^{18}F-Labeled N-(4-fluorobenzylidene)oxime-VENK[homoC]NKEMRNRYWEAALDPNLNNQQKRAKIRSIYDDP[homoC]-NH_2 with a disulfide bridge between the two homoC

^{18}F-FBO-MUT-DS

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Chemical name:	^{18}F-Labeled N-(4-fluorobenzylidene)oxime-VENK[homoC]NKEMRNRYWEAALDPNLNNQQKRAKIRSIYDDP[homoC]-NH_2 with a disulfide bridge between the two homoC	
Abbreviated name:	^{18}F-FBO-MUT-DS	
Synonym:		
Agent Category:	Affibody, antibody	
Target:	HER2	
Target Category:	Receptor	
Method of detection:	Positron emission tomography (PET)	
Source of signal / contrast:	^{18}F	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure is not available.

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Background

[PubMed]

The ^{18}F-labeled N-(4-fluorobenzylidene)oxime (FBO)-VENK[homoC]NKEMRNRYWEAALDPNLNNQQKRAKIRSIYDDP[homoC]-NH_2 (MUT-DS) conjugate with a disulfide bridge between the two homocysteines, abbreviated as ^{18}F-FBO-MUT-DS, is a 2-helix affibody derivative that was synthesized by Miao et al. for positron emission tomography (PET) of HER2-expressing tumors (1).

Affibody molecules are a group of nonimmunogenic scaffold proteins that are derived from the B-domain of staphylococcal surface protein A (2, 3). These molecules have only 58 amino acid residues (~7 kDa), which form a 3-α-helical bundle structure (3, 4). Helices 1 and 2 bundles are responsible for the high binding affinity and specificity of affibodies to their targets, while helix 3 contributes to the affibody stabilization and is not involved in receptor recognition (4). Studies have further confirmed that the binding domain in the helices 1 and 2 bundles includes 13 amino acid residues that are surface-exposed (2). Therefore, large affibody libraries have been constructed by randomization of the 13 amino acid residues, and a large set of affibody molecules against a wide variety of targets have been selected from those libraries. Of them, the affibodies specific to HER2, including $Z_{HER2:342}$ and $Z_{HER2:477}$, have been intensively investigated in recent years (3, 5). These molecules have been radiolabeled and tested for molecular imaging of HER2-expressing tumors.

The investigators at Stanford University first tested the feasibility of the monomeric (~7 kDa) and dimeric (~14 kDa) forms of affibody $Z_{HER2:477}$ for molecular imaging (6, 7). Both forms have been labeled with various radiouclides through chelating agents. Studies have shown that smaller affibody constructs perform better *in vivo* in terms of tumor uptake and clearance, which prompts them to generate smaller proteins with only α-helices 1 and 2 bundles (~4 kDa) (4, 6, 7). However, simple deletion of the helix 3 leads to significantly decreased binding affinity of the proteins because of decreased helix conformation (4). The investigators then applied various strategies to improve the helix conformation of the affibody molecules, including sequence mutation, placement of disulfide bridges, and inclusion of helix-promoting amino acids (4). Although the helix conformation (~15%, the amount of α-helix represented in the secondary structure of the affibodies) of the 2-helix molecules is still much lower than that of the parent 3-helix affibodies, the investigators successfully obtained a class of 2-helix small proteins with HER2-binding affinity up to 5 nM with these strategies (1, 8, 9). One of these 2-helix proteins is MUT-DS, which has α-helices 1 and 2 bundles with a disulfide bridge being formed between the two inserted homocysteines (4). The radiolabeled MUT-DS derivatives exhibited favorable pharmacokinetics for imaging HER2-expressing tumors. ^{68}Ga-DOTA-MUT-DS, ^{64}Cu-DOTA-MUT-DS, ^{111}In-DOTA-MUT-DS, and ^{18}F-FBO-MUT-DS are examples of the MUT-DS derivatives (1, 8, 9).

This chapter summarizes the data obtained with ^{18}F-FBO-MUT-DS (1).

Related Resource Links:

- Anti-HER2 affibody conjugates in MICAD:

^{68}Ga-DOTA-$Z_{HER2:342-pep2}$, ^{111}In-DOTA-$Z_{HER2:342-pep2}$, Alexa750-$Z_{HER2:342}$, ^{111}In-benzyl-DOTA-$Z_{HER2:342}$, ^{111}In-benzyl-DTPA-$Z_{HER2:342}$, Eaff800, DY-682-ABD-$Z_{HER2:342}$ (Haff682), ^{99m}Tc-Z_{2395}-C, [^{111}In]-ABY-025, ^{111}In-BZ-DTPA-$Z_{EGFR:1907}$.

- HER2-related clinical trials for imaging and therapy

Synthesis

[PubMed]

The linear aminooxy-functionalized (AO)-MUT-DS was synthesized with standard solid-phase peptide synthesis (purity, >95%) (1). Cyclization of the linear peptides was achieved with I_2 oxidation of the two L-homocysteines to form a disulfide bridge. The measured molecular weight (MW) of AO-MUT-DS was 4,823.9 (calculated 4,823.4). The AO-MUT-DS was then reacted with 4-fluorobenzaldehyde to prepare nonradioactive FBO-MUT-DS as a standard compound. Recovery yield was 70%–90%, and no starting cyclized peptide was detected. The measured MW (4,926.9) of the purified FBO-MUT-DS was consistent with the expected MW (4,928.4).

^{18}F-FBO-MUT-DS was prepared by conjugating 4-^{18}F-fluorobenzaldehyde with AO-MUT-DS (1). The purity was >95%. The overall yield ranged from 13% to 18% (non-decay-corrected) and the specific activities were 20–32 MBq/nmol (0.54–0.86 mCi/nmol) at the end of synthesis. The total time needed for the radiosynthesis was ~100 min.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The binding affinities of AO-MUT-DS and FBO-MUT-DS with the extracellular domain of HER2 antigen were measured *in vitro* with surface plasmon resonance detection (1). The binding affinity of FBO-MUT-DS was slightly higher than that of AO-MUT-DS (2 nM and 1 nM, respectively). The off-rate of FBO-MUT-DS was found to be similar as the AO-MUT-DS with a dissociation constant of $7 \times 10{-}^{4}$ 1/s. The on-rate of FBO-MUT-DS was only approximately one-fold higher than that of AO-MUT-DS, with association constants of 7.5×10^{6} 1 M/s (association time, >2 min).

The HER2-targeting ability for cultured cells was evaluated with SKOV3 human ovarian cancer cells over 1 h incubation with ^{18}F-FBO-MUT-DS (1). ^{18}F-FBO-MUT-DS quickly accumulated in SKOV3 cells and reached a value of 14% of applied activity at 0.25 h. The uptake was maintained at almost the same level until 1 h. When the probe was co-incubated with a large excess (final concentration 4 μg/ml) of nonradioactive $Z_{HER2:342}$, the probe uptake dropped to only ~5% of applied activity after 0.25 h incubation at 37°C (1).

Animal Studies

Rodents

[PubMed]

The biodistribution of ^{18}F-FBO-MUT-DS was examined with mice bearing SKOV3 human ovarian tumors (n = 3 mice/time point) after tail vein injection of ^{18}F-FBO-MUT-DS (1.11–1.85 MBq (30–50 μCi)) (1). Mice were euthanized at 1 h and 3 h after injection. ^{18}F-FBO-MUT-DS showed rapid and high accumulation in the tumors (6.9 ± 3.8% of injected dose per gram of tissue (ID/g) at 1 h). ^{18}F-FBO-MUT-DS also displayed relatively rapid blood clearance (2.4 ± 0.8% ID/g) at 1 h. Both renal and liver uptake values were ~7% ID/g at 1 h, which suggested that ^{18}F-FBO-MUT-DS was cleared through the hepatobiliary and renal systems. Distribution at 3 h after injection was not described in detail. Co-injection of ^{18}F-FBO-MUT-DS (1.11–1.85 MBq (30–50 μCi), 0.3 μg) and blocking agent $Z_{HER2:342}$ (500 μg)blocked the tumor uptake of ^{18}F-FBO-MUT-DS (1.8 ± 1.1% ID/g at 1 h after injection) (n = 3 mice).

The tumor targeting and imaging abilities were further confirmed with PET imaging in mice bearing SKOV3 tumors (n = 3) (1). Good tumor imaging contrast was observed at 1 h and 2 h after injection of ^{18}F-FBO-MUT-DS. The washout rate of the probe from tumors was slow. After 3 h, high tumor accumulation could still be seen in the PET images, while the uptake values for normal organs such as the liver and kidney declined dramatically. There was no significant difference for the tumor uptake at 1 h and 2 h after injection ($P > 0.05$), but the difference was significant for the liver uptakes ($P < 0.05$). These results suggest the good tumor retention and fast normal organ clearance of the ^{18}F-FBO-MUT-DS.

The *in vivo* metabolic stability of ^{18}F-FBO-MUT-DS was analyzed at 1 h after tail vein injection of ^{18}F-FBO-MUT-DS into the mice bearing SKOV3 tumors (1). Studies showed that 80% of the probe remained intact in tumor, but only a small percentage of the intact probe was observed from urine samples, indicating relatively rapid degradation of the probe to more polar molecules cleared from the blood by the kidney–urinary system. The result also showed that a small fraction of the probe was metabolized to a more lipophilic fragment in liver.

Other Non-Primate Mammals

[PubMed]

No references are available.

Non-Human Primates

[PubMed]

No references are available.

Human Studies

[PubMed]

No references are available.

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