T.C
REPUBLIC OF TURKEY
HACETTEPE UNIVERSITY
GRADUATE SCHOOL OF HEALTH SCIENCES

EVALUATION OF RECOMBINATIONAL DNA DAMAGE RESPONSE IN CASTRATION-RESISTANT PROSTATE CANCER AND INVESTIGATION OF THE COMBINED EFFECT OF RAD51 AND WEE1 INHIBITORS ON RADIOSENSITIVITY

RABİA ŞEYMA NUR TAŞDEMİR

Biochemistry Program

MASTER OF SCIENCE THESIS

ANKARA

2024

T.C
REPUBLIC OF TURKEY
HACETTEPE UNIVERSITY
GRADUATE SCHOOL OF HEALTH SCIENCES

EVALUATION OF RECOMBINATIONAL DNA DAMAGE RESPONSE IN CASTRATION-RESISTANT PROSTATE CANCER AND INVESTIGATION OF THE COMBINED EFFECT OF RAD51 AND WEE1 INHIBITORS ON RADIOSENSITIVITY

RABİA ŞEYMA NUR TAŞDEMİR

Biochemistry Program

MASTER OF SCIENCE THESIS

SUPERVISOR

Doç. Dr. Oytun Portakal Akçin

ANKARA

2024

EVALUATION OF RECOMBINATIONAL DNA DAMAGE RESPONSE IN CASTRATION-RESISTANT PROSTATE CANCER AND INVESTIGATION OF THE COMBINED EFFECT OF RAD51 AND WEE1 INHIBITORS ON RADIOSENSITIVITY

Rabia Şeyma Nur Taşdemir

Supervisor: Assoc. Prof. Oytun Portakal Akçin

This thesis study has been approved and accepted as a Master dissertation in "Biochemistry Program" by the assesment committee, whose members are listed below, on 14.08.2024

Chairman of the Committee:	*Prof. Dr. İmge Ergüder* *Ankara University Faculty of Medicine* *Department of Biochemistry*
Advisor of the Dissertation:	*Assoc. Prof. Oytun Portakal Akçin* *Hacettepe University Faculty of Medicine* *Department of Biochemistry*
Member:	*Prof. Dr. Mukaddes Gürler* *Hacettepe University Faculty of Medicine* *Department of Biochemistry*
Member:	*Prof. Dr. Yeşim Er Öztaş* *Hacettepe University Faculty of Medicine* *Department of Biochemistry*
Member:	*Prof. Dr. Tuba Tüylü Küçükkılınç* *Hacettepe University Faculty of Pharmacy Department of Biochemistry*

This dissertation has been approved by the above committee in conformity to the relatedissues of Hacettepe University Graduate Education and Examination Regulation.

09 Eylül 2024

Prof. Müge YEMİŞCİ ÖZKAN, MD, PhD

Director

YAYIMLAMA VE FİKRİ MÜLKİYET HAKLARI BEYANI

Enstitü tarafından onaylanan lisansüstü tezimin/raporumun tamamını veya herhangi bir kısmını, basılı (kağıt) ve elektronik formatta arşivleme ve aşağıda verilen koşullarla kullanıma açma iznini Hacettepe Üniversitesine verdiğimi bildiririm. Bu izinle Üniversiteye verilen kullanım hakları dışındaki tüm fikri mülkiyet haklarım bende kalacak, tezimin tamamının ya da bir bölümünün gelecekteki çalışmalarda (makale, kitap, lisans ve patent vb.) kullanım hakları bana ait olacaktır.

Tezin kendi orijinal çalışmam olduğunu, başkalarının haklarını ihlal etmediğimi ve tezimin tek yetkili sahibi olduğumu beyan ve taahhüt ederim. Tezimde yer alan telif hakkı bulunan ve sahiplerinden yazılı izin alınarak kullanılması zorunlu metinlerin yazılı izin alınarak kullandığımı ve istenildiğinde suretlerini Üniversiteye teslim etmeyi taahhüt ederim.

Yükseköğretim Kurulu tarafından yayınlanan ***"Lisansüstü Tezlerin Elektronik Ortamda Toplanması, Düzenlenmesi ve Erişime Açılmasına İlişkin Yönerge"*** kapsamında tezim aşağıda belirtilen koşullar haricince YÖK Ulusal Tez Merkezi / H.Ü. Kütüphaneleri Açık Erişim Sisteminde erişime açılır.

o Enstitü / Fakülte yönetim kurulu kararı ile tezimin erişime açılması mezuniyet tarihimden itibaren 2 yıl ertelenmiştir.(1)
o Enstitü / Fakülte yönetim kurulunun gerekçeli kararı ile tezimin erişime açılması mezuniyet tarihimden itibaren ... ay ertelenmiştir.(2)
o Tezimle ilgili gizlilik kararı verilmişti

09/09/2024

Rabia Şeyma Nur Taşdemir

1–Lisansüstü Tezlerin Elektronik Ortamda Toplanması, Düzenlenmesi ve Erişime Açılmasına İlişkin Yönerge‖

(1) Madde 6. 1. Lisansüstü tezle ilgili patent başvurusu yapılması veya patent alma sürecinin devam etmesi durumunda, tez **danışmanının** önerisi ve **enstitü anabilim dalının** uygun görüşü üzerine **enstitü veya fakülte yönetim kurulu** iki yıl süre ile tezin erişime açılmasının ertelenmesine karar verebilir.

(2) Madde 6. 2. Yeni teknik, materyal ve metotların kullanıldığı, henüz makaleye dönüşmemiş veya patent gibi yöntemlerle korunmamış ve internetten paylaşılması durumunda 3. şahıslara veya kurumlara haksız kazanç imkanı oluşturabilecek bilgi ve bulguları içeren tezler hakkında tez **danışmanının** önerisi ve **enstitü anabilim dalının** uygun görüşü üzerine **enstitü** veya **fakülte yönetim kurulunun** gerekçeli kararı ile altı ayı aşmamak üzere tezin erişime açılması engellenebilir.

(3) Madde 7. 1. Ulusal çıkarları veya güvenliği ilgilendiren, emniyet, istihbarat, savunma ve güvenlik, sağlık vb. konulara ilişkin lisansüstü tezlerle ilgili gizlilik kararı, **tezin yapıldığı kurum** tarafından verilir*. Kurum ve kuruluşlarla yapılan işbirliği protokolü çerçevesinde hazırlanan lisansüstü tezlere ilişkin gizlilik kararı ise, **ilgili kurum ve kuruluşun** önerisi ile **enstitü** veya **fakültenin** uygun görüşü üzerine **üniversite yönetim kurulu** tarafından verilir. Gizlilik kararı verilen tezler Yükseköğretim Kuruluna bildirilir. Madde 7.2. Gizlilik kararı verilen tezler gizlilik süresince enstitü veya fakülte tarafından gizlilik kuralları çerçevesinde muhafaza edilir, gizlilik kararının kaldırılması halinde Tez Otomasyon Sistemine yüklenir.

* Tez **danışmanının** önerisi ve **enstitü anabilim dalının** uygun görüşü üzerine **enstitü** veya **fakülte yönetim kurulu tarafından karar verilir.**

ETHICAL DECLARATION

In this thesis study, I declare that all the information and documents have been obtained in the base of the academic rules and all audio-visual and written information and results have been presented according to the rules of scientific ethics. I did not do any distortion in data set. In case of using other works, related studies have been fully cited in accordance with the scientific standards. I also declare that my thesis study is original except cited references. It was produced by myself in consultation with Doç. Dr. Oytun Portakal Akçin (Evaluation Of Recombinational Dna Damage Response In Castration-Resistant Prostate Cancer And Investigation Of The Combined Effect Of Rad51 And Wee1 Inhibitors On Radiosensitivity, Rabia Şeyma Nur TAŞDEMİR) and written according to the rules of thesis writing of Hacettepe University Institute of Health Sciences.

ACKNOWLEDGEMENT

I would like to thank Hacettepe University Institute of Health Sciences, the head of the Department, the faculty members and students of Biochemistry Department, and Scientific Research Council of Hacettepe University for their support during this thesis study.

I am grateful for all the support of Hacettepe University Faculty of Medicine, Department of Biochemistry; Hacettepe University Faculty of Medicine, Department of Radiation Oncolcogy and Department of Basic Oncology during my master education and my thesis. I would like to thank to Head of the Department of Biochemistry Prof. Dr. Asuman Özkara.

I would like to thank to my supervisor Assoc. Prof. Dr. Oytun Portakal Akçin for all of her support.

I am thankful to Dr. Sibel Gökşen and Prof. Dr. Güneş Esendağlı for their helps during evaluating the results.

I would like to thank radiology staff in Hacettepe University Oncology Hospital for their help during the experiments and Prof. Dr. Gökhan Özyiğit for supervising them.

Special thanks to Dr. Esra Büber, Onur Aktan, and Ateş Kutay Tenekeci for their help and support with their instructions.

My biggest thanks go to my mother Ayşegül Öner for her amazing motherhood and support for all my life, and of course her patience while raising me and my dearie brother Burak Kaan Taşdemir. I would also like to thank my friends that would be a bit long if I wrote all of their names here, for their emotional support during this education. Also, I miss my cat Pıtır with all my heart, who died while I was doing my experiments and not able to go to see her.

Lastly, I want to express my gratitude to our country's founder and father Mustafa Kemal Atatürk. If it was not for him, I would not be able to get education and dream of being a scientist as a woman in my own country. I am forever thankful to his Republic of Türkiye like all the other Turkish women.

ABSTRACT

Taşdemir SN. Evaluation Of Recombinational Dna Damage Response In Castration-Resistant Prostate Cancer And Investigation Of The Combined Effect Of Rad51 And Wee1 Inhibitors On Radiosensitivity. Hacettepe University Graduate School of Health Sciences, Department of Medical Biochemistry, Thesis of Master Degree, Ankara, 2024.
Castration-resistant prostate cancer (mCRPC) is a disease with a poor prognosis. Recently, DNA repair mechanisms have been targeted in new treatment regimens, in particular inpatients carrying mutations in the genes of DNA repair proteins. Rad51 recombinase is the key protein of recombinational repair process. It is one of the proteins targeted in cancer research in recent years. Wee1 is a protein kinase in nuclei, which is a negative regulator of mitosis by inactivating Cdc2-cyclinB1 complex. So it is an important regulatory protein in the G2/M phase of cell cycle. In vitro studies on Wee1 inhibition have intensified in the last decade. The aim of this study was to determine the effectiveness of Rad51 and Wee1 inhibition on radiosensitivity in mCRPC. For this aim, PC-3 cells were expose to ionizing radiation with or without pre-treatment with Rad51 and Wee1 inhibitors. Cell survival was determined by MTT and colony formation assays. The apoptotic rate was measured by flow cytometry method following Annexin V-FITC/PI staining. The synergism between AZD1775 and B02 was investigated by the median-effect method of Chou and Talalay. AZD1775 and B02 are promising radiosensitizing agents for prostate cancer cells both single treatment and in combination. AZD1775 and B02 increases radiosensitivity of PC-3 cells as a single treatment. In this study, it’s observed that combination of AZD1775 and B02 also increased radiosensitivity in PC-3 cells. Combined treatment of AZD1775 and B02’s decreased cell viability more than single treatment. In conclusion, as a single or combined therapy of Rad51 recombinase inhibitor and Wee1 kinase inhibitor may be a novel strategy to improve the clinical outcome in CRPC.

Key words: Wee1 kinase, Rad51, DNA Damage Response, Recombinational DNA Repair, Radiotherapy, Castration-Resistant Prostate Cancer

This thesis was supported by Hacettepe University Scientific Research Coordination Unit. Project No: 20286

ÖZET

Taşdemir SN. Kastrasyona Dirençli Prostat Kanserinde Rekombinasyonel DNA Hasar Yanıtının Değerlendirilmesi ve Rad51 ve Wee1 İnhibitörlerinin Radyosensitivite Üzerindeki Kombine Etkisinin Araştırılması. Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü, Tıbbi Biyokimya Anabilim Dalı, Yüksek Lisans Tezi, Ankara, 2024. Kastrasyon dirençli prostat kanseri (mCRPC), kötü prognoza sahip bir hastalıktır. Son zamanlarda, özellikle DNA onarım proteinlerinin genlerinde mutasyon taşıyan hastalarda, yeni tedavi rejimlerinde DNA onarım mekanizmaları hedeflenmektedir. Rad51 rekombinaz, rekombinasyon onarım sürecinin anahtar proteinidir. Son yıllarda kanser araştırmalarında hedeflenen proteinlerden biridir. Wee1, çekirdeklerde bulunan ve Cdc2-siklinB1 kompleksini inaktive ederek mitozun negatif düzenleyicisi olan bir protein kinazdır. Bu nedenle hücre döngüsünün G2/M fazında önemli bir düzenleyici proteindir. Son on yılda Wee1 inhibisyonu üzerine in vitro çalışmalar yoğunlaşmıştır. Bu çalışmanın amacı, mCRPC'de radyosensitivite üzerinde Rad51 ve Wee1 inhibisyonunun etkinliğini belirlemekti. Bu amaçla PC-3 hücreleri, Rad51 ve Wee1 inhibitörleri ile ön tedavi ile veya olmadan iyonize radyasyona maruz bırakıldı. Hücre sağkalımı MTT ve koloni formasyon analizleriyle belirlendi. Apoptotik oran, Annexin V-FITC/PI boyama sonrasında akış sitometrisi yöntemi ile ölçüldü. AZD1775 ve B02 arasındaki sinerji, Chou ve Talalay'ın medyan etki yöntemi ile araştırıldı. AZD1775 ve B02, hem tek tedavi hem de kombinasyon halinde prostat kanseri hücreleri için umut vadeden radyosensitize edici ajanlardır. AZD1775 ve B02, tek tedavi olarak PC-3 hücrelerinin radyosensitivitesini artırır. AZD1775 ve B02'nin kombinasyonunun da aynı zamanda PC-3 hücrelerinde radyosensitiviteyi artırdığı görüldü. AZD1775 ve B02'nin kombine tedavisi, hücre canlılığını tek tedaviden daha fazla azalttı. Sonuç olarak, Rad51 rekombinasyon inhibitörü ve Wee1 kinaz inhibitörünün tek veya kombine tedavisi, CRPC'de klinik sonucu iyileştirmek için yeni bir strateji olabilir.

Anahtar kelimeler: Wee1 kinaz, Rad51, DNA Hasar Yanıtı, Rekombinasyonel DNA Onarımı, Radyoterapi, Kastrasyona Dirençli Prostat Kanseri

Bu tez Hacettepe Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimince desteklenmiştir. Proje No: 20286

TABLE OF CONTENTS

LIST OF ABBREVIATION

5-FU: 5-fluorouracil
Abs: Absorbance
ADT: Androgen Deprivation Therapy
AP: Apurinic/Apyrimidinic site
APE1: Apurinic/Apyrimidinic Endonuclease
AR: Androgen Receptor
ARv7: Androgen Receptor Variant 7
ASCO: American Society of Clinical Oncology
ATCC: American Type Cell Culture,
ATM: Ataxia Telangiectasia Mutated
ADP: Adenosine Diphosphate
ATP: Adenosine Triphosphate
ATR: Ataxia Telangiectasia and RAD3-related Protein
AUA: American Urological Association
BER: Base Excision Repair
Bp: Base Pair
BRCA1: Breast Cancer Type 1 Susceptibility Protein
CHK1: Checkpoint Kinase 1
CHK2: Checkpoint Kinase 2
CRPC: Catstration Resistant Prostate Cancer
DDR: DNA damage repair
DMEM: Dulbecco's Modified Eagle's Medium
DNA: Deoxyribonucleic Acid
DNA-PKcs: DNA Protein Kinase
DSBs: Double-strand Break
FDA: Food and Drug Administration
ɣH2AX: Phosphorylated H2A Histone Family Member X
H2AX: H2A Histone Family Member X
HR: Homologous Recombination

mCRPC: Metastatic Castration Resistant Prostate Cancer

MMR: Mismatch Repair

Mre11: Meiotic Recombination 11 Homolog A

MTT: (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-tetrazoliumbromide)

NHEJ: Non-homologous End Joining

PARP1: Poly [adenosine diphosphate – ribose] polymerase 1

PCa: Prostate Cancer

PBS: Phosphate Buffer Saline

SSBP: Single Strand Binding Protein

SSB: Single-strand Break

FIGURES

TABLES

1. INTRODUCTION

1.1. Aim

The purpose of this study was to determine the effectiveness of Rad51 and Wee1 inhibitors on radiosensitivity in mCRPC cells, alone or in combination. The study has been carried on in vitro, therefore PC-3 cell line was used because of its' properties of being androgen receptor (AR) and prostate specific antigen (PSA) deficient, p53 defected and androgen-independent proliferation. Rad51 is a protein employed in homolog recombination, which is a DNA repair mechanism. Wee1 is a kinase that controls checkpoint during cell cycle and inhibits DNA damaged cell to go through mitosis. Ionized radiation therapy is commonly used for cancers and it affects through damaging DNA, creating double strand breaks. Since radiotherapy is a commonly used therapy for prostate cancer; enhancing the radiation therapy's effect was aimed by inhibiting Wee1 and Rad51.

The following hypotheses were created and attempted to be proven in this study with prostate cancer cells:

1. Wee1 inhibition may increase susceptibility of the cells to irradiation,
2. Rad51 inhibition may enhance sensitivity of the cells to irradiation,
3. Combination of Wee1 and Rad51 inhibition may further increase susceptibility of the cells to irradiation.

2. GENERAL INFORMATION

2.1. Prostate Cancer

2.1.1. Definition and Incidence

Prostate cancer (PCa) is a specific tumor to prostate tissue, which is a gland of male reproductive system. It is the second most frequent cancer in males worldwide and the third leading cause of cancer-related mortality (1). Studies reported a prevalence of PCa as 59% (CI 48–71%) by age > 79 years; it is increased by an odds ratio of 1.7 (1.6–1.8) per decade (2).

According to the 2018 "Unified Database" of the Public Health Institute in Turkey, the incidence of PCa was reported as 40.3 per 100,000 people and it is the second most common cancer following lung cancer (3). According to the same data, the PCa incidence rate in men based on age was reported to be 88.9 per 100,000 at the age of 55, Therefore, the incidence begins to increase after the age of 50 and peaks between the ages of 70-80.

About 7% of disease is metastatic at diagnosis worldwide (4). Although the new treatment regimen, it is still considered an important health burden for all populations in the world (5).

2.1.2. Etiology

Ethnical backgrounds and geographical areas are important in etiology. In the West, PCa is generally diagnosed at progressive stage (6). Hereditary PCa shows earlier onset 6-7 years with a relative risk of 2.30 for diagnosis (7).

About 15.6% of PCa patients were shown to be pathogenic variants in p53, ATM, CHEK2, BRCA1, BRCA2, HOXB13, MLH1, MSH2, MSH6 and 10.9% of them

in DNA repair genes (8). In addition, 11.8% of metastatic PCa patients and 16.2% of mCRPC patients had germline mutations in DNA-repair processing genes (9).

Increasing age, having genetic mutations and a family history were reported as risk factors for development of prostate cancer (4-6). Additionally, coffee, lycopene, phytoestrogen, soy food and 5-alpha-reductase inhibitors were reported as negative correlators with prostate cancer development (6-9).

2.2. Castration-Resistant Prostate Cancer (CRPC)

Castration-resistant prostate cancer (CRPC) can be defined as disease progression despite medical or surgical castration. It is the development of resistance to ADT and AR blockade. The term CRPC was first defined by the Prostate Cancer Working Group-2 (PCWG2) in 2008 (10). Androgen axis and androgen receptor (AR) variants play an important role in the development of CRPC. The European Association of Urology (11) PCWG 2 and RECIST 1.1 criteria (12) for CRPC are based on biochemical and/or clinical progression; serum testosterone should be below 50 ng/mL (13).

Three consecutive increases in plasma PSA levels (at least one week apart resulting in two 50% increases and a PSA > 2 ng/mL) and the presence of new lesions (bone lesions on bone scan or a soft tissue lesion using RECIST (Response Evaluation Criteria in Solid Tumors) are defined as progression parameters (12,13). More than 84% of patients are estimated to display metastatic disease at diagnosis of CRPC (13) Metastatic CRPC is a poor disease with a short overall survival rate.

2.2.1. Mechanisms for CRPC

a. Mutations on Androgen Receptor

A 10-20% of cases have been reported to have mutations, which are mostly localized in ligand binding domain (LBD). This leads to a decrease in androgen binding to the receptor. Also, it leads to a decrease in binding of AR antagonist agents to the

receptor and cause the resistance (14-16). Splicing mutations of AR such as AR-V7, AR4 and AR5 are also common in CRPCs, which leads to lack of LBD. Therefore, those activates AR pathways. Overexpression of splice variant ARV7 has been reported to be associated with poor prognosis in CRPC (17). Translocation mutations of AR have been associated with early-onset, very aggressive prostate cancer (18). It has been reported that point mutations in AR may lead to signaling of the receptor by non-androgen steroids (19).

b. Amplification of androgen receptor

Androgen receptor (AR) amplification has been detected in 30-80% of CRPC cell lines (20), this makes androgen receptors hypersensitive to low androgen levels (21-24). It is common in patients who underwent ADT; however, histone acetylation, DNA methylation and also miRNAs may lead to AR overexpression in CRPC (25, 26). It was also reported that AR may amplified independent to ADT response (27)

c. Activation or repression of androgen receptor

The change in co-activators and co-repressors in AR signaling, may lead to CRPC by increasing survive of tumor cells during ADT. (28, 29)

d. Other factors

A polymorphism in coding gene of 5-reductase enzyme in CRPC has been reported; this causes androgen retention within the prostate (30).

Growth factors, such as insulin-like growth factor-I (IGF-I), epidermal growth factor (EGF) may stimulate AR transcription activity in the absence of androgens. IGF1 also facilitate AR translocation. Cytokines, such as IL-6 and IL-8 may increase AR activation without ligand-binding (31,32).

Other signaling pathways such as receptor tyrosine kinase (RTKs), Akt pathway may lead to cellular growth and proliferation in CRPC (33) it was shown that

HER2/ERBB2 are overexpressed in CRPC to regulate AR signaling in low-androgen-concentrations (34-36).

2.3. Diagnosis of CRPC

2.3.1. Serum prostate specific antigen (PSA) assay

Diagnosis of CRPC based on serum prostate-specific antigen (PSA) measurement along with digital rectal examination (DRE), and MRI. Serum PSA measurement is an essential test. Biopsy is required for confirmation. (37-39)

2.3.2. Genetic testing

Somatic mutations in prostate epithelial cell are important for PCa development. Germline testing including cancer-related genes such as p53, RB1, PTEN, BRCA1, BRCA2, ATM, CHEK2, MLH1, MSH2, MSH6, PMS2 is performed for both diagnosis and prediction of recurrence and metastasis (36-39).

2.3.3. Magnetic resonance imaging (MRI)

MRI was more sensitive method that is recommended for pre biopsy of PCa. It is recommended for any individual with a PSA >3 ng/mL to discriminate from benign hyperplasia, with repeating serum PSA and DRE. Magnetic resonance imaging (MRI) is also used to scan bone metastasis and recurrence of disease (20,35).

2.3.4. PSMA-PET

Since F-FDG application is not successful method because of the low glucose usage of prostate cancer cells, the PSMA PET method has come into use. In this method, the antigen glutamate carboxypeptidase II expressed on PCa cell membrane is radioactively identified. Thus, it is beneficial for lymph nodes, distant organ metastasis, and recurrence (4, 5, 21).

2.3.5. Biopsy

Image-guided biopsy is the recommended method for prostate biopsy. Biopsy, Gleason scoring and staging are gold standard for diagnosis. Adenocarcinoma is the most common type of PCa. Adenocarcinoma can transform into neuroendocrine tumor which is aggressive tumor and resistant to treatments (40-42).

2.4. Treatment Regimens for CRPC

Since the rate of localized disease at diagnosis is high (72-73%), curative treatment options are possible including radical prostatectomy, brachytherapy and external beam radiotherapy (EBRT). Metastasis is observed in 7% of patients at diagnosis. Biochemical recurrence develops in 30-40% of patients after surgery and radiotherapy (1, 3-6). Most of these patients become resistant to ADT and AR blockade; the disease progresses to metastatic castration-resistant prostate cancer (mCRPC) in 18-36 months. The long-term prognosis of mCRPC is poor, OS is short (43,44).

2.4.1. Androgen Deprivation Therapy (ADT)

National Comprehensive Cancer Network (NCCN), European Association of Urology (EAU), and the American Urological Association (AUA) guidelines recommend to maintain androgen deprivation therapy for CRPC patients. ADT leads to medical castration by using LHRH (GnRH) agonists (leuprolide) or LHRH antagonists (degarelix) that inhibits testosterone synthesis by blockage LH secretion (3, 44-47). Today ADT is recommended to use in combination with androgen synthesis inhibitors (such as abiraterone acetate) or antiandrogen therapies (such enzalutamide) or chemotherapies (docetaxel) (48).

2.4.2. Chemotherapy

It contains taxane-based therapy, including docetaxel and cabazitaxel, which lead to mitotic arrest and apoptotic cell death. The former is the first-line therapy and

Docetaxel plus Prednisone is the standard of care for mCRPC, and upon Docetaxel failure.

Cabazitaxel is preferred as a second-line therapy to overcome docetaxel resistance. (49-51). Mitoxantrone, approved by the FDA, for CRCP treatment was more succeed than prednisone therapy (3,52). Now, other platinum-derived drugs, such as cisplatin, and carboplatin, are evaluated clinically (53) (Figure 2.1.).

Figure 2.1: Treatment agents for CRPC on a time scale

2.4.3. Androgen-receptor signaling inhibitors

Androgen receptor (AR) signaling inhibitors contains Abiraterone acetate, enzalutamide and apalutamide, which are called ARPI. Those are generally preferred to use in asymptomatic, mildly symptomatic patients and in elderly patients with comorbidities, because of their higher tolerability as a first line therapy (4).

2.4.4. Immunotherapy

Sipuleucel-T and checkpoint inhibitors are approved by FDA. Sipuleucel-T, an immune-cell-based vaccine, leads to T-cell immune responses against the PAP antigen leads to an increase in patient survival (54,55) Immune checkpoint inhibitors, pembrolizumab and dostarlimab, inhibit the programmed death-1 pathway (PD-1) by

blocking PD-1 receptor. (56) immunotherapy is preferred to inoperable patients and the CRPC patients with mismatch repair-deficient (dMMR).

2.4.5. Radiological treatment

a. ^{177}Lu-PSMA

Prostate-specific membrane antigen (PSMA) is highly expressed in patients with PCa (57), so PSMA-617 can be used for targeting therapy. ^{177} Lutetium-PSMA-617 was approved by the FDA and EMA for CRPC patients treated with ARPI or taxane-based chemotherapy (58).

b. Radium-223 Dichloride

Radium-223 dichloride (223Ra) is a radioactive molecule that emit four high energy- alpha particles, which leads to DNA damage in cancer cells. 223Ra therapy was approved by FDA for CRPC patients (59,60) It was shown that 223Ra improved OS in bone-metastatic CRPC (61).

c. Bone-targeting agents

Bone metastasis is a major cause of death. Bisphosphonates (Zoledronic acid) as a first line drug for CRPC patients with painful bone, and RANK-L inhibitors have also been used. (62)

d. PARP inhibitors

Poly(ADP-ribose) polymerase (PARP) family, including PARP1 and PARP2, play a role in DNA repair. Those are involved in single-strand break repair of DNA by recruiting related proteins and cell survive. PARP inhibition leads to forming and accumulation of double strand breaks, and result in cell death in the presence of recombinational repair deficiency (63,64).

PARP inhibitors have been developed as a first line drugs for CRPC affecting by synthetic lethality. In this mechanism there is a non-lethal mutation in a single gene of the cells, which are subject to recombinational DNA repair; so further inhibition of PARP activity results in cell death. Two PARP inhibitors, olaparib and rucaparib, have been approved by the FDA, for mCRPC patients carrying mutations in the genes of BRCA for monotherapy. In 2023, the FDA approved PARP inhibitor (olaparib) in combination with abiraterone and prednisone for the initial therapy (65-67).

All of these treatments increase survival rate and comfort of CRPC patients. However, mCRPC is not curable, its prognosis is poor; survival has been reported as 9-13 months (9).

2.5. Ionizing radiation

Ionizing radiation is frequently used to diagnose and treat a variety of illnesses, particularly cancer. Electromagnetic radiation is classified into two types: ionizing and non-ionizing. Nonionizing radiation is defined as having insufficient energy per quantum to generate ionization directly; examples include ultraviolet and visible radiation (69). However, ionizing radiation has enough energy to remove tightly bound electrons from an atom, leading it to become charged or ionized (9).

Radiation can cause cell damage through several ways (Figure 2.2), however DNA damage is the primary cause of tumor cell death. This damage can be caused by radiation's direct action on DNA molecules, which accounts for 30%-40% of lesions, or by the production of free radicals, which damage DNA, accounting for 60%-70% (70). DNA lesions, especially double-strand breaks (DSBs), activate complex and tightly controlled DNA damage response and repair (DRR) processes (71). As an instance, it is reported that when 1 Gy IR exposure to human cell, approximately 20 DSBs are accumulated in G0/G1 phase and 40 DSBs in the G2 phase (Table 2.1) (72).

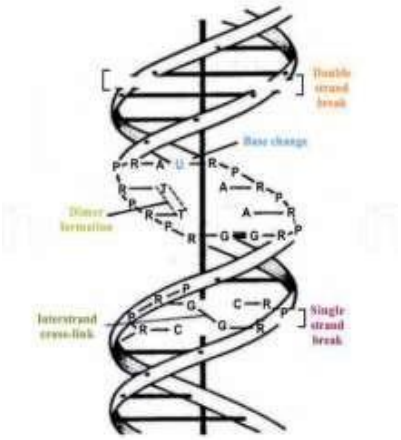


Figure 2.2: Several DNA lesions induced by ionizing radiation (73)

Table 2.1: Estimated numbers of DNA damages in human cell exposed to 1 Gy of ionizing radiation (72)

Type of damage	**Number of per cell per Gy**
Biochemical damage	
Single-strand breaks	600-1000
Hydroxyadenine	700
T* (thymine damage)	250
Sugar damage	800-1600
DNA-double-strand breaks	40
DNA-DNA crosslinks	30
DNA-protein cross links	150

Cellular damage	
Celll death	0.2-0.8
Chromosome aberrations	1
Mutation of HPRT	10^{-5}

2.5.1. Ionizing Radiaition Therapy

For numerous cancers, radiation therapy, either alone or in conjunction with other treatments, is an effective therapeutic approach. When tumors are exposed to a high-energy radiation beam immediately, they are destroyed. Nevertheless, there isn't a distinct division to safeguard the nearby noncancerous cells (74). Patient may develop radioresistance, but normal tissue damage prevents dosage increases. Prostate cancer is a good candidate for radiation therapy (75).

2.5.2. DNA damage repair after ionizing radiation treatment

ATR, a serine/threonine-specific protein kinase, is triggered by DNA single-strand breaks (SSBs) caused by the uncoupling of helicase and DNA polymerase during replication fork stalling (76). Likewise, DNA double-strand breaks activate the serine/threonine-specific kinase ATM (77). When these lesions are recognized, ATR and ATM phosphorylate and activate several downstream transducers and effectors (78). For instance, when ATR activates Chk1 or ATM activates Chk2, intra-S cell cycle arrest gets started by Cdc25 inhibition/nuclear export (79). Chk2 functions as well to stop the cell cycle through p53-dependent mechanisms (80). Tumor suppressor BRCA1, which protects the genome, affects both cell cycle checkpoints and DSB repair (81). ATR and ATM activates BRCA1 by phosphorylating several serine residues. As a result, the kinases have unique but overlapping control on BRCA1's role in managing the G2/M checkpoint (80,81). Furthermore, ATM-mediated phosphorylation and activation of Chk2 allows it to phosphorylate BRCA1, which is critical for BRCA1's role in homologous recombination (HR)-mediated DSB repair (82). Chk2 works to stop the cell

cycle through p53-dependent mechanisms (83). As a result, DNA strand break-mediated activation of ATM and ATR promotes HR repair and cell cycle arrest (81).

A similar reaction mechanism is present at the G1 restriction point (84). The p53-p21 pathway is the earliest and best understood signaling mechanism that regulates G1 arrest (85). ATM, the main DRR protein kinase, phosphorylates p53 and regulates the G1 checkpoint, which is controlled by p53 and p21 (86). Ku, a DNA-binding protein heterodimer (made up of Ku70 and Ku80), was best known for its role in V(D)J recombination and DSB repair (87). ATM has been shown to phosphorylate and activate Ku in response to DNA damage (86, 87).

When DSBs occur in human cell, it stimulates checkpoint signaling and repair as previously said. The MRN (MRE11/RAD50/NBS1) complex binds to DSBs and activates ATM (Ataxia Telangiectasia Mutated) (88). Following its attachment to the damage site, ATM autophosphorylates, starting the phosphorylation processes of ATM substrates, including H2AX, in the surrounding chromatin (88,89). One of the first signs of DNA double-strand break repair is the phosphorylation of H2AX, which plays a role in the process (90,91). Amplification of the H2AX phosphorylation process occurs through MDC1 engagement (88,91). Multiple proteins are drawn in as a result of MDC1 activation. Phosphorylation of downstream effectors including CHK1, CHK2 leads to activation of WEE1, p53, BRCA1 and PARP is crucial for maintaining the replication fork (Figure 2.3) (85, 90). Hence, to provide the cell enough time to repair its DNA, cell cycle arrest happens in G1/S and/or G2/M (90,91).

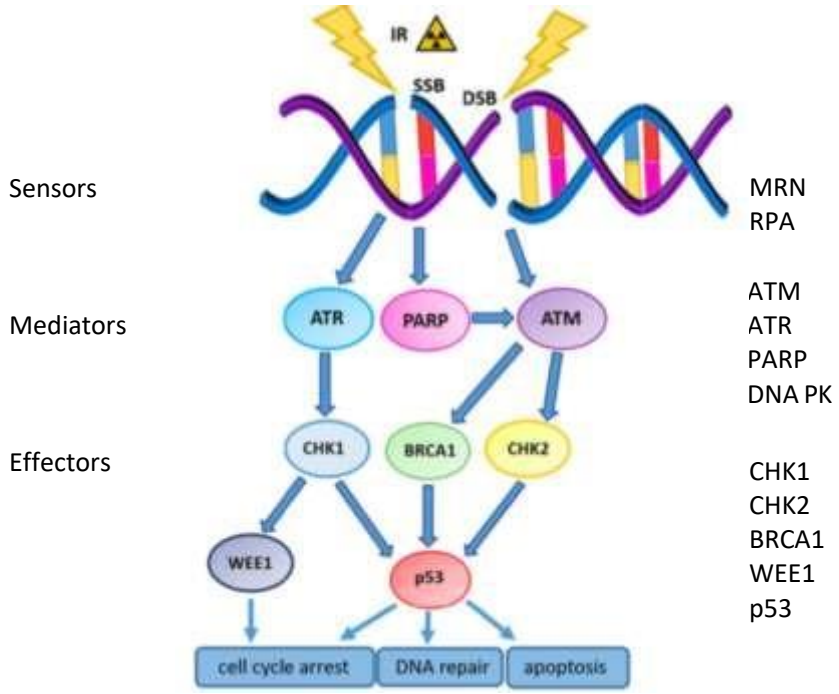


Figure 2.3: DNA damage response to ionizing radiation (edited from 85)

2.6 DSB repair mechanisms

The deadliest lesions are DSBs, which can cause cell death and genetic information loss if they multiply or remain unrepaired. Non-homologous end-joining repair (NHEJ) and homologous recombination repair (HR) are the two mechanisms by which DSBs can be repaired in human cells (92).

Small insertions or deletions at the break site are often handled using the quick but inaccurate NHEJ method (92,93). NHEJ has an effect on the cell cycle at every stage (93). Nevertheless, the more precise HR pathway uses homologous DNA sequences as a template and only activates during the late S/G2 phase of the cell cycle when the sister chromatids are near together (94).

The major pathway in humans is the NHEJ pathway. After IR, when the replication fork collapses at unresolved single-stranded DNA damage, HR is primarily involved in repairing DSBs (95).

2.6.1. NHEJ

NHEJ involves recognizing double-strand breaks and removing un-ligatable groups from damaged DNA ends. The Ku70/Ku80 heterodimer attaches to DNA ends first. A complex consisting of XLF, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), and XRCC4/DNA Ligase IV (X4LIG4) is recruited (93). DNA-PKcs autophosphorylates upon binding to Ku80, causing a conformational shift that activates the nucleases (94). Ligation occurs at the end of the process (Figure 2.4).

2.6.2. Homolog Recombination

For more precise repair, HR needs a template, or sister chromatid sequence. A DNA end is cut in the first step (95). The recombination mediators (such as BRCA2 and PALB2) then push RPA out of the 3' tails of the ssDNA and promote the RAD51-nucleoprotein filament formation. After strand invasion and homology search, RAD51 is necessary to construct the D-loop structure (96). Non-crossover products could be caused via D-loop dissociation. Specific endonucleases can dissolve the Double Holiday junction to produce crossover and non-crossover products, or BLM helicase can create and dissolve it to form crossover intermediates (Figure 2.5) (95, 97).

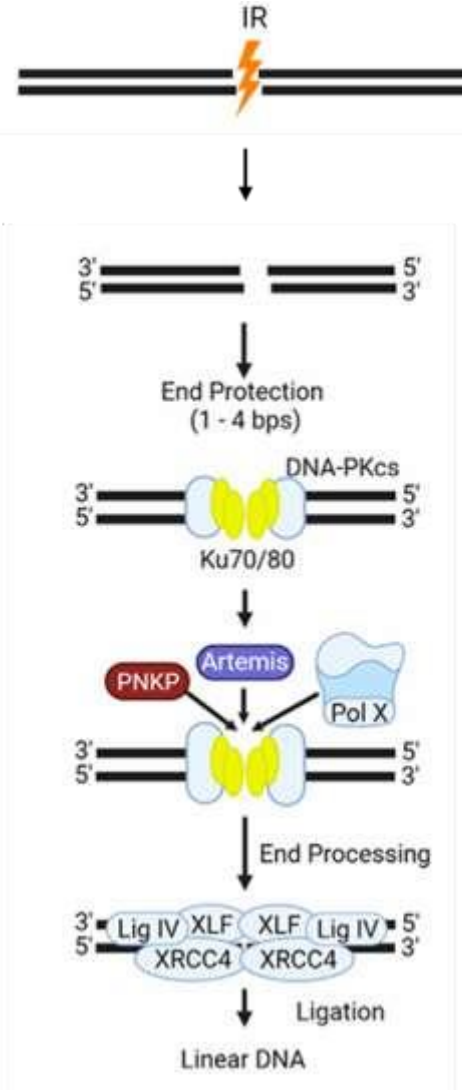


Figure 2.4: NHEJ mechanism. It is initiated by the binding of the Ku70/80 heterodimer to the ends, which recruits DNA protein kinases. Those can ligate the broken ends by recruiting proteins play a role in end processing (94).

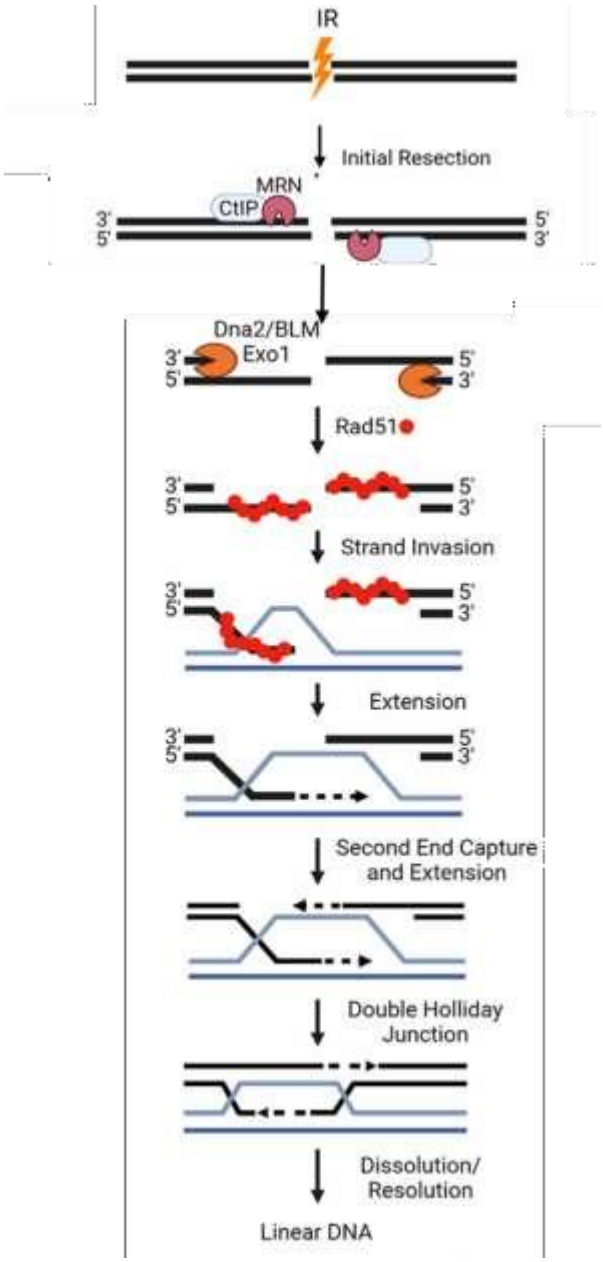


Figure 2.5: In S and G2 phases, CDK/ cyclin complex phosphorylates CtIP , which activates MRN complex. Then the ends are trimmed to produce single-stranded 3'DNA ends, which are not repaired by NHEJ mechanism. RAD51 /RPA complex forms a pre-synaptic filament. RAD51 scan for homology. After finding homologous sequence, pre-synaptic filament leads to invasion to the duplex DNA. After strands exchange the strand is extended. Holliday junction them undergoes resolution (95)

2.7. Targeting Therapy

2.7.1. ATM and ATR inhibitors

Central regulatory functions are mostly carried out by ATM and ATR, which are tied to both ATM and Rad3 (90). The ATR and ATM pathways cross across. ATM reacts to double-strand breaks in DNA mostly. ATM inhibitors are being used in clinical trials (96).

ATR promotes replication fork advancement and, in the event of single-strand breakage, stops cells in the G2/M phase (97,98). In ATM-deficient cells, ATR inhibition specifically causes cell death (99). Certain medicines are being investigated both as single treatments and in conjunction with immunotherapy, chemotherapy, radiation therapy, or PARP inhibitors (100).

2.7.2. PARP inhibitors

Poly(ADP-ribose) polymerase (PARP) inhibitors as a novel treatment have been worked on (101). The first class of authorized medications based on synthetic lethality are PARP inhibitors. In this approach, a single gene in the cells has a non-lethal mutation that makes them susceptible to recombinant DNA repair. As a result, further inhibition of PARP activity causes cells death (102). The FDA has approved olaparib and rucaparib, two PARP inhibitors, for use as monotherapy in patients with mCRPC who have mutations in the homologous recombination repair protein genes (103,104). The FDA has approved olaparib, a PARP inhibitor, along with prednisone and abiraterone for use as the first line of treatment for patients with BRCA-mutated mCRPC in 2023 (105). It was noted that in mCRPC, PARP inhibitors raised OS (105).

2.7.3. CHK1 and CHK2 inhibitors

CHK1 and CHK2 kinases regulate the cell cycle; ATR targets CHK1 and ATM targets CHK2 (106). Through BRCA1-mediated processes, CHK2 causes double-strand break repair and modulates the p53-dependent G1/S phase checkpoint. Cell cycle arrest

occurs at the G2/M checkpoint when replication forks are stopped in the S phase by CHK1, which controls single-strand break repair (107). Preclinical research has demonstrated the synergistic effects of CHK1 inhibitors with ionizing radiation therapy, antimetabolites, and chemotherapy (108). Clinical research on first-generation CHK1 inhibitors, however, has revealed cytotoxic side effects and poor selectivity (109).

It was discovered that second-generation CHK1 inhibitors were more successful; for example, patients with mCRPC were able to overcome docetaxel resistance thanks to the CHK1 inhibitor MU380 (110). Furthermore, in patients with this resistance, it was observed to improve the efficacy of the antimetabolite gemcitabine (110,111).

mCRPC patients are undergoing testing for the CHK1/CHK2 dual inhibitor (111). Experiments conducted in vitro have evaluated the efficacy of CHK2 inhibitors. Compared to combined ATR/CHK1 inhibitors, selective CHK2 inhibitors seem to be less efficacious (112).

2.7.4. Outcomes of the cell

2.7.4.1 Apoptosis

It is important tool to control cell death and proliferation is in normal cells. Programmed cell death or apoptosis is principal concern of this subject in higher eukaryotes. It is a normal process, which requires active p53. DNA damaging agents, IR exposure, oncogenic signals induce the p53 and then lead to cell cycle arrest through p21 activation to allow the cell to repair of DNA. (112) If DNA repair can not be performed, p53 stimulates the apoptosis by suppression of anti-apoptotic factors and by activating proapoptotic factors, which causes mitochondrial depolarization and releasing of cytochrome C and then caspase cleavage and activation, stimulation of proteases and destruction of subcellular organelles (113). The most important structural variations are cell reduction, membrane blebbing, chromatin condensation and nuclear fragmentation. apoptotic bodies are formed which are cleared by phagocytes.

Initiation phase comprises to make decision for death and BCL-2 protein family is responsible. Execution phase is application phase in which caspases are functional that play a role degradation of cytoskeleton of the cell, subcellular organelles and DNA (114).

The degraded components are packaged into membrane compartments, that prevents the release of materials outside. Then macrophages and other scavenger cells engulf the membrane packages. Apoptotic markers are as follows:

- Stimulation of apoptotic signals such a Bcl-2 family
- Phosphatidylserine exposure on the outer leaflet of the plasma membrane
- Releasing of cytochrome C from mitochondria
- Caspase activation
- Cleavage of substrates by caspases
- DNA fragmentation

However, cancer cells escape apoptosis, because p53 is non-functional in most cancer cells due to the overexpression of Mdm2 or some mutations in inhibitors of mdm2 or p53. It results in prolonged tumor cell survival (112).

2.7.4.2. Apoptosis by ionizing radiation

The cell exposed to IR, if DNA repair is not successful (112) and a SSBs and DSBs are detected, then cell initiates the apoptotic pathways. Irradiation typically activates releasing of mitochondrial cytochrome c and then apoptosome formation and cell death, as known intrinsic apoptotic pathway. Briefly, p53 is activated, and the cell prefers to intrinsic apoptosis instead of cell arrest. Increased p53 leads to the activation of pro-apoptotic genes including BAX (BCL2-associated X protein), PUMA (p53-

upregulated modulator of apoptosis), which then (112,113) proapoptotic events are produced as follow:

- loss of mitochondrial membrane potential (109, 110)

- release of proapoptotic mitochondrial proteins [111]

- increasing membrane permeability [112];

- releases of cytochrome c from the mitochondria [111 ,113].

- formation of the apoptosome complex (cytochrome c, APAF1 (apoptotic protease activating factor 1) and caspase-9) [114].

- Caspase-9 activates effector caspases-3 and-7 that activate the post-mitochondrial- caspase cascade [115].

Ionizing radiation also activates extrinsic apoptotic pathway through death receptors (DRs). IR activates p53, then it activates CD95 and its' ligands CD95L or CD178, and DR5 receptors. CD95-CD178 binding recruits the adapter protein FADD (FAS associated death domain). Then procaspase-8 binds FADD, and forms DISC complex Activation of the caspase-8 leads to activation of procaspase-3 and procaspase-7.

2.7.4.3.Senescence

Cellular senescence is a response to damage stimulation which includes cell cycle arrest. Ionizing radiation induces the p53/p21 and the p16/RB1 pathways, which result in transient cell-cycle arrest in the G1 phase and subsequent senescence.

2.7.4.4. Mitotic catastrophe

Ionizing irradiation leads to mitotic catastrophe, which is the result of premature mitosis of S and G2 phases. Abnormal mitosis leads to atypical chromosome segregation and cell division, resulting in the formation of giant cells. In p53-deficient cells, CDK2-

cyclinA/E is active; thus, centrosome hyper-amplification may occur. This is needed for mitotic catastrophe.

2.8. WEE1

2.8.1.Structure

Wee1 (Wee1A), Wee2 (Wee1B), and Myt1 (PKMYT1) are members of the Wee family, a protein kinase family that controls the cell cycle by blocking the CDK1-cyclin B complex (113).

Wee1 protein has a 94 kDa of molecular weight. It contains a N-terminal regulatory domain, a central kinase domain and a C-terminal regulatory domain (Figıre 2.6) (114). The N-terminal domain has two phosphorylation sites (S53 and S123) that are involved in protein degradation (115).

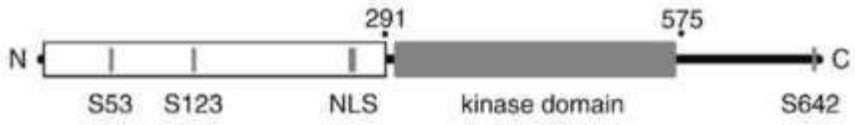


Figure 2.6: Structure for human Wee1 kinase (114)

2.8.2. Functions of WEE1

Wee1 controls G2/M checkpoint in cell cycle and hampers mitotic initiation in the presence of DNA damage (113).

The primary mechanism, the cell cycle, is strictly regulated by G1/S, intra-S, and G2/M checkpoints. Checkpoints provide cells a chance to fix DNA damage before they undergo mitosis (113, 114). This process involves cyclin-dependent kinases (CDKs), which are members of the serine/threonine kinase family (114). These proteins are

extremely conserved. CDK1/cyclin B regulates the transmission of G2/M (112). When checkpoints are inhibited, damage spreads and cells die.

G1/S arrest repairs damages in DNA. G1/S checkpoint deficits, however, can arise in tumor cells, especially in p53-deregulated cells (112, 113). The G2/M checkpoint becomes more significant in these cells' DNA damage repairment (114). Consequently, focusing on the G2/M checkpoint may be a novel approach to cancer treatment (112-114).

Tyrosine 15 on CDK1 is phosphorylated by WEE1, which inactivates cyclin-dependent kinase CDK1/Cyclin B (115). In response to double strand DNA breaks, WEE1 also triggers the G2/M checkpoint. To control this step in reverse, CDC25 dephosphorylates CDK1 (116). When mitosis begins, CDC25 is triggered. Recent evidence has demonstrated WEE1's involvement in the intra-S checkpoint (117). Since DNA replication and mitosis are related processes, a replication error causes the cell cycle to stall in the intra-S phase (118).

2.8.3. WEE1 inhibitors

WEE1 inhibition is a feasible therapeutic target for cancer, as preclinical studies have proven its significance in DNA damage repair and replication fork stabilization (115). The first WEE1 inhibitor was found to be AZD-1775 (Adavosertib) (Figure 2.7), a strong and specific small molecule inhibitor of Wee1 kinase that is presently being studied in patients with metastatic solid tumors (119).

Pyrazole-pyrimidine derivative AZD1775 is a strong and ATP-competitive selective small-molecule inhibitor of Wee1 kinase (120). Its' IC50 value was reported as 5.2 nmol/L (121). AZD1775 exhibits a comparatively brief terminal half-life (t1/2) in vivo, with a range of 9 to 12 hours. It is shown that radiosensitization by Wee1 inhibition was observed in both p53-mutant and wild type cells (121).

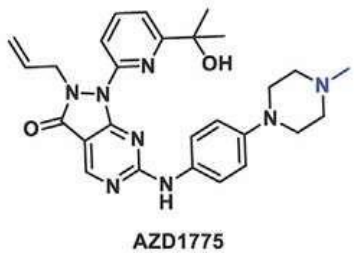


Figure 2.7: Wee1 inhibitor AZD1775's structure (121).

Wee1 inhibition has been shown to be useful with minimal cytotoxicity in recent investigations (122). In clinical trials, showed promise anticancer activity and was proven to be tolerated when paired with radiation or medicines that destroy DNA (123).

Patients with hepatocellular carcinoma were reported to have elevated levels of Wee1 kinase activity (120). It was discovered to be overexpressed in ovarian cancer, gliomas (120,121) and medulloblastomas (121). Furthermore, Wee1 levels were shown to rise during chemotherapy exposure, indicating a potential function for this kinase in modulating the course of the illness (122). As a result, elevated Wee1 expression could be an adaptive reaction to the chemotherapy, enabling tumor cells to repair damaged DNA and endure (123,124).

2.9. Rad51

2.9.1. RecA/Rad51 Superfamily

The homologous recombination process depends on Rad51, a member of the recombinase superfamily in eukaryotes (125). The highly conserved RecA protein in E. coli is likewise a member of this superfamily. Three categories can be identified from it: recA, RADα, and RADβ (126). RecA from bacteria makes up the majority of the first group, while DMC1 and eukaryotic Rad51 exhibit substantial sequence identity in the

second group. The final group, which represents a good deal of variation in eukaryotes, consists of RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 (127,128).

2.9.2. Rad51 Functions

Rad51 is a strand exchange protein. Presynaptic, synaptic, and postsynaptic stages of the HR process are all involved (125). In the initial stage, Rad51 creates a Rad51-ssDNA filament by binding to ssDNA. A single monomer can bind three DNA nucleotides. D-loop formation occurs when Rad51 nucleofilament invades a homologous template during the synaptic phase. Rad51 separates from dsDNA during postsynaptic phase to allow for DNA synthesis. ATP hydrolysis is required for homology search, strand invasion, and joint molecule synthesis (126).

2.9.3. Rad51 Inhibitors

A key target for the creation of novel anti-cancer treatment options is Rad51 recombinase (126-128). B02, B02 isomers, IR-1, and other small molecule inhibitors and antibodies that target Rad51 have been produced and are being investigated in vitro (129).

2.9.4. B02

B02 (Figure 2.8) inhibits the Rad51 in humans (Figure 2.9). B02 sensitized human cells to chemotherapeutic medicines and suppressed HR in them. It was demonstrated that the PARP inhibitor olaparib sensitizes triple-negative breast cancer (MDA-MB-231) cells to B02-isomer (130).

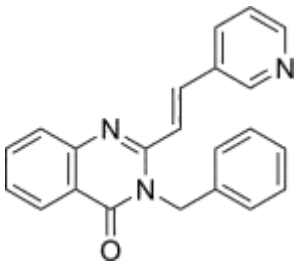


Figure 2.8: Rad51 inhibitor B02's structure (edited from Wikipedia)

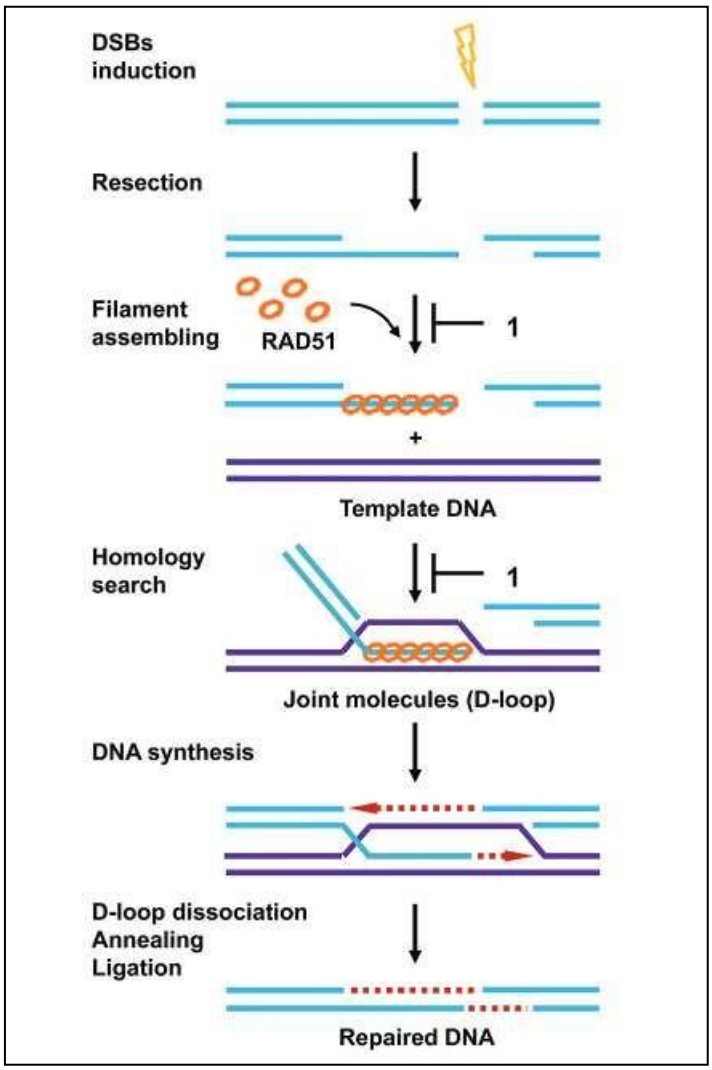


Figure 2.9: B02's inhibition mechanism of Rad51 (130).

2.10. PC-3 Cell Line

The PC-3 cell line is originated from a bone metastasis of a grade IV prostatic cancer of a 62-year-old white male (ATCC). It's doesn't express AR or PSA, so it's not sensitive to AR. It's even shown that female nude mice can grow prostate tumor from PC-3 since it's androgen-independent (131). This cell line doesn't express p53, either (132). There are 3 main prostate cancer cell lines that are commonly used and they are differ from PC-3 based on their AR and p53 status. DU145 cell line is isolated from a

brain metastasis and it's the first prostate cancer cell line to be isolated (732,133). This cell line doesn't express AR either but it expresses mutant p53 (134). LNCaP cell line is originated from a metastatic lesion of a lymph node and this cell line expresses AR and has a wild type p53 (134, 135). PC-3's AR-free and p53-null properties make it an interest in related studies.

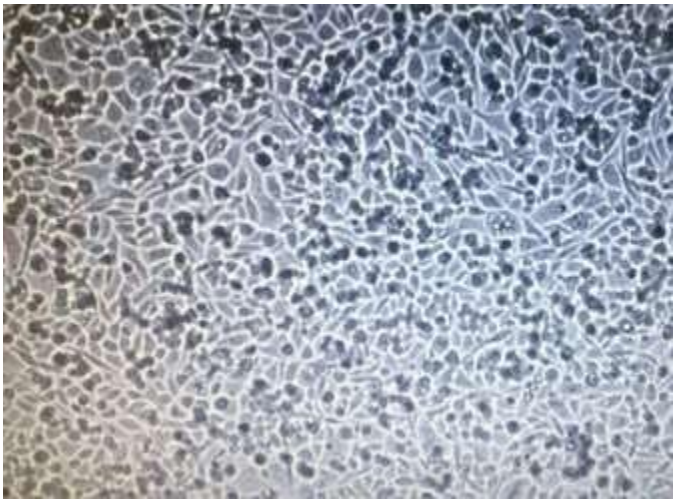


Figure 2.10: PC-3 cell image from our laboratory

3. MATERIALS AND METHODS

This is a prospective in vitro study. The study was carried out in Department of Biochemistry, Department of Radiation Oncology and Department of Basic Oncology, Faculty of Medicine, Hacettepe University.

3.1. Materials

3.1.1. Chemicals, Reagents and Consumables

- Dulbecco's modified Eagle's Medium (DMEM) [LOT:13010123MCL], Penicillin-Streptomycin [LOT:43030723RAL], Fetal Bovine Serum (FBS) [LOT: 46010323FBSP] and Trypsin-EDTA [LOT: 223601012] used for the cell culture were purchased from Serana, Germany.
- Phosphate buffered saline (PBS) was prepared in laboratory by dissolving PBS tablet in distilled water (1 tablet/200 mL) according to the manufacturer's protocol (Merck, Germany) [Product No: P4417].
- MTT kit (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-tetrazoliumbromide) was purchased from Elabscience (USA) [LOT: E-CK-A341].
- Annexin V kit and Propidium Iodide, containing 300 µg/ml RNase, was purchased from Elabscience (USA) [LOT: E-CK-A211].
- 96-well plates with F bottom and 6-well plates and serological pipettes were provided by Serana (Germany).
- T25 cell culture flasks with filter cap were purchased from Serana (Germany).

3.1.2. Equipment

- JuliBr Cell Analyzer (Chincilla Life Sciences, USA) was used for cell monitoring and cell counting.
- Elekta Synergy linear accelerator (Elekta Oncology Systems, Stockholm, Sweden) was used for irradiation.

- BD FACSVerse flow cytometer (Becton-Dickinson, San Jose, CA, USA) was used for apoptosis assay.
- Oven, shaker and spectrometer used are belong to Hacettepe University Biochemistry Department laboratories.

3.1.3. Inhibitors

The defined WEE1 kinase and RAD51 inhibitors were used in inhibition experiments. WEE1 kinase inhibitor AZD1775 (Adavosertib) was purchased from Cell Signaling Technology (USA) [Product No: 69589] and RAD51 inhibitor B02 was obtained from Cayman Chemical (USA) [Product No: 1290541-46-6]. All drugs were kept frozen at -20°C until use.

Inhibitors were prepared as 10 mmol/L stock solutions in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany). Before application, it was checked that DMSO is <0.1% due to toxic effects. All stock solutions were kept at -20°C to store.

Inhibitors were diluted with complete medium when they were diluted to smaller concentrations and treated to the cells.

3.2. Methods

3.2.1. Cell Culture

Prostate cancer cell line PC-3 was obtained from ATCC (American Type Culture Collection, Manassas, VA, USA) [CRL-1435]. PC-3 cell line was cultured in DMEM (Biological Industries, Israel), supplemented with 10% fetal bovine serum (Biological Industries, Israel) and 1% penicillin-streptomycin (Biological Industries, Israel). Cells were grown at 37°C atmosphere humidified with 5% CO_2 . The medium has been changed every 1-2 days and the cells were passaged when the confluence in the flask reached 80%. Cell imaging and cell counting were all done with JuliBr Cell Analyzer (Chincilla Life Sciences, USA).

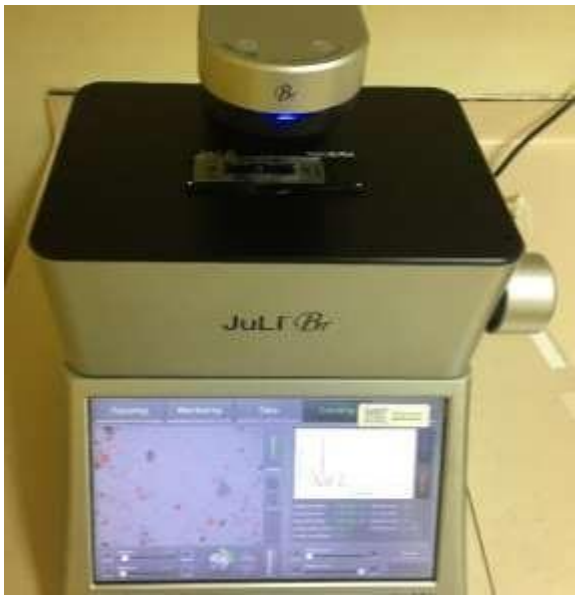


Figure 3.1: JuliBr Cell Analyzer

3.2.2. Study groups

Total of 8 groups were formed for this study:

- **Group 1:** Control group (no treatment)
- **Group 2:** Irradiation
- **Group 3:** Irradiation with Wee1 inhibitor
- **Group 4:** Irradiation with Rad51 inhibitor
- **Group 5:** Irradiation with both Wee1 and Rad51 inhibitors
- **Group 6:** Only Wee1 inhibitor treated
- **Group 7:** Only Rad51 inhibitor treated
- **Group 8:** Wee1 and Rad51 inhibitor combined treatment

3.2.3. Irradiation treatment

IR was performed with Varian DHX linear accelerator (Varian, MA, USA). Cells were treated with various doses of 6 MV photons at 3.96 Gy/min rate. The distance between IR source and cells were 100 cm and the field size was 20 x 20 cm. Cell plates were placed under a 5-cm-thick platform.

Figure 3.2: Varian DHX Linear Accelerator

3.2.4. MTT Assay

Cytotoxicity and cell viability assays were done by MTT assay.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) is a tetrazolium salt containing one tetrazole ring, one thiazolyl ring and two phenyl rings. It

is positively charged and lipophilic molecule, and can pass through cell membrane and mitochondrial membrane and undergoes the reduction reaction (136).

The MTT assay is a colorimetric assay based on the conversion of tetrazolium (yellow) into formazan (blue-purple) by the mitochondrial oxidoreductase of the living cells (137).

The absorbance of colored molecules is measured at 570 nm by spectrophotometer (a microplate reader).

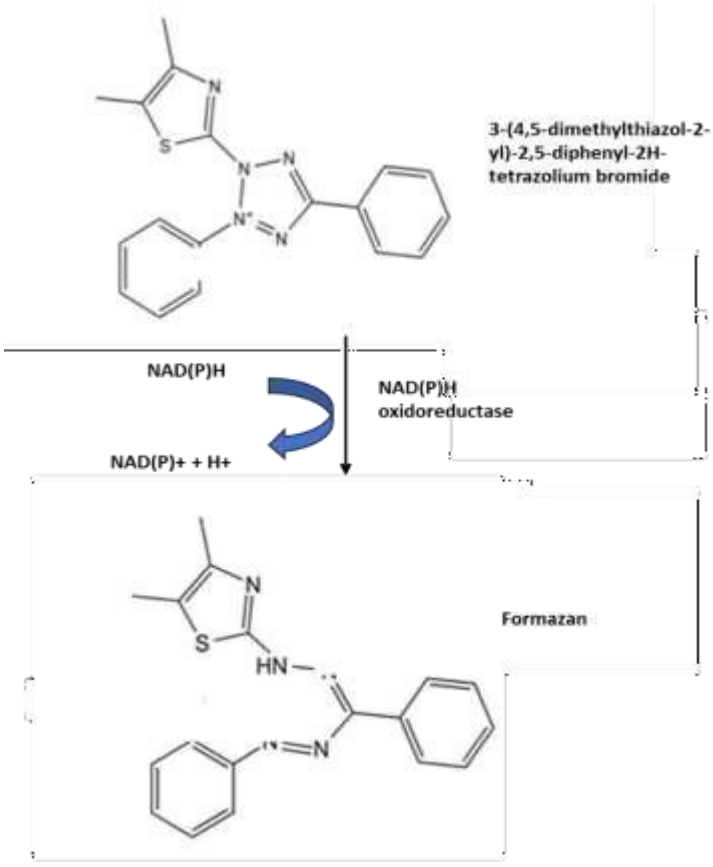


Figure 3.3: MTT mechanism (edited from 136)

3.2.5. Cell Optimization

To determine the amount of cells that will be used for cell viability assay, cell optimization was done.

- Cells were seeded in 96-well plates with varying amounts between 2500-40000 cells/well.
- Cells were incubated for 48 hours at 37 °C and 5% CO_2 .
- 5 mg/mL stock MTT solution was prepared with PBS. 1 mg/mL MTT solution was prepared by diluting the stock solution with complete medium.
- 100 µL of diluted MTT solution was added to each cell and incubated for 3-4 hours.
- 200 µL 1:1 DMSO-ethanol mixture was added to the wells after formazan crystals were formed.
- The absorbance was read at 570 nm and the graph was plotted to determine the cell number that will be used.

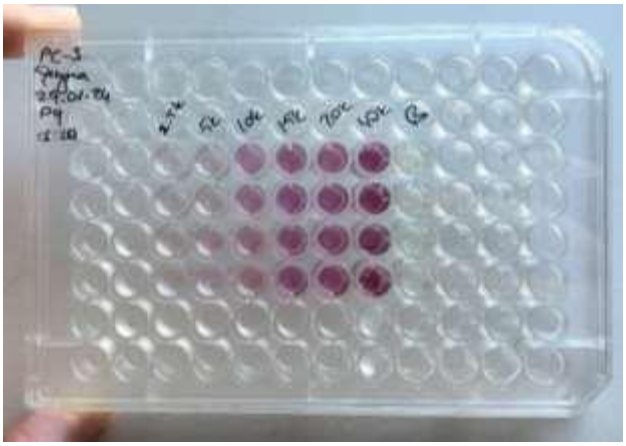


Figure 3.4: Cell optimization MTT assay

3.2.6. Cytotoxicity Assays

Cytotoxicity of AZD1775 and B02 was found by MTT assay. 20000 cells/well were seeded in 96-well plates and incubated for 24 hours. Then, the cells were treated with various concentrations of AZD1775 and B02. After 24-hour and 48-hour incubations for each inhibitor, 100 mL of 1 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazoliumbromide) solution was added to each cell and incubated for 3-4 hours. After incubation, MTT solution was removed from cells and then 200 mL of 1:1 mixture of DMSO and ethanol was added. The absorbance was readed at 570 nm.

3.2.7. Cell Viability Assay

MTT assay was done for measuring cell viability. 20000 cells/well were seeded in 96-well plates and incubated for 24 hours for each group. After 24-hour incubation of cultured cells in 96-well plates, following treatments were done to the groups:

- **Only IR:** Cells were treated with 2, 4, 6, 8, 10 Gy IR and incubated for 48 hours.
- **Only Wee1 inhibition:** Cells were treated with 4 μM AZD1775 and incubated for 48 hours.
- **Wee1 inhibition and IR:** Cells were treated with 4 μM AZD1775 and 1 hour later, irradiated with 2, 6 and 10 Gy IR. Then they were incubated for 48 hours.
- **Only Rad51 inhibition:** Cells were treated with 10 μM B02 and incubated for 48 hours.
- **Rad51 inhibition and IR:** Cells were treated with 10 μM B02 and 1 hour later, irradiated with 2, 6 and 10 Gy IR. Then they were incubated for 48 hours.
- **Wee1 and Rad51 inhibition:** Cells were treated with 4 μM AZD1775 and 10 μM B02 with a 1:1 ratio. Then the cells were incubated for 48 hours.
- **Wee1 and Rad51 inhibition and IR:** Cells were treated with 4 m AZD1775 and 10 M B02 with a 1:1 ratio. 1 hour later, they were irradiated with 2, 6 and 10 Gy IR. Then the cells were incubated for 48 hours.

Cell viability is calculated by the Formula 3.1:

$$\text{Cell viability } \% = \frac{\text{Absorbance (treated cells)}}{\text{Absorbance (untreated cells)}} \times 100$$

Formula 3.1: Cell viability percentage

3.2.8. Colony Formation Assay

- 1500 cells/well were seeded in 6-well plates and incubated for 24 hours.
- For only inhibitor-treated groups; inhibitors were added to the wells and plates were incubated for 12 days.
- For only irradiation group; plates were irradiated and then incubated for 12 days.
- For inhibitor and irradiation combined groups; cells were treated with inhibitor(s) 1 hour prior to irradiation. Then they were incubated for 12 days.
- After incubation, medium was discarded from plates and the cells were washed with PBS.
- PBS was discarded. Then a mixture of 6.0% glutaraldehyde and 0.5% crystal violet was added to the wells in 2-3 mL volumes.
- The stained cells were left for 30 minutes.
- Then the mixture was removed and cells were washed with tap water.
- Plates were left to dry outside at room temperature.
- Colonies were counted with ProMega Colony Counter application software.

- Plating efficiency (PE) was calculated with Formula 3.2:

$$PE = \frac{\text{the number of viable colonies}}{\text{the number of cells plated}}$$

Formula 3.2: Plating efficiency

- PE was expressed as a percentage (PE%)
- Then survival fraction (SF) was calculated with Formula 3.3:

$$SF\ \% = \frac{\text{the PE of the treated cells}}{\text{the PE of the control}} \times 100$$

Formula 3.3: Survival fraction percentage

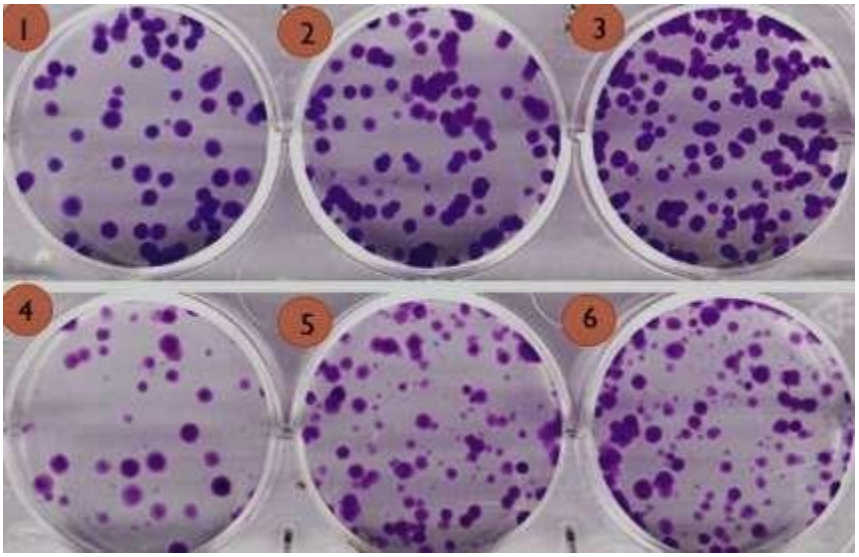


Figure 3.5: Colony formation assay plate

3.2.9. Apoptosis Assay

Apoptosis assay was done by flow cytometry. Annexin V/PI Kit (Elabscience, USA) was used for the protocol.

- 50000 cells/well were seeded in 6-well plates for each group.
- For the groups that included both inhibitor and IR treatment, cells were treated with inhibitors 1 hour prior to the IR treatment.
- After treatments, cells were incubated for 96 hours for each group.
- After 96-hour incubation, media was discarded from the plates and put in FACS tubes.
- Wells were washed with PBS and then it was put in the tubes as well.
- Cells were removed with trypsin, then trypsin was inactivated with complete medium, the cell suspensions were put in the FACS tubes to centrifuge at 300 g for 5 minutes.
- Supernatant was discarded after centrifuge, the pellet was vortexed and 50 µL of it was put in AF tubes for each sample (200-250 µL of cell pellet remained in the tubes).
- The remained pellet was washed with PBS to avoid false positive results.
- They were centrifuged at 300 g for 5 minutes again. Supernatant was discarded.
- The buffer solution was prepared using the buffer in the kit and diluting it 10x with distilled water. The amount of Annexin V and PI stains were calculated (2,5 µL Annexin V and 2,5 µL PI for each FACS tube) and added to the buffer solution.
- 105 µL of buffer-stain mixture was added to the tubes except the AF ones.
- Tubes were vortexed and then incubated in the dark for 20 minutes.
- Samples were read with the flow cytometry and BDAccuri software.

Figure 3.6: Flow Cytometer

3.2.10. Statistical Analysis

GraphPad Prism Version 8 software was used to analyze cytotoxicity, cell viability and survival fraction datas. One-way ANOVA with post hoc Tukey's test was used for intergroup comparison.

4. RESULTS

4.1. Cell number optimization

Optimization studies were firstly performed to determine the optimal number of PC-3 cells for the further cell viability and cytotoxicity experiments. For this aim, cells were cultured in the 96-well plates in the range of 2.5 x 10^3 cells to 4.0 x10^4 cells, and incubated for 24-hour and 48-hour. Cell viability was evaluated by MTT method (Figure 4.1).

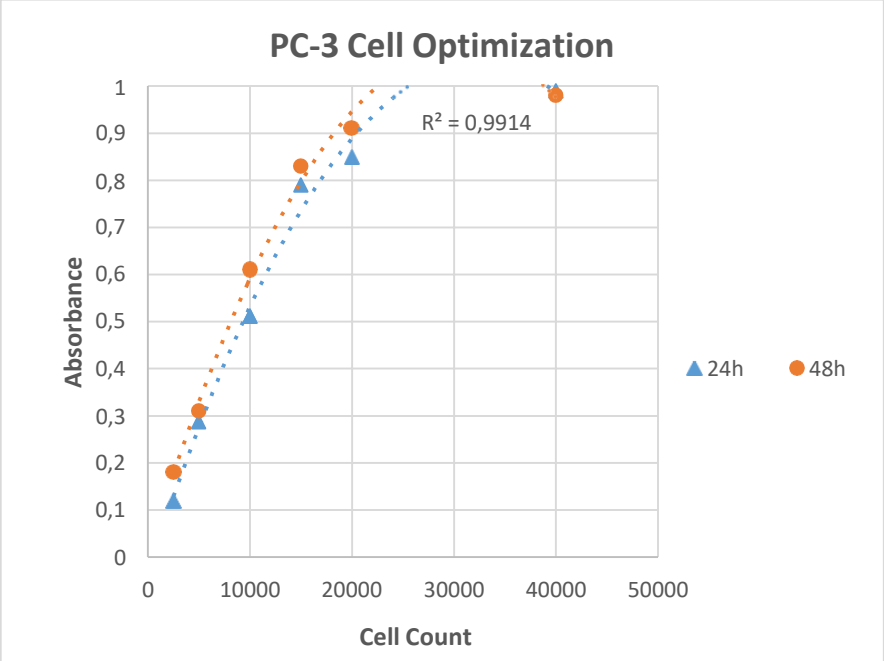


Figure 4.1: Cell number optimization of PC-3.

Based on the graph, the maximum point of the linear part of the line was determined as 20000 cells for both 24-hour and 48-hour. It was decided to continue subsequent cell viability experiments with this number of cells.

4.2. Evaluation of IR Exposure On Cell Viability of PC-3 Cells

The effect of IR exposure on PC-3 cells were evaluated; cells were plated into 96-well plates and incubated for 48 hours, then they were exposed to 2 Gy-10 Gy irradiation. The cell viability of IR-exposed cells were calculated (Figure 4.2).

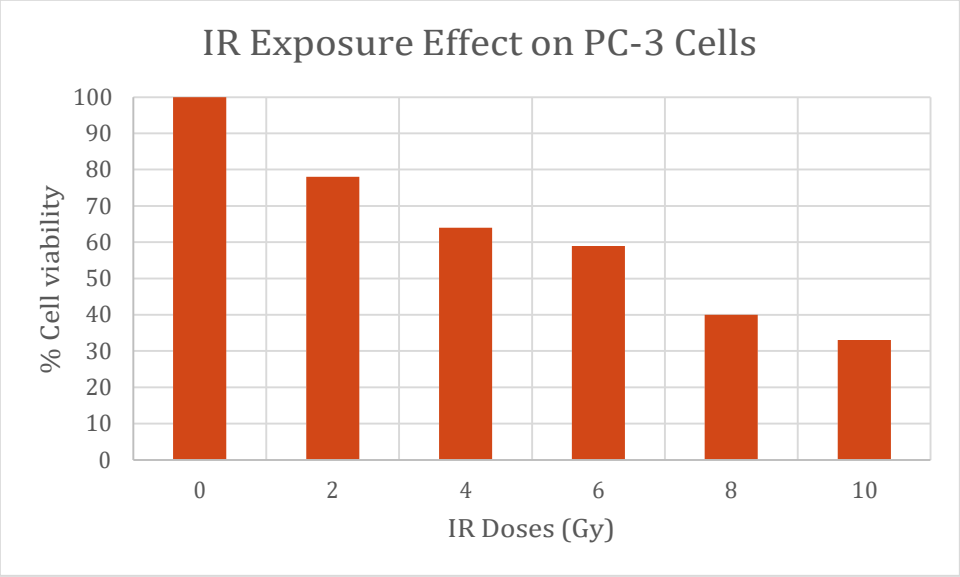


Figure 4.2: The effect of IR on PC-3 cell viability.

The control cells which are the untreated cells (0 Gy) were accepted as showing 100% cell viability according to the Formula 3.1. It was found that cell viability was decreased to 78% after exposure to 2 Gy. The cell viability was 64% after 4 Gy treatment and 59% after 6 Gy. By 8 Gy the viability of the PC-3 cells was 40% and by 10 Gy it was 33%. Since the difference between 4 Gy and 6 Gy, and the difference between 8 Gy and 10 Gy are not too much; 2, 6 and 10 Gy were chosen as treatment doses for the further experiments.

4.3. Determining IC_{50} values of the inhibitors

IC_{50} values of the inhibitors were determined by MTT assay for both 24 and 48 hours. Concentration range was between 0.1-32 µM for AZD1775 and 5-30 µM for B02. Absorbance values were read at 570 nm and the results were analyzed on GraphPad Prism 8 software (Figure 4.3. and Figure 4.4). IC_{50} values were shown on the Table 4.1.

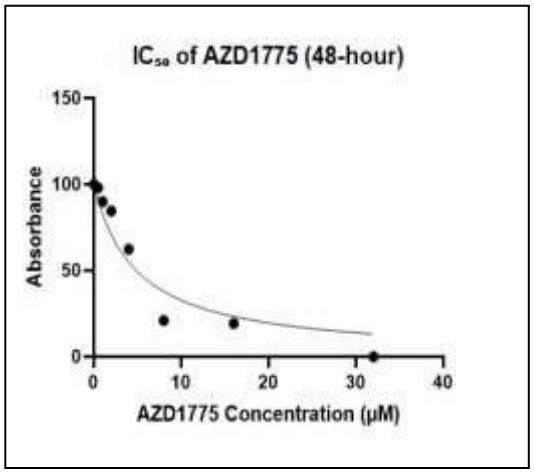


Figure 4.3: IC_{50} graph of AZD1775 for 48 hours

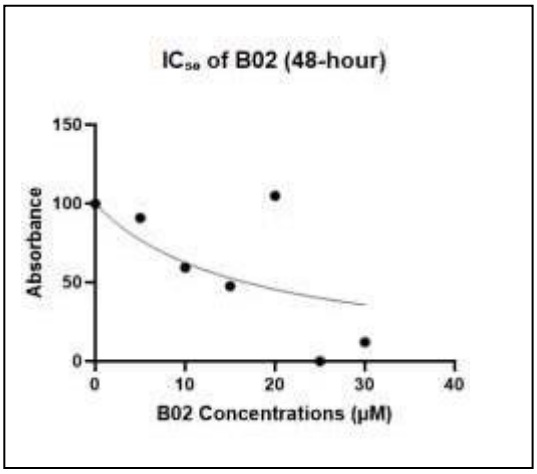


Figure 4.4: IC_{50} graph of B02 for 48 hours

Inhibitor	IC_{50} (24h)	IC_{50} (48h)
AZD1775	2,5 µM	4,9 µM
B02	14,1 µM	16,3 µM

Table 4.1: IC_{50} values of AZD1775 and B02

Treatment concentrations for both inhibitors were chosen below the IC_{50} values for the experiments where the inhibitors were treated alone.

4.4. Cell viability of the PC-3 cells after treatments

Cell viability was assessed by MTT assay. Incubation time was determined as 48 hours since radiation is a static energy so its' effect enhances as the time goes by. All experiments were done at least twice.

4.4.1. Evaluation of the Wee1 inhibitor AZD1775's effect on radiosensitivity via cell viability

The effect of AZD1775 on radiosensitization of prostate cancer cells was assessed. For cell viability evaluation, MTT assay was done and the assessments were done after 48 hours. The concentration of AZD1775 was 4 μM in all experiments.

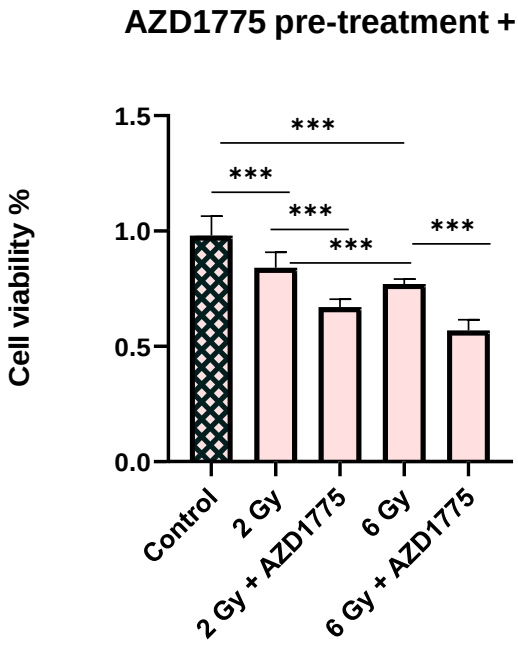


Figure 4.5: The effect of AZD1775 pre-treatment on radiosensitivity of PC-3 cells. Data are expressed as mean ± SD, from five independent experiments ***$p < 0.001$, one-way ANOVA with post hoc Tukey's test for intergroup comparison.

As shown in the Figure 4.5.; it was observed in cell viability experiments that 2 Gy of IR exposure led to 15% decrease in cell viability whereas 32% decrease was observed by AZD1775 pre-treatment and 2 Gy IR exposure combined, compared to the control cells (accepted as 100% viability). The difference between them statistically

significant ($p<0.001$). AZD1775 pre-treatment with 6 Gy IR exposure caused 42% decrease compared to the control cells. The difference between 6 Gy and AZD1775 pre-treatment combined with 6 Gy IR exposure was significant ($p<0.001$).

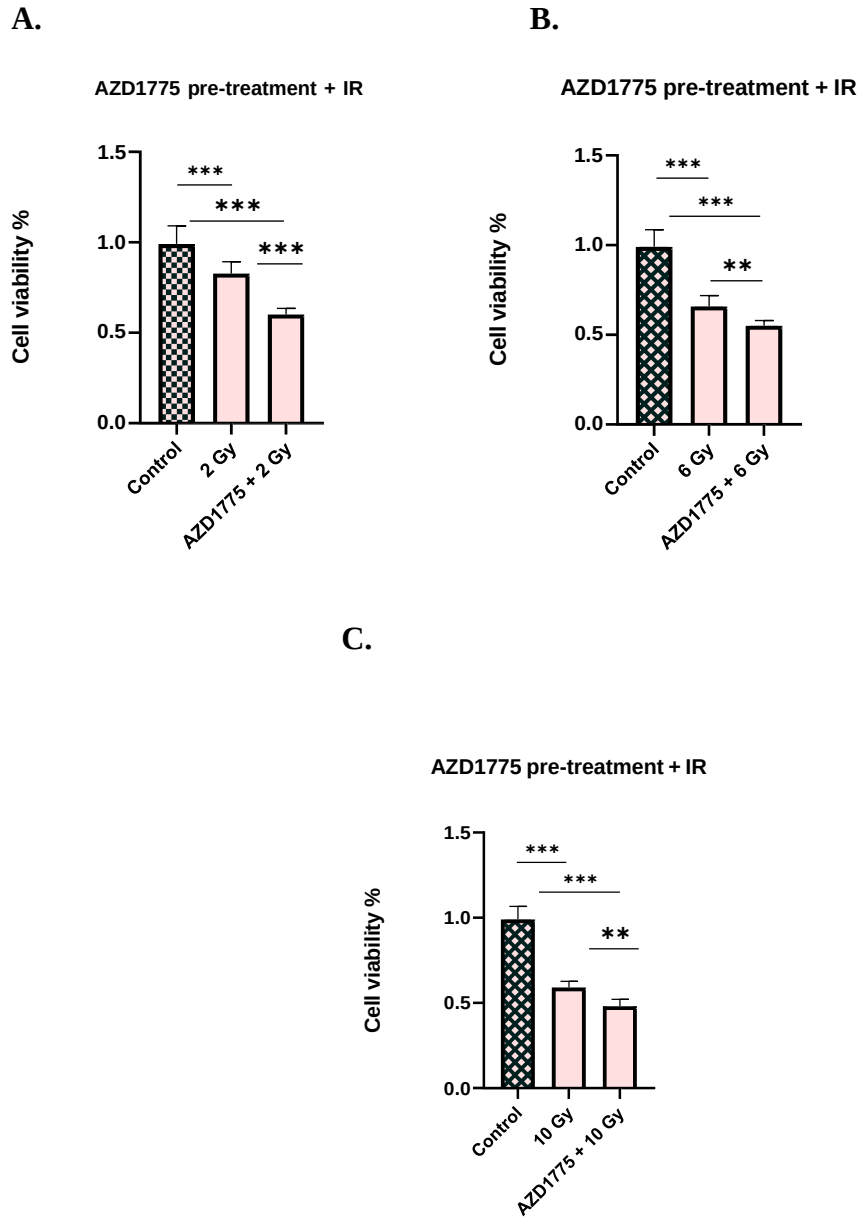


Figure 4.6: The effect of AZD1775 pre-treatment on IR at different doses. **A.** 2 Gy **B.** 6 Gy **C.** 10 Gy IR doses. Data are expressed as mean ± SD, from five independent experiments ***$p < 0.001$ and **$p < 0.05$, one-way ANOVA with post hoc Tukey's test for intergroup comparison.

As shown in the Figure 4.6.; compared to the 100% viable control cells the cell viability difference between 2 Gy IR and AZD1775 pre-treated 2 Gy IR is 17%. the cell viability difference between 6 Gy IR and AZD1775 pre-treated 6 Gy IR is 10% which is also statistically significant ($p<0.05$). 10 Gy IR exposure led to 40% decrease of cell viability compared to the control cells and the cell viability difference between 10 Gy IR and AZD1775 pre-treated 10 Gy IR is 12% which is significant statistically ($p<0.05$). Therefore, it can be said that AZD1775 pre-treatment increased the radiosensitivity of PC-3 cells.

4.4.2. Evaluation of RAD51 inhibitor B02's effect on radiosensitivity via cell viability

The effect of B02 on radiosensitization of prostate cancer cells was assessed. For cell viability evaluation, MTT assay was done and the assessments were done after 48 hours. The concentration of B02 was 15 μM in these experiments.

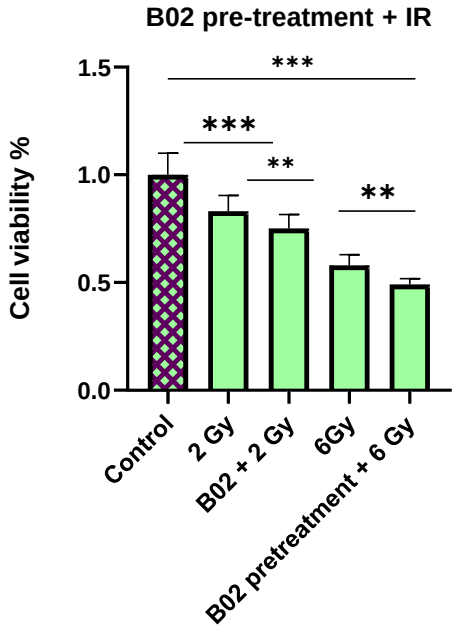


Figure 4.7: The effect of AZD1775 pretreatment on radiosenitivity of PC-3 cells. Data are expressed as mean ± SD, from five independent experiments ***$p < 0.001$ and **$p < 0.05$, one-way ANOVA with post hoc Tukey's test for intergroup comparison.

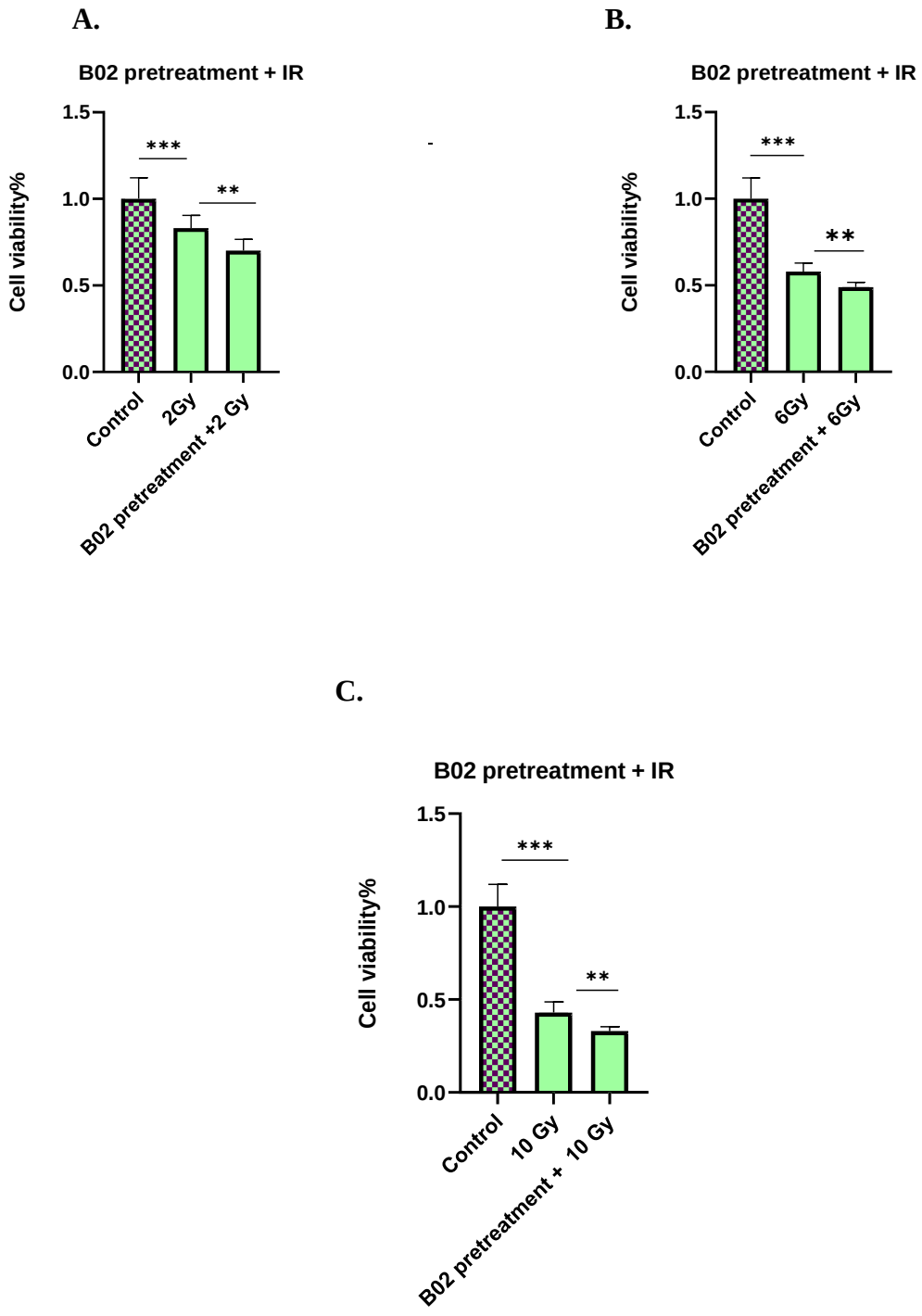


Figure 4.8: The effect of B02 pre-treatment on radiosenitivity of PC-3 cells. **A**. 2Gy **B.** 6 Gy **C.** 10 Gy IR doses. Data are expressed as mean ± SD, from five independent experiments ***$p < 0.001$ and **$p < 0.05$, one-way ANOVA with post hoc Tukey's test for intergroup comparison.

As shown in the Figure 4.7; it was observed that B02 pre-treatment significantly increased IR susceptibility of PC-3 cells with both 2 Gy ($p<0.05$) and 6 Gy ($p<0.001$). The cell viability decrease between 2 Gy and 2 Gy with B02 pre-treatment is 10%. Also, pre-treatment of B02 with 6 Gy decreased cell viability 12% compared to 6 Gy alone.

B02's effect on radiosensitivity were shown separately for each IR dose in Figure 4.8. Control cells' viability is accepted as 100% since they were untreated. Compared to the control cells, 2 Gy IR decreased cell viability by 20% and pre-treatment with B02 combined with 2 Gy IR decreased cell viability by 30%. 6 Gy IR exposure decreased cell viability by 55% while 6 Gy IR with B02 pre-treatment decreased it by 67%. Lastly, 10 Gy IR exposure decreased cell viability by 65% while pre-treatment with B02 and 10 Gy IR combined decreased it by 72%. The differences between all the IR doses alone and B02 combined treatments are statistically significant ($p<0.05$).

4.4.3 Evaluation of Wee1 Inhibitor AZD1775's and Rad51 Inhibitor B02's effect on radiosensitivity by survival fraction

Colony formation assay was done to evaluate survival fraction of prostate cancer cells after AZD1775 and B02 treatments prior to irradiation. AZD1775's concentration was 4 μM and B02's concentration was 15 μM in these experiments. Only 6 Gy of IR exposure was done. Colony formation experiments were done at least twice with n=2.

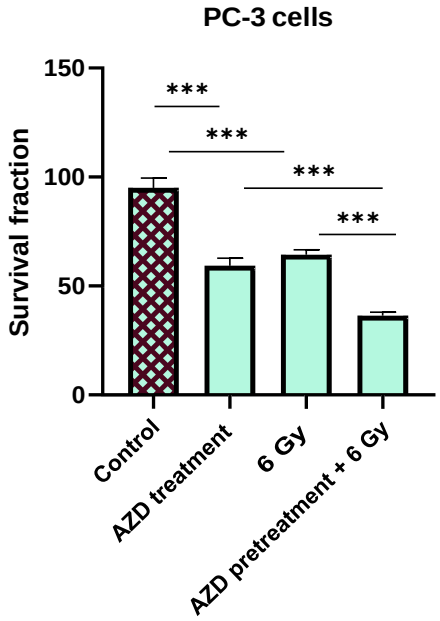


Figure 4.9: The effect of AZD1775 treatment alone and combined with IR on survival fraction of PC-3 cells. Data are expressed as mean ± SD, from five independent experiments ***p < 0.001, one-way ANOVA with post hoc Tukey's test for intergroup comparison.

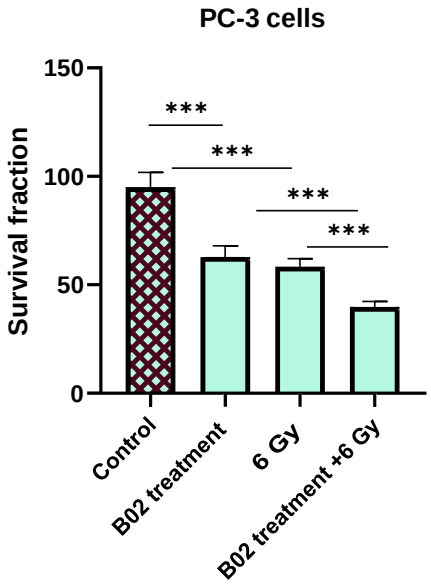


Figure 4.10: The effect of B02 treatment alone and combined with IR on survival fraction of PC-3 cells. Data are expressed as mean ± SD, from five independent experiments ***p < 0.001, one-way ANOVA with post hoc Tukey's test for intergroup comparison.

Control cells' survival fraction was calculated as 95% as average in this experiment. As shown in the Figure 4.9; AZD1775 decreased survival fraction by 32% when was treated alone on the PC-3 cells. 6 Gy IR exposure is slightly less effective than AZD1775 since it decreased survival fraction by 29% compared to the control cells. When AZD1775 was pre-treated with 6 Gy IR, the survival fraction was evaluated as 35% which is significant statistically when compared to AZD1775 or 6 Gy IR alone ($p<0.001$) which indicates that AZD1775 enhances PC-3 cells' radiosensitivity.

In Figure 4.10, B02's effect on survival fraction alone and combined with IR is shown. Control cells' survival fraction was calculated as 94% as average in this experiment. B02 decreased survival fraction by 30% alone compared to the control cells, 6 Gy IR exposure is slightly more effective alone which is decreasing survival fraction by 33%. The survival fraction is 37% when B02 was combined with 6 Gy IR, which is significantly less than both control cells, B02 and IR treatment alone ($p<0.001$). It can be said that B02 radiosensitizes prostate cancer cells to radiation.

4.5. Evaluation of the Effect of Combined Wee1 Inhibitor and Rad51 Inhibitor Treatment on PC-3 Cells

The synergism between AZD1775 and B02 was investigated by the median-effect method of Chou and Talalay (27). The combination index (CI) is defined as the degree of drug interaction as quantitatively. Based on IC_{50} values, synergistic ($CI < 1$) and antagonistic ($CI > 1$) effect were investigated between AZD1775 and B02.

For this aim, PC-3 cells were cultured for 48 hours and then treated with constant ratio of AZD1775 and B02. By using IC_{50} values of 4.9 μM and 16.3 μM of AZD1775 and B02, respectively, for 48 hours, combined drug application was performed at 1/8 IC_{50}, ¼ IC_{50}, ½ IC_{50}, IC_{50}, 2x IC_{50}, 4x IC_{50} concentrations. In this study, a 1:4 dilution was performed for AZD1775 and B02.

The percent of dead cells was determined by MTT assay which was validated by CompuSyn program. The dead/affected cell fraction (Fa) was calculated, and dose-effect curves, median effect plots, CI plots and isobolograms were drawn. (Table 4.3)

Table 4.2: CI data from CompuSyn program.

CI Data for Drug Combo: k004 (azd+b02 [1:4])

Fa	**CI Value**	**Total Dose**
0.05	0.68622	306.302
0.1	0.73816	175.606
0.15	0.77236	124.437
0.2	0.79919	96.0118
0.25	0.82209	77.5002
0.3	0.84265	64.2747
0.35	0.86180	54.2291
0.4	0.88012	46.2566
0.45	0.89806	39.7149
0.5	0.91602	34.2032
0.55	0.93436	29.4564
0.6	0.95346	25.2906
0.65	0.97382	21.5725
0.7	0.99608	18.2009
0.75	1.02120	15.0949
0.8	1.05077	12.1845
0.85	1.08777	9.40121
0.9	1.13902	6.66185
0.95	1.22718	3.81929
0.97	1.29422	2.57082

It was found CI = 0.91 with Fa=0.5 (16,06 uM of AZD and 55,81 uM of B02), CI = 1,02 with Fa=0.75 (6,2uM of AZD and 22,6 uM of B02) and CI = 1.13 with Fa = 0.9 (2.4 uM of AZD and 9.12 uM of B02).

For the 48h combined drug application, it was determined that a synergistic effect (CI<1) could be detected in the region where (Fa) was lower than 0.75, and an antagonistic effect (CI>1) could be determined for the region where Fa was higher than 0.70 (Table 4.3).

The term Dm shows of the potency a drug. Dm was found to be 16,06 μM for AZD1775 and 55,81 μM for B02. Based on CompuSyn data, it was calculated that the DRI value for AZD1775 would provide dose reduction with the use of B02 in combination with the Fa value expected to be equal to or higher than 50% for 48 h incubation (condition where the AZD1775 dose is higher than 16,06μM) (DRI>2.34). (Table 4.4)

Table 4.3: DRI data from CompuSyn program.

DRI Data for Drug Combo: k004 (azd+b02 [1:4])

Fa	Dose azd	Dose b02	DRI azd	DRI b02
0.05	205.201	632.064	3.34965	2.57941
0.1	107.508	341.415	3.06105	2.43027
0.15	72.0485	233.173	2.89498	2.34228
0.2	53.3041	174.983	2.77592	2.27815
0.25	41.5599	138.043	2.68128	2.22650
0.3	33.4389	112.216	2.60125	2.18234
0.35	27.4468	92.9702	2.53063	2.14299
0.4	22.8165	77.9635	2.46629	2.10682
0.45	19.1119	65.8539	2.40614	2.07271
0.5	16.0660	55.8146	2.34862	2.03982
0.55	13.5056	47.3058	2.29247	2.00745
0.6	11.3128	39.9581	2.23656	1.97495
0.65	9.40430	33.5083	2.17970	1.94161
0.7	7.71909	27.7615	2.12052	1.90660
0.75	6.21074	22.5673	2.05723	1.86879
0.8	4.84236	17.8033	1.98709	1.82642
0.85	3.58255	13.3603	1.90537	1.77641
0.9	2.40092	9.12458	1.80199	1.71210
0.95	1.25788	4.92873	1.64674	1.61310

Fa	Dose azd	Dose b02	DRI azd	DRI b02
0.97	0.79412	3.17993	1.54449	1.54617

4.6. Evaluation of combined effect of Wee1 Inhibitor and Rad51 Inhibitor on Cell Viability

Based on the calculated synergism data of the two inhibitors, for the experiments where the both inhibitors applied together, AZD1775's concentration was chosen as 4 µM and B02's concentration was chosen as 20 µM (even though it was higher than the inhibitor's IC_{50} value, 20 µM was the most effective concentration when combined with AZD1775).

Cell viability of the prostate cancer cells were evaluated as described before in Methods 3.2.5. Control cells' cell viability was accepted as 100% since they were the untreated group.

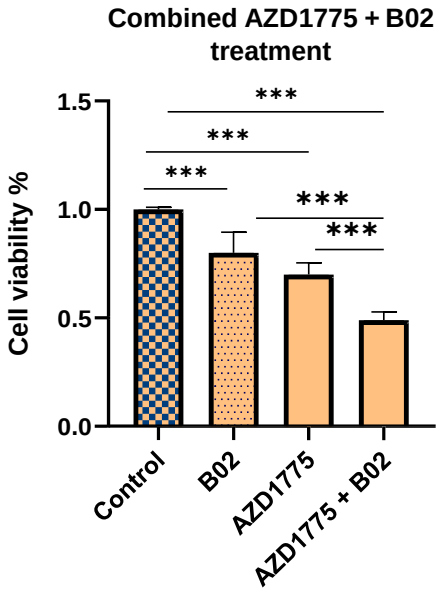


Figure 4.11: Cell viability graph of PC-3 cells treated with AZD1775 and B02 alone and in combination for 48 hours. Data are expressed as mean ± SD, from five independent experiments ***p < 0.001, one-way ANOVA with post hoc Tukey's test for intergroup comparison.

As shown in Figure 4.11; B02 decreased cell viability by 26% compared to the control cells. AZD1775 alone showed 63% cell viability. When the both inhibitors were treated to the PC-3 cells in combination, the cell viability decreased by 53%, exhibiting 47% of cell viability. The difference both inhibitors displayed alone and in combination are all statistically significant ($p<0.001$), indicating the combined effect is also successful to kill prostate cancer cells even without the IR treatment.

4.7. The Effect of Combined AZD1775 and B02 Pre-treatment on Radiosensitivity of PC-3 cells

4.7.1. Cell viability assay results

AZD1775 and B02's combined effect with irradiation was evaluated by cell viability assay (MTT assay) for 48 hours of treatment incubation time. Control cells are the untreated cells, so the cell viability is 100% for them. AZD1775's concentration was 4 μM and B02's concentration was 20 μM in these experiments. All experiments were done at least twice.

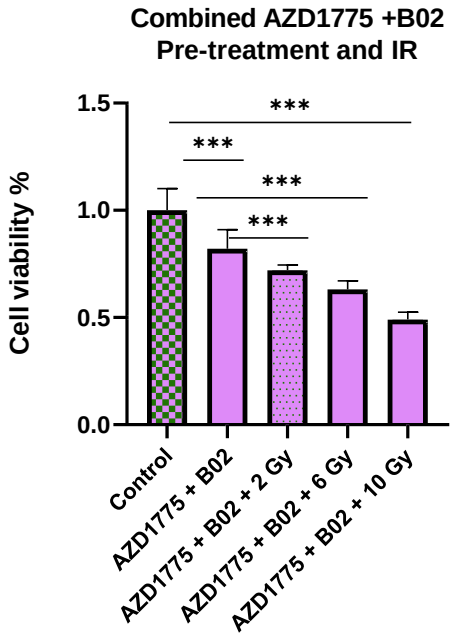


Figure 4.12: Effect of AZD1775 and B02's combination with irradiation. Data are expressed as mean ± SD, from five independent experiments ***p < 0.001, one-way ANOVA with post hoc Tukey's test for intergroup comparison.

In the Figure 4.12, it can be observed that AZD1775 and B02's combined pre-treatment decreases cell viability with IR exposure. Inhibitors combined showed 78% cell viability. When they were both pre-treated with 2, 6 and 10 Gy IR treatment, the cell viability percentages were 65%, 55% and 48% respectively. The differences between the groups and the difference compared to the control cells were significant statistically ($p<0.001$).

4.7.2. Colony formation assay results

Survival fraction of the combined inhibitors' treatment and their pre-treatment with irradiation was evaluated. All experiments were done at least twice. Control group's survival fraction was 94% averagely (Figure 4.13).

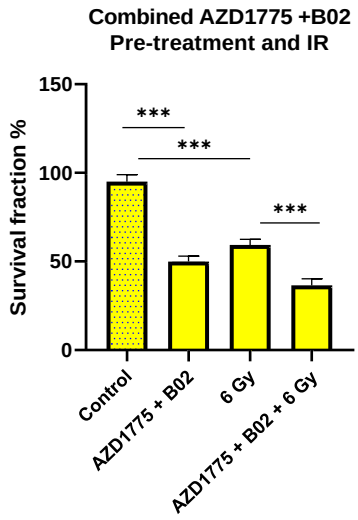


Figure 4.13: The effect of combined pre-treatment of inhibitors on radiosensitivity of PC-3 cells. Data are expressed as mean ± SD, from five independent experiments ***p < 0.001, one-way ANOVA with post hoc Tukey's test for intergroup comparison.

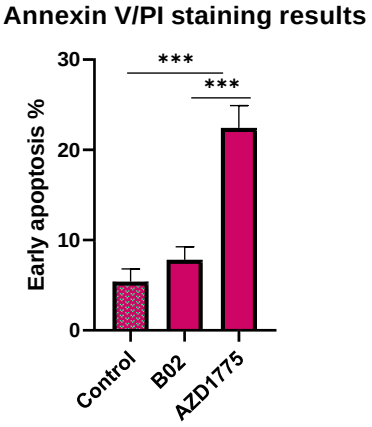
6 Gy IR decreased survival fraction by 40% alone compared to the control cells. AZD1775 and B02 combined treatment group exhibited a survival fraction of 47%.

When both of the inhibitors were treated prior to the IR exposure, survival fraction was calculated as 32%. The differences between the treatment groups compared to the control cells are statistically significant ($p<0.001$), indicating the combined treatment is highly effective.

4.8. Evaluation of the effect of AZD1775 and B02 Treatment on Apoptosis in PC-3 cells

To determine whether AZD1775 and B02 induces PC-3 cell apoptosis; the apoptotic rate was measured by flow cytometry method with Annexin V-FITC/PI staining (Figure 4.15). Since late apoptotic cells are secondary necrotic cells, early apoptotic cell percentage was more important to observe if the inhibitors can make cancer cells go to early apoptosis by alone or in combination. Seven groups were studied for determination of early apoptosis including control group, IR group, AZD1775 treatment group, B02 treatment group, AZD1775 + IR group, B02 + IR group and AZD1775 + B02 + IR group (Figure 4.14). All experiments were done at least twice.

A.



B.

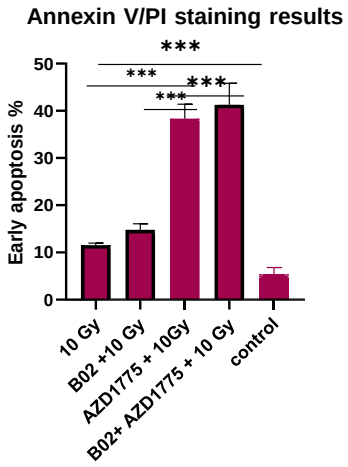


Figure 4.14: AZD1775 increases apoptosis in PC-3 cells. **A.** Individual treatments **B.** Pre-treatment with AZD1775, B02 and both. Data are expressed as mean ± SD, from five independent experiments ***p < 0.001, one-way ANOVA with post hoc Tukey's test.for intergroup comparison.

AZD1775 treatment alone showed the presence of 22% early apoptotic cells when B02 treatment gave 8% early apoptotic cells alone. Compared to the control group which showed 5% early apoptotic cells, there is no significant difference between B02 alone treatment. AZD1775 is very effective alone (Figure 4.14. A).

When compared to the control cells, 10 Gy IR exposure increases early apoptotic cells alone by 5%. When B02 treatment is combined with IR exposure, early apoptotic cells are 13%. AZD1775 treatment with IR exposure increases early apoptotic cells significantly, with a value of 35%. B02 and AZD1775 combined treatment prior to IR exposure showed a 38% of early apoptotic cells which is significantly higher compared to the control group and IR alone group. AZD1775 is more effective as a radiosensitizer compared to B02 alone according to these results (Figure 4.14. B).

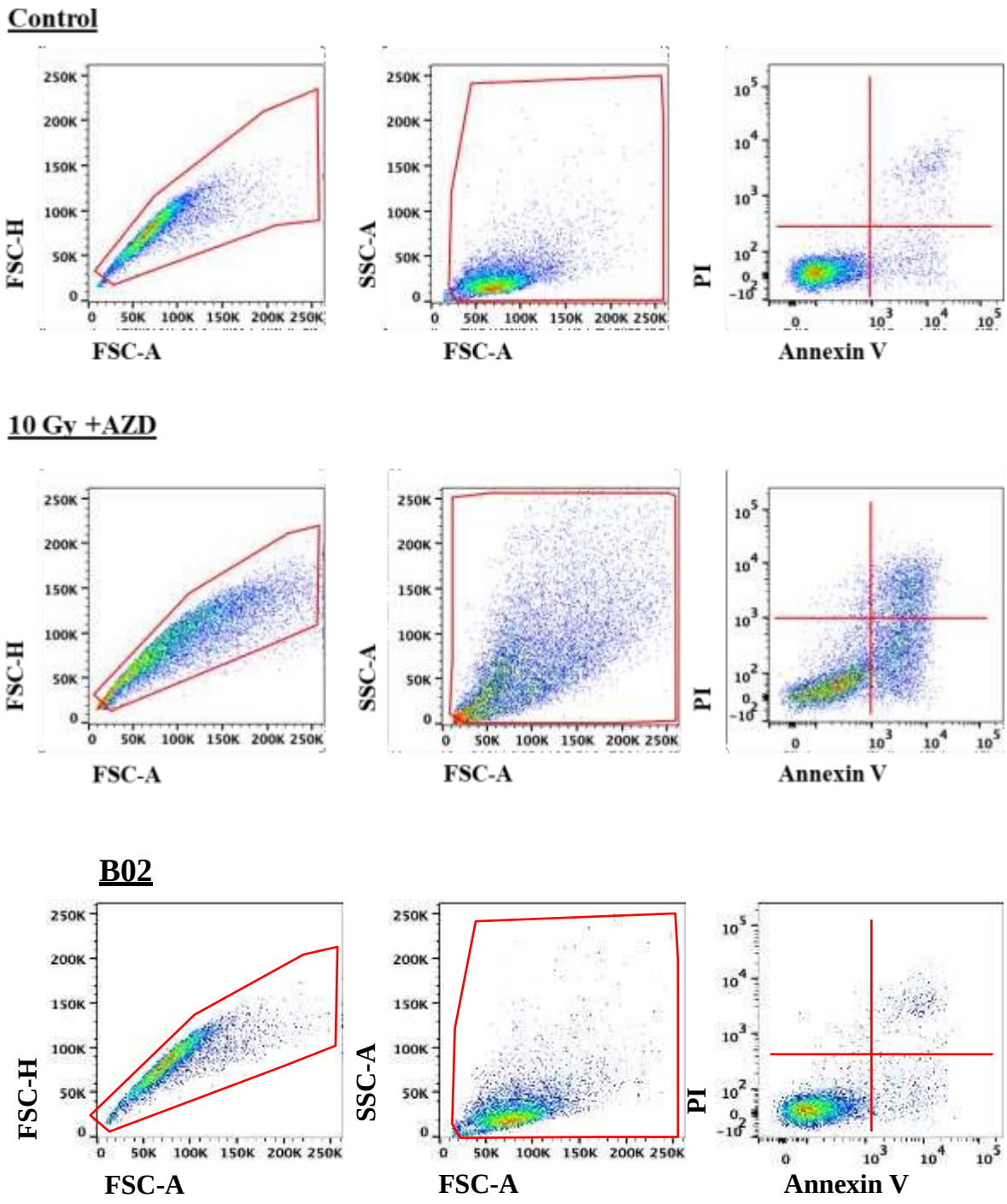


Figure 4.15: Representative histograms are shown. Gating of PC-3 cells treated with AZD1775 or B02 or IR exposure compare to control cells.

5. DISCUSSION

Prostate cancer is one of the most common cancers in males. Radiotherapy is often used as treatment along with chemotherapy. Radiotherapy's function as creating DNA damage on cancer cells makes it the target for studies to enhance the effect of it. Therefore, there have been studies on chemotherapeutics and some DDR inhibitors to radiosensitize tumor cells.

DDR inhibitors have been the interest of such studies these past years since stopping the cell response to DNA damages would be more effective on the treatment that's aiming mechanism is through DNA damages. Studies on inhibiting DDR elements such as PARP, ATM/ATR, Chk1 and Chk2 have been done on different cancer types either as a sole target or combining it with other therapeutics and/or radiotherapy (70-80). The results so far have shown that targeting DDR mechanism is promising for radiosensitizing tumor cells, so that we can say radiotherapy would be more effective as a treatment in the future for cancers that have radiotherapy in their treatment regimen.

Targeted therapy by small molecule inhibitors with irradiation has been suggested a new strategy for some cancer types to improve treatment regimen including head and neck, pancreas, esophagus, prostate and cervical cancers in particular the cancers treated with irradiation. In this study we tested the enhancer effect of WEE1 inhibitor and RAD51 inhibitor on efficacy of irradiation on prostate cancer cells in vitro. We showed that:

1. inhibition of Wee1 remarkably increased the efficiency of irradiation on prostate cancer cells,
2. inhibition of RAD51 increased the efficacy of irradiation on prostate cancer cells, but it was lower than that of Wee1 inhibition, so both act as radiosensitizer,
3. and Wee1 inhibitor and RAD51 inhibitor showed a significant antitumor effect alone and synergistic antitumor effect when combined on prostate cancer cells.

To our knowledge, this is the first study to detect the anticancer effects of AZD1775 and B02, alone and in combination, in prostate cells in vitro.

In this study, PC-3 cells were exposed to IR with or without pretreatment of inhibitors. The typical properties of PC-3 cells are being p53 deficient cells, which means that only G2/M checkpoint is functional following exposure to irradiation to repair DNA damage. However, pretreatment with inhibitors eliminates this effect. In this study, we found that Wee1 inhibitor led to inhibition of PC-3 cell growth and colony formation. Bridges et al showed that Wee1inhibitor MK-1775 abrogated G2/M checkpoint induced by irradiation in p53-defective lung cancer cells (H1299) (140). It was shown that inhibition of Wee1 led to sensitize HPV-positive head and neck squamous cell carcinoma (HNSCC) to cytotoxic agents, including cisplatin since Wee1 is crucial for cell survival in p53 mutant HNSCC cells (141). These results were comparable with our results.

Small molecule inhibitors took place in cancer therapy since 2000s as proposed by clinical guidelines. Higher than eighty agents, approved by FDA are on the market (142). Most of small molecule inhibitors belong to protein kinase inhibitors. such as Wee1 inhibitors. Selective small molecule inhibitors bind to the target protein and inhibit down-stream cell signaling which are related to cell growth, differentiation, apoptosis, immune system regulation (143).

It was reported that the expression of Wee1 in prostate cancer cells is low. This allows them to be more sensitive to DNA-damaging agents, which is a treatment strategy for today for prostate cancer. It may be used as a monotherapy or in combination with other chemotherapeutics or radiation therapy. Furthermore, it can be more effective in cancer cells that have chromosomal instability (144)

It was also shown that Wee1 protein was not expressed in 66% of patients with non–small cell lung cancer (145). Wee1-negative NSCLC are associated with poor survival and high recurrence rate (146).

Wee1 inhibitors are selective inhibitor, that bind to ATP binding pocket of the protein and inhibits phosphorylation. It may contain a pyrimidine core or not (147).

The first approved small molecule inhibitor of Wee1 kinase was AZD1775 (MK1775), which is a powerful agent. It inhibits Wee1 kinase selectively. It binds to ATP binding site and blocks phosphorylation. Many clinical trials reported the on different cancer cells (148).

Bi et al showed the antitumor effect of AZD1775 on human esophageal cancer cells (ESCC). They suggested that premature mitosis plays an important role and increased apoptosis in ESCC cells in time and dose -dependent manner (149).

The present study showed that Wee1 inhibition repressed PC-3 cell proliferation and survival and also colony formation. It led to entering premature apoptosis. Murrow et al reported that Wee1 inhibition as monotherapy, reduced cell viability, and induced apoptosis in ER positive, HER positive, and triple-negative breast cancer cell lines (150) It was also shown that a Wee1 kinase inhibitor reduced viability and induced apoptosis in breast and cervical cancer cells (151) both are compatible with our results.

Cancer cells with genomic instability, those are p53 deficient cells, need Wee1 for survival. Wee1 can be considered a cancer conserving oncogene, inhibition of which holds potential as an effective sensitizer in combination with DNA-damaging therapy.

In early studies it was reported that MK1775 enhanced the cytotoxic effects of 5-fluorouracil (5-FU) at in p53-deficient colon cancer cells and pancreatic cancer cells (151).

It was also demonstrated that Wee1 inhibition by MK1775 at nanomolar concentrations in combination with gemcitabine induced premature mitotic entry and cell death in p53-deficient colon and lung carcinoma cells. (152)

Recent studies showed that Wee1 inhibition combined with ATR inhibition caused the decrease of cell proliferation in acute myeloid leukemia (AML) cell lines, which was associated with increased replication stress and DNA damage (153).

We demonstrated that Wee1 inhibition increased the sensitivity of PC-3 cells to irradiation. Karnak et al reported that Wee1 inhibition decreased pCdk11 and therefore increased the radiosensitivity of pancreatic cells (154). It was shown that Wee1 kinase inhibitor PD0166285 caused cell death in glioblastoma cell lines in combination with irradiation or the alkylating agents. but not in normal human fibroblasts and astrocytes (155). Lee et al reported that Wee1 inhibition in combination with irradiation led to diminish of clonogenic survival and increasing of apoptosis in cervical cancer cells that was convenient to our results (155).

Additionally, it was reported that AZD1775 radio-sensitizes oral tongue squamous cell carcinoma, irrespective of TP53 status. (157)

In this study, we demonstrated that the AZD1775 strongly inhibits Wee1 kinase and significantly increased the efficacy of RT, so act as a radiosensitizer in PC-3 cells. The inhibitory effect of Wee1 kinase inhibitor (Figure 5.1.) and IR on PC-3 cells was higher than that of monotherapy. Briefly, we can suggest that Wee1 inhibitor, AZD1775, abrogates the radiation induced G2/M block and allow the prostate cells to enter premature mitosis and then undergo to apoptosis, that we determined by flow cytometry.

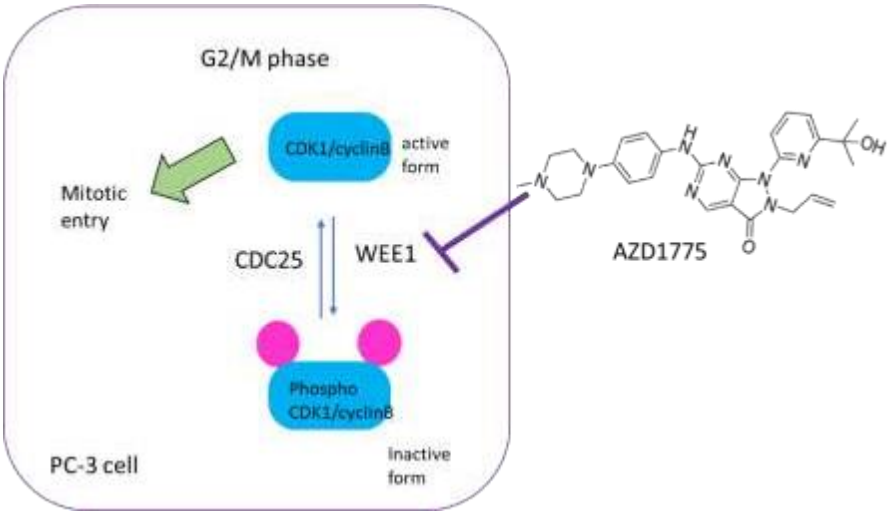


Figure 5.1: AZD1775 inhibiting Wee1 mechanism

HR is essential for genomic integrity. If the DNA damage can not be repaired it leads to apoptosis or mitotic catastrophe. Targeting Rad51, an important player in HR, is an promising therapy regimen. In this study we investigated the effect of Rad51 inhibitor on the prostate cancer cell survival and on their IR sensitivity.

In some cancer cells, such as pancreatic and triple-negative breast cancer high expression levels of Rad51 were observed (158), which are associated with resistant to chemotherapeutics and irradiation and poor prognosis for survival (159). It was shown that the median survival of colon cancer patients with high Rad51 tumor expression is short than that of patients with weak expression (160) so diminish Rad51 expression is required to increase the sensitivity of cancer cells to chemotherapeutics (160)

Additionally, knockdown mouse models of Rad51 showed diminish metastasis rate in breast cancer (161). It was shown that Rad51 knockdown led to increase the sensitivity of cancer cells to DNA-damaging agents (162).

Therefore, Rad51 is an important target for anti-cancer therapies (163). Many small-molecule inhibitors for Rad51 have been developed (164).

Chemical inhibitors of Rad51 (e.g., B02, IBR2, RI-1/2) have been reported to either interfere with Rad51 oligomerization, filament formation or DNA binding, and, ultimately, to induce HR deficiency (160-164).

B02 is a small molecule inhibitor for RAD51. B02, ((E)-3-benzyl-2-(2-(pyridin-3-yl) vinyl) quinazolin-4(3H)- one) specifically inhibited binding Rad51 to ssDNA, and therefore Rad51-mediated DNA strand exchange activity and homologues recombination.

Huang et al also showed that B02 enhanced the effect of cisplatin on triple-negative breast cancer cells in mouse xenograft model (165) Treatment with 4 mg/kg cisplatin caused a 33% inhibition of tumor growth, whereas treatment with 50 mg/kg B02 and 4 mg/kg cisplatin led to a 66% inhibition of tumor growth.

Wiegmans et al. (2016) demonstrated that the combination of B02, a PARP inhibitor and a p38 kinase inhibitor significantly reduced tumor growth in triple negative breast cancer in xenograft model (166), all of them were consistent to our results.

Lim et al reported that homologous recombination abnormalities and also cell cycle checkpoint abnormalities may lead to the radioresistance of glioma cells; so those were suggested as appropriate targets for therapy. then we examined the effects of WEE1 inhibitor and RAD51 inhibitor alone and in combination on radiation sensitivity in PC-3 cells.

In this study B02 treatment alone showed the modest effect on PC-3 cell growth ($p < 0.05$), but increases IR effect. Huang et al reported that B02 increased sensitivity of cancer cells to IR as well as MMC (Huang et al., 2012), which was compatible to our results.

King et al (167) investigated RAD51 expression in glioblastoma stem cells and found that small-molecule inhibitors for RAD51 were effective GSC radiosensitizers

and suggested that. RAD51-dependent repair was effective, so could be used specific target.

Recent study (168) showed that high Rad51 mRNA expression in head and neck squamous cell carcinoma (HNSCC) cell line associated with worse survival in particular HPV-positive types.

Rad51 is essential part of HR for genome stability. Therefore, we can suggest that Rad51 inhibitor, B02, can inhibit homologous recombination by preventing Rad51 focus formation (Figure 5.2.), so double strand breaks can not be repaired and causes cell cycle arrest in S phase. Therefore, the inhibitor enhances PC-3 radiosensitization.

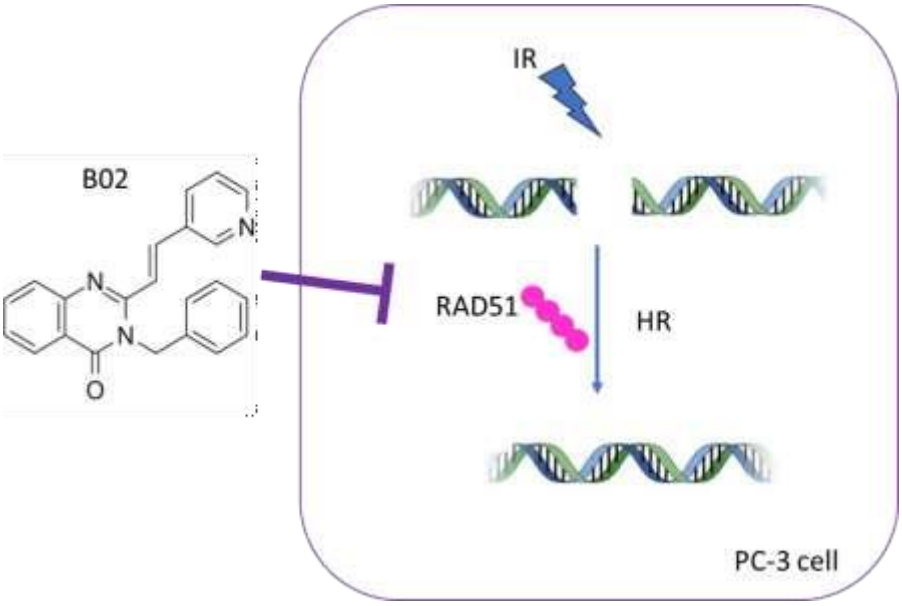


Figure 5.2: B02 inhibiting Rad51 mechanism

Our results indicate that Rad51-dependent HR repair of DNA may be suggested as a specific target for PC-3 cells. Rad51 inhibition might be efficient to sensitize these cells to ionizing radiation. This is constant with the results of Lim et al whom

investigated the role of HR in glioma cells and suggested as a new approach to increase cell survival (169).

Since ionizing radiation are often associated with developing radio-resistance during treatment, using recombinase inhibitor and cell cycle checkpoint inhibitor in combination is an alternate treatment method. In the present study we tested the synergistic inhibition role of Rad51 recombinase inhibitor and Wee1 inhibitor on human prostate carcinoma cell proliferation.

Combination of B02 and AZD1775 demonstrated modest effect on tumor growth in PC-3 cells. Our results showed that there was a synergism B02 in combination with AZD1775 in vitro Fa was higher than 0.50. this is important for anticancer drugs, because higher Fa levels lead to more effective results in that combination.

Combination of these drugs significantly inhibited tumor growth, when compared with single AZD1775 ($p<0.001$), single B02 ($p<0.05$) therapy, in PC-3 cells. Furthermore, combined treatment with B02 and AZD1775 improved cell survival compared with controls and single treatment groups.

Rad51 and Wee1 are involved in different cellular response to DNA damage. In our study when we combined Rad51 and Wee1 inhibition, more DNA damage was induced, but the cells could not respond to and repair it. We also showed that the combination of a Rad51 inhibitor and a Wee1 inhibitor led to antiproliferative effect in vitro.

There is no data to show combined Rad51 inhibition and Wee1 inhibition led to enhanced cytotoxicity in p53 deficient PC-3 cells when treated with IR yet. So these results are valuable.

In a previous study, Havelek et al. (170) examined the effects of Wee1 kinase II inhibitor (681641) and Rad51 inhibitor (RI-1) on cell cycle progression and apoptosis in human leukemic T-cells after exposure to ionizing radiation. They found that pre-

treatment with RI-1 had no effect on apoptosis stimulation whereas 681641 enhanced ionizing radiation-induced cell death.

Lindeman et al (171) reported that the combination of B02 and AZD1775 significantly inhibited tumor growth in vivo in mice with HPV-positive head and neck squamous cell carcinoma. They also showed high synergistic effect of B02 and AZD1775 in HNSCC cells in vitro by clonogenic survival assay and suggested that the Cdk1 stimulation leads to extreme DNA damage and replication stress, resulted in early mitosis and apoptosis. These results are compatible with our findings.

In the present study, we demonstrated for the first time that a combination of Rad51 inhibitor and Wee1 inhibitor synergistically inhibited the proliferation of PC-3 cancer cells. Both inhibitors stopped the S phase arrest and G2/M arrest, respectively in PC-3 cells. It was concluded that this combination may be promising as an effective treatment strategy for prostate cancer.

In conclusion, the evidence of the study showed the first time that targeting homologous recombination by Rad51 recombinase inhibitor and G2/M checkpoint by Wee1 kinase inhibitor, as a single or combined therapy, may be a novel strategy to improve the clinical outcome in CRPC. Both can be used to create the radiosensitizer effect in prostate cancer cells.

6. CONCLUSION AND SUGGESTIONS

AZD1775's and B02's IC_{50} values are found as 4,9 μM and 16,29 μM respectively for the PC-3 prostate cancer cell line. According to the CI results, the used concentration of AZD1775 was 4 μM and B02's was 20 μM when they were treated as a combination. Cell viability assay results show that AZD1775 and B02 both decreases cell viability of prostate cancer cells solely when compared to the untreated cells. Their effects on cell viability when they're treated per se is similar to only IR treatments. AZD1775 and B02's combined treatment on the cells decreases cell viability more than the sole inhibitors. AZD1775 and B02 increases radiosensitivity of PC-3 cells separately, as well as their combination does. AZD1775 and B02 combination effect on radiosensitivity is observed significantly in this study, too. Survival fractions from colony formation assay shows similar outcomes to the cell viability assay. Apoptosis assay via flow cytometry showed that AZD1775 and B02 helps prostate cancer cells undergo early apoptosis; when these inhibitors were treated before IR, early apoptotic cell percentage increased.

The evidence of the study showed the first time that targeting homologous recombination by RAD51 recombinase inhibitor and G2/M checkpoint by WEE1 kinase inhibitor, as a single or combined therapy, may be a novel strategy to improve the clinical outcome in CRPC. Both can be used to create the radiosensitizer effect in prostate cancer cells.

For the further experiments, the combination effect can be observed on other prostate cancer cell lines and the results can be compared to see the outcomes when cell lines are not AR-independent or p53-defected. To assess the DNA damage properly; DNA damage and repair assay and cell cycle assay can be done by flow cytometry. Incubation times can be changed to observe its' effect on cell viability. We treated cells with inhibitors 1 hour prior to IR treatment in this study; the pre-treatment time can be changed to see if it affects the results or not. Moreover, in vivo studies can be done to see combination effect on the tumor itself.

7. REFERENCES

1. Seisen T, Rouprêt M, Faix A, et al (2012). The prostate gland: a crossroad between the urinary and the seminal tracts. Prog Urol, 22, 66-80.
2. Barrett, Kim E.; Barman, Susan M.; Brooks, Heddwen L.; Yuan, Jason X.-J.; Ganong, William F. (2019). *Ganong's review of medical physiology* (26th ed.). New York: McGraw-Hill Education. pp. 411, 415.
3. European Union. Prostate cancer burden in EU-27. 2021.
4. EAU guideline 2024
5. Parker C, Castro E, Fizazi K, Heidenreich A, Ost P, Procopio G, et al. Prostate cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020 Sep. 31 (9):1119-113
6. Bell, K.J., et al. Prevalence of incidental prostate cancer: A systematic review of autopsy studies. Int J Cancer, 2015. 137: 174
7. TR Ministry of Health, General Directorate of Public Health, Türkiye Cancer Statistics 2018
8. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D.M.; Forman, D.; Bray, F. Cancer Incidence and Mortality Worldwide: Sources, Methods and Major Patterns in GLOBOCAN 2012. Int. J. Cancer 2015, 136, E359–E386. [CrossRef]
9. Siegel, R.L.; Miller, K.D.; Wagle, N.S.; Jemal, A. Cancer Statistics, 2023. CA. Cancer J. Clin. 2023, 73, 17–48. [CrossRef]
10. [Guideline] American Cancer Society Recommendations for Prostate Cancer Early Detection. American Cancer Society. Available at https://www.cancer.org/Cancer/ProstateCancer/MoreInformation/ProstateCancerEarlyDetection/prostate-cancer-early-detection-acs-recommendations. February 24, 2023; Accessed: May 28, 2023.
11. 16. Karami, S., et al. Earlier age at diagnosis: another dimension in cancer disparity? Cancer Detect Prev, 2007. 31: 29.
12. Hemminki, K. Familial risk and familial survival in prostate cancer. World J Urol, 2012. 30: It was found
13. Beebe-Dimmer, J.L., et al. Risk of Prostate Cancer Associated With Familial and Hereditary Cancer Syndromes. J Clin Oncol, 2020. 38: 1807
14. Amin Al Olama, A., et al. Multiple novel prostate cancer susceptibility signals identified by finemapping of known risk loci among Europeans. Hum Mol Genet, 2015. 24: 5589.
15. Gulati, R., et al. Screening Men at Increased Risk for Prostate Cancer Diagnosis: Model Estimates of Benefits and Harms. Cancer Epidemiol Biomarkers Prev, 2017. 26: 222.
16. Giri, V.N., et al. Germline genetic testing for inherited prostate cancer in practice: Implications for genetic testing, precision therapy, and cascade testing. Prostate, 2019. 79: 333. https://pubmed.ncbi.nlm.nih.gov/30450585 27. Pritchard, C.C., et

al. Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer. N Engl J Med, 2016. 375: 443.
17. Castro, E., et al. PROREPAIR-B: A Prospective Cohort Study of the Impact of Germline DNA Repair Mutations on the Outcomes of Patients With Metastatic Castration-Resistant Prostate Cancer. J Clin Oncol, 2019. 37: 490.
18. Leitzmann, M.F., et al. Risk factors for the onset of prostatic cancer: age, location, and behavioral correlates. Clin Epidemiol, 2012. 4: 1.
19. Scher, H.I.; Halabi, S.; Tannock, I.; Morris, M.J.; Sternberg, C.N.; Carducci, M.A.; Eisenberger, M.A.; Higano, C.; Bubley, G.J.; Dreicer, R.; et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: Recommendations of the Prostate Cancer Clinical Trials Working Group. *J. Clin. Oncol.* **2008**, *26,* 1148–1159
20. Kirby M, Hirst C, Crawford ED. Characterising the castration- resistant prostate cancer population: a systematic review. Int J Clin Pract 2011;65:1180–92.
21. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–249. doi:10.3322/caac.21660
22. Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res. 1941;1:293–297.
23. Taplin, M.-E.; Bubley, G.J.; Shuster, T.D.; Frantz, M.E.; Spooner, A.E.; Ogata, G.K.; Keer, H.N.; Balk, S.P. Mutation of the Androgen-Receptor Gene in Metastatic Androgen-Independent Prostate Cancer. N. Engl. J. Med. 1995, 332, 1393–1398. [CrossRef] [PubMed]
24. Newmark,J.R.; Hardy, D.O.; Tonb, D.C.; Carter, B.S.; Epstein, J.I.; Isaacs, W.B.; Brown, T.R.; Barrack, E.R. Androgen Receptor Gene Mutations in Human Prostate Cancer. Proc. Natl. Acad. Sci. USA 1992, 89, 6319–6323. [CrossRef] [PubMed]
25. Wyatt, A.W.; Gleave, M.E. Targeting the Adaptive Molecular Landscape of Castration-Resistant Prostate Cancer. EMBO Mol. Med. 2015, 7, 878–894. [CrossRef] [PubMed]
26. Hörnberg, E.; Ylitalo, E.B.; Crnalic, S.; Antti, H.; Stattin, P.; Widmark, A.; Bergh, A.; Wikström, P. Expression of Androgen Receptor Splice Variants in Prostate Cancer Bone Metastases is Associated with Castration-Resistance and Short Survival. PLoS ONE 2011, 6, e19059
27. Liu W, Xie CC, Zhu Y, et al. Homozygous deletions and recurrent amplifications implicate new genes involved in prostate cancer. Neoplasia 2008;10:897-907.
28. Gregory CW, Johnson RT Jr, Mohler JL, et al. Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. Cancer Res 2001;61:2892-8.
29. Feldman, B.J.; Feldman, D. The development of androgen-independent prostate cancer. Nat. Rev. Cancer 2001, 1, 34–45.
30. Saraon, P.; Jarvi, K.; Diamandis, E.P. Molecular Alterations during Progression of Prostate Cancer to Androgen Independence. Clin. Chem. 2011, 57, 1366–1375. [CrossRef]

31. Powell, S.M.; Christiaens, V.; Voulgaraki, D.; Waxman, J.; Claessens, F.; Bevan, C.L. Mechanisms of Androgen Receptor Signalling via Steroid Receptor Coactivator-1 in Prostate. Endocr. Relat. Cancer 2004, 11, 117–130. [CrossRef]
32. Zhou,X.E.; Suino-Powell, K.M.; Li, J.; He, Y.; Mackeigan, J.P.; Melcher, K.; Yong, E.-L.; Xu, H.E. Identification of SRC3/AIB1 as a Preferred Coactivator for Hormone-Activated Androgen Receptor. J. Biol. Chem. 2010, 285, 9161–9171.
33. Nacusi, L.P.; Tindall, D.J. Targeting 5-Reductase for Prostate Cancer Prevention and Treatment. Nat. Rev. Urol. 2011, 8, 378–384.
34. Culig, Z.; Hobisch, A.; Cronauer, M.V.; Radmayr, C.; Trapman, J.; Hittmair, A.; Bartsch, G.; Klocker, H. Androgen Receptor Activation in Prostatic Tumor Cell Lines by Insulin-Like Growth Factor-1,Keratinocyte Growth Factor and Epidermal Growth Factor. Eur. Urol. 2017, 27, 45–47.
35. Malinowska, K.; Neuwirt, H.; Cavarretta, I.T.; Bektic, J.; Steiner, H.; Dietrich, H.; Moser, P.L.; Fuchs, D.; Hobisch, A.; Culig, Z. Interleukin-6 Stimulation of Growth of Prostate Cancer in Vitro and in Vivo through Activation of the Androgen Receptor. Endocr. Relat. Cancer 2009, 16, 155–169.
36. Saraon, P.; Drabovich, A.P.; Jarvi, K.A.; Diamandis, E.P. Mechanisms of Androgen-Independent Prostate Cancer. EJIFCC 2014, 25, 42–54.
37. Wen, Y.; Hu, M.C.; Makino, K.; Spohn, B.; Bartholomeusz, G.; Yan, D.H.; Hung, M.C. HER-2/Neu Promotes Androgen Independent Survival and Growth of Prostate Cancer Cells through the Akt Pathway. Cancer Res. 2000, 60, 6841–6845.
38. Graff, J.R.; Konicek, B.W.; McNulty, A.M.; Wang, Z.; Houck, K.; Allen, S.; Paul, J.D.; Hbaiu, A.; Goode, R.G.; Sandusky, G.E.; et al. Increased AKT Activity Contributes to Prostate Cancer Progression by Dramatically Accelerating Prostate Tumor Growth and Diminishing p27Kip1 Expression. J. Biol. Chem. 2000, 275, 24500–24505
39. Lavallee LT, Binette A, Witiuk K, et al. Reducing the harm of prostate cancer screening: repeated prostate-specific antigen testing. Mayo Clin Proc 2016;91:17-22.
40. Thompson IM, Pauler DK, Goodman PJ, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. N Engl J Med 2004;350:2239-2246.
41. Thompson IM, Ankerst DP, Chi C, et al. Operating characteristics of prostate-specific antigen in men with an initial PSA level of 3.0 ng/ml or lower. JAMA 2005;294:66-70.
42. Rebbeck TR, Devesa SS, Chang BL, et al. Global patterns of prostate cancer incidence, aggressiveness, and mortality in men of African descent. Prostate Cancer 2013;2013:1–12.
43. Dinnes, J.; Ferrante di Ruffano, L.; Takwoingi, Y.; Cheung, S.T.; Nathan, P.; Matin, R.N.; Chuchu, N.; Chan, S.A.; Durack, A.; Bayliss, S.E.; et al. Ultrasound, CT, MRI, or PET-CT for Staging and Re-Staging of Adults with Cutaneous Melanoma. Cochrane Database Syst. Rev. 2019, 7, CD012806. [CrossRef]
44. Ceci, F.; Fiorentino, M.; Castellucci, P.; Fanti, S. Molecular Imaging and Precision Medicine in Prostate Cancer. PET Clin. 2017, 12, 83–92. [CrossRef]

45. Pianou, N.K.; Stavrou, P.Z.; Vlontzou, E.; Rondogianni, P.; Exarhos, D.N.; Datseris, I.E. More Advantages in Detecting Bone and Soft Tissue Metastases from Prostate Cancer Using 18F-PSMA PET/CT. Hell. J. Nucl. Med. 2019, 22, 6–9.
46. Kweldam,C.F.; van Leenders, G.J.; van der Kwast, T. Grading of Prostate Cancer: A Work in Progress. Histopathology 2019, 74, 146–160.
47. Gleason, D.F. Histologic Grading of Prostate Cancer: A Perspective. Hum. Pathol. 1992, 23, 273–279.
48. Stark, J.R.; Perner, S.; Stampfer, M.J.; Sinnott, J.A.; Finn, S.; Eisenstein, A.S.; Ma, J.; Fiorentino, M.; Kurth, T.; Loda, M.; et al. Gleason Score and Lethal Prostate Cancer: Does 3 + 4 = 4 + 3? J. Clin. Oncol. 2009, 27, 3459–3464.
49. Humphrey,P.A.; Moch, H.; Cubilla, A.L.; Ulbright, T.M.; Reuter, V.E. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs—Part B: Prostate and Bladder Tumours. Eur. Urol. 2016, 70, 106–119. 4
50. Sumanasuriya S, De Bono J. Treatment of advanced prostate cancer-are view of current therapies and future promise. Cold Spring Harb Perspect Med. 2018;8(6).
51. Yap TA, Smith AD, Ferraldeschi R, Al-Lazikani B, Workman P, de Bono JS. Drug discovery in advanced prostate cancer: translating biology into therapy. Nat Rev Drug Discov. 2016;15(10):699–718.
52. Heidenreich, A.; Bastian, P.J.; Bellmunt, J.; Bolla, M.; Joniau, S.; van der Kwast, T.; Mason, M.; Matveev, V.; Wiegel, T.; Zattoni, F.; et al. EAU Guidelines on Prostate Cancer. Part II: Treatment of Advanced, Relapsing, and Castration-Resistant Prostate Cancer. Eur. Urol. 2014, 65, 467–479. [CrossRef] [PubMed]
53. Cookson, M.S.; Roth, B.J.; Dahm, P.; Engstrom, C.; Freedland, S.J.; Hussain, M.; Lin, D.W.; Lowrance, W.T.; Murad, M.H.; Oh,W.K.; et al. Castration-Resistant Prostate Cancer: AUA Guideline. J. Urol. 2013, 190, 429–438. [CrossRef] [PubMed]
54. Mohler, J.L.; Kantoff, P.W.; Armstrong, A.J.; Bahnson, R.R.; Cohen, M.; D'Amico, A.V.; Eastham, J.A.; Enke, C.A.; Farrington, T.A.;Higano, C.S.; et al. Prostate Cancer, Version 1.2014. J. Natl. Compr. Cancer Netw. 2013, 11, 1471–1479. [CrossRef] [PubMed]
55. Merseburger, A.S.; Hammerer, P.; Rozet, F.; Roumeguère, T.; Caffo, O.; da Silva, F.C.; Alcaraz, A. Androgen Deprivation Therapy in Castrate-Resistant Prostate Cancer: How Important Is GnRH Agonist Backbone Therapy? World J. Urol. 2015, 33, 1079–1085. [CrossRef]
56. Martin, S.K.; Kyprianou, N. Chapter Three—Exploitation of the Androgen Receptor to Overcome Taxane Resistance in Advanced Prostate Cancer. In Advances in Cancer Research; Fisher, P.B., Tew, K.D., Eds.; Academic Press: Cambridge, MA, USA, 2015; Volume 127, pp. 123–158
57. Cai M, Song XL, Li XA, et al. Current therapy and drug resistance in metastatic castration-resistant prostate cancer. Drug Resist Updates 2023;68:100962.
58. Seruga, B.; Tannock, I.F. Chemotherapy-Based Treatment for Castration-Resistant Prostate Cancer. J. Clin. Oncol. 2011, 29, 3686–3694.y

59. Hager, S.; Ackermann, C.J.; Joerger, M.; Gillessen, S.; Omlin, A. Anti-Tumour Activity of Platinum Compounds in Advanced Prostate Cancer—A Systematic Literature Review. Ann. Oncol. 2016, 27, 975–984.
60. Rehman,L.U.; Nisar, M.H.; Fatima, W.; Sarfraz, A.; Azeem, N.; Sarfraz, Z.; Robles-Velasco, K.; Cherrez-Ojeda, I. Immunotherapy for Prostate Cancer: A Current Systematic Review and Patient Centric Perspectives. J. Clin. Med. 2023, 12, 1446.
61. Kantoff, P.W.; Higano, C.S.; Shore, N.D.; Berger, E.R.; Small, E.J.; Penson, D.F.; Redfern, C.H.; Ferrari, A.C.; Dreicer, R.; Sims, R.B.; et al. Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer. N. Engl. J. Med. 2010, 363, 411–4224
62. Iranzo, P.; Callejo, A.; Assaf, J.D.; Molina, G.; Lopez, D.E.; Garcia-Illescas, D.; Pardo, N.; Navarro, A.; Martinez-Marti, A.; Cedres,S.; et al. Overview of Checkpoint Inhibitors Mechanism of Action: Role of Immune-Related Adverse Events and Their Treatment on Progression of Underlying Cancer. Front. Med. 2022, 9, 875974.
63. Israeli, R.S.; Powell, C.T.; Corr, J.G.; Fair, W.R.; Heston, W.D. Expression of the Prostate-Specific Membrane Antigen. Cancer Res. 1994, 54, 1807–1811.
64. Gupta, N.; Devgan, A.; Bansal, I.; Olsavsky, T.D.; Li, S.; Abdelbaki, A.; Kumar, Y. Usefulness of Radium-223 in Patients with Bone Metastases. Proc. Bayl. Univ. Med. Cent. 2017, 30, 424–426.
65. Parker, C.; Nilsson, S.; Heinrich, D.; Helle, S.I.; O'Sullivan, J.M.; Fosså, S.D.; Chodacki, A.; Wiechno, P.; Logue, J.; Seke, M.; et al. Alpha Emitter Radium-223 and Survival in Metastatic Prostate Cancer. N. Engl. J. Med. 2013, 369, 213–223.4
66. Congregado, B.; Rivero, I.; Osmán, I.; Sáez, C.; López, R.M. PARP Inhibitors: A New Horizon for Patients with Prostate Cancer.Biomedicines 2022, 10, 1416.
67. De Bono, J.; Mateo, J.; Fizazi, K.; Saad, F.; Shore, N.; Sandhu, S.; Chi, K.N.; Sartor, O.; Agarwal, N.; Olmos, D.; et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. N. Engl. J. Med. 2020, 382, 2091–2102.
68. US Food and Drug Administration. FDA Approves Olaparib with Abiraterone and Prednisone (or Prednisolone) for BRCA-Mutated Metastatic Castration-Resistant Prostate Cancer; US Food and Drug Administration: Silver Spring, MD, USA, 2023.
69. F. Hutchinson, ―Chemical changes induced in DNA by ionizing radiation,‖ Progress in Nucleic Acid Research and Molecular Biology, 1985.vol. 32, pp. 115–154,
70. Goodhead HT. Initial events in the cellular effects of ionizing radiations : clustered damage in DNA Int. J. Radiat. Biol., 1994, Vol. 65, No. 1, 7-17
71. Pearson, Dustin D., Provencher, Luc, PeterBrownlee, M., and Goodarzi, Aaron A. (2021). ―Modern Sources of Environmental Ionizing Radiation Exposure and Associated Health Consequences,‖ in Genome Stability. 2nd Editio (Cambridge, US: Academic Press), 603–619. doi:10.1016/b978-0-323 85679-9.00032-5

72. Asaithamby, A., Hu,B., and Chen, D.J.(2011).UnrepairedClustered DNALesions Induce Chromosome Breakage in Human Cells. Proc. Natl. Acad. Sci. U.S.A. 108 (20), 8293–8298. doi:10.1073/pnas.1016045108
73. Bee, L., Fabris, S., Cherubini, R., Mognato, M., and Celotti, L. (2013). The Efficiency of Homologous Recombination and Non-homologous End Joining Systems in Repairing Double-Strand Breaks during Cell Cycle Progression. PLoS ONE 8 (7), e69061. doi:10.1371/journal.pone.006906
74. Friedberg, Errol C., Walker, Graham C., Siede, Wolfram, Wood, Richard D., Schultz, Roger A., and Ellenberger, Tom (2006). ‑DNA Damage,‖ in DNA Repair and Mutagenesis. 2nd ed. (Washington D.C.: ASM Press), 9–69.
75. Polo SE, Jackson SP. Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. Genes Dev 2011;25:409–33.
76. Uziel T, Lerenthal Y, Moyal L, Andegeko Y, Mittelman L, Shiloh Y. Requirement of the MRN complex for ATM activation by DNA damage. EMBO J 2003;22:5612–21. [
77. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 1998;273:5858–68.
78. Thompson LH. Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: the molecular choreography. Mutat Res 2012;751:158–246.
79. Wang C, Lees-Miller SP. Detection and repair of ionizing radiation-induced DNA double strand breaks: New developments in nonhomologous end joining. Int J Radiat Oncol Biol Phys. 2013;86:440–9..
80. .Beucher A, Birraux J, Tchouandong L, Barton O, Shibata A, Conrad S, et al. ATM and Artemis promote homologous recombination of radiation induced DNA double-strand breaks in G2. EMBO J. 2009;28:3413–27.
81. Shibata A, Conrad S, Birraux J, Geuting V, Barton O, Ismail A, et al. Factors determining DNA double-strand break repair pathway choice in G2 phase. EMBO J. 2011;30:1079–92.
82. Jeggo PA, Geuting V, Löbrich M. The role of homologous recombination in radiation-induced double-strand break repair. Radiother Oncol. 2011;101:7–12.
83. Sartori AA, Lukas C, Coates J, et al. Human CtIP promotes DNA end resection. Nature 2007;450:509–14.
84. Nimonkar AV, Genschel J, Kinoshita E, et al. BLM-DNA2-RPA-MRN and EXO1 BLM-RPA-MRNconstitute two DNA end resection machineries for human DNA break repair. Genes Dev 2011;25:350–62.
85. Svendsen JM, Harper JW. GEN1/Yen1 and the SLX4 complex: solutions to the problem of Holliday junction resolution. Genes Dev 2010;24:521–36.
86. Makharashvili, Nodar, Tubbs, Anthony T., Hyun Yang, Soo, Wang, Hailong, Barton, Olivia, Zhou, Yi, et al. (2014). Catalytic and Noncatalytic Roles of the CtIP Endonuclease in Double-Strand Break End Resection. Mol. Cell 54 (6), 1022–1033. doi:10.1016/j.molcel.2014.04.011
87. Huertas, Pablo, Cortés-Ledesma, Felipe, Sartori, Alessandro A., Aguilera, Andrés, and Jackson, Stephen P. (2008). CDK Targets Sae2 to Control DNA-End

Resection and Homologous Recombination. Nature 455 (7213), 689–692. doi:10.1038/nature07215.CDK
88. Williams, R. Scott, Dodson, Gerald E., Limbo, Oliver, Yamada, Yoshiki, Williams, Jessica S., Guenther, Grant, et al. (2009). Nbs1 Flexibly Tethers Ctp1 and Mre11-Rad50toCoordinateDNADouble-StrandBreakProcessingandRepair. Cell 139 (1), 87–99. doi:10.1016/j.cell.2009.07.033
89. Thorslund, Tina, McIlwraith, Michael J., Compton, Sarah A., Lekomtsev, Sergey, Petronczki, Mark, Griffith, Jack D., et al. (2010). The Breast Cancer Tumor Suppressor BRCA2 Promotes the Specific Targeting of RAD51 to Single Stranded DNA. Nat. Struct. Mol. Biol. 17 (10), 1263–1265. doi:10.1038/ nsmb.1905
90. Ma, Chu Jian, Gibb, Bryan, Kwon, Youngho, Sung, Patrick, and Greene, Eric C. (2017). Protein Dynamics of Human RPA and RAD51 on SsDNA during Assembly and Disassembly of the RAD51 Filament. Nucleic Acids Res. 45 (2), 749–761. doi:10.1093/nar/gkw1125
91. Bizard, A. H., and HicksonHickson, I. D. (2014). The Dissolution of Double Holliday Junctions. Cold Spring Harb. Perspect. Biol. 6, a016477–14. doi:10. 1101/cshperspect.a016477
92. Wyatt, Haley D. M., Sarbajna, Shriparna, Matos, Joao, and West, Stephen C. (2013). Coordinated Actions of SLX1-SLX4 and MUS81-EME1 for Holliday Junction Resolution in Human Cells. Mol. Cell 52 (2), 234–247. doi:10.1016/j.molcel.2013. 08.035
93. Wyatt, Haley D. M., and West, Stephen C. (2014). Holliday Junction Resolvases. Cold Spring Harb. Perspect. Biol. 6, 1–29. doi:10.1101/cshperspect.a023192
94. Russell P, Nurse P. Negative regulation of mitosis by Wee1+, a gene encoding a protein kinase homo log. Cell 1987; 49:559-67; PMID:3032459; http:// dx.doi.org/10.1016/0092-8674(87)90458-2
95. C.H. McGowan, P. Russell, Cell cycle regulation of human WEE1, EMBO J. 14 (10) (1995) 2166–2175, https://doi.org/10.1002/j.1460-2075.1995.tb07210.x.
96. Y. Tominaga, C. Li, R.-H. Wang, C.-X. Deng, Murine Wee1 plays a critical role in cell cycle regulation and pre-implantation stages of embryonic development, Int. J. Biol. Sci. 2 (4) (2006) 161–170, https://doi.org/10.7150/ijbs.2.161.
97. P. Nurse, P. Thuriaux, Regulatory genes controlling mitosis in the fission yeast, Genetics 96 (3) (1980) 627 LP – 637.
98. C.H. McGowan, P. Russel, Human Wee1 kinase inhibits cell division by phosphor ylating p34cdc2 exclusively on Tyr15, EMBO J. 12 (1) (1993) 75–85.
99. L.L. Parker, H. Piwnica-Worms, Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase, Science 1992; 257:1955 7; PMID:1384126; science.1384126
100. Do K, Doroshow JH, Kummar S. Wee1 kinase as a target for cancer therapy. Cell Cycle. 2013;12(19):3159–64.
101. Shkundina IR. Et al. New RAD51 Inhibitors to Target Homologous Recombination in Human Cells. Genes **2021**, 12, 920. https://doi.org/10.3390/genes12060920

102. Harris, P.S.; Venkataraman, S.; Alimova, I.; Birks, D.K.; Balakrishnan, I.; Cristiano, B.; Donson, A.M.; Dubuc, A.M.; Taylor, M.D.; Foreman, N.K.; et al. Integrated genomic analysis identifies the mitotic checkpoint kinase WEE1 as a novel therapeutic target in medulloblastoma. Mol. Cancer 2014, 13, 72.
103. Mueller, S.; Hashizume, R.; Yang, X.; Kolkowitz, I.; Olow, A.K.; Phillips, J.; Smirnov, I.; Tom, M.W.; Prados, M.D.; James, C.D.; et al. Targeting Wee1 for the treatment of pediatric high-grade gliomas. Neuro Oncol. 2014, 16, 352–360.
104. Music, D.; Dahlrot, R.; Hermansen, S.K.; Hjelmborg, J.; De Stricker, K.; Hansen, S.; Kristensen, B.W. Expression and prognostic value of the WEE1 kinase in gliomas. J. Neuro Oncol. 2016, 127, 381–389.
105. Moran NA,Mira A.2001The process of genome shrinkage in the obligate symbiont Buchnera aphidicola. Genome Biol. 2:research0054.1
106. Shigenobu S, Watanabe H, Hattori M, Sakaki Y, Ishikawa H. 2000 Genome sequence of the endocellular bacterial symbiont of aphids Buchnera sp. APS. Nature 407:81–86
107. Lin Z, Kong H, Nei M, Ma H. 2006 Origins and evolution of the recA/RAD51 gene family: evidence for ancient gene duplication and endosymbiotic gene transfer. PNAS 103:10328–33 [PubMed: 16798872]
108. Sullivan MR, Bernstein KA. 2018 RAD-ical new insights into RAD51 regulation. Genes 9:629
109. Bell JC, Plank JL, Dombrowski CC, Kowalczykowski SC. 2012 Direct imaging of RecA nucleation and growth on single molecules of SSB-coated ssDNA. Nature 491:274–78 [PubMed: 23103864]
110. Hilario J, Amitani I, Baskin RJ, Kowalczykowski SC. 2009 Direct imaging of human Rad51 nucleoprotein dynamics on individual DNA molecules. PNAS 106:361–68 [PubMed: 19122145]
111. Short JM, Liu Y, Chen S, Soni N, Madhusudhan MS, et al. 2016 High-resolution structure of the presynaptic RAD51 filament on single-stranded DNA by electron cryo-microscopy. Nucleic Acids Res. 44:9017–30 [PubMed: 27596592]
112. Subramanyam S, Ismail M, Bhattacharya I, Spies M. 2016 Tyrosine phosphorylation stimulates activity of human RAD51 recombinase through altered nucleoprotein filament dynamics. PNAS 113:E6045–54 [PubMed: 27671650]
113. Qiu Y, Antony E, Doganay S, Koh HR, Lohman TM, Myong S. 2013 Srs2 prevents Rad51 filament formation by repetitive motion on DNA. Nat. Commun 4:2281 [PubMed: 23939144]
114. Schipler A, Iliakis G. DNA double-strand-break complexity levels and their possible contributions to the probability for error-prone processing and repair pathway choice. Nucleic Acids Res. 2013;41(16):7589–605.
115. Mladenov E, Iliakis G. Induction and repair of DNA double strand breaks: the increasing spectrum of non-homologous end joining pathways. Mutat Res. 2011; 711(1–2):61–72.
116. Shkundina IR. Et al. New RAD51 Inhibitors to Target Homologous Recombination in Human Cells. Genes **2021**, 12, 920. https://doi.org/10.3390/genes12060920

117. Tanaka, N. et al. Wee-1 Kinase Inhibition Sensitizes High-Risk HPV+ HNSCC to Apoptosis Accompanied by Downregulation of MCl-1 and XIAP Antiapoptotic Proteins. Clin. Cancer Res. 21, 4831–4844, https://doi.org/10.1158/1078-0432.CCR-15-0279 (2015). +
118. Liu G.H. et al. Small molecule inhibitors targeting the cancers. Med Comm. 2022;3:e181. https://doi.org/10.1002/mco2.181
119. Roskoski R Properties of FDA-approved small molecule protein kinase inhibitors: A 2024 update. Pharmacological Research Volume 200, February 2024, 107059
120. Vriend, L. E. M.; De Witt Hamer, P. C.; Van Noorden, C. J. F.; Würdinger, T. WEE1 Inhibition and Genomic Instability in Cancer. *Biochim. Biophys. Acta* **2013**, *1836* (2), 227– 235, DOI: 10.1016/j.bbcan.2013.05.002
121. Esposito, F.; Giuffrida, R.; Raciti, G.; Puglisi, C.; Forte, S. Wee1 Kinase: A Potential Target to Overcome Tumor Resistance to Therapy. *Int. J. Mol. Sci*. **2021**, *22* (19), 10689, DOI: 10.3390/ijms221910689
122. Kiviharju-af H€ allstr€ om TM, J€ a€ amaa S, M€ onkk€ onen M, Peltonen K, Andersson LC, Medema RH, et al. Human prostate epithelium lacks Wee1A-mediated DNA damage-induced checkpoint enforcement. Proc Natl Acad Sci U S A 2007;104:7211–6.
123. Yoshida T, Tanaka S, Mogi A, Shitara Y, Kuwano H. The clinical significance of Cyclin B1 and Wee1 expression in non-small-cell lung cancer. Ann Oncol 2004;15:252–6
124. Alli et al. Synthetic and Medicinal Chemistry Approaches Toward WEE1 Kinase Inhibitors and Its Degraders. ACS Omega 2023, 8, 20196−20233 https://doi.org/10.1021/acsomega.3c01558
125. Pfister, S. X., Markkanen, E., Jiang, Y., Sarkar, S., Woodcock, M., Orlando, G., et al. (2015). Inhibiting Wee1 selectively kills histone H3K36me3-deficient cancers by dNTP starvation. Cancer Cell 28 (5), 557–568. doi: 10.1016/j. ccell.2015.09.015
126. Richer, A. L., Cala, J. M., O'Brien, K., Carson, V. M., Inge, L. J., and Whitsett, T. G. (2017). Wee1 kinase inhibitor AZD1775 has preclinical efficacy in LKB1 deficient non-small cell lung cancer. Cancer Res. 77 (17), 4663–4672. doi: 10.1158/0008-5472.CAN-16-3565Webster et al., 2017
127. Do, K., Wilsker, D., Ji, J., Zlott, J., Freshwater, T., Kinders, R. J., et al. (2015). Phase I study of single-agent AZD1775 (MK-1775), a Wee1 kinase inhibitor, in patients with refractory solid tumors. J. Clin. Oncol. 33 (30), 3409–3415. doi: 10.1200/ JCO.2014.60.4009 Lewis et al., 2017;
128. Webster, P. J., Littlejohns, A. T., Gaunt, H. J., Prasad, K. R., Beech, D. J., and Burke, D. A. (2017). AZD1775 induces toxicity through double-stranded DNA breaks independently of chemotherapeutic agents in p53-mutated colorectal cancer cells. Cell Cycle 16 (22), 2176–2182. doi: 10.1080/15384101.2017.1301329
129. Bi. Wee1 Inhibitor AZD1775 Effectively Inhibits the Malignant Phenotypes of Esophageal Squamous Cell Carcinoma *In Vitro* and *In VivoPharmacology of Anti-Cancer Drugs Volume 10 - 2019* | *https://doi.org/10.3389/fphar.2019.00864*

130.35.Murrow LM, Garimella SV, Jones TL, Caplen NJ, Lipkowitz S. Identi fication of WEE1 as a potential molecular target in cancer cells by RNAi screening of the human tyrosine kinome. Breast Cancer Res Treat 2010;122:347–57
131. Iorns E, Lord CJ, Grigoriadis A, McDonald S, Fenwick K, Mackay A, et al. Integrated functional, gene expression and genomic analysis for the identification of cancer targets. PLoS ONE 2009;4:e5120
132. Hirai H,Arai T,OkadaM,NishibataT,KobayashiM,SakaiN,etal.MK 1775, a small molecule Wee1 inhibitor, enhances anti-tumor efficacy of various DNA-damaging agents, including 5-fluorouracil. Cancer Biol Ther 2010;9:514–22.
133. Hirai H, Iwasawa Y, Okada M, Arai T, Nishibata T, Kobayashi M, et al. Small-molecule inhibition of Wee1 kinase by MK-1775 selectively sensitizes p53-deficient tumor cells to DNA-damaging agents. Mol Cancer Ther 2009;8:2992–3000
134. Qi, W., et al., Inhibition of Wee1 sensitizes AML cells to ATR inhibitor VE-822 induced DNA damage and apoptosis. Biochem Pharmacol, 2019. 164: p. 273-282.
135.Bukhari, A.B., et al., Inhibiting Wee1 and ATR kinases produces tumor-selective synthetic lethality and suppresses metastasis. J Clin Invest, 2019. 129(3): p. 1329 1344.
136. Bahuguna, Ashutosh & Khan, Imran & Bajpai, Vivek K. & Chul, Sun. (2017). MTT assay to evaluate the cytotoxic potential of a drug. Bangladesh Journal ofPharmacology. 12. 8. 10.3329/bjp.v12i2.30892.
137. Davies K et al. Chk1 inhibition and Wee1 inhibition combine synergistically to impede cellular proliferation Chk1 inhibition and Wee1 inhibition combine synergistically to impede cellular proliferation
138. Fang, Y., et al., Sequential Therapy with PARP and WEE1 Inhibitors Minimizes Toxicity while Maintaining Efficacy. Cancer Cell, 2019. 35(6): p. 851-867 e7.
139. Karnak, D. et al. Combined inhibition of Wee1 and PARP1/2 for radiosensitization in pancreatic cancer. Clinical cancer research: an official journal of the American Association for Cancer Research.. Clin Cancer Res. 2014 Oct 1;20(19):5085-96. doi: 10.1158/1078-0432.CCR-14-1038.
140. Mir SE, De Witt Hamer PC, Krawczyk PM, Balaj L, Claes A, Niers JM, et al. In silico analysis of kinase expression identifies WEE1 as a gatekeeper against mitotic catastrophe in glioblastoma. Cancer Cell 2010;18:244–57.
141. Lee YY. Anti-Tumor Effects of Wee1 Kinase Inhibitor with Radiotherapy in Human Cervical Cancer.Nature .com Scientific Reports. 2019:9:15394 | https://doi.org/10.1038/s41598-019-51959-3
142.l-Jamaei, A. H.; de Visscher, J. G. A. M.; Subramanyam, V. R.; Forouzanfar, T.; Sminia, P.; Doulabi, B. Z.; Helder, M. N. WEE1 Kinase Inhibitor MK-1775 Sensitizes Oral Tongue Squamous Cell Carcinoma Cells to Radiation Irrespective of TP53 Status. Oral Dis 2022,
143. Raderschall, E.; Stout, K.; Freier, S.; Suckow, V.; Schweiger, S.; Haaf, T. Elevated levels of Rad51 recombination protein in tumor cells. Cancer Res. 2002, 62, 219–225.

144. Maacke, H.; Jost, K.; Opitz, S.; Miska, S.; Yuan, Y.; Hasselbach, L.; Luttges, J.; Kalthoff, H.; Sturzbecher, H.W. DNA repair and recombination factor Rad51 is over-expressed in human pancreatic adenocarcinoma. Oncogene 2000, 19, 2791–2795.
145. Maacke, H.; Opitz, S.; Jost, K.; Hamdorf, W.; Henning, W.; Kruger, S.; Feller, A.C.; Lopens, A.; Diedrich, K.; Schwinger, E.; et al. Over-expression of wild-type Rad51 correlates with histological grading of invasive ductal breast cancer. Int. J. Cancer 2000, 88, 907–913.
146. Barbano, R.; Copetti, M.; Perrone, G.; Pazienza, V.; Muscarella, L.A.; Balsamo, T.; Storlazzi, C.T.; Ripoli, M.; Rinaldi, M.; Valori, V.M.; et al. High RAD51 mRNA expression characterize estrogen receptor-positive/progesteron receptor-negative breast cancer and is associated with patient's outcome. Int. J. Cancer 2011, 129, 536–545.
147. Tennstedt et al. 2013. RAD51 overexpression is a negative prognostic marker for colorectal adenocarcinoma. Int J Cancer. 2013 May 1;132(9):2118-26.
148. Wiegmans, A.P.; Al-Ejeh, F.; Chee, N.; Yap, P.Y.; Gorski, J.J.; Da Silva, L.; Bolderson, E.; Chenevix-Trench, G.; Anderson, R.; Simpson, P.T.; et al. Rad51 supports triple negative breast cancer metastasis. Oncotarget 2014, 5, 3261–3272.
149. Li, L.; Zhang, X.; Zhang, Z.T. Genetic Polymorphisms in the RAD51 Gene with a Risk of Head and Neck Cancer and Esophageal Cancer: A Meta-Analysis. Int. J. Genom. 2019, 2019, 2789035.
150. Huang,F.; Mazin, A.V. Targeting the homologous recombination pathway by small molecule modulators. Bioorg. Med. Chem. Lett. 2014, 24, 3006–3013.
151. Chen,Q.; Cai, D.; Li, M.; Wu, X. The homologous recombination protein RAD51 is a promising therapeutic target for cervical carcinoma. Oncol. Rep. 2017, 38, 767–774.
152. Budke, B.; Lv, W.; Kozikowski, A.P.; Connell, P.P. Recent Developments Using Small Molecules to Target RAD51: How to Best Modulate RAD51 for Anticancer Therapy? ChemMedChem 2016, 11, 2468–2473.
153. Hengel SR, Spies MA, Spies M. Small-molecule inhibitors targeting DNA repair and DNA repair deficiency in research and cancer therapy. Cell Chem Biol. (2017) 24:1101–19. doi: 10.1016/j.chembiol.2017.08.027
154. Budke B, Kalin JH, Pawlowski M, Zelivianskaia AS, Wu M, Kozikowski AP, et al. An optimized RAD51 inhibitor that disrupts homologous recombination without requiring michael acceptor reactivity. J Med Chem. (2012) 56:254–63. doi: 10.1021/jm301565b
155. Budke B, Logan HL, Kalin JH, Zelivianskaia AS, Cameron McGuire W, Miller LL, et al. RI-1: a chemical inhibitor of RAD51 that disrupts homologous recombination in human cells. Nucleic Acids Res. (2012) 40:7347–57. doi: 10.1093/nar/gks353
156. Huang F, Motlekar NA, Burgwin CM, Napper AD, Diamond SL, Mazin AV. Identification of specific inhibitors of human RAD51 recombinase using high-throughput screening. ACS Chem Biol. (2011) 6:628–35. doi: 10.1021/cb100428c

157. Ward A, Khanna KK, Wiegmans AP. Targeting homologous recombination, new pre-clinical and clinical therapeutic combinations inhibiting RAD51. Cancer Treat Rev. (2015) 41:35–45. doi: 10.1016/j.ctrv.2014.10.006
158. Zhu J, Zhou L, Wu G, Konig H, Lin X, Li G, et al. A novel small molecule RAD51 inactivator overcomes imatinibresistance in chronic myeloid leukaemia. EMBO Mol Med. (2013) 5:353–65. doi: 10.1002/emmm.201201760
159. Budke, B.; Logan, H.L.; Kalin, J.H.; Zelivianskaia, A.S.; Cameron McGuire, W.; Miller, L.L.; Stark, J.M.; Kozikowski, A.P.; Bishop, D.K.; Connell, P.P. RI-1: A chemical inhibitor of RAD51 that disrupts homologous recombination in human cells. Nucleic Acids Res. 2012, 40, 7347–7357
160. Grundy, M.K.; Buckanovich, R.J.; Bernstein, K.A. Regulation and pharmacological targeting of RAD51 in cancer. NAR Cancer 2020, 2, zcaa024.
161. Roberti, M.; Schipani, F.; Bagnolini, G.; Milano, D.; Giacomini, E.; Falchi, F.; Balboni, A.; Manerba, M.; Farabegoli, F.; De Franco, F.; et al. Rad51/BRCA2 disruptors inhibit homologous recombination and synergize with olaparib in pancreatic cancer cells. Eur. J. Med. Chem. 2019, 165, 80–92.
162. Bagnolini, G.; Milano, D.; Manerba, M.; Schipani, F.; Ortega, J.A.; Gioia, D.; Falchi, F.; Balboni, A.; Farabegoli, F.; De Franco, F.; et al. Synthetic Lethality in Pancreatic Cancer: Discovery of a New RAD51-BRCA2 Small Molecule Disruptor That Inhibits Homologous Recombination and Synergizes with Olaparib. J. Med. Chem. 2020, 63, 2588–2619
163. Falchi, F.; Giacomini, E.; Masini, T.; Boutard, N.; Di Ianni, L.; Manerba, M.; Farabegoli, F.; Rossini, L.; Robertson, J.; Minucci, S.; et al. Synthetic Lethality Triggered by Combining Olaparib with BRCA2-Rad51 Disruptors. ACS Chem. Biol. 2017, 12, 2491–2497.
164. Huang,F., Mazina,O.M., Zentner,I.J., Cocklin,S. and Mazin,A. V. (2012) Inhibition of homologous recombination in human cells by targeting RAD51 recombinase. J. Med. Chem., 55, 3011–3020.
165. Alagpulinsa, D.A., Ayyadevara, S., and Shmookler Reis, R.J. (2014). A smallmolecule inhibitor of RAD51 reduces homologous recombination and sensitizes multiple myeloma cells to doxorubicin. Front. Oncol. 4
166. Huang, F., and Mazin, A.V. (2014). A small molecule inhibitor of human RAD51 potentiates breast cancer cell killing by therapeutic agents in mouse xenografts. PLoS One e100993.
167. Wiegmans AP, Miranda M, Wen SW, Al-Ejeh F, Möller A. RAD51 inhibition in triple negative breast cancer cells is challenged by compensatory survival signaling and requires rational combination therapy. Oncotarget. (2016) 7:60087–100. doi: 10.18632/oncotarget.11065.
168. King H et al. RAD51 Is a Selective DNA Repair Target to Radiosensitize Glioma Stem Cells Stem Cell Reports j Vol. 8 j 125–139 j January 10, 2017
169. Lynch et al. First-in-human phase I/II study of CYT-0851, a first-in-class inhibitor of RAD51-mediated homologous recombination in patients with advanced solid and hematologic cancers. Journal of Clinical Oncology Volume 39, Number 15_suppl

170. Lim, Y.C., Roberts, T.L., Day, B.W., Stringer, B.W., Kozlov, S., Fazry,S., Bruce, Z.C., Ensbey, K.S., Walker, D.G., Boyd, A.W., et al. (2014).Increased sensitivity to ionizing radiation by targeting the homologous recombination pathway in glioma initiating cells. Mol. Oncol. 8, 1603–1615.
171. Havelek et al. Specific inhibition of Wee1 kinase and Rad51 recombinase: A strategy to enhance the sensitivity of leukemic T-cells to ionizing radiation-induced DNA double-strand breaks Biochemical and Biophysical Research Communications 453 (2014) 569–575.
172. Lindeman et al. Combined Inhibition of Rad51 and Wee1 Enhances Cell Killing in HNSCC Through Induction of Apoptosis Associated With Excessive DNA Damage and Replication Stress . Mol Cancer Ther 2021;20:1257–69

8. SUPPLEMENTS

SUPPLEMENT-1 Turnitin Digital Receipt

turnitin

Dijital Makbuz

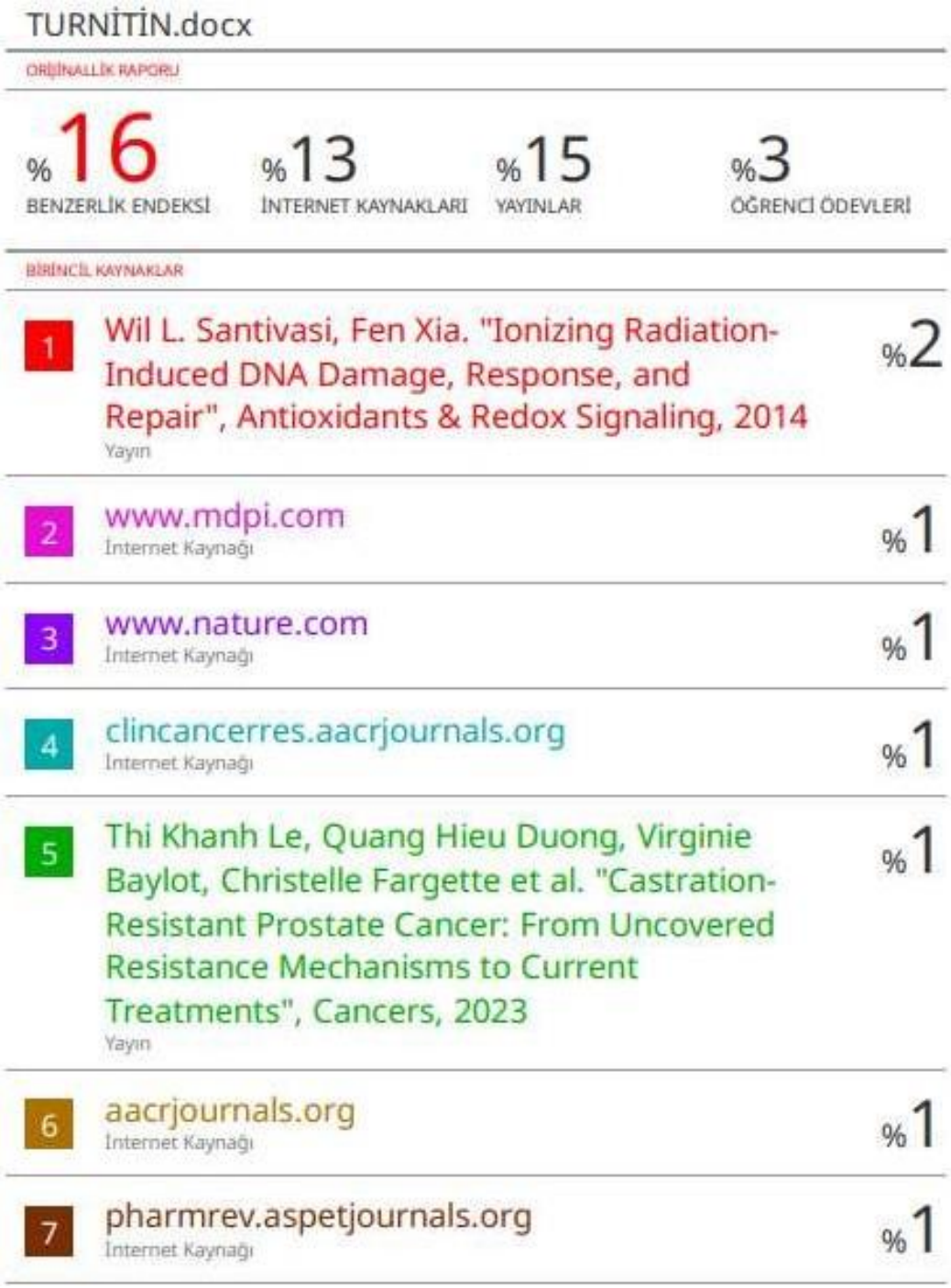
Bu makbuz ödevinizin Turnitin'e ulaştığını bildirmektedir. Gönderiminize dair bilgiler şöyledir:

Gönderinizin ilk sayfası aşağıda gönderilmektedir.

Gönderen:	Rabia Şeyma Nur Taşdemir
Ödev başlığı:	şeyma tez
Gönderi Başlığı:	TURNİTİN.docx
Dosya adı:	TURNİTİN.docx
Dosya boyutu:	4.64M
Sayfa sayısı:	64
Kelime sayısı:	11,022
Karakter sayısı:	60,030
Gönderim Tarihi:	05-Eyl-2024 08:29ÖS (UTC+0300)
Gönderim Numarası:	2445823544

Copyright 2024 Turnitin. Tüm hakları saklıdır.

SUPPLEMENT-2 Turnitin Originality Report



TURNİTİN.docx

ORİJİNALLİK RAPORU

%16	%13	%15	%3
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	Wil L. Santivasi, Fen Xia. "Ionizing Radiation-Induced DNA Damage, Response, and Repair", Antioxidants & Redox Signaling, 2014 Yayın	%2
2	www.mdpi.com İnternet Kaynağı	%1
3	www.nature.com İnternet Kaynağı	%1
4	clincancerres.aacrjournals.org İnternet Kaynağı	%1
5	Thi Khanh Le, Quang Hieu Duong, Virginie Baylot, Christelle Fargette et al. "Castration-Resistant Prostate Cancer: From Uncovered Resistance Mechanisms to Current Treatments", Cancers, 2023 Yayın	%1
6	aacrjournals.org İnternet Kaynağı	%1
7	pharmrev.aspetjournals.org İnternet Kaynağı	%1

9. CURRICULUM VITAE