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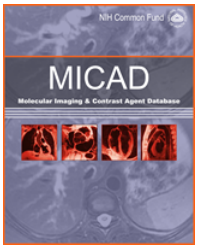

^{99m}Tc-Hydrazinonicotinamide-anti-TAG-72 CC49 tetravalent single-chain Fv monoclonal antibody

^{99m}Tc-HYNIC-CC49 [sc(Fv)$_2$]$_2$ MAb

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Chemical name:	^{99m}Tc-Hydrazinonicotinamide-anti-TAG-72 CC49 tetravalent single-chain Fv monoclonal antibody	
Abbreviated name:	^{99m}Tc-HYNIC-CC49 [sc(Fv)$_2$]$_2$ MAb	
Synonym:	^{99m}Tc-CC49 [sc(Fv)$_2$]$_2$ MAb, ^{99m}Tc-CC49 MAb	
Agent Category:	Tetravalent single-chain Fv monoclonal antibody ([sc(Fv)$_2$]$_2$ MAb)	
Target:	TAG-72	
Target Category:	Antibody to antigen binding	
Method of detection:	Single-photon emission computed tomography (SPECT), planar gamma imaging	
Source of signal:	^{99m}Tc	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about TAG-72

Background

[PubMed]

^{99m}Tc-Hydrazinonicotinamide-anti-TAG-72 CC49 tetravalent single-chain Fv monoclonal antibody (^{99m}Tc-HYNIC-CC49 [sc(Fv)$_2$]$_2$ MAb), which is formed by the conjugation of ^{99m}Tc with a bioengineered anti–tumor-associated glycoprotein 72 (TAG-72) antibody construct, has been developed for single-photon emission computed tomography (SPECT) imaging of cancers that express TAG-72 (1). ^{99m}Tc is a gamma emitter with a half-life ($t_{½}$) of 6.02 h.

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 was initially generated by immunization of mice with a membrane-enriched

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fraction of a human breast carcinoma (7). With use of affinity-purified TAG-72 from LS-174T as an immunogen, CC49 and other anti–TAG-2 monoclonal antibodies with higher affinity constants (K_a) have been produced and characterized (1-3, 7).

Radiolabeled MAbs have been developed for both the diagnosis and treatment of tumors (8). Radiolabeled B72.3 and CC49 have shown excellent tumor localization capabilities with potential diagnostic and therapeutic applications in the clinical setting (9, 10). Because of their relatively large size, radiolabeled intact monoclonal antibodies tend to have unfavorable imaging kinetics, poor tumor penetration, and high potential for human anti-mouse antibody response (1, 11-13). One approach to minimize these problems is reducing intact antibodies to antibody fragments such as F(ab')$_2$ and Fab'. (14). Another approach is the development of genetic engineering methods to obtain single-chain Fv constructs (scFv) and multivalent scFv constructs (1, 15, 16). 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. (17) constructed the monomeric CC49 scFv MAb (~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 (16, 18). To further improve the imaging kinetics, Pavlinkova et al. (18) 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. (19) 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.

MAbs can be labeled with ^{99m}Tc, a gamma emitter with ideal SPECT imaging properties, by direct or indirect labeling. Direct labeling involves reduction of ^{99m}Tc-pertechnetate and nonspecific binding of the reduced ^{99m}Tc to donor atoms, namely thiol, amide, amino, and carboxylate (20). Indirect labeling uses a bifunctional chelating agent, which can be more binding site–specific on the MAb molecule. Goel et al. (1) used hydrazinonicotinamide (HYNIC) as a bifunctional coupling agent to label divalent CC49 sc(Fv)$_2$ and tetravalent CC49 [sc(Fv)$_2$]$_2$ with ^{99m}Tc. Both ^{99m}Tc-HYNIC-CC49 sc(Fv)$_2$ MAb and ^{99m}Tc-HYNIC-CC49 [sc(Fv)$_2$]$_2$ MAb showed good tumor targeting and *in vivo* biodistribution properties.

Synthesis

[PubMed]

Goel et al. (1) reported the construction and radiolabeling of the ^{99m}Tc-HYNIC-CC49 [sc(Fv)$_2$]$_2$ MAb 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. The bacterial scFv construct was used as the template DNA for the expression of the scFv in competent methylotrophic *P. 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 linker in a *P. pastoris* expression system. On expression as a secreted protein by the *P. pastoris*, 20–30% of the divalent form was found to spontaneously associate through noncovalent interactions into the tetravalent [sc(Fv)$_2$]$_2$ or higher aggregates (>200 kDa) (19). 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/liter. 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. Competitive solid-phase competition enzyme-linked immunosorbent assay (ELISA) with bovine submaxillary gland mucin (BSM) confirmed the immunoreactivity of the tetravalent construct with $K_a = 1.0 \times 10^8$ M-1. To radiolabel the agent, the hydrazino-modification of CC49 [sc(Fv)$_2$] was achieved by reacting the construct with the *N*-hydroxysuccinimide ester of succinimidyl-6-hydrazinonicotinate hydrochloride (SHNH) at a molar ratio of 10:1 in sodium phosphate buffer (pH 7.8) in the dark at 4°C overnight. It was estimated that there were ~2.8 SHNH

groups per [sc(Fv)$_2$]$_2$. In the radiolabeling procedure, sodium ^{99m}Tc-pertechnetate, tricine, and stannous chloride were first mixed, then SHNH-derivatized CC49 [sc(Fv)$_2$]$_2$ was added, and the reaction mixture was incubated at room temperature for 45 min. The final ^{99m}Tc-HYNIC-CC49 [sc(FV)$_2$]$_2$ MAb was purified on a Sephadex G-25 column. The final radiochemical yield was not reported. The specific activity was 74–111 MBq/mg (2–3 mCi/mg) or 8.88–13.32 MBq/μmol (0.24–0.36 mCi/μmol) on the basis of the estimated 120-kDa molecular mass with a radiochemical purity ≥95% (HPLC analysis).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The immunoreactivity of ^{99m}Tc-HYNIC-CC49 [sc(Fv)$_2$]$_2$ MAb by solid-phase ELISA with immobilized BSM was 85–95% with a nonspecific binding of 0.8–1.5% (1).

In vitro stability studies of ^{99m}Tc-HYNIC-CC49 [sc(Fv)$_2$]$_2$ MAb were conducted by incubating the radiolabeled MAb construct in 1% bovine serum albumin (BSA) or 1% mouse serum at 37°C for 24 h (1). By HPLC analysis, <20% loss of ^{99m}Tc label was detected in 1% BSA. In the 1% mouse serum, 65.2% of ^{99m}Tc-HYNIC-CC49 [sc(Fv)$_2$]$_2$ MAb remained intact, and 6.4% and 18.2% were associated with >130-kDa and <50-kDa proteins, respectively; ~10.2% of the ^{99m}Tc radioactivity was associated with a 60-kDa protein. This suggested the possibility of dissociation of the tetramers to the dimmers.

Using the Scatchard plot and surface plasmon resonance technique to measure the real-time interactions, Goel et al. (19) reported the K_a of unlabeled CC49 [sc(Fv)$_2$]$_2$ to be 1.02×10^8 M^{-1} in binding to the immobilized BSM. 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.

Animal Studies

Rodents

[PubMed]

Biodistribution studies of ^{99m}Tc-HYNIC-CC49 [sc(Fv)$_2$]$_2$ MAb were performed in nude mice bearing LS-174T s.c. human colon carcinomas (~250–300 mm^3) (1). Each mouse received 0.37 MBq (10 μCi) of ^{99m}Tc-HYNIC-CC49 sc(Fv)$_2$ MAb by i.v. administration. The blood elimination $t_{½}$ was 307 min, and the whole-body clearance $t_{½}$ was 265 ± 39 min (n = 3). The radioactivity levels (n = 3 × 2) in percentage injected dose per gram (% ID/g) of the tumors were 13.3 ± 1.4 (0.5 h), 14.5 ± 0.9 (1 h), 15.0 ± 1.2 (4 h), 19.1 ± 1.1 (6 h), 5.6 ± 0.2 (16 h), and 2.5 ± 0.0 (24 h). At 16 h, the tumor/blood ratio was 12.7:1 with tumor localization approximately three-fold higher than that of ^{99m}Tc-sc(Fv$_2$). At 0.5 h, the radioactivity levels (% ID/g) of major organs were 27.3 ± 1.5 (blood), 18.0 ± 1.3 (liver), 13.2 ± 0.8 (spleen), 17.2 ± 0.5 (kidneys), and 7.2 ± 1.1 (lungs). At 4 h, these levels changed to 7.5 ± 0.5 (blood), 20.1 ± 1.1 (liver), 10.9 ± 0.5 (spleen), 14.3 ± 1.9 (kidneys), and 2.7 ± 0.5 (lungs). By 24 h, these levels declined to 0.1 ± 0.0 (blood), 1.6 ± 0.0 (liver), 1.0 ± 0.0 (spleen), 1.1 ± 0.0 (kidneys), and 0.1 ± 0.0 (lungs). Macroautoradiography studies performed in mice at 6 h and 16 h after radioactivity administration confirmed a high degree of tumor localization and negligible retention in the blood and normal organs (1). The exceptions were the liver and pancreas. The tumor remained positive with decreased background radioactivity at 16 h after injection. In comparison, the radioactivity level of ^{99m}Tc-HYNIC-CC49 [sc(Fv)$_2$]$_2$ MAb was about three-fold less than that of the divalent ^{99m}Tc-HYNIC-CC49 sc(Fv)$_2$ MAb

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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