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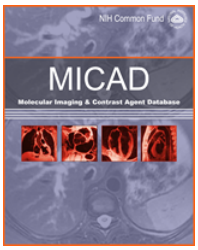


# [$^{18}$F]Fluorobenzoyl anti-HER2 Cys-diabody

## [$^{18}$F]FB-Cys-Db

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| | | |
|---|---|---|
| **Chemical name:** | [$^{18}$F]Fluorobenzoyl anti-HER2 Cys-diabody | |
| **Abbreviated name:** | [$^{18}$F]FB-Cys-Db | |
| **Synonym:** | | |
| **Agent Category:** | Antibodies | |
| **Target:** | HER2 | |
| **Target Category:** | Receptors | |
| **Method of detection:** | Positron emission tomography | |
| **Source of signal / contrast:** | $^{18}$F | |
| **Activation:** | No | |
| **Studies:** | • *In vitro*<br>• Rodents | No structure available. |

# Background

[PubMed]

[$^{18}$F]Fluorobenzoyl (FB) anti-HER2 Cys-diabody (Cys-Db), abbreviated as [$^{18}$F]FB-Cys-Db, was synthesized by Olafsen et al. for use in positron emission tomography (PET) of tumors expressing HER2 (1).

Antibodies have long been considered to be the most attractive agents for molecular imaging because of their high specificity and binding affinity (2, 3). However, the usefulness of full antibodies as imaging agents is limited by their long circulation time in blood (several days to weeks), which requires a long time to optimally accumulate in tumors (1–2 days) (2). Because of their large molecular size (150 kDa), antibodies also exhibit poor tumor-penetrating ability, which leads to poor signal/noise ratio. To improve antibody pharmacokinetics without compromising the affinity and specificity, a promising solution is to reduce the antibody size or alter the Fc receptor-binding domain with protein engineering (2, 3). Indeed, various antibody fragments have been generated, such as single-chain variable fragment (scFv; 25–30 kDa), Fab (~50 kDa), and F(ab')$_2$ (~110 kDa) fragments, bivalent scFv (tandem scFv and diabody, 50–60 kDa), and scFv-fusion proteins (80 kDa for minibody and 105 kDa for scFv-Fc) (2). These fragments exhibit good tumor penetration, fast clearance kinetics, high affinity, and high tumor/blood ratio, which are desirable for imaging agents. However, several issues with the use

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of antibody fragments as imaging agents remain to be solved, one of which is the diverse effects of labeling chemistry on the binding affinity of antibody fragments (4, 5).

To reduce the diverse effects of labeling chemistry, various strategies have been designed. For example, Vaidyanathan and Zalutsky prepared $^{18}$F-labeled antibody fragments using *N*-succinimidyl-4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB), which reacts with the ε-amino group of surface-exposed lysine residues on proteins (6). Labeling with this approach showed no loss of the antibody fragment affinity. Olafsen et al. further improved efficiency and speed of [$^{18}$F]SFB production by adapting the synthesis to a three-step, one-pot, microwave-assisted method, followed by purification using either a single cartridge or high-performance liquid chromatography (HPLC) (1, 6). Alternatively, McCabe et al. first conjugated the diabody to DOTA, followed by $^{64}$Cu-radiolabeling through DOTA (7). All these strategies have been reported to be efficient and have fewer diverse effects on the binding affinity of antibody fragments. This chapter summarizes the data obtained with [$^{18}$F]FB-Cys-Db. In another chapter, the data obtained with $^{64}$Cu-DOTA-CysDb are summarized.

## Related Resource Links:

HER2-related imaging agents in MICAD

Protein and nucleotide sequences of HER2

Structure of HER2

Bioassays of HER2 in PubChem

# Synthesis

[PubMed]

Olafsen et al. synthesized [$^{18}$F]SFB using a facile, one-pot synthesis, which was based on a three-step, microwave-assisted, nonaqueous radiochemical process (1). Microwave heating enables a rapid transformation in each step of the [$^{18}$F]fluorination, deprotection, and activation. Each step was completed within 1–2 min. The total synthesis time for the microwave-assisted, one-pot process was 35–40 min. The decay-corrected RCY of [$^{18}$F]SFB ranged from 17% to 31%, with a radiochemical purity of >95% and specific activity of 7.4–37 GBq/μmol (0.2–1 Ci/μmol) after purification. An improved method of microwave-assisted, one-pot synthesis was later established and resulted in an increased RCY of 35 ± 5%. [$^{18}$F]SFB was stable in phosphate-buffered saline (pH 7.4) for at least 2 h based on radio-HPLC.

Cys-Db labeling with [$^{18}$F]SFB was achieved by incubating the anti-HER2 Cys-Db in sodium borate buffer (pH 8.5) with [$^{18}$F]SFB for 30–45 min at room temperature, followed by HPLC separation (1). The total synthesis time (including [$^{18}$F]SFB synthesis) was ~100 min, and the RCY of [$^{18}$F]FB-Cys-Db was 1.8%–2.5%. The ratio of SFB to Cys-Db in the intact [$^{18}$F]FB-Cys-Db was not reported. Purification with spin column chromatography and HPLC yielded radiochemical purities of 67.9 ± 13.0% ($n$ = 11) and 94.7 ± 4.8% ($n$ = 8), respectively. Although the HPLC method yielded [$^{18}$F]FB-Cys-Db with high radiochemical purity, the investigators concluded that this approach was not practical because it led to significant dilution, requiring concentration of the product prior to injection into the mice (1).

The radiolabeled fraction associated with [$^{18}$F]FB-Cys-Db was determined by measurement of the activity in the bands presented on an SDS-PAGE gel after electrophoresis of an HPLC-purified [$^{18}$F]FB-Cys-Db with a radiochemical purity of 83.7%. Counting revealed that 82.5% of the activity was associated with the band that represented the Cys-Db, and that the remaining radioactivity was associated with higher (3.3%) and lower (14.2%) molecular weight bands.

# *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

After binding with MCF-7/HER2 and C6 (HER2-negative) cells for 1 h at room temperature, the immunoreactivity of [18F]FB-Cys-Db was determined to be 32.2% (1). The specificity was also demonstrated with competitive cell binding, showing a 75% decrease in binding. When the immunoreactivity was determined with the agents obtained in later improved conjugation reactions, this value was 59.0 ± 10.0% ($n$ = 4) and 52.3 ± 21.9% ($n$ = 5) for the agents purified with spin column chromatography and HPLC, respectively. Less than 1% activity was associated with the C6 control cells.

# Animal Studies

## Rodents

[PubMed]

MicroPET images with [18F]FB-Cys-Db were obtained from five mice bearing MCF-7/HER2 breast cancer xenografts (1). Two of these mice also carried HER2-negative C6 control tumors. All of the mice were injected with the [18F]FB-Cys-Db purified with spin column chromatography. The amount of radioactivity administered ranged from 1.147 to 3.034 MBq (from 31 to 82 μCi).

The MCF-7/HER2 breast cancer xenografts were clearly delineated at 2 h, and the time-activity curves showed retention and a slight increase of activity in the tumor during the first 2 h after injection, whereas a steady decrease was observed in the kidneys and other organs (Table 1). The tumor signal increased further at 4 h and remained strong at 6 h after injection, with the overall background activity reduced. [18F]FB-Cys-Db cleared rapidly from the blood during the first 2 h. The half-life times $T_{1/2\alpha}$ and $T_{1/2\beta}$ calculated from the images were 0.91 min and 58.95 min, respectively.

Low radioactivity was also visible in the HER2-negative C6 tumors, and the radioactivity became invisible at 6 h. Nonspecific background activity, predominantly in the kidneys and bladder, was seen in all animals at 2 h and 6 h after injection. An approximately two-fold increase in positive/negative tumor signal ratio was obtained at 4 h (~1.5:1) and at 6 h (~3:1) after injection. As expected for a rapidly clearing antibody fragment, the ratios of tumor/normal tissues were highest at 6 h in all tissues, except for bladder (Table 1). No blocking studies were reported.

Table 1. Tumor/normal tissue ratios at 4 h and 6 h after injection of [18F]FB-Cys-Db.

| Ratio | 4 h after injection | 6 h after injection |
|---|---|---|
| Tumor/blood | 1.25 | 1.83 |
| Tumor/liver | 2.09 | 3.13 |
| Tumor/kidney | 0.83 | 1.19 |
| Tumor/muscle | 5.50 | 6.87 |
| Tumor/bladder | 0.01 | 0.07 |

Biodistribution data were obtained from four mice at 6 h after injection. The mean tumor uptake values were 2.87 ± 0.43% injected dose per gram tissue (ID/g) for the positive tumors and ~1% ID/g for the negative tumors. The kidneys had the next highest uptake. The blood and lung had 1%–2% ID/g, whereas uptake values in the liver, spleen, and carcass were all <1% ID/g. The positive tumor uptake was significantly higher ($P < 0.05$) than

the uptakes in all normal organs and negative tumors, with the exception of the kidney ($P = 0.3214$). No blocking studies were reported.

## Other Non-Primate Mammals

[PubMed]

No references are currently available.

## Non-Human Primates

[PubMed]

No references are currently available.

# Human Studies

[PubMed]

No references are currently available.

# References

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