Radioiodinated anti-TAG-72 CC49 tetravalent single-chain Fv antibody

$^{125}I/^{131}I$-CC49 $[sc(Fv)_2]_2$ Ab

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Chemical name:	Radioiodinated anti-TAG-72 CC49 tetravalent single-chain Fv antibody	
Abbreviated name:	$^{125}I/^{131}I$-CC49 $[sc(Fv)_2]_2$ Ab	
Synonym:	$^{125}I/^{131}I$-CC49 Ab, $^{125}I/^{131}I$-CC49 tetravalent single-chain Fv recombinant antibody, $^{125}I/^{131}I$-tetrameric CC49 $[sc(Fv)_2]_2$	
Agent Category:	Tetravalent single-chain Fv antibody construct ($[sc(Fv)_2]_2$ Ab)	
Target:	TAG-72	
Target Category:	Antibody to antigen binding	
Method of detection:	Single-photon emission tomography (SPECT). planar gamma imaging	
Source of signal:	^{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 CC49 tetravalent single-chain Fv antibody ($^{125}I/^{131}I$-CC49 $[sc(Fv)_2]_2$ Ab), which is formed by the conjugation of ^{125}I or ^{131}I with a bioengineered

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recombinant anti–tumor-associated glycoprotein 72 (TAG-72) antibody construct, has been developed for gamma imaging of cancers that express TAG-72 (1, 2). ^{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.

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 (3-6). 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 (7). The murine monoclonal antibody (MAb) B72.3 against TAG-72 was initially generated by immunization of mice with a membrane-enriched fraction of a human breast carcinoma (8). 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 (3, 4, 8, 9).

Radiolabeled MAbs have been developed for both the diagnosis and treatment of tumors (10). Radiolabeled B72.3 and CC49 have shown excellent tumor localization capabilities with potential diagnostic and therapeutic applications in the clinical setting (11, 12). Because of their relatively large size, radiolabeled intact MAbs tend to have unfavorable imaging kinetics, poor tumor penetration, and high potential for human anti-mouse antibody response (9, 13-15). One approach to minimize these problems is reducing intact antibodies to antibody fragments such as F(ab')$_2$ and Fab' (16). Another approach is the development of genetic engineering methods to obtain single-chain Fv constructs (scFv) and multivalent scFv constructs (9, 17, 18). 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. (19) constructed the monomeric CC49 scFv Ab (~27 kDa), which selectively recognizes a unique sialyl-Tn epitope of TAG-72. The radioiodinated CC49 scFv appeared to clear rapidly from the blood with good tumor penetration (18, 20). To further improve the imaging kinetics, Pavlinkova et al. (20) constructed the high-affinity dimer CC49 sc(Fv)$_2$ (~60 kDa). The radioiodinated CC49 sc(Fv)$_2$ showed good stability and increased avidity *in vivo* compared with the radioiodinated CC49 scFv construct. Goel et al. (1) formed the tetravalent [sc(Fv)$_2$]$_2$ construct (~120 kDa) that exhibited four potentially active antigen-binding sites and showed improved *in vitro* binding properties. Radioiodinated CC49 [sc(Fv)$_2$]$_2$ (^{125}I/^{131}I-CC49 [sc(Fv)$_2$]$_2$) was studied and evaluated for potential cancer therapy and diagnosis.

Synthesis

[PubMed]

Goel et al. (1) and Wittel et al. (2) reported the construction and radiolabeling of the ^{125}I/^{131}I-CC49 [sc(Fv)$_2$]$_2$ Ab. The CC49 scFv (V_L-linker-V_H) was derived from the murine MAb CC49 cloned in the yeast expression vector pPICZαA and constructed with

the 205C linker with 25 amino acids (LSADDAKKDAAKKDDAKKDDAKKDL). The bacterial scFv construct was used as the template DNA for the expression of the scFv in competent methylotrophic *Pichia pastoris* KM71 cells. The construction of the divalent sc(Fv)$_2$ (V_L-linker-V_H-linker-V_L-linker-V_H-His_6) was performed as described by Goel et al. (21) using the 205C helical linker in a *P. pastoris* expression system. Upon expression as a protein secreted by *P. pastoris*, 20–30% of the divalent form was found to spontaneously associate through noncovalent interactions into tetravalent [sc(Fv)$_2$]$_2$ or higher aggregates (>200 kDa) (1). Purification of scFv was expedited by the use of a hexahistidine tag attached to the COOH-terminal of the construct. The construct was purified from the secreted medium with immobilized metal-affinity chromatography, and the [sc(Fv)$_2$]$_2$ was separated on a Superdex 200 column. The yield was reported to be 2.0–3.5 mg/L. The preparation was shown to be >95% pure by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE study also confirmed that the two polypeptide chains of the tetramer were noncovalently linked. Size-exclusion high-performance liquid chromatography (HPLC) showed the molecular mass to be 120 kDa (9).

Radioiodination of CC49 [sc(Fv)$_2$]$_2$ Ab was performed with the use of 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (IodoGen) as the oxidizing agent (1). Briefly, 20–100 μg of CC49 [sc(Fv)$_2$]$_2$ Ab in sodium phosphate buffer (pH 7.2) was added to a glass tube coated with IodoGen. Approximately 0.5–1 mCi ^{125}I- or ^{131}I-labeled sodium iodide was added and the mixture was incubated for 3 min at room temperature (2). Free radioactive iodine was removed by a Sephadex 25 column. Instant thin-layer chromatography or gel filtration HPLC was used to determine the radiochemical purity of the radiolabeled antibody. Goel et al. (1) reported the specific activity of ^{125}I- or ^{131}I-CC49 [sc(Fv)$_2$]$_2$ Ab was ~111–333 MBq/mg (3–9 mCi/mg) or 13.3–40.0 MBq/nmol (0.36–1.1 mCi/nmol) on the basis of the molecular mass of 120 kDa. Wittel et al (2) reported that the specific activity of ^{125}I/^{131}I-CC49 [sc(Fv)$_2$]$_2$ Ab was only 29.6 MBq/mg (0.8 mCi/mg). The SDS-PAGE showed a radiochemical purity of ≥95%. The size-exclusion HPLC profiles were not altered by the radioiodination procedure.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The immunoreactivity of CC49 [sc(Fv)$_2$]$_2$ Ab was assessed by solid-phase competition enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay using bovine submaxillary gland mucin as the antigen (1, 2). The specific binding for CC49 [sc(Fv)$_2$]$_2$ Ab was 85–95%, and the nonspecific binding was 0.8–1.5%. Using the surface plasmon resonance technique to measure the real-time interactions, Goel et al. (1) reported the association constant (K_a) of unlabeled CC49 [sc(Fv)$_2$]$_2$ to be 1.02×10^8 M^{-1}. In comparison, the K_a for the intact CC49 MAb and CC49 sc(Fv)$_2$ constructs were 1.14×10^8 M^{-1} and 2.75×10^7 M^{-1}, respectively. Wittel et al. (2) evaluated [sc(Fv)$_2$]$_2$ by the solid-phase competitive enzyme-linked immunosorbent assay with the binding of biotinylated CC49 IgG. The [sc(Fv)$_2$]$_2$ antibody construct was able to inhibit 50% (IC_{50})

of the CC49 IgG binding at the concentration of 5.65 x 10^{-8} M. In comparison, the IC_{50} of the whole intact CC40 IgG was 5.0 x 10^{-9} M,

In vitro stability studies of $^{125}I/^{131}I$-CC49 $[sc(Fv)_2]_2$ Ab were conducted by incubating the radiolabeled MAb construct in 1% bovine serum albumin at 37°C (1). By HPLC analysis, the $^{125}I/^{131}I$-CC49 $[sc(Fv)_2]_2$ MAb was stable *in vitro* up to 3 days at 37°C, with ≥90% radioactivity associated with the antibody until 24 h. By 72 h, there was 5.6% radioactivity associated with the smaller $sc(Fv)_2$ and 2.9% radioactivity associated with low molecular weight proteins (M_r = 45,000 and 30,000). Goel et al. (1) also reported that the unlabeled antibody could be stored at −70°C for 6 months without any loss of immunoreactivity or evidence of breakdown products.

Animal Studies

Rodents

[PubMed]

Biodistribution studies of $^{125}I/^{131}I$-CC49 $[sc(Fv)_2]_2$ Ab were performed in nude mice bearing s.c. LS-174T human colon carcinomas (~200–300 mm^3) (1). Dual-label biodistribution studies were conducted with simultaneous i.v. administration of 0.185 MBq (5 μCi) ^{125}I-CC49 $sc(Fv)_2$ Ab and 0.0925 MBq (2.5 μCi) ^{131}I-CC49 $[sc(Fv)_2]_2$ Ab in one group (n = 6) of mice. Another group of mice (n = 6) received 0.185 MBq (5 μCi) ^{125}I-CC49 $[sc(Fv)_2]_2$ Ab and 0.0925 MBq (2.5 μCi) ^{131}I-CC49 IgG Ab. The elimination half-life ($t_{½}$) of radioiodinated CC49 $[sc(Fv)_2]_2$ Ab was 170 min. The blood clearance had an α-phase and a β-phase. Wittel et al. (2) determined the $t_{½\beta}$ to be 116.30 min. Goel et al. (1) also found a third phase that corresponded to the clearance of free radioiodine. The whole-body clearance $t_{½}$ was 8.9 ± 1.3 h. At 48 h, ~95% of the radioactivity was cleared from the body. In comparison, only 75% of CC49 IgG radioactivity was cleared in 48 h. There was also a 2-fold increase in the blood biological $t_{½}$ of radioiodinated CC49 $[sc(Fv)_2]_2$ Ab as compared with the dimeric CC49 $sc(Fv)_2$. The tumor radioactivity levels of radioiodinated CC49 $[sc(Fv)_2]_2$ Ab in percent injected dose per gram (% ID/g) were 6.2 ± 1.0 (0.5 h), 9.0 ± 1.2 (1 h), 12.3 ± 1.6 (4 h), 21.3 ± 1.3 (6 h), 17.0 ± 1.6 (16 h), 10.5 ± 1.1 (24 h), and 8.2 ± 0.1 (48 h) (1). However, Wittel et al. (2) reported only ~2-7% ID/g tumor radioactivity levels (extrapolated from Figure 3) from 0.5-48 h for the dose of 0.185 MBq (5 μCi) ^{125}I-CC49 $[sc(Fv)_2]_2$ Ab. In the study of Goel et al. (1), the radioactivity levels of ^{125}I-CC49 $sc(Fv)_2$ and ^{131}I-CC49 IgG Ab at 6 h were 9.8 ± 1.3% ID/g and 17.3 ± 1.1, respectively. There were also significant radioactivity levels of radioiodinated CC49 $[sc(Fv)_2]_2$ Ab in the spleen, liver, and kidneys. The radioactivity levels (% ID/g) for major organs at 0.5 h were 22.0 ± 0.6 (blood), 7.9 ± 0.6 (liver), 9.0 ± 1.4 (spleen), 8.5 ± 0.6 (kidneys), 4.7 ± 0.3 (heart), and 5.6 ± 0.1 (lungs). At 6 h, these levels changed to 4.5 ± 0.4 (blood), 4.9 ± 0.6 (liver), 5.1 ± 1.5 (spleen), 2.9 ± 0.2 (kidneys), 1.7 ± 0.1 (heart), and 0.7 ± 0.2 (lungs). The tumor/liver, tumor/blood, and tumor/spleen ratios at 24 h were 6.2:1, 35:1, and 10.5:1, respectively.

The *in vivo* stability of radioiodinated CC49 $[sc(Fv)_2]_2$ Ab in blood plasma was also analyzed with HPLC (1). The $^{125}I/^{131}I$-CC49 $[sc(Fv)_2]_2$ Ab fraction of the radioactivity was >70% at 1 h but only 49% at 2 h. There was 38% radioactivity at 2 h in higher molecular weight forms. Wittel et al. (2) reported that radioiodinatedI-CC49 $[sc(Fv)_2]_2$ Ab remained intact with 60.27% at 1 h, 61.84% at 2 h, and 38.75% at 12 h. The amounts of the higher molecular weight forms (>200 kDa aggregates) were 18.65% at 1 h, 20.78% at 2 h, and 20.00% at 12 h.

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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