Preparation of BAY 864367

The radiolabeling was performed by using an Eckert & Ziegler Module (Modular Lab, EN-039) and has been described previously (*1*). In brief, no-carrier-added ^{18}F-fluoride was produced via the ^{18}O(p,n)^{18}F nuclear reaction by irradiation of enriched ^{18}O-water in a fixed-energy Cyclone 18/9 cyclotron (IBA). ^{18}F-fluoride was immobilized on an anion-exchange cartridge (QMA Light; Waters) and eluted with a solution of Kryptofix K2.2.2. (10 mg; Merck KGaA) and Cs_2CO_3 (2.3 mg; Fluka) in acetonitrile (1.5 mL) and water (0.4 mL). The fluoride was dried by azeotropic distillation of acetonitrile at 120°C under vacuum with a stream of nitrogen. The azeotropic drying process was repeated with 1 mL of acetonitrile. To the dried ^{18}F-fluoride complex (typically 80 GBq) was added a solution of the bombesin peptide precursor (4 mg, synthesized by NeoMPS) in 0.5 mL of dimethyl sulfoxide [DMSO]). The reaction mixture was heated at 90°C for 10 min. The reaction mixture was diluted with 3 mL of 0.1% TFA in water, and the reactor was rinsed with another 3 mL of 0.1% TFA in water and injected into a semipreparative HPLC ACE column (C18-300, 5 μm, 250 × 10 mm). The conditions were as follows: 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile:water (9:1) (solvent B), isocratic elution with 23% solvent B, and a flow rate of 4 mL/min. The radiolabeled product peak fraction (∼6 mL) was collected at 25 min, diluted with water (18 mL), passed through a preconditioned C18 light cartridge (Waters, preconditioned with 5 mL of EtOH and 10 mL of water), washed with water (5 mL), and eluted with EtOH (1.5 mL) into 12.5 mL of 0.9% NaCl. The product solution (typically ∼5–7 GBq) containing 10% ethanol was then passed through a sterile filter (0.2 μm) and used for patient studies.

Quality Control of BAY 864367

The identity of the tracer (BAY 864367) was confirmed by coinjection of the authentic standard reference compound into the same analytic HPLC system ACE C18 column (50 ×

4.6 mm, 3 μm). The elution conditions were as follows: 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B); 0 min, 0.5% B; 0–2.0 min, 0.5%–23% B; 2.0–4.5 min, 23% B; 4.5–8.8 min, 90% B; and 8.8–9.0 min, 90%–0.5% B; and a flow rate of 2 mL/min. ^{18}F-fluoromethylcholine (^{18}F-fluorocholine) was a routine production of Zurich University Hospital.

Safety Monitoring

Vital signs (blood pressure, body temperature, and heart beat), ECG, and adverse effects were assessed in all patients. Laboratory blood analysis and urine testing was performed before, on the day of, and 2 times (day 2 and days 4–8) after the scan. The following laboratory parameters were assessed: sodium (mmol/L), potassium (mmol/L), chloride (mmol/L), urea (mmol/L), creatinine (μmol/L), glomerular filtration rate (mL/min), bilirubin (μmol/L), protein (g/L), albumin (g/L), aspartate aminotransferase (U/L), alanine aminotransferase (U/L), gamma-glutamyltransferase (U/L), alkaline phosphatase (U/L), C-reactive protein (mg/L), prostate-specific antigen (PSA; μg/L), complete blood count, hemogram, international normalized ratio (INR), partial thromboplastin time, and in the urine sample: pH, erythrocytes, leucocytes, nitrite, protein, and bilirubin.

Physical examinations were performed and included the following organ systems: head, ear, eye, nose, and throat; respiratory; cardiovascular; gastrointestinal; hepatic; genitourinary; musculoskeletal; endocrine; neurologic; psychiatric; skin/dermatologic; lymph nodes; and allergies.

Data Acquisition

After injection of 302 ± 11 MBq of the ^{18}F-labeled tracer BAY 864367 with a peptide mass dose of less than 40 μg per patient, all patients underwent standardized dynamic and static PET/CT imaging. Nine of ten patients had a static PET/CT scan with ^{18}F-fluorocholine

before BAY 864367 PET/CT. Because of an urgently scheduled operation, in patient 1 no ^{18}F-fluorocholine PET/CT was performed.

PET/CT procedure: Imaging with BAY 864367 was performed using an integrated PET/CT system (Discovery VCT; GE Healthcare). Patients were encouraged to take oral hydration. In total, 6 emission scans were assessed at time points 30, 45, 60, 75, 90, and 110 min after injection of BAY 864367. The scan duration at each time point was 15 min. Before the beginning of the PET series, a standard low-dose CT scan was performed for attenuation correction of the PET scan. Scans were corrected und reconstructed using the integrated software algorithms of the manufacturer. ^{18}F-fluorocholine PET/CT was assessed as previously described (*2*).

References

1. Honer M, Mu L, Stellfeld T, et al. ^{18}F-labeled bombesin analog for specific and effective targeting of prostate tumors expressing gastrin-releasing peptide receptors. *J Nucl Med.* 2011;52:270–278.

2. Oprea-Lager DE, Vincent AD, van Moorselaar RJ, et al. Dual-phase PET-CT to differentiate [^{18}F]fluoromethylcholine uptake in reactive and malignant lymph nodes in patients with prostate cancer. *PLoS One.* 2012;7:e48430.