

# Radioiodinated anti-TAG-72 CC49 Fab' antibody fragment

<sup>125</sup>I-CC49 Fab'

Kenneth T. Cheng, PhD<sup>1</sup>

Created: March 10, 2008; Updated: April 9, 2008.

|                                   |   |   |
|-----------------------------------|---|---|
| <b>Chemical name:</b>             | Radioiodinated anti-TAG-72 CC49 Fab' antibody fragment                          |   |
| <b>Abbreviated name:</b>          | <sup>125</sup> I-CC49 Fab'  |   |
| <b>Synonym:</b>                   | <sup>125</sup> I-CC49 Ab, <sup>125</sup> I-CC49                                 |   |
| <b>Agent Category:</b>            | Fab' antibody fragment  |   |
| <b>Target:</b>                    | (Sialyl-Tn (STn)) TAG-72  |   |
| <b>Target Category:</b>           | Antibody to antigen binding   |   |
| <b>Method of detection:</b>       | Single-photon emission computed tomography (SPECT), planar gamma imaging        |   |
| <b>Source of signal/contrast:</b> | <sup>125</sup> I  |   |
| <b>Activation:</b>                | No  |   |
| <b>Studies:</b>                   | <ul style="list-style-type: none"><li><i>In vitro</i></li><li>Rodents</li></ul> | Click on <a href="#">protein</a> , <a href="#">nucleotide</a> (RefSeq), and <a href="#">gene</a> for more information about TAG-72. |

## Background

[PubMed]

Radioiodinated anti-TAG-72 CC49 Fab' antibody fragment (<sup>125</sup>I-CC49 Fab'), which is formed by the conjugation of <sup>125</sup>I with an anti-tumor-associated glycoprotein 72 (TAG-72) Fab' antibody fragment, has been developed for gamma imaging of cancers that express TAG-72 (1-3). <sup>125</sup>I has a relatively long physical half-life (*t*<sub>1/2</sub>) of 60 days and a gamma energy that makes it suitable for imaging only in small animals. <sup>123</sup>I, another

---

<sup>1</sup> National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.nih.gov.

radioiodine, has better physical properties for single-photon emission computed tomography (SPECT) and planar gamma imaging in humans.

The TAG-72 antigen was isolated from the LS-174T human colon cancer xenograft as a high molecular weight glycoprotein (molecular mass of  $10^3$  kDa) with mucin-like characteristics (4-7). The TAG-72 antigen 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 (8). 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 (9). 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 have been produced and characterized (4, 5, 9, 10). CC49 MAb appears to react with a unique disaccharide sialyl-Tn (STn) epitope on TAG-72 (11, 12).

Radiolabeled MAbs have been developed for both the diagnosis and treatment of tumors (13). Radiolabeled B72.3 and CC49 exhibit excellent tumor localization capabilities with potential diagnostic and therapeutic applications in the clinical setting (14, 15). 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 (10, 16-18). One possible approach to minimize these problems is reducing intact antibodies to smaller antibody fragments such as  $F(ab')_2$  and Fab' (19). Another approach is the development of genetic engineering methods to obtain single-chain Fv constructs (scFv) and multivalent scFv constructs (10, 20, 21). 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 a molecular weight of 100,000 or the Fab' fragment with a molecular weight of 50,000 (1). Because of the smaller size, the Fab' fragment has faster blood clearance and better tumor penetration than intact IgG (2, 22). The removal of the Fc portion during the enzymatic cleavage also reduces nonspecific binding of Fab' to Fc receptors. The *in vitro* and *in vivo* properties of the radioiodinated CC49 Fab' fragment have been studied (1-3, 23, 24).

## Synthesis

[PubMed]

CC49 MAb IgG was developed by the immunization of mice with a membrane-enriched fraction of a human breast tumor carcinoma or purified TAG-72 (23, 25). CC49 IgG was purified from ascetic fluid obtained from immunized mice. CC49 Fab' was prepared by pepsin digestion of the purified CC49 IgG (1, 2, 23). Briefly, 10 mg of IgG was first adjusted to 10 mM dithiothreitol in 150 mM Tris-HCl:150 mM NaCl:2 mM EDTA (pH 8.0) and incubated at room temperature for 1 h. The thiol group was blocked using 21–22 mM iodoacetamide at room temperature for 15 min. The IgG was then dialyzed against 100 mM sodium acetate (pH 4.5); 2% pepsin was then added, and the mixture was incubated at 37°C for 16 h (1, 2, 23). The mixture was adjusted to pH 7.5–8.0, and the Fab'

fragment was separated by molecular sieving. Further purification was performed by ion-exchange chromatography.

Radioiodination of CC49 Fab' was performed with the use of 1,3,4,6-tetrachloro-3 $\alpha$ ,6 $\alpha$ -diphenylglycoluril (IodoGen) as the oxidizing agent (1, 23). Briefly, CC49 Fab' in 0.1 M sodium phosphate buffer (pH 7.2) was added to a glass tube coated with 20  $\mu\text{g}$  IodoGen. Approximately 18.5 MBq (0.5 mCi)  $^{125}\text{I}$ -sodium iodide was added, and the mixture was incubated for 2 min at room temperature. Gel filtration chromatography was performed immediately to remove the unincorporated  $^{125}\text{I}$ . The specific activity of  $^{125}\text{I}$ -CC49 Fab' was 111–185 kBq/ $\mu\text{g}$  (3–5  $\mu\text{Ci}/\mu\text{g}$ ) or 5.55–9.25 MBq/nmol (0.15–0.25 mCi/nmol) on the basis of the molecular weight of 50,000. The labeling efficiency and radiochemical purity were not reported, but the molecular weight and integrity of  $^{125}\text{I}$ -CC49 Fab' was confirmed by sodium dodecyl sulfate:polyacrylamide gel electrophoresis. Yokota et al. (3) prepared  $^{125}\text{I}$ -CC49 Fab' with a specific activity of 340.4–399.6 kBq/ $\mu\text{g}$  (9.2–10.8  $\mu\text{Ci}/\mu\text{g}$ ) or 17.02–19.98 MBq/nmol (0.46–0.54 mCi/nmol). The radiochemical purity was >90% in this preparation. Yokota et al. (2) also reported a specific activity of 81.4–432.9 kBq/ $\mu\text{g}$  (2.2–11.7  $\mu\text{Ci}/\mu\text{g}$ ) or 4.07–21.83 MBq/nmol (0.11–0.59 mCi/nmol).

## In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Milenic et al. (1) used different sources of antigen to perform solid-phase radioimmunoassays. The binding percentages of  $^{125}\text{I}$ -CC49 Fab' were 5.1%, 11.3%, 8.2%, and 0.3% for purified TAG-72, LS-174T colon carcinoma xenograft, colon carcinoma biopsy, and melanoma xenograft, respectively. The relative affinity constant of  $^{125}\text{I}$ -CC49 Fab' was  $\sim 2.6 \times 10^8 \text{ M}^{-1}$ , which was similar to that of intact  $^{125}\text{I}$ -CC49 IgG ( $4.3 \times 10^8 \text{ M}^{-1}$ ). In another study, Yokota et al. (2) reported immunoreactivity of 29% for  $^{125}\text{I}$ -CC49 Fab' to an extract positive for TAG-72. In comparison, the immunoreactivity of  $^{125}\text{I}$ -CC49 IgG in this study was 23%.

## Animal Studies

### Rodents

[PubMed]

Biodistribution studies of  $^{125}\text{I}$ -CC49 Fab' were performed in nude mice bearing subcutaneous LS-174T human colon carcinomas (0.5–0.8 cm in diameter) on the back (1). Mice were given i.v. injections of 0.28 MBq (7.5  $\mu\text{Ci}$ )  $^{125}\text{I}$ -CC49 Fab' and then were euthanized at various time points in groups ( $n = 4$ –6 mice in each group). The mean tumor radioactivity levels of  $^{125}\text{I}$ -CC49 Fab' in percent injected dose per gram (% ID/g) were 6.2 (6 h), 3.7 (24 h), 2.7 (48 h), and 2.0 (72 h) with a standard error of the mean <15%. The mean liver radioactivity levels (% ID/g) were 2.3 (6 h), 0.9 (24 h), 0.4 (48 h), and 0.2 (72 h). The mean kidney radioactivity levels (% ID/g) were 37.0 (6 h), 4.4 (24 h), 0.3 (48 h), and 0.1 (72 h). The tumor/blood radiolocalization indexes (ratios of % ID/g in

tumor to % ID/g in normal tissue) were 7.8 (6 h), 48.4 (24 h), 144.2 (48 h), and 242.6 (72 h). In comparison, the tumor/blood radiolocalization indexes for mice injected with  $^{131}\text{I}$ -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  $^{125}\text{I}$ -CC49 Fab' had a plasma clearance  $t_{1/2\alpha}$  of 9.1 min and  $t_{1/2\beta}$  of 88 min, whereas  $^{131}\text{I}$ -CC49 IgG had a plasma clearance  $t_{1/2\alpha}$  of 39 min and  $t_{1/2\beta}$  of 113 h. Gamma imaging of a mouse bearing the tumor with a dose of 3.7 MBq (100  $\mu\text{Ci}$ )  $^{131}\text{I}$ -CC49 Fab' clearly visualized the tumor at 30 h after injection (1).

Yokota et al. (2) conducted microautoradiography studies of  $^{125}\text{I}$ -CC49 Fab' (a dose of 4.1 MBq (112  $\mu\text{Ci}$ ) or 9.6  $\mu\text{g}$  (192 pmol)) in mice bearing LS-174T tumors (0.5–1 cm diameter). The microautoradiography showed that  $^{125}\text{I}$ -CC49 Fab' had intermediate tumor penetration in a size-related manner. It provided greater tumor penetration than  $^{125}\text{I}$ -CC49 IgG during the early time points. This greater penetration decreased after 24 h. In another report, Yokota et al. (3) found that the  $^{125}\text{I}$ -CC49 Fab' radioactivity was primarily associated with the cortical tubules in the kidney and Kupffer cells in the liver. In the spleen, the radioactivity was localized to the marginal zone surrounding the lymphoid follicles. The authors suggested that the localization in the liver and spleen in the mice bearing LS-174T tumors was antigen-mediated. In comparison,  $^{125}\text{I}$ -CC49 IgG radioactivity was found in the renal vasculature and associated with hepatocytes in the liver.

## 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.

## References

1. Milenic D.E., Yokota T., Filpula D.R., Finkelman M.A., Dodd S.W., Wood J.F., Whitlow M., Snoy P., Schlom J. Construction, binding properties, metabolism, and tumor targeting of a single-chain Fv derived from the pancreatic carcinoma monoclonal antibody CC49. *Cancer Res.* 1991;**51**(23 Pt 1):6363–71. PubMed PMID: 1933899.
2. Yokota T., Milenic D.E., Whitlow M., Schlom J. Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. *Cancer Res.* 1992;**52**(12):3402–8. PubMed PMID: 1596900.

3. Yokota T., Milenic D.E., Whitlow M., Wood J.F., Hubert S.L., Schlom J. Microautoradiographic analysis of the normal organ distribution of radioiodinated single-chain Fv and other immunoglobulin forms. *Cancer Res.* 1993;**53**(16):3776–83. PubMed PMID: 8339291.
4. Muraro R., Kuroki M., Wunderlich D., Poole D.J., Colcher D., Thor A., Greiner J.W., Simpson J.F., Molinolo A., Noguchi P. et al. Generation and characterization of B72.3 second generation monoclonal antibodies reactive with the tumor-associated glycoprotein 72 antigen. *Cancer Res.* 1988;**48**(16):4588–96. PubMed PMID: 3396010.
5. Johnson V.G., Schlom J., Paterson A.J., Bennett J., Magnani J.L., Colcher D. Analysis of a human tumor-associated glycoprotein (TAG-72) identified by monoclonal antibody B72.3. *Cancer Res.* 1986;**46**(2):850–7. PubMed PMID: 3940648.
6. Katari R.S., Fernsten P.D., Schlom J. Characterization of the shed form of the human tumor-associated glycoprotein (TAG-72) from serous effusions of patients with different types of carcinomas. *Cancer Res.* 1990;**50**(16):4885–90. PubMed PMID: 2379152.
7. Xiao J., Horst S., Hinkle G., Cao X., Kocak E., Fang J., Young D., Khazaeli M., Agnese D., Sun D., Martin E. Pharmacokinetics and clinical evaluation of 125I-radiolabeled humanized CC49 monoclonal antibody (HuCC49deltaC(H)2) in recurrent and metastatic colorectal cancer patients. *Cancer Biother Radiopharm.* 2005;**20**(1):16–26. PubMed PMID: 15778575.
8. Paterson A.J., Schlom J., Sears H.F., Bennett J., Colcher D. A radioimmunoassay for the detection of a human tumor-associated glycoprotein (TAG-72) using monoclonal antibody B72.3. *Int J Cancer.* 1986;**37**(5):659–66. PubMed PMID: 3699929.
9. Colcher D., Hand P.H., Nuti M., Schlom J. A spectrum of monoclonal antibodies reactive with human mammary tumor cells. *Proc Natl Acad Sci U S A.* 1981;**78**(5): 3199–203. PubMed PMID: 6789331.
10. Goel A., Baranowska-Kortylewicz J., Hinrichs S.H., Wisecarver J., Pavlinkova G., Augustine S., Colcher D., Booth B.J., Batra S.K. 99mTc-labeled divalent and tetravalent CC49 single-chain Fv's: novel imaging agents for rapid in vivo localization of human colon carcinoma. *J Nucl Med.* 2001;**42**(10):1519–27. PubMed PMID: 11585867.
11. Beresford G.W., Pavlinkova G., Booth B.J., Batra S.K., Colcher D. Binding characteristics and tumor targeting of a covalently linked divalent CC49 single-chain antibody. *Int J Cancer.* 1999;**81**(6):911–7. PubMed PMID: 10362138.
12. Colcher D., Pavlinkova G., Beresford G., Booth B.J., Batra S.K. Single-chain antibodies in pancreatic cancer. *Ann N Y Acad Sci.* 1999;**880**:263–80. PubMed PMID: 10415872.
13. Kowalsky R.J., Falen S.W. and Radiopharmaceuticals in nuclear pharmacy and nuclear medicine, American Pharmacists Association: Washington, D.C. p. 733-752. 2004.
14. Colcher D., Minelli M.F., Roselli M., Muraro R., Simpson-Milenic D., Schlom J. Radioimmunolocalization of human carcinoma xenografts with B72.3 second generation monoclonal antibodies. *Cancer Res.* 1988;**48**(16):4597–603. PubMed PMID: 3396011.

15. Colcher D., Esteban J., Carrasquillo J.A., Sugarbaker P., Reynolds J.C., Bryant G., Larson S.M., Schlom J. Complementation of intracavitary and intravenous administration of a monoclonal antibody (B72.3) in patients with carcinoma. *Cancer Res.* 1987;**47**(15):4218–24. PubMed PMID: 3607761.
16. Britton K.E. The development of new radiopharmaceuticals. *Eur J Nucl Med.* 1990;**16**(4-6):373–85. PubMed PMID: 2190837.
17. Jain R.K. Transport of molecules across tumor vasculature. *Cancer Metastasis Rev.* 1987;**6**(4):559–93. PubMed PMID: 3327633.
18. Primus F.J., Bennett S.J., Kim E.E., DeLand F.H., Zahn M.C., Goldenberg D.M. Circulating immune complexes in cancer patients receiving goat radiolocalizing antibodies to carcinoembryonic antigen. *Cancer Res.* 1980;**40**(3):497–501. PubMed PMID: 7008935.
19. Behr T., Becker W., Hannappel E., Goldenberg D.M., Wolf F. Targeting of liver metastases of colorectal cancer with IgG, F(ab')<sub>2</sub>, and Fab' anti-carcinoembryonic antigen antibodies labeled with <sup>99m</sup>Tc: the role of metabolism and kinetics. *Cancer Res.* 1995;**55**Suppl(23):5777s–5785s. PubMed PMID: 7493346.
20. Bird R.E., Hardman K.D., Jacobson J.W., Johnson S., Kaufman B.M., Lee S.M., Lee T., Pope S.H., Riordan G.S., Whitlow M. Single-chain antigen-binding proteins. *Science.* 1988;**242**(4877):423–6. PubMed PMID: 3140379.
21. Colcher D., Bird R., Roselli M., Hardman K.D., Johnson S., Pope S., Dodd S.W., Pantoliano M.W., Milenic D.E., Schlom J. In vivo tumor targeting of a recombinant single-chain antigen-binding protein. *J Natl Cancer Inst.* 1990;**82**(14):1191–7. PubMed PMID: 2362290.
22. Goldenberg D.M. Monoclonal antibodies in cancer detection and therapy. *Am J Med.* 1993;**94**(3):297–312. PubMed PMID: 8452154.
23. Colcher D., Zalutsky M., Kaplan W., Kufe D., Austin F., Schlom J. Radiolocalization of human mammary tumors in athymic mice by a monoclonal antibody. *Cancer Res.* 1983;**43**(2):736–42. PubMed PMID: 6848189.
24. Abergel C., Padlan E.A., Kashmiri S.V., Milenic D., Calvo B., Schlom J. Crystallographic studies and primary structure of the antitumor monoclonal CC49 Fab'. *Proteins.* 1993;**17**(4):438–43. PubMed PMID: 8108385.
25. Pavlinkova G., Beresford G.W., Booth B.J., Batra S.K., Colcher D. Pharmacokinetics and biodistribution of engineered single-chain antibody constructs of MAb CC49 in colon carcinoma xenografts. *J Nucl Med.* 1999;**40**(9):1536–46. PubMed PMID: 10492377.