^{99m}Tc- anti-EGFR monoclonal antibody h-R3

^{99m}Tc-hR3

The MICAD Research Team

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Chemical name:	^{99m}Tc-labeled humanized anti-EGFR monoclonal antibody h-R3	
Abbreviated name:	^{99m}Tc-hR3	
Synonym:	DiaCIM	
Agent Category:	anti-EGFR monoclonal antibody h-R3	
Target:	Epidermal growth factor receptor	
Target Category:	Binding	
Method of detection:	SPECT	
Source of signal:	^{99m}Tc	
Activation:	Not required	
Studies:	• *In vitro* • Rodents • Humans	View human EGFR protein and nucleotide sequences.

Background

[PubMed]

Overexpression of the epidermal growth factor receptor (EGFR) is a characteristic feature of a variety of cancers such as squamous cell lung carcinoma and breast, ovarian, head and neck, bladder, and colon cancers (1). The EGFR is a 170-kDa transmembrane protein that promotes cell proliferation by the specific binding of the autocrine epidermal growth factor (EGF) and transforming growth factor α (TGFα). The activity of these factors is believed to contribute to the progression of cancers. This EGFR operates through a receptor-associated tyrosine kinase–mediated signal transduction pathway. In an effort to develop therapy against cancer, a variety of EGFR inhibitors have been developed that either inhibit activation of the receptor kinase or inhibit the binding of EGF and TGFα, e. g., monoclonal antibodies (MAb) to the receptor (2).

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A murine MAb ior egf/r3, with a high affinity for an extracellular domain of the EGFR, was developed and tested for the treatment of cancers (3). This MAb was also labeled with radioactive technetium (^{99m}Tc) for molecular imaging of the EGFR in tumors to possibly predict responses to a variety of therapeutic agents under development for the treatment of cancers (4). However, because of its immunogenicity, the ^{99m}Tc-labeled ior egf/r3 MAb had limited clinical utility, particularly if repeated imaging investigations were necessary (3). To circumvent this problem, a humanized form of ior egf/r3 was developed by combining the complimentary determining regions of the murine antibody with a human IgG_1 framework (5). This humanized antibody had a low affinity for the receptor, but with modification of the structure hR3, an antibody with a similar receptor affinity as the original murine MAb, was obtained.

The hR3 was subsequently labeled with ^{99m}Tc and investigated for its tissue distribution and imaging properties (3, 6).

Synthesis

[PubMed]

To produce the humanized MAb h-R3, a murine IgG_{2a} antibody ior egf/r3 secreting hybridoma was initially obtained by the fusion of murine myeloma SP2/Ag14 cells with splenocytes from Balb/c mice immunized with a partially purified human placental tissue EGFR as described elsewhere (7). The variable regions of the ior egf/e3 were cloned and a reshaped antibody was constructed using the heavy and light chains of REI and Eu, respectively, as the human immunoglobulin framework to graft the complimentary determining regions (5). The antibodies were reduced with mercaptoethanol in phosphate-buffered saline at room temperature for 30 min and purified on a Sephadex G-50 gel filtration column.

Aliquots of the reduced mAb (3 mg each) formulated in 15 mg glucose were dispensed in 10-ml vials and instantly frozen with liquid nitrogen (8). To each vial 150 µl of the methyl diphosphonate (MDP) bone scanning kit (dissolved in normal saline) was added to obtain 50 µg MDP, 3.4 µg stannous acid, and 20 µg p-aminobenzoic acid per mg MAb. The vials were once again frozen with liquid nitrogen, lyophilized for 24 h, sealed under vacuum, and stored as a kit at 4°C until required (8).

To radiolabel the agent, the lyophilized MAb was reconstituted with 1–2 ml (30 mCi) pertechnetate (TcO_4^-) and the contents were dissolved by gently swirling the vial. To complete the labeling reaction, the vial was left at room temperature for 10 min. The average number of –SH groups per molecule, as measured by a cysteine assay, was 5.28 ± 0.06, and the specific activity of the labeled MAb, as determined by paper chromatography, was 10 mCi/58.8 nmol protein with a labeling efficiency of 99.21 ± 0.42% (8).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The immunorectivity of ^{99m}Tc-hR3 was compared to the murine ior egf/r3 antibody by use of a radioreceptor assay (8). The humanized MAbs were determined to have an inhibition constant (K_i) that was similar to the original mouse antibody.

Animal Studies

Rodents

[PubMed]

The biodistribution of ^{99m}Tc-hR3 was determined in nude mice bearing A431 human epidermoid carcinoma cell tumors (8). The mice were sacrificed 4 and 24 h after administration of ^{99m}Tc-hR3. Radioactivity levels in the tumors, heart, liver, kidney, lungs, stomach, spleen, and intestines were determined. In general, accumulation of radioactivity was evident in the serum, the kidneys, and the spleen. A significant accumulation of radioactivity as compared to the other organs was observed in the kidneys and the spleen both at 4 and 24 h post injection.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

The safety, localization in normal tissue and tumor, pharmacokinetics, and radiation dosimetry of ^{99m}Tc-hR3 in patients with recurrent or metastatic epithelial malignancies were evaluated (3). Either 3.0 mg/505 MBq (3.0 mg/13.64 mCi) or 6.0 mg/1,010 MBq (6.0 mg/27.29 mCi) ^{99m}Tc-hR3 was administered to the patients. Whole-body imaging was performed up to 24 h after administration and the normal organ uptake was quantified. Patients showed no adverse effects and no changes in biochemical/hematological indices or immune response to ^{99m}Tc-hR3. Tissue uptake was found mainly in the liver, spleen, and the kidneys. One patient was found by use of single-photon emission computed tomography (SPECT) analysis to be positive for squamous cell carcinoma of the mouth involving the lymph nodes. It was concluded ^{99m}Tc-hR3 had an excellent safety profile

and that a larger group of patients pre-selected for EGFR positivity should be included in a clinical trial for further study.

In another clinical trial, the biodistribution, organ radiation dosimetry, and toxicity of ^{99m}Tc-hR3 were investigated in humans (6). Patients with suspected epithelium-derived tumors were divided into two groups: patients in group 1 received a dose of 3 mg/1,110 MBq (3 mg/30 mCi) of ^{99m}Tc-hR3, and patients in group 2 received a dose of 6 mg/2,220 MBq (6 mg/60 mCi) of the labeled antibody. Biodistribution of the antibody was monitored by use of SPECT, and multiple samples of blood and urine were collected for up to 24 h. The liver, spleen, kidneys, bladder, and heart were identified as the main organs for accumulation of radioactivity in these patients. The liver had the highest absorbed dose, followed by the kidneys and the urinary bladder wall with an excretion of ~22% of the injected dose in the urine. A preliminary analysis of the results suggested that the labeled antibody had a sensitivity of 76.5% and a specificity of 100%. It was concluded that ^{99m}Tc-hR3 was safe for use in humans and could be used for diagnosis of epithelium-derived tumors at the two doses used in the study.

References

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