Construction of ^{124}I -trastuzumab for noninvasive PET imaging of HER2 expression: from patient-derived xenograft models to gastric cancer patients

Xiaoyi Guo¹ · Nina Zhou¹ · Zuhua Chen² · Teli Liu¹ · Xiaoxia Xu¹ · Xia Lei¹ · Lin Shen² · Jing Gao² · Zhi Yang¹ · Hua Zhu¹

Received: 15 August 2019 / Accepted: 23 December 2019 / Published online: 9 January 2020
© The International Gastric Cancer Association and The Japanese Gastric Cancer Association 2020

Abstract

Purpose Here, we sought to develop a PET radioligand based on trastuzumab labeled with ^{124}I , ^{124}I -trastuzumab, to evaluate its distribution, internal dosimetry, and initial PET images of HER2-positive lesions in gastric cancer (GC) patients.

Methods In animal studies, micro-PET imaging and bio-distribution were performed to examine the specificity of ^{124}I -trastuzumab in HER2-positive and HER2-negative mouse models. Subsequently, ^{124}I -trastuzumab was applied in human clinic trial. Six gastric cancer patients with metastases underwent ^{124}I -trastuzumab PET imaging, with ^{18}F -FDG PET/CT in each to compare.

Results In animal studies, PET imaging of ^{124}I -trastuzumab showed significant higher tumor uptake than that of ^{124}I -IgG1 in HER2-positive PDX mouse models at 24 h. The low tumor uptake of ^{124}I -trastuzumab in HER2-negative PDX models further confirmed the specificity. In human clinical studies, 18 HER2-positive lesions and 11 HER2-negative lesions were evaluated in PET imaging analysis. The detection sensitivity of ^{124}I -trastuzumab was 100% (18/18) at 24 h. The PET images showed significant difference in tumor uptake between HER2-positive and HER2-negative lesions at 24 h ($\text{SUV}_{\text{max}} 7.83 \pm 0.55$ vs. 1.75 ± 0.29 , $p < 0.0001$). Quite striking difference in tumor uptake was observed between ^{124}I -trastuzumab and ^{18}F -FDG ($\text{SUV}_{\text{max}} 1.75 \pm 0.29$ vs. 6.46 ± 0.44 , $p < 0.0001$) in HER2-negative lesions, further confirming the specific binding of ^{124}I -trastuzumab in HER2-positive lesions. The radiation-absorbed dose was calculated to be 0.3011 ± 0.005 mSv/MBq. No toxicities or adverse effects were observed in any of the patients.

Conclusion The findings described here demonstrated that ^{124}I -trastuzumab was feasible to detect HER2-positive lesions in primary and metastatic gastric cancer patients and to differentiate HER2-positive and HER2-negative lesions quantitatively.

Xiaoyi Guo, Nina Zhou, and Zuhua Chen have contributed equally to this work.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10120-019-01035-6>) contains supplementary material, which is available to authorized users.

✉ Zhi Yang
pekyz@163.com

✉ Hua Zhu
zhuhuananjin@163.com

¹ Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Nuclear Medicine, Peking University Cancer Hospital and Institute, No. 52 Fucheng Rd., Beijing 100142, China

² Department of Gastrointestinal Oncology, Peking University Cancer Hospital and Institute, Beijing 100142, China

Graphic abstract

Keywords ^{124}I -trastuzumab · PET/CT · Gastric cancer · HER2

Abbreviations

HER2	Human epidermal receptor type 2
GC	Gastric cancer
GEC	Gastroesophageal junction cancer
IHC	Immunohistochemistry
i.v.	Intravenous injection
<i>h</i>	Hour
SISH/CISH/FISH	Silver, chromogenic, or fluorescent in situ hybridization
SPECT	Single-photon emission computed tomography
PET	Positron emission tomography
DFO	Desferrioxamine
NOTA	S-2-(4-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid
PDX	Patient-derived tumor xenograft
radio-TLC	Radioactive thin-layer chromatography scanner
HPLC	High-performance liquid chromatography
p.i.	Post-injection
<i>d</i>	Day
SUVmax	The maximum single-voxel standardized uptake value

ROIs
%ID/g

Regions of interest
Percent injected dose per gram

Introduction

Gastric cancer (GC) ranks fourth in tumor incidence and second in cancer death, and it occurs most commonly in East Asia areas [1]. In China, over 70% of GC patients are diagnosed at advanced stages, and this limits their effective treatments [2]. What's worse, the resistance of gastric cancer toward chemotherapy renders this disease with no effective therapeutic options. The overall survival (OS) rate for 5 years is less than 20% [3–5].

Human epidermal growth factor receptor 2 (HER2) is overexpressed in 7–34% of GC patients [6]. Its inhibitor, trastuzumab, has been demonstrated to increase OS for HER2-positive advanced gastric cancer (AGC) and gastroesophageal junction cancer (GEC) patients [6, 7]. On October 2010, FDA approved the combination therapy of trastuzumab and chemotherapy as the first-line treatment of HER2-positive AGC and GEC. Essentially, accurate determination of HER2 status in vivo is essential in effective treatment. The most frequently used analytical methods of HER2 levels are immunohistochemistry (IHC) and fluorescent in situ hybridization

(FISH). However, pathological samples taken from patients may not represent the large bulky mass of lesions, and the absence of tumor may result in false negative diagnosis. In addition, tumor heterogeneity may exist between primary tumors and metastases, among different metastases, and even in primary tumor alone, and within the same lesion before and after treatment [6, 8–12]. It is difficult to use IHC/FISH to accurately reflect systemic lesions, to monitor HER2 levels, and to measure heterogeneity of tumors [13]. To overcome these problems, a systemic *in vivo* techniques, such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) imaging, are required to monitor HER2 expression in patients noninvasively.

Many SPECT/PET imaging agents have been developed to determine HER2 expression and localize the primary tumors and/or metastases. In 2006, Perik et al. reported that ^{111}In -DTPA-trastuzumab could predict the cardiotoxicity and therapeutic efficacy of trastuzumab [14]. In 2009, Dijkers et al. reported that ^{89}Zr -trastuzumab PET imaging agent exhibited excellent tumor uptake and visualization of HER2-positive metastases, and its detection sensitivity was equivalent to that of ^{111}In -trastuzumab; ^{89}Zr -trastuzumab gave better images than ^{111}In -trastuzumab because of high spatial resolution and sensitivity of PET equipment [15, 16]. Many PET radionuclide-labeled trastuzumab agents have been suggested to be effective for HER2-positive tumor detection, such as those labeled with ^{124}I and ^{64}Cu [17–22].

Our laboratory has a long-standing interest in radio-immunotherapy (RIT) to treat solid tumors using antigen-selective tumor-targeting radiolabeled antibodies and the corresponding PET imaging agents. We have successfully developed ^{64}Cu -NOTA-trastuzumab as PET/CT imaging agent in clinical imaging of patients with gastric cancer [20]. It exhibited comparable detection sensitivity as that of ^{18}F -FDG, even in liver metastases. The long physical decay half-life and low liver background of ^{124}I -labeled agents were demonstrated to be useful to observe small lesions of tumor. Here, we describe our synthesis and evaluation of ^{124}I -trastuzumab in patient-derived xenograft (PDX) mouse models of GC. In addition, it was successfully applied in PET/CT imaging of GC patients in a clinical trial with Ethics Approval License: 2018KT48. In the clinical studies, we evaluated the feasibility and plausible conditions for PET imaging of ^{124}I -trastuzumab to detect HER2-positive lesions in HER2-positive GC patients and to differentiate quantitatively between HER2-positive and HER2-negative GC lesions.

Materials and methods

Preparation of ^{124}I -trastuzumab

Trastuzumab was labeled with ^{124}I by the N-bromosuccinimide (NBS) method. The details of the radiolabeling and quality control conditions are described in the supplemental materials.

Micro-PET imaging of ^{124}I -trastuzumab in PDX mouse models

All animal experiments were performed in accordance with the guidelines of the Animal Care and Use Committee of Peking University (Ethics Approval License: 2015KT08). The details to establish the mouse models can be found in the supplemental materials. Micro-PET images were acquired at 2, 24, 48, 72, and 96 h post-injection on a SuperArgus PET scanner (Sedecal, Spain) with 14.8 MBq ^{124}I -trastuzumab or ^{124}I -hIgG1 in HER2-positive and HER2-negative PDX mouse models [23, 24]. HER2 levels in PDX xenograft tumors were confirmed by IHC stain: HER2-positive models were HER2 3+, HER2-negative models were HER2 1+.

Patient enrollment

This study was performed under a single-center prospective imaging protocol and was approved by the Medical Ethics Committee of Peking University Cancer Hospital (Ethics Approval License: 2018KT48). The patients included in this study had pathologically confirmed HER2-positive (IHC 3+ or IHC 2+/FISH+) or HER2-negative (IHC 2+/FISH-, IHC 1+) advanced GC/GEC and were aged between 20 and 75 years. Patients' specimens for pathological examination came from gastroscopy or surgical procedures, mainly at primary lesions. According to the size and invasion range of the primary tumor, we took 1~4 specimens in tumor bodies, 2~4 specimens in tumor-free gastric walls at a distance from the tumor, and 1~2 upper and 1~2 lower broken ends, respectively. The main exclusion criteria were that the patients were without congestive heart failure, severe liver or kidney dysfunction, pregnant or lactating, had no known hypersensitivity to trastuzumab or could not lie in the scan bed for more than 1 h. Two HER2-positive (IHC 3+) patients and four HER2-negative (IHC 2+/FISH-) patients from June 2018 to December 2018 were included. All patients provided written informed consent before participating in the study.

^{124}I -trastuzumab and ^{18}F -FDG PET/CT imaging in GC/GEC patients

Patients underwent ^{18}F -FDG PET/CT 3 days before ^{124}I -trastuzumab PET/CT imaging studies. For ^{124}I -trastuzumab PET

imaging, a patient was initiated a thyroid iodine uptake blockade by oral administration of Lugol's potassium iodide (ten drops each time, 3 times a day) 3 days before and continued for 7 days after the administration of the radiotracer. To reduce the nonspecific uptake in the liver, patient 1 and patient 6 were co-injected with 5 mg "cold" trastuzumab and ^{124}I -trastuzumab, and the other four patients were co-injected with 10 mg "cold" trastuzumab and ^{124}I -trastuzumab. For all patients, $74 \pm 17 \text{ MBq } ^{124}\text{I}$ -trastuzumab was injected intravenously. PET imaging was performed at 1, 24, 48, 72, and 96 h post-injection for patient 2 and at 1, 24, and 48 h post-injection for the other 5 patients.

Image analysis

Tumors uptake analyses were restricted to lesions of $\geq 1 \text{ cm}$ diameter on a PET image of ^{18}F -FDG PET/CT relative to the adjacent tissues. After the imaging features of the lesions were analyzed, the regions of interest (ROI) were drawn to measure the standard uptake value (SUV). Based on ^{18}F -FDG imaging results, a total of 29 lesions were identified and were analyzed correspondingly by PET imaging of ^{124}I -trastuzumab. The analysis showed that the SUVmax of 18 lesions from HER2-positive patients (patient 1 and patient 3) was higher than 11 lesions from HER2-negative patients (patient 2, 4, 5, 6). So, we observed lesions with ^{18}F -FDG high uptake and ^{124}I -trastuzumab high uptake in HER2-positive lesions, and lesions with ^{18}F -FDG high uptake and ^{124}I -trastuzumab low uptake in HER2-negative lesions. The mean SUV of solid organs (liver, spleen, and kidney) was calculated by the layered boundary delineation method. The mean SUV of solid organs, such as liver, spleen, and kidney, was calculated using the layered boundary delineation method. The mean SUV of blood was obtained from the PET images of the cardiac ventricles, and the heart wall was visualized as a region of relatively low uptake adjacent to the ventricles. The internal radiation dose was calculated on the basis of radioactivity data from the blood, urinary excretions, and normal tissues of the heart, liver, spleen, kidneys, and other parts of the body at each time point using the OLINDA/EXM software.

Radiation dosimetry

The organ radioactivities and their data analysis are described in the supplemental materials. The absorbed radiation doses in each individual organ were calculated using the OLINDA/EXM software [16, 25, 26].

Statistical analysis

The data are presented as the mean $\pm$ SD. The micro-PET images were quantified using ROI-based quantification,

and those data were analyzed using two-tailed, unpaired Student's *t*-tests, and *p* values less than 0.05 as statistically significant. These statistical computations were performed using the Excel software program (Microsoft Corporation, Redmond, WA) or GraphPad software (GraphPad Prism 5).

Results

Synthesis, quality control, and micro-pet imaging of ^{124}I -trastuzumab

The radiolabeling efficiency of ^{124}I -trastuzumab was $95\% \pm 0.5\%$ ($n=5$), and the radiochemical purity after purification reached $99.2\% \pm 0.3\%$ ($n=5$). The specific activity was calculated to be $1.3 \text{ GBq}/\mu\text{mol}$. The results of quality control are shown in Table S1. Radio-TLC and HPLC analysis (Figure S1) of the radioligand revealed no aggregates, fragments, or radioactive impurities. The ^{124}I -trastuzumab was produced as sterile and endotoxin-free injectable solution, which was stable both in saline and 5% human serum albumin (HSA) at 4°C for up to 7 d (Figure S2).

The micro-PET imaging of ^{124}I -trastuzumab versus ^{124}I -hIgG1 in HER2-positive and HER2-negative PDX mouse models were performed at different time point after injection. Both ^{124}I -trastuzumab and ^{124}I -hIgG1 in HER2-positive PDX mouse models mainly resided in circulatory system within 2 h post-injection. Along with time, the tumor increased and could be visualized on the imaging of ^{124}I -trastuzumab at 24 h with uptake at $11.91 \pm 1.08 \text{ ID \%g}$ and T/M ratio of 9.13 ± 2.52 (Fig. 1) and remained to be clearly visible up to 96 h post-injection. Low tumor uptake was observed during the entire development period using ^{124}I -hIgG1, with uptake of $3.83 \pm 0.09 \text{ ID \%g}$, $n=5$, $p=0.0002$ and T/M ratios of 3.49 ± 0.31 at 24 h (Fig. 1). In comparison, ^{124}I -trastuzumab in HER2-negative PDX mouse models did not show clear tumor uptake, under the same conditions, with uptake of $5.05 \pm 0.17 \text{ ID \%g}$, $n=5$, $p=0.0004$ and T/M ratios of 2.73 ± 0.30 at 24 h (Fig. 1). Although the uptake of ^{124}I -trastuzumab in HER2-negative PDX models was slightly higher than that of ^{124}I -IgG1 in HER2-positive PDX models at 24 h post-injection, the T/M ratios of ^{124}I -trastuzumab in the former were lower than those of ^{124}I -IgG1 in the latter.

Patient characteristics

We had 6 patients in this study, with 2 patients HER2-positive based on IHC 3+/FISH+ and 4 patients HER2-negative based on HER2 2+/FISH- (Table 1). All 6 patients were stage IV cancer, and 5 patients had liver, bone, and lymph node metastases. Based on the selection criteria for lesions described above, we selected a total of 29 lesions

Fig. 1 **a** Micro-PET images of ^{124}I -trastuzumab in HER2-positive PDX models (^{124}I -tras. (HER2+)), ^{124}I -hIgG1 in HER2-positive PDX models (^{124}I -hIgG1. (HER2+)) and ^{124}I -trastuzumab in HER2-negative PDX models (^{124}I -Tras. (HER2-)) at 2, 24, 48, 72, and 96 h post-injection; **b** Region of interest (ROI) analysis of the tumor uptake

values in micro-PET images with ^{124}I -Tras. (HER2+), ^{124}I -hIgG1. (HER2+), and ^{124}I -tras. (HER2-) models; **c** the tumor-to-muscle (T/M) ratios of ^{124}I -trastuzumab in ^{124}I -Tras. (HER2+), ^{124}I -hIgG1. (HER2+), and ^{124}I -Tras. (HER2-) models. Yellow arrows indicate the tumors. **, $P < 0.05$, ***, $P < 0.001$

(18 HER2-positive lesions and 11 HER2-negative lesions) for image analysis. No infusion-related reactions or adverse events were observed during the study.

The regional time-activity curves

The regional time-activity curves of ^{124}I -trastuzumab in 5 patients were collected at 1, 24, and 48 h (Fig. 2). At 1 h, ^{124}I -trastuzumab mainly resided in the circulatory system and well-perfused organs (including liver, spleen, and kidney). The SUVmax of ^{124}I -trastuzumab in blood was 7.3–11.2, 3.6–11.9, and 2.2–8.2 at 1, 24, and 48 h, respectively, showing long-term high activity in vivo. Liver and kidney also had high radioactivity uptake due to the clearance of the radioligand, such as degradation into small molecular compounds in the liver, kidney, and other organs and excretion through the urine or feces. The SUVmax values were 4.6–7.4, 2.6–5.4, and 2.5–3.9 in the liver, 0.9–3.3, 0.5–4.5, and 0.4–3.6 in the kidneys, and 0.4–2.8, 3.3–14.7, and 3.9–19.5 in the urinary bladder, at 1 h, 24 h, and 48 h, respectively. The uptake in the kidney slightly increased at

1 h and decreased at 24 h and 48 h. The uptake in the urinary bladder was visualized at 1 h, increased rapidly at approximately 24 h, and decreased at 48 h. The spleen had high uptake values of 4.6–6.9, 2.2–6.3, and 0.8–4.6. The whole-body distribution of ^{124}I -trastuzumab in patient 2 showed normal radiation dose compared with other analogous radioligands (Figure S5 and Figure S6). The uptake in the thyroid was a special concern for iodinated radioligand. ^{124}I -trastuzumab showed low uptake at 1 h and 24 h but increased at 48, 72, and 120 h, with the SUVmax of 1.9–2.3, 4.1–8.4, and 4.8–11.0 at 1, 24 and 48 h post-injection, respectively. Within 24 h, the nonspecific uptake in the thyroid was pre-blocked, but the thyroid uptake increased after 48 h. Because the SUVmax values of patient 6 were 64.5 and 76.1 at 24 and 48 h, respectively, the regional time-activity curves of patient 6 were not shown.

Radiation dosimetry

Assessments of internal organ dosimetry were performed in 5 patients, with injected radioactivity at 74 ± 17 MBq. The

Table 1 Patient characteristics

Patient no.	Age (y)	Sex	Histology	Stage	HER2 score of IHC/ FISHm primary lesion	Status of HER2 in primary lesion	Co-injection Trastuzumab	Type of pathology	Primary or meta- static lesions' loca- tion or number	Treatment
1	52	Male	GC	IV	3+/+	Positive	5 mg	Adenocarcinoma postoperative recur- rence	Liver: 3 Bone: right humerus 1. Iliac pubic: 4. sacral bone: 1, spinal: 7	Chemother- apy + trastuzumab one cycle
2	62	Male	EGC	IV	2+/-	Negative	10 mg	Moderate Differentiated AD	Lymph node: 3	Chemotherapy
3	38	Female	GC	IV	3+/+	Positive	10 mg	Moderate Differentiated AD	Primary Lymph node: 1	Chemotherapy
4	70	Male	EGC	IV	2+/-	Negative	10 mg	Moderate Differentiated, postoperative recurrence	Primary Lymph node: 2	Chemotherapy
5	61	Male	GC	IV	2+/-	Negative	10 mg	Poorly moderate Differentiated with signet ring cell carcinoma	Primary	Chemotherapy
6	69	Male	EGC	IV	2+/-	Negative	5 mg	Moderate Differentiated AD Lauren diffuse intestine	Primary Liver: 3 Lymph node: 1	No

Bold indicates the main item classification, italic indicates HER2-Negative patients

Fig. 2 Regional time-activity curves of ^{124}I -trastuzumab in 5 patients. **a** The SUVs in the blood, thyroid, liver, spleen, kidneys, and bladder were calculated from ROI-based analyses of each tissue. The SUV in the blood was measured from a region of interest in the

heart. In patient 6, the SUV in the thyroid was extremely high. **b** The SUVmax in the tumor and tumor-to-normal tissue ratio (TNR) in 5 patients

highest absorbed dose was at the heart wall, followed by the liver, spleen, and kidneys (Table 2). The average effective dose was estimated to be 0.3011 ± 0.005 mSv/MBq.

Time of lesion detection

The SUVmax values at left iliac metastasis were 5.1, 9.2, and 7.4, and those at right iliac metastasis were 6.5, 7.7, and 6.6 at 1 h, 24 h, and 48 h post-injection, respectively (Fig. 3a), maximized at 24 h. At 1 h, the radioligand mainly resided in the circulatory system and was not fully distributed. At 48 h, both radioactive decay and physiological

metabolism of the radiotracer led to a low image quality because of insufficient radioactivity counting. The optimal time for tumor imaging was at 24 h.

Comparison of direct lesion uptake between ^{124}I -trastuzumab and ^{18}F -FDG at 24 h

The correlation between the PET imaging of ^{124}I -trastuzumab and ^{18}F -FDG in HER2-positive lesions (Fig. 3b) was established in patient 1, who also underwent through GC resection to establish his IHC score of 3+. ^{18}F -FDG PET imaging was used to establish qualified tumor lesions, showing humeral metastasis, iliac metastases, and spinal

Table 2 Organ-absorbed doses and effective dose estimated from whole-body ^{124}I -Trastuzumab PET

Organ	Absorbed dose (mGy/Mbq)
Adrenals	0.35 + 0.001
Brain	0.088 ± 0.005
Esophagus	0.269 ± 0.014
Gall bladder	0.388 + 0.002
Left colon	0.027 + 0.025
Small intestine	0.025 + 0.004
Right colon	0.026 + 0.001
Gastric	0.039 + 0.004
Heart	0.741 + 0.006
Kidney	0.396 + 0.004
Liver	0.651 + 0.022
Lung	0.17 + 0.005
Pancreas	0.24 + 0.017
Red marrow	0.183 + 0.03
Cortex of bone	0.177 + 0.004
Spleen	0.617 + 0.023
Thyroid	0.463 + 0.027
Bladder	0.346 + 0.005
Total body	0.201 + 0.003

Effective dose = 0.3011 ± 0.005 mSv/MBq

metastases in this patient. In the 18 HER2-positive lesions established in the ^{18}F -FDG PET image, the ^{124}I -trastuzumab PET imaging showed higher uptake at 24 h than that at 48 h (SUVmax 7.83 ± 0.55 vs. 5.73 ± 0.47 , $p = 0.0065$). The detection sensitivity of ^{124}I -trastuzumab was 77.8% (14/18) at 1 h, 100% (18/18) at 24 h, but 88.9% (16/18) at 48 h. The correlation between the PET imaging of ^{124}I -trastuzumab and ^{18}F -FDG in HER2-negative lesions (Fig. 3c) was established in patient 3, who also underwent through GEC resection to establish his IHC score of 2+, FISH-. ^{18}F -FDG PET imaging was used to establish that the patient had lymph node metastasis, with SUVmax 6.7. The PET imaging of ^{124}I -trastuzumab showed low uptake of SUVmax 1.9, 1.5, and 1.4 at 1, 24, and 48 h, respectively. In the 11 HER2-negative lesions established in the ^{18}F -FDG PET image, the ^{124}I -trastuzumab PET imaging showed lower uptake at 24 h than that with ^{18}F -FDG (SUVmax 1.75 ± 0.29 vs. 6.46 ± 0.44 , $p < 0.0001$).

PET imaging could help resolve ambiguity in CT images in identifying tumors. For one patient, we observed a 0.5 cm cortical defect in the left transverse process of the 5th cervical spine, which was not obvious on CT and was easily misdiagnosed (Fig. 4a). However, significant radioactivity uptake in both ^{18}F -FDG PET imaging and ^{124}I -trastuzumab imaging was observed at the same location, with SUVmax value of 5.31 and 7.09, respectively. Another lesion was

observed in the third lumbar vertebra (L3), and the morphological changes of the lesions were also vague on the CT image. Both ^{18}F -FDG and ^{124}I -trastuzumab PET imaging showed significant radioactive accumulation with SUVmax value of 10.42 and 6.42, respectively (Fig. 4b).

Discussion

Radioligands based on ^{124}I should be suitable to investigate lengthy biological processes since iodine-124 has relatively long physical half-life ($t_{1/2} = 4.08$ days), and it is a positron-emitting radionuclide with high-energy γ -emissions (0.6 MeV, 61%) and positron emissions (2.14 MeV, 24%). Direct radioiodination of surface-exposed tyrosine residues is effective and convenient way to synthesize these ligands, and several studies have shown the added iodine minimal impact on plasma clearance of the radiolabeled antibody.

Compared with the ^{89}Zr - and ^{64}Cu -labeled antibodies, ^{124}I -trastuzumab achieved higher imaging contrast because of lower nonspecific uptake and better tumor-to-soft-tissue radioactivity ratio. However, after ^{124}I -trastuzumab is degraded upon internalization, the released ^{124}I -iodide may somehow diffuse freely from the tissues, resulting in the loss of specific signal in target tissues and in the increase in background signal over time. In contrast, when ^{64}Cu - and ^{89}Zr -labeled antibodies are processed, the radionuclides are trapped intracellularly in lysosomes and radioactivity remain in cell, leading to high uptake in normal clearance organs (liver and kidney) and high background uptake of other tissues that passively absorb antibodies [21, 22, 27]. When ^{124}I cleaves with antibody upon degradation, it enters the blood circulation and gradually enters the thyroid and stomach organ, and it clears from kidney, instead of liver, leading to higher tumor to liver ratios, which are obviously beneficial in imaging resolution.

^{124}I -trastuzumab gave higher imaging contrast than ^{64}Cu -NOTA-trastuzumab because of lower nonspecific uptake and better tumor-to-soft-tissue ratios. Compared with our previously reported ^{64}Cu -NOTA-trastuzumab, we observed that both ^{124}I - and ^{64}Cu -labeled trastuzumab gave specific signals in PDX mouse tumors and high-contrast images [20]. However, they differ in tumor and background uptake, resulting in different levels of imaging contrast in the PDX mouse model. For example, ^{124}I -trastuzumab gave lower liver uptake at 3.62 ± 1.18 percent injected dose per gram [%ID/g] than the residualizing ^{64}Cu -NOTA-trastuzumab ($7.87 \pm 0.52\%$ ID/g, $p < 0.0001$ for each at 24 h, and in consequence, the tumor to liver ratios reached 1.93 ± 0.017 for ^{124}I -trastuzumab at 24 h and 0.95 ± 0.029 for ^{64}Cu -NOTA-trastuzumab at 14 h ($p < 0.0001$, $n = 3$), and 2.26 ± 0.069 for ^{124}I -trastuzumab

Fig. 3 **a** The ^{124}I -trastuzumab PET images at 1 h, 24 h, and 48 h of patient 1 who had bilateral iliac metastases; **b** The ^{124}I -trastuzumab PET image at 24 h and ^{18}F -FDG PET image at 1 h of the HER2-positive lesions in patient 1 who had a right humeral metastasis, bilateral ilium metastases, and multiple spinal metastases; **c** The ^{18}F -FDG PET image at 1 h and ^{124}I -trastuzumab PET images at 1 h, 24 h, and 48 h of the HER2-negative lesions in patient 3 who had lymph node metastases. The red arrow indicates the tumors

at 72 h and 1.14 ± 0.094 for ^{64}Cu -NOTA-trastuzumab at 36 h ($p < 0.0001$, $n = 3$). In addition, the tumor-to-muscle ratios reached 9.87 ± 0.095 for ^{124}I -trastuzumab at 24 h and 10.59 ± 0.025 for ^{64}Cu -NOTA-trastuzumab at 14 h ($p = 0.003$, $n = 3$), and 17.27 ± 0.76 for ^{124}I -trastuzumab at 72 h and 7.13 ± 0.49 for ^{64}Cu -NOTA-trastuzumab at 36 h ($p < 0.0001$, $n = 3$). In the Xenograft mouse model, we confirmed that the HER2 levels in tumors of HER2-positive models were HER2 3+ and those in HER2-negative models were HER2 1+ [20], with details in the supplemental materials.

We had successfully applied ^{124}I -trastuzumab in human clinical PET imaging. In order to observe the complete pharmacokinetics of ^{124}I -trastuzumab in vivo, we did PET imaging at 1, 24, 48, 72, and 96 h post-injection for patient 2. After ^{124}I -trastuzumab degrades upon internalization, the released ^{124}I -iodide may diffuse freely within the tissues, resulting in the loss of signal in target tissues and in the increase in background signal over time. Better contrast images of tumor were obtained at 24 and 48 h than those at 72 h and 96 h, confirming the degradation hypothesis described above. The effective dose of ^{124}I -trastuzumab was estimated to be 0.3011 ± 0.005 mSv/MBq. For a normal

Fig. 4 **a** The ^{124}I -trastuzumab PET image at 24 h and ^{18}F -FDG PET image at 1 h of the HER2-positive lesions in patient 1 in the left transverse process of the 5th cervical spine; **b** The ^{124}I -trastuzumab

PET image at 24 h and ^{18}F -FDG PET image at 1 h of the HER2-positive lesions in patient 1 in the third lumbar spine. The red arrows indicate the tumors

injection dose of 74 ± 17 MBq, the internal radiation dose of ^{124}I -trastuzumab absorbed by patient was estimated to be 17.16–27.4 mSv per PET scan, not significantly different

from the estimated dose of 7.0 mSv for injected dose of 370 MBq per ^{18}F -FDG PET scan.

^{18}F -FDG PET imaging could be used to detect lesions based on the changes in glucose metabolism in tumor cells, while ^{124}I -trastuzumab PET imaging could help to monitor the HER2 expression levels in lesions, resulting in accurate diagnosis and early treatment. We measured the tumor SUVmax values in both HER2-positive and HER2-negative patients using both ^{124}I -trastuzumab and ^{18}F -FDG PET imaging (Fig. 5). Stronger PET signals were observed using ^{124}I -trastuzumab in HER2-positive lesions than those in HER2-negative lesions, with SUVmax of 7.83 ± 0.55 versus 1.75 ± 0.29 , $p < 0.0001$ at 24 h. If we use a SUVmax cutoff value of 4.0 between HER2-positive and HER2-negative, the diagnosis sensitivity was 94.4%, and the specificity was 100%. More samples are needed to verify an exact threshold. With ^{18}F -FDG, all 29 lesions in both HER2-positive and HER2-negative patients had SUVmax values greater than 4, and the range of SUVmax values in HER2-negative lesions overlaps with that of SUVmax values of the HER2-positive lesions (SUVmax 4.4–8.9, 4.7–14.1). In

HER2-positive lesions, the SUVmax values of ^{124}I -trastuzumab were 3.0–10.5, and those of ^{18}F -FDG were 4.7–14.1. In HER2-negative lesions, the average of SUVmax values of ^{124}I -trastuzumab was 1.75 ± 0.29 , and that of ^{18}F -FDG was 6.46 ± 0.44 ($p < 0.0001$). Thus, ^{18}F -FDG PET imaging cannot be used to differentiate HER2-positive tumor tissues even with scores of 3+ from the test of the patients. The CT images only cannot detect the small and less morphological changes in lesions of patients, while the combination of PET imaging using ^{18}F -FDG and ^{124}I -trastuzumab could be used to identify abnormal metabolic signals in tissues with less morphological changes.

Although patient 1 received trastuzumab 4 days before the ^{124}I -trastuzumab PET/CT scan, the lesions also showed clear uptake of ^{124}I -trastuzumab. This shows that the radioligand could be useful to evaluate therapeutic responses, consistent with that of ^{89}Zr -pertuzumab [27].

When iodinated radioligand is used, absorption into thyroid is always a concern. The SUVmax values of

Fig. 5 **a** SUVmax values of the ^{124}I -trastuzumab in HER2-positive (IHC 3+ or 2+ and FISH+) and HER2-negative (IHC 2+/FISH-) patients; **b** SUVmax values of the ^{18}F -FDG in HER2-positive and HER2-negative patients; **c** comparison of SUVmax values between

^{124}I -trastuzumab and ^{18}F -FDG images in HER2-negative patients; **d** comparison of SUVmax values between ^{124}I -trastuzumab and ^{18}F -FDG images in HER2-positive patients

^{124}I -trastuzumab in the thyroid were 1.9–2.3, 4.1–8.4, and 4.8–11.0 at 1, 24, and 48 h post-injection, respectively. Within 24 h, the nonspecific uptake in the thyroid was well blocked, but after 48 h, the thyroid uptake was high. We attempted to block the thyroid uptake of ^{124}I by orally administering Lugol's potassium iodide, and the thyroid uptake was still observed; this finding indicates that more Lugol's potassium iodide may be needed. We concluded that it might be difficult for patients to adhere to the scheduled dosage of Lugol's potassium iodide and that the dosage of Lugol's potassium iodide may not sufficient to block nonspecific thyroid uptake. When ^{124}I is introduced into ^{124}I -trastuzumab under oxidative conditions, it is generally randomly conjugated to tyrosine residues present in the antibody. Once ^{124}I -trastuzumab is internalized and degraded, the released ^{124}I -iodide or ^{124}I -iodotyrosine may diffuse from the tissue, resulting in high uptake in normal thyroid tissues.

Liver uptake of ^{124}I -trastuzumab may be modulated with therapeutic drugs. With a 10 mg cold trastuzumab preinjection, all patients had relatively low liver backgrounds at 24 h, and the SUVmax of the liver was 2.6, 2.9, 4.2, 4.4, and 9.9 in 5 trastuzumab-naïve patients and 5.4 in one patient on trastuzumab therapy. Previous PET imaging studies on ^{89}Zr -trastuzumab and ^{64}Cu -DOTA-trastuzumab for HER2 patients demonstrated that 50 mg cold trastuzumab was needed to decrease the high liver uptake in trastuzumab-naïve patients. Our results indicated that 10 mg cold trastuzumab preinjection was adequate for HER2 PET imaging in trastuzumab-naïve patients, and ^{124}I -trastuzumab may have the advantage of low liver uptake, consistent with the results obtained in the PDX mouse models.

The limitation of present human clinical PET study is the small number of patients with limited diversity in HER2 status. Among the 6 patients studied, only two types of patients were enrolled, HER2-positive (IHC 3+) and HER2-negative (IHC 2+/FISH-), which were measured using HE staining, IHC and FISH (Figure S7). Even for the limited number of patients, heterogeneity of HER2 status between primary and metastatic lesions was not established. In addition, the HER2 levels of all metastatic lesions through pathological examination were not attempted.

Conclusion

In this study, we have demonstrated that ^{124}I -trastuzumab PET/CT imaging is safe without any adverse effect on patients and acceptable level of radiation dosage in whole body of human and can measure HER2 levels in HER2-positive patients with GC/GEC. The potential clinical applications of the imaging include assessing the HER2 status of lesions before therapy to guide selection of therapy, or

monitoring the changes of HER2 levels during therapy to monitor the effect of therapy. In addition, HER2-targeted PET/CT imaging may also be used in prospective clinical trials of patients with GC/GEC to analyze the ability of ^{124}I -trastuzumab to detect unsuspected HER2-positive metastatic disease arising from HER2-negative GC and to help predict the response of HER2-positive GC to HER2-targeted therapy. If combined with ^{18}F -FDG imaging, ^{124}I -trastuzumab imaging may also help the studies on the mechanism of tumor metastases and drug resistance.

Funding The current research was financially supported by the National Key Research and Development Program of China (No. 2017YFC1308900), National Natural Science Foundation of China (81571705, 81671733, and 81871386), Beijing Municipal Administration of Hospitals-Yangfan Project (ZYLX201816), Beijing Natural Science Foundation, Jing-Jin-Ji special projects for basic research cooperation (H2018206600), Beijing Nova Program (Z171100001117020), and Beijing Excellent Talents Funding (2017000021223ZK33).

Compliance with ethical standards

Conflicts of interests The authors declare no potential conflict of interests.

Ethical approval All procedures in this study were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later versions. Informed consent, or its equivalent, was obtained from all patients included in the study. The clinical study was approved by the Ethics Committee of the Beijing Cancer Hospital (Ethics Approval License: 2018KT48).

Human and animal rights All institutional and national guidelines for the care and use of laboratory animals were followed.

Informed consent Informed consent was obtained from the 6 individual participants included in this study.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. *CA Cancer J Clin*. 2017;67(1):7.
2. Lin S, Yan-Shen S, Huang-Ming H, et al. Management of gastric cancer in Asia: resource-stratified guidelines. *Lancet Oncol*. 2013;14(12):e535–47.
3. Van CE, Sagaert X, Topal B, et al. Gastric cancer. *J Natl Cancer Inst*. 2016;57(1):2654–64.
4. Shah MA. Update on metastatic gastric and esophageal cancers. *J Clin Oncol*. 2015;33(16):1760–9.
5. Stella M, Serventi A, Friedman D. Right portal vein thrombosis after splenectomy for trauma. *J Gastrointest Surg*. 2005;9(5):646–7.
6. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet*. 2010;376(9742):687–97.

7. Okines AF, Cunningham D. Trastuzumab in gastric cancer. *Eur J Cancer*. 2010;46(11):1949–59.
8. Niikura N, Liu J, Hayashi N, et al. Loss of human epidermal growth factor receptor 2 (HER2) expression in metastatic sites of HER2-overexpressing primary breast tumors. *J Clin Oncol*. 2012;30(6):593–9.
9. Pectasides D, Gaglia A, Arapantonidadioti P, et al. HER-2/neu status of primary breast cancer and corresponding metastatic sites in patients with advanced breast cancer treated with trastuzumab-based therapy. *Anticancer Res*. 2006;26(1B):647.
10. Altundag K. Role of endocrine therapy in weak estrogen/progesterone receptor expression in HER2 negative breast cancer. *Eur J Surg Oncol*. 2018;44(4):539.
11. Altundag K. Digital breast tomosynthesis findings may be different in HER2 positive breast cancer patients according to hormone receptor status. *Br J Radiol*. 2018;91(1082):20170730.
12. Di Leo A, Johnston S, Lee KS, et al. Buparlisib plus fulvestrant in postmenopausal women with hormone-receptor-positive, HER2-negative, advanced breast cancer progressing on or after mTOR inhibition (BELLE-3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol*. 2018;19(1):87–100.
13. Chao WR, Lee MY, Ruan A, et al. Assessment of HER2 status using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) techniques in mucinous epithelial ovarian cancer: a comprehensive comparison between ToGA biopsy method and ToGA surgical specimen method. *PLoS ONE*. 2015;10(11):e0142135.
14. Perik PJ, Lub-De Hooge MN, Gietema JA, et al. Indium-111-labeled trastuzumab scintigraphy in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer. *J Clin Oncol*. 2006;24(15):2276–82.
15. Dijkers EC, Kosterink JG, Rademaker AP, et al. Development and characterization of clinical-grade 89Zr-trastuzumab for HER2/neu immunoPET imaging. *J Nucl Med*. 2009;50(6):974–81.
16. Dijkers EC, Oude Munnink TH, Kosterink JG, et al. Biodistribution of 89Zr-trastuzumab and PET imaging of HER2-positive lesions in patients with metastatic breast cancer. *Clin Pharmacol Ther*. 2010;87(5):586–92.
17. Tamura K, Kurihara H, Yonemori K, et al. 64Cu-DOTA-trastuzumab PET imaging in patients with HER2-positive breast cancer. *J Nucl Med*. 2013;54(11):1869–75.
18. Kurihara H, Hamada A, Yoshida M, et al. (64)Cu-DOTA-trastuzumab PET imaging and HER2 specificity of brain metastases in HER2-positive breast cancer patients. *EJNMMI Res*. 2015;5:8.
19. Woo SK, Jang SJ, Seo MJ, et al. Development of 64Cu-NOTA-Trastuzumab for HER2 targeting: radiopharmaceutical with improved pharmacokinetics for human study. *J Nucl Med*. 2018. <https://doi.org/10.2967/jnumed.118.210294>.
20. Guo X, Zhu H, Zhou N, et al. Noninvasive detection of HER2 expression in gastric cancer by (64)Cu-NOTA-trastuzumab in PDX mouse model and in patients. *Mol Pharm*. 2018;15(11):5174–82.
21. Knowles SM, Zettlitz KA, Richard T, et al. Quantitative immunoPET of prostate cancer xenografts with 89Zr- and 124I-labeled anti-PSCA A11 minibody. *J Nucl Med*. 2014;55(3):452–9.
22. Divgi CR, Pandit-Taskar N, Jungbluth AA, et al. Preoperative characterisation of clear-cell renal carcinoma using iodine-124-labelled antibody chimeric G250 (124I-cG250) and PET in patients with renal masses: a phase I trial. *Lancet Oncol*. 2007;8(4):304–10.
23. Hamilton EP, Patel MR, Armstrong AC, et al. A first in human study of the new oral selective estrogen receptor degrader AZD9496 for HR +/HER2- advanced breast cancer. *Clin Cancer Res*. 2018.
24. Chen Z, Huang W, Tian T, et al. Characterization and validation of potential therapeutic targets based on the molecular signature of patient-derived xenografts in gastric cancer. *J Hematol Oncol*. 2018;11(1):20.
25. Wong JYC, Raubitschek A, Yamauchi D, et al. A Pretherapy biodistribution and dosimetry study of indium-111-radiolabeled trastuzumab in patients with human epidermal growth factor receptor 2-overexpressing breast cancer. *Cancer Biother Radiopharm*. 2010;25(4):387.
26. O'Donoghue JA, Lewis JS, Pandit-Taskar N, et al. Pharmacokinetics, biodistribution, and radiation dosimetry for 89Zr-trastuzumab in patients with esophagogastric cancer. *J Nucl Med Off Publ Soc Nucl Med*. 2017. <https://doi.org/10.2967/jnumed.117.194555>.
27. Van Dongen GA, Visser GW, Lub-de Hooge MN, et al. ImmunoPET: a navigator in monoclonal antibody development and applications. *Oncologist*. 2007;12(12):1379–89.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.