

Article

PARP Inhibitors Combined with Abiraterone Overcome Resistance in Metastatic Castration-Resistant Prostate Cancer Independently of Androgen Receptor

Hamza Mallah ^{1,2}, Sina Soultani ^{1,2}, Zania Diabasana ^{1,2}, Véronique Lindner ³ , Philippe Barthélémy ⁴, Ysia Idoux-Gillet ^{1,2} and Thierry Massfelder ^{1,2,*} 

- ¹ French National Institute of Health and Medical Research (INSERM), UMR 1260, Regenerative Nanomedicine (RNM), FMST, 1 Rue Eugène Boeckel, 67000 Strasbourg, France; hamza.mallah@etu.unistra.fr (H.M.); sina.soultani@etu.unistra.fr (S.S.); z.dibasana@unistra.fr (Z.D.); yidouxgillet@unistra.fr (Y.I.-G.)
- ² Université de Strasbourg, UMR 1260, CRBS, 1 Rue Eugène Boeckel, 67000 Strasbourg, France
- ³ Department of Pathology, Strasbourg University Hospital, 67200 Strasbourg, France; veronique.lindner@chru-strasbourg.fr
- ⁴ Hôpitaux Universitaires de Strasbourg, 1 Avenue Molière, 67200 Strasbourg, France; philippe.barthelemy@chru-strasbourg.fr
- * Correspondence: massfeld@unistra.fr

Simple Summary

This study investigated the therapeutic potential of combining Abiraterone with PARP inhibitors (Niraparib or Olaparib) in Abiraterone-resistant metastatic castration-resistant prostate cancer (mCRPC). Resistant PC3 and DU145 cell lines were analyzed using 2D and 3D cultures and cell-derived xenograft (CDX) mouse models. Our results show that combining Abiraterone with PARP inhibitors enhances therapeutic efficacy and overcomes the acquired resistance in mCRPC with BRCA1/2 or HRR mutations. Thus, combining Abiraterone with PARP inhibitors is more effective than using PARP inhibitors alone in Abiraterone-resistant mCRPC. The combination therapy significantly reduces tumor growth in cell and mouse models, suggesting a promising strategy to overcome treatment resistance in advanced PC. These results support continued use of PARPi with Abiraterone to improve clinical outcomes.

Abstract

Background: PC is the second most common malignancy in men, and progression to metastatic castration-resistant prostate cancer (mCRPC) after androgen deprivation therapy (ADT) remains incurable. Current treatments for mCRPC include chemotherapy, immunotherapy, radiopharmaceuticals, and second-line androgen receptor signaling inhibitors (ARSIs) such as Abiraterone. PARP inhibitors (PARPis) have recently shown clinical benefits in tumors with homologous recombination repair (HRR) deficiencies, particularly BRCA1/2 mutations. Combining PARPi with ARSIs has improved progression-free (PFS) and overall survival (OS), especially in ARSI-naïve patients, but limited data exist for resistant disease. **Objectives:** This work focuses on intrinsically hormone-insensitive, AR-negative, BRCA-wildtype models, representing a clinically distinct population with limited therapeutic options. We thus investigated the therapeutic potential of combining Abiraterone with PARPis (Niraparib or Olaparib) in Abiraterone-resistant prostate cancer. **Methods:** Resistant PC3 and DU145 cell lines were analyzed using 2D and 3D cultures and cell-derived xenograft (CDX) mouse models. **Results:** Cytotoxicity assays revealed significantly reduced cell viability with combination therapy compared to single



Academic Editor: Kathryn E. Meier

Received: 7 January 2026

Revised: 30 January 2026

Accepted: 5 February 2026

Published: 9 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

agents. These findings were supported by RT-qPCR, Western blot, and immunofluorescence analyses of xenograft tumors, demonstrating enhanced antitumor activity with the combination. Conclusions and significance: Overall, the results indicate that maintaining Abiraterone treatment in combination with PARPis after resistance develops provides superior therapeutic efficacy compared to PARP inhibition alone, offering a promising strategy for managing Abiraterone-resistant prostate cancer. Combining Abiraterone with PARPis enhances therapeutic efficacy and overcomes the acquired insensitivity in mCRPC with BRCA1/2 or HRR mutations. These results support continued use of PARPis with Abiraterone to improve clinical outcomes.

Keywords: prostate cancer; mCRPC; abiraterone resistance; PARP inhibitors; combination therapy; BRCA/HRR deficiency

1. Introduction

Prostate cancer (PC) is the second most commonly diagnosed cancer in men and a major cause of cancer-related mortality worldwide. It exhibits a wide spectrum of clinical behavior, ranging from slow-growing tumors requiring minimal intervention to aggressive forms needing systemic therapy. Management must be tailored based on disease stage and progression rate. While early-stage PC can often be managed with active surveillance, surgery, or radiation, advanced cases typically require a multimodal treatment approach [1,2].

PC development is driven by both genetic predisposition and environmental factors. A common genetic alteration is the fusion of the androgen-regulated TMPRSS2 gene with the ERG oncogene. This fusion leads to overexpression of ERG, a transcription factor that promotes tumor growth and progression by altering gene expression in cancer cells [3,4]. PC treatment is guided by factors such as cancer spread, castration status, tumor characteristics, and prior chemotherapy exposure. Diagnosis commonly involves prostate-specific antigen (PSA) testing, transrectal ultrasound guided biopsy, and rectal examination. However, PSA screening is controversial, as it may detect low-risk tumors and does not always reflect cancer presence or severity [5,6]. At diagnosis, 80–90% of PCs are androgen-dependent, and ADT is the standard initial treatment for metastatic hormone-sensitive prostate cancer (mHSPC). While ADT effectively lowers testosterone levels, 20–30% of patients progress to mCRPC, where the disease advances despite castrate-level androgens due to continued androgen receptor (AR) activity [7].

When PC becomes resistant to initial ADT, second-line hormonal therapies like Enzalutamide, Abiraterone Acetate, and Apalutamide are used to more effectively target AR signaling. Enzalutamide and Apalutamide inhibit AR activity, while Abiraterone blocks androgen biosynthesis. These agents improve survival and delay progression in mCRPC, but resistance to them frequently emerges [8,9]. This resistance arises through various mechanisms, such as mutations in the AR gene (e.g., T878A), the upregulation of AR splice variants like AR-V7, increased intratumoral androgen synthesis (e.g., through CYP17A1), and cellular reprogramming that allows tumors to shift away from dependence on AR signaling [10,11].

In advanced PC, resistance to AR-targeted therapies arises through alternative survival pathways. These include glucocorticoid receptor upregulation, activation of PI3K/AKT/mTOR and Wnt/ β -catenin pathways, and loss of tumor suppressors such as PTEN, TP53, and RB1. Some tumors develop AR independence through neuroendocrine transdifferentiation (NEPC). Epigenetic changes and transcription factor reprogramming

(e.g., MYC, FOXA1, HOXB13) also contribute to resistance. Meanwhile, PARPis like Olaparib, Niraparib, and Rucaparib have shown clinical benefit in BRCA1/2-mutated mCRPC by targeting defective DNA repair mechanisms and improving survival [10–14].

PARPi are targeted therapies that block the PARP enzyme, impairing DNA repair in cancer cells. They are particularly effective in mCRPC patients with BRCA1/2 mutations or other DNA repair defects, leading to cancer cell death by exploiting synthetic lethality [15]. The Food and Drug Administration has approved two PARPis, Olaparib and Rucaparib, for use in these cases, as they have been shown to improve both PFS and OS. Ongoing research aims to expand the benefits of PARPis to patients without known BRCA or HRR gene mutations [15,16]. One promising approach is combining PARPis with other treatments, including ARSIs, radiation or radioligand therapy, chemotherapy, and immunotherapy [16]. Combining PARPis with ARSIs, such as Abiraterone and Enzalutamide, is particularly encouraging because androgen receptor signaling affects DNA repair gene expression, potentially creating a BRCA-like state (“BRCAness”) that increases cancer cell sensitivity to PARP inhibition [17–19]. Nevertheless, resistance to PARPis is increasingly observed in clinical settings. Resistance mechanisms include restoration of homologous recombination, drug efflux, mutations in PARP1 or DNA repair pathway reactivation and epigenetic modifications, highlighting the need for better biomarkers and combination strategies to enhance efficacy [20,21].

Preclinical studies using models such as organoids and xenografts have shown that combining PARPis with ARSIs leads to a greater reduction in tumor cell viability and tumor volume than either treatment alone. This effect is observed in both HRR-proficient tumors and those with BRCA2 mutations [19,22]. Mechanistic studies using RNA sequencing and immunohistochemistry show that combining PARP inhibitors with ARSIs increases DNA damage markers like γ H2AX, indicating synergistic activity [22]. Large trials (PROpel, MAGNITUDE, TALAPRO-2) have demonstrated significant improvements in PFS in mCRPC patients, regardless of HRR mutation status [17,19,23]. Furthermore, meta-analyses further support this combination, showing reduced risk of progression and death, making it a strong first-line option even in biomarker-unselected populations [23].

In this study, we used PC3 and DU145 human prostate cancer cell lines, derived from bone and brain metastases, respectively, to model advanced, treatment-resistant disease. Both are AR-negative and resistant to androgen deprivation therapies, including Abiraterone, making them valuable for investigating therapeutic resistance and vulnerabilities related to DNA damage response (DDR) alterations [24]. Genomic profiling has shown that PC3 cells lack functional BRCA1/2 mutations but have multiple alterations in other DDR genes, including ATM, BARD1, and BRIP1, as well as a confirmed frameshift mutation in TP53, indicating a distinct DDR profile. In contrast, DU145 cells exhibit potentially pathogenic missense mutations in both BRCA1 and BRCA2, suggesting a partially reduced HRR capacity [25]. This genetic divergence between PC3 and DU145 makes them useful supplementary models for studying the effects of combining Abiraterone with PARPi. Their distinct DNA repair profiles provide insight into the mechanisms of treatment resistance and therapeutic targets in metastatic-resistant PC.

PC3 and DU145 cells exhibit intrinsic hormone insensitivity, which is partly mediated by suppression of AR expression. In PC3 cells, AR promoter methylation contributes to low AR levels, and restoring AR expression (PC3-AR9) led to downregulation of stemness markers CD44 and SOX2, decreased invasion in bone lesion assays, and reduced tumor growth in vivo, suggesting a potential tumor-suppressive role for AR in this context. Similarly, in DU145 cells, FOXC2-mediated transcriptional repression of AR contributes to intrinsic insensitivity to androgen signaling. Together, these findings indicate that both cell lines maintain low AR activity through distinct mechanisms, emphasizing that the study

interrogates intrinsic hormone insensitivity rather than acquired abiraterone resistance, a distinction that is critical for interpreting the observed treatment responses [26–28].

2. Methods

2.1. 2D Culture

Human PC cell lines PC3 (ATCC, LGC Standards, Middlesex, UK, CRL-1435) and DU145 (ATCC, HTB-81) were seeded in 96-well plates at a density of 5000 cells per well in a total volume of 200 μ L of culture medium DMEM/F12 (Thermo Fisher Scientific, Villebon-sur-Yvette, France, 11320033). The cells were allowed to adhere and grow for 24 h before treatment, and the plates were incubated at 37 °C, 5% CO₂.

2.2. Treatment of 2D Cells

Two days after seeding, the cells were treated with the following compounds: Abiraterone 10 μ M (Thermo Fisher Scientific, 466732500), Olaparib (5, 10, and 20 μ M, Abmole Bioscience, Europe Branch, Huissen, The Netherlands, M1664) and Niraparib (5 and 10 μ M, Abmole Bioscience, M2215).

After 3 days of treatment, cell viability was assessed using the MTT assay (Roche, Switzerland, Basel, 11465007001). Absorbance was measured using an absorbance plate reader to determine the effects of the treatments on cell viability.

2.3. 3D Culture and Treatments

Human PC cell lines PC3 and DU145 were plated in 96-well ultra-low attachment (ULA) plates (S-bio, Tokyo, Japan, MS-9096UZ) at a density of 5000 cells per well, with a total volume of 200 μ L of culture medium DMEM/F12 (Thermo Fisher Scientific, 11320033). The cells were cultured for 3 days to allow organoid formation. After the 3-day period, the organoids were treated with the following compounds: Abiraterone (10 μ M, Thermo Fisher Scientific, 466732500), Olaparib (20 and 50 μ M, Abmole Bioscience, M1664) and Niraparib (10, 20, 30, and 50 μ M, Abmole Bioscience, M2215).

Following a 3-day treatment period, cell viability was assessed using the CellTiter-Glo assay (Promega, Charbonnières-les-Bains, France, G9681). Luminescence measurements were performed using a luminescence plate reader to evaluate the impact of the treatments on cell viability.

2.4. MTT Assay

Cell viability was assessed using the MTT assay (Roche, Basel, Switzerland; 11465007001) following the manufacturer's protocol. PC3 and DU145 cells cultured in 2D monolayers were treated with Abiraterone, Niraparib, Olaparib, or their combinations for 72 h. Cells were incubated with MTT labeling reagent (10 μ L per well) for 4 h, followed by addition of solubilization solution (100 μ L per well) and overnight incubation to dissolve formazan crystals. Absorbance was measured at 570 nm using a microplate reader to determine cell viability.

2.5. Cell Titer Glo Assay

After a 3-day treatment period with Abiraterone, Niraparib, Olaparib, or their combinations, 100 μ L of culture medium was carefully removed from each well of 96-well plates containing 3D organoids derived from PC3 and DU145 cells. Subsequently, 100 μ L of CellTiter-Glo[®] 3D Reagent (Promega; G9681) was added to each well to match the volume of the remaining medium. The plate contents were mixed vigorously for 5 min to lyse cells and release ATP. Following mixing, the plate was incubated at room temperature for 25 min to stabilize the luminescent signal. Luminescence was then measured using a

luminescence plate reader to evaluate cell viability, as ATP levels correlate with the number of metabolically active cells.

2.6. PC Tumor Model

Tumor Implantation and Growth

All animal studies were in compliance with the French animal use regulations. The results obtained *in vitro* were analyzed *in vivo* in the CDX model in male nude mice (Swiss nude mice, 5–6 weeks old, Charles River, France). Two PC models were developed from the 2 cell lines, PC3 and DU145. Five million viable cells (trypan blue exclusion) were injected subcutaneously into the interscapular region into 200 µL of PBS under gaseous general anesthesia (isoflurane). When tumors reached ~200 mm³ ((Width × length²) × 0.5), as measured using an electronic caliper, mice were randomized to 4 groups of 6–7 mice per cell line. For PC3, the groups included a control group, a group treated with Abiraterone, a group receiving Niraparib, and a group receiving a combination of Abiraterone and Niraparib. For DU145, the groups consisted of a control group, a group treated with Abiraterone, a group receiving Olaparib, and a group receiving a combination of Abiraterone and Olaparib. Abiraterone acetate (200 mg/kg per day) (Thermo Fisher Scientific, 466732500) was administered via intraperitoneal injection every day for up to 15 days in a solution consisting of 0.1 mL of 5% benzyl alcohol and 95% safflower oil. Olaparib (50 mg/kg) (Thermo Fisher Scientific, 466290010) was administered daily by gavage for 15 days, using a solution containing 5% dimethylacetamide, 10% Soluthol HS15, and 85% PBS. Niraparib (50 mg/kg) (Thermo Fisher Scientific, 30117144) was prepared in 10% PBS and 0.5% methylcellulose in water and was given daily by gavage for 15 days. For this, the water was heated to just below boiling point, at 98 °C, and then allowed to cool to room temperature; after 20 min, 0.5% methylcellulose was added and stirred at room temperature for 45 min [29].

During treatments, tumors were measured 2 times per week. Post-treatment tumor volumes were then normalized to the baseline volume for each animal.

2.7. Quantitative RT-PCR

2.7.1. RNA Extraction and cDNA Synthesis

Total RNA was extracted from tumor tissue samples using TRIzol reagent (Thermo Fisher Scientific, 15596026) according to the manufacturer's instructions. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific), with acceptable A260/A280 ratios between 1.8 and 2.0. Complementary DNA (cDNA) was synthesized from 500 ng to 1 µg of total RNA using the iScript™ Reverse Transcription Supermix (Bio-Rad, Marnes-la-Coquette, France; 1708840) following the manufacturer's protocol.

2.7.2. qPCR Amplification

Quantitative PCR was performed using a SsoAdvanced™ Universal SYBR® Green Supermix, Bio-Rad, 1725271) detection system on a real-time PCR instrument (Applied Biosystems StepOnePlus, Villebon-sur-Yvette, France or Bio-Rad CFX96). Each reaction was carried out in a 10 µL volume containing: 5 µL of Syber Green, 0.5 µL of each primer (final concentration 20 µM), 2 µL of diluted cDNA template, and 2 µL of nuclease-free water.

Primers were designed using Primer-BLAST(https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC%20=%20BlastHome (accessed on 4 February 2026)) to span exon–exon junctions and were synthesized (Eurogentec, Seraing, Belgium). Primer specificity was confirmed by single melting peaks and agarose gel electrophoresis.

AR1-F1: 5' ATG-GTG-AGC-AGA-GTG-CCC-TAT-C 3'

AR1-R1: 5' ATG-GTC-CCT-GGC-AGT-CTC-CAA-A 3'
AR2-F2: 5' AGA-TGA-AGC-TTC-TGG-GTG-TC 3'
AR2-R2: 5' GGA-CAT-TCA-GAA-AGA-TGG-GC 3'

2.8. Immunofluorescence Analysis

Tumor tissue samples were fixed in paraformaldehyde, embedded in paraffin, and sectioned at 7 µm thickness. For immunofluorescence, antigen retrieval was performed by immersing slides in 10 mM sodium citrate buffer (pH 6.0) followed by heat-induced epitope retrieval using a pressure cooker. Slides were then cooled for 60 min at room temperature before staining.

2.8.1. Assessment of Cell Proliferation

To assess cell proliferation, the tissue sections were incubated overnight at 4 °C with a primary monoclonal antibody against Ki-67 (Thermo Fisher Scientific, MA5-14520 clone SP6, diluted 1:250 in 0.1% BSA). The following day, detection was performed using an Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody (Thermo Fisher Scientific; A-11008, diluted 1:500). Quantification of cell proliferation in each treatment group for both CDX models was performed by counting the number of proliferating (marker-positive) cells and the total number of cells in five randomly selected microscopic fields per tumor section at 10× magnification. Staining was quantified in a blinded fashion. The proliferative index was calculated as the percentage of positive cells relative to the total number of cells.

2.8.2. TUNEL Assays

Apoptotic cell death was detected using the Click-iT™ Plus TUNEL Assay with Alexa Fluor™ Dyes (Thermo Fisher Scientific, Cat. No. C10617, C10618, or C10619), following the manufacturer's instructions with minor modifications. Samples were fixed in 4% paraformaldehyde for 15 min at room temperature, then permeabilized using proteinase K (1:25 dilution in PBS, 15 min at 37 °C for tissue sections). After washing, DNA breaks, characteristic of apoptosis, were marked by incorporation 5-ethynyl-2'-deoxyuridine triphosphate (EdUTP) using terminal deoxynucleotidyl transferase (TdT). The labeling reaction was carried out at 37 °C for 60 min. Detection of incorporated nucleotides was achieved via a copper-catalyzed azide-alkyne cycloaddition (Click) between the alkyne-modified EdUTP and a fluorescent Alexa Fluor™ picolyl azide (AF-594). The Click reaction was carried out for 30 min at room temperature in the dark. Nuclei were counterstained with DAPI (7 min), and samples were mounted using ibidi. Imaging was performed using a fluorescence microscope (Zeiss, Oberkochen, Germany) equipped with the appropriate filter sets for Alexa Fluor dyes. Negative controls (absence of TdT enzyme) and positive controls (DNase I-treated samples) were included to confirm labeling specificity.

Quantification of TUNEL positive cell in each group of treatment was performed as indicated above for cell proliferation. The ratio corresponded to the apoptotic index, as expressed in percent positive cells/total number of cells.

2.9. Western Blot Analysis

2.9.1. Protein Extraction

For total protein extraction, RIPA lysis buffer (Thermo Fisher Scientific; 89901) was supplemented with 10 µL of Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific; 78442, 1:100 dilution). Tissue samples were washed twice with ice-cold PBS to remove any residual blood or debris. After washing, 200 µL of the prepared RIPA buffer was added to each sample, followed by thorough homogenization using a tube homogenizer until the tissue was completely lysed.

The homogenized samples were then incubated on ice for 30 min to ensure complete lysis. After incubation, the samples were briefly sonicated and then centrifuged at 4 °C for 15 min. The resulting supernatant, containing the total protein extract, was carefully collected and stored for downstream analysis.

2.9.2. Blotting

Western blotting was performed using standard protocols with optimizations to preserve signal quality and reduce background. Protein lysates were prepared in RIPA buffer, denatured, and separated on SDS-PAGE gels (8–15% acrylamide) based on target size. Gels were transferred to membranes by wet transfer at 350 mA for 1.5 h, with transfer confirmed by Ponceau S staining. Membranes were handled carefully to avoid contamination or drying. Blocking and antibody incubations included 0.05–0.1% Tween[®]-20; sodium azide was excluded to preserve HRP activity. Chemiluminescent detection reagents were protected from light throughout the process.

Membranes were blocked with 5% BSA in TBS-T for 1 h at room temperature or overnight at 4 °C, then incubated overnight at 4 °C with the following primary antibodies: Rabbit monoclonal anti-androgen receptor recombinant (Euromedex, Souffelweyersheim, France, PR-81844-1-RR-100 µL, dilution 1/5000). After washing in TBS-T, membranes were incubated with HRP-conjugated secondary antibodies (anti-Rabbit IgG, Abcam ab288151, Cambridge, UK, dilution 1:10,000) for 1 h at room temperature. Detection was performed via enhanced chemiluminescence, using a luminol–peroxide substrate and exposure to X-ray film for 1–5 min based on signal intensity.

For re-probing or detection of loading controls, membranes were stripped and incubated with a second primary antibody (β-tubulin, mouse monoclonal, Thermo Fisher Scientific, BT7R, 1:3000), followed by HRP-conjugated anti-Mouse IgG (Abcam, ab205719, 1:10,000) and chemiluminescence-based detection using freshly prepared substrate.

2.9.3. Data Analysis

All samples were run in triplicate. Relative gene expression was quantified using the $2^{-\Delta\Delta C_t}$ method, with (reference gene, GAPDH) used as the internal control. Negative controls without template (NTC) and without reverse transcriptase were included to confirm the absence of contamination or genomic DNA amplification.

2.10. Statistical Analysis

All in vitro experiments data are presented as mean ± standard error of the mean (SEM) from at least three independent experiments. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. Statistical analyses were conducted using GraphPad Prism 8.4 software, and *p*-values < 0.05 were considered statistically significant.

In vivo experimental data are presented as mean ± standard error of the mean (SEM). Differences between two groups were analyzed using an unpaired parametric *t*-test. Statistical analyses were performed using GraphPad Prism software, and *p*-values < 0.05 were considered statistically significant.

3. Results

3.1. Effects of Abiraterone on Cell Viability in 2D Models

We first examined the effects of Abiraterone at increasing concentrations on cell viability in both cell lines cultured in 2D. As depicted in Figure 1 both PC3 (Figure 1A) and DU145 cells (Figure 1B) exhibited resistance to Abiraterone within the normal therapeutic concentration range, i.e., up to 10 µM. As expected, at higher concentrations (30–100 µM), a

significant reduction in cell viability was observed in both cell lines, probably related to cell toxicity. These results show that both cell lines are resistant to Abiraterone.

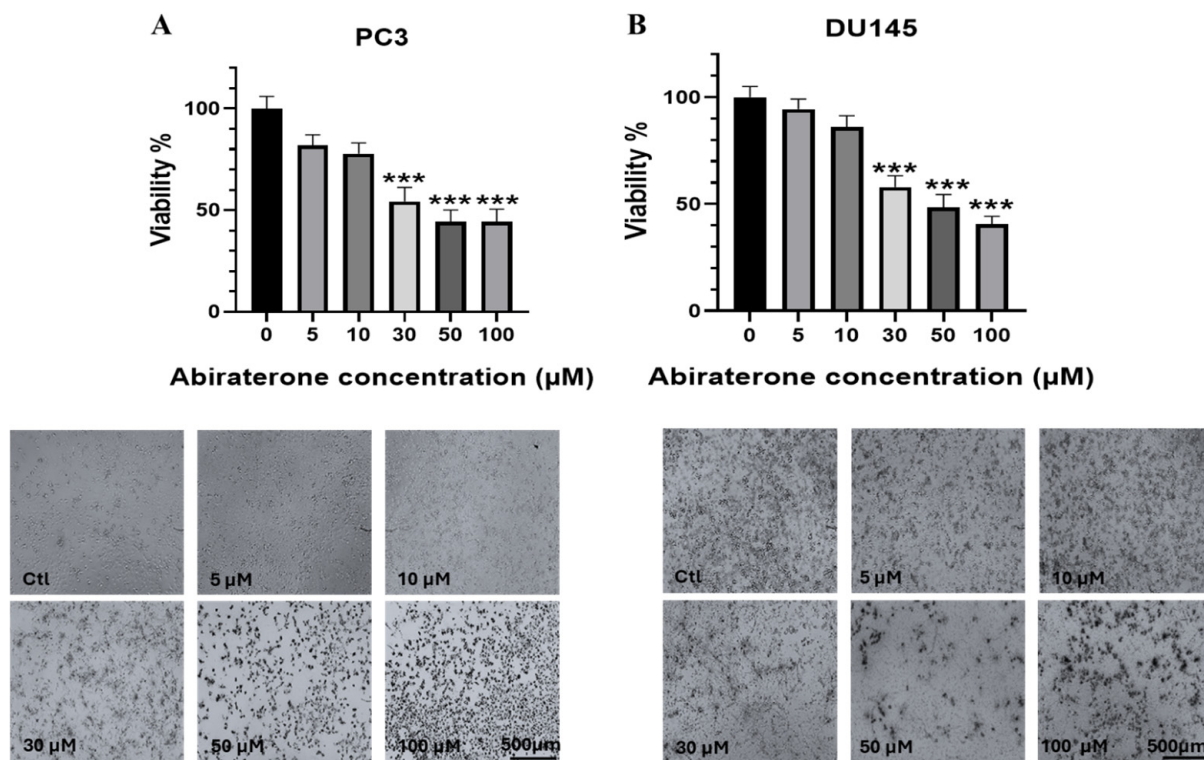


Figure 1. Concentration-dependent effect of Abiraterone on cell viability in 2D models. (A, Top) Effects of Abiraterone (Abi) at various concentrations (0 to 100 µM) on PC3 cells viability ($n = 8$). Data are presented as mean $\pm$ SEM and were analyzed using one-way ANOVA. ***, $p < 0.001$ from Abi at 0 µM. (A, Bottom) Representative microphotographs depicting cells in each experimental condition. (B, Top) Effects of Abiraterone (Abi) at various concentrations on DU145 cells viability ($n = 8$). Data are expressed as the mean $\pm$ SEM. ***, $p < 0.001$ from Abi at 0 µM. (B, Bottom) Representative microphotographs depicting cells in each experimental condition.

3.2. Effects of the Combination of PARPis with Second-Generation Hormonotherapy on Cell Viability in 2D Models

We next assessed whether the PARPis Olaparib and Niraparib may influence the effects of Abiraterone on cell viability in cells cultured in 2D. In PC3 cells (Figure 2A, left), treatment with Olaparib, either alone or in combination with Abiraterone, did not result in a statistically significant change in cell viability compared to Abiraterone alone, suggesting that PC3 cells are not sensitive to Olaparib, whether used as a monotherapy or in combination with Abiraterone. However, in DU145 cells (Figure 2B, left), treatment with the combination of Abiraterone and Olaparib resulted in a statistically significant decrease in cell viability compared to each agent alone, suggesting a potential synergistic effect of Abiraterone and Olaparib at both concentrations.

In PC3 cells (Figure 2A, right), treatment with Niraparib alone or in combination with Abiraterone led to a statistically significant decrease in cell viability compared to Abiraterone alone, which was greater for the higher dose of the PARPi. However, the combination treatments did not exhibit a clear dose-dependent enhancement of cytotoxicity, suggesting that the observed effects are likely due to Niraparib monotherapy rather than a synergistic interaction. Similar results were obtained in DU145 cells (Figure 2B, right).

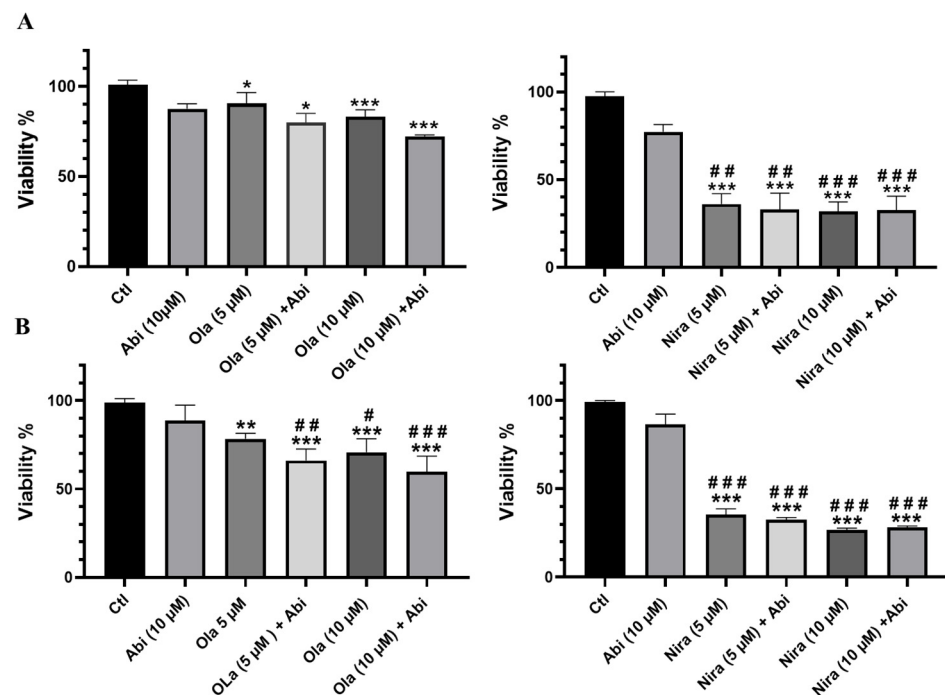


Figure 2. Effects of the PARPi/ Abiraterone combination on cell viability in 2D models. **(A, left)** Effects of Abiraterone (Abi) and Olaparib (Ola) at different concentrations, either alone or in combination, on PC3 cell viability ($n = 4$). Data are presented as mean $\pm$ SEM and were analyzed using one-way ANOVA. *, $p < 0.05$; ***, $p < 0.001$ from Control (Ctl). **(A, right)** Effects of Abiraterone (Abi) and Niraparib (Nira) at different concentrations, either alone or in combination, on PC3 cell viability ($n = 4$). Data are expressed as the mean $\pm$ SEM. ***, $p < 0.001$ from Control (Ctl). ##, $p < 0.01$; ###, $p < 0.001$ from Abi alone. **(B, left)** Effects of Abiraterone (Abi) and Olaparib (Ola) at different concentrations, either alone or in combination, on DU145 cell viability ($n = 4$). Data are expressed as the mean $\pm$ SEM. **, $p < 0.01$; ***, $p < 0.001$ from Control (Ctl). #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$ from Abi alone. **(B, right)** Effects of Abiraterone (Abi) and Niraparib (Nira) at different concentrations, either alone or in combination, on DU145 cell viability ($n = 4$). Data are expressed as the mean $\pm$ SEM. ***, $p < 0.001$ from Control (Ctl). ###, $p < 0.001$ from Abi alone.

3.3. Effects of the Combination of PARPis with Hormonotherapy on Cell Viability in 3D Models

Results from 2D experiments showed that combining Niraparib or Olaparib with Abiraterone did not decrease PC3 cell viability relative to treatment with each drug alone, whereas the combination of Olaparib and Abiraterone significantly reduced viability in DU145 cells compared to either agent alone.

We then assessed cell viability in 3D experiments in order to analyze whether the effects of PARPi/ Abiraterone combination were also observed in more complex 3D cell models. The effects of increasing concentrations of each PARPi, either alone or in combination with Abiraterone on cell viability, were measured.

In PC3 cells, Abiraterone did not affect cell viability, indicating resistance to the hormonotherapy agent in 3D models as well (Figure 3). The combination of Abiraterone with Niraparib significantly decreased cell viability at 20 μ M (Figure 3A, left) but almost blunted cell viability at 30 μ M (Figure 3A, middle) and 50 μ M (Figure 3A, right). Interestingly, as observed in 2D models, the combination of Niraparib with Abiraterone significantly decreased cell viability for the lowest concentration of the PARPi (Figure 3A, left), while neither Abiraterone alone nor Niraparib alone showed any effects. These synergistic effects could not be observed at higher concentrations of the PARPi due to cell toxicity (Figure 3A, middle and right). Olaparib decreased cell viability in a concentration-dependent manner

in PC3 cells but, as observed in 2D models, no synergistic effects were observed when it was combined with Abiraterone (Figure 3B, left and right).

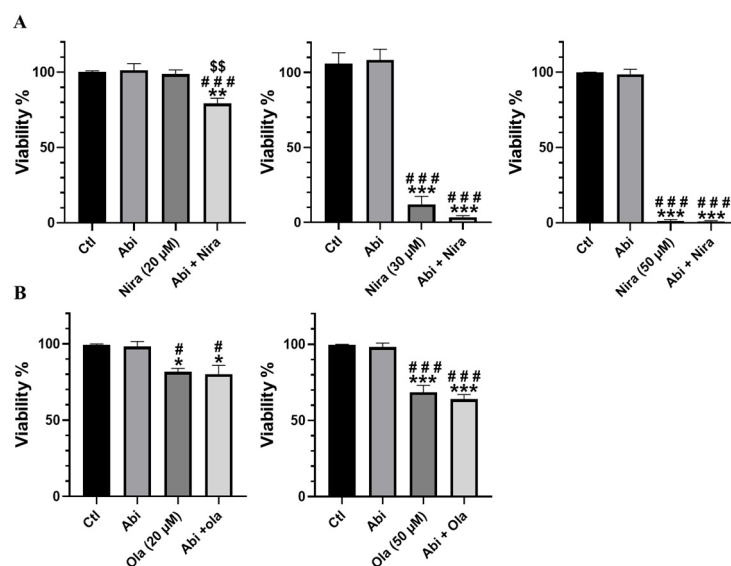


Figure 3. Effects of the PARPi/Abiraterone combination on cell viability in PC3 3D models. **(A)** Effects of Abiraterone (Abi) and Niraparib (Nira) at different concentrations (**left**, 20 µM; **middle**, 30 µM; **right**, 50 µM), either alone or in combination, on PC3 cell viability ($n = 5$). Data are presented as mean $\pm$ SEM and were analyzed using one-way ANOVA. **, $p < 0.01$; ***, $p < 0.001$ from Control (Ctl). ###, $p < 0.001$ from Abi alone. \$\$, $p < 0.01$ from Nira alone. **(B)** Effects of Abiraterone (Abi) and Olaparib (Ola) at different concentrations (**left**, 20 µM; **right**, 50 µM), either alone or in combination, on PC3 cell viability ($n = 5$). Data are expressed as the mean $\pm$ SEM. *, $p < 0.05$; ***, $p < 0.001$ from Control (Ctl). #, $p < 0.05$; ###, $p < 0.001$ from Abi alone.

Similar results were obtained in DU145 cells but with the synergistic effects observed for the combination Olaparib/Abiraterone (Figure 4A) and not for the combination Niraparib/Abiraterone (Figure 4B).

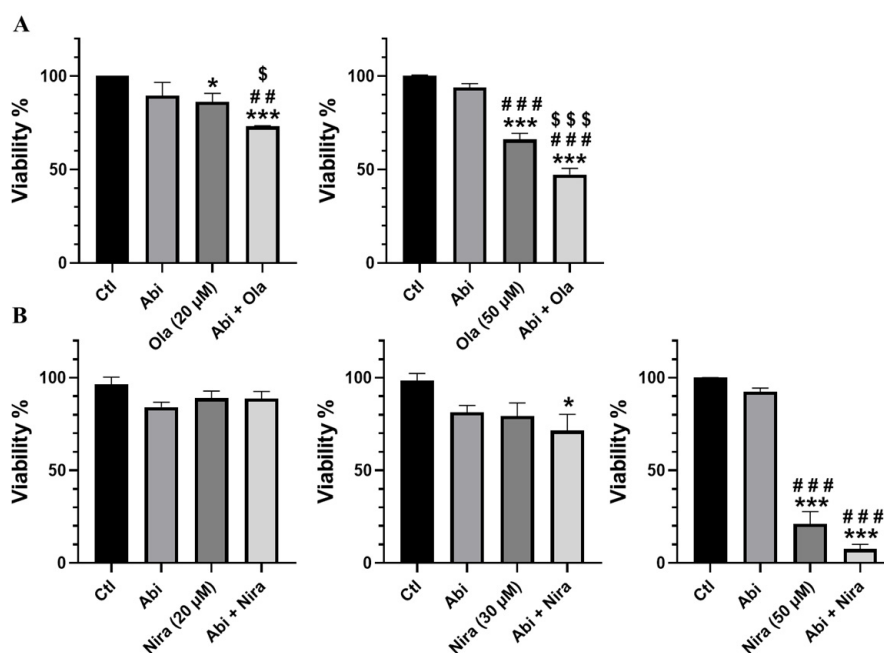


Figure 4. Effects of the PARPi/Abiraterone combination on cell viability in DU145 3D models. **(A)** Effects of Abiraterone (Abi) and Olaparib (Ola) at different concentrations (**left**, 20 µM; **right**, 50 µM), either alone

or in combination, on DU145 cell viability ($n = 5$). Data are presented as mean $\pm$ SEM and were analyzed using one-way ANOVA. *, $p < 0.05$; ***, $p < 0.001$ from Control (Ctl). ##, $p < 0.01$; ###, $p < 0.001$ from Abi alone. \$, $p < 0.05$; \$\$\$, $p < 0.001$ from Ola alone. (B) Effects of Abiraterone (Abi) and Niraparib (Nira) at different concentrations (left, 20 μ M; middle, 30 μ M; right, 50 μ M), either alone or in combination, on DU145 cell viability ($n = 5$). Data are expressed as the mean $\pm$ SEM. *, $p < 0.05$; ***, $p < 0.001$ from Control (Ctl). ###, $p < 0.001$ from Abi alone.

Overall, the effects of each agent alone and in combination were largely comparable between 2D and 3D models for DU145 cells, underscoring the robustness of these results. However, PC3 cells exhibited differential responses between 2D and 3D cultures, indicating that the treatment effects are model-dependent in this cell line and the importance of 3D model.

3.4. Effects of PARPi/Abiraterone Combination on PC Tumor Growth In Vivo

To evaluate the biological significance of PARPi/Abiraterone combination in PC, we used the xenograft athymic nude mouse model. Data obtained in vitro in 2D and 3D models showed that synergistic effects on cell viability were observed for the Niraparib/Abiraterone combination in PC3 cells and for Olaparib/Abiraterone in DU145 cells. We thus evaluated the effects of each agent alone and following these combinations in mice xenografted with either the PC3 cell line or with the DU145 cell line. Doses and methods of administrations of each agent were obtained from previously published data from other investigators [29–33]. The 15-day treatments, despite everyday administration, were well tolerated by the mice. Since the growth of tumors was irregular in shape, we decided to show the effects of the treatments on tumor weights at the end of the experimental period. Indeed, many of the tumors had irregular shapes, which made volume estimates using calipers less reliable and sometimes misleading. Tumor weight, in contrast, provides a straightforward and accurate measure of the actual tumor burden at the end point, making comparisons between groups more dependable. These effects are depicted for PC3 cells (Figure 5A) and for DU145 cells (Figure 5B). As observed in 2D and 3D models, we also observed a significant and synergistic effect on the inhibition of tumor growth when mice were treated with the PARPi/Abiraterone combination, while each agent alone did not have significant effects on this model. Notably, the combination therapy did not affect the body weight of the mice, suggesting limited systemic toxicity.

Taken together with the in vitro results obtained in 2D and 3D models, these results clearly show the antitumoral therapeutic benefit of combining a PARPi with Abiraterone in clinical settings.

Although it was not the main purpose of the present preclinical work, in order to gain an insight into the mechanism of the synergistic effects observed with the combination therapy, we performed additional experiments aimed at investigating whether the effects involve regulation of AR expression and whether they are a consequence of inhibition of cell proliferation and/or induction of cell apoptosis. Thus, for each tumor type, tumors harvested from the four groups of mice were processed for (i) RT-qPCR and Western blot of the AR and (ii) immunofluorescence to assess cell proliferation and death. Using specific forward and reverse primer sets (two for human AR) and a positive control cell line (human LnCaP cell line), we showed that AR is not expressed in mRNA at measurable levels in either the PC3 or the DU145 cell line, whatever the experimental group (Figure 6A). To confirm these results at the protein level, we then performed Western blot analysis. As shown in Figure 6B and Supplemental Figure S1 for PC3 and DU145 cells, human AR was not detectable at the protein level, while a clear band was observed in the AR-expressing human LnCaP cell line. We then assessed whether cell proliferation and/or cell apoptosis were involved in the effect of the combination therapy on tumor cell growth through Ki67

and TUNEL staining, respectively. Figure 7A–C shows that the treatments did not affect cell proliferation at all experimental conditions tested and for both cell lines. However, as shown in Figure 8A–C, apoptosis was significantly increased when mice were treated with the PARPi/Abiraterone combination for both cell lines.

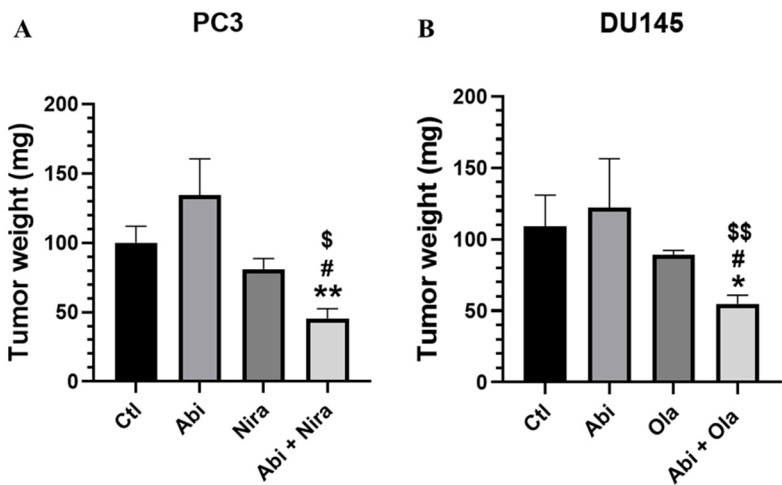


Figure 5. Effects of Abiraterone, PARPis, and the PARPi/Abiraterone combination on human PC tumor growth in vivo in nude mice. When tumors reached around 200 mm³, treatments were administered daily for 15 days, and tumors harvested at the end of the experimental period. (A) Effects of Abiraterone (Abi), Niraparib (Nira) and their combination on PC3 tumor weights measured at the end of the experimental period (*n* = 4). Data are presented as mean ± SEM and were analyzed using Unpaired parametric *t*-test. **, *p* < 0.01 from Control (Ctl). #, *p* < 0.05 from Abi alone. \$, *p* < 0.05 from Nira alone. (B) Effects of Abiraterone (Abi), Olaparib (Ola) and their combination on DU145 tumor weights measured at the end of the experimental period (*n* = 3–6). Data are expressed as the mean ± SEM. *, *p* < 0.05 from Control (Ctl). #, *p* < 0.05 from Abi alone. \$\$, *p* < 0.01 from Ola alone.

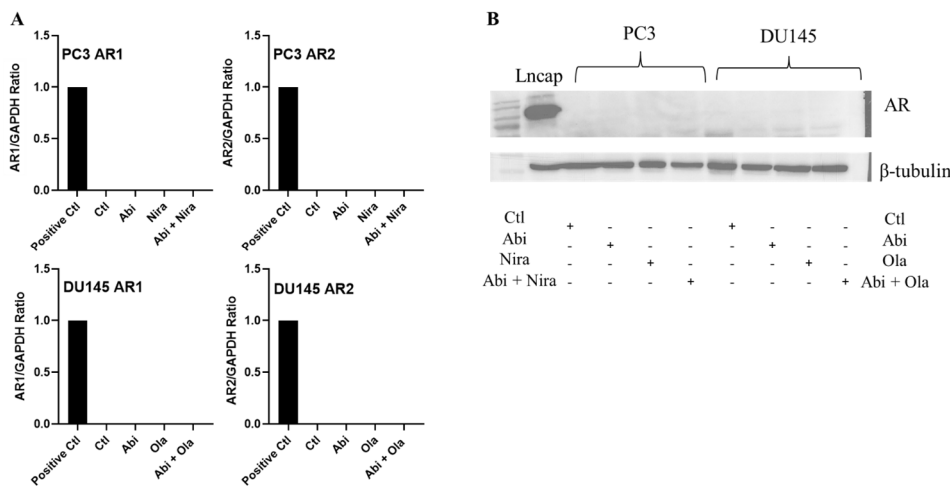


Figure 6. AR (androgen receptor) expression in PC3 and DU145 tumors following Abiraterone, PARPi and combination treatments. (A) RT-qPCR analysis of the expression of the AR at the mRNA level in PC3 (top, left and right) and DU145 (bottom, left and right) treated in the various experimental conditions using two different sets of forward and reverse primers and compared with the AR-positive LnCaP. No significant differences were observed for either PC3 tumors or DU145 tumors. (B) Western blot of PC3 and DU145 tumor cell lysates incubated with a human specific antibody against human AR, and β-tubulin for each experimental conditions in mice (Ctl, Abi, Nira or Ola, and combination treatments). The LnCaP cell lysate was used as a positive AR expression control. Representative radiographs of at least three independent experiments.

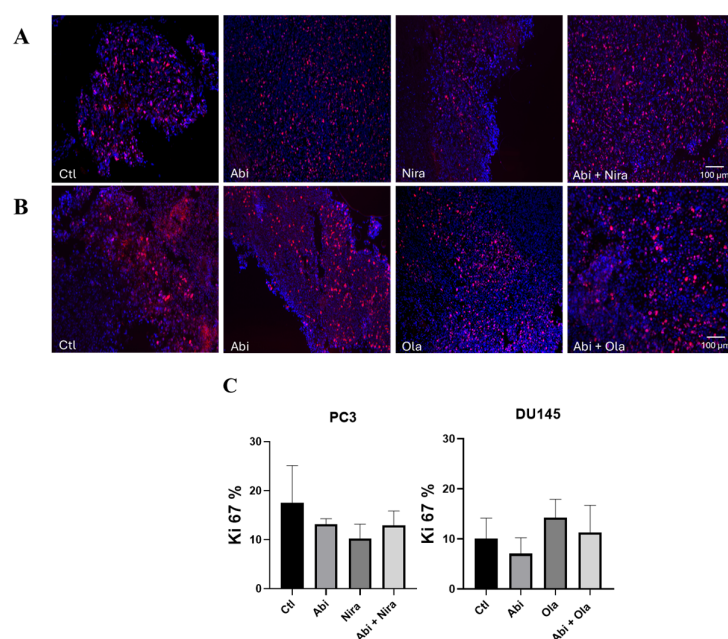


Figure 7. Effects of Abiraterone, PARPis, and the PARPi/Abiraterone combination on tumor cell proliferation. Data are presented as mean $\pm$ SEM and were analyzed using one-way ANOVA. (A) Effects of Abiraterone (Abi), Niraparib (Nira) and their combination on Ki67 staining in PC3 tumors. Nuclei were stained with DAPI. Merged representative microphotographs are depicted. (B) Effects of Abiraterone (Abi), Olaparib (Ola) and their combination on Ki67 staining in DU145 tumors. Nuclei were stained with DAPI. Merged representative microphotographs are depicted. (C) The proliferative indices in PC3 and DU145 tumors were calculated as the number of Ki67 positive cells on the total number of cells in each field. No significant differences were observed for either PC3 tumors (left) or DU145 tumors (right).

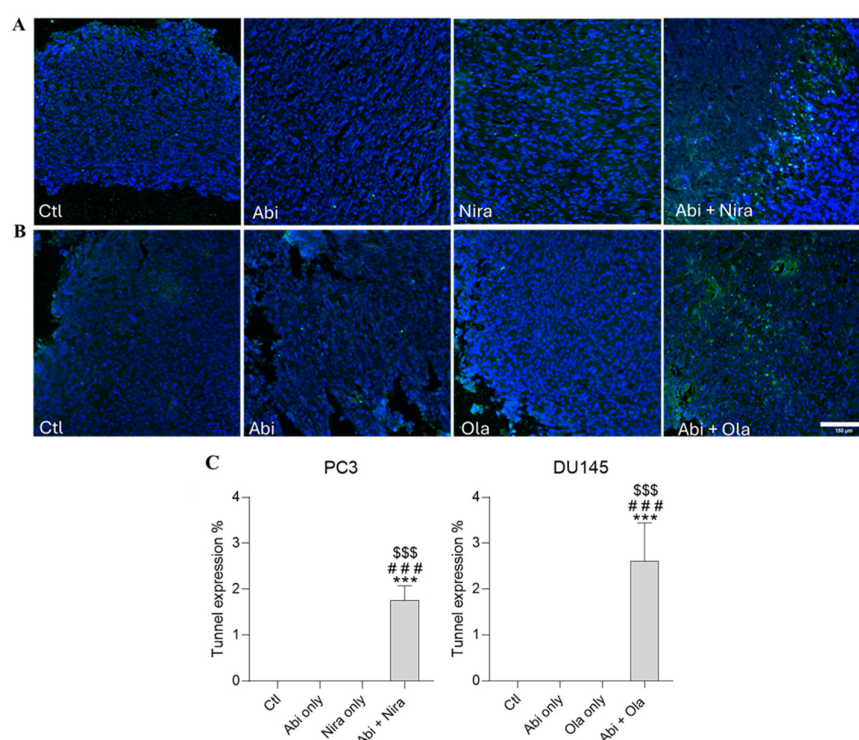


Figure 8. Effects of Abiraterone, PARPis and the PARPi/Abiraterone combination on tumor cell death. (A) Effects of Abiraterone (Abi), Niraparib (Nira) and their combination on TUNEL staining in

PC3 tumors. Nuclei were stained with DAPI. Merged representative microphotographs are depicted. **(B)** Effects of Abiraterone (Abi), Olaparib (Ola) and their combination on TUNEL staining in DU145 tumors. Nuclei were stained with DAPI. Merged representative microphotographs are depicted. **(C)** The apoptotic indices in PC3 and DU145 tumors were calculated as the number of TUNEL positive cells on the total number of cells in each field. Data are presented as mean $\pm$ SEM and were analyzed using one-way ANOVA. **(Left)** In PC3 tumors, $***, p < 0.001$ from Ctl; $###, p < 0.001$ from Abi alone; $$$$, p < 0.001$ from Nira alone. **(Right)** In DU145 tumors, $***, p < 0.001$ from Ctl; $###, p < 0.001$ from Abi alone; $$$$, p < 0.001$ from Ola alone.

Taken together, these latter results show that the synergistic effects of the PARPi/Abiraterone combinations in both cell lines are not dependent on AR expression and cell proliferation but that they are a consequence of cell death.

4. Discussion

Our results demonstrate that both PC3 and DU145 prostate cancer cell lines are highly resistant to Abiraterone at clinically relevant concentrations, with significant cytotoxicity observed only at $\geq 30 \mu\text{M}$, suggesting a non-specific effect. This supports the limited efficacy of Abiraterone in AR-negative models. Consistently with this, prior studies have shown that Abiraterone's cytotoxicity is closely linked to AR expression, and it is more effective in AR-positive cell lines like LNCaP than in AR-negative ones like DU145 [34]. Indeed, in this study, Abiraterone was significantly more effective in LNCaP cells, likely due to its strong interaction with the T877A-mutated AR, whereas DU145 cells showed minimal sensitivity to the drug. Furthermore, a study by Xu et al. [35] reported that Abiraterone induces autophagy in PC3 cells, potentially contributing to drug resistance. Notably, combining Abiraterone with autophagy inhibitors enhanced cytotoxicity, suggesting a promising strategy to overcome resistance in AR-negative prostate cancer models. These findings reinforce the conclusion that PC3 and DU145 cells are intrinsically resistant to Abiraterone monotherapy and may benefit from combination therapies.

Currently, for patients with mCRPC, the standard of care is androgen deprivation combined with chemotherapy or second-generation hormone therapy (or both of them together). The majority of patient cases are those following androgen deprivation in addition to Abiraterone. However, there is a severe lack of data regarding the efficacy of combining second-generation hormone therapy and PARPis after patients with mCRPC have progressed in the disease following androgen deprivation. The current standard of care is then mainly chemotherapy. But, tomorrow, will we have arguments for adding a PARPi to Abiraterone in order to overcome hormone insensitivity based on HRR recombination and BRCA mutations? This crucial question related to improving the clinical setting for mCRPC patients led us to perform the present study using various in vitro and in vivo models. To the best of our knowledge, the results reported here are clearly innovative.

As detailed in the Introduction Section, special characteristics of PC3 and DU145 cells in terms of Abiraterone resistance, AR expression, and BRCA mutations make them among the best models to handle this question. Both cell lines are AR-negative and resistant to androgen deprivation therapies. In 2D and 3D models, in PC3 cells, which lack functional BRCA1/2 mutations, Niraparib appeared as the best PARPi choice in combination therapy with Abiraterone. In DU145, which carries missense mutations in both BRCA1 and BRCA2, Olaparib appeared as the best PARPi choice in combination therapy with Abiraterone. The synergistic effects of these PARPi/Abiraterone combinations on tumor growth were confirmed in in vivo CDX models as well, highlighting the robustness of all results obtained in three different but complementary models.

In the PC3 CDX model in mice, the combination therapy Niraparib/Abiraterone led to a greater reduction in tumor weight compared to monotherapies, indicating synergistic

antitumor activity *in vivo*. The MAGNITUDE phase III clinical trial provides compelling evidence for the efficacy of the Niraparib/Abiraterone combination for HRR positive cases, especially in BRCA1/2-mutated mCRPC, with significant PFS gains from 13.7 to 16.5 months and OS benefits [36]. The BEDIVERE phase Ib clinical trial established the feasibility and tolerability of this combination and defined a recommended dose for further investigation [37]. This body of evidence strongly supports using this combination in preclinical models such as PC3 CDX to study mechanisms of synergy and therapeutic potential.

Similarly, in the DU145 CDX model, the Olaparib/Abiraterone combination therapy led to a greater reduction in tumor growth compared to monotherapies, also indicating synergistic antitumor activity *in vivo*. A screening of PC cell lines treated with various PARPi (e.g., Olaparib) found that DU145 cells, despite being AR-negative, exhibited sensitivity particularly to Olaparib with significant reductions in cell viability and induction of apoptosis compared to other lines [38]. In a DU145 subcutaneous tumor mouse model, combining the PARPi Olaparib with the MET inhibitor Crizotinib significantly reduced tumor growth compared to either treatment alone [39]. Tumors treated with the Olaparib and Crizotinib combination were smaller, with reduced proliferation (Ki-67, RAD51) and increased apoptosis (cleaved caspase-3, γ H2AX), demonstrating enhanced efficacy despite the absence of AR inhibition. While DU145-specific studies combining PARP and AR inhibitors are limited, clinical data support their potential. In the Phase II trial (NCT01972217), Olaparib combined with Abiraterone significantly improved PFS (13.8 vs. 8.2 months) in post-docetaxel mCRPC patients, though with increased grade ≥ 3 adverse events, particularly anemia [40,41]. The Phase III PROpel trial (NCT03732820) confirmed these findings in the first-line mCRPC setting, showing a significant rPFS benefit (24.8 vs. 16.6 months), regardless of HRR mutation status [42].

Our findings support the intrinsic antitumor activity of PARP inhibitors and highlight the therapeutic potential of combining them with AR inhibitors. In CDX models, the enhanced tumor cell death observed with PARPi plus Abiraterone occurred through AR-independent mechanisms, as no AR expression was detected at the mRNA or protein level. These results align with emerging clinical and preclinical data indicating that such combinations can be effective regardless of tumor AR or HRR status [23,43]. Potential mechanisms include synthetic lethality leveraging DNA damage repair deficits, induction of ‘BRCAness,’ or induction of mitotic defects independently of AR signaling [16,44]. Studies suggest that AR and PARP pathways interact in complex ways within the tumor microenvironment. Even if tumor cells themselves lack AR, interactions with host stroma or immune cells may still influence responses to therapy. Also, there is evidence that Abiraterone can induce mitotic defects independently of AR signaling, especially when combined with other inhibitors (even Plk1 inhibitors) [44,45]. Inhibition of both AR and PARP leads to synthetic lethality in PC cells, suggesting a potential therapeutic strategy [46]. These findings support continued investigation into AR-independent pathways and suggest that patients with AR-resistant or AR-negative PC regardless of BRCA1/2 mutation status but with other HRR alterations may still benefit from combination therapy with PARPi and Abiraterone. Immunofluorescence analyses of the tumor samples (DU145, PC3) revealed no significant differences in Ki67 staining between the different treatment conditions for CDX models, strongly suggesting that the inhibitory effect on tumor growth do not involve cell proliferation. In contrast, the combination therapies in both CDX models increased significantly tumor cell death through apoptosis, as evidenced by TUNEL immunofluorescence staining. Thus, the inhibitory effects of the combination therapies on tumor cell growth are independent on AR expression and cell proliferation but involve cell death. The more intimate mechanism was not the purpose of the present preclinical study but will be the aim of upcoming work.

Recent clinical trials have demonstrated the efficacy of combining Abiraterone with PARPi in mCRPC, most notably the PROpel study evaluating Abiraterone plus Olaparib, which showed improved clinical outcomes compared with Abiraterone alone [47]. These trials have primarily focused on treatment-naïve mCRPC patients, with the greatest benefit observed in AR-positive tumors and those harboring BRCA or other HRR alterations. While these studies establish the clinical feasibility and benefit of upfront PARPi–ARSI combinations, they do not directly address therapeutic strategies for patients who have already progressed on abiraterone or who exhibit AR-independent disease, a population with limited treatment options.

5. Conclusions

In this context, the present study explored the therapeutic potential of combining abiraterone with PARP inhibition in intrinsically hormone-insensitive, AR-negative prostate cancer models representing a distinct and advanced disease state. Using PC3 and DU145 cells, which differ in their HRR- and BRCA-related alterations, we show that PARPi can enhance antitumor activity when combined with Abiraterone, even in the absence of canonical AR signaling. Rather than providing direct clinical proof, these findings offer preclinical support for extending the biological rationale of PARPi–Abiraterone combinations to Abiraterone-resistant and AR-independent prostate cancer, complementing existing clinical data and motivating further mechanistic and translational studies.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cancers18040560/s1>, Figure S1: AR (androgen receptor) expression in PC3 and DU145 tumors following Abiraterone, PARPi and combination treatments.

Author Contributions: H.M.: methodology, conceptualization, formal analysis, validation, investigation, writing the first original draft. S.S.: investigation, methodology. Z.D.: investigation, methodology. V.L.: investigation, methodology. P.B.: conceptualization, validation. Y.I.-G.: conceptualization, formal analysis, validation, investigation, methodology, writing—review and editing. T.M.: conceptualization, supervision, resources, validation, project administration, funding acquisition, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: Institut National de la Sante et de la Recherche Médicale, Université de Strasbourg, annual dotation (T. Massfelder); Technological platform TiBioC (Tissue Biofabrication and Characterization, annual prestations (Y. Idoux-Gillet, T. Massfelder).

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee CREMEAS - CE035 on 31 January 2024 and then approved by the French Ministry of Higher Education and Research through the APAFIS authorization process (Reference dossier 2023112814087177) on 13 February 2023. The study was conducted at the Faculté de Médecine de Strasbourg (approval number of the animal facility, F-67-482-35), in accordance with applicable national and institutional guidelines for animal welfare.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data that support the findings of this study are shared by the corresponding author upon request.

Conflicts of Interest: The authors declare no conflict of interest. PB: Receipt of honoraria or consultation fees, Speaker bureau, Advisory board: BMS, Merck, MSD, IPSEN, Johnson & Johnson, Eisai, Novartis, Astellas, Gilead, Pfizer, Astra Zeneca, Bayer; Receipt of grants/research supports: Ipsen, Astra Zeneca, BMS.

Abbreviations

PC	Prostate cancer
mCRPC	Metastatic castration-resistant prostate cancer
ADT	Androgen deprivation therapy
ARSI	Androgen receptor signaling inhibitor
PARPi	PAR inhibitor
HRR	Homologous recombination repair
PFS	Progression-free survival
OS	Overall survival
CDX	Cell-derived xenograft
PSA	Prostate-specific antigen
AR	Androgen receptor
NEPC	Neuroendocrine prostate cancer
DDR	DNA damage response

References

1. Rawla, P. Epidemiology of Prostate Cancer. *World J. Oncol.* **2019**, *10*, 63–89. [[CrossRef](#)]
2. Leslie, S.W.; Soon-Sutton, T.L.; Skelton, W.P. Prostate Cancer. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2025. Available online: <http://www.ncbi.nlm.nih.gov/books/NBK470550/> (accessed on 24 August 2025).
3. Adamo, P.; Ladomery, M.R. The oncogene ERG: A key factor in prostate cancer. *Oncogene* **2016**, *35*, 403–414.
4. Treppiedi, D.; Marra, G.; Di Muro, G.; Catalano, R.; Mangili, F.; Esposito, E.; Barbieri, A.M.; Arosio, M.; Mantovani, G.; Peverelli, E. TMPRSS2 Expression and Activity Modulation by Sex-Related Hormones in Lung Calu-3 Cells: Impact on Gender-Specific SARS-CoV-2 Infection. *Front. Endocrinol.* **2022**, *13*, 862789.
5. Mallah, H.; Diabasana, Z.; Soultani, S.; Idoux-Gillet, Y.; Massfelder, T. Prostate Cancer: A Journey Through Its History and Recent Developments. *Cancers* **2025**, *17*, 194. [[CrossRef](#)]
6. Teo, M.Y.; Rathkopf, D.E.; Kantoff, P. Treatment of Advanced Prostate Cancer. *Annu. Rev. Med.* **2019**, *70*, 479–499. [[CrossRef](#)] [[PubMed](#)]
7. Aurilio, G.; Cimadamore, A.; Mazzucchelli, R.; Lopez-Beltran, A.; Verri, E.; Scarpelli, M.; Massari, F.; Cheng, L.; Santoni, M.; Montironi, R. Androgen Receptor Signaling Pathway in Prostate Cancer: From Genetics to Clinical Applications. *Cells* **2020**, *9*, 2653. [[CrossRef](#)]
8. Virgo, K.S.; Basch, E.; Loblaw, D.A.; Oliver, T.K.; Rumble, R.B.; Carducci, M.A.; Nordquist, L.; Taplin, M.-E.; Winkquist, E.; Singer, E.A. Second-Line Hormonal Therapy for Men With Chemotherapy-Naïve, Castration-Resistant Prostate Cancer: American Society of Clinical Oncology Provisional Clinical Opinion. *J. Clin. Oncol.* **2017**, *35*, 1952–1964. Erratum in *J. Clin. Oncol.* **2017**, *35*, 2591–4096. [[CrossRef](#)] [[PubMed](#)]
9. Liu, J.-M.; Lin, C.-C.; Liu, K.-L.; Lin, C.-F.; Chen, B.-Y.; Chen, T.-H.; Sun, C.-C.; Wu, C.-T. Second-line Hormonal Therapy for the Management of Metastatic Castration-resistant Prostate Cancer: A Real-World Data Study Using a Claims Database. *Sci. Rep.* **2020**, *10*, 4240. [[CrossRef](#)]
10. Einstein, D.J.; Arai, S.; Balk, S.P. Targeting the androgen receptor and overcoming resistance in prostate cancer. *Curr. Opin. Oncol.* **2019**, *31*, 175–182. [[CrossRef](#)] [[PubMed](#)]
11. Watson, P.A.; Arora, V.K.; Sawyers, C.L. Emerging mechanisms of resistance to androgen receptor inhibitors in prostate cancer. *Nat. Rev. Cancer* **2015**, *15*, 701–711. [[CrossRef](#)]
12. Westaby, D.; Fenor De La Maza, M.D.L.D.; Paschalis, A.; Jimenez-Vacas, J.M.; Welti, J.; De Bono, J.; Sharp, A. A New Old Target: Androgen Receptor Signaling and Advanced Prostate Cancer. *Annu. Rev. Pharmacol. Toxicol.* **2022**, *62*, 131–153. [[CrossRef](#)] [[PubMed](#)]
13. Boudadi, K.; Antonarakis, E.S. Resistance to Novel Antiandrogen Therapies in Metastatic Castration-Resistant Prostate Cancer. *Clin. Med. Insights Oncol.* **2016**, *10*, CMO-Ss34534. [[CrossRef](#)]
14. Jacob, A.; Raj, R.; Allison, D.B.; Myint, Z.W. Androgen Receptor Signaling in Prostate Cancer and Therapeutic Strategies. *Cancers* **2021**, *13*, 5417. [[CrossRef](#)] [[PubMed](#)]
15. Taylor, A.K.; Kosoff, D.; Enamekhoo, H.; Lang, J.M.; Kyriakopoulos, C.E. PARP inhibitors in metastatic prostate cancer. *Front. Oncol.* **2023**, *13*, 1159557. [[CrossRef](#)]
16. Bourlon, M.T.; Valdez, P.; Castro, E. Development of PARP inhibitors in advanced prostate cancer. *Ther. Adv. Med. Oncol.* **2024**, *16*, 17588359231221337. [[CrossRef](#)]
17. Panebianco, M.; Cereda, V.; D’Andrea, M.R. Combination of the PARPi and ARSi in advanced castration resistant prostate cancer: A review of the recent phase III trials. *Explor. Target. Anti-Tumor Ther.* **2024**, *5*, 997–1010. [[CrossRef](#)]

18. Giesen, A.; Baekelandt, L.; Devlies, W.; Devos, G.; Dumez, H.; Everaerts, W.; Claessens, F.; Joniau, S. Double trouble for prostate cancer: Synergistic action of AR blockade and PARPi in non-HRR mutated patients. *Front. Oncol.* **2023**, *13*, 1265812. [\[CrossRef\]](#)
19. Fenor De La Maza, M.D.; Pérez Gracia, J.L.; Miñana, B.; Castro, E. PARP inhibitors alone or in combination for prostate cancer. *Ther. Adv. Urol.* **2024**, *16*, 17562872241272929. [\[CrossRef\]](#)
20. Wang, C.; Han, X.; Kong, S.; Zhang, S.; Ning, H.; Wu, F. Deciphering the mechanisms of PARP inhibitor resistance in prostate cancer: Implications for precision medicine. *Biomed. Pharmacother.* **2025**, *185*, 117955. [\[CrossRef\]](#)
21. Mweempwa, A.; Wilson, M.K. Mechanisms of resistance to PARP inhibitors—An evolving challenge in oncology. *Cancer Drug Resist.* **2019**, *2*, 608. [\[CrossRef\]](#)
22. Ranasinghe, W.; Delavar, M.; Lawrence, M.G.; Choo, N.; Niranjani, B.; Papagiris, M.; Kraska, J.; Wang, H.; Keerthikumar, S.; Manyam, G.C.; et al. Targeting highly aggressive ductal prostate tumours with poly ADP ribose polymerase inhibitor (PARPi) and androgen receptor signaling inhibitor (ARSi) combination therapy. *J. Clin. Oncol.* **2025**, *43*, 420. [\[CrossRef\]](#)
23. Messina, C.; Giunta, E.F.; Signori, A.; Rebuzzi, S.E.; Banna, G.L.; Maniam, A.; Buti, S.; Cattrini, C.; Fornarini, G.; Bauckneht, M.; et al. Combining PARP Inhibitors and Androgen Receptor Signalling Inhibitors in Metastatic Prostate Cancer: A Quantitative Synthesis and Meta-analysis. *Eur. Urol. Oncol.* **2024**, *7*, 179–188. [\[CrossRef\]](#)
24. Tai, S.; Sun, Y.; Squires, J.M.; Zhang, H.; Oh, W.K.; Liang, C.; Huang, J. PC3 is a cell line characteristic of prostatic small cell carcinoma. *Prostate* **2011**, *71*, 1668–1679. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Beatson, E.L.; Risdon, E.N.; Napoli, G.C.; Price, D.K.; Chau, C.H.; Figg, W.D. Genomic Characterization of Preclinical Prostate Cancer Cell Line Models. *Int. J. Mol. Sci.* **2024**, *25*, 6111. [\[CrossRef\]](#)
26. Litvinov, I.V.; Antony, L.; Dalrymple, S.L.; Becker, R.; Cheng, L.; Isaacs, J.T. PC3, but not DU145, human prostate cancer cells retain the coregulators required for tumor suppressor ability of androgen receptor. *Prostate* **2006**, *66*, 1329–1338. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Srinivasan, D.; Senbanjo, L.; Majumdar, S.; Franklin, R.B.; Chellaiah, M.A. Androgen receptor expression reduces stemness characteristics of prostate cancer cells (PC3) by repression of CD44 and SOX2. *J. Cell. Biochem.* **2019**, *120*, 2413–2428. [\[CrossRef\]](#)
28. Niu, Y.; Altuwaijri, S.; Lai, K.-P.; Wu, C.-T.; Ricke, W.A.; Messing, E.M.; Yao, J.; Yeh, S.; Chang, C. Androgen receptor is a tumor suppressor and proliferator in prostate cancer. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 12182–12187. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Sambade, M.J.; Van Swearingen, A.E.D.; McClure, M.B.; Deal, A.M.; Santos, C.; Sun, K.; Wang, J.; Mikule, K.; Anders, C.K. Efficacy and pharmacodynamics of niraparib in BRCA-mutant and wild-type intracranial triple-negative breast cancer murine models. *Neuro-Oncol. Adv.* **2019**, *1*, vdz005. [\[CrossRef\]](#)
30. Qin, S.; Gao, H.; Kim, W.; Zhang, H.; Gu, Y.; Kalari, K.R.; Sinnwell, J.P.; Scholz, J.A.; Xie, F.; Yin, P.; et al. Biomarkers for Predicting Abiraterone Treatment Outcome and Selecting Alternative Therapies in Castration-Resistant Prostate Cancer. *Clin. Pharma. Ther.* **2022**, *111*, 1296–1306. [\[CrossRef\]](#)
31. Sun, R.; Luo, H.; Su, J.; Di, S.; Zhou, M.; Shi, B.; Sun, Y.; Du, G.; Zhang, H.; Jiang, H.; et al. Olaparib Suppresses MDSC Recruitment via SDF1 α /CXCR4 Axis to Improve the Anti-tumor Efficacy of CAR-T Cells on Breast Cancer in Mice. *Mol. Ther.* **2021**, *29*, 60–74. [\[CrossRef\]](#)
32. Zhu, S.; Yang, Z.; Zhang, Z.; Zhang, H.; Li, S.; Wu, T.; Chen, X.; Guo, J.; Wang, A.; Tian, H.; et al. HOXB3 drives WNT-activation associated progression in castration-resistant prostate cancer. *Cell Death Dis.* **2023**, *14*, 215. [\[CrossRef\]](#)
33. Miao, C.; Tsujino, T.; Takai, T.; Gui, F.; Tsutsumi, T.; Sztupinszki, Z.; Wang, Z.; Azuma, H.; Szallasi, Z.; Mouw, K.W.; et al. RB1 loss overrides PARP inhibitor sensitivity driven by RNASEH2B loss in prostate cancer. *Sci. Adv.* **2022**, *8*, eabl9794. [\[CrossRef\]](#)
34. Pan, W.; Zhang, Z.; Kimball, H.; Qu, F.; Berlind, K.; Stopsack, K.H.; Lee, G.-S.M.; Choueiri, T.K.; Kantoff, P.W. Abiraterone Acetate Induces CREB1 Phosphorylation and Enhances the Function of the CBP-p300 Complex, Leading to Resistance in Prostate Cancer Cells. *Clin. Cancer Res.* **2021**, *27*, 2087–2099. [\[CrossRef\]](#)
35. Ma, X.; Zou, L.; Li, X.; Chen, Z.; Lin, Z.; Wu, X. Inhibition of Autophagy Improves the Efficacy of Abiraterone for the Treatment of Prostate Cancer. *Cancer Biother. Radiopharm.* **2019**, *34*, 181–188. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Chi, K.N.; Rathkopf, D.; Smith, M.R.; Efstathiou, E.; Attard, G.; Olmos, D.; Lee, J.Y.; Small, E.J.; Pereira De Santana Gomes, A.J.; Roubaud, G.; et al. Niraparib and Abiraterone Acetate for Metastatic Castration-Resistant Prostate Cancer. *J. Clin. Oncol.* **2023**, *41*, 3339–3351. [\[CrossRef\]](#)
37. Saad, F.; Chi, K.N.; Shore, N.D.; Graff, J.N.; Posadas, E.M.; Lattouf, J.-B.; Espina, B.M.; Zhu, E.; Yu, A.; Hazra, A.; et al. Niraparib with androgen receptor-axis-targeted therapy in patients with metastatic castration-resistant prostate cancer: Safety and pharmacokinetic results from a phase 1b study (BEDIVERE). *Cancer Chemother. Pharmacol.* **2021**, *88*, 25–37. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Feiersinger, G.E.; Trattinig, K.; Leitner, P.D.; Guggenberger, F.; Oberhuber, A.; Peer, S.; Hermann, M.; Skvortsova, I.; Vrbkova, J.; Bouchal, J.; et al. Olaparib is effective in combination with, and as maintenance therapy after, first-line endocrine therapy in prostate cancer cells. *Mol. Oncol.* **2018**, *12*, 561–576. [\[CrossRef\]](#)
39. Zhou, S.; Dai, Z.; Wang, L.; Gao, X.; Yang, L.; Wang, Z.; Wang, Q.; Liu, Z. MET inhibition enhances PARP inhibitor efficacy in castration-resistant prostate cancer by suppressing the ATM/ATR and PI3K/AKT pathways. *J. Cell. Mol. Med.* **2021**, *25*, 11157–11169. [\[CrossRef\]](#)

40. Saad, F.; Armstrong, A.J.; Shore, N.; George, D.J.; Oya, M.; Sugimoto, M.; McKay, R.R.; Hussain, M.; Clarke, N.W. Olaparib Monotherapy or in Combination with Abiraterone for the Treatment of Patients with Metastatic Castration-Resistant Prostate Cancer (mCRPC) and a BRCA Mutation. *Target. Oncol.* **2025**, *20*, 445–466. [[CrossRef](#)]
41. Saad, F.; Clarke, N.W.; Oya, M.; Shore, N.; Procopio, G.; Guedes, J.D.; Arslan, C.; Mehra, N.; Parnis, F.; Brown, E.; et al. Olaparib plus abiraterone versus placebo plus abiraterone in metastatic castration-resistant prostate cancer (PROpel): Final prespecified overall survival results of a randomised, double-blind, phase 3 trial. *Lancet Oncol.* **2023**, *24*, 1094–1108. Erratum in *Lancet Oncol.* **2024**, *25*, e180. [[CrossRef](#)] [[PubMed](#)]
42. Saad, F.; Armstrong, A.J.; Oya, M.; Vianna, K.; Özgüroğlu, M.; Gedye, C.; Buchschacher, G.L.; Lee, J.Y.; Emmenegger, U.; Navratil, J.; et al. Tolerability of Olaparib Combined with Abiraterone in Patients with Metastatic Castration-resistant Prostate Cancer: Further Results from the Phase 3 PROpel Trial. *Eur. Urol. Oncol.* **2024**, *7*, 1394–1402. [[CrossRef](#)] [[PubMed](#)]
43. Nientiedt, C.; Duensing, A.; Zschäbitz, S.; Jäger, D.; Hohenfellner, M.; Stenzinger, A.; Duensing, S. PARP inhibition in prostate cancer. *Genes Chromosom. Cancer* **2021**, *60*, 344–351. [[CrossRef](#)] [[PubMed](#)]
44. Patterson, J.C.; Varkaris, A.; Croucher, P.J.P.; Ridinger, M.; Dalrymple, S.; Nouri, M.; Xie, F.; Varmeh, S.; Jonas, O.; Whitman, M.A.; et al. Plk1 Inhibitors and Abiraterone Synergistically Disrupt Mitosis and Kill Cancer Cells of Disparate Origin Independently of Androgen Receptor Signaling. *Cancer Res.* **2023**, *83*, 219–238. [[CrossRef](#)]
45. Garofoli, M.; Maiorano, B.A.; Bruno, G.; Giordano, G.; Di Tullio, P.; Maselli, F.M.; Landriscina, M.; Conteduca, V. Androgen receptor, PARP signaling, and tumor microenvironment: The ‘perfect triad’ in prostate cancer? *Ther. Adv. Med. Oncol.* **2024**, *16*, 17588359241258443. [[CrossRef](#)]
46. Kelsey, R. Potential of PARP inhibition plus ADT in prostate cancer. *Nat. Rev. Urol.* **2017**, *14*, 640. [[CrossRef](#)]
47. Clarke, N.W.; Armstrong, A.J.; Thiery-Vuillemin, A.; Oya, M.; Shore, N.; Lored, E.; Procopio, G.; de Menezes, J.; Girotto, G.; Arslan, C.; et al. Abiraterone and Olaparib for Metastatic Castration-Resistant Prostate Cancer. *NEJM Evid.* **2022**, *1*, EVIDo2200043. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.