Supplementary Materials

In vivo tissue pharmacokinetics of ERBB2-specific binding oligonucleotide based drugs by PET imaging

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Radiosynthesis of ERBB2-cODN-idT-APs-[^{18}F]F ([^{18}F]1)

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Fluorine-18 in O-18 water from the cyclotron was extracted on a pre-activated QMA carbonate Sep-Pak cartridge using a solution of tetra-n-butylammonium bicarbonate ($TBAHCO_3$, 40 wt%, 5.0 μL) in MeOH (1 mL). The eluted solution containing fluorine-18 was dried azeotropically at 100 °C under a nitrogen stream; this process was repeated with the subsequent addition of acetonitrile (CH_3CN, 0.4 mL) at 100 °C (× 2). The precursor, 11-azido-3,6,9-trioxa-1-undecanol mesylate (**2**, N_3-PEG_3-OMs, 2.5 μL) was dissolved in acetonitrile (0.5 mL) and added in a reaction vial (4 mL size) which contained the $TBA^+[^{18}F]F^-$ complex. The reaction mixture was then stirred at 100 °C for 10 min. After cooling to room temperature, the labeling yield was checked by radio-thin layer chromatography (TLC) (100% EtOAc, R_f = 0.7~0.8, Figure S1). The reaction solution was diluted with 15 mL of water, loaded on a C18 plus Sep-Pak cartridge, washed with 5 mL of water, and eluted with 1.5 mL of acetonitrile. The pretreated product by a C18 plus Sep-Pak cartridge, N_3-PEG_3-[^{18}F]F ([^{18}F]**3**), was found to be over 99% of radiochemical purity (Figure S2). The eluted acetonitrile solution was diluted with 4 mL of water, filtered using a syringe filter (GHP, 13 mm, 0.45 μm), and purified using a reverse-phase HPLC system A [Gilson 321 System equipped with a UV detector (UV-220 nm) and gamma-ray detector (Lablogic systems, Sheffield, UK); column: XBridge RP18 (Waters, 10 × 250 mm) with a guard column (Phenomenex, CA, USA; 10 × 10 mm); eluent: 20% CH_3CN:H_2O; flow rate: 3 mL/min; Figure S3]. The eluate was collected at a retention time of approximately 26 min and diluted with 40 mL of water, slowly trapped on a C18 plus Sep-Pak cartridge, washed with 5 mL of water, and eluted with 1.0 mL of acetonitrile. The eluted solution was used for the subsequent click reactions without further treatment. In this step, the radiochemical purity was above 99%, as checked by an analytical HPLC system B [Agilent 1260 system equipped with a UV detector (UV-220 nm) and gamma-ray detector (Elysia-Raytest, Straubenhardt, Germany); column: Xterra RP18 (Waters, 4.6 × 250 mm); flow rate: 1 mL/min; eluent: 10% CH_3CN:0.1 M triethylamine acetate (TEAA, 0 min); 10% CH_3CN:0.1 M TEAA (5 min); 80% CH_3CN:0.1 M TEAA (15 min); Figure S4].

For the synthesis of cODN-PEG_3-[^{18}F]F ([^{18}F]**5**), 0.1 M CuI in acetonitrile (20 μL), 1 M DIPEA in acetonitrile (10 μL), and N_3-PEG_3-[^{18}F]F in CH_3CN ([^{18}F]**3**, 0.7 mL) were added in a 4 mL vial in order; finally 3'-GTC GGT GTG GTG GTC-5'-hexynyl (**4**, cODN-5'-hexynyl) in water (200 μg in 0.2 mL) was added to the vial. The mixture was then stirred at 70 °C for 20 min. After cooling to room temperature, the conversion yield was checked by radio-TLC (100% EtOAc, R_f = 0.0~0.1 for cODN-PEG_3-[^{18}F]F and R_f = 0.7~0.8 for N_3-$(PEG)_3$-[^{18}F]F; Figure S5). The mixture was diluted with 10 mL of 0.05 M TEAA which contained 10 μL of 0.5 M ethylenediaminetetraacetic acid (EDTA), loaded on a C18 plus Sep-Pak cartridge, washed with 10 mL of water, and then eluted with 1 mL of acetonitrile (Figure S6). In this step, most of the N_3-PEG_3-[^{18}F]F ([^{18}F]**3**) was removed. The eluted solution was diluted with 4 mL of 0.1 M TEAA, filtered using a syringe filter (GHP, 13 mm, 0.45 μm), and purified using a reverse-phase HPLC System C [Gilson 321 System equipped with a UV detector (UV-260 nm)

and gamma-ray detector (Lablogic systems, UK); column: XBridge RP18 (10 × 250 mm) with a guard column (Phenomenex, CA, USA; 10 × 10 mm); eluent: 5% CH_3CN:0.1 M TEAA (0 min); 5% CH_3CN:0.1 M TEAA (5 min); 30% CH_3CN:0.1 M TEAA (25 min); 80% CH_3CN:0.1 M TEAA (30 min); flow rate: 3 mL/min; Figure S7]. The eluate was collected at a retention time of approximately 22 min, diluted with 10 mL of water, slowly trapped on a C18 plus Sep-Pak cartridge, washed with 10 mL of water, and eluted with 1.0 mL of ethanol. The collected solution was completely evaporated under a nitrogen stream at 95 °C. In this step, the radiochemical purity (over 99%, Figure S8) and identity (Figure S9) were checked using an analytical HPLC system D [Agilent 1260 system equipped with a UV detector (UV-260 nm) and gamma-ray detector (Elysia-Raytest, Germany); column: Xterra RP18 (Waters, 4.6 × 250 mm); flow rate: 1 mL/min; eluent: 10% CH_3CN:0.1 M TEAA (0 min); 10% CH_3CN:0.1 M TEAA (5 min); 80% CH_3CN:0.1 M TEAA (15 min)].

To prepare the hybridized aptamers, the prepared cODN-PEG_3-[^{18}F]F ([^{18}F]**5**) was dissolved in annealing buffer (0.2 mL; 10 mM Tris pH 7.5, 1 mM EDTA, 50 mM NaCl, 10 mM $MgCl_2$) containing ERBB2-cODN-idT-APs (**6**, 100 nmol). The mixture was heated to 95 °C for 5 min and cooled slowly to 45 °C at a rate of 1 °C/10 sec; the temperature was maintained at 45 °C for 5 min, and then slowly cooled to 25 °C. The hybridization efficiency and radiochemical purity were checked using an analytical HPLC system D (Figure S10). The identity was confirmed by co-injection with standard ERBB2-cODN-idT-APs-F (**1**, Figure S11). The final solution was diluted with 2 mL of saline and used for further PET imaging studies without further purification.

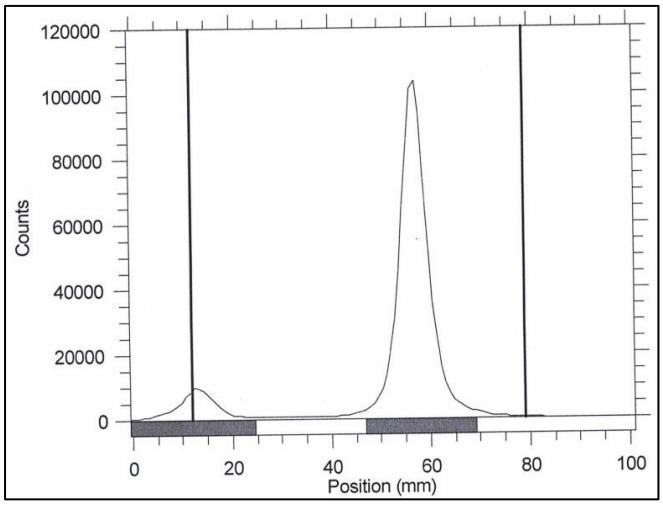

Figure S1. Radio-TLC chromatogram for fluorine-18 incorporation of N_3-PEG_3-[^{18}F]F ([^{18}F]**3**).

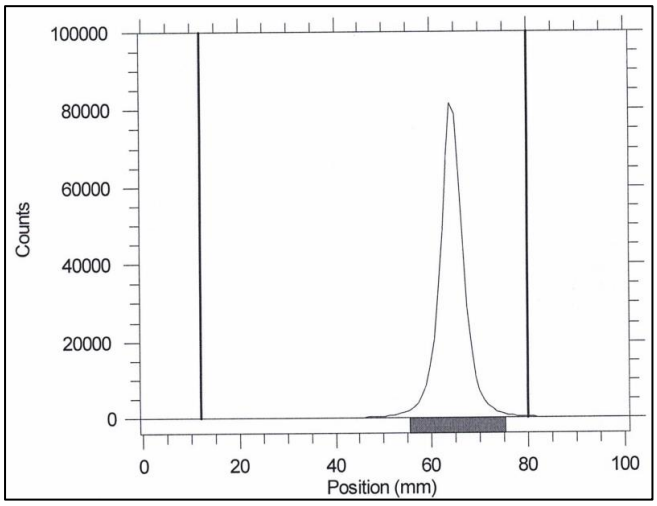

Figure S2. Radio-TLC chromatogram of the N_3-PEG_3-[^{18}F]F mixture obtained after pretreatment with a C18 plus Sep-Pak cartridge.

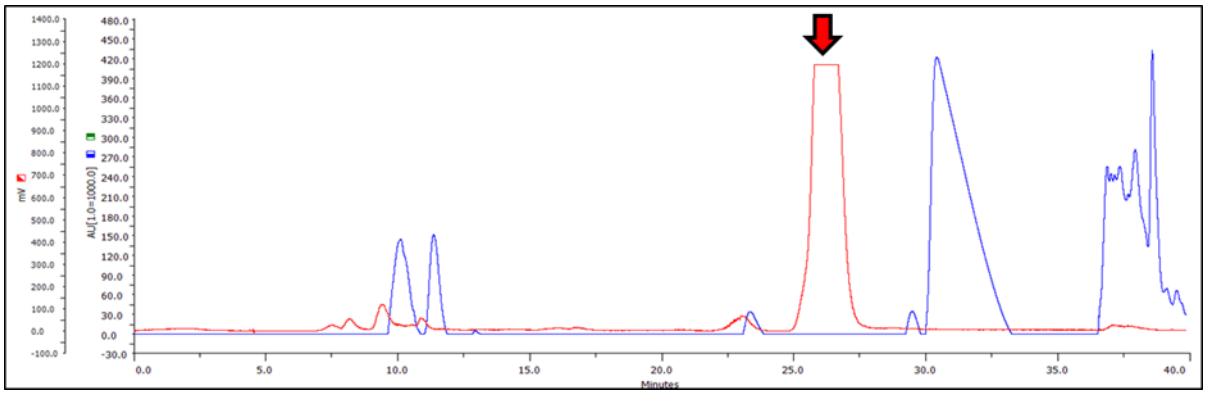

Figure S3. Preparative HPLC chromatogram of the reaction mixture, [^{18}F]**3** (red: gamma ray; blue: UV-220 nm)

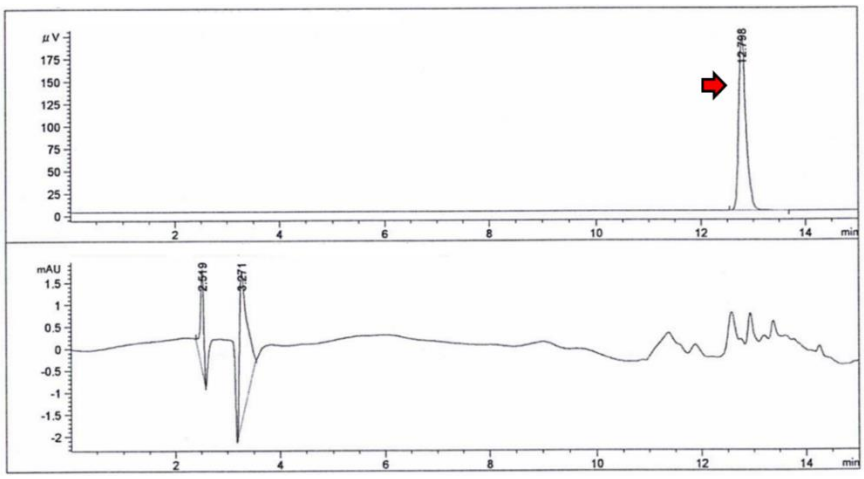

Figure S4. Analytical HPLC chromatogram for determining the radiochemical purity of [^{18}F]**3** (upper: gamma ray, bottom: UV-220 nm).

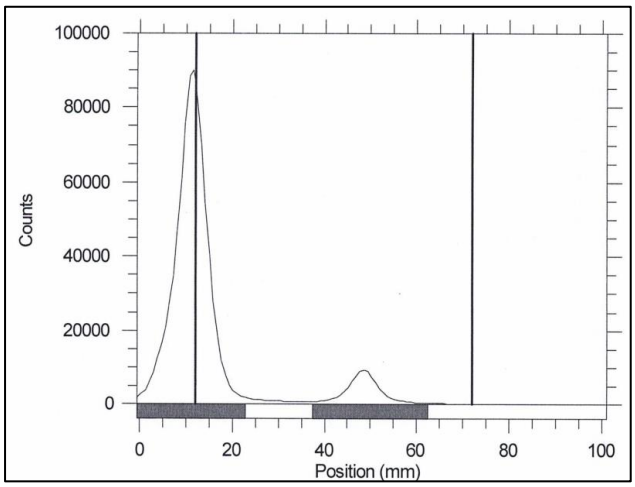

Figure S5. Radio-TLC chromatogram for determining the conversion yield from [^{18}F]**3** to [^{18}F]**5**.

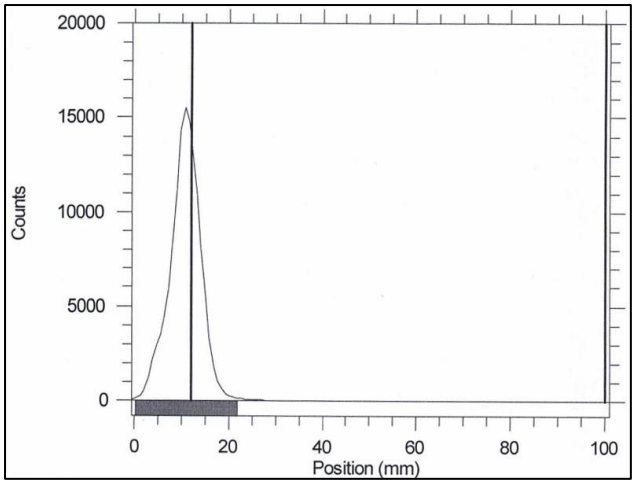

Figure S6. Radio-TLC chromatogram obtained after pretreatment of [^{18}F]**5** with a C18 Sep-Pak cartridge.

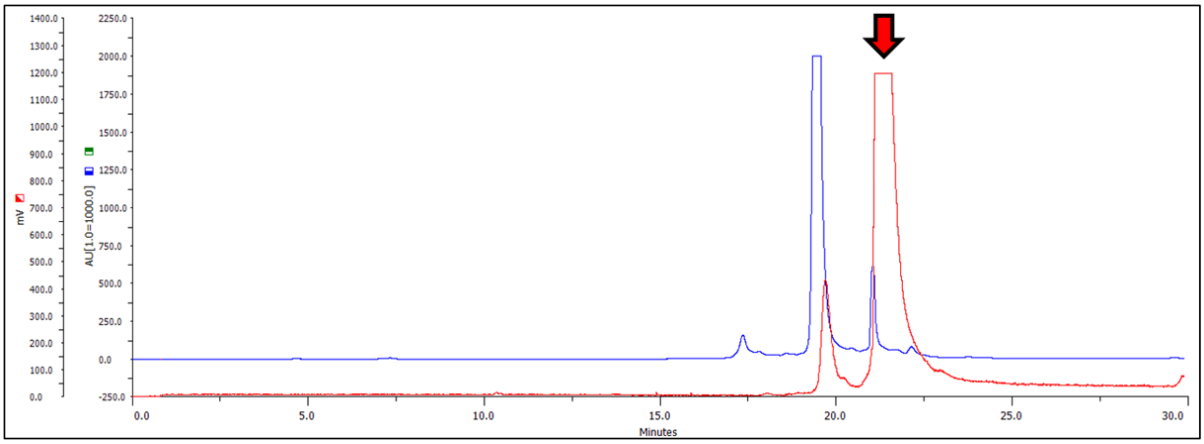

Figure S7. Preparative HPLC chromatogram of the reaction mixture, [^{18}F]**5** (red: gamma ray; blue: UV-260 nm).

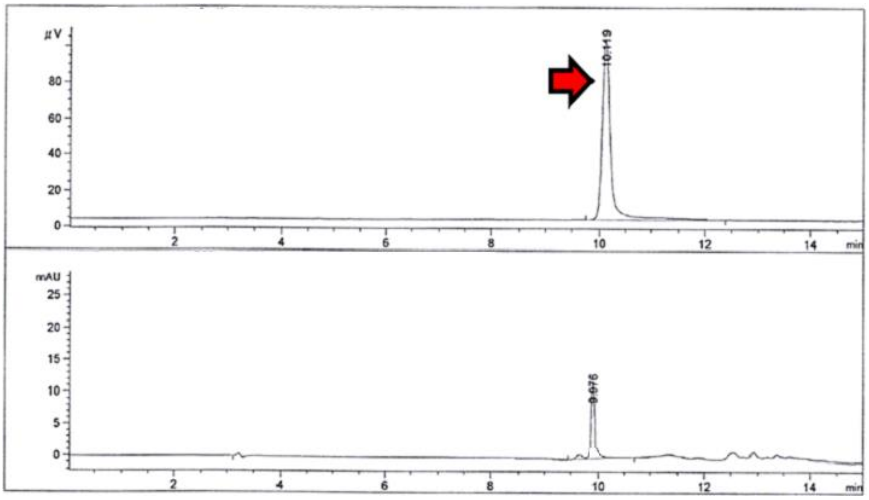

Figure S8. Analytical HPLC chromatogram obtained to determine the radiochemical purity of [^{18}F]**5** (upper: gamma ray; bottom: UV-260 nm).

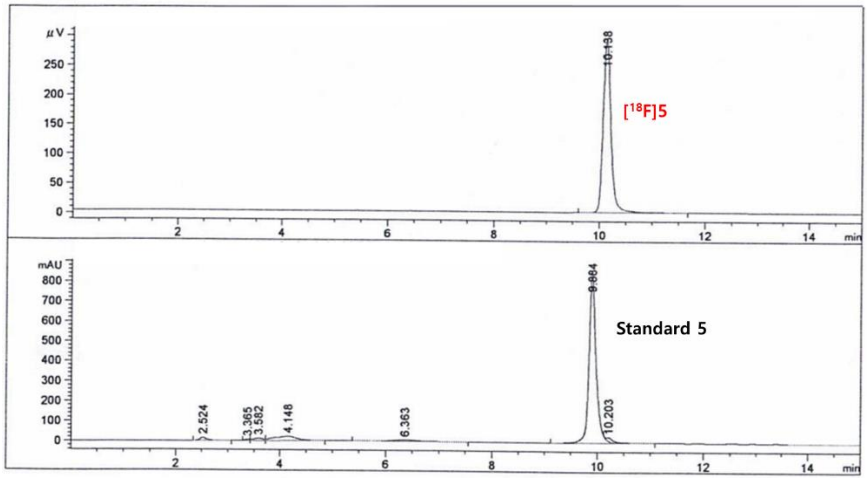

Figure S9. Co-injection HPLC chromatogram with standard **5** for determining the identity of [^{18}F]**5** (upper: gamma ray; bottom: UV-260 nm).

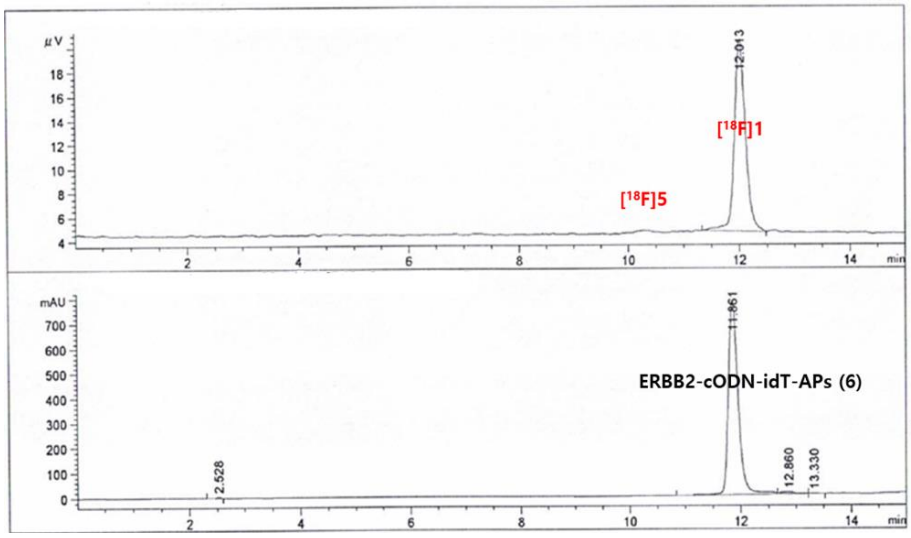

Figure S10. Analytical HPLC chromatogram obtained for determining the hybridization efficiency of [^{18}F]**1** (upper: gamma ray; bottom: UV-260 nm).

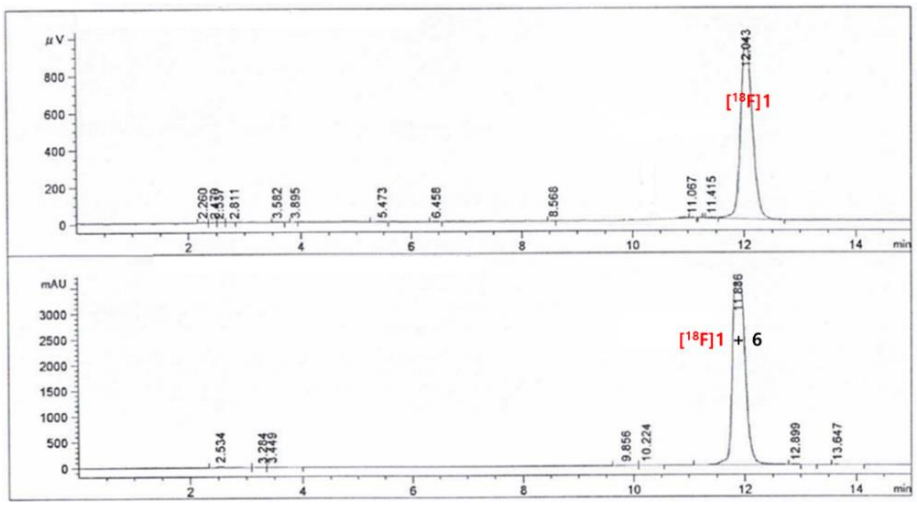

Figure S11. Co-injection HPLC chromatogram with standard **1** for determining the identity of [^{18}F]**1** (upper: gamma ray; bottom: UV-260 nm).

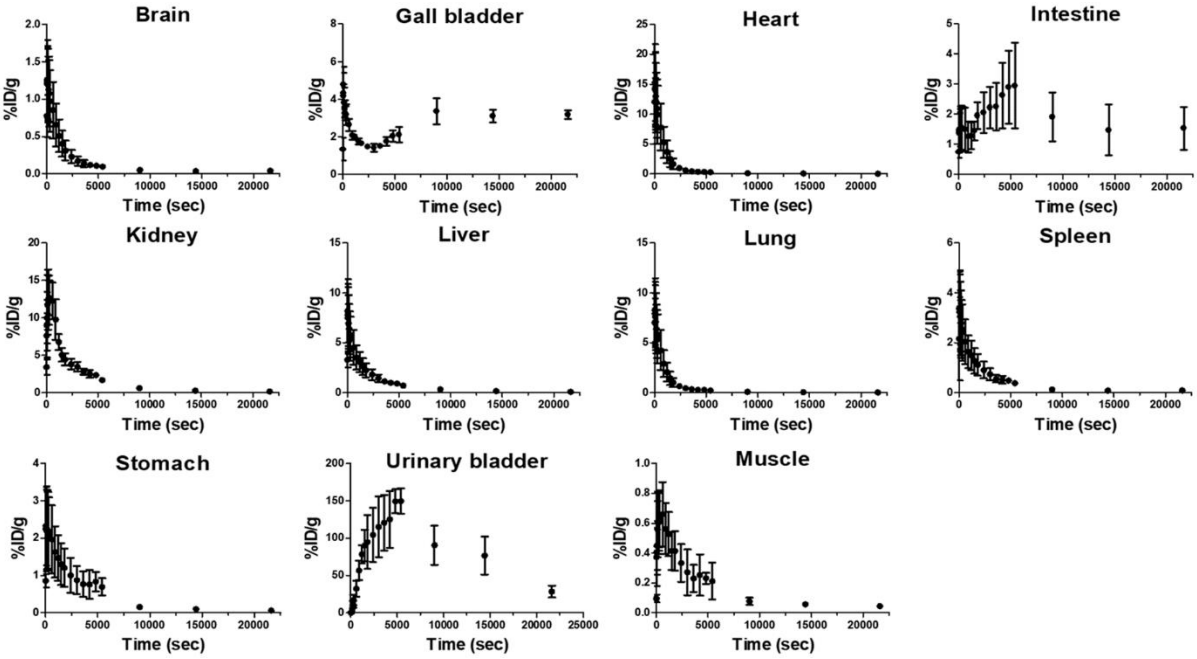

Figure S12. Time-activity curves of ERBB2-cODN-idT-APs-[^{18}F]F ([^{18}F]**1**) for internal dosimetry calculated from whole-body PET images.

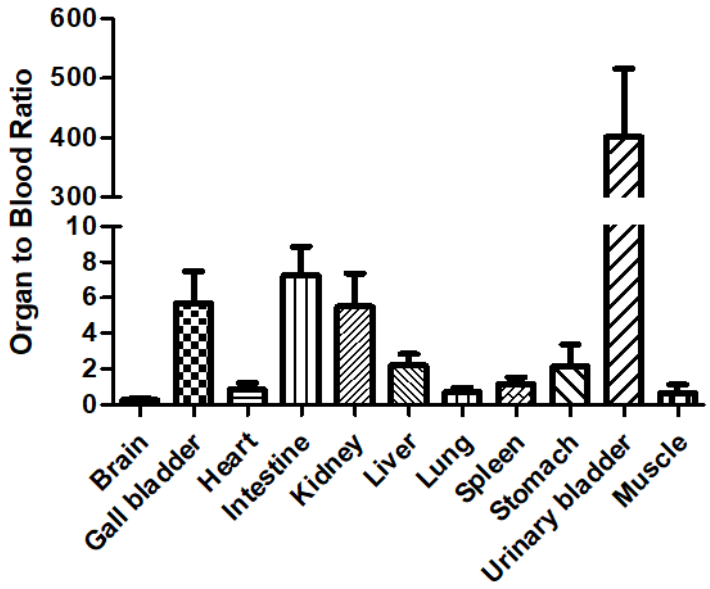

Figure S13. The ratio of AUCs for various organs to the blood over a late time-interval (70-90 min).

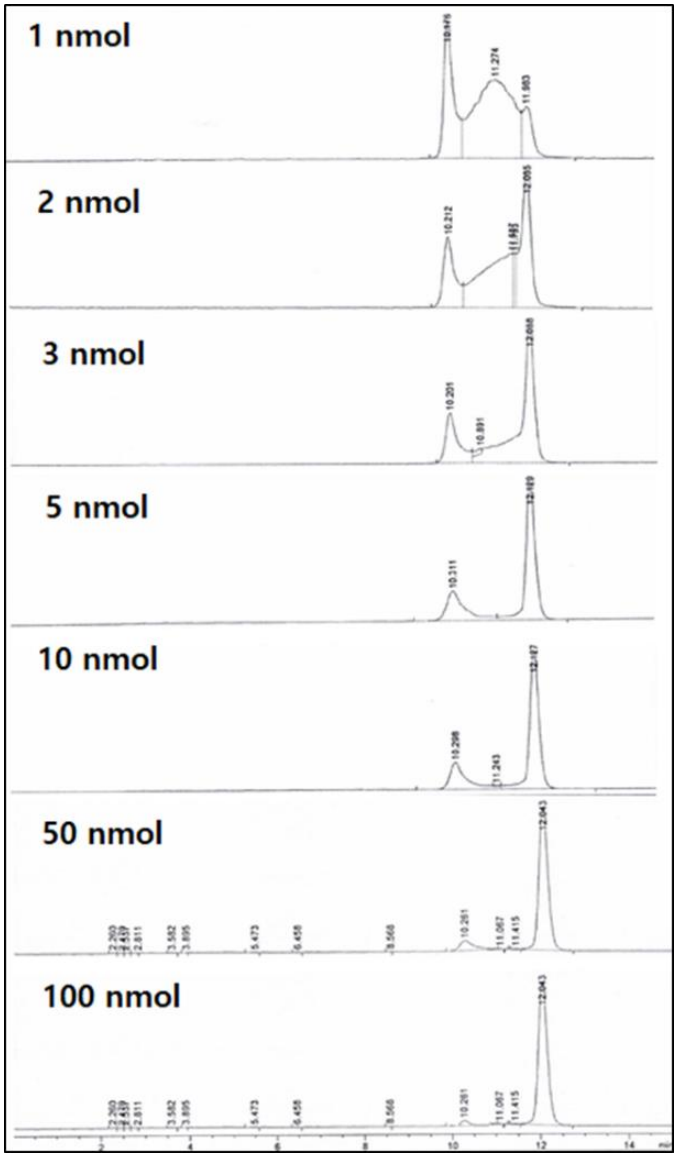

Figure S14. Investigation of changes for optimal hybridization efficiency at various concentrations (1, 2, 3, 5, 10, 50, and 100 nmol) of ERBB2-cODN-idT-AP (**6**) (gamma ray).

Table S1. Pharmacokinetic parameters of [^{18}F]**1** after intravenous administration in healthy mice

PK parameter	T_{max} (sec)	C_{max} (%ID/g)		$AUC_{0\text{-}5,400\ sec}$ (%ID/g·sec)		$T_{1/2}$ (sec)	
Organ	median (min-max)	mean	GSD	mean	GSD	mean	GSD
Brain	45.0 (45.0-60.0)	1.2	1.7	1,684.5	1.7	734.3	1.3
Gall bladder	30.0 (30.0-30.0)	4.7	1.2	10,420.8	1.1	290.4	1.2
Heart	30.0 (30.0-30.0)	13.9	1.7	11,718.7	1.9	505.8	1.3
Intestine	5,100.0 (240.0-5,400.0)	3.0	1.4	11,090.9	1.3	-	-
Kidney	240.0 (120.0-600.0)	14.0	1.1	26,519.2	1.2	666.5	1.3
Liver	30.0 (30.0-30.0)	7.6	1.7	11,333.4	1.4	628.1	1.1
Lung	30.0 (30.0-45.0)	7.7	1.7	6,995.2	1.7	497.1	1.3
Spleen	37.5 (15.0-60.0)	3.1	1.9	5,280.9	1.6	697.2	1.2
Stomach	37.5 (30.0-180.0)	2.1	1.7	5,723.2	1.6	1,025.0	1.7
Urinary bladder	5,400.0 (4,800-5,400.0)	149.2	1.1	524,726.6	1.3	-	-
Muscle	450.0 (240.0-600.0)	0.6	1.4	1,863.6	1.4	1,019.1	1.3
Blood	15.0 (15.0-60.0)	132.2	1.8	37,922.4	1.1	146.6	1.7

Table S2. Pharmacokinetic parameters of [^{18}F]**1** after intravenous administration in ERBB2-positive tumor (KPL4)-bearing mice.

PK parameter	T_{max} (sec)	C_{max} (%ID/g)		$AUC_{0\text{-}5,400sec}$ (%ID/g·sec)		$T_{1/2}$ (sec)	
Organ	median (min-max)	mean	GSD	mean	GSD	mean	GSD
Tumor	210.0 (120.0-300.0)	0.86	1.06	3,385.6	1.1	3,666.3	1.8

Table S3. Internal absorbed dose in normal mice (mGy/MBq)

Organs	Absorbed dose (mGy/MBq)
Brain	6.69E00
Heart	2.64E01
Lung	2.95E01
Liver	4.49E00
Kidney	3.73E01
Stomach	6.35E01
Spleen	2.67E01
Intestine	3.17E-01
Urinary bladder	7.52E-02
Total Body	1.83E00