Radiolabeled pertuzumab for imaging of human epidermal growth factor receptor 2 expression in ovarian cancer

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Abstract

Purpose Human epidermal growth factor receptor 2 (HER2) is over-expressed in over 30% of ovarian cancer cases, playing an essential role in tumorigenesis and metastasis. Non-invasive imaging of HER2 is of great interest for physicians as a mean to better detect and monitor the progression of ovarian cancer. In this study, HER2 was assessed as a biomarker for ovarian cancer imaging using 64Cu-labeled pertuzumab for immunoPET imaging.

Methods HER2 expression and binding were examined in three ovarian cancer cell lines (SKOV3, OVCAR3, Caov3) using in vitro techniques, including western blot and saturation binding assays. PET imaging and biodistribution studies in subcutaneous models of ovarian cancer were performed for non-invasive in vivo evaluation of HER2 expression. Additionally, orthotopic models were employed to further validate the imaging capability of 64Cu-NOTA-pertuzumab.

Results HER2 expression was highest in SKOV3 cells, while OVCAR3 and Caov3 displayed lower HER2 expression. 64Cu-NOTA-pertuzumab showed high specificity for HER2 (Ka = 3.1 ± 0.6 nM) in SKOV3. In subcutaneous tumors, PET imaging revealed tumor uptake of 41.8 ± 3.8, 10.5 ± 3.9, and 12.1 ± 2.3%ID/g at 48 h post-injection for SKOV3, OVCAR3, and Caov3, respectively (n = 3). In orthotopic models, PET imaging with 64Cu-NOTA-pertuzumab allowed for rapid and clear delineation of both primary and small peritoneal metastases in HER2-expressing ovarian cancer.

Conclusions 64Cu-NOTA-pertuzumab is an effective PET tracer for the non-invasive imaging of HER2 expression in vivo, rendering it a potential tracer for treatment monitoring and improved patient stratification.

Keywords Positron emission tomography (PET) · Human epidermal growth factor receptor 2 (HER2) · Pertuzumab · Ovarian cancer · Molecular imaging

Introduction

Human epidermal growth factor receptor 2 (HER2) is over-expressed in 15–40% of patients with ovarian cancer, and plays a major role in cell growth, proliferation, and differentiation [1–3]. HER2-directed therapy was found to reduce tumor progression in a xenograft model of ovarian cancer [4]. In addition, it is the current standard of care for HER2-positive breast cancer and is under clinical evaluation in ovarian cancer [5, 6]. HER2 status is typically evaluated by fluorescence in situ hybridization or immunohistochemistry using tumor biopsy samples.
However, the intra-individual heterogeneous expression of HER2 in patients may lead to unpredictable biopsy results [7, 8]. Non-invasive imaging of HER2 expression can allow physicians to monitor HER2-directed therapies in patients, and can also be of assistance in patient stratification.

Pertuzumab is a HER2-targeting monoclonal antibody which was approved by the US Food and Drug Administration after a survival benefit was proven in a clinical trial of patients with HER2-positive metastatic breast cancer [9]. In combination with trastuzumab, pertuzumab has also shown enhanced antitumor effects in xenograft models of human ovarian cancer [10]. The potential of pertuzumab as an imaging probe for HER2-positive cancer has also been reported using fluorescent labeling of pertuzumab [11]. More importantly, pertuzumab can be utilized as an imaging tracer in patients treated with trastuzumab, because trastuzumab and pertuzumab each have no effect on the binding of the other [12]. However, pertuzumab has not been well investigated as a tracer for positron emission tomography (PET) imaging, which has higher sensitivity and quantification abilities than optical imaging.

In 2016, there will be an estimated 22,280 new cases of ovarian cancer, along with 14,240 attributable deaths [13]. This highly aggressive disease will result in 54% of patients succumbing to ovarian cancer within 5 years. The increased mortality attributed to ovarian malignancies is related to the fact that most patients (>85%) are diagnosed during late disease stages with widely spread metastatic tumors in the peritoneal cavity. Herein, we describe the development and evaluation of a $^{64}$Cu-labeled PET tracer ($^{64}$Cu-NOTA-pertuzumab) for the in vivo imaging of HER2-positive ovarian cancer using pertuzumab. We hypothesized that PET imaging using $^{64}$Cu-NOTA-pertuzumab could allow for in vivo evaluation of relative HER2 expression and sensitive detection of HER2-positive tumors in both subcutaneous and orthotopic models of ovarian cancer.

**Materials and methods**

**Cell culture**

Human ovarian adenocarcinoma cell lines (SKOV3, OVCAR3, and Caov3) were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS; HyClone, GE Healthcare, Chicago, IL, USA) at 37 °C in a humidified incubator with 5% CO$_2$.

**Animal models**

All animal procedures were carried out under a protocol approved by the University of Wisconsin Institutional Animal Care and Use Committee. Subcutaneous tumors (SKOV3, OVCAR3, and Caov3) were engrafted into 4–6-week-old female athymic nude mice [Crl: NU(NCr)-Foxn1nu] obtained from Envigo (Envigo RMS, Indianapolis, IN, USA). For implantation, $2 \times 10^6$ cancer cells in 100 μL Matrigel (BD Biosciences, San Jose, CA, USA) were subcutaneously injected into the lower right flank of each mouse. For orthotopic implantation of SKOV3 and OVCAR3 tumors, mice were anesthetized with 2–4% isoflurane and placed dorsal side up. An incision was made to the right of the midline and above the ovaries. Next, $1 \times 10^6$ cancer cells in 10 μL of Matrigel were slowly injected into the ovaries before the incision was closed with absorbable 6-0 sutures (Ethicon, Cincinnati, OH, USA).

**Western blot**

For western blot, cancer cells were harvested, and total protein concentration was measured with the Pierce Coomassie protein assay kit (Thermo Fisher Scientific). Thirty micrograms of total protein was loaded into the wells of a 4–12% Bolt Bis-Tris Plus gel (Thermo Fisher Scientific), along with the Chameleon Duo ladder (LI-COR Biosciences, Lincoln, NE, USA). After electrophoresis at 120 mV for 60 min at 4 °C, proteins were transferred to a nitrocellulose membrane using the iBlot 2 (Thermo Fisher Scientific). Next, the membrane was blocked with Odyssey blocking buffer (LI-COR Biosciences) and incubated with 1:200 pertuzumab antibody and 1:200 β-actin antibody (Novus Biologicals, Littleton, CO, USA) for 12 h at 4 °C. The membrane was then washed three times with PBS-T (phosphate-buffered saline with Tween 20) before secondary antibody incubation with goat anti-human DyLight 680 and donkey anti-mouse DyLight 680 (Novus Biologicals). The membrane was scanned using the LI-COR Odyssey infrared imaging system (LI-COR Biosciences).

**Flow cytometry**

Cells were suspended in cold phosphate-buffered saline (PBS) with 2% bovine serum albumin (BSA) at a concentration of $\sim 1 \times 10^6$ cells/mL. Cells were incubated with 200 μL of pertuzumab (25 μg/mL in PBS with 2% BSA) on ice for 30 min, and then washed three times with cold PBS. After re-suspending in cold PBS, cells were incubated with fluorescence isothiocyanate (FITC)-labeled goat anti-human secondary antibody (Novus Biologicals) on ice for 30 min. The cells were analyzed using the MACSQuant cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany), and mean fluorescence intensities were processed using the FlowJo software (Tree Star, Ashland, OR, USA).
NOTA conjugation and \(^{64}\text{Cu}\) labeling of pertuzumab

The methods for 1,4,7-triazacyclononane-triacetic acid (NOTA) conjugation and \(^{64}\text{Cu}\) labeling with antibodies were previously reported in detail \([14, 15]\). Briefly, pertuzumab was mixed with p-SCN-Bn-NOTA in dimethyl sulfoxide (DMSO) and incubated at pH 8.5–9.0 at room temperature for 2 h. NOTA-pertuzumab was purified using a PD-10 desalting column with 1 × PBS as the elution buffer. \(^{64}\text{Cu}\) was produced by a PETtrace cyclotron (GE Healthcare) using the \(^{64}\text{Ni}(p,n)^{64}\text{Cu}\) reaction. For the radiolabeling of NOTA-pertuzumab, \(^{64}\text{Cu}\) (∼37 MBq) was diluted with sodium acetate and mixed with NOTA-pertuzumab (∼1 mg/mL). Following 1 h incubation at 37 °C with constant shaking, the mixed solution was purified using a PD-10 column. Human serum IgG was used as nonspecific antibody and labeled using the same method to obtain \(^{64}\text{Cu}\)-NOTA-IgG\text{Nonspecific}.

Saturation binding assay

SKOV3 cells were seeded in a 96-well plate at \(1 \times 10^5\) cells per well. \(^{64}\text{Cu}\)-NOTA-pertuzumab solution was prepared in PBS (containing 0.1% BSA) with a concentration gradient from 0.03 to 100 nM. After adding antibody solutions to each well, cells were incubated at room temperature for 2 h. To determine the nonspecific binding of \(^{64}\text{Cu}\)-NOTA-pertuzumab, 1 μmol of unlabeled pertuzumab was added. At the end of the 2 h incubation, cells were washed three times with PBS and harvested for gamma counter analysis. Based on the overall and nonspecific ligands bound to SKOV3 cells, the \(K_a\) and \(B_{\text{max}}\) values were determined, and the HER2 receptor density on SKOV3 cells was determined using GraphPad Prism software (La Jolla, CA, USA).

PET/CT imaging and biodistribution

PET imaging was performed on an Inveon micro-PET/CT rodent model scanner (Siemens Medical Solutions, Erlangen, Germany). Tumor-bearing mice were intravenously (i.v.) injected with 5–10 MBq of \(^{64}\text{Cu}\)-NOTA-pertuzumab or \(^{64}\text{Cu}\)-NOTA-IgG\text{Nonspecific}, and PET scans were conducted at 3, 24, and 48 h after injection. For orthotopic groups, PET/CT scans were performed at the last time point. Following the terminal PET or PET/CT scan, mice were sacrificed, and organs of interest were harvested to validate the imaging data. A gamma counter (PerkinElmer, Waltham, MA, USA) was used to quantify tracer uptake in various tissues and organs, and accumulations were denoted by percentage of injected dose per gram of tissue (%ID/g; ≥3 mice per group).

Immunofluorescence staining

Tumors were extracted, and sectioning was performed by the University of Wisconsin Carbone Cancer Center Experimental Pathology Laboratory. Frozen tumor tissue slices of 5-mm thickness were fixed with cold acetone for 10 min, washed with PBS, and then blocked with 10% donkey serum for 45 min at room temperature. Slices were then incubated overnight with pertuzumab (10 μg/mL) and rat anti-mouse CD31 (2 μg/mL; Thermo Fisher Scientific) at 4 °C. Next, sections were further stained with Alexa Fluor 488-labeled goat anti-human antibody and Cy3-labeled donkey anti-rat antibodies (Thermo Fisher Scientific). A cover glass was applied to each slide using Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA), and imaging was performed using a Nikon A1R confocal microscope (Nikon Instruments, Melville, NY, USA).

Ultrasoundography detection of orthotopic tumors

Ultrasound imaging (Vevo 2100; FUJIFILM VisualSonics, Toronto, Canada) was performed weekly in mice to monitor tumor development. Two-dimensional images of mouse ovaries were acquired using a linear array transducer (MS-400) in B-mode with 30-MHz center frequency.

Dosimetry

Dosimetry analysis was performed using OLINDA/EXM software \([16]\]. Estimated human dosimetry was calculated using %ID/g values from the serial PET scans on mice injected with \(^{64}\text{Cu}\)-labeled pertuzumab. It was assumed that the biodistribution in adult humans was the same as in the animal models, and a triexponential model was utilized for the time–activity curves. OLINDA provides effective dose outputs; thus, weighting factors from ICRP 103 were employed to convert to absorbed dose in each organ \([17]\).

Statistical analysis

Quantitative data were displayed as mean ± standard deviation (SD). Means were compared using the Student t-test, and \(p\)-values < 0.05 were considered statistically significant.

Results

HER2 expression in ovarian cancer cell lines

Relative expression levels of HER2 in three ovarian cancer cell lines were determined using western blot with pertuzumab as the primary antibody. A 110-kDa band was seen in each lane representing HER2, while β-actin was visible at 38 kDa.
SKOV3 cells showed the strongest band intensity at 110 kDa, indicating the highest expression of HER2, while OVCAR3 and Caov3 cells both displayed low levels of HER2 expression.

Cell binding affinity of pertuzumab to HER2

The binding affinity of pertuzumab with cellular HER2 was examined through flow cytometry studies. A significant shift along the x-axis denoted strong binding of pertuzumab to the cell line. A strong fluorescent signal shift was shown in the SKOV3 group, indicating enhanced binding of pertuzumab to SKOV3 (Fig. 1b). The shifts for the OVCAR3 and Caov3 groups were smaller than that of the SKOV3 group, suggesting lower binding of HER2.

Saturation binding assay with $^{64}$Cu-NOTA-pertuzumab for SKOV3 cells

A saturation binding assay was performed to quantify the maximum binding and HER2-affinity constant ($K_a$) of $^{64}$Cu-NOTA-pertuzumab with SKOV3 cells. Rapid saturation of HER2 was achieved using 30 nM $^{64}$Cu-NOTA-pertuzumab, and a $K_a$ value of 3.1 ± 0.6 nM was calculated (Fig. 1c). The receptor density in SKOV3 was determined to be $(1.63 ± 0.12) \times 10^6$ HER2 receptors per cell.

ImmunopET imaging and biodistribution of $^{64}$Cu-NOTA-pertuzumab in subcutaneous tumors

Serial PET scans were completed with $^{64}$Cu-NOTA-pertuzumab at 3, 24, and 48 h after injection of the tracer. Maximum intensity projections (MIPs) of PET images are shown in Fig. 2a. The quantitative data were obtained from the region-of-interest (ROI) analyses and are shown in Fig. 2b. At all imaging time points, the SKOV3 group showed the highest tumor accumulation among the three study groups, with $16.8 ± 3.9$, $38.6 ± 6.7$, and $41.8 ± 3.8$%ID/g at 3, 24, and 48 h after injection, respectively ($n = 3$). Tumor accumulations of both the OVCAR3 and Caov3 groups were significantly lower than that of the SKOV3 group, with the highest tumor accumulation of $13.6 ± 4.7$%ID/g for the OVCAR3 model and $10.2 ± 4.5$%ID/g for the Caov3 model at 48 h after injection ($n = 3$; $p < 0.01$ at each time point).

To ensure that the uptake of $^{64}$Cu-NOTA-pertuzumab was specific, a nonspecific antibody ($^{64}$Cu-NOTA-IgGNonspecific) was injected into SKOV3 tumor-bearing mice (Fig. S1). While blood uptake of the two tracers was comparable at each
time point, the tumor uptake of $^{64}$Cu-NOTA-IgGNonspecific was significantly lower than that of $^{64}$Cu-NOTA-pertuzumab, with uptake values of 7.2 ± 1.6 and 41.8 ± 3.8%ID/g at 48 h after injection ($n=4$, $p<0.01$), respectively, thus confirming the in vivo binding specificity of $^{64}$Cu-NOTA-pertuzumab for HER2-expressing tumors (Fig. S1).

Biodistribution studies were performed to validate PET analyses. The ex vivo biodistribution of $^{64}$Cu-NOTA-pertuzumab in SKOV3 tumors was 42.3 ± 10.3%ID/g, significantly higher than the activity values of 10.5 ± 3.9 and 12.1 ± 2.3%ID/g for the OVCAR3 and Caov3 tumors, respectively (Fig. S2; $p<0.05$, $n=3$). Tracer uptake in other organs, including the liver, spleen, lung, and kidneys, was similar among the models. In addition, $^{64}$Cu-NOTA-IgGNonspecific exhibited minimal tumor accumulation, with activity values of 5.0 ± 0.5%ID/g at 48 h after injection (Fig. S3, $n=4$; $p<0.01$). Overall, the ex vivo biodistribution study confirmed the excellent in vivo HER2-targeting ability of $^{64}$Cu-NOTA-pertuzumab.

**Immunofluorescence co-staining of HER2 and CD31**

SKOV3 tumor sections showed the highest expression of HER2, while tumor sections from the OVCAR3 and Caov3 groups displayed lower levels (Fig. 3). HER2 staining intensity correlated well with tumor uptake in all three groups, further supporting the utility of $^{64}$Cu-NOTA-pertuzumab PET for the noninvasive assessment of HER2 expression in ovarian cancer.

**ImmunoPET imaging and biodistribution of $^{64}$Cu-NOTA-pertuzumab in orthotopic tumors**

The presence of ovarian tumors was verified using ultrasonography (Fig. S4). Primary tumors were clearly identifiable in both the SKOV3 and OVCAR3 orthotopic models of ovarian cancer using PET/CT imaging (Fig. 4). SKOV3 orthotopic tumors showed enhanced accumulation of $^{64}$Cu-NOTA-pertuzumab that increased from 11.4 ± 2.8 to 29.8 ± 13.3%ID/g at 3 and 48 h after injection, respectively, whereas OVCAR3 orthotopic tumors showed persistent yet low tumor uptake of 8–9%ID/g at all time points (Fig. S5).

The primary tumors and peritoneal metastases were visually verified through autopsy. Primary tumors could be easily delineated from PET/CT imaging in both orthotopic tumor models (Fig. 5). In the images, additional focal uptake was found in the peritoneal space of the SKOV3 group, suspected to be peritoneal implants (Fig. 5a). The white nodular tissues with high tracer uptake were extracted for further evaluation (Fig. 5b). The tissues were confirmed as HER2-positive peritoneal...
implants by ex vivo PET imaging. Five peritoneal implants from 2 to 5 mm in size were discovered in three SKOV3 mice with high uptake from 14.4 to 18.6%ID/g (Fig. 5c). Peritoneal implants were not found in the OVCAR3 orthotopic tumor model, suggesting that PET imaging using $^{64}$Cu-NOTA-pertuzumab may allow for sensitive detection of small peritoneal implants in HER2-positive ovarian cancer.

Ex vivo biodistribution studies showed 23.3 ± 4.8%ID/g tumor uptake in the SKOV3 group, which was significantly higher than that in the OVCAR3 group, with tumor uptake of 8.7 ± 3.0%ID/g ($n = 3, p < 0.05$). Normal ovaries showed tracer uptake of 4.08 ± 1.6 and 4.5 ± 0.6%ID/g for the SKOV3 and OVCAR3 models, respectively (Fig. 6). Overall, imaging with $^{64}$Cu-NOTA-pertuzumab enabled non-invasive assessment of HER2 expression.

**Radiation dosimetry of $^{64}$Cu-NOTA-pertuzumab**

Human absorbed doses to normal organs were estimated based on the biodistribution of $^{64}$Cu-NOTA-pertuzumab ($n = 4$); organs with high absorbed doses were the liver (1.2 ± 0.1 × $10^{-1}$ mSv/MBq), ovary (6.6 ± 0.4 × $10^{-2}$ mSv/MBq), heart wall (3.9 ± 0.2 × $10^{-2}$ mSv/MBq), and lung (4.3 ± 0.7 × $10^{-2}$ mSv/MBq), as shown in Table S1. The total whole-body effective dose was (2.9 ± 0.1) × $10^{-2}$ mSv/MBq, which was far lower than that previously determined using $^{89}$Zr-labeled antibodies [18, 19].

**Discussion**

In the present study, $^{64}$Cu-NOTA-pertuzumab was developed and investigated for HER2-targeted imaging of ovarian cancer. Three ovarian cancer cell lines were selected and were confirmed to have different relative levels of HER2 expression (SKOV3: high, OVCAR3: low, Caov3: low). All tumors from three subcutaneous models were clearly visualized with SKOV3 tumors, showing nearly 42%ID/g, about four times as high as that of the OVCAR3 and Caov3 groups, and eight times that of nonspecific IgG. In addition, the specific

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**Fig. 3** Immunofluorescence staining of tumor tissue sections. Pertuzumab and FITC-labeled anti-human secondary antibody were used for HER2 staining (green), CD31 was stained to expose locations of the vasculature (red), and DAPI was used for the visualization of cell nuclei locations (blue). Scale bar = 20 μm.
HER2-targeting ability of $^{64}$Cu-NOTA-pertuzumab enabled sensitive and specific tumor detection in the orthotopic models of ovarian cancer. We could readily recognize the location of the primary tumors in the SKOV3 and OVCAR3 orthotopic models and small peritoneal implants (2–5 mm) in SKOV3 tumor-bearing mice. These findings suggest that $^{64}$Cu-NOTA-pertuzumab may be utilized for the sensitive detection of HER2-positive ovarian cancer.

Non-invasive imaging methods for evaluating HER2 expression status have been explored using monoclonal antibodies, antibody fragments, and affibodies [20–22]. Trastuzumab, a HER2-specific monoclonal antibody, is the most widely investigated platform for HER2-targeted imaging tracers [22]. Preclinical studies of $^{89}$Zr-labeled trastuzumab revealed high uptake in HER2-expressing cancers in vivo [23]. Pertuzumab is unique in that it inhibits the dimerization process of HER2 with other HER family members, a mechanism that can obstruct tumor growth in combination with other chemotherapeutic drugs [10]. However, pertuzumab has not been well evaluated as a molecular imaging tracer. In 2009, McLarty et al. reported that $^{111}$In-labeled pertuzumab could detect HER2 downregulation in a xenograft model of breast cancer [24]. Marquez et al. recently evaluated $^{89}$Zr-pertuzumab for imaging of HER2 in breast cancer xenografts, showing enhanced uptake (47.5 ± 32.9%ID/g) in BT474 tumor xenografts [25]. Consistent with their study, we confirmed that radiolabeled pertuzumab allowed for the non-invasive imaging of HER2 expression in ovarian cancer models. Furthermore, our study revealed that radiolabeled pertuzumab could selectively and specifically target HER2 expression in both subcutaneous and orthotopic models of ovarian cancer.

ImmunopET imaging is a relatively new field of study that relies on the innate specificity of antibodies towards their corresponding antigens. Molecular imaging of HER2 expression was first assessed over a decade ago using radiolabeled trastuzumab, yet the formation of intrinsic resistance mechanisms effectively limited the clinical translation of the tracer. Despite these pitfalls with trastuzumab, researchers have made significant progress in the development and characterization of antibody-based imaging tracers. There are several differences between pertuzumab and trastuzumab that may affect the potential clinical translation of these antibodies as imaging tracers. First, both antibodies show distinct binding properties to HER2, with trastuzumab binding to domains IV and II of the extracellular HER2 receptor, respectively. Additionally, trastuzumab is a recombinant humanized IgG1 with a half-life of 25.5 days, while pertuzumab is a fully humanized IgG1κ with a much shorter half-life of only 10 days. Hence, the rapid clearance of radiolabeled
pertuzumab from circulation may lead to improved tumor-to-blood ratios. Also, the mechanism of action varies greatly between the two antibodies [26], which may impact the overall tumor accumulation. Lastly, pertuzumab-based HER2-targeting agents may have a better chance for clinical translation because of the lower immunogenicity and the lower chance that the tumor will form resistance to pertuzumab versus trastuzumab [26–28].

To ease concerns regarding radioactivity absorbed by patients, we utilized $^{64}$Cu to label pertuzumab to minimize radiation dosage to patients. As expected, dosimetry data extrapolated to human subjects revealed a lower level of absorbed dose with $^{64}$Cu-NOTA-pertuzumab than with $^{89}$Zr, which is attributable to the short half-life of $^{64}$Cu. Lam et al. recently developed $^{64}$Cu-NOTA-pertuzumab F(ab)'$_2$ for monitoring changes in HER2 expression associated with trastuzumab therapy [29]. The radiolabeled antibody fragment resulted in a twofold reduction in predicted total-body doses in humans compared to the intact antibody (determined in this study), with values of 0.029 and 0.015 mSv/MBq for the intact and fragmented antibody, respectively. While $^{64}$Cu limited the amount of time for longitudinal PET imaging, we obtained similar tumor uptake values compared to $^{89}$Zr-labeled pertuzumab in tumors with high HER2 expression. This rapid and enhanced uptake at an earlier time point is a distinct advantage for the successful clinical translation of a tracer.

Fig. 5 Radiological–surgical correlation of SKOV3 orthotopic ovarian cancer model. a In PET/CT imaging, a primary tumor with strong uptake was found in the area of the right ovary (yellow arrow), and there were numerous foci of uptake in the peritoneal space (white arrow). b Surgical exploration was performed in the same animal after terminal PET/CT imaging. The primary tumor mass was found in the right ovary (yellow arrow), and there was white nodular tissue (white arrow) at the location of focal uptake on the PET/CT imaging, suggesting peritoneal implants. c Ex vivo PET imaging of excised peritoneal implants was performed. The small peritoneal implants showed high PET uptake values.

Fig. 6 Ex vivo biodistribution studies for the validation of PET/CT results. Biodistribution of $^{64}$Cu-NOTA-pertuzumab in SKOV3 and OVCAR3 orthotopic ovarian cancer models at 48 h after injection (n = 4). SKOV3 tumors showed significantly higher tracer accumulation than OVCAR3 tumors, while normal ovaries in both groups displayed low levels of $^{64}$Cu-NOTA-pertuzumab uptake.
Standard imaging methods such as CT and magnetic resonance imaging are greatly limited for the detection of peritoneal metastasis, with per-lesion sensitivity of only 20% [30]. Diffusion-weighted magnetic resonance imaging (DW-MRI) shows higher sensitivity for small lesions, but specificity may be limited by diffusion restriction [31]. Also, ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) PET/CT imaging is used to evaluate peritoneal metastasis in advanced ovarian cancer. However, nonspecific uptake of ¹⁸F-FDG has been problematic in ovarian cancer, effectively limiting the detection of small or diffusely infiltrative lesions [32]. The present study demonstrated that ⁶⁴Cu-NOTA-pertuzumab allowed for clear delineation of small peritoneal implants (2–5 mm) in an orthotopic model of ovarian cancer. These findings support further evaluation of the tracer in patients with advanced ovarian cancer with peritoneal metastasis.

Conclusions

We investigated ⁶⁴Cu-labeled pertuzumab in PET imaging for the non-invasive evaluation of HER2 expression in ovarian cancer. ⁶⁴Cu-NOTA-pertuzumab displayed specific, rapid, and persistent accumulation in tumors with high HER2 expression (SKOV3), and the quantified uptake correlated well with HER2 expression levels. The enhanced targeting capability and high detection sensitivity of ⁶⁴Cu-NOTA-pertuzumab were further validated using orthotopic models, suggesting that the tracer could be employed for initial diagnosis and characterization of HER2-expressing malignancies and for the sensitive detection of HER2-positive peritoneal metastases.

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