**Primary Evaluation of 68Ga-DOTA-TMVP1 as a novel VEGFR-3 PET Imaging Radiotracer in Gynecological Cancer: First Preclinical and Clinical Results**

SUPPLEMENTAL METHODS

**Ridiolabeling**

The protocol was followed: 68GaCl3 was eluted with 6 mL of 0.1 M HCl from a 68 Ge/ 68 Ga generator (IGG-100 [1,850 MBq]; Puriﬁed 68GaCl 3 was added into a precharged reaction vial containing the precursor DOTA-TMVP1 (10 μ g) in sodium acetate buffer (pH 4.5). The mixture was heated at 95 ℃ for 180 s. Crude 68Ga-DOTA-TMVP1 was diluted with saline and loaded onto a Sep-pak c18 cartridges (Waters Company, America). The loaded cartridge was subsequently washed with saline and eluted with a 50% ethanol solution. The puriﬁed product was then diluted with saline and passed through a sterilizing membrane ﬁlter (0.2 mm) to afford the ﬁnal formulated product. Instant thin-layer chromatography silica-gel paper purity and reversed-phase radio-HPLC(RP-HPLC) were used to detect the radiochemical purity. 18F-FDG was obtained based on clinical standard procedure.

**In vitro stability and the plasma protein binding measurement**

68Ga-DOTA-TMVP1 was dissolved in 1 mL of saline or cysteine solution (1 mg/mL), incubated at room temperature and analyzed using instant thin layer chromatography medium impregnated with silica gel (iTLC-SG) at 0, 1, 3, 6, and 18 h post-incubation. Human serum from healthy donors was incubated at 37 °C with 68Ga-DOTA-TMVP1 for different time points (1, 3, 6, and 18 h). After incubation, a sample of 250 μL was precipitated with 750 μL of acetonitrile/ethanol (V acetonitrile /V ethanol = 1:1) and centrifuged (3 min at 3000 rpm). The supernatants were passed through a ﬁlter and analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) and iTLC-SG, and the results were plotted as the radiochemical purity (RCP) at different time points.

In brief, plasma samples (centrifugated from fresh heparinized blood) were incubated with 100 MBq/mL of 68Ga-DOTA-TMVP1 for 10 min, 30 min, 1 h or 2 h. Afterward, 50 μL of samples were added into pretreatment microspinTM G-50 columns. After 10 minutes， the columns were centrifuged at 4500 rpm for 1min. The protein binding activity was calculated from the measured radioactivity of the liquid divided by the total radioactivity value.

**Cold complexes of DOTA-TMVP1 with natGa**

To a solution of DOTA-TMVP1 (10.0 mg, 0.0069 mmol, 1.00 eq) and deionized H2O (1.00 mL) was added GALLIUM(III)CHLORIDE (1.20 mg, 0.0069 mmol, 1.00 eq). The reaction was adjusted to pH = 6 with NaOH solution (1N) at 25°C. The solution was stirred at 25°C for 1 h.  LCMS indicated the desired MS was detected.  The reaction was filtered and the filtrate was purified by prep HPLC (Gemini, 10um, C18,110A + luna, C18, 10um, 100A; mobile phase:[water(0.075%TFA)-ACN]; B%:0%-21%, 36.5min) to give natGa-DOTA-TMVP1 (4.20 mg, 0.0026 mmol, 38.2% yield) as a white solid.

**Surface Plasmon Resonance (SPR) Protocol**

In brief, the VEGFR-3 protein (30 μg/mL) was covalently attached to amino Au colloidal nanoparticles (N- AuNPs) chip (Nicoya, Canada) by the EDC/NHS chemistry. The running buffer (PBS, PH 7.4) was allowed to flow through the COOH-sensor chip until a smooth testing baseline was obtained. Then, natGa-DOTA-TMVP1 were serially diluted into several different concentrations using the running buffer and severally injected into the chip from low to high concentrations. In each cycle, 250 μL sample was flowed through the chip for 5min at a constant flow rate of 20 μL /min. After detection, 0.25% SDS was added to dissociate the peptides from the target protein. Finally, the kinetic parameters of the binding reactions were calculated and analyzed by Trace Drawer software (Nicoya, Canada). In SPR assay, based on the different curves, the relevant kinetic data such as the association constant (ka), the dissociation constant (kd) and equilibrium dissociation constant (K D =k d /k a ) were obtained, respectively.