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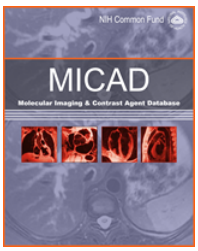


# Radioiodinated anti–TAG-72 CC49 (Fab')$_2$ antibody fragment

$^{131}$I/$^{125}$I-CC49 (Fab')$_2$

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| **Chemical name:** | Radioiodinated anti–TAG-72 CC49 (Fab')$_2$ antibody fragment | |
| **Abbreviated name:** | $^{131}$I/$^{125}$I-CC49 (Fab')$_2$ | |
| **Synonym:** | $^{131}$I-CC49 Ab, $^{125}$I-CC49 | |
| **Agent Category:** | (Fab')$_2$ antibody fragment | |
| **Target:** | (Sialyl-Tn (STn)) TAG-72 | |
| **Target Category:** | Antibody to antigen binding | |
| **Method of detection:** | Single-photon emission tomography (SPECT), planar gamma imaging | |
| **Source of signal:** | $^{131}$I, $^{125}$I | |
| **Activation:** | No | |
| **Studies:** | • *In vitro*<br>• Rodents | Click on protein, nucleotide (RefSeq), and gene for more information about TAG-72. |

## Background

[PubMed]

Radioiodinated anti–TAG-72 CC49 (Fab')$_2$ antibody fragment ($^{131}$I-CC49 (Fab')$_2$), which is formed by the conjugation of $^{125}$I/$^{131}$I with an anti–tumor-associated glycoprotein 72 (TAG-72) (Fab')$_2$ antibody fragment, has been developed for gamma imaging of cancers that express TAG-72 (1-4). $^{125}$I has a physical half-life ($t_{½}$) of 60 days, and $^{131}$I has a $t_{½}$ of 8 days. Although both can be used for *in vivo* gamma imaging, $^{125}$I has a gamma energy that is too low and $^{131}$I has a gamma energy that is too high. Another radioiodine, $^{123}$I, is more ideal for single-photon emission tomography (SPECT) and planar gamma imaging.

The TAG-72 antigen was isolated from the LS-174T human colon cancer xenograft as a high molecular weight glycoprotein (molecular mass of $10^6$ Da) with mucin-like characteristics (5-8). It is expressed on a variety of human adenocarcinomas such as pancreatic, breast, colorectal, prostate, endometrial, and ovarian cancers. This antigen has also been shown to be shed into the serum of cancer patients (9). The murine monoclonal antibody B72.3 (MAb B72.3) against TAG-72 mucin was initially generated by immunization of mice with a membrane-

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enriched fraction of a human breast carcinoma (10). With the use of affinity-purified TAG-72 from LS-174T as an immunogen, CC49 and other anti–TAG-72 MAbs with higher affinity constants ($K_a$) have been produced and characterized (5, 6, 10, 11). CC49 MAb appears to react with a unique disaccharide sialyl-Tn (STn) epitope on TAG-72 (12, 13).

Radiolabeled MAbs have been developed for both the diagnosis and treatment of tumors (14). Radiolabeled B72.3 and CC49 exhibit excellent tumor localization capabilities with potential diagnostic and therapeutic applications in the clinical setting (15, 16). Because of their relatively large size, intact radiolabeled MAbs tend to have unfavorable imaging kinetics, poor tumor penetration, and high potential for human anti-mouse antibody response (11, 17-19). One possible approach to minimize these problems is reducing intact antibodies to smaller antibody fragments such as F(ab')$_2$ and Fab' (20). Another approach is the development of genetic engineering methods to obtain single-chain Fv constructs (scFv) and multivalent scFv constructs (11, 21, 22). The F(ab')$_2$ and Fab' fragments can generally be prepared by simple enzymatic cleavage. Pepsin digestion of the intact IgG removes the antibody constant region and produces the F(ab')$_2$ fragment with bivalent antigen-binding capability and a molecular weight of 100,000 (14). Because of the smaller size, the F(ab')$_2$ fragment has a faster blood clearance and a better tumor penetration than the intact IgG (2, 23). The removal of the Fc portion during the enzymatic cleavage also reduces nonspecific binding of F(ab')$_2$ to Fc receptors. The *in vitro* and *in vivo* properties of radioiodinated CC49 (Fab')$_2$ fragments have been studied and compared to those of the single-chain Fv constructs (1-4, 24).

## Synthesis

[PubMed]

CC49 MAb IgG was developed by the immunization of mice with purified TAG-72 (4). CC49 IgG was purified from ascetic fluid obtained from immunized mice. CC49 (Fab')$_2$ was prepared by 2% pepsin digestion of the purified CC49 IgG at 37°C for 16 h (1, 2). The digested fragments were dialyzed and purified using ion-exchange chromatography. Radioiodination of CC49 (Fab')$_2$ was performed with the use of 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (IodoGen) as the oxidizing agent. Briefly, 100 µg of CC49 (Fab')$_2$ in 0.1 M sodium phosphate buffer (pH 7.2) was added to a glass tube coated with 20 µg IodoGen. Approximately 18.5 MBq (0.5 mCi) $^{125}$I-sodium iodide or $^{131}$I-sodium iodide was added and the mixture was incubated for 2 min at room temperature. Size-exclusion chromatography was performed immediately to purify the radiolabeled antibody. The labeling efficiency was 50%. SDS-PAGE analysis identified the $^{125}$I/$^{131}$I-CC49 (Fab')$_2$ fragment molecular weight as ~100,000. The specific activity of $^{125}$I/$^{131}$I-CC49 (Fab')$_2$ was ~111–185 kBq/µg (3–5 µCi/µg) or ~11.1–18.5 MBq/nmol (0.3–0.5 mCi/nmol) on the basis of the molecular weight of 100,000. Yokota et al. (2) reported a specific activity of 81.4–432.9 kBq/µg (2.2–11.7 µCi/µg) or 8.1–43.3 MBq/nmol (0.2–1.2 mCi/nmol). In another study, Yokota et al. (3) reported a radiochemical yield and purity of 30–50% with a specific activity of 0.34–0.4 MBq/µg (9.2–10.8 µCi/µg) or 34–40 MBq/nmol (0.9–1.1 mCi/nmol). Pavlinkova et al. (4) reported a specific activity of 111–333 kBq/µg (3–9 µCi/µg) or 11.1–33.3 MBq/nmol (0.3–0.9 mCi/nmol).

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

[PubMed]

The radioimmunoreactivity of $^{125}$I-CC49 (Fab')$_2$ was determined by a solid-phase radioimmunoassay using a TAG-72–positive extract of a LS-174T human colon carcinoma xenograft (1). $^{125}$I-CC49 was used as the competitor. The binding of $^{125}$I-CC49 (Fab')$_2$ was 39.1%, and the relative $K_a$ was ~$4 \times 10^8$ $M^{-1}$, which was similar to that of $^{125}$I-CC49 IgG. The binding of $^{125}$I-CC49 (Fab')$_2$ to purified TAG-72 was 22.1%. Yokota et al. (2) reported a binding of 35% to a TAG-72–positive extract. In another study, Yokota et al. (3) found >90% binding of purified $^{125}$I-CC49 (Fab')$_2$ to bovine submaxillary gland mucin (BSM)-coated beads. Pavlinkova et al. (4) used the surface plasmon resonance technique to measure the real-time interactions, and the investigators

reported that the $K_a$ of unlabeled CC49 $(Fab')_2$ was $1.04 \times 10^8$ $M^{-1}$ in binding to the immobilized BSM. In comparison, the $K_a$ for the intact CC49 IgG was $1.14 \times 10^8$ $M^{-1}$. In the same study, the binding of $^{125}I$-CC49 $(Fab')_2$ to BSM was 81%.

# Animal Studies

## Rodents

[PubMed]

Biodistribution studies of $^{125}I$-CC49 $(Fab')_2$ were performed in nude mice bearing s.c. LS-174T human colon carcinomas (0.5–0.8 cm in diameter) on the back (1). The studies were conducted with i.v. administration of 0.278 MBq (7.5 μCi) $^{125}I$-CC49 $(Fab')_2$, and the mice were euthanized at various time points in groups ($n$ = 3–4 in each group). The mean tumor radioactivity levels of $^{125}I$-CC49 $(Fab')_2$ in percent injected dose per gram (% ID/g) were 20.8 (6 h), 19.2 (24 h), 13.9 (48 h), and 9.1 (72 h) with the standard error of the mean (SEM) <15%. The mean liver radioactivity levels (% ID/g) were 6.7 (6 h), 11.1 (24 h), 5.3 (48 h), and 1.2 (72 h). The mean kidney radioactivity levels (% ID/g) were 15.8 (6 h), 2.2 (24 h), 0.8 (48 h), and 0.2 (72 h). The radiolocalization indexes (RI; ratios of % ID/g in tumor to % ID/g in normal tissue) of tumor/blood were 3.7 (6 h), 21.7 (24 h), 82.9 (48 h), and 277.0 (72 h). In comparison, the tumor/blood RIs for mice injected with $^{131}I$-CC49 IgG were 0.3 (4 h), 1.6 (24 h), and 4.1 (48 h). A separate pharmacokinetic study ($n$ = 4 mice) showed that $^{125}I$-CC49 $(Fab')_2$ had a plasma clearance $t_{½\alpha}$ of 26 min and $t_{½\beta}$ of 12 h, whereas $^{131}I$-CC49 IgG had a plasma clearance $t_{½\alpha}$ of 39 min and $t_{½\beta}$ of 113 h. Gamma imaging of a mouse bearing the tumor with a dose of 1.85 MBq (50 μCi) $^{131}I$-CC49 $(Fab')_2$ clearly visualized the tumor at 30 h after injection (1). The bladder was also visualized. Yokota et al. (2) conducted microautoradiography studies of $^{125}I$-CC49 $(Fab')_2$ in mice bearing LS-174T tumors (0.5–1 cm diameter) with a dose of 0.56 MBq (15 μCi) or 6.8 μg (68 pmol). The autoradiography showed that CC49 $(Fab')_2$ gave a greater penetration of tumor than CC49 IgG. This greater penetration decreased after 24 h.

Pavlinkova et al. (4) performed dual-label studies by injecting 0.185 MBq (5 μCi) $^{125}I$-CC49 $(Fab')_2$ and 0.0925 MBq (2.5 μCi) $^{131}I$-CC49-$(scFv)_2$ constructs simultaneously into nude mice ($n$ = 6) bearing s.c. LS-174T tumors on the back. The mean tumor radioactivity levels (% ID/g) with <20% SEM were 14.63 (0.5 h), 12.48 (1 h), 25.82 (4 h), 35.74 (6 h), 28.06 (24 h), 19.42 (48 h), 13.11 (72 h), and 12.55 (120 h). The blood radioactivity levels were 30.15 (0.5 h), 27.88 (1 h), 16.32 (4 h), 10.09 (6 h), 1.68 (24 h), 0.36 (48 h), 0.16 (72 h), and 0.15 (120 h). The mean liver radioactivity levels were 7.47 (0.5 h), 6.79 (1 h), 7.84 (4 h), 6.03 (6 h), 2.24 (24 h), 1.21 (48 h), 1.21 (72 h), and 1.49 (120 h). The mean kidney radioactivity levels were 11.48 (0.5 h), 11.31 (1 h), 9.78 (4 h), 7.44 (6 h), 2.10 (24 h), 0.52 (48 h), 0.25 (72 h), and 0.23 (120 h). The RI of tumor/blood at 24 h was 16.7. In comparison, the RIs of tumor/blood for $^{131}I$-CC49 IgG and $^{131}I$-CC49 $(scFv)_2$ were 3.4 and 80.3, respectively.

## Other Non-Primate Mammals

[PubMed]

No publication is currently available.

## Non-Human Primates

[PubMed]

No publication is currently available.

# Human Studies

[PubMed]

No publication is currently available.

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