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Application of ^{68}Ga-FXX489 (NNS309) Imaging in the Diagnosis of Tumour Diseases

I. Basic Project Information:

Self-selected research topic, prospective study, project duration: 1 August 2025 to 31 July 2027.

II. Research Background:

Tumours represent a major threat to human health. In the management of cancer patients, 18F-FDG PET/CT serves as a valuable imaging modality, though certain limitations warrant attention. Firstly, 18F-FDG PET/CT occasionally demonstrates reduced sensitivity in detecting primary tumour lesions, owing to significant variations in 18F-FDG uptake across different tumour histological types. Secondly, its low to moderate sensitivity in lymph node staging limits its utility for tumour staging and surgical planning. Furthermore, 18F-FDG PET occasionally demonstrates reduced sensitivity for primary or metastatic tumours in organs such as the liver and brain. Consequently, developing an effective PET tracer holds promise for enhancing tumour detection and facilitating personalised patient care. Fibroblast activation protein is overexpressed in tumour-associated fibroblasts, which constitute the primary component of the epithelial tumour stroma. Consequently, a novel radiotracer, ^{68}Ga-FXX489 (NNS309), has been developed to target fibroblast activation protein and visualise the tumour stroma. It exhibits favourable in vivo pharmacokinetics and biodistribution, enabling clear delineation of both primary tumours and their metastases. Phase I studies of [^{68}Ga]Ga-FXX489 (NNS309) and [^{177}Lu]Lu-FXX489 (NNS309) are currently underway in patients with pancreatic, lung, breast, and colorectal cancers. Units undertaking the clinical application of the ^{68}Ga-FXX489 (NNS309) imaging agent must possess a Class IV licence for the use of radiopharmaceuticals. Our department currently holds the requisite qualifications to

undertake this work and is positioned to pioneer the implementation of this technology.

III. Research Objectives:

The objective of this study is to investigate the diagnostic value of ^{68}Ga-FXX489 (NNS309) imaging in oncological diseases.

IV. Research Tools

General Information Questionnaire: A questionnaire for collecting general demographic data on study subjects was designed independently through literature review. It included: patient age, gender, educational attainment, marital status, occupation, and current medical history.V. Study Population

Prospective collection of case records from 80 patients newly diagnosed with or treated for oncological conditions at the First Affiliated Hospital of China Medical University between 1 August 2025 and 31 July 2027, who underwent ^{68}Ga-FXX489 (NNS309) and ^{68}Ga-FAP-2286 PET/CT examinations.

Inclusion Criteria: 1-2 are mandatory requirements

1. Patients with a high suspicion of neoplastic disease;
2. Age: 18-80 years, no gender restrictions, able to express themselves independently, willing to participate in this study and sign the informed consent form.

Exclusion Criteria: 1-6 are mandatory exclusion requirements

1. Failure to sign the informed consent form;
2. Patients with severe visual or hearing impairment, cognitive impairment, or claustrophobia unable to communicate effectively;
3. Severe cardiac insufficiency, NYHA class III–IV;
4. Renal failure (serum creatinine > 1.2 mg/dl);
5. Individuals with alcohol allergy;
6. Use of medications within one week prior to examination that may induce a

disulfiram-like reaction with alcohol, such as penicillins, cephalosporins, or cephamycins.

Exclusion criteria:

1. Participants unable to complete the examination and forced to discontinue the trial prematurely;
2. Subjects requesting withdrawal from the clinical trial;
3. Images rendered unusable for clinical diagnosis due to non-equipment-related factors such as subject movement during scanning or leakage of radiopharmaceutical.

VI. Clinical Research Instruments, Equipment and Premises

1. Siemens Biograph MCT PET/CT positron emission and X-ray computed tomography imaging equipment;
2. The research project was conducted in the Nuclear Medicine PET/CT Department of the First Affiliated Hospital of China Medical University.

VII. Clinical Research Methods

(I) Clinical Research Process

1. Prior to study commencement, subjects undergo preliminary screening to identify those meeting inclusion criteria and exclude ineligible candidates. Selected subjects sign informed consent forms, typically completed by the subject themselves. In exceptional circumstances, a designated proxy may sign on the subject's behalf.
2. The investigator recorded relevant clinical information (e.g., subject age, serum biomarkers, or tumour-related symptoms) and equipment operational status.
3. ^{68}Ga-FXX489 (NNS309) and ^{68}Ga-FAP-2286 were produced in-house by this department. Drug synthesis utilised GE's cyclotron, with radiochemical purity exceeding 95%.

The ^{68}Ga-FXX489 (NNS309) and ^{68}Ga-FAP-2286 PET/CT imaging equipment comprises Siemens Biograph MCT PET/CT positron emission and X-ray computed tomography systems. No specific preparations (such as fasting or normal blood

glucose levels) are required on the day of the PET/CT examination. Prior to the scan, administer ^{68}Ga-FXX489 (NNS309) intravenously at a dose of 1.8–2.2 MBq/kg. Following administration, instruct the patient to rest, drink plenty of fluids, and urinate frequently. [0.05–0.06 mCi]/kg intravenously. Following administration, patients are instructed to rest, drink ample fluids, urinate frequently, and empty their bladder prior to imaging. PET/CT scanning commences 60 minutes later with the patient raising both arms above their head, arms pressed against the ears, breathing calmly. The scan range extends from the top of the head to the groin. Low-dose CT: 120 kV, 120 mA·s scan. Matrix: 512×512. Slice thickness: 5 mm. PET/CT acquisition: 5–6 gantry positions, 2–3 minutes per position.

4. Reconstruction methods for ^{468}Ga-FXX489 (NNS309) and ^{68}Ga-FAP-2286 PET/CT imaging: TrueX+TOF (ultraHD-PET) with 3 iterations and 21 subsets. Image fusion was performed via the Xeleris workstation to generate coronal, sagittal, and transverse CT, PET, and PET/CT fusion images.

5. Both ^{68}Ga-FXX489 (NNS309) and ^{68}Ga-FAP-2286 images were randomly interpreted by physicians with over 10 years' experience in nuclear oncology. Disagreements were resolved by consensus. Lesions exhibiting uptake above the visually identifiable background were considered positive. Regions of interest were manually delineated for semi-quantitative analysis. The maximum standardised uptake value (SUVmax) was automatically calculated by the dominant workstation to quantify tracer uptake in primary tumours, involved lymph nodes, and distant metastases. SUVmax, median standard uptake value, and range were recorded.

6. Statistics and Analysis: All data analysis was performed using SPSS (version 22.0; IBM, Amonk, New York). Visual interpretation of PET/CT images was compared with histopathological findings (biopsy or surgical specimens) as the reference standard. The sensitivity, specificity, and accuracy of ^{68}Ga-FXX489 (NNS309) and ^{68}Ga-FAP-2286 PET/CT examinations were calculated, with diagnostic performance evaluated using the McNemar test. A two-tailed t-test with $P < 0.05$ was considered statistically significant.

(II) Quality Control in Clinical Research

Throughout this study, all participating personnel must undergo standardised training, employing uniform recording methods and assessment criteria. The entire clinical research process shall be conducted under strict operational protocols. Investigators shall complete the General Information Questionnaire truthfully, thoroughly, and conscientiously, ensuring all entries are complete, accurate, and reliable. All observations and findings within the research project shall be verified to guarantee data reliability, ensuring that conclusions are derived from original data. Corresponding data management measures shall be implemented during both the project execution and data processing phases.

VIII. Evaluation Methods and Statistical Analysis for Clinical Research

Evaluation of Equipment and Medications: Technicians and nurses operating the equipment and administering medications shall conduct assessments, with summary statistics compiled upon trial completion.

Image Quality Evaluation: Obtained images shall be assessed by physicians holding the title of Attending Physician or above within the Nuclear Medicine Department, employing a triple-blind evaluation approach.

Statistical Processing Methods: Statistical analysis shall be conducted using SPSS 22.0 statistical software, with receiver operating characteristic curves plotted for subjects.