Antiandrogen Therapy Radiosensitizes Androgen Receptor–Positive Cancers to ^{18}F-FDG

Indulekha Singaravelu[1], Henry Spitz[2], Mary Mahoney[3], Zhongyun Dong[4], and Nalinikanth Kotagiri[1]

[1]Division of Pharmaceutical Sciences, James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, Ohio; [2]Department of Nuclear and Mechanical Engineering, University of Cincinnati, Cincinnati, Ohio; [3]Department of Radiology, University of Cincinnati College of Medicine, Cincinnati, Ohio; and [4]Division of Hematology and Oncology, University of Cincinnati College of Medicine, Cincinnati, Ohio

A subset (35%) of triple-negative breast cancers (TNBCs) expresses androgen receptor (AR) activity. However, clinical trials with antiandrogen drugs have shown limited efficacy, with about a 19% clinical benefit rate. We investigated the therapeutic enhancement of antiandrogens as radiosensitizers in combination with ^{18}F-FDG in TNBC. **Methods:** We screened 5 candidate drugs to evaluate shared toxicity when combined with either ^{18}F-FDG, x-rays, or ultraviolet radiation, at doses below their respective half-maximal inhibitory concentrations. Cytotoxic enhancement of antiandrogen in combination with ^{18}F-FDG was evaluated using cell proliferation and DNA damage assays. Finally, the therapeutic efficacy of the combination treatment was evaluated in mouse tumor models of TNBC and prostate cancer. **Results:** Bicalutamide, an antiandrogen drug, was found to share similar toxicity in combination with either ^{18}F-FDG or x-rays, indicating its sensitivity as a radiosensitizer to ^{18}F-FDG. Cell proliferation assays demonstrated selective toxicity of combination bicalutamide-^{18}F-FDG in AR-positive 22RV1 and MDA-MB-231 cells in comparison to AR-negative PC3 cells. Quantitative DNA damage and cell cycle arrest assays further confirmed radiation-induced damage to cells, suggesting the role of bicalutamide as a radiosensitizer to ^{18}F-FDG–mediated radiation damage. Animal studies in MDA-MB-231, 22RV1, and PC3 mouse tumor models demonstrated significant attenuation of tumor growth through combination of bicalutamide and ^{18}F-FDG in the AR-positive model in comparison to the AR-negative model. Histopathologic examination corroborated the in vitro and in vivo data and confirmed the absence of off-target toxicity to vital organs. **Conclusion:** These data provide evidence that ^{18}F-FDG in conjunction with antiandrogens serving as radiosensitizers has utility as a radiotherapeutic agent in the ablation of AR-positive cancers.

Key Words: antiandrogen therapy; bicalutamide; radiosensitization; ^{18}F-FDG

J Nucl Med 2022; 63:1177–1183
DOI: 10.2967/jnumed.121.262958

Concomitant chemotherapy and radiation therapy are an established treatment regimen for many neoplasms (*1–3*). For example, paclitaxel (Taxol; Bristol-Myers Squibb), besides having antiangiogenic properties, is also known to enhance the therapeutic effects of ionizing radiation as revealed in clinical trials (*4*). Thus, the risks associated with use of both radiotherapy and drugs are considerably high because of unintentional radiation–drug interactions (*5*). Therefore, there is a need for systematic examination of radiation–drug interactions and, more fundamentally, photochemical and biologic interactions between different classes of drugs and radiation in order to enhance therapeutic outcomes.

Recent studies have shown that radionuclides, in contrast to external-beam radiation, can provide the flexibility and extended reach in vivo to trigger therapeutic events in niches where metastatic cancer cells tend to localize, such as bone marrow and lungs (*6,7*). Typically, emissions from radionuclides include ionizing radiation, comprising electron (β^-) particles, positron (β^+) particles, α-particles, Auger electrons, x-rays, and γ-photons, as well as nonionizing radiation, comprising luminescence and ultraviolet–blue light emitted by β-particles, known as Cherenkov radiation. The various types of emissions from a single radionuclide can drive new opportunities in determining beneficial effects in combination with drugs. Because of the associated complexity in dosimetry, these combinations can also be challenging, as they can lead to unintended consequences if the treatment strategy is not optimized. For example, a recent phase III trial of vaginal cuff brachytherapy in combination with paclitaxel/carboplatin chemotherapy in patients with high-risk, early-stage endometrial cancer resulted in acute toxicities to the patients (*8*). Therefore, in our study we sought to identify and characterize compounds that are capable of efficiently harvesting the different energy emissions from diagnostic radionuclides to trigger therapeutic outcomes through synergistic action, without causing radiation or drug toxicity.

Since ^{18}F-FDG PET scans are routinely used in the evaluation of breast cancer patients, we postulated that by leveraging this technique for activating light-sensitive Food and Drug Administration–approved drugs, we could potentially transform toxic chemotherapeutics to nontoxic targeted therapy of untargetable cancers, such as triple-negative breast cancer (TNBC). In this study, we screened various anticancer drugs and explored synergy with different types of radiation sources to identify drugs that exhibit significantly higher toxicity against cancer cells when used in combination with ^{18}F-FDG. Previous studies have explored how nonionizing radiation from radionuclides can be harnessed by drugs and nanoparticles for photoactivated therapy (*6,9*). In this study, we explored how ionizing radiation from diagnostic radionuclides can be used in combination with certain drugs for enhanced cancer therapy while minimizing off-target effects.

Received Jul. 23, 2021; revision accepted Nov. 8, 2021.
For correspondence or reprints, contact Nalinikanth Kotagiri (kotaginh@ucmail.uc.edu).
Published online Nov. 12, 2021.

MATERIALS AND METHODS

Drug Screening in Combination with ^{18}F-FDG

All drugs (bicalutamide, flutamide, dacarbazine, 5-fluorouracil, and titanocene) were purchased from Apexbio Inc. The drugs were resuspended in dimethylsulfoxide, and aliquots were prepared. The in vitro efficacy of the drugs was investigated in the MDAMB231 cell line (ATCC). The cells were treated with drugs at a subtoxic dose, which was followed by the addition of 3 different activities (7.4, 14.8, and 29.6 MBq) of the radiopharmaceutical, ^{18}F-FDG, procured in phosphate-buffered saline. Medium was replaced after 1 half-life of ^{18}F, approximately 110 min of incubation, to remove excess ^{18}F-FDG that did not internalize in cells. Cell viability was evaluated after 72 h by measuring the mitochondrial activity in live cells using an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell proliferation agent (Promega) and absorbance measurement at 490 nm by Cytation I (BioTek Instruments) in triplicate. Similarly, cell viability was also measured for the cells pretreated with drugs with ultraviolet light (portable mini ultraviolet lamp, 4 W, 365 and 254 nm) for 1 h and ionizing radiation (50–220 kV x-rays, XenX irradiator; Xstrahl). An exposure of 2 Gy was used, which is a clinically used exposure limit for therapeutic purposes. The assays were repeated 3 times.

Androgen Receptor (AR) Expression on Cancer Cells and Cytotoxicity Assessment

AR expression of 22RV1, PC3, MDAMB231, and MCF10A cells (ATCC) was measured by flow cytometry. The cells were stained using standard procedures as outlined in the supplemental materials (available at http://jnm.snmjournals.org). The stained cells were analyzed for AR expression in flow cytometry (LSRII; BD Biosciences). The assays were repeated 3 times for each cell line. The toxicity of bicalutamide combined with ^{18}F-FDG treatment was evaluated using MTS assays in the cell lines following the manufacturer's instructions.

DNA Damage Assessment

DNA damage caused by bicalutamide-^{18}F-FDG in MDAMB231 was determined by cell death detection assays and γH2AX phosphorylation assays. A dose of 7.4 MBq of ^{18}F-FDG was henceforth used for all in vitro and in vivo experiments. Additional details on the cell death detection assay are outlined in the supplemental materials.

To measure γH2AX phosphorylation of histones, cells were harvested in a 96-well plate with a seeding density of 10^4 per well, treated with bicalutamide-^{18}F-FDG, bicalutamide, or ^{18}F-FDG, or left untreated, in triplicate using an EMD H2A.X phosphorylation assay kit (Millipore). Details are in the supplemental materials.

Evaluation of Cell Death Mechanism

Apoptotic cell death and autophagy were quantified by flow cytometry analysis using annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining and an autophagy detection kit (Enzo Life Sciences), respectively. Cell cycle analysis was performed by staining the fixed cells with PI dye. Details are in the supplemental materials.

Efficacy of Combined Bicalutamide-^{18}F-FDG Treatment in Animal Models

MDAMB231, 22RV1, and PC3 cultured cancer cells (2×10^6 cells) admixed with Matrigel (Corning Life Sciences) were injected subcutaneously in the flank regions of 6- to 8-wk-old female and male athymic nude mice (Jackson Laboratory). When the tumors reached 200–250 mm^3, the mice were randomized into different treatment groups (5 animals per group). Bicalutamide was administered orally every week at a dose of 60 mg/kg of body weight, and 7.4 MBq of ^{18}F-FDG were injected intraperitoneally for 4 wk. The mice were monitored every 2 d for 30–60 d for survival, adverse effects, and weight loss. Treatment efficacy was evaluated in comparison with no treatment, treatment with bicalutamide alone, and treatment with ^{18}F-FDG alone as control groups. An additional discussion on small-animal ^{18}F-FDG activity can be found in the supplemental materials (*10*).

Statistical Analysis

All experiments were performed in triplicate, and statistical analyses were performed using Prism software (version 8; GraphPad). *P* values were determined by 1-way ANOVA, and a *P* value of less than 0.01 was considered statistically significant in all experiments whenever applicable. The results of in vitro and in vivo analyses are shown as mean ± SD or as the SEM of 3 or more independent experiments.

RESULTS

Evaluation of Toxicity of Drugs in Combination with ^{18}F-FDG

We evaluated 5 anticancer drugs—5-fluorouracil, dacarbazine, flutamide, bicalutamide, and titanocene—on the basis of their already-established photosensitive properties. 5-fluorouracil is known to induce protein degradation through superoxide radical and photooxidation mechanisms on ultraviolet irradiation (*11*). Dacarbazine is a photosensitive drug known to synergize with light to produce increased toxicity through a photodegradation mechanism (*12*). Flutamide and bicalutamide are known to be photoreactive and to induce photosensitive drug eruptions in patients (*13,14*). Titanocene is an ultraviolet light–sensitive compound that exhibits enhanced phototoxicity through a photodegradation mechanism (*6,7*). Although all 5 drugs are known to be light-sensitive, it is unclear to which type of radiation—ionizing or nonionizing—they are sensitive. Since ^{18}F-FDG generates both ultraviolet and ionizing radiation, we sought to identify specifically which radiation, between the two, triggered cell death in combination with drugs. Our first objective was to estimate their dark toxicity by identifying their half-maximal inhibitory concentrations in MDAMB231 cells, a representative TNBC cell line (Supplemental Fig. 1). The drugs were then screened and evaluated for their ability to induce cytotoxicity, at doses below their respective half-maximal inhibitory concentrations, in combination with ^{18}F-FDG, x-rays, or ultraviolet radiation. On the basis of the estimated half-maximal inhibitory concentrations, doses of 3.12, 50, 25, 12.5, and 25 μM were selected for 5-fluorouracil, titanocene, dacarbazine, flutamide, and bicalutamide, respectively.

All 5 drugs showed enhanced toxicity when treated with ^{18}F-FDG. However, the toxicity profile for the drugs was unique with respect to sensitivity to ultraviolet light, radiation, or both (Figs. 1A and 1B). Besides ^{18}F-FDG, 5-fluorouracil and titanocene exhibited cytotoxicity in combination with ultraviolet light. Similarly, besides ^{18}F-FDG, flutamide and bicalutamide exhibited cytotoxicity exclusively in combination with x-rays. Dacarbazine, however, exhibited cytotoxicity in combination with both ultraviolet light and x-rays, as well as ^{18}F-FDG. These results suggest that ionizing radiation (flutamide, bicalutamide, and dacarbazine) or nonionizing radiation (5-fluorouracil, titanocene, and dacarbazine) emitted by the radioactive decay of ^{18}F could be playing a role in triggering these drugs. The lower cytotoxicity of ultraviolet light–treated cultures treated with flutamide and bicalutamide, compared with drug alone, likely suggests drug photoinactivation (*15*).

Bicalutamide Radiosensitizes Cancer Cells to ^{18}F-FDG and X-Rays

Bicalutamide is a molecularly targeted antiandrogen drug that also has radiosensitizing properties. Therefore, we hypothesized that bicalutamide in combination with ^{18}F-FDG will likely enhance cytotoxicity and provide greater control in spatiotemporal modulation

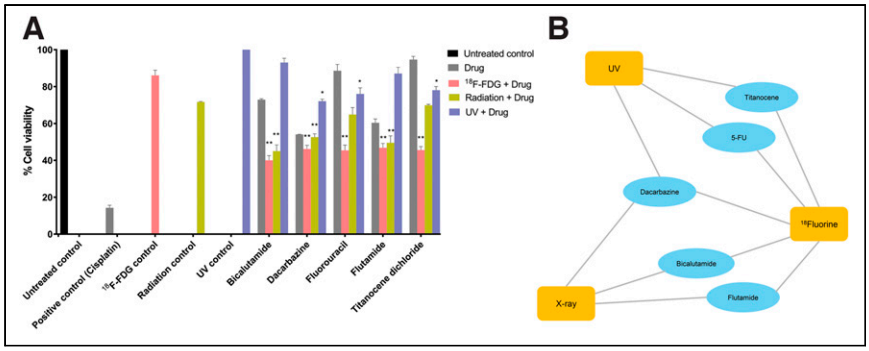

FIGURE 1. (A) Screening of Food and Drug Administration–approved drugs in MDAMB231 cells and evaluation of cell toxicity in combination with ^{18}F-FDG, ultraviolet light, and x-ray exposure. (B) Schematic representation of drugs classified by response to ^{18}F-FDG, ultraviolet light, and x-rays. *$P < 0.01$. **$P < 0.001$. UV = ultraviolet.

of this therapeutic approach. First, we investigated the photophysics behind the reactants, bicalutamide, and ^{18}F-FDG. Photoproducts formed by photoreaction of the drugs with ultraviolet light typically causes type IV hypersensitive (cell-mediated) reactions (*16*). However, we noted earlier that bicalutamide in the presence of ultraviolet light did not induce cytotoxicity. To further confirm whether ultraviolet irradiation of bicalutamide induces the generation of photoproducts, we performed mass spectrometry and Fourier-transform infrared spectroscopy analysis. We observed no change in the structure (Supplemental Fig. 2) or molar mass (Supplemental Fig. 3) of bicalutamide, suggesting that the parent compound was stable even after irradiation with ultraviolet light. These results suggest that irradiating bicalutamide with ^{18}F-FDG likely results in minimal photodegradation. However, to explain the enhanced toxicity observed with bicalutamide-^{18}F-FDG, it is possible that there is biologic cooperation, with bicalutamide blocking the AR and inhibiting DNA damage repair, thereby increasing the susceptibility of cancer cells to radiation damage by ^{18}F-FDG (*17*).

Cytotoxic Enhancement of Bicalutamide-^{18}F-FDG Combination in AR-Positive Cancer Cells

We analyzed 4 human cancer cell lines, 2 prostate and 2 breast cancers, for AR expression by flow cytometry. The prostate cancer cell lines were 22RV1 and PC3, and the breast cancer cell lines were MDAMB231 and MCF10A, the latter being a normal breast epithelial cell line. Although MCF10A and PC3 cells did not display any significant AR expression (Supplemental Figs. 4A and 4B), an estimated 85% of the cultured 22RV1 cells and 81% of the MDAMB231 cells expressed AR (Supplemental Figs. 4C and 4D). We then evaluated the cytotoxicity of the bicalutamide-^{18}F-FDG combination on AR-positive 22RV1 and MDAMB231 cells and AR-negative MCF10A and PC3 cells. We observed that even at a subcytotoxic dose of 25 μM, cytotoxicity (20% inhibitory concentration) of bicalutamide was significantly enhanced after exposure to activity of as low as 7.4 MBq in both MDAMB231 and 22RV1 cells, whereas the 20% inhibitory concentration of bicalutamide alone was 65 μM (Figs. 2A and 2B). We observed an activity-dependent increase in cytotoxicity with 7.4, 14.8, and 29.6 MBq of ^{18}F-FDG. Both MCF10A cells and PC3 cells were unresponsive to either bicalutamide alone or bicalutamide-^{18}F-FDG treatment even at a dose of 50 μM (Figs. 2C and 2D). These results suggest that the combination of bicalutamide-^{18}F-FDG selectively inhibits proliferation of AR-positive cells over non-AR–expressing cells at subcytotoxic monotherapy doses.

Bicalutamide-^{18}F-FDG Treatment Causes DNA Damage

We first investigated whether the cells were experiencing oxidative damage to their DNA, as is typical in radiotherapies. Relative quantification of the DNA fragments obtained in the cell lysate after treatment with bicalutamide and 7.4 MBq of ^{18}F-FDG in MDAMB231 cells was correlated with DNA damage, which is generally considered an early indicator of apoptosis. Using a quantitative enzyme-linked immunosorbent assay–based assay to detect mono- and oligonucleosomes, we observed that bicalutamide-^{18}F-FDG treatment induced increased DNA damage to the cells compared with bicalutamide alone,^{18}F-FDG alone, or no treatment (Fig. 3A). DNA damage was observed to be higher at 5 h after treatment than at 48 h, mainly because nucleosomes are detected before manifestation of the cascade of other apoptotic events and because there are likely to be fewer intact viable cells at 48 h as a consequence of apoptosis.

Phosphorylation of H2AX histone protein typically occurs when DNA double-stranded breaks are induced during exposure of cancer cells to chemotherapeutics or radiation, resulting in DNA rearrangement or apoptosis. Bicalutamide-^{18}F-FDG treatment resulted in a significantly higher chemiluminescence measurement, which correlates with high amounts of γH2AX protein from increased double-stranded breaks in the DNA (Fig. 3B).

Cell cycle and DNA content analysis showed no significant increase in cells occupying the G1/S phase 24 h after treatment. However, 32.8% of the cells were occupying the G_2/M phase when treated with bicalutamide-^{18}F-FDG, compared with 18.3% with bicalutamide alone and 17.3% with ^{18}F-FDG–treated cells and untreated cells (Fig. 3C). This study indicates that the antiproliferative characteristics of bicalutamide-^{18}F-FDG treatment in cancer cells is caused by G_2/M cell cycle arrest after induction of DNA damage, thus preventing the cells with DNA damage from entering the M phase and undergoing mitosis.

Cell Death Mechanism: Apoptosis and Autophagy

Apoptotic cell death is characterized by distinct morphologic changes such as cell shrinkage, DNA condensation, and cell blebbing. These biochemical processes, involving various signaling pathways, are triggered when cells undergo DNA damage and cell cycle arrest. MDAMB231 cells treated with bicalutamide-^{18}F-FDG (7.4 MBq of ^{18}F-FDG, 25 μM of bicalutamide) were evaluated using flow cytometric analysis of annexin V-FITC/PI staining. Figure 4 shows that 32.7% of bicalutamide-^{18}F-FDG–treated cells were found to be in the late apoptotic state, since they stained positively for both annexin V-FITC and PI. In contrast, only 10.6% of untreated and 18.6% of bicalutamide-alone–treated cells stained with both annexin V-FITC and PI. Annexin V-FITC stains cells undergoing apoptosis by staining phosphatidylserine molecules that have translocated to the outside of the cell membrane, whereas PI stains necrotic cells or late apoptotic cells, which are characterized by loss of the integrity of the plasma and nuclear membranes. The high degree of DNA fragmentation and double staining of cells with annexin V and PI suggests apoptosis, rather than necrosis, to be the dominant cell death mechanism as a result of bicalutamide-^{18}F-FDG treatment.

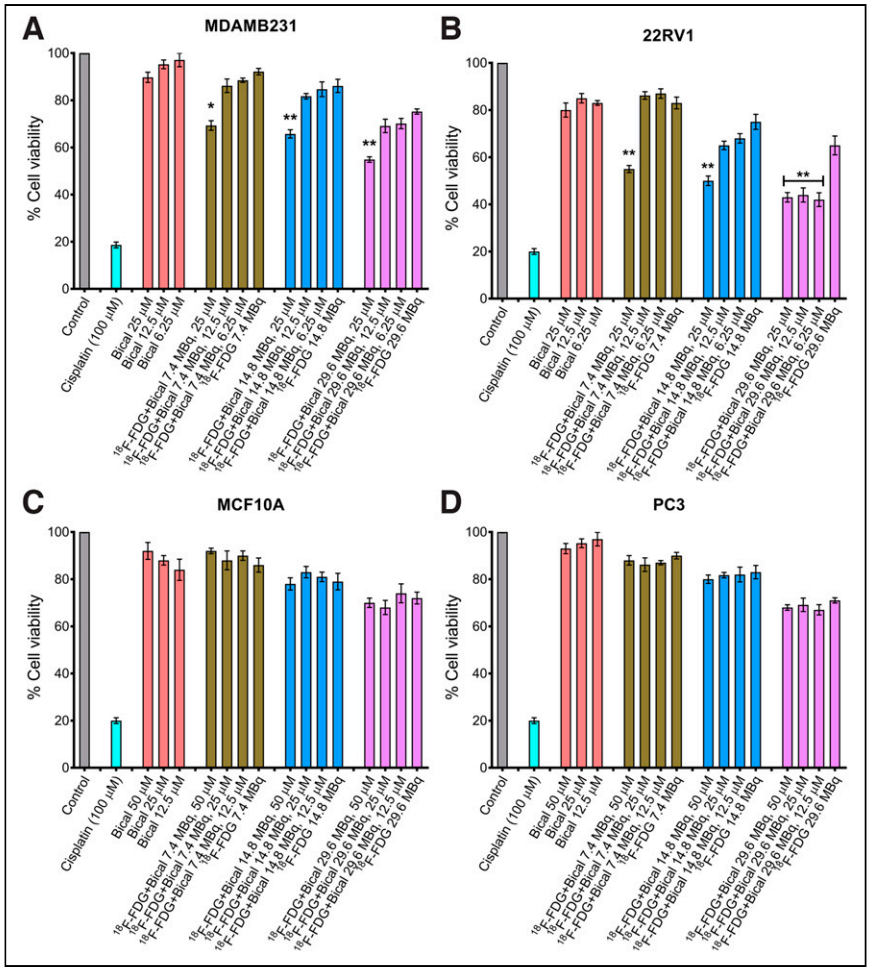

FIGURE 2. Cytotoxicity of bicalutamide-^{18}F-FDG treatment for 72 h in MDAMB231 (A), 22RV1 (B), MCF10A (C), and PC3 (D) cells. *$P < 0.01$. **$P < 0.001$. Bical = bicalutamide.

We next examined whether cells were undergoing autophagy, since ionizing radiation has been shown to induce autophagy in cancer cells (*18*). The autophagic process is a regulated mechanism involving swelling of the cell membrane and formation of autophagosomes to degrade and recycle the intracellular contents. Cell death by the autophagic process was quantified by analyzing cells stained with Cyto-ID green dye (Enzo Life Sciences) using a flow cytometer. Cyto-ID stains acidic vesicular organelles green at different stages in the process of autophagy before it fuses with lysosomes and is internalized. Figure 5 and Supplemental Figure 5 clearly show a higher fluorescence intensity and upregulation of autophagic activity in cells treated with combined bicalutamide-^{18}F-FDG than in cells treated with bicalutamide alone or ^{18}F-FDG alone or in cells left untreated. Rapamycin- and chloroquine-treated cells were used as positive controls.

Efficacy of Combination Bicalutamide-^{18}F-FDG Treatment in Mouse Xenograft Models

After initiation of treatment, we started observing significant separation between the tumor volume curve of the bicalutamide-^{18}F-FDG and the others, as shown in Figures 6A and 6B. Overall, combination treatment with bicalutamide and ^{18}F-FDG exhibited significant attenuation in tumor growth, compared with bicalutamide alone or ^{18}F-FDG alone, which did not significantly differ from the untreated group.

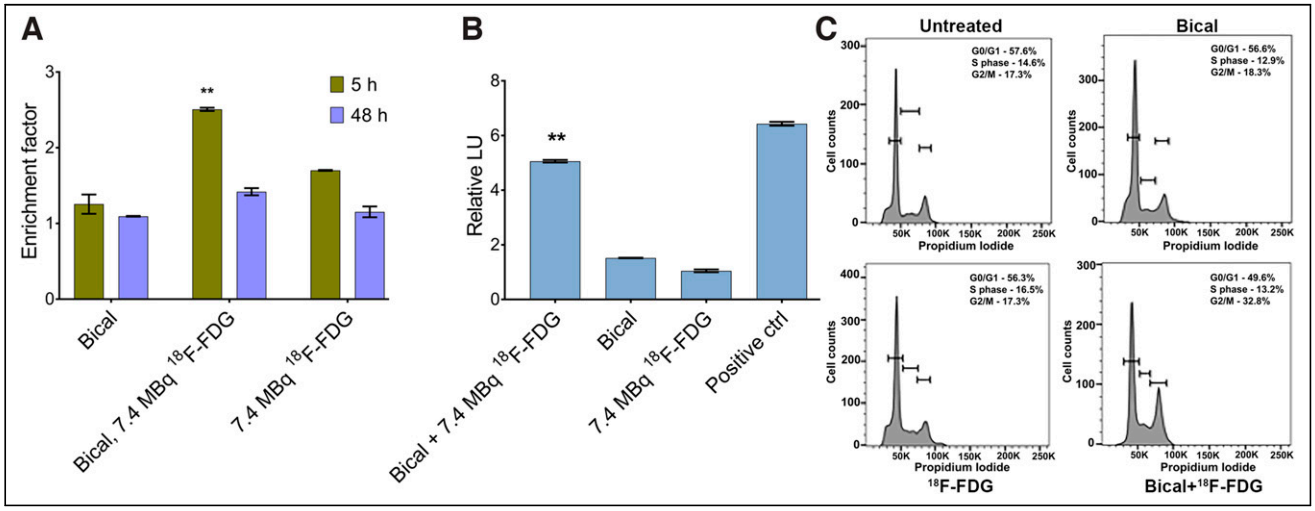

FIGURE 3. (A) Evaluation of DNA damage in bicalutamide-^{18}F-FDG–treated MDAMB231 cells by enzyme-linked immunosorbent assay–based detection of mono- and oligonucleosomes (DNA fragments) as result of DNA fragmentation. (B) Luminescent measurement of phosphorylated γH2AX protein that occurred as result of DNA damage within cells. (C) Determination of cell cycle arrest in various phases due to DNA damage in bicalutamide-^{18}F-FDG–treated cells. **$P < 0.001$. Bical = bicalutamide; ctrl = control; LU = luminescence.

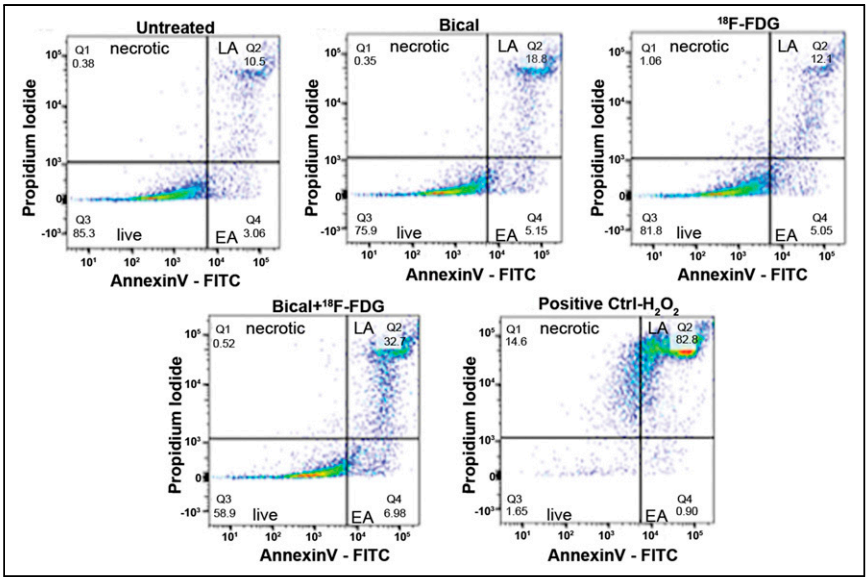

FIGURE 4. Flow cytometric quantification of bicalutamide-^{18}F-FDG–treated MDAMB231 cells undergoing apoptosis in various apoptotic stages. EA = early apoptosis; LA = late apoptosis.

Histopathologic analysis of immunostained MDAMB231 tumor tissues (Supplemental Fig. 6A) revealed that tumor tissues treated with bicalutamide-^{18}F-FDG stained strongly positive for cleaved caspase-3, which is an expression marker for apoptosis and DNA damage. Similarly, with Ki-67 staining, which is a marker for highly proliferating cells, there was a significant reduction in Ki-67 staining in tumors treated with bicalutamide-^{18}F-FDG. We also observed a decreased number of cells staining positively for CD68, suggesting lower degrees of macrophage infiltration within the tumor. Typically, increased tumor growth is also characterized by the presence of CD68-expressing tumor-infiltrating macrophages, playing a key role in promoting tumor initiation and malignant progression. In addition, hematoxylin and eosin staining of brain, heart, liver, and kidney confirmed no evidence of metastatic spread to these organs or of any treatment-related off-target toxicity (Supplemental Fig. 6B).

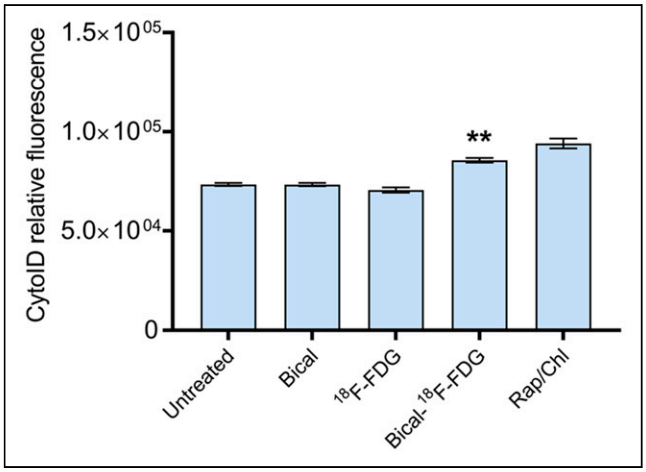

FIGURE 5. Flow cytometric quantification of bicalutamide-^{18}F-FDG–treated MDAMB231 cells undergoing autophagy. Rapamycin/chloroquine-treated cells were used as positive control. $^{**}P < 0.001$. Bical = bicalutamide; Rap/Chl = rapamycin/chloroquine.

To elucidate the role that AR-targeted bicalutamide plays in this dual-specificity strategy, a similar experiment was performed with an AR-negative prostate cancer xenograft model in male nude mice using PC3 cells. Unlike the AR-positive cancer models, the bicalutamide-^{18}F-FDG treated group did not significantly differ from the ^{18}F-FDG or bicalutamide control arms (Fig. 6C). Interestingly, there was a significant increase in PC3 tumor attenuation in mice treated with ^{18}F-FDG, compared with untreated mice or mice treated with bicalutamide alone, further confirming the critical role that AR plays in sensitizing cells to ^{18}F-FDG in TNBC. Histopathologic analyses using staining with terminal deoxynucleotidyl transferase 2′-deoxyuridine, 5′-triphosphate nick-end labeling (DNA damage causing cell death) and CD68 staining for tumor-infiltrating macrophages did not indicate any degree of tumor ablation at the tissue level, as shown in Supplemental Figures 6C and 6D. Because of the nontumorigenic nature of MCF10A cells, tumor induction was not possible for this model.

DISCUSSION

TNBC is an aggressive cancer that lacks estrogen, progesterone, and human epidermal growth factor receptor 2 receptors and therefore cannot be treated with targeted hormone therapies or anti–human epidermal growth factor receptor 2 agents (*19*). As a result, conventional always-on chemotherapy remains the only effective systemic treatment available for these patients. Recent studies have reported that approximately 35% of TNBC patients have shown expression of membrane AR (*20*). Although the role of AR in TNBC is an area of active research, there is evidence that this cancer subset may respond to therapeutic targeting of AR (*21*). Bicalutamide functions by blocking and inhibiting AR and is widely used in the treatment of prostate cancer subtypes that overexpress AR (*22*). However, clinical trials with bicalutamide and other antiandrogen drugs in TNBC have shown limited efficacy, with about a 19% clinical benefit rate (*21*). Therefore, we hypothesized that bicalutamide-activated cytotoxic enhancement of ^{18}F-FDG would improve the outcomes of the combination treatment in TNBC and provide spatiotemporal modulation of therapy as well. This hypothesis is supported by the influence that AR activity has on cell cycle checkpoint inhibition and the DNA damage repair pathway (*23,24*). Prior research has also demonstrated that antiandrogen drugs that block AR activity confer radiosensitivity to traditionally radioresistant AR-positive TNBC cell lines by significantly impairing resolution of double-stranded DNA breaks (*17*). Although TNBCs are chemosensitive, they metastasize rapidly and are characterized by poor prognosis and few therapeutic options. Therefore, use of radionuclides, in contrast to external-beam radiation, extends the in vivo reach to trigger therapeutic events in niches where metastatic cancer cells tend to localize, such as bone marrow and lungs.

Although both targeted hormonal therapy and radiosensitizing roles for bicalutamide would be highly desired in a clinical setting, for our proof-of-concept studies we sought the effective separation of both these properties in order to evaluate the singular role of

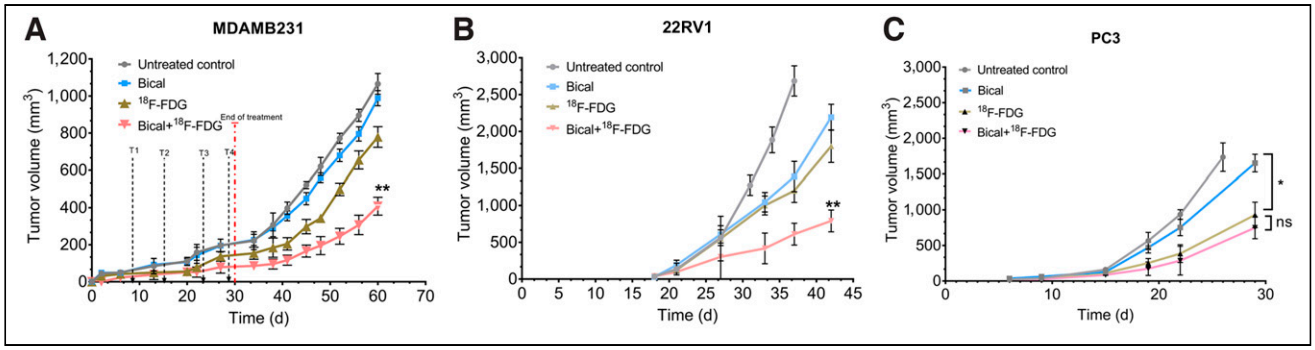

FIGURE 6. Tumor growth demonstrating therapeutic efficacy of combined treatment of bicalutamide-^{18}F-FDG in athymic nude xenograft tumor model induced with subcutaneous MDAMB231 cancer cells (A), 22RV1 cancer cells (B), or PC3 cancer cells (C). n = 5/group. *$P < 0.01$. **$P < 0.001$. Bical = bicalutamide.

bicalutamide as a radiosensitizer. In vitro studies revealed that a subcytotoxic dose of bicalutamide in combination with ^{18}F-FDG inhibited cell proliferation, induced double-stranded DNA breaks, and caused cell cycle arrest at the G_2/M phase, leading to apoptotic cell death. Therefore, we can infer that bicalutamide is a potent radiosensitizer because it selectively inhibits DNA damage repair of AR-positive cells caused by ^{18}F-FDG.

Previous reports have shown that apoptosis accounts for approximately only 20% of radiation-induced cell death (*25*). Therefore, we explored other cell death pathways, such as autophagy, to determine whether multiple pathways are involved in inducing cell death. We observed significant cell death through autophagy, implicating the role of endoplasmic reticulum stress and aberrant protein in the formation of autophagosomes. Although there is still no consensus on attributing specific mechanisms that link radiation and autophagy, there is some evidence that double-stranded DNA breaks may induce endoplasmic reticulum stress (*26*). The enhanced therapeutic efficacy of the combination treatment in TNBC cells appears to contribute significantly to both the autophagy and the apoptosis cell death pathways.

In vivo therapy studies on AR-positive TNBC and 22RV1 mouse models showed significant attenuation of tumor growth and enhanced therapeutic efficacy in mice treated with bicalutamide-^{18}F-FDG compared with control groups. The therapeutic effect of bicalutamide alone was minimal, as can be attributed to the subtherapeutic concentration used for these studies. However, in an AR-negative PC3 mouse model, we observed no significant attenuation of tumor growth, supporting the critical role bicalutamide plays in radiosensitizing AR-positive tumors to radiation-induced damage from ^{18}F-FDG. Further studies are needed to elucidate the mechanism of enhanced therapeutic efficacy—synergistic versus additive—before translation of this strategy can potentially be realized.

With ^{18}F-FDG, we avoided using an excessively high or nondiagnostic imaging activity, not only to demonstrate the efficiency of bicalutamide as a radiosensitizer in reducing the threshold for radiation damage but also to allay any concerns about potential toxicity during scale-up for translational studies, in which higher activities of ^{18}F-FDG might be deemed unrealistic for use in humans (*10*). However, additional studies are needed to understand the dosage expectancy of ^{18}F-FDG for potential clinical translation. Gross morphologic analysis, as well as hematoxylin and eosin analysis of major organs, did not reveal any off-target toxicity from treatment. Indeed, further studies are required to estimate absorbed dose in normal organs and collect additional evidence to confirm the absence of off-target toxicity in various vital organs such as brain and bone marrow.

CONCLUSION

We have demonstrated the sensitivity of AR-positive cancer to radiation damage using the diagnostic radiopharmaceutical ^{18}F-FDG in conjunction with bicalutamide, an antiandrogen that serves as a targeted radiosensitizer. These findings open opportunities to investigate other antiandrogens and radionuclides, as synergistic pairs, to enhance therapeutic outcomes. Given the wide range of radionuclides available, studies exploring the mixed radiation emissions and interaction with drugs to identify synergistic enhancement of cell killing would be intriguing.

DISCLOSURE

Nalinikanth Kotagiri receives funding from the CDMRP BRCA Breakthrough Award (W81XWH-18-1-0609), the NIH (U54 CA199092), and a University of Cincinnati Strategic collaborative grant. No other potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

We thank the radiation safety staff at the University of Cincinnati for assistance with the handling of radioactive materials. We also acknowledge the help provided by Shindu Thomas and Tushar Madaan with animal experiments, Sithara Raju Ponny with data analysis, Betsy DiPasquale and Jessica Webster with preparation of samples for IHC, Larry Salans with mass spectrometry studies, Mallikarjuna Nadagouda with FTIR studies, and the flow cytometry core at the Cincinnati Children's Hospital with training and flow analysis.

KEY POINTS

QUESTION: Can antiandrogen hormonal therapy radiosensitize AR-positive cancers to ^{18}F-FDG?

PERTINENT FINDINGS: We observed a significant attenuation, 2-fold, of tumor growth in AR-positive compared with AR-negative cancers.

IMPLICATIONS FOR PATIENT CARE: We envisage that this strategy will benefit AR-positive cancer patients with metastasis by providing an effective image-guided targeted therapy with minimal side effects.

REFERENCES

1. Apisarnthanarax S, Dhruva N, Ardeshirpour F, et al. Concomitant radiotherapy and chemotherapy for high-risk nonmelanoma skin carcinomas of the head and neck. *Int J Surg Oncol.* 2011;2011:464829.
2. Glatzer M, Elicin O, Ramella S, Nestle U, Putora PM. Radio(chemo)therapy in locally advanced nonsmall cell lung cancer. *Eur Respir Rev.* 2016;25:65–70.
3. Calais G, Alfonsi M, Bardet E, et al. Randomized trial of radiation therapy versus concomitant chemotherapy and radiation therapy for advanced-stage oropharynx carcinoma. *J Natl Cancer Inst.* 1999;91:2081–2086.
4. Dicker AP, Williams TL, Iliakis G, Grant DS. Targeting angiogenic processes by combination low-dose paclitaxel and radiation therapy. *Am J Clin Oncol.* 2003;26: e45–e53.
5. Niyazi M, Maihoefer C, Krause M, Rodel C, Budach W, Belka C. Radiotherapy and "new" drugs: new side effects? *Radiat Oncol.* 2011;6:177.
6. Kotagiri N, Sudlow GP, Akers WJ, Achilefu S. Breaking the depth dependency of phototherapy with Cerenkov radiation and low-radiance-responsive nanophotosensitizers. *Nat Nanotechnol.* 2015;10:370–379.
7. Kotagiri N, Cooper ML, Rettig M, et al. Radionuclides transform chemotherapeutics into phototherapeutics for precise treatment of disseminated cancer. *Nat Commun.* 2018;9:275.
8. Randall ME, Filiaci V, McMeekin DS, et al. Phase III trial: adjuvant pelvic radiation therapy versus vaginal brachytherapy plus paclitaxel/carboplatin in high-intermediate and high-risk early stage endometrial cancer. *J Clin Oncol.* 2019;37:1810–1818.
9. Pratt EC, Shaffer TM, Zhang Q, Drain CM, Grimm J. Nanoparticles as multimodal photon transducers of ionizing radiation. *Nat Nanotechnol.* 2018;13:418–426.
10. Taylor K, Lemon JA, Boreham DR. Radiation-induced DNA damage and the relative biological effectiveness of ^{18}F-FDG in wild-type mice. *Mutagenesis.* 2014;29: 279–287.
11. Miolo G, Marzano C, Gandin V, et al. Photoreactivity of 5-fluorouracil under UVB light: photolysis and cytotoxicity studies. *Chem Res Toxicol.* 2011;24:1319–1326.
12. Dorr RT, Alberts DS, Einspahr J, Mason-Liddil N, Soble M. Experimental dacarbazine antitumor activity and skin toxicity in relation to light exposure and pharmacologic antidotes. *Cancer Treat Rep.* 1987;71:267–272.
13. Yokote R, Tokura Y, Igarashi N, Ishikawa O, Miyachi Y. Photosensitive drug eruption induced by flutamide. *Eur J Dermatol.* 1998;8:427–429.
14. Lee K, Oda Y, Sakaguchi M, Yamamoto A, Nishigori C. Drug-induced photosensitivity to bicalutamide: case report and review of the literature. *Photodermatol Photoimmunol Photomed.* 2016;32:161–164.
15. Sortino S, Giuffrida S, De Guldi G, et al. The photochemistry of flutamide and its inclusion complex with beta-cyclodextrin: dramatic effect of the microenvironment on the nature and on the efficiency of the photodegradation pathways. *Photochem Photobiol.* 2001;73:6–13.
16. Sasada K, Sakabe J, Tamura A, et al. Photosensitive drug eruption induced by bicalutamide within the UVB action spectrum. *Eur J Dermatol.* 2012;22:402–403.
17. Speers C, Zhao SG, Chandler B, et al. Androgen receptor as a mediator and biomarker of radioresistance in triple-negative breast cancer. *NPJ Breast Cancer.* 2017;3:29.
18. Zois CE, Koukourakis MI. Radiation-induced autophagy in normal and cancer cells: towards novel cytoprotection and radio-sensitization policies? *Autophagy.* 2009;5:442–450.
19. Schneider BP, Winer EP, Foulkes WD, et al. Triple-negative breast cancer: risk factors to potential targets. *Clin Cancer Res.* 2008;14:8010–8018.
20. Park S, Koo J, Park HS, et al. Expression of androgen receptors in primary breast cancer. *Ann Oncol.* 2010;21:488–492.
21. Gucalp A, Tolaney S, Isakoff SJ, et al. Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic breast cancer. *Clin Cancer Res.* 2013;19:5505–5512.
22. Labrie F, Bélanger A, Luu-The V, et al. Gonadotropin-releasing hormone agonists in the treatment of prostate cancer. *Endocr Rev.* 2005;26:361–379.
23. Goodwin JF, Schiewer MJ, Dean JL, et al. A hormone-DNA repair circuit governs the response to genotoxic insult. *Cancer Discov.* 2013;3:1254–1271.
24. Polkinghorn WR, Parker JS, Lee MX, et al. Androgen receptor signaling regulates DNA repair in prostate cancers. *Cancer Discov.* 2013;3:1245–1253.
25. Verheij M, Bartelink H. Radiation-induced apoptosis. *Cell Tissue Res.* 2000;301: 133–142.
26. He L, Kim SO, Kwon O, et al. ATM blocks tunicamycin-induced endoplasmic reticulum stress. *FEBS Lett.* 2009;583:903–908.