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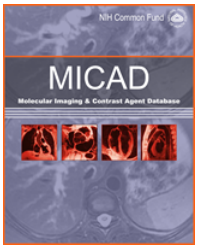

Radioiodinated anti–TAG-72 non-covalently linked CC49 divalent single-chain Fv antibody

$^{125}I/^{131}I$-CC49 $(scFv)_2$ Ab

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Chemical name:	Radioiodinated anti–TAG-72 non-covalently linked CC49 divalent single-chain Fv antibody	
Abbreviated name:	$^{125}I/^{131}I$-CC49 $(scFv)_2$ Ab	
Synonym:	$^{125}I/^{131}I$-CC49 Ab, $^{125}I/^{131}I$-CC49 divalent single-chain Fv recombinant antibody, $^{125}I/^{131}I$-CC49 non-covalent $(scFv)_2$ Ab, $^{125}I/^{131}I$-dimeric single-chain Fv antibody construct	
Agent Category:	Non-covalently linked CC49 divalent single-chain Fv antibody construct ($(scFv)_2$ Ab)	
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/ contrast:	$^{125}I/^{131}I$	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about TAG-72.

Background

[PubMed]

Radioiodinated anti–TAG-72 non-covalently linked CC49 divalent single-chain Fv antibody ($^{125}I/^{131}I$-CC49 $(scFv)_2$ Ab), which is formed by the conjugation of $^{125}I/^{131}I$ with a bioengineered recombinant anti–tumor-associated glycoprotein 72 (TAG-72) antibody construct, 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 with a gamma energy that is not ideal for *in vivo* imaging. ^{131}I has a physical half-life ($t_{½}$) of 8.02 days with a gamma energy that is high but acceptable for *in vivo* imaging.

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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-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 (2, 4).

Radiolabeled MAbs have been developed for both the diagnosis and treatment of tumors (12). Radiolabeled B72.3 and CC49 exhibit excellent tumor localization capabilities with potential diagnostic and therapeutic applications in the clinical setting (13, 14). 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, 15-17). One approach to minimize these problems is reducing intact antibodies to antibody fragments such as $F(ab')_2$ and Fab' (18). Another approach is the development of genetic engineering methods to obtain single-chain Fv constructs (scFv) and multivalent scFv constructs (11, 19, 20). These scFv constructs contain the variable regions of the light chain (V_L) and heavy chain (V_H) connected by a flexible linker. Colcher et al. (21) constructed the monomeric CC49 scFv Ab (~27 kDa), which selectively recognizes a unique STn epitope of TAG-72. The radioiodinated CC49 scFv appeared to clear rapidly from the blood with good tumor penetration (1, 20). To further improve the imaging kinetics by use of multivalency as a means of increasing the functional affinity, a non-covalently linked dimeric scFv was isolated on the basis of the spontaneous formation of the dimer from the monomeric CC49 scFv (1, 2, 4). This dimeric scFv was designated $(scFv)_2$ to distinguish it from the covalently-linked dimer $sc(Fv)_2$, in which two repeating chains of V_L and V_H are covalently linked in tandem (4). The radioiodinated CC49 $(scFv)_2$ showed good stability and improved *in vivo* pharmacokinetics compared with the intact CC49 IgG.

Synthesis

[PubMed]

Beresford et al. (4) and Pavlinkova et al. (1, 3) reported the construction and radiolabeling of the $^{125}I/^{131}I$-CC49 $(scFv)_2$ Ab. The covalent CC49 scFv (V_L-linker-V_H) was derived from the murine MAb CC49 and constructed with the 205C linker with 25 amino acids (LSADDAKKDAAKKDDAKKDDAKKDL) (22). The sequence was confirmed by deoxyribonucleic acid sequencing. The fragment was cloned into the pRW83 vector, which contained a chloramphenicol resistance gene for recombination selection, the *pen P* gene with a penP promoter and terminator, and a pe1B signal peptide that directed the recombinant protein to the periplasmic space to produce the biologically active protein (4). The expression plasmid was transformed into *Escherichia coli* strain AG1. When expressed, this construct spontaneously formed the monomeric CC49 scFv and dimeric CC49 $(scFv)_2$. These soluble proteins were purified by ion-exchange chromatography, and CC49 $(scFv)_2$ was separated from CC49 scFv by gel filtration chromatography on a Superdex 75 column. The preparation was shown to be >90% pure by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and the protein migrated to its theoretical molecular weight of 60,000 (3).

Radioiodination of CC49 $(scFv)_2$ Ab was performed with the use of 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (IodoGen) as the oxidizing agent (1, 3, 4). Briefly, 20–100 μg of CC49 $(scFv)_2$ Ab in 0.1 M sodium phosphate buffer (pH 7.2) was added to a glass tube coated with 20 μg IodoGen. Approximately 0.014–0.027 MBq (0.5–1.0 mCi) ^{131}I-labeled sodium iodide ($[^{131}I]NaI$) or ^{125}I-NaI was added and the mixture was incubated for 3 min. Size-exclusion chromatography on a Sephadex G-25 column was performed immediately to purify the radiolabeled antibody. The labeling efficiency was ~90% by high-performance liquid chromatography (HPLC)

(4). The specific activity of $^{125}I/^{131}I$-CC49 $(scFv)_2$ Ab was ~111–333 MBq/mg (3–9 mCi/mg) or 6.66–19.98 MBq/nmol (0.18–0.54 mCi/nmol) on the basis of the molecular mass of 60 kDa. The SDS-PAGE analysis showed that the non-covalently linked ^{131}I-CC49 $(scFv)_2$ was dissociated by the SDS and appeared only as its 30-kDa form. The radiochemical purity was >90% (3).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The immunoreactivity of radioiodinated CC49 $(scFv)_2$ Ab was assessed by solid-phase radioimmunoassay (RIA), with bovine submaxillary gland mucin (BSM) as the protein antigen attached to a solid-phase matrix (1, 2, 4). The specific binding for $^{125}I/^{131}I$-CC49 $(scFv)_2$ Ab was 88–94%. The binding kinetics of $^{125}I/^{131}I$-CC49 $(scFv)_2$ Ab was assessed by the real-time kinetic analysis with use of a surface plasmon resonance detector (4). The study reported the association constant (K_a) of unlabeled CC49 $(scFv)_2$ to be 4.46×10^7 M^{-1} (1-4). In comparison, the K_a for the intact CC49 MAb was 1.14×10^8 M^{-1}. The relative affinity constant of $^{125}I/^{131}I$-CC49 $(scFv)_2$ calculated from the Scatchard plot of the data obtained from the competition enzyme-linked immunosorbent assay was 1.70×10^9 M^{-1} (1).

In vitro stability studies of $^{125}I/^{131}I$-CC49 $(scFv)_2$ Ab showed that it was stable for at least 35 days when stored at 4°C (1, 4). No significant protein degradation or radioactive iodine release was determined by HPLC and radioimmunoassay. Only ~2–3% loss in immunoreactivity was exhibited (1). Unlabeled $(scFv)_2$ was stable at −70°C for >10 months without loss of immunoreactivity (1). When $^{125}I/^{131}I$-CC49 $(scFv)_2$ Ab was incubated in murine serum or 1% bovine serum albumin and incubated at 37°C for up to 4 days, HPLC analysis showed that radioiodinated CC49 $(scFv)_2$ maintained its full integrity (3).

Animal Studies

Rodents

[PubMed]

Biodistribution studies of ^{131}I-CC49 $(scFv)_2$ Ab were performed in nude mice bearing s.c. LS-174T human colon carcinomas (2, 4). Dual-label biodistribution studies were conducted with simultaneous i.v. administration of 0.0925 MBq (2.5 μCi) ^{131}I-CC49 $(scFv)_2$ Ab and 0.185 MBq (5 μCi) ^{125}I-covalently-linked CC49 $sc(Fv)_2$ Ab. The groups of mice (n = 5–6 per group) were euthanized at various time points. Similar separate experiments were performed for intact, radioiodinated CC49 IgG. The average tumor radioactivity levels of ^{131}I-CC49 $(scFv)_2$ Ab in percent injected dose per gram (% ID/g) were 5.94 (0.5 h), 6.37 (1 h), 6.91 (4 h), 6.45 (6 h), 5.52 (9 h), 4.48 (16 h), 4.29 (24 h), 2.56 (48 h), 1.92 (72 h), and 1.20 (120 h) with standard errors (SEM) <20% ID/g. There were also high radioactivity levels in the kidneys, spleen, blood, lungs, and liver at 0.5 h. By 24 h, these major organ radioactivity levels (% ID/g) decreased to 0.10 (blood), 0.42 (kidneys), 0.45 (spleen), 0.30 (liver), 0.09 (lungs), and 0.04 (heart). The rapid clearance from the major organs and the blood was favorable when compared with intact CC49 IgG. The tumor/blood ratios of ^{131}I-CC49 $(scFv)_2$ Ab were 4.19 (6 h), 44.05 (24 h), 41.09 (48 h), 29.75 (72 h), and 26.90 (120 h). In comparison, the tumor/blood ratios of radioiodinated CC49 IgG were 1.34 (6 h), 4.19 (24 h), 8.53 (48 h), and 51.52 (120 h). The radiolocalization indexes (ratios of % ID/g in tumor to % ID/g in normal tissue) at 24 h for ^{131}I-CC49 $(scFv)_2$ Ab were 42.9 (tumor/blood), 14.3 (tumor/liver), 9.5 (tumor/spleen), 10.2 (tumor/kidneys), and 47.7 (tumor/lungs). The 24-h radiolocalization indexes for radiolabeled CC49 IgG were 3.4 (tumor/blood), 6.1 (tumor/liver), 9.1 (tumor/spleen), 17.3 (tumor/kidneys), and 7.2 (tumor/lungs). The ^{125}I-covalently linked CC49 $sc(Fv)_2$ Ab had similar tumor localization, but there were differences in the degree of clearance from other normal tissues.

Pharmacokinetic studies of ^{131}I-CC49 $(scFv)_2$ Ab were conducted in mice bearing s.c. LS-147 tumors with a dose of 0.37 MBq (10 μCi) (4). ^{131}I-CC49 $(scFv)_2$ Ab showed rapid blood clearance with >50% cleared from the blood pool in <40 min. ^{131}I-CC49 $(scFv)_2$ Ab appeared to be eliminated by the kidneys and not retained by the extravascular space. Whole-body retention studies with a dose of 0.056 MBq (1.5 μCi) in mice bearing the tumors (n = 3) also showed rapid blood clearance. The ^{125}I-CC49 $sc(Fv)_2$ Ab showed similar pharmacokinetics and blood clearance. In another study, Pavlinkova et al. (3) reported that ^{131}I-CC49 $(scFv)_2$ had faster blood and whole-body clearances than those of CC49 IgG and CC49 $(Fab')_2$.

Pavlinkova et al. (1, 3) also compared CC49 $(scFv)_2$ with CC49 $F(ab')_2$ in mice (n = 6) bearing s.c. LS-174T carcinomas. Dual-label biodistribution studies were conducted with simultaneous i.v. administration of 0.0925 MBq (2.5 μCi) ^{131}I-CC49 $(Fab')_2$ and 0.185 MBq (5 μCi) ^{125}I-CC49 $(scFv)_2$. In this study, the tumor radioactivity levels of ^{125}I-CC49 $(scFv)_2$ were higher than those reported by Beresford et al. (4). The radiolocalization indexes at 24 h for ^{125}I-CC49 $(scFv)_2$ were 80.3 (tumor/blood), 25.5 (tumor/liver), 31.2 (tumor/spleen), 21.6 (tumor/kidneys), and 80.3 (tumor/lungs). In comparison, the radiolocalization indexes for ^{131}I-CC49 $(Fab')_2$ were 16.7 (tumor/blood), 12.5 (tumor/liver), 20.5 (tumor/spleen), 13.4 (tumor/kidneys), and 29.5 (tumor/lungs).

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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