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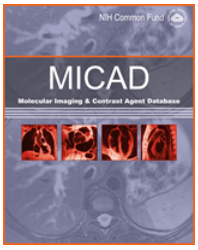

^{99m}Tc-Anti-ED-B fibronectin single-chain antibody fragment L19-His

^{99m}Tc-L19-His

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Chemical name:	^{99m}Tc-Anti-ED-B fibronectin single-chain antibody fragment L19-His	
Abbreviated name:	^{99m}Tc-L19-His	
Synonym:	^{99m}Tc-scFv L19-His, ^{99m}Tc-anti-ED-B FN scFv Ab	
Agent Category:	Single-chain Antibody fragment (scFv)	
Target:	ED-B Fibronectin (ED-B 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 single-chain antibody fragment L19-His (^{99m}Tc-L19-His) is a radiolabeled molecular imaging agent developed for single-photon emission computed tomography (SPECT) imaging of tumor angiogenesis and guidance for antiangiogenic treatment (1). ^{99m}Tc is a gamma emitter with a half-life ($t_{½}$) of 6.01 h.

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 from 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 solid and hematologic tumors in humans (3). Fibronectins (FNs) are a family of universal cell-adhesion molecules that are widely distributed (1). FN is a polymorphic

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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 matrices (4, 5). Both forms are dimers composed of a series of repeating units of three types and joined by two disulfide bonds at the C-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 regions [extra domain A (ED-A), extra domain B (ED-B), and type III homology connecting segment (IIICS)] 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 cancers (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 an 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, which has a high affinity for 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) genetically introduced a $(His)_6$ peptide sequence at the C-terminus of L19 to produce L19-His molecules. Two other L19 derivatives, AP39 and L19-Hi20, were also prepared for radiolabeling. These ^{99m}Tc-labeled L19 derivatives appeared to have favorable biodistribution and imaging properties in mice bearing murine embryonal teratocarcinomas (F9). However, the study did not provide data to confirm that the binding was a result of angiogenesis.

Synthesis

[PubMed]

Pini et al. (11) constructed and used a large synthetic phage display human antibody library (>3×10^8 clones) to produce L19 with a very high affinity (dissociation constant (K_d) = 54 pM) for 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 $(His)_6$ domain at the C-terminal end of the V_L chain. The DNA sequence encoding this LP19 derivative was cloned into the prokaryotic expression vector pDN5 with isopropyl-1-thio-β-D-galactoside–inducible promoter and ampicillin resistance marker. L19-His was purified by affinity chromatography before radiolabeling. Radiolabeling was performed according to the tricarbonyl method (1, 14, 15). In this method, L19-His was labeled with ^{99m}Tc by the formation of the precursor $[^{99m}Tc(OH_2)_3(CO)_3]^+$. $[^{99m}Tc(OH_2)_3(CO)_3]^+$ was prepared *via* one-pot synthesis with potassium boranocarbonate and ^{99m}Tc-pertechnetate (16), and it was purified by high-performance liquid chromatography (HPLC). Approximately 100 µg of L19-His in 10 mmol/L *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) buffer (pH 7.5) was added to 37 MBq (1 mCi) $[^{99m}Tc(OH_2)_3(CO)_3]^+$ solution. The mixture was incubated for 1 h at 37°C. ^{99m}Tc-L19-His was purified by affinity chromatography. HPLC analysis showed that ^{99m}Tc- L19-His was a dimer. The radiochemical yield was >93%, and the specific activity was 9 MBq/nmol (dimer; 0.24 mCi/nmol). No yield was reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Berndorff et al. (1) determined the *in vitro* immunoreactivity of ^{99m}Tc-L19-His by using affinity chromatography with ED-B FN–conjugated Sepharose. ^{99m}Tc-L19-His showed an immunoreactivity of 89%.

Animal Studies

Rodents

[PubMed]

Biodistribution studies (n = 3) of ^{99m}Tc-L19-His were performed in nude mice bearing murine F9 tumors (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-L19-His (percentage of injected dose per g (% ID/g)) in the tumors were 6.3 ± 2.0 (0.25 h), 8.4 ± 0.9 (1 h), 9.4 ± 1.4 (3 h), 8.1 ± 2.0 (5 h), and 5.7 ± 2.0 (24 h). The tumor/blood ratios were 0.4 ± 0.1 (0.25 h), 2.0 ± 0.2 (1 h), 5.7 ± 0.5 (3 h), 9.5 ± 2.6 (5 h), and 23.6 ± 8.3 (24 h). ^{99m}Tc-L19-His appeared to be excreted primarily by the kidneys, radioactivity levels in the kidneys were 87.2 ± 4.2% ID/g and 72.4 ± 11.3% ID/g at 0.25 and 24 h, respectively. Only ~32.4% ID was excreted *via* the urine after 24 h. ^{99m}Tc-L19-His radioactivity also accumulated in the ED-B FN-expression reproductive organs (ovaries, and uterus). At 0.25 h, the radioactivity levels (% ID/g) in other major organs were 27.2 ± 18.8 (ovaries), 18.7 ± 2.0 (blood), 10.4 ± 1.8 (lungs), 6.1 ± 0.7 (liver), 5.5 ± 0.6 (spleen), 4.9 ± 1.5 (thyroid), 3.2 ± 1.7 (uterus), and 2.0 ± 0.2 (stomach). By 24 h, these radioactivity levels (% ID/g) decreased to 2.8 ± 0.5 (ovaries), 5.7 ± 2.0 (blood), 2.0 ± 0.6 (lungs), 2.5 ± 0.7 (liver), 0.8 ± 0.2 (spleen), 4.4 ± 2.4 (uterus), 1.1 ± 0.0 (thyroid), and 1.1 ± 0.2 (stomach). No specific blocking study was performed.

Gamma imaging was performed in mice bearing s.c. F9 tumors (80–100 mm^2) (1). Each mouse was injected with 4–7 MBq (0.11–0.19 mCi) ^{99m}Tc-L19-His. Imaging with ^{99m}Tc-L19-His produced clear tumor images at 5 and 24 h. The background was low except for high radioactivity accumulation in the kidneys. The kidneys were visualized even at 24 h. The liver was slightly detectable after 5 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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