

Preclinical studies of a PARP targeted, Meitner-Auger emitting, theranostic radiopharmaceutical for metastatic ovarian cancer

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Abstract: Advanced ovarian cancer currently has few therapeutic options. Poly(ADP-ribose) polymerase (PARP) inhibitors bind to nuclear PARP and trap the protein-inhibitor complex to DNA. This work investigates a theranostic PARP inhibitor for targeted radiopharmaceutical therapy of ovarian cancer *in vitro* and PET imaging of healthy mice *in vivo*. **Methods:** [⁷⁷Br]RD1 was synthesized and assessed for pharmacokinetics and cytotoxicity in human and murine ovarian cancer cell lines. [⁷⁶Br]RD1 biodistribution and organ uptake in healthy mice were quantified through longitudinal PET/CT imaging and *ex vivo* radioactivity measurements. Organ-level dosimetry following [^{76/77}Br]RD1 administration was calculated using RAPID, an in-house platform for absorbed dose in mice, and OLINDA for equivalent and effective dose in human. **Results:** The maximum specific binding ($B_{\max}$), equilibrium dissociation constant (K_d), and nonspecific binding slope (NS) were calculated for each cell line. These values were used to calculate the cell specific activity uptake for cell viability studies. The half maximal effective concentration (EC_{50}) was measured as 0.17 (95% CI: 0.13–0.24) nM and 0.46 (0.13–0.24) nM for PARP(+) and PARP(-) expressing cell lines, respectively. The EC_{50} was 0.27 (0.21–0.36) nM and 0.30 (0.22–0.41) nM for BRCA1(-) and BRCA1(+) expressing cell lines, respectively. When measuring the EC_{50} as a function

of cellular activity uptake and nuclear dose, the EC_{50} ranges from 0.020–0.039 Bq/cell and 3.3–9.2 Gy, respectively. Excretion through the hepatobiliary and renal pathways were observed in mice, with liver uptake of 2.3 ± 0.4 %ID/g after 48 h, contributing to estimated absorbed dose values in mice of 19.3 ± 0.3 mGy/MBq and 290 ± 10 mGy/MBq for [^{77}Br]RD1 and [^{76}Br]RD1, respectively. **Conclusion:** [^{77}Br]RD1 cytotoxicity was dependent on PARP expression and independent of BRCA1 status. The *in vitro* results suggest that [^{77}Br]RD1 cytotoxicity is driven by the targeted Meitner-Auger electron (MAe) radiotherapeutic effect of the agent. Further studies investigating the theranostic potential, organ dose, and tumor uptake of [$^{76/77}\text{Br}$]RD1 are warranted.

Key Words: bromine-77, bromine-76, Meitner-Auger radionuclide therapy, positron emission tomography, PARP-1 inhibitor, theranostic, ovarian cancer therapy

1. Introduction

An estimated 20,000 new cases of ovarian cancer will be diagnosed in 2023 with approximately 50% of patients presenting with distant-staged disease [1]. Upon metastasis of ovarian cancer, patients have a 5-year relative survival rate of 31%, only a 1–3% increase over the last 20 years [1], [2]. The current standard of care for advanced ovarian cancer includes cytoreductive surgery and platinum-based chemotherapy with curative intent [3]. Platinum-based chemotherapy drugs are known for their lack of selectivity, high systemic toxicity, and drug resistance [4]. Patients may have good initial response to this treatment, but approximately 75% of patients with advanced stage ovarian cancer will relapse, requiring further chemotherapy treatments. Furthermore, 30% of these patients will develop chemotherapy drug resistance, at which point, treatment shifts from curative intent to maximizing quality of life [5].

Comprehensive molecular characterization of epithelial ovarian cancer (EOC) and especially high-grade serous ovarian cancer demonstrated an over-representation of *BRCA1/2* pathogenic variants [6]. This demonstrates the importance of double strand DNA repair pathways, such as homologous recombination (HR), with further studies confirming that dysfunction in this pathway leads to a higher

risk of development of EOC [7], [8]. Drugs targeting the DNA damage response systems provides an opportunity to target this advanced disease for patients who otherwise have poor prognosis and few treatment options. Poly(ADP-ribose) polymerase (PARP) enzymes are nuclear proteins with a critical role in DNA damage response. PARP inhibitors exploit synthetic lethality through single-strand break and double-strand break repair pathways, specifically the HR impaired pathway [9]–[12]. FDA approved PARP inhibitors olaparib, rucaparib, and talazoparib trap the PARP-drug complex nanometers from target DNA [13] making them viable diagnostic and therapeutic radiopharmaceutical candidates in cancers with PARP expression [14].

The use of positron emission tomography (PET) imaging to assess physiologic PARP expression in patients has been established as a noninvasive imaging tool [14]. Two ^{18}F -labeled PARP inhibitors are in the process of being translated from preclinical research to clinical PET application [15]–[17]. The first agent, [^{18}F]FTT, is a rucaparib analog which has shown potential for imaging PARP [18]. Multiple studies have demonstrated [^{18}F]FTT's efficacy in preclinical models, and human trials are currently underway [16], [19]. The second agent is an olaparib analog, [^{18}F]PARPi, that targets PARP and has shown promise in imaging breast, ovarian and brain cancers [17], [20]. [^{18}F]PARPi has also been shown to have potential in imaging other DNA damage response pathways, including DNA-PK, ATR, and ATM [21] and is currently undergoing clinical trials investigating its potential as a diagnostic tool for cancer. Additional ^{18}F -labeled isotopologues that have been preclinically investigated include [^{18}F]rucaparib [22], [23], [^{18}F]talazoparib [24], [25], and [^{18}F]olaparib [26], [27].

PARP has been targeted in radiopharmaceutical therapy using olaparib- and rucaparib-derived agents labeled with various radionuclides, including β^- emitter ^{131}I [28], alpha-emitter ^{211}At [29]–[31], and Meitner-Auger electron (MAe) emitters ^{125}I [32]–[35], ^{123}I [28], [35] and ^{77}Br [36]. These preclinical studies have investigated responses in ovarian cancer [35] as well as glioblastoma [28], [37], [38], neuroblastoma [32], colon [33], breast [34], prostate cancers [36]. The biological mechanism of PARP

that brings its inhibitors within nanometers of DNA is well suited for use with the low energy (eV–keV), short range (0.001–100 μm), and high linear energy transfer (LET, 1–23 keV/ μm) of MAe [39]. *In vitro* cell cytotoxicity studies show MAe PARP inhibitors to be highly cytotoxic across several cancer cell lines [32], [34], [35], [37], [40], and tumor-bearing mice treated with MAe PARP inhibitors were observed to have prolonged survival *in vivo* [28], [33], [36]. Higher cytotoxicity was observed compared to radioinert PARP inhibitors with no dependence on *BRCA1* expression [35]. These results show promising radiotherapeutic potential that warrants further preclinical and clinical investigation across a multitude of cancers.

Rucaparib is FDA approved for both treatment and maintenance therapy of ovarian cancer [41] and a derivative of this molecule was investigated as a theranostic radiopharmaceutical labeled with MAe-emitting ^{77}Br ($t_{1/2}=57$ h, 6-7 MAe per decay) [42] and positron emitting ^{76}Br ($t_{1/2}=16.2$ h, 55% β^+). The short-range MAe from ^{77}Br deposits their energy locally [43], providing low dose to off-target cells. Bromine forms a more stable bond with carbon compared with iodine, resulting in less dehalogenation of radiopharmaceuticals *in vivo*. Additionally, unlike radioiodine, free radiobromine does not accumulate in the thyroid, diffusing evenly throughout the body with only the stomach reaching higher uptake than blood [44], [45]. The PARP inhibitor mechanism provides a biological approach to localize the radioactive drug to the DNA. The imaging analog, labeled with ^{76}Br , allows for noninvasive *in vivo* PET imaging [46], [47] of radiopharmaceutical biodistribution. This study examines the *in vitro* radiopharmacology and toxicology of rucaparib derived [^{77}Br]RD1. Additionally, it investigated the potential for *in vivo* PET imaging of its diagnostic analog, [^{76}Br]RD1, in healthy mice.

2. Materials and Methods

2.1 Radiosynthesis of [$^{76/77}\text{Br}$]RD1

Production of $^{76/77}\text{Br}$ and $[^{76/77}\text{Br}]\text{RD1}$ synthesis and quality control were performed according to published procedures as detailed in the supplementary methods [48], [49]. Proton irradiation of $\text{Co}^{76/77}\text{Se}$ yielded $^{76/77}\text{Br}$ which was isolated via dry distillation. $[^{76/77}\text{Br}]\text{RD1}$ was synthesized by Cu-mediated bromo deborylation chemistry of a boronic acid pinacol ester precursor (Figure 1). $[^{76/77}\text{Br}]\text{RD1}$ was isolated by preparative high performance liquid chromatography (HPLC), and subsequently formulated for *in vitro* and *in vivo* studies in sterile phosphate buffered saline (PBS). Molar activity and radiochemical purity of $[^{76/77}\text{Br}]\text{RD1}$ were evaluated by analytical HPLC and autoradiography-visualized thin layer chromatography (radioTLC, Fig. S1–2) with methods further described in the supplementary materials.

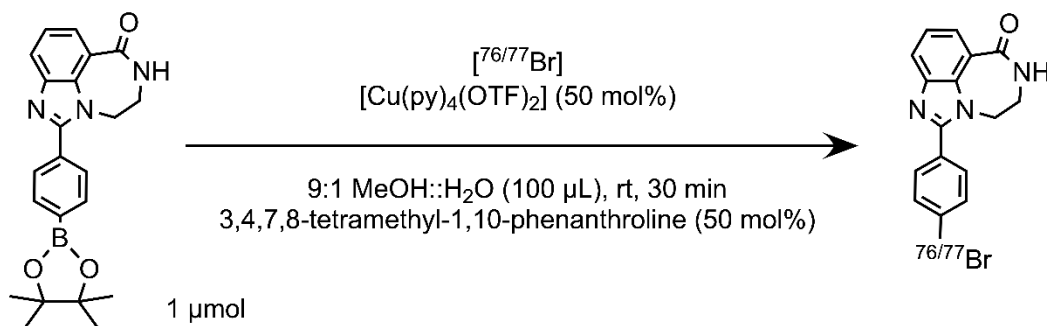


Figure 1: Radiosynthesis of $^{76/77}\text{Br}$ -labeled PARP1 inhibitor $[^{76/77}\text{Br}]\text{RD1}$.

2.2 Cell Culture

Murine ID8 ovarian cancer cells were gifted from Dr. Katherine Roby (University of Kansas, KS) and maintained in Dulbecco's modified Eagle's medium with 4.5 g/L glucose, L-glutamine, and sodium pyruvate (Corning) supplemented with 5% fetal bovine serum (FBS, Corning), 1% penicillin-streptomycin (Corning) and 0.2% 500X insulin-transferrin-selenium supplement (BioWhittaker). OVCAR8 and OVCAR8 PARP1-KO G1 (isogenic pair to OVCAR8 with PARP1 knocked out, [50]) human ovarian cancer cells were maintained in RPMI-1640 medium (Gibco) supplemented with 10% FBS and 1% penicillin-streptomycin. OVCAR8 PARP1-KO G1 had an additional 2 μg/mL puromycin (Gibco) added to the medium. UWB1.289

and UWB1.289+BRCA1 (isogenic pair to UWB1.289 with BRCA1 restored) human ovarian cancer cells were purchased from American Type Culture Collection and maintained in 1:1 RPMI-1640 (Gibco) and mammary epithelial growth medium (Lonza) supplemented with 3% FBS. UWB1.289+BRCA1 media also had 200 µg/mL Geneticin selective antibiotic (G418 Sulfate, Gibco). All cells were cultured in a humidified environment at 37°C and 5% CO₂.

2.3 [⁷⁷Br]RD1 *in vitro* binding assays

All cell lines were grown in clear, sterile, tissue-culture (TC)-treated 96-well plates (Corning) at a seeding density between 5,000–50,000 cells/well in a volume of 180 µL cell medium for 15–24 hours. The cells were treated in triplicate with 0.0086–128 nM of [⁷⁷Br]RD1 (122–500 MBq/µmol) with and without a blocking dose of 1 µM non-radioactive BrRD1. [⁷⁷Br]RD1 in PBS (BioWhittaker) was added to the wells in a volume of 20 µL for a total volume in wells of 200 µL for 2 hours.

A hemocytometer was used to verify the cell density per well at the time of contact in triplicate using wells with zero activity. The medium containing [⁷⁷Br]RD1 was removed and placed in corresponding polypropylene cluster tubes (Corning). The wells were rinsed with 100 µL of PBS and added to the same corresponding cluster tubes. The cells were then dispersed with 50 µL of 0.25% trypsin-EDTA (Corning) for 20 minutes at 5% CO₂ and 37°C and this volume collected. The wells were rinsed with 100 µL of PBS and collected in the same corresponding vial. The radioactivity in the cells+rinses and the medium+rinses was measured using a Wizard² Automatic Gamma Counter (PerkinElmer) calibrated by high purity germanium (HPGe) gamma spectroscopy (HPGe, Canberra C1519). Background and dead time were manually measured. For dead times higher than 10%, measurements were retaken after a period of decay. Cellular molar uptake (in attomol/cell) was calculated by dividing the cell-associated radioactivity (MBq) by the measured number of cells per well and the measured molar activity (MBq/nmol).

The total binding curve measured from the cells contacted with [^{77}Br]RD1 and the nonspecific binding curve measured from the cells contacted with [^{77}Br]RD1 + 1 μM BrRD1 were fitted using GraphPad Prism v9 (GraphPad Software, San Diego, CA, USA). The maximum number of binding sites per cell (B_{max}), equilibrium dissociation constant (K_d), and nonspecific binding slope (NS) were calculated (Eq. S1–S3).

2.4 Cytotoxicity following exposure to [^{77}Br]RD1

Cells were plated at a seeding density of 200 cells/well in 96-well white, opaque TC-treated plates (Falcon) for 2 hours before contact with varying concentrations of [^{77}Br]RD1 from 0.001–60 MBq/mL (0.0005–450 nM) for 7 days. Cell viability was assayed using Cell-Titer Glo 2.0 (Promega) by measuring adenosine triphosphate (ATP) with luminescence. Dose response curves were fitted using GraphPad Prism v9 and the half maximal effective concentrations (EC_{50}) were calculated as a function of molar concentration, activity concentration, and specific uptake using Equation S4. Radiation dose to the cell nucleus was calculated using Monte Carlo simulation with Medical Internal Radiation Dosimetry Cell (MIRDcell) v3.12, as previously described [51]. Further protocol details are provided in the supplementary methods.

2.5 PET/CT imaging and biodistribution of [^{76}Br]RD1

Imaging and biodistribution studies were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin—Madison (IACUC M006459). Four female C57BL/6 mice (Jackson Laboratory, ME) aged 12 weeks were used for *in vivo* studies. Mice were imaged prone two at a time on an Inveon microPET/CT scanner (Siemens Medical Solutions) under anesthesia, with 1.5–3.5% isoflurane at a rate of 1–2 L/min of oxygen via nose cone after an induction with 3–5% isoflurane. X-ray computed tomography (CT) imaging lasted 10 minutes, followed by PET image acquisition. Mice

received 5.1 ± 0.1 MBq of 23 MBq/nmol [^{76}Br]RD1 via tail vein (IV) injections at the beginning of a 1-h dynamic PET scan. Images were histogrammed into 1-minute bins between 1–5 minutes post injection (p.i.), 5-minute bins between 5–30 minutes p.i., and 10-minute bins between 30–60 minutes p.i. Static PET scans were performed at 4 h, 24 h, and 48 h p.i. and histogrammed into one frame (50 million counts/scan for all the time points except 20 million counts/scan at 48 h). PET images for static and dynamic scans were reconstructed using ordered-subset expectation maximization of 3 dimensions followed by the maximum a posteriori algorithm (18 iterations and 16 subsets). The corresponding CT was used for attenuation correction. PET/CT images were automatically coregistered and analyzed using Inveon Research Workplace 4.2 (Siemens Medical Solutions).

Regions of interest (ROIs) were drawn in the heart, lungs, brain, kidneys, bladder, liver, stomach, intestines, femur, and quadricep muscle. The mean percentage injected dose per volume (%ID/g) was measured after decay-correcting to time of injection. After the final PET/CT image at 48 h, mice were euthanized via CO_2 asphyxiation followed by exsanguination. The blood, heart, lungs, pancreas, spleen, kidneys, liver, gallbladder, large intestine, small intestine, stomach, femur bone, quadriceps muscle, and enteric (combined stomach and small and large intestine) contents were harvested and weighed. The radioactivity was measured using an automated HPGe-calibrated gamma counter (PerkinElmer). Measurements were background- and decay-corrected to time of injection to calculate the %ID/g. A partial volume (PV) correction phantom (Phantech, WI) with sphere volumes from 8–900 mm^3 filled with 142 ± 6 kBq/mL of [^{76}Br]RD1 was PET imaged as described above, and the recovery coefficient (RC) curves were calculated.

2.6 [$^{76/77}\text{Br}$]RD1 three-dimensional dosimetry

PET/CT images were used to define the activity distribution and geometry for use in an in-house Monte Carlo (MC) dosimetry platform, RAPID [52]. PET/CT were coregistered and resampled in Amira

(v5.3.3, Mercury Computer Systems, Berlin, Germany) and used to calculate the mean absorbed dose rate for each voxel after decay correction. This was done for each organ region at each timepoint. This was imported into the MC framework (Geant4, v9.6.p2) and the Auger electron function (ARMflag) was utilized for calculations using data from the Evaluated Atomic Data Library [53]. CT data were transformed from Hounsfield units into mass density using a CT scanner specific calibration curve. Evaluated Nuclear Structure Data Files (ENSDF, Brookhaven National Laboratory) data, which includes all MAe (>1 keV), β , and γ radiation emitted per decay, were used to calculate the source decay sampled uniformly in a voxel. The energy deposition was tracked to create a 3D cumulative dose distribution with the PET contours defining organ regions to calculate organ specific dose. To calculate the dose distribution for [^{77}Br]RD1, the PET images acquired with [^{76}Br]RD1 were corrected for physical decay differences between the two radionuclides.

The PET quantified [^{76}Br]RD1 organ %ID/g uptake in mice was used to estimate the human organ dose using OLINDA/EXM v1.1 [54] as described previously [55]. Briefly, the mouse biodistribution was assumed to be equal to human biodistribution. The mouse organ [^{76}Br]RD1 uptakes (%ID/g) were multiplied by the ratio of the average mouse mass in the study to the average human woman mass to get the estimated human organ %ID/g uptakes, which were multiplied by average human organ masses [56] to obtain organ-specific %ID in human. These values were used in OLINDA/EXM to calculate organ-level equivalent dose.

3. Statistical analysis

All data were obtained in technical and biological triplicate unless otherwise stated. Statistical analyses and nonlinear regression were performed using GraphPad Prism v9. Results are reported as mean $\pm$ standard deviation (SD) or with stated 95% confidence interval (CI).

4. Results

4.1 Radiosynthesis

The average cyclotron physical yield was 16 ± 2 MBq· μ A⁻¹·h⁻¹ (n = 7) for ⁷⁷Br, 59 ± 4 MBq· μ A⁻¹·h⁻¹ (n = 4) for ⁷⁶Br and the distillation yield was $67 \pm 13\%$ (n=11). The non-decay corrected radiochemical yield (RCY) and molar activity (MA) for [⁷⁷Br]RD1 was $58 \pm 25\%$ and 300 ± 120 MBq/nmol (n = 7). The RCY and MA for [⁷⁶Br]RD1 was $5 \pm 3\%$ and 23 MBq/nmol (n=2), respectively, when GBq-quantity [⁷⁶Br]bromide was stored in solution overnight prior to reaction. When stored dry, it was 73% and 740 MBq/nmol (n=1), respectively.

4.2 In vitro analysis

The total, nonspecific (Figure 2A–B) and specific (Figure 2C) binding were used to calculate the B_{max} , K_d and NS, which are summarized in Table 1. For the OVCAR8 PARP1-KO cell line, there was no statistical difference between the nonspecific and total binding measurements for molar concentrations above 25 nM (n = 4). This indicated a low number of specific binding sites that were small compared with the nonspecific binding above 25 nM. Thus, values measured above 25 nM were excluded from the B_{max} , K_d and NS fitting analysis for this cell line.

Table 1: The [⁷⁷Br]RD1 pharmacological parameters calculated from total and nonspecific binding studies in the various ovarian cancer cell lines and their corresponding 95% CI.

Ovarian Cancer Cell Line	B_{max} (95% CI) (attomol/cell)	K_d (95% CI) (nM)	NS (95% CI) (attomol/cell/nM)
ID8	3.0 (2.4–3.9)	31 (20–49)	0.049 (0.047–0.052)
OVCAR8	1.6 (1.4–1.8)	6.0 (4.2–8.2)	0.050 (0.048–0.052)
OVCAR8 PARP1-KO	0.83 (0.50–1.5)	7.5 (2.7–20)	0.039 (0.033–0.046)

UWB1.289	11	(7.9–16)	24	(10–57)	0.20	(0.19–0.21)
UWB1.289+BRCA1	8.6	(6.6–11)	25	(13–48)	0.26	(0.25–0.26)

4.3 *In vitro* cytotoxicity

The cell survival curves of the various ovarian cancer cell lines are summarized in Figure 2D–F as a function of activity concentration (Figure 2D), molar concentration (Figure 2E and S3), and specific uptake (Figure 2F). The EC₅₀ and the corresponding 95% confidence intervals (CI) are summarized in Table 2.

Table 2: The [⁷⁷Br]RD1 toxicological EC₅₀ values of the various ovarian cancer cell lines and their corresponding 95% CI.

Ovarian Cancer Cell Line	Molar Concentration (nM)		Activity Concentration (10 ⁻² MBq/mL)		Cellular Activity Uptake (10 ⁻² Bq/cell)		Nuclear Dose (Gy)
	EC ₅₀	95% CI	EC ₅₀	95% CI	EC ₅₀	95% CI	D ₅₀
ID8	2.3	(1.8–2.9)	44	(36–53)	3.9	(3.3–4.6)	9.2±1.0
OVCAR8	0.17	(0.13–0.24)	7.0	(6.2–8.0)	1.8	(1.6–2.0)	4.3±0.3
OVCAR8 PARP1-KO	0.46	(0.34–0.62)	20	(15–28)	2.0	(1.5–2.7)	3.4±0.6
UWB1.289	0.27	(0.21–0.36)	6.1	(4.4–8.6)	2.7	(2.0–3.7)	3.9±0.8
UWB1.289+BRCA1	0.30	(0.22–0.41)	6.8	(5.5–8.3)	2.3	(1.9–2.8)	3.3±0.4

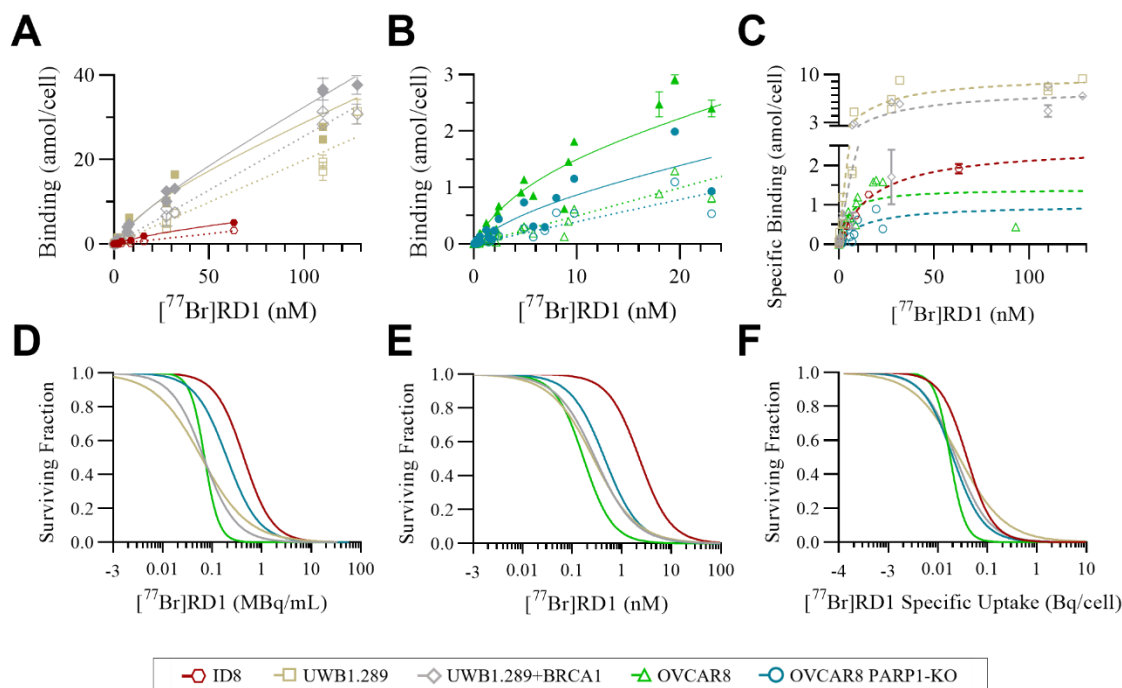


Figure 2: Total (closed symbols, solid lines) and nonspecific (open symbols, dotted lines) binding of (A) UWB1.289 (gold, $n=3$), UWB1.289 + BRCA1 (gray, $n=3$), ID8 (red, $n=2$) (B) OVCAR8 (green, $n=4$), and OVCAR8 PARP1-KO (blue, $n=4$) and (C) the specific binding (dashed lines) of $[^{77}\text{Br}]\text{RD1}$ as a function of radiopharmaceutical molar concentration. (D) The cell survival of the same cell lines as a function of radiopharmaceutical activity concentration, (E) molar concentration, and (F) specific uptake (Bq/cell) inherent to the cell lines.

4.4 PET imaging, biodistribution, and dosimetry

Partial volume correction phantom results and recovery coefficient calibration curves are summarized in Figure S4. Significant $[^{76}\text{Br}]\text{RD1}$ uptake in the liver (brown arrow) occurred within 5 minutes p.i. and the gallbladder (blue arrow) between 1-4 hours p.i. (Figure 3A). The 48 h p.i. *in vivo* PET quantification was compared to the *ex vivo* biodistribution measurements (Figure 3B, Table S2). Time activity curves showed biphasic blood clearance with a calculated α -phase half-life of 1.5 min (95% CI: 1.2–1.9 min) and β -phase half-life of 85 min (95% CI: 67–107 min, Figure 3C). Based on the organ-

averaged PET results from 1–48 h, $85 \pm 10\%$ of $[^{77}\text{Br}]\text{RD1}$ was excreted from 1–24 hours and $69 \pm 15\%$ of that remaining excreted from 24–48 hours. Dosimetry analyses of $[^{76/77}\text{Br}]\text{RD1}$ following injection in mice and humans are summarized in Figure 4 and Table S3.

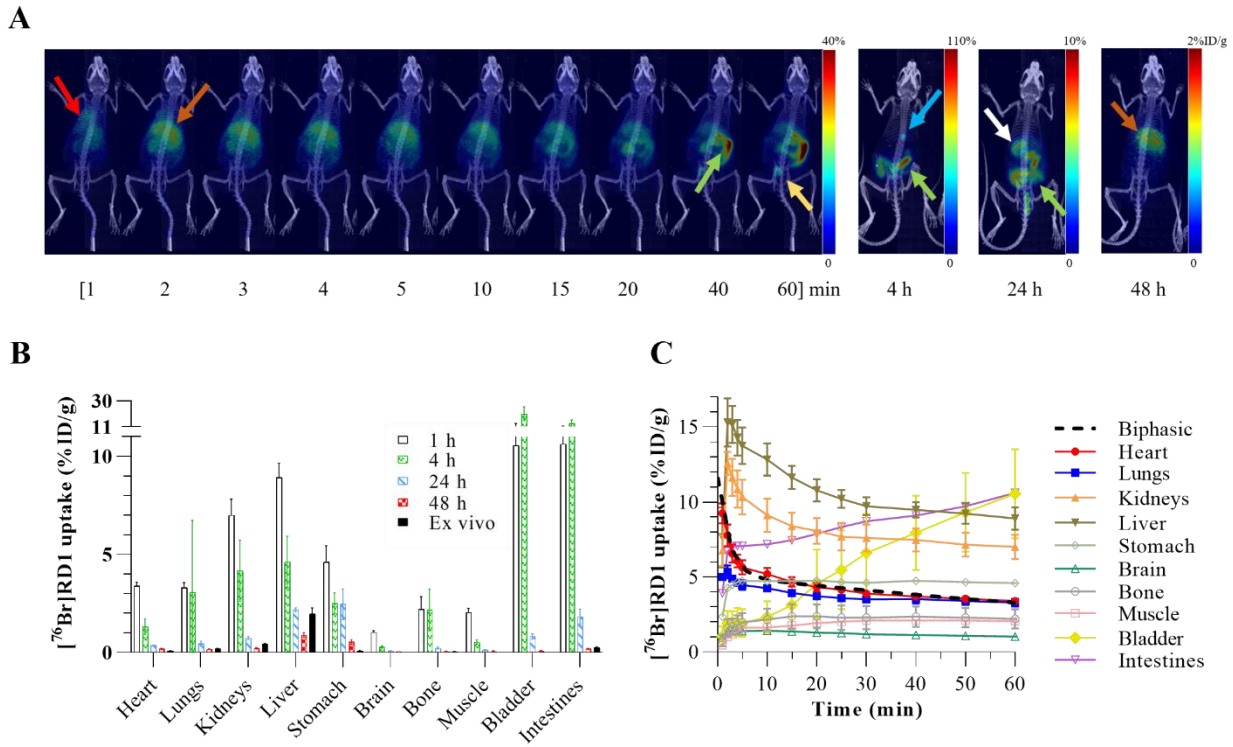


Figure 3: (A) PET maximum intensity projection (MIP) images of a representative mouse injected intravenously with $[^{76}\text{Br}]\text{RD1}$. Red arrow: heart; brown arrow: liver; green arrow: intestines; yellow arrow: bladder; blue arrow: gallbladder; white arrow: stomach contents. (B) A comparison between the *in vivo* measurements at four time points p.i. and the *ex vivo* measurements ($n=4$). *Ex vivo* intestinal uptake was the average measurement of the small intestine, large intestine, and enteric contents. (C) Time activity curves based on ROI analysis of dynamic PET images.

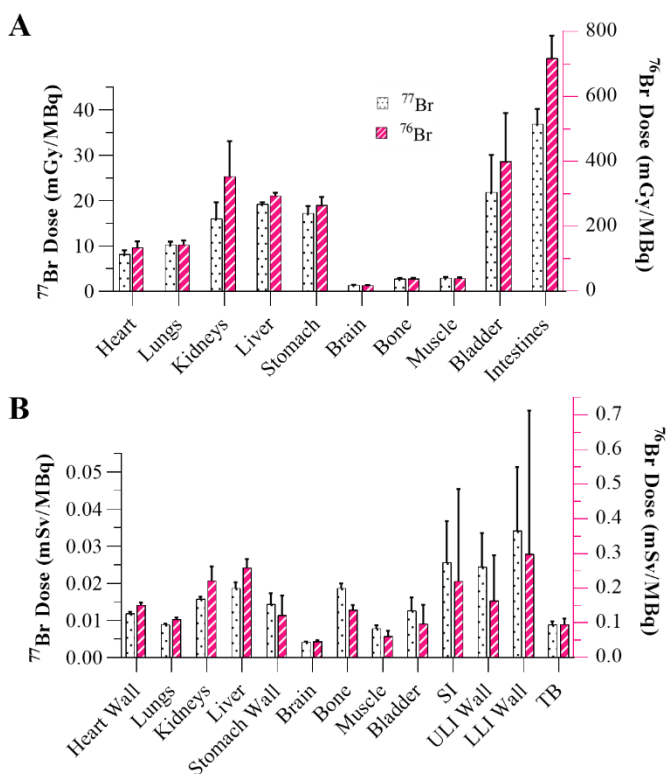


Figure 4: Dosimetry analysis showing (A) organ-level absorbed dose following $[\text{}^{76/77}\text{Br}]\text{RD1}$ administration in mice using RAPID and (B) equivalent dose in female humans using OLINDA. SI: small intestine; ULI: upper large intestine; LLI: lower large intestine; TB: total body.

5. Discussion

Radiopharmacological and toxicological properties of a PARP-targeted MAe emitting radiopharmaceutical $[\text{}^{77}\text{Br}]\text{RD1}$ were measured in ovarian cancer cell lines. $[\text{}^{77}\text{Br}]\text{RD1}$ demonstrated specific, blockable uptake with nanomolar binding affinity and attomoles of binding sites per cell. The binding affinity, represented by the equilibrium dissociation constant K_d , ranged from 6–30 nM with cell line variability potentially due to differences in relative expression levels of the various PARP isoforms. The number of specific binding sites per cell, represented by B_{max} , ranged from 0.8 – 11 amol/cell (0.5–6.6 million sites per cell). Radiotracer studies utilizing structurally analogous $[\text{}^{125}\text{I}]\text{KX1}$ have shown B_{max} to

be strongly positively correlated to PARP1 expression, as determined by Western blot analysis [57]. Supporting this, the $B_{\max}$ of the OVCAR8 cell line (1.6 amol/cell) is a factor of two higher than the analogous PARP1 knock out cell line (0.83 amol/cell).

The EC_{50} (in nM) of [^{77}Br]RD1 was thousands of times lower compared to rucaparib for OVCAR8 (2,400x), OVCAR8 PARP1-KO (6,700x) and UWB1.289+BRCA1 (3,000x), and 70x lower for UWB1.289 [50]. These results show the radiotoxicity of [^{77}Br]RD1 is significantly greater than the chemotoxicity of structurally-related rucaparib in ovarian cancer. Compared to the radioiodinated MAe-emitting analog, [^{125}I]KX1, the EC_{50} (in MBq/mL) of [^{77}Br]RD1 is 5x and 4x higher for OVCAR8 and OVCAR8 PARP1-KO, respectively, and the D_{50} (Gy) is approximately 3x higher for OVCAR8 and OVCAR8 PARP1-KO, 1.7x higher for UWB1.289, and approximately the same for UWB1.289+BRCA1 [35]. This difference may be due to the smaller average number of MAes emitted with ^{77}Br (6-7 MAe/decay) compared with ^{125}I (~23 MAe/decay) [42]. When measured in terms of activity concentration, the OVCAR8 PARP1-KO cell line was significantly more [^{77}Br]RD1-tolerant ($P=0.0140$) compared to OVCAR8 (Figure 2D), with EC_{50} of 0.20 (95% CI: 0.15–0.28) MBq/mL and 0.070 (0.062–0.080) MBq/mL (Table 2), respectively. This agrees with Makvandi, et al. [57], which demonstrates that PARP expression is essential to the efficacy of MAe-emitting PARP inhibitors, where lower $B_{\max}$ values were associated with higher EC_{50} values. However, after accounting for the cell line's lower number of specific binding sites, this differential sensitivity was no longer observed ($P=0.6228$), with EC_{50} of 0.020 (0.015–0.027) Bq/cell and 0.018 (0.016–0.020) Bq/cell for OVCAR8 PARP1-KO and OVCAR8 cell lines, respectively (Figure 2F, Table 2). These results indicate that the PARP-expression dependence of [^{77}Br]RD1 radiotoxicity is driven by differences in specific binding site expression, in which the loss of PARP1 did not change the radiosensitivity of the cancer cell line.

[^{77}Br]RD1 had similar cytotoxic effects regardless of BRCA1 gene expression (Figure 2D–E). The chemotherapeutic effect of rucaparib and other PARP inhibitors results in increased cytotoxicity in

homologous recombination deficient BRCA1 mutated cells [50], [58]. When adding the radiotherapeutic effect of the MAe emitting isotope ^{77}Br , this biomarker-dependency was no longer apparent—the $[^{77}\text{Br}]\text{RD1}$ showed similar cytotoxicity in UWB1.289 cells even with upregulated BRCA1 expression (Figure 2D–E) with EC_{50} values of 0.061 (0.044–0.086) MBq/mL and 0.068 (0.055–0.083) MBq/mL for UWB1.289 and UWB1.289+BRCA cell lines, respectively.

Hepatobiliary and renal excretion pathways were observed for $[^{76}\text{Br}]\text{RD1}$ (Figure 3A) in agreement with other radiolabeled PARP inhibitors [23], [36], [59], [60]. The excretion rate of $[^{76}\text{Br}]\text{RD1}$ (70–85% per day) was significantly faster than that of $[^{82}\text{Br}]\text{bromide}$ (5% per day) [45], supporting the hypothesis that minimal debromination occurred *in vivo*. Future metabolite analyses are necessary to confirm this. Terminal *in vivo* and *ex vivo* biodistributions differed for organs with contents that were removed for *ex vivo* measurement, e.g., blood and enteric contents, and in organs with inhomogeneous radiopharmaceutical distribution, e.g., liver. At 48 h, $[^{76}\text{Br}]\text{RD1}$ resides mainly in the liver region. This prolonged retention of $[^{77}\text{Br}]\text{RD1}$ may cause toxicity in the liver, with dose estimates of 19.3 ± 0.3 mGy/MBq and 19 ± 2 mSv/GBq in mice and humans, respectively (Fig. 4, Table S3). The liver dose limit for external-beam radiotherapy is 30 Gy, which would limit the $[^{77}\text{Br}]\text{RD1}$ activity to approximately 1,600 GBq (Table S4). However, liver uptake of an olaparib-based fluorescent PARP inhibitor was observed to be cytoplasmic, significantly decreasing the equivalent dose to liver cell DNA compared to the nuclear uptake observed in tumor cells [27]. In mice, stomach and intestinal absorbed doses were 270 ± 30 and 720 ± 70 mGy/MBq, respectively, for $[^{76}\text{Br}]\text{RD1}$. However, as mentioned above, the *ex vivo* and *in vivo* differences indicate that most of the activity and thus dose is in the contents of the organ. Human dose estimates were compared to organ dose reported for $^{18}\text{F}\text{-FTT}$ [16]. All $[^{76}\text{Br}]\text{RD1}$ human equivalent organ dose estimates were 7 ± 2 times larger compared to PARP-targeted imaging agent, $^{18}\text{F}\text{-FTT}$, except for lower large intestine (20 times larger). Dose limits for the reported organs [61] show that the dose limiting organ is the bone marrow, which limits $[^{77}\text{Br}]\text{RD1}$ injected activity to approximately 110 GBq.

6. Conclusion

The cytotoxicity of [^{77}Br]RD1 was found to be PARP expression dependent and BRCA1 status independent. These results together indicate the radiotherapeutic effect of the MAe emitting radionuclide is driving the cytotoxicity of [^{77}Br]RD1 beyond the PARP inhibitor chemotherapeutic effect. This study offers insights into the fundamental radiation biology of low energy electron emitting radiopharmaceuticals targeting the nuclear DNA damage response system. Future imaging and therapeutic studies should be conducted with further refined *in vivo* tumor models to show the efficacy of [^{76}Br]RD1 as a theranostic agent for ovarian cancer.

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8. Declaration of competing interest

The authors have no competing interests to report.

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Declaration of interests

Preclinical studies of a PARP targeted, Meitner-Auger emitting, theranostic radiopharmaceutical for metastatic ovarian cancer.

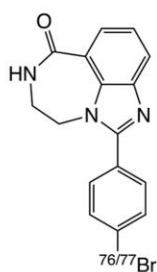
SLV Hoffman, JC Mixdorf, O Kwon, TR Johnson, M Makvandi, H Lee, E Aluicio-Sarduy, TE Barnhart, JJ Jeffery, MS Patankar, JW Engle, BP Bednarz, and PA Ellison

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

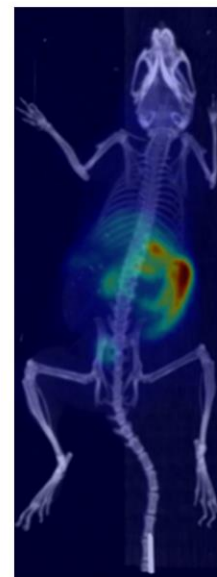
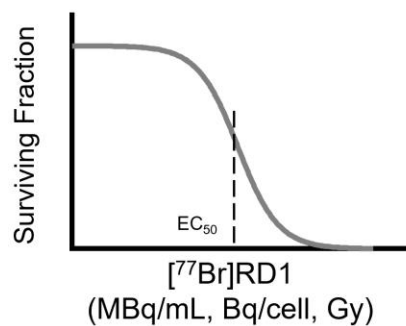
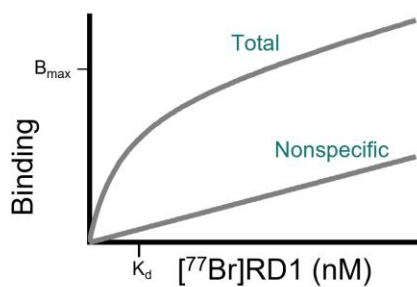
☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Graphical abstract



Ovarian Cancer Cell Line	Species	Gene Expression	
		<i>BRCA1</i>	<i>PARP1</i>
ID8	Murine	WT	WT
OVCAR8	Human	Methylated	WT
OVCAR8 PARP1-KO	Human	Methylated	KO
UWB1.289	Human	Null	WT
UWB1.289+BRCA1	Human	Restored	WT



PET/CT