^{99m}Tc-Anti-ED-B fibronectin L19-(Gy)$_3$-Cys-Ala scFv antibody fragment

^{99m}Tc-AP39

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Created: February 6, 2007; Updated: January 14, 2008.

Chemical name:	^{99m}Tc-Anti-ED-B fibronectin L19-(Gy)$_3$-Cys-Ala scFv antibody fragment	
Abbreviated name:	^{99m}Tc-AP39	
Synonym:	^{99m}Tc-scFv L19, ^{99m}Tc-anti-ED-B FN scFv Ab	
Agent Category:	Single-chain Antibody fragment (scFv)	
Target:	ED-B Fibronectin (FN)	
Target Category:	Antibody-antigen binding	
Method of detection:	Single-photon emission computed tomography (SPECT), gamma planar imaging	
Source of signal:	^{99m}Tc	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about ED-B fibronectin.

Background

[PubMed]

^{99m}Tc-Anti-ED-B fibronectin L19-(Gly)$_3$-Cys-Ala scFv antibody fragment (^{99m}Tc-AP39) is a radiolabeled molecular imaging agent developed for single-photon emission computed tomography (SPECT) imaging of tumor angiogenesis and guidance in antiangiogenic treatment (1). ^{99m}Tc is a gamma emitter with a half-life ($t_{½}$) of 6.02 h.

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NLM Citation: Cheng KT. ^{99m}Tc-Anti-ED-B fibronectin L19-(Gy)$_3$-Cys-Ala scFv antibody fragment. 2007 Feb 6 [Updated 2008 Jan 14]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

Angiogenesis is a process of development and growth of new blood vessels from pre-existing vessels (2). Tumor growth depends on the formation of new blood vessels via this process. Normal angiogenesis is orderly and highly regulated, whereas tumor angiogenesis is chaotic and irregular. Abnormal angiogenesis is important in the carcinogenesis, growth and progression of human solid and hematologic tumors (3). Fibronectins (FNs) are a widely distributed family of universal cell-adhesion molecules (1). FN is a polymorphic glycoprotein of ~2500 amino acids and has a high molecular mass of 250–280 kDa. FN occurs in soluble form in plasma and other body fluids and in insoluble form in the extracellular matrixes (4, 5). Both forms are dimers composed of a series of repeating units of three types and joined by two disulfide bonds at the carboxyl terminus of the molecule. FN polymorphism arises from alternative splicing patterns of the pre-mRNA or post-translational modifications of FN itself (5). Alternative splicing in three different regions may generate 20 different FN subunit isoforms. The splice variant ED-B FN, which is highly expressed during angiogenesis in both neoplastic and normal tissues (6), is an oncofetal antigen expressed at different levels in the stroma associated with the neovasculature of solid tumors. High levels of ED-B expression have been found in primary and metastatic tumors in breast, colorectal, and non-small cell lung cancer (1, 7-9).

Molecular imaging of angiogenesis offers serial non-invasive evaluation of both location and growth dynamics of tumors (10) SPECT or positron emission tomography imaging with the appropriate radiolabeled tracer targeted to angiogenic pathways may allow the evaluation of specific aspects of tumor vascular biology (9). A molecular probe that targets ED-B FN can be both an early tumor marker and a tool to monitor the success of antiangiogenic cancer therapy. The single-chain antibody fragment (scFv), L19, with a high affinity to ED-B FN was developed by Pini et al. (11). Radioiodinated L19 showed specific accumulation around tumor neovasculature and tumor stroma with high ED-B expression (12, 13). In an effort to prepare a stable ^{99m}Tc-labeled L19, Berndorff et al. (1) inserted the amino acid sequence (Gly)3-Cys-Ala at the C terminus of L19 to produce the recombinant protein, AP39 The authors suggested that a ^{99m}Tc(V) oxo metal complex could be formed. ^{99m}Tc-AP39 appeared to have favorable biodistribution and imaging properties in mice bearing murine embryonal teratocarcinoma (F9).

Synthesis

[PubMed]

Pini et al. (11) constructed and used a large synthetic phage display human antibody library (>3 x 10^8 clones) to produce L19 with a very high affinity of dissociation constant (K_d) of 54 pM to the ED-B domain of FN. L19 was cloned in scFv configuration in the novel phagemid vector, pDN332. Berndorff et al. (1) prepared the L19 derivative by modifying the sequence of scFv LP19 to encode the ^{99m}Tc binding motif $(Gly)_3$-Cys-Ala at the C-terminal end of the VL chain. The DNA sequence encoding this LP19 derivative was cloned into the prokaryotic expression vector pDN5 with isopropyl-1-thio-β-D-galactoside (IPTG)-inducible promoter and the ampicillin resistance marker.AP39 was

isolated and purified from the soluble fraction of the French press lysate of the *Escherichia coli* culture. Before radiolabeling, AP39 was reduced by Tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) in sodium phosphate (Na_2HPO_4) buffer (pH 7.4). The reaction mixture was gently shaken for 1 h at room temperature. TCEP was then removed by chromatography. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of the purified product confirmed the transformation of the S-S-dimeric AP39 to the SH-monomeric AP39 and associative dimer form. For radiolabeling, reduced AP39 in phosphate- buffered saline was added to disodium-L-tartrate and diluted with Na_2HPO_4 buffer. ^{99m}Tc pertechnetate and tin-II chloride ($SnCl_2$ in 0.1 mol/L hydrochloric acid) were added. The reaction mixture was shaken for 30 min at 37°C. ^{99m}Tc-AP39 was purified by gel chromatography. The radiochemical yield was >50% after purification. The radiochemical purity was >95%. By high-performance liquid chromatography analysis, ^{99m}Tc-AP39 appeared to be a mixture of predominantly dimeric (~80%) and monomeric (~16%) ^{99m}Tc-AP39. The specific activity of ^{99m}Tc-AP39 was 35 MBq/nmol [0.95 mCi/nmol(dimer)].

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Berndorff et al. (1) determined the *in vitro* immunoreactivity of ^{99m}Tc-AP39 by using affinity chromatography with ED-B FN-conjugated Sepharose. ^{99m}Tc-AP39 showed an immunoreactivity of 96%.

Animal Studies

Rodents

[PubMed]

Biodistribution studies (n = 3) of ^{99m}Tc-AP39 were performed in nude mice bearing the murine teratocarcinoma F9 (1). F9 tumors were previously reported to express high levels of ED-B FN (13). ^{99m}Tc-AP39 showed rapid blood clearance and radioactivity localization in the tumor. The radioactivity levels of ^{99m}Tc-AP39 in percentage of the injected dose per g (% ID/g) in the tumor were 5.2 ± 2.5 (0.25 h), 6.1 ± 2.0 (1 h), 8.3 ± 3.2 (3 h), 8.7 ± 52.5 (5 h), and 2.8 ± 1.4 (24 h). The tumor/blood ratios were 0.4 ± 0.2 (0.25 h), 1.6 ± 0.2 (1 h), 6.4 ± 2.9 (3 h), 9.0 ± 6.5 (5 h), and 17.2 ± 6.8 (24 h). ^{99m}Tc-AP39 appeared to be excreted primarily by the kidneys. The radioactivity levels (% ID/g) in the kidneys were 48.2 ± 4.2 (0.25 h), 21.8 ± 2.5 (1 h), 8.6 ± 0.6 (3 h), 7.9 ± 0.4 (5 h), and 2.6 ± 0.4 (24 h). About 64.5% ID was excreted *via* the urine after 24 h. There was little radioactivity accumulation in other nontarget organs. The authors suggested that the absence of radioactivity in the thyroid indicated no significant release of unlabeled ^{99m}Tc from ^{99m}Tc-AP39.

Gamma imaging was performed in mice bearing 80–100 mm^2 s.c.F9 tumors (1). Each mouse was injected with 4–7 MBq (0.11–0.19 mCi) ^{99m}Tc-AP39. Imaging with ^{99m}Tc-

AP39 gave clear tumor images for at least 24 h with low background radioactivities. The kidneys were visualized at 3 and 5 h. The liver and bladder were the only other organs weakly detected at early time points.

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. Berndorff D., Borkowski S., Moosmayer D., Viti F., Muller-Tiemann B., Sieger S., Friebe M., Hilger C.S., Zardi L., Neri D., Dinkelborg L.M. Imaging of tumor angiogenesis using 99mTc-labeled human recombinant anti-ED-B fibronectin antibody fragments. J Nucl Med. 2006;**47**(10):1707–16. PubMed PMID: 17015908.
2. Shinkaruk S., Bayle M., Lain G., Deleris G. Vascular endothelial cell growth factor (VEGF), an emerging target for cancer chemotherapy. Curr Med Chem Anticancer Agents. 2003;**3**(2):95–117. PubMed PMID: 12678905.
3. Ranieri G., Patruno R., Ruggieri E., Montemurro S., Valerio P., Ribatti D. Vascular endothelial growth factor (VEGF) as a target of bevacizumab in cancer: from the biology to the clinic. Curr Med Chem. 2006;**13**(16):1845–57. PubMed PMID: 16842197.
4. Carnemolla B., Balza E., Siri A., Zardi L., Nicotra M.R., Bigotti A., Natali P.G. A tumor-associated fibronectin isoform generated by alternative splicing of messenger RNA precursors. J Cell Biol. 1989;**108**(3):1139–48. PubMed PMID: 2646306.
5. Kosmehl H., Berndt A., Katenkamp D. Molecular variants of fibronectin and laminin: structure, physiological occurrence and histopathological aspects. Virchows Arch. 1996;**429**(6):311–22. PubMed PMID: 8982375.
6. Castellani P., Viale G., Dorcaratto A., Nicolo G., Kaczmarek J., Querze G., Zardi L. The fibronectin isoform containing the ED-B oncofetal domain: a marker of angiogenesis. Int J Cancer. 1994;**59**(5):612–8. PubMed PMID: 7525495.
7. Kaczmarek J., Castellani P., Nicolo G., Spina B., Allemanni G., Zardi L. Distribution of oncofetal fibronectin isoforms in normal, hyperplastic and neoplastic human breast tissues. Int J Cancer. 1994;**59**(1):11–6. PubMed PMID: 7927891.

8. Pujuguet P., Hammann A., Moutet M., Samuel J.L., Martin F., Martin M. Expression of fibronectin ED-A+ and ED-B+ isoforms by human and experimental colorectal cancer. Contribution of cancer cells and tumor-associated myofibroblasts. Am J Pathol. 1996;**148**(2):579–92. PubMed PMID: 8579120.
9. Santimaria M., Moscatelli G., Viale G.L., Giovannoni L., Neri G., Viti F., Leprini A., Borsi L., Castellani P., Zardi L., Neri D., Riva P. Immunoscintigraphic detection of the ED-B domain of fibronectin, a marker of angiogenesis, in patients with cancer. Clin Cancer Res. 2003;**9**(2):571–9. PubMed PMID: 12576420.
10. Laking, G.R. and P.M. Price, Positron emission tomographic imaging of angiogenesis and vascular function. Br J Radiol, 2003. 76 Spec No 1: p. S50-9.
11. Pini A., Viti F., Santucci A., Carnemolla B., Zardi L., Neri P., Neri D. Design and use of a phage display library. Human antibodies with subnanomolar affinity against a marker of angiogenesis eluted from a two-dimensional gel. J Biol Chem. 1998;**273**(34):21769–76. PubMed PMID: 9705314.
12. Tarli L., Balza E., Viti F., Borsi L., Castellani P., Berndorff D., Dinkelborg L., Neri D., Zardi L. A high-affinity human antibody that targets tumoral blood vessels. Blood. 1999;**94**(1):192–8. PubMed PMID: 10381513.
13. Borsi L., Balza E., Bestagno M., Castellani P., Carnemolla B., Biro A., Leprini A., Sepulveda J., Burrone O., Neri D., Zardi L. Selective targeting of tumoral vasculature: comparison of different formats of an antibody (L19) to the ED-B domain of fibronectin. Int J Cancer. 2002;**102**(1):75–85. PubMed PMID: 12353237.