of olaparib or AZD6738 to twice a day can completely regress the tumor [7]. Thus, the ATR inhibitor AZD6738 not only acts as a monotherapy but also has anti-tumor activity when combine it with chemotherapy aswellas olaparib(the PARP inhibitor).

In a single group phase II study targeting TNBC patients, researchers attempted drug therapy for some TNBC patients [9]. The treatment consists of 300 milligrams of olaparib twice a day and 160 milligrams of celecoxib from day 1 to day 7, with a cycle of 28 days, until the disease progresses. The evaluation criteria are objective response rate (ORR). Among them, 10 patients (14%) had germline BRCA1/2 mutations. Additionally, 3 patients (4%) were found to have somatic BRCA mutations [9]. The ORR is 12 out of 70, corresponding to 17.1% (95% confidence interval, 10.4-25.5). Patients who do not have somatic or germline BRCA1/2 mutations had a response observed in the experimental results, including those patients who have other gene mutations caused by homologous recombination repair in RAD51 lesions and tumors with defects that are functional homologous recombination [9]. Contrary to the predetermined activity criteria, the reaction rates of olaparib and AZD6738 did not meet

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the expected values in the entire experimental population. However, signs of response were observed in the experimental population who did not respond to Olaparib monotherapy as expected [9].

In addition, there is evidence to suggest that when faced with the combined effects of PARP inhibitors as well as inhibitors of DNA damage response kinase like ATR inhibitors, patients with TNBC, ATM deficient, or BRCA mutations cancers may have increased sensitivity, which can be observed in preclinical models.

Table 1. Applications of AZD6738 in breast cancer.

Model	Treatment	Results	Conclusions	Ref
Human breast cancer cells (TNBC and hormone receptor positive breast cancer)	AZD0156, AZD6738 and Olaparib	Processed cells are more sensitive to ionizing radiation	Provided preclinical evidence for the role of AZD0156, AZD6738, and olaparib as radiosensitizers	[6]
Human patient derived tumor explant (PDX) model in immunodeficient mice	AZD6738, Carboplatin, Irinotecan, Olaparib	The tumor was regressed	Provided a theoretical basis for the clinical evaluation of the use of only AZD6738 as a therapeutic agent or combination therapy	[7]
Immortal human mammary epithelial cell line (hMEC)	AZD6738, Rabusertib (CHK1 inhibitor), B02 (RAD51 inhibitor)	LMW-E overexpression of hMECs and significant reduction of breast cancer cell viability	Provided a new treatment strategy for LMW-E overexpression breast cancer	[8]
TNBC patients	Olapari, AZD6738	The results did not meet the predetermined activity criteria. Unexpected reactions were observed in patients who were not originally planned to respond to Olaparib monotherapy.	Identified biomarkers with potential benefits	[9]

5. Conclusion

Breast cancer and other cancers pose a significant threat to human health, and the research and development of AZD6738 drug brings new ideas for cancer treatment. AZD6738 inhibits ATR kinase, thereby suppressing the activation of ATR-mediated DNA damage checkpoints and halting DNA damage repair. Further research can be conducted to better understand the mechanisms of AZD6738. The clinical trial of AZD6738 in breast cancer shows its significance when used together with other treatments, such as drugs and chemotherapy. In addition, it still has shortcomings in some aspects, such as the treatment effect was not as expected in some cases. In the future, in-depth research can be conducted on the different doses of AZD6738 used in combination with different treatment methods to enhance its application in cancer treatment.

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