Preclinical Evaluation of ^{64}Cu-Labeled Cetuximab Immuno-PET for Detecting Sentinel Lymph Node Metastasis and Assessing Therapeutic Potential in EGFR-Positive Breast Cancer

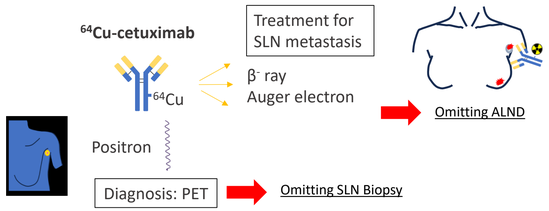
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Abstract

Background: Despite advances in breast cancer imaging, reliable detection of sentinel lymph node (SLN) metastasis remains challenging. We evaluated immuno-PET using ^{64}Cu-labeled cetuximab for SLN detection and therapeutic potential in an Epidermal growth factor receptor(EGFR) -positive model.
Methods: The SLN metastasis model was established using the EGFR-strongly-expressing MDA-MB-468. [^{64}Cu]Cu-PCTA-cetuximab was administered intravenously (IV) or intradermally/subdermally (ID/SD), followed by PET/CT. SLNs were confirmed with dye and pathology. A comparison with ^{18}FDG-PET was also conducted. Therapeutic efficacy was tested with escalating IV doses.
Results: IV administration detected SLN metastases with high accuracy (sensitivity 89%, specificity 100%). ID/SD showed strong tracer uptake but low specificity. ^{18}F-FDG PET failed to detect SLN metastases. Therapeutically, IV [^{64}Cu]Cu-PCTA-cetuximab suppressed tumor growth; the highest dose caused weight loss.
Conclusions: IV [^{64}Cu]Cu-PCTA-cetuximab enables accurate SLN metastasis detection and shows therapeutic promise in EGFR-positive breast cancer. ID/SD delivery may be useful for localized therapy.

Introduction

SLN biopsy is standard method for axillary staging in breast cancer but still causes complications such as shoulder dysfunction and lymphedema. Non-invasive imaging methods such as MRI, US and CT have been explored, these imaging techniques cannot replace SLN biopsy in current practice. Immuno-PET, using radiolabeled antibodies, enables precise detection of cancer-specific targets. EGFR is expressed in ~15% of breast cancers and ~50% of TNBC cases. Cetuximab, an anti-EGFR antibody, shows promise when labeled with ^{64}Cu, a theranostic radionuclide emitting both β^+ and β^- particles. Preclinical studies suggest that ^{64}Cu-labeled cetuximab can detect SLN metastases and deliver targeted radiotherapy to micro-metastatic disease, potentially reducing the need for invasive axillary procedures in EGFR-positive cN0 breast cancer.



Methods and Materials

SLN metastasis model was established using MDA-MB-468. SLNs were identified using blue dye and pathologically evaluated for metastases(Fig.1).
[^{64}Cu]Cu-PCTA-cetuximab was administered either intravenously (IV; 5.8 ± 0.9 MBq; n=12) or intradermally/subdermally (ID/SD; 4.3 ± 0.4 MBq; n=11) into the parapapillary region of the left third mammary gland. PET/CT scans were performed 24 h after tracer injection, with delayed imaging at 48 h. Following SLN biopsy, PET confirmed the absence of residual disease. For comparison, ^{18}F-FDG PET was also performed via IV (9.1 ± 1.4 MBq, n = 4) or ID/SD (5.4 ± 2.2 MBq, n = 3) routes in the same cohort (Fig.2).
To evaluate therapeutic efficacy, additional mice received IV [^{64}Cu]Cu-PCTA-cetuximab at 9, 18, 28 or 37 MBq (n = 3 per group), and tumor volumes and body weight were monitored over time in comparison to untreated controls.

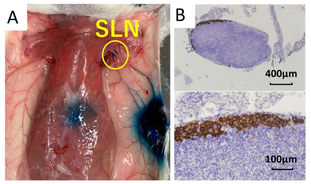


Figure 1.
(A). SLN identified by blue dye (yellow arrow).
(B). SLN metastasis detected by EGFR IHC (upper, [×40]; bottom, [×200]pathological diagnosis)【1】.

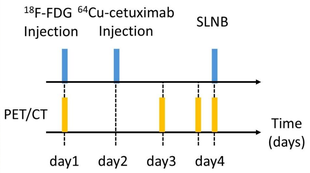


Figure 2. Summary of the study protocol for administration of radiotracers and PET/CT imaging【1】.

Results

After IV administration of [^{64}Cu]Cu-PCTA-cetuximab (n = 12), accumulation was detected in the axilla of eight mice (67%, SUVmax 1.24 ± 0.51), all of which were found to have SLNs with histologically confirmed metastasis. The sensitivity, specificity and positive predictive values for PET with IV [^{64}Cu]Cu-PCTA-cetuximab were 89%, 100%, 75% and 100%, respectively. In contrast, all mice with ID/SD administration (n = 11) showed high accumulation in axillary lymph nodes (SUVmax 4.28 ± 1.19), with six mice (55%, SUVmax 5.01 ± 1.12) having histologically confirmed metastasis.
The sensitivity, accuracy, and positive predictive values for PET with ID/SD administered [^{64}Cu]Cu-PCTA-cetuximab were 100%, 55% and 55%, respectively. SLN metastasis was not detectable by IV or ID/SD ^{18}F-FDG PET. Therapeutically, IV [^{64}Cu]-PCTA-cetuximab suppressed tumor growth dose-dependently. The 37 MBq group showed the greatest reduction but also significant weight loss, indicating toxicity risk at higher doses(Fig.4).

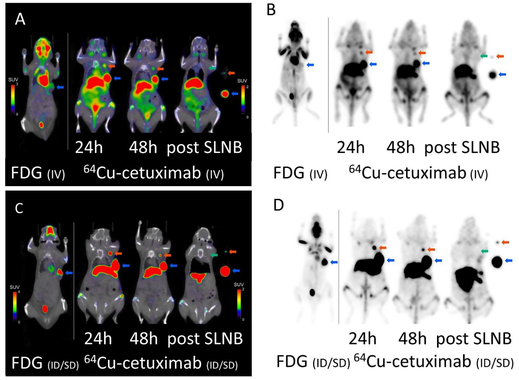


Figure 3. [^{64}Cu]-cetuximab PET and MIP images compared with FDG PET after IV (A, B) and ID/SD (C, D) administration. Red arrows indicate metastatic SLNs and the green arrow denotes disappearance of the lymph node with accumulation of [^{64}Cu]-cetuximab. The blue arrow denotes the primary tumor【1】.

A

PET/CT	Pathology: Positive	Pathology: Negative
[^{64}Cu]-cetuximab (IV) PET/CT		
Positive	8	0
Negative	1	3
^{18}F-FDG (IV) PET/CT		
Positive	0	0
Negative	9	3

B

PET/CT	Pathology: Positive	Pathology: Negative
[^{64}Cu]-cetuximab (ID/SD) PET/CT		
Positive	6	5
Negative	0	0
^{18}F-FDG (ID/SD) PET/CT		
Positive	0	0
Negative	6	5

Table 1. Comparison of PET/CT detection of SLN metastasis using [^{64}Cu]-cetuximab and ^{18}-FDG PET after IV (A) and ID/SD (B) administration【1】.

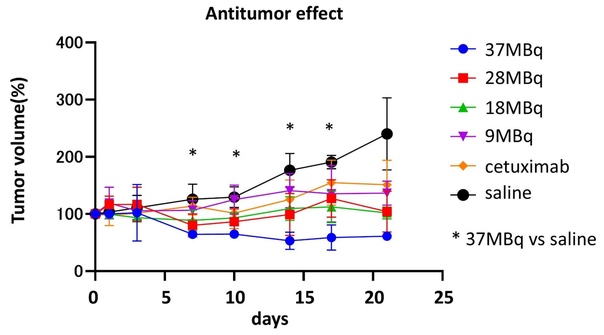


Figure4. Antitumor effect of [^{64}Cu]-cetuximab on primary tumors with different doses, cetuximab alone, and saline (significant difference for 37 MBq vs. saline, $p < 0.05$ (*t*-test)).

Discussion

IV [^{64}Cu]-PCTA-cetuximab PET/CT demonstrated higher precision for diagnosis of SLN metastasis than ^{18}F-FDG PET. Although diagnostic accuracy via ID/SD administration was inferior to that of IV delivery, the SUVmax in SLNs was notably higher after ID/SD administration than after IV administration, likely due to the large molecular weight of [^{64}Cu]-cetuximab causing non-specific lymphatic accumulation. Nevertheless, its high retention suggests potential for localized therapy. IV administration also suppressed tumor growth dose-dependently, though toxicity at high doses underscores the need for optimization. These findings highlight the theranostic promise of [^{64}Cu]Cu-PCTA-cetuximab PET/CT, especially for EGFR-positive TNBC, and warrant further clinical investigation including broader breast cancer subtypes.

Conclusions

PET with IV [^{64}Cu]Cu-PCTA-cetuximab demonstrated high precision for diagnosis of SLN metastasis and showed dose-dependent antitumor efficacy in a xenograft model of EGFR-positive human breast cancer. Although further evaluation is necessary, ID/SD administration could be a useful therapeutic approach owing to its high accumulation in SLNs. Overall, [^{64}Cu]Cu-PCTA-cetuximab immuno-PET represents a promising theranostic strategy for EGFR-positive breast cancer.

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References

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Figures 1–3 and Table 1 are adapted from this publication.

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