Journal of Nuclear Medicine, published on February 12, 2021 as doi:10.2967/jnumed.120.253096

A Prospective Randomized, Double-blind Study to Evaluate the Safety, Biodistribution, and Dosimetry of ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 in Patients with Well-differentiated Neuroendocrine Tumors

Wenjia Zhu[1], Yuejuan Cheng[2], Ru Jia[3], Hong Zhao[4], Chunmei Bai[2], Jianming Xu[3], Shaobo Yao[5], Li Huo[1]

1. Department of Nuclear Medicine, Beijing Key Laboratory of Molecular Targeted Diagnosis and Therapy in Nuclear Medicine, Peking Union Medical College Hospital, CAMS & PUMC, Beijing, 100730, China
2. Department of Oncology, Peking Union Medical College Hospital, Beijing, 100730, China
3. Department of Gastrointestinal Oncology, the fifth Medical Center, General Hospital of PLA, Beijing, China
4. Department of Hepatobiliary Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100021, China.
5. Department Nuclear Medicine, The First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, 350005, China

First author: Wenjia Zhu, 1 Shuaifuyuan, Dongcheng District, Beijing, China. Telephone: +86 18614080164. Email: zhuwenjia_pumc@163.com

Corresponding author: Li Huo, 1 Shuaifuyuan, Dongcheng District, Beijing, China. Telephone: +86 13910801986. Email: huoli@pumch.cn

Word count: 5046

Short running title: ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 in NET

ABSTRACT

Purpose:

^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 are somatostatin receptor subtype 2 (SSTR2) specific antagonists used for PET/CT imaging. The purpose of this study was to evaluate the safety, biodistribution, and dosimetry of ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 in patients with well-differentiated neuroendocrine tumors (NETs).

Methods:

Patients were equally randomized into two arms: Arm A, ^{68}Ga-NODAGA-LM3; Arm B, ^{68}Ga-DOTA-LM3. Serial PET scans were acquired at 5, 15, 30, 45, 60, and 120 minutes after ^{68}Ga-NODAGA-LM3 (200 MBq ± 11 MBq/40 μg total peptide mass) or ^{68}Ga-DOTA-LM3 (172 MBq ± 21 MBq/40 μg total peptide mass) injection. The biodistribution in normal organs, tumor uptake, and safety were assessed. Radiation dosimetry was calculated using OLINDA/EXM (version 1.0).

Results:

Sixteen patients, 8 in each arm, were recruited in the study. Both tracers were well tolerated in most patients. Two patients in Arm B had nausea (G2) and one of them had vomiting (G1). The PET images of other fourteen patients were further analyzed. Significantly lower organ uptake was observed in the pituitary, parotids, liver, spleen, pancreas, adrenal, stomach, small intestine, and kidneys with ^{68}Ga-DOTA-LM3 compared to ^{68}Ga-NODAGA-LM3. A total of 38 lesions were analyzed, including 18 lesions on ^{68}Ga-NODAGA-LM3 and 20 lesions on ^{68}Ga-DOTA-LM3. Both tracers showed good tumor uptake and retention. With ^{68}Ga-NODAGA-LM3, the tracer accumulation in tumor lesions increased by 138%, from an average SUVmax of 31.3 ± 19.7 at 5 minutes to 74.6 ± 56.3 at 2h. With ^{68}Ga-DOTA-LM3, the tumor uptake rapidly reached a high level at 5 minutes after injection, with an average SUVmax of 36.6 ± 23.6, and continued to increase to 45.3 ± 29.3 until 30 minutes post-injection. Urinary bladder wall is the organ receiving the highest absorbed dose in both arms. The mean effective dose was 0.026 ± 0.003 mSv/MBq for ^{68}Ga-NODAGA-LM3 and 0.025 ± 0.002 mSv/MBq for ^{68}Ga-DOTA-LM3.

Conclusion:

Both ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 show favorable biodistribution, high tumor uptake, and good tumor retention, resulting in high image contrast. The dosimetric data is comparable to other ^{68}Ga-labeled SSTR2 antagonists. Further studies are required to look into the potential antagonistic effects of ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3.

Key Words:

Somatostatin receptor antagonist, ^{68}Ga-NODAGA-LM3, ^{68}Ga-DOTA-LM3, neuroendocrine tumor, PET/CT

INTRODUCTION

Neuroendocrine tumors (NETs) are a family of heterogeneous tumors featured by overexpression of somatostatin receptor (SSTR), especially SSTR subtype 2 (SSTR2), which could be a target for molecular imaging and radionuclide therapy. The role of radiolabeled somatostatin analogues, such as TOC, TATE, NOC, in staging and restaging of NETs has been widely discussed (*1*). All these agents are SSTR agonists, which will be internalized into tumor cells after ligand–receptor interaction (*2*).

Nowadays, somatostatin receptor antagonists emerge as another type of somatostatin analogues characterized by low internalization rate and high tumor affinity (*3-7*). They bind to significantly more receptor sites than agonists (*4*). Previous clinical studies have demonstrated higher sensitivity and better image contrast of ^{68}Ga-NODAGA-JR11 compared to ^{68}Ga-DOTATOC (*8*). Data from our group suggested better performance of ^{68}Ga-DOTA-JR11 in the detection of liver metastases compared with ^{68}Ga-DOTATATE (*9*). Nevertheless, ^{68}Ga-DOTA-JR11 showed an overall lower tumor uptake than ^{68}Ga-DOTATATE.

p-Cl-Phe- cyclo(D-Cys-Tyr-D-4-amino-Phe(carbamoyl)-Lys-Thr-Cys)D-Tyr- NH_2 (LM3) is a novel somatostatin receptor antagonist developed by Prof. Helmut R. Maecke et al (*10*). It was coupled with different chelators (NODAGA, DOTA, and CB-TE2A) and radiometals (^{68}Ga, ^{64}Cu, and ^{177}Lu). In vitro studies have shown high SSTR2 affinities of ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 with a 50% inhibitory concentration (IC_{50}) of 1.3 nmol/L and 12.5 nmol/L, respectively (*10*). In animal models, both tracers showed good image contrast at 1 hour after injection, which can be blocked by cold peptides. Richard P.Baum et al reported a case with ^{68}Ga-DOTATOC-negative high-grade liver metastases (*11,12*). The patient was successfully imaged with ^{68}Ga-NODAGA-LM3 PET/CT and subsequently treated with ^{177}Lu-DOTA-LM3. Nearly complete remission was achieved after 3 cycles of intra-arterial peptide receptor radionuclide therapy.

Given the promising preclinical results and preliminary clinical data, we designed this prospective, randomized, double-blind study to evaluate the safety, biodistribution, dosimetry (Phase I), and diagnostic efficacy (Phase II) of Gallium-68 labelled LM3 in patients with well-differentiated NETs. Both ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 were investigated in this study and they were designed as two parallel arms. The results of Phase I study are presented and discussed in this paper.

MATERIALS AND METHODS

Study Design

This study was designed as a prospective two-armed, randomized, double-blind phase I/II single center study (ClinicalTrials.gov identifier NCT04318561). It was approved by the institutional review board of Peking Union Medical College Hospital and all patients signed a written informed consent before study participation. The inclusion and exclusion criteria can be found in supplemental Table 1. The primary objectives of the Phase I study were the safety, biodistribution, and dosimetry of ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3. Sixteen patients with well-differentiated NETs were prospectively and consecutively recruited in this study (Figure 1). Patients were equally randomized into two arms and they were unaware of their arms.

Synthesis and Radiolabeling

GMP-grade precursor, NODAGA-LM3 and DOTA-LM3 were supplied by CS Bio Co. (20 Kelly Court Menlo Park, CA94025 USA). The radiolabeling procedure was performed manually in a hot cell. Briefly, ^{68}Ga was eluted from a ^{68}Ge/^{68}Ga generator (Eckert & Ziegler, Germany) using 5ml of 0.1 mol/L hydrochloric acid directly into a reaction vial containing 40 μg precursor dissolved in sodium acetate buffer, for a final reaction mixture pH of 4. The mixture was heated to 100 °C for 10 min to allow for radionuclide incorporation. After cooling to room temperature, the reaction mixture was diluted with 5 mL water and then loaded onto an Oasis HLB light cartridge (preconditioned with 5 mL ethanol and 5 mL water) and washed with normal saline to remove unincorporated radionuclide. Finally, the product was eluted off the cartridge with 0.5ml 75% ethanol solution followed by 5 ml normal saline through a Millipore filter (0.22 μm, 25 mm) into a sterile product vial. The radiochemical purity was >95%. The final product was composed of 150-200 MBq radiopharmaceutical and approx. 0.38 ml ethanol and approx. 40 μg total peptide mass.

PET/CT Imaging

The study was carried out on a time-of-flight PET/CT scanner (Polestar m660, SinoUnion Healthcare Inc., China). Patients received an intravenous injection of ^{68}Ga-NODAGA-LM3 (200 MBq ± 11 MBq) or ^{68}Ga-DOTA-LM3 (172 MBq ± 21 MBq) according to their arms. The radiotracers were administered to subjects by

quick bolus injection (5 ml over 15 seconds). A low-dose CT scan (120KeV; 100 mAs; 1.3 pitch; 2.5 mm slice thickness; 0.5 s rotation time) from head to proximate thigh was obtained for anatomical localization and attenuation correction. Serial PET scans were acquired at 5, 15, 30, 45, 60, and 120 minutes after injection. Patients were required to lay still on the exam table during the first hour. Images were reconstructed using an ordered subsets expectation maximization algorithm (2 iterations, 10 subsets, 192×192 matrix) and corrected for CT-based attenuation, dead time, random events, and scatter.

Safety Assessment

Heart rate, blood pressure, pulse oximetry, and 3-lead electrocardiography were recorded within 1 h before and at 24 h after LM3 injection. Clinical symptoms were monitored and graded according to the Common Terminology Criteria for Adverse Events (AEs, v4.03).

Image Analysis

The images of ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 PET/CT were anonymized and reviewed by one experienced nuclear medicine expert, masked to the medical history of the patients, on MIM software (MIM Software Inc., Cleveland, OH).

The physiologic uptake was evaluated in the following organs at all time points: pituitary gland, parotids, thyroids, lungs, blood pool, liver, spleen, pancreas (uncinate process), stomach, small intestine, kidneys, and adrenal glands. Regions of interest were drawn over these organs to exclude focal lesions and the maximum standardized uptake value (SUVmax) normalized to patients' body weight was recorded. In case of bilateral organs such as parotids, thyroids, lungs, and kidneys, the average SUVmax were calculated. For adrenal glands, only left adrenal gland was measured because the uptake of right adrenal gland could be easily influenced by adjacent liver uptake.

Any focal accumulations not explained by physiologic uptake were interpreted as focal lesions. Up to four lesions were chosen in each patient, including two hepatic lesions and two extrahepatic lesions. The lesion uptake was measured using SUVmax. Tumor-to-background ratio (TBR) was quantified using blood pool, kidney, and liver as reference tissues.

Radiation Dosimetry

Whole organ volumes of interest were manually drawn over the source organs, including spleen, liver, kidneys, pituitary glands, vertebral bodies L1-L5, and urinary bladder, at each time point. The non-decay corrected activities at different time points were documented as percentage of injected dosage and fitted with mono-exponential curves. The area under the time-activity curve between time 0 and the first time point was calculated assuming a linear increase from 0 to first measured activity. The area under the time-activity curve after the first time point was calculated by trapezoidal integration from the first time point to the last time point and extrapolation from the last data point using the fitted mono-exponential function. For bone marrow, the residence time was derived using an image-based integration of L1-L5 vertebre, assuming L1-L5 have 12.3% of the whole-body bone marrow (*13*). Urinary bladder residence time was determined using the voiding bladder model implemented in OLINDA/EXM software, setting a 2-h bladder-voiding interval. The residence time in the remainder of the body was calculated as the maximum possible residence time (based on physical decay only) minus the sum of the residence time of all source organs. Absorbed dose for target organs and whole-body effective dose were determined with OLINDA/EXM software (version 1.0) using adult male models.

Statistical Analysis

Data were expressed as mean ± SD values. The differences between ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 were evaluated using students' t-test (SPSS, version 22). Because of the 2 drop-outs in arm B (see below), PET analyses were done in 14 patients, while safety evaluation was done in all 16 patients. P value < 0.05 was considered to indicate statistical significance.

RESULTS

Patients and Safety

A total of 16 patients, 8 in each arm, were recruited in this phase. There were two drop-outs due to AEs at 10 to 15 minutes post-injection. The demographic and clinical characteristics are summarized in Table 1 (including the 2 drop-outs).

There was a mild decrease in the blood pressure at 24 h post-injection compared to baseline (127.5/82.4 mmHg versus 133.6/86.3mmHg, $P<0.05$). No patients presented symptoms related to hypotension after LM3 injection. No significant change in other vital signs or 3-lead electrocardiography was recorded.

There were two AEs (patient 3 and 8). The first patient (patient 3) is a 69-year-old man with functional pancreatic NET (insulinoma, G1, Ki67 index 2%, primary tumor resected) and multiple hepatic metastases. He had G2 nausea 10 minutes after tracer injection. The second patient (patient 8) is a 33-year-old woman also with functional pancreatic NET (insulinoma, G3, Ki67 index 30%) as well as multiple hepatic, lymph node, and a solitary bone metastasis. She had G2 nausea and G1 vomiting 15 minutes after tracer injection. The scans were discontinued after the AEs and both patients withdrew from the study. The symptoms relieved after a few hours without any intervention. Vital signs were stable during that period and also at 24 h post-injection. Both patients were from arm B (^{68}Ga-DOTA-LM3). No other AEs were reported.

Biodistribution in Normal Organs

Figure 2 shows the biodistribution of ^{68}Ga-NODAGA-LM3 at 5, 15, 30, 45, 60, and 120 minutes post-injection in a patient with pancreatic NET. Figure 3 shows the biodistribution of ^{68}Ga-DOTA-LM3 in another patient with pancreatic NET. Significantly lower organ uptake was observed in the pituitary, parotids, liver, spleen, pancreas, adrenal, stomach, small intestine, and kidneys with ^{68}Ga-DOTA-LM3 compared to ^{68}Ga-NODAGA-LM3. The biodistribution in normal organs is summarized in Figure 4. The SUVmax at 1 hour post-injection is compared in Table 2.

Tumor Uptake

A total of 38 lesions were analyzed in 14 patients, including 18 lesions (13 hepatic, 2 pancreatic, 1 lymph node, 1 bone, and 1 stomach) on ^{68}Ga-NODAGA-LM3 and 20 lesions (12 hepatic, 4 pancreatic, 2 lymph node, 1 bone, and 1 brain) on ^{68}Ga-DOTA-LM3. With ^{68}Ga-NODAGA-LM3, the tracer accumulation in tumor lesions increased by 138%, from an average SUVmax of 31.3 ± 19.7 at 5 minutes to 74.6 ± 56.3 at 2 h. With ^{68}Ga-DOTA-LM3, the tumor uptake rapidly reached a high level at 5 minutes after injection, with an average SUVmax of 36.6 ± 23.6, and continued to increase to 45.3 ± 29.3 until 30 minutes post-injection, remaining at

plateau thereafter. The SUVmax and TBRs are summarized in Figure 5 (data available in supplemental Table 2). Due to the relatively lower kidney and liver background, ^{68}Ga-DOTA-LM3 showed significantly higher tumor-to-kidney and tumor-to-liver ratios than ^{68}Ga-NODAGA-LM3 at all time points. There were no significant differences in SUVmax or TBRs between hepatic and extrahepatic lesions in either arm ($P > 0.05$).

Radiation Dosimetry

The residence time of source organs and absorbed dose of target organs are summarized in Table 3 and 4, respectively. Urinary bladder wall received the highest radiation dose, 0.162 mGy/MBq for ^{68}Ga-NODAGA-LM3 and 0.202 mGy/MBq for ^{68}Ga-DOTA-LM3. Patients with fulminant liver diseases showed higher liver residence time and absorbed doses than those without fulminant liver diseases (Supplemental Table 3). The effective dose was 0.026 ± 0.003 mSv/MBq for ^{68}Ga-NODAGA-LM3 and 0.025 ± 0.002 mSv/MBq for ^{68}Ga-DOTA-LM3.

DISCUSSION

Antagonists ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 show high SSTR2 affinities in both in vitro and in vivo preclinical studies. To our knowledge, this is the first clinical study to evaluate these two tracers in patients with NETs. The results show favorable biodistribution and dosimetry features and both tracers were well tolerated in most patients.

One important finding of our study is the high tumor accumulation of ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3. Both tracers showed high tumor uptakes with the highest SUVmax up to 231.9 on ^{68}Ga-NODAGA-LM3 and 126.9 on ^{68}Ga-DOTA-LM3. The average SUVmax at 1 hour post-injection was 57.5 ± 39.4 for ^{68}Ga-NODAGA-LM3 and 47.2 ± 32.6 for ^{68}Ga-DOTA-LM3, which is certainly comparable to SSTR agonists and other SSTR2 antagonists (*8,14-18*). Furthermore, both tracers show excellent tumor retention. Our data agrees with the previous finding that radioantagonists show long tumor retention despite few internalization (*6,10*). High tumor retention is a key feature for peptide receptor radionuclide therapy. Richard P. Baum et al provided preliminary evidence of efficacy using ^{177}Lu-DOTA-LM3 treatment in a patient with ^{68}Ga-DOTATOC-negative liver metastases (*11,12*). The patient was in nearly complete remission after 3

cycles of intra-arterial peptide receptor radionuclide therapy, with a total of 20.4 GBq ^{177}Lu-DOTA-LM3. Our finding suggests that antagonist LM3 may be another available peptide for peptide receptor radionuclide therapy in the future and both ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 could be promising diagnostic companions.

The biodistribution of ^{68}Ga-NODAGA-LM3 is similar to that of SSTR2 agonists where we can see moderate or high uptake in SSTR2-positive organs (*19*). The highest organ SUVmax (except for kidneys and urinary bladder) was observed in spleen, followed by adrenal and pituitary glands. ^{68}Ga-DOTA-LM3, on the other hand, shows minimal uptake in almost all organs apart from the urinary tract. Only liver and spleen show slightly higher ^{68}Ga-DOTA-LM3 accumulation than the blood pool, while all other organs show either comparable or lower uptake. Interestingly, the differences in organ uptake between these two tracers can not only be observed in SSTR2 positive organs, such as pituitary, spleen, and adrenals, but also in liver, reputed to be a SSTR2 negative organ. The lower background of ^{68}Ga-DOTA-LM3 was further translated into significantly higher tumor-to-kidney and tumor-to-liver ratio. The reason for the differences in organ uptake is currently not well understood and requires further studies. SSTR2 antagonists are sensitive to chelator appended. With different chelators attached, a previous study has shown a 10-fold higher SSTR2 affinity of ^{68}Ga-NODAGA-LM3 (1.3 nmol/L) than ^{68}Ga-DOTA-LM3 (12.5 nmol/L) (*10*). Our study suggests that the chelators not only affect the tumor uptake and retention but also biodistribution in normal organs. It should be noted, however, that our data is partially in contrast to that published by Fani et al (*10*). They found a 65% higher kidney uptake of ^{68}Ga-DOTA-LM3 compared to ^{68}Ga-NODAGA-LM3 (32.50 %ID/g versus 19.68 %ID/g) in animal models, while our study showed a 72% lower kidney uptake of ^{68}Ga-DOTA-LM3 compared with ^{68}Ga-NODAGA-LM3 (SUVmax 5.1 versus 17.9). The differences may result from different species and peptide amount used.

The dosimetry data of ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 showed slightly higher yet comparable effective dose compared to that of other SSTR2 antagonists (0.024 mSv/MBq for ^{68}Ga-NODAGA-JR11 and 0.022 mSv/MBq for ^{68}Ga-DOTA-JR11) (*18,20*). We also observed a higher liver dose of ^{68}Ga-DOTA-LM3 despite lower liver background. This is mainly attributed to dosimetry methodology. We used whole organ volumes of interest to calculate the whole organ activity (including disease activity). In our study, several

patients (patient #1 in arm A, and patient #2, #7, 16# in arm B) had fulminant hepatic metastases (Supplemental Figure 1), which led to much higher liver residence time and absorbed dose than other patients (Supplemental Table 3). It explains the higher liver dose of ^{68}Ga-DOTA-LM3 despite lower liver background. It is also responsible for comparable effective dose between these two tracers in spite of faster wash-out of ^{68}Ga-DOTA-LM3.

The administration of ^{68}Ga-NODAGA-LM3 was well tolerated in all patients in arm A. However, two patients with functional pancreatic NET (insulinoma) in arm B reported AEs at 10-15 minutes after ^{68}Ga-DOTA-LM3 injection. Both patients experienced nausea (G2) and one of them had vomiting (G1). These two AEs were considered to be related to ^{68}Ga-DOTA-LM3 injection. In a previous study investigating the safety of another antagonist ^{68}Ga-NODAGA-JR11 (^{68}Ga-OPS202), no pharmacologic response to radiopharmaceutical was reported (*20*). However, Simone Krebs et al. reported potential SSTR2 antagonistic properties of ^{68}Ga-DOTA-JR11 (*18*). In their study, two patients with functional NETs (the type of tumor was not specified) experienced symptoms such as flushing, hypotension (G3), nausea, and lightheadedness. Our study suggests that administration of SSTR2 antagonist might trigger side effects such as nausea and vomiting. However, due to the limited number of patients recruited, it is too early to tell whether it is related to tumor functional status, antagonist peptides, or chelating agents. In fact, nausea and vomiting are common side effects of injecting somatostatin analogues. Patients with neuroendocrine tumors, functional or nonfunctional, could have nausea and vomiting after administration of a therapeutic dose of Sandostatin. The AEs observed in our study may be related to quick bolus administration. Slow bolus injection or infusion might help to relieve the symptom. Though the two patients with AEs in our study discontinued the scan because they were not able to lay still on the exam table for the first hour, the AEs are usually mild and won't affect image acquisition at 60 min post-injection.

The blood pressure measured at 24 h post-injection was significantly lower than baseline (127.5/82.4 mmHg versus 133.6/86.3 mmHg, $P<0.05$). However, this finding was not translated into any clinically relevant events. Blood pressure change is not a specific finding and may be influenced by many conditions like patients' emotional status, body temperature, exercise, and caffeine consumption (*21*). Given the low peptide dose (40

μg) used in our study and fast clearance of radiopharmaceuticals (median biological half life of 5.18 hours), the change in blood pressure at 24 hours post-injection was probably not related to antagonist injection.

Our study was limited by the small patient number, which is typical for dosimetry evaluation of radiopharmaceuticals. Besides, neither blood or urine samples were collected in our study. The blood and urine samples could allow us to search for metabolites. Lastly, the ideal comparison between ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 should be conducted in the same group of patients. Further head-to-head comparison study is required.

CONCLUSION

Both ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 show favorable biodistribution, high tumor uptake and good tumor retention, resulting in high image contrast. The dosimetry data is comparable to other ^{68}Ga-labeled SSTR2 antagonists. Further studies are required to look into the potential antagonistic effects of ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3.

DISCLOSURE

This work was sponsored in part by the CAMS Initiative for Innovative Medicine (2017-I2M-4-002, 2018-I2M-3-001) and Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2019PT310026). No other potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

We want to thank Prof. Richard P.Baum for inspiring us on the LM3 project. We also thank all the patients who participated in this study, Yue Zhang (SinoUnion Healthcare Inc., China) for image acquisition, and Dr. Chengyan Dong (GE Healthcare, China) for critical proofreading and figure suggestions.

KEY POINTS

QUESTION: Do ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 show suitable biodistribution and dosimetry data in NET and are they safe?

PERTINENT FINDINGS: Both ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 show favorable biodistribution, high tumor uptake and good tumor retention. Few AEs were reported using ^{68}Ga-DOTA-LM3.

IMPLICATIONS FOR PATIENT CARE: Both ^{68}Ga-NODAGA-LM3 and ^{68}Ga-DOTA-LM3 are promising in NET imaging.

REFERENCES

1. Virgolini I, Ambrosini V, Bomanji JB, et al. Procedure guidelines for PET/CT tumour imaging with 68Ga-DOTA-conjugated peptides: 68Ga-DOTA-TOC, 68Ga-DOTA-NOC, 68Ga-DOTA-TATE. *Eur J Nucl Med Mol Imaging.* 2010;37:2004-2010.

2. Reubi JC, Waser B, Cescato R, Gloor B, Stettler C, Christ E. Internalized somatostatin receptor subtype 2 in neuroendocrine tumors of octreotide-treated patients. *J Clin Endocrinol Metab.* 2010;95:2343-2350.

3. Fani M, Nicolas GP, Wild D. Somatostatin receptor antagonists for imaging and therapy. *J Nucl Med.* 2017;58:61S-66S.

4. Ginj M, Zhang H, Waser B, et al. Radiolabeled somatostatin receptor antagonists are preferable to agonists for in vivo peptide receptor targeting of tumors. *Proc Natl Acad Sci U S A.* 2006;103:16436-16441.

5. Wild D, Fani M, Behe M, et al. First clinical evidence that imaging with somatostatin receptor antagonists is feasible. *J Nucl Med.* 2011;52:1412-1417.

6. Nicolas GP, Mansi R, McDougall L, et al. Biodistribution, pharmacokinetics, and dosimetry of (177)Lu-, (90)Y-, and (111)In-labeled somatostatin receptor antagonist OPS201 in comparison to the agonist (177)Lu-DOTATATE: the mass effect. *J Nucl Med.* 2017;58:1435-1441.

7. Dalm SU, Nonnekens J, Doeswijk GN, et al. Comparison of the therapeutic response to treatment with a 177Lu-labeled somatostatin receptor agonist and antagonist in preclinical models. *J Nucl Med.* 2016;57:260-265.

8. Nicolas GP, Schreiter N, Kaul F, et al. Sensitivity comparison of (68)Ga-OPS202 and (68)Ga-DOTATOC PET/CT in patients with gastroenteropancreatic neuroendocrine tumors: a prospective phase II imaging study. *J Nucl Med.* 2018;59:915-921.

9. Zhu W, Cheng Y, Wang X, et al. Head-to-head comparison of (68)Ga-DOTA-JR11 and (68)Ga-DOTATATE PET/CT in patients with metastatic, well-differentiated neuroendocrine tumors: a prospective study. *J Nucl Med.* 2020;61:897-903.

10. Fani M, Del Pozzo L, Abiraj K, et al. PET of somatostatin receptor-positive tumors using 64Cu- and 68Ga-somatostatin antagonists: the chelate makes the difference. *J Nucl Med.* 2011;52:1110-1118.

11. Zhang J, Kulkarni HR, Singh A, Baum RP. Successful intra-arterial peptide receptor radionuclide therapy of DOTATOC-negative high-grade liver metastases of a pancreatic neuroendocrine neoplasm using 177Lu-DOTA-LM3. *Clin Nucl Med.* 2020;45:e165-e168.

12. Zhang J, Singh A, Kulkarni HR, et al. From bench to bedside-The Bad Berka experience with first-in-human studies. *Semin Nucl Med.* 2019;49:422-437.

13. Basic anatomical and physiological data for use in radiological protection: reference values. A report of age- and gender-related differences in the anatomical and physiological characteristics of reference individuals. ICRP Publication 89. *Ann ICRP.* 2002;32:5-265.

14. Aalbersberg EA, de Wit-van der Veen BJ, Versleijen MWJ, et al. Influence of lanreotide on uptake of (68)Ga-DOTATATE in patients with neuroendocrine tumours: a prospective intra-patient evaluation. *Eur J Nucl Med Mol Imaging.* 2019;46:696-703.

15. Wild D, Bomanji JB, Benkert P, et al. Comparison of 68Ga-DOTANOC and 68Ga-DOTATATE PET/CT within patients with gastroenteropancreatic neuroendocrine tumors. *J Nucl Med.* 2013;54:364-372.

16. Brogsitter C, Zophel K, Hartmann H, Schottelius M, Wester HJ, Kotzerke J. Twins in spirit part II: DOTATATE and high-affinity DOTATATE--the clinical experience. *Eur J Nucl Med Mol Imaging.* 2014;41:1158-1165.

17. Velikyan I, Sundin A, Sorensen J, et al. Quantitative and qualitative intrapatient comparison of 68Ga-DOTATOC and 68Ga-DOTATATE: net uptake rate for accurate quantification. *J Nucl Med.* 2014;55:204-210.

18. Krebs S, Pandit-Taskar N, Reidy D, et al. Biodistribution and radiation dose estimates for (68)Ga-DOTA-JR11 in patients with metastatic neuroendocrine tumors. *Eur J Nucl Med Mol Imaging.* 2019;46:677-685.

19. Moradi F, Jamali M, Barkhodari A, et al. Spectrum of 68Ga-DOTA TATE uptake in patients with neuroendocrine tumors. *Clin Nucl Med.* 2016;41:e281-287.

20. Nicolas GP, Beykan S, Bouterfa H, et al. Safety, biodistribution, and radiation dosimetry of (68)Ga-OPS202 in patients with gastroenteropancreatic neuroendocrine tumors: a prospective phase I imaging study. *J Nucl Med.* 2018;59:909-914.

21. Jin J. Checking blood pressure at home. *Jama.* 2017;318:310.

Table 1. Demographic and clinical characteristics of patients

Patients*	Arm†	Age	Gender	Grade	Ki67	Primary Tumor	Tumor function
1	A	39	Female	2	5	Unknown	Yes(gastrinoma)
2	B	64	Male	2	20	Pancreas	No
3	B	69	Male	1	2	Pancreas	Yes(insulinoma)
4	B	58	Male	1	1	Lung	No
5	A	69	Male	2	3	Pancreas	No
6	A	58	Male	2	10	Pancreas	No
7	B	37	Female	2	3	Pancreas	No
8	B	33	Female	3	30	Pancreas	Yes(insulinoma)
9	A	33	Male	2	3	Stomach	No
10	A	18	Female	3	30	Pancreas	Yes(gastrinoma)
11	A	58	Female	2	5	Rectus	No
12	B	38	Female	1	1	Pancreas	No
13	A	54	Female	3	40	Pancreas	No
14	A	57	Female	2	10	Small intestine	No
15	B	40	Male	3	25	Pancreas	No
16	B	48	Male	2	5	Rectus	No

* Patients were numbered according to the recruiting sequence.

†Arm A: ^{68}Ga-NODAGA-LM3. Arm B: ^{68}Ga-DOTA-LM3.

Table 2. The uptake of normal organs at 1 hour post-injection

Organs	SUVmax		P value
	^{68}Ga-NODAGA-LM3	^{68}Ga-DOTA-LM3	
Pituitary	9.6 ± 3.5	1.5 ± 1.0	< 0.001
Parotids	2.4 ± 0.9	1.3 ± 0.3	0.012
Thyroids	1.9 ± 0.6	1.3 ± 0.4	0.072
Lungs	1.0 ± 0.3	0.8 ± 0.2	0.062
Blood pool	1.3 ± 0.5	1.3 ± 0.4	0.973
Liver	6.4 ± 1.8	2.5 ± 0.7	< 0.001
Spleen	17.5 ± 7.7	2.6 ± 0.8	0.012
Pancreas	3.7 ± 1.6	0.8 ± 0.5	0.005
Adrenal	11.2 ± 4.8	1.9 ± 0.6	0.001
Stomach	3.0 ± 0.9	1.3 ± 0.9	0.005
Small intestine	3.2 ± 0.7	1.2 ± 0.4	< 0.001
Kidneys	17.9 ± 2.7	5.1 ± 1.9	< 0.001

Table 3. Residence time in source organs

Source organs	Residence time (h)	
	^{68}Ga-NODAGA-LM3	^{68}Ga-DOTA-LM3
Kidneys	0.097 ± 0.025	0.025 ± 0.008
Red marrow	0.041 ± 0.013	0.029 ± 0.017
Liver	0.194 ± 0.105	0.357 ± 0.278
Spleen	0.079 ± 0.059*	0.011 ± 0.008
Urinary bladder	0.132 ± 0.038	0.168 ± 0.062
Remainder body	1.105 ± 0.089	1.004 ± 0.196
Whole body	1.63	1.63

* n=5. Splenectomy in three patients.

Table 4. Absorbed doses to target organs and effective dose.

Target organs	Organ doses (mGy/MBq)	
	^{68}Ga-NODAGA-LM3	^{68}Ga-DOTA-LM3
Adrenals	0.014 ± 0.001	0.014 ± 0.001
Brain	0.009 ± 0.001	0.008 ± 0.002
Breasts	0.009 ± 0.000	0.009 ± 0.001
Gallbladder wall	0.015 ± 0.001	0.017 ± 0.004
Lower large intestine wall	0.012 ± 0.001	0.012 ± 0.002
Small intestine	0.012 ± 0.001	0.013 ± 0.003
Stomach wall	0.012 ± 0.000	0.011 ± 0.001
Upper large intestine wall	0.012 ± 0.000	0.012 ± 0.001
Heart wall	0.011 ± 0.000	0.011 ± 0.001
Kidneys	0.136 ± 0.061	0.064 ± 0.052
Liver	0.056 ± 0.028	0.098 ± 0.075
Lungs	0.011 ± 0.000	0.010 ± 0.001
Muscle	0.010 ± 0.000	0.010 ± 0.001
Ovaries	0.013 ± 0.001	0.012 ± 0.002
Pancreas	0.015 ± 0.002	0.013 ± 0.000
Red marrow	0.016 ± 0.003	0.013 ± 0.003
Osteogenic cells	0.019 ± 0.002	0.016 ± 0.003
Skin	0.009 ± 0.000	0.008 ± 0.001
Testes	0.010 ± 0.001	0.010 ± 0.002
Spleen	0.132 ± 0.151	0.034 ± 0.022
Thymus	0.010 ± 0.001	0.010 ± 0.001
Thyroid	0.010 ± 0.001	0.009 ± 0.001
Urinary bladder wall	0.162 ± 0.045	0.202 ± 0.073
Uterus	0.015 ± 0.002	0.015 ± 0.003
Total body	0.013 ± 0.000	0.013 ± 0.000
Effective dose (mSv/MBq)	0.026 ± 0.002	0.025 ± 0.002

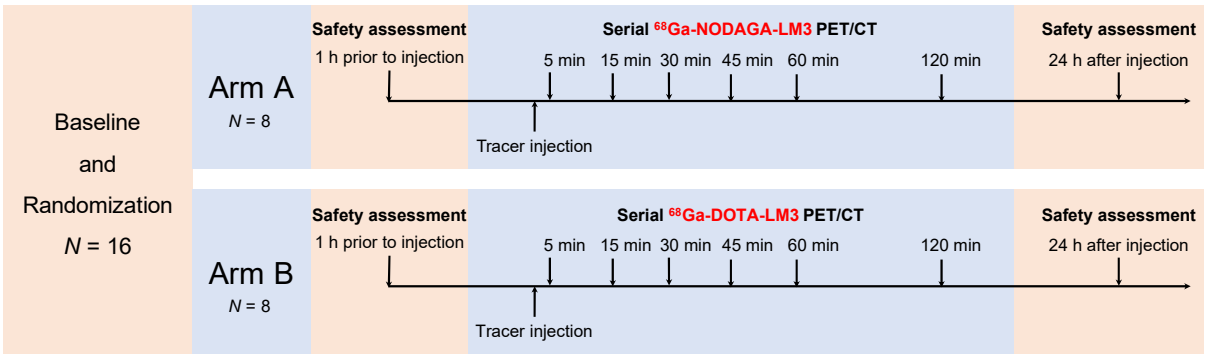


Figure 1. The diagram of study design. Sixteen patients were prospectively recruited in this study and equally randomized into two arms. Arm A, eight patients underwent serial PET/CT scans at 5, 15, 30, 45, 60, and 120 minutes after ^{68}Ga-NODAGA-LM3 injection. Arm B, eight patients (anticipated) underwent serial PET/CT scans at 5, 15, 30, 45, 60, and 120 minutes after ^{68}Ga-DOTA-LM3 injection. Two patients from Arm B withdrew from the study due to adverse events.

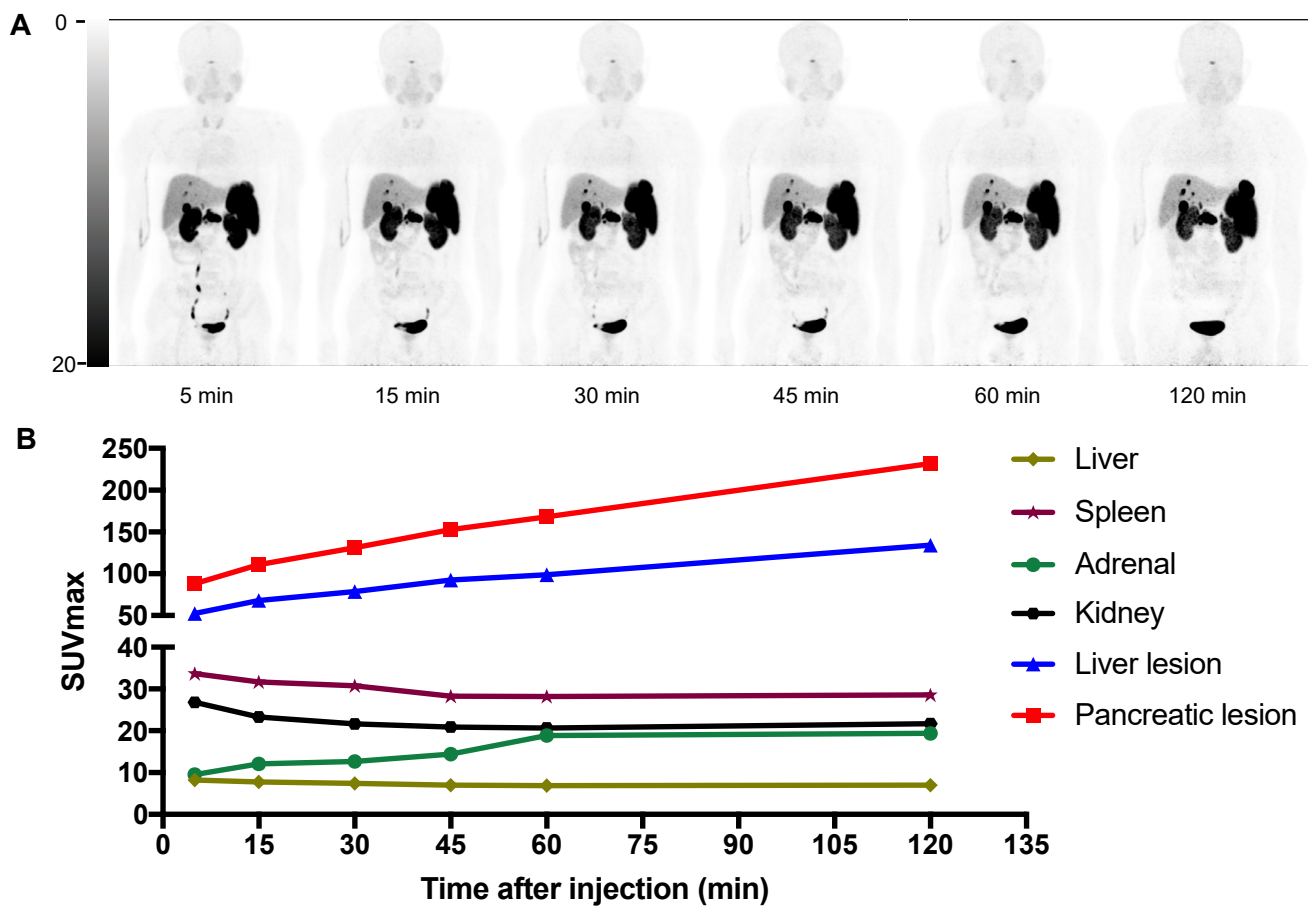


Figure 2. Patient #6 with grade 2 pancreatic NET as well as multiple hepatic and lymph node metastases. A. Biodistribution of ^{68}Ga-NODAGA-LM3 at 5, 15, 30, 45, 60, and 120 minutes post-injection. Physiological uptake could be visualized in somatostatin receptor positive organs such as pituitary, adrenals, and spleen. Liver demonstrated moderate accumulation of ^{68}Ga-NODAGA-LM3. B. SUVmax-time curves showed an excellent tumor retention in both pancreatic tumor and hepatic metastases.

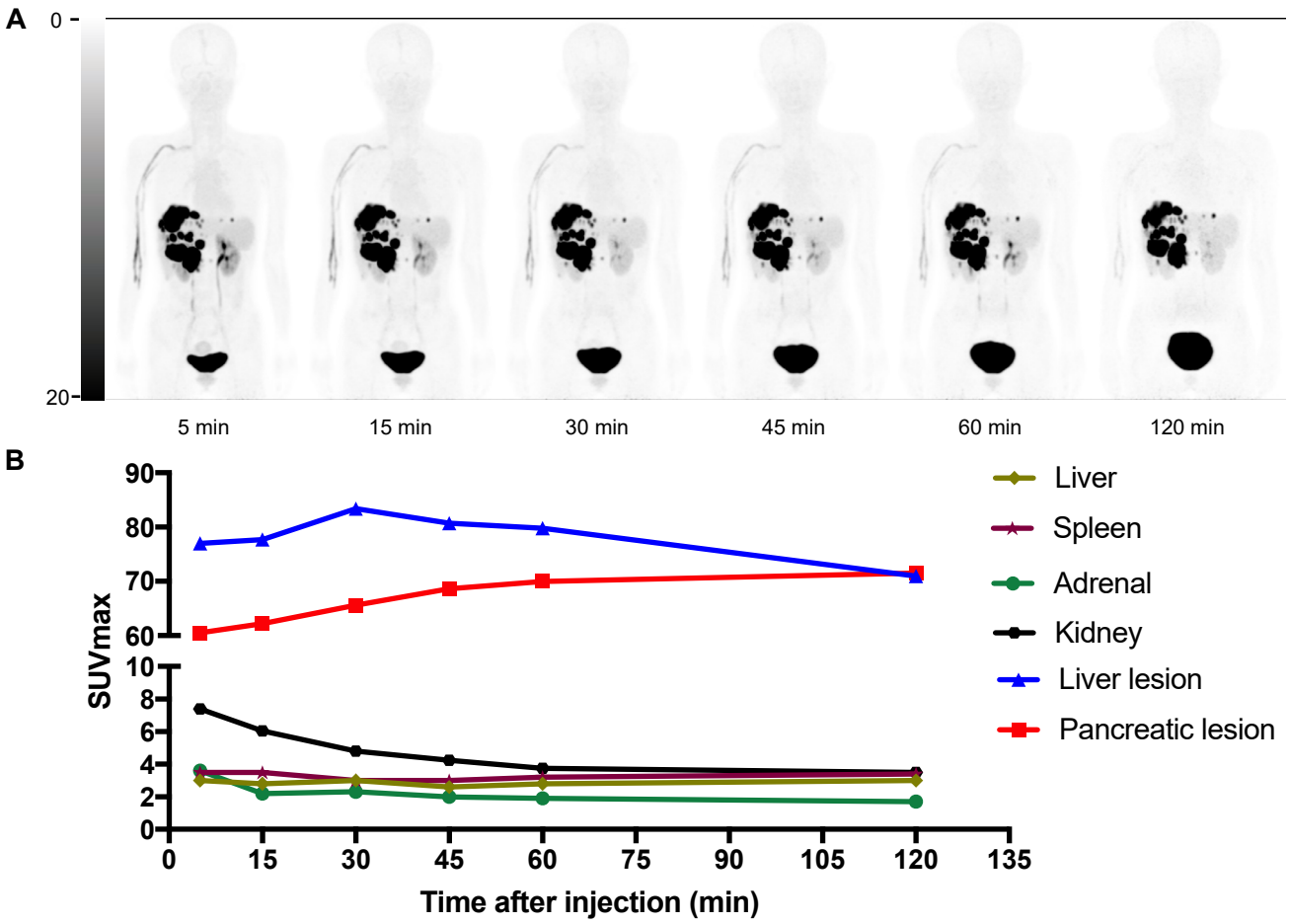


Figure 3. Patient #12 with grade 1 pancreatic NET as well as multiple hepatic and lymph node metastases. A. Biodistribution of ^{68}Ga-DOTA-LM3 at 5, 15, 30, 45, 60, and 120 minutes post-injection. No significant uptake is noted in any normal organs except for urinary tracts. Spleen demonstrated only mild ^{68}Ga-DOTA-LM3 accumulation. B. SUVmax-time curves. The SUVmax of normal organs remained at a low level after ^{68}Ga-DOTA-LM3 administration. Both the primary and metastatic lesions showed good tracer accumulation, leading to high image contrast.

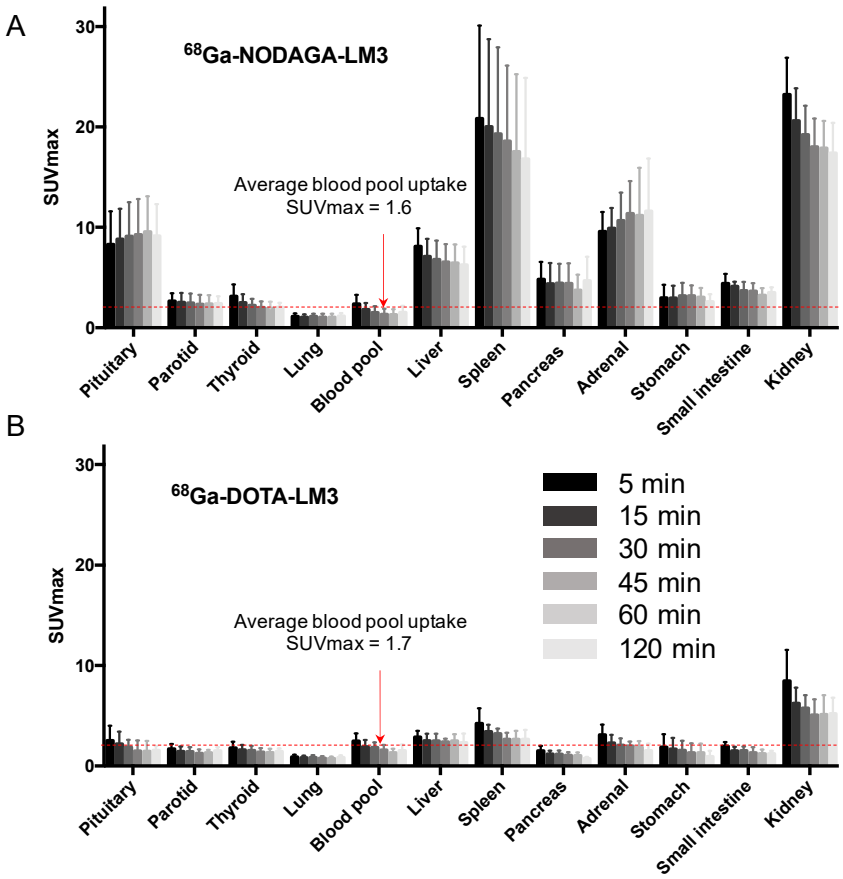


Figure 4. The biodistribution of ^{68}Ga-NODAGA-LM3 (A) and ^{68}Ga-DOTA-LM3 (B) in normal organs at 5, 15, 30, 45, 60, and 120 minutes post-injection.

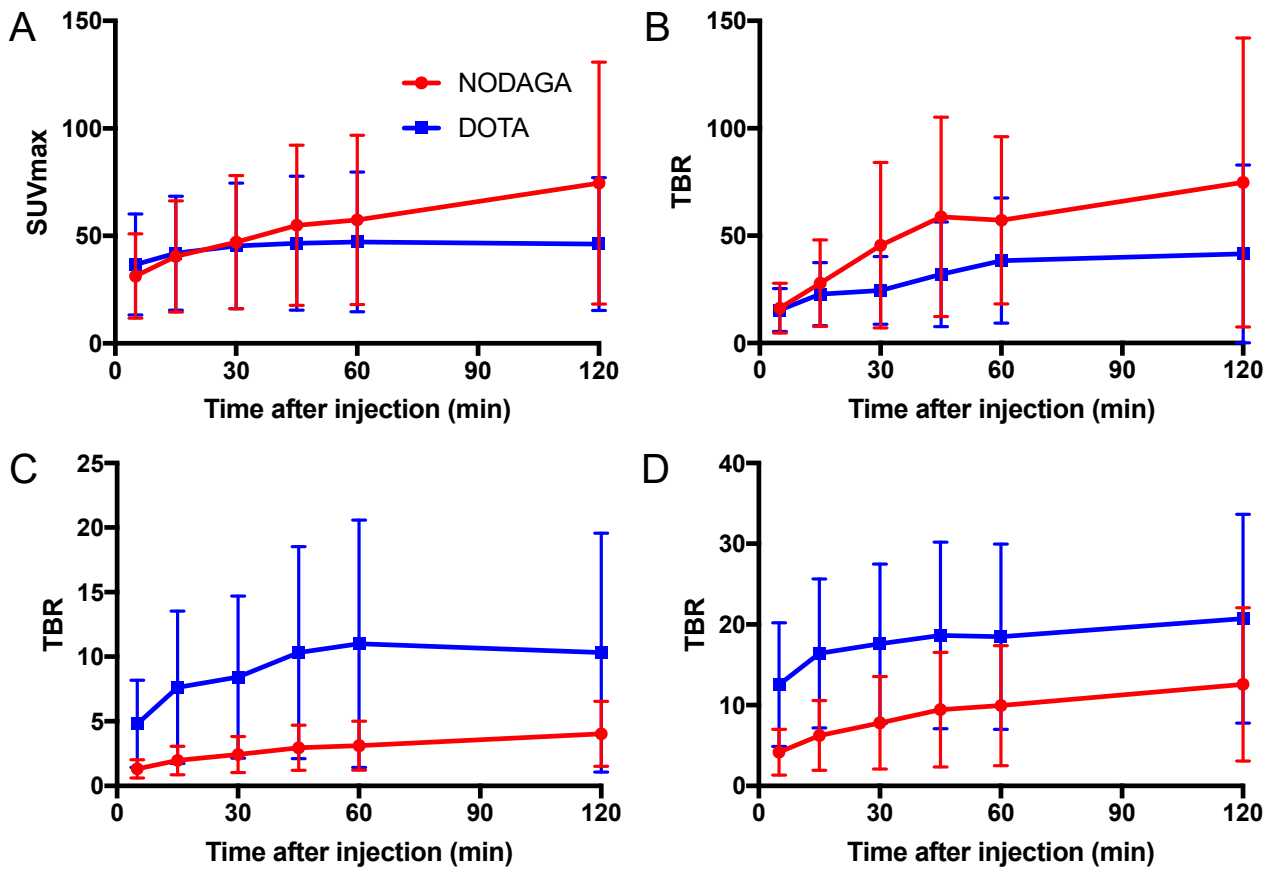
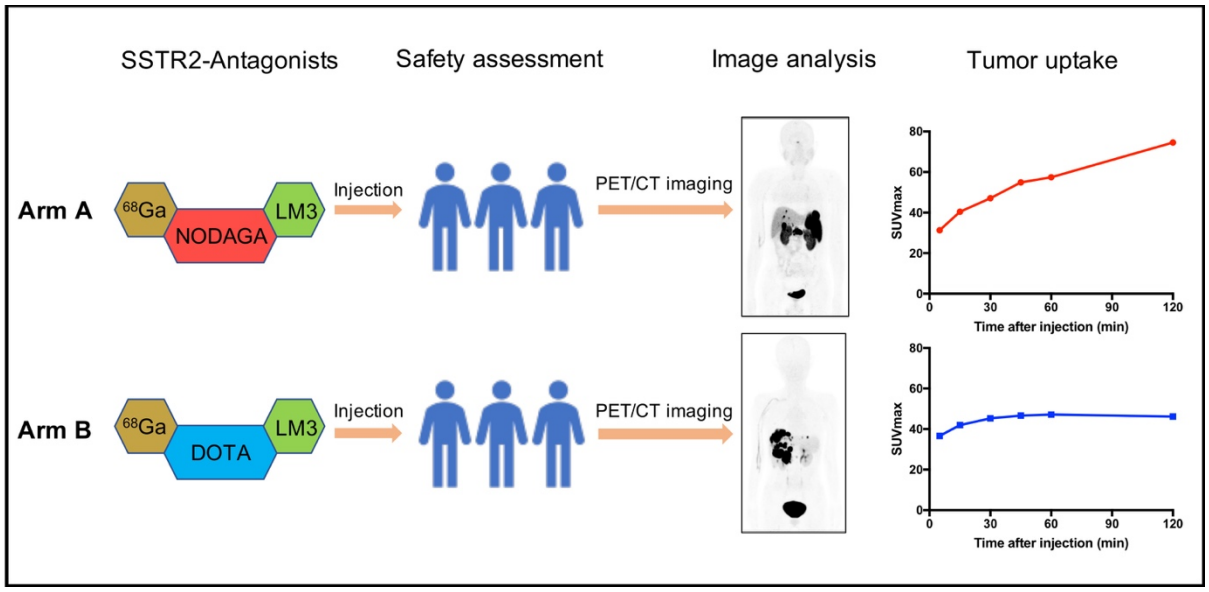
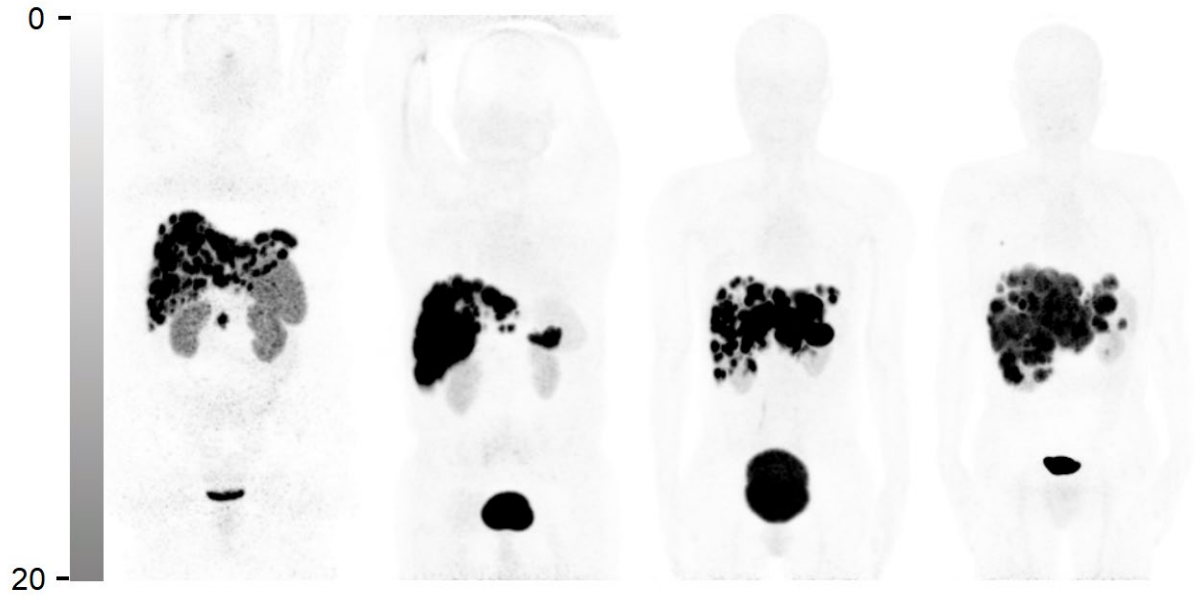


Figure 5. The SUVmax (A), tumor-to-blood-pool ratio (B), tumor-to-kidney ratio (C), and tumor-to-liver ratio (D) of 38 reference lesions, including 18 lesions on ^{68}Ga-NODAGA-LM3 PET and 20 lesions on ^{68}Ga-DOTA-LM3 PET. TBR: tumor-to-background ratio.



Graphical Abstract



Supplemental Figure 1. Four patients (from left to right: patient #1 in arm A, and patient #2, #7, #16 in arm B) with fulminant hepatic metastases.

Supplemental Table 1. Inclusion and exclusion criteria

Inclusion Criteria
• Written informed consent. • Patients of either gender, aged ⩾ 18 years. • Histologically confirmed diagnosis of Metastatic, well-differentiated neuroendocrine tumor. • A diagnostic computed tomography (CT) or magnetic resonance imaging (MRI) of the tumor region within the previous 6 months prior to dosing day is available. • At least 1 measurable lesion based on RECIST v1.1. • Blood test results as follows (White blood cell: ⩾ 3*10^9/L, Hemoglobin: ⩾ 8.0 g/dL, Platelets: ⩾ 50x10^9/L, Alanine aminotransferase / Aspartate aminotransferase / Alkaline phosphatase: ⩽ 5 times upper limit of normal (ULN), Bilirubin: ⩽ 3 times ULN) • Serum creatinine: within normal limits or < 120 μmol/L for patients aged 60 years or older. • Calculated Glomerular filtration rate (GFR) ⩾ 45 mL/min.
Exclusion Criteria
• Known hypersensitivity to Gallium-68, to NODAGA, to DOTA, to LM3, or to any of the excipients of Gallium-68 DOTA-LM3 or Gallium-68 NODAGA-LM3. • Presence of active infection at screening or history of serious infection within the previous 6 weeks. • Therapeutic use of any somatostatin analog, including long-acting Sandostatin (within 28 days) and short-acting Sandostatin (within 2 days) prior to study imaging. If a patient is on long-acting Sandostatin, then a wash-out phase of 28 days is required before the injection of the study drug. If a patient is on short-acting Sandostatin, then a wash-out phase of 2 days is required before the injection of the study drug. • Prior or planned administration of a radiopharmaceutical within 8 half-lives of the radionuclide used on such radiopharmaceutical including at any time during the current study. • Pregnant or breast-feeding women. • Current history of any malignancy other than neuroendocrine tumor; patients with a secondary tumor in remission of > 5 years can be included. • Any mental condition rendering the patient unable to understand the nature, scope and possible consequences of the study, and/or evidence of an uncooperative attitude.

Supplemental Table 2. The SUVmax and tumor-to-background ratios of 38 reference lesions

Time after injection (minutes)	^{68}Ga-NODAGA-LM3 (N = 18)	^{68}Ga-DOTA-LM3 (N = 20)	P value
	SUVmax		
5	31.3 ± 19.7	36.6 ± 23.6	0.455
15	40.4 ± 25.9	41.9 ± 26.5	0.860
30	47.1 ± 31.0	45.3 ± 29.3	0.858
45	54.9 ± 37.3	46.6 ± 31.2	0.461
60	57.5 ± 39.4	47.2 ± 32.6	0.385
120	74.6 ± 56.3	46.1 ± 30.9	0.058
	Tumor-to-blood-pool ratio		
5	16.4 ± 11.7	15.5 ± 10.0	0.803
15	28.0 ± 20.1	22.8 ± 14.7	0.366
30	45.5 ± 38.6	24.6 ± 15.8	0.044
45	58.8 ± 46.4	32.0 ± 24.4	0.038
60	57.1 ± 38.9	38.4 ± 29.1	0.099
120	74.8 ± 67.2	41.5 ± 41.4	0.071
	Tumor-to-kidney ratio		
5	1.3 ± 0.7	4.8 ± 3.4	<0.001
15	2.0 ± 1.1	7.6 ± 5.9	<0.001
30	2.4 ± 1.4	8.4 ± 6.3	<0.001
45	3.0 ± 1.8	10.3 ± 8.2	0.001
60	3.1 ± 1.9	11.0 ± 9.6	0.002
120	4.0 ± 2.5	10.3 ± 9.2	0.008
	Tumor-to-liver ratio		
5	4.2 ± 2.8	12.5 ± 7.7	<0.001
15	6.2 ± 4.3	16.4 ± 9.2	<0.001
30	7.8 ± 5.8	17.6 ± 9.9	0.001
45	9.4 ± 7.1	18.6 ± 11.6	0.006
60	10.0 ± 7.4	18.5 ± 11.5	0.011
120	12.6 ± 9.5	20.7 ± 12.9	0.035

Supplemental Table 3. Residence times and absorbed doses of liver.

	Patient #*	Arm	Residence time (h)	Absorbed doses (mGy/MBq)
Patients with fulminant liver diseases	1	A	0.448	0.124
	2	B	0.536	0.147
	7	B	0.546	0.150
	16	B	0.719	0.196
Patients without fulminant liver diseases	4	B	0.092	0.028
	5	A	0.144	0.042
	6	A	0.172	0.050
	9	A	0.160	0.046
	10	A	0.165	0.048
	11	A	0.110	0.033
	12	B	0.198	0.052
	13	A	0.169	0.049
	14	A	0.181	0.052
	15	B	0.053	0.017

* Patient #3 and #8 were dropped out.