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

^{125}I-CC49 sc(Fv)$_2$ Ab

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Chemical name:	Radioiodinated-anti–TAG-72 covalently linked CC49 divalent single-chain Fv antibody	
Abbreviated name:	^{125}I-CC49 sc(Fv)$_2$ Ab	
Synonym:	^{125}I-CC49 Ab, ^{125}I-CC49 divalent single-chain Fv recombinant antibody, ^{125}I-CC49 covalent sc(Fv)$_2$ Ab	
Agent Category:	Divalently linked CC49 divalent single-chain Fv antibody construct sc(Fv)$_2$ 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:	^{125}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 covalently linked CC49 divalent single-chain Fv antibody (^{125}I-CC49 sc(Fv)$_2$ Ab), which is formed by the conjugation of ^{125}I with a bioengineered recombinant anti–tumor-associated glycoprotein 72 (TAG-72) antibody construct, has

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been developed for gamma imaging of cancers that express TAG-72 (1). ^{125}I has a physical half-life ($t_{½}$) of 60 days with a gamma energy that is not ideal for *in vivo* imaging, but there are other radioactive iodines (^{123}I, ^{131}I) available that are more suitable 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 (2-5). 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 (6). 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 (7). 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 (2, 3, 7, 8). CC49 MAb appears to react with a unique disaccharide sialyl-Tn (STn) epitope on the TAG-72 (1, 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 (8, 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 (8, 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 STn 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 by use of multivalency as a means of increasing the functional affinity, Beresford et al. (1) reported the construction of a high-affinity covalently linked dimer CC49 $sc(Fv)_2$ (~58 kDa). This dimeric structure does not rely on non-covalent interactions and is designated as $sc(Fv)_2$ to distinguish it from the non-covalently linked dimer $(scFv)_2$ derived from the monomeric construct. CC49 $sc(Fv)_2$ does not require additional chemical processing for *in vivo* studies. The radioiodinated CC49 $sc(Fv)_2$ showed good stability and improved *in vivo* pharmacokinetics compared with the radioiodinated non-covalently linked CC49 $(scFv)_2$ construct and intact CC49 IgG.

Synthesis

[PubMed]

Beresford et al. (1) reported the construction and radiolabeling of the ^{125}I-CC49 sc(Fv)$_2$ 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) (21). The dimer sc(Fv)$_2$ (V_L-linker-V_H-linker-V_L-linker-V_H) was constructed by covalently linking the two repeating chains of V_L and V_H in tandem with use of the same helical linker between each region. The nucleotide sequence of the sc(Fv)$_2$ construct was cloned into the pRW83 vector (1). The expression plasmid was transformed into *Escherichia coli* strain AG1 or BL21. The CC49 sc(Fv)$_2$ protein was prepared from the peri-plasmic fraction of 16- to 18-hr cultures. After separation, sc(Fv)$_2$ was purified by ion-exchange and size-exclusion chromatography. 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.

Radioiodination of CC49 sc(Fv)$_2$ 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)$_2$ 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) ^{125}I-labeled sodium iodide was added and the mixture was incubated for 3 min. Size-exclusion chromatography was performed immediately to purify the radiolabeled antibody. The labeling efficiency was 50%. The specific activity of ^{125}I-CC49 sc(Fv)$_2$ Ab was ~111–333 MBq/mg (3–9 mCi/mg) or 6.44–19.3 MBq/nmol (0.17–0.522 mCi/nmol) on the basis of the molecular mass of 58 kDa. The SDS-PAGE analysis showed a radiochemical purity of ~90%.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The immunoreactivity of radioiodinated CC49 sc(Fv)$_2$ Ab was assessed by the solid-phase radioimmunoassay (RIA), with bovine submaxillary gland mucin (BSM) as the protein antigen attached to a solid-phase matrix (1). The specific binding for radioiodinated CC49 sc(Fv)$_2$ Ab was 94%, and the nonspecific binding was 2%. In a competitive RIA, the ^{125}I-CC49 sc(Fv)$_2$ Ab was completely inhibited by CC49 IgG, F(ab')2 and Fab' for binding to BSM (1). The binding kinetics of ^{125}I-CC49 sc(Fv)$_2$ Ab was assessed by the real-time kinetic analysis with use of a surface plasmon resonance detector (1). The study reported the association constant (K_a) of unlabeled CC49 sc(Fv)$_2$ to be 3.34×10^7 M-1. In comparison, the K_a for the intact CC49 MAb and the non-covalently linked CC49 (scFv)$_2$ construct were 1.14×10^8 M-1 and 4.46×10^7 M-1, respectively.

In vitro stability studies of ^{125}I-CC49 sc(Fv)$_2$ Ab showed that it was stable for at least 14 days when stored at 4°C. No significant protein degradation or radioactive iodine release was observed. Only a 5–10% loss in immunoreactivity was observed over a 2-week period. Unlabeled sc(Fv)$_2$ was stable at −70°C for 5 months without loss of immunoreactivity. When ^{125}I/^{131}I-CC49 sc(Fv)$_2$ Ab was incubated in murine serum or 1% BSA and incubated at 37°C for up to 48 h, high-performance liquid chromatography

analysis of 24–48 hr samples showed only a small increase in high molecular weight forms.

Animal Studies

Rodents

[PubMed]

Biodistribution studies of ^{125}I-CC49 sc(Fv)$_2$ Ab were performed in nude mice bearing s.c. LS-174T human colon carcinomas (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-non-covalently linked CC49 (scFv)$_2$Ab and euthanized at various time points in groups of mice (n = 5–6). The average tumor radioactivity levels of ^{125}I-CC49 sc(Fv)$_2$Ab in percent injected dose per gram (% ID/g) were 6.12 (0.5 h), 6.63 (1 h), 6.78 (4 h), 6.27 (6 h), 5.74 (9 h), 4.67 (16 h), 4.29 (24 h), 2.62 (48 h), 1.94 (72 h), and 1.21 (120 h) with standard errors (SEM) <20% ID/g. There were also high radioactivity levels in the kidneys, spleen, and liver. At 0.5 h, the major organ radioactivity levels (% ID/g) were 18.30 (blood), 27.85 (kidneys), 10.01 (spleen), 5.65 (liver), 7.25 (lungs), and 4.31 (heart). By 24 h, these levels decreased to 0.07 (blood), 0.36 (kidneys), 0.18 (spleen), 0.20 (liver), 0.07 (lungs), and 0.03 (heart). The ^{131}I-non-covalently linked CC49 (scFv)$_2$ Ab had similar tumor localization, but there were differences in the degree of clearance from other normal tissues. As early as 0.5–1 h, the ^{131}I-CC49 (scFv)$_2$ Ab radioactivity levels in major organs were greater than those of ^{125}I-CC49 sc(Fv)$_2$Ab. This trend was maintained up to 48 h. The biggest differences were in the radioactivity levels in the liver and spleen. At 6 h, ^{125}I-CC49 sc(Fv)$_2$ Ab had radioactivity levels of 1.63% ID/g and 1.53% ID/g for the liver and spleen, respectively. In comparison, ^{131}I-CC49 (scFv)$_2$ Ab had radioactivity levels of 2.25% ID/g and 2.28% ID/g for the liver and spleen, respectively. The tumor/blood ratios of ^{125}I-CC49 sc(Fv)$_2$ Ab were 4.79 (6 h), 60.19 (24 h), 48.29 (48 h), 28.93 (72 h), and 27.79 (120 h). The radiolocalization indexes (ratios of % ID/g in tumor to % ID/g in normal tissue) at 24 h of ^{125}I-CC49 sc(Fv)$_2$ Ab were 61.3 (tumor/blood), 21.5 (tumor/liver), 23.8 (tumor/spleen), 11.9 (tumor/kidneys), and 61.3 (tumor/lungs). In comparison, the values 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 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).

Pharmacokinetic studies of ^{125}I-CC49 sc(Fv)$_2$ Ab were conducted in mice bearing s.c. LS-147 tumors with a dose of 0.37 MBq (10 μCi) (1). ^{125}I-CC49 sc(Fv)$_2$ Ab showed rapid blood clearance with >50% cleared from the blood pool in <40 min. ^{125}I-CC49 sc(Fv)$_2$ 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.

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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