^{131}I-NP-4 anti-CEA monoclonal antibody

^{131}I-NP-4 MAb

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Chemical name:	^{131}I-NP-4 anti-CEA monoclonal antibody	
Abbreviated name:	^{131}I-NP-4 MAb	
Synonym:		
Agent Category:	Antibody	
Target:	Carcinoembryonic antigen (CEA)	
Target Category:	Antibody to antigen binding	
Method of detection:	Single Photon Emission Computed Tomography (SPECT), planar gamma imaging	
Source of signal /contrast:	^{131}I	
Activation:	No	
Studies:	• *In vitro* • Rodents • Non-primate non-rodent mammals • Non-human primates • Humans	Click on protein, nucleotide (RefSeq), and gene for more information about CEA.

Background

[PubMed]

^{131}I-NP4 MAb, which is formed by the conjugation of ^{131}I with an intact murine anti-carcinoembryonic antigen (CEA) monoclonal antibody (MAb), can be used for imaging and therapy of CEA- expressing cancers (1, 2). ^{131}I has a physical $t_{½}$ of 8 days.

CEA was first identified from extracts of human adenocarcinoma of the colon (3). It is a β-glycoprotein, and its predominant expression on the cell surface is increased in a variety

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of carcinomas, particularly of the gastrointestinal tract, as well as in fetal gastrointestinal tissues and in certain inflammatory states, such as inflammatory bowel disease (4, 5). CEA, which exhibits extensive heterogeneity in its physicochemical and immunologic properties (2, 3), has a molecular weight of 200 kDa and can be shed and detected in the serum (2). It has been used as a serum marker for monitoring disease status in patients who have various CEA- secreting tumors (gastrointestinal, lung, medullary, thyroid, uterine, ovarian, and bladder carcinomas). Other cross-reactive, but genetically distinct, CEA variants have been identified, including nonspecific cross-reactive antigen (NCA) and meconium antigen (MA) (6).

Radiolabeled MAbs have been developed for both the diagnosis and treatment of tumors (4). Primus et al. (2) studied the immunologic heterogeneity of CEA by use of four MAbs to differentiate the antigenic sites on colonic cancer CEA. They classified the MAbs into three general classes based on their reactivity with CEA, NCA, and MA. The class I antibody, NP-1, had high affinity for CEA and MA but low affinity for NCA. The class II antibodies, NP-2 and NP-3, had moderate affinities for CEA and MA. The class III antibody, NP-4, appeared to recognize determinants unique to CEA and had no affinity for NCA or MA. Because of this specificity, NP-4 MAb in the form of intact IgG has been labeled with radioisotopes for CEA tumor imaging and therapy (7). NP-4 MAb is covalently linked to ^{131}I by radioiodination with $Na^{131}I$. ^{131}I emits both gamma and beta radiation, and it can be used for imaging at low doses and for therapy at high doses. NP-4 MAb in the form of Fab' fragment has also been labeled with ^{99m}Tc for imaging (8).

Synthesis

[PubMed]

NP-4 MAb is an IgG_1 subtype and has a CEA affinity in the range of 10^8 M^{-1} (2). NP-4 MAb was initially produced by Primus et al. (5, 9) in 1983 with the use of the hybridoma technique and CEA isolated from liver metastases of a colonic adenocarcinoma. The NP-4 MAb was produced in mice and purified from ascites fluid by protein A and ion-exchange column chromatography at 4 ^oC (10). The final purity and identity were confirmed by immunoelectrophoresis, sodium dodecyl sulfate (SDS) gel electrophoresis, and size-exclusion high-performance liquid chromatography (HPLC). Direct radioiodination of the intact whole MAb with ^{131}I was performed by the chloramines-T or IodoGen method. The specific activity obtained was 444-592 MBq/mg (12-16 mCi/mg), or 67.3-89.7 GBq/μmol (1.8-2.4 Ci/μmol) based on a molecular weight of 150,000 for the MAb. in 1983 with the use of the hybridoma technique and CEA isolated from liver metastases of a colonic adenocarcinoma). The radioimmunoreactivity was >70% based on binding of ^{131}I-NP-4 MAb to a CEA-immunoadsorbent column. The radiochemical purity was >95%, and 85-95% of the radioactivity was native-size IgG as determined by size-exclusion HPLC.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Primus et al.(2) first reported the production of NP-4 anti-CEA MAb. Using a competitive radioimmunoassay method, they obtained an affinity constant (*K*) for CEA of 8.9 x 10^8 M^{-1}. NP-4 MAb did not appear to bind to NCA or MA.

Animal Studies

Rodents

[PubMed]

Sharkey et al.(11) studied the biodistribution of ^{131}I-NP-4 MAb in nude mice bearing the CEA-producing human colorectal cancer, GW-39. NP-4 MAb was radiolabeled at a ratio of 0.06 mg MAb/mCi to achieve a specific activity of 370-518 MBq (10-14 mCi)/mg (56.1-78.5 GBq (1.5-2.1 Ci)/μmol). Maximum tumor localization of ^{131}I-NP-4 MAb occurred within 3 days and reached about 30% injected dose (ID)/g. The tumor radioactivity decreased between days 7 and 14. The tumor/nontumor tissue ratios on day 1 for the kidney, liver, lung, spleen, and blood were 2.5 ± 0.9, 3.8 ± 1.3, 2.1 ± 0.8, 4.8 ± 2.2, and 0.9 ± 0.3, respectively. By day 14, these ratios increased to 50.0 ± 20.0, 59.0 ± 21.3, 36.2 ± 13.0, 101.0 ± 57.0, and16.6 ± 7.0,

Boerman et al. (12) studied the influence of antibody protein dose on the biodistribution of ^{131}I-NP-4 MAb in small GW-39 tumors (0.1-0.4 g) implanted in nude mice. NP-4 MAb was labeled with ^{131}I by the chloramine-T method with a specific activity of 444-555 MBq (12-15 mCi)/mg (67.3-84.1 GBq (1.8-2.3 Ci)/μmol). Increasing amounts of unlabeled antibody were added to 1 μg of ^{131}I-NP-4 MAb preparations to achieve 5 different protein doses. Seven days after injection, tumor radioactivity of ^{131}I-NP-4 MAb remained constant up to an antibody protein dose of 100 μg/animal. The tumor radioactivities (in %ID/g) were 20.4 ± 1.9, 27.1 ± 9.3, 25.0 ± 4.2, 11.4 ± 0.2, and 7.2 ± 1.6 for protein doses of 1, 10, 100, 500, and 1000 μg/animal, respectively. In comparison, the radioactivities in the normal tissues were not decreased at higher protein doses.

Blumenthal et al. (13) evaluated the localization of ^{125}I-NP-4 MAb in 4 size-matched human colonic carcinomas (LSi74T, GW-39, GS-2, and Moser) of varying histopathology grown s.c. in nude mice. They found that, unless serum CEA levels became very high (>150 ng/ml), ^{125}I-NP-4 MAb did not complex with circulating CEA. Differences in tumor vascularity did not appear to have a strong influence on the antibody tumor localization. Intra-tumoral distribution of antigen and subcellular accessibility of antigen for radiolabeled antibody appeared to have a bigger influence. Low ^{125}I-NP-4 MAb targeting was found in tumors with 90% CEA within the tumor cells and slow ^{125}I-NP-4 MAb internalization.

Other Non-Primate Mammals

[PubMed]

Sharkey et al. (14) studied the biodistribution of ^{131}I-NP-4 MAb in hamsters bearing the GW-39 tumor. NP MAbs (NP-2, NP-3, and NP-4) were radioiodinated with ^{131}I by the chloramines-T method to achieve a specificity activity of 444-555 MBq (12-15 mCi)/mg (67.3-84.1 GBq (1.8-2.3 Ci)/μmol). An irrelevant mouse myeloma IgG (Ag8) labeled with ^{125}I was also used to indicate the nonspecific localization of a MAb. Each tumor bearing hamster received intracardiac injections containing 5550-7400 kBq (150-200 μCi)/10-15 μg of ^{131}I-labeled antibody and 1850 kBq (50 μCi)/5 μg of ^{125}I-Ag8. A tumor localization index was calculated from the ratio of specific *versus* irrelevant antibody in the tumor divided by the ratio of specific *versus* irrelevant antibody in the blood. All ^{131}I-labeled NP MAbs showed a preferential localization for GW-39 compared with ^{125}I-Ag8. The tumor radioactivities (%ID/g) for ^{131}I-NP-4 MAb (n = 5) were 1.3 ± 0.09, 1.0 ± 0.06, and 0.7 ± 0.04 on days 1, 3, and 7 after injection, respectively. The tumor localization indices were 0.9-1.5, 2-2.5, and 3.5-4.5 on days 1, 3, and 7, respectively. The tumor/blood, tumor/liver, tumor/spleen, tumor/kidney, and tumor/lung ratios of ^{131}I-NP-4 MAb on day 7 were 1.60 ± 0.16, 6.5 ± 0.6, 7.1 ± 0.6, 5.1 ± 0.5, and 3.9 ± 0.4, respectively.

Wahl et al. (5) performed imaging of s.c. GW-39 bearing Syrian hamsters. Doses containing 1850 kBq (50 μCi)/14 μg of ^{131}I-NP-4 MAb were given by intracardiac injection. One to 2 days after injection, the tumor was visible. By 6 days, there was a gradual decrease in background activity with preservation of most radioactivity in the tumor. After 7 days, the organ radioactivities in %ID/g for the tumor, blood, liver, kidney, spleen, and lung (n = 4 for each) were 1.21 ± 0.14, 0.75 ± 0.12, 0.16 ± 0.02, 0.17 ± 0.03, 0.18 ± 0.02, and 0.26 ± 0.04, respectively. A specific localization ratio (ratio of ^{131}I-NP-4 MAb %ID/g to a nonspecific MAb %ID/g) based on the localization of a nonspecific ^{125}I-MOPC MAb in the same animal was calculated as 1.97 ± 0.125 for the tumor at 7 days after injection. On day 11, the total radioactivity left in the tumor was 8.87 ± 1.38% ID, about 63% of the whole-body radioactivity.

Non-Human Primates

[PubMed]

Losman et al. (15) immunized a baboon with NP-4 MAb to produce anti-NP-4 antibody. This baboon antibody inhibited specifically the binding between NP-4 and CEA in enzyme-linked immunosorbent assay (ELISA). Mice immunized with this baboon anti-idiotype antibody produced antibody to the CEA epitope recognized by NP-4 MAb. The study suggested that baboon anti-idiotype antibodies functionally mimic a CEA epitope.

Human Studies

[PubMed]

The immunologic, pharmacokinetic, and targeting properties of ^{131}I-NP-4 MAb were studied in patients with colorectal carcinoma (7). NP-4 MAb was radiolabeled with ^{131}I by the chloramine-T method. Radiolabeling efficiencies were >70% with a specific activity of 444-666 MBq (12-18 mCi)/mg (67.3-100.9 GBq (1.8-2.7 Ci)/μmol). Immunoreactivities were typically 80-90%. Patients were given 92.5-185 MBq (2.5-5 mCi) of ^{131}I-NP-4 MAb in less than 1 mg for imaging. The blood clearance of ^{131}I-NP-4 MAb had an elimination $t_{½}$ of 29 ± 14 h. For additional patients who received protein doses of >1 mg, the blood clearance was prolonged. Most patients had less than 20% complexing with CEA at 1 or 24 h after injection. The sensitivity, specificity, and accuracy of detecting tumor sites in 21 patients were 73.5%, 88.6%, and 81.2%, respectively. Losman et al. (16) produced anti-idiotype antibody from a cancer patient treated with^{131}I-NP-4 and suggested that human anti-idiotype antibody to NP-4 could antigenically mimic the CEA epitope recognized by NP-4. Murray et al. (17) compared the tumor localization of ^{125}I-NP-4 MAb (whole antibody) with its ^{131}I-NP-4 MAb F(ab´)$_2$ fragment in 5 patients with colorectal cancer. All 5 patients received a combination of 74 MBq (2 mCi)/1 mg of ^{125}I-NP-4 MAb and 370 MBq (10 mCi)/1 mg of ^{131}I-NP-4 MAb F(ab´)$_2$ fragment. The tumor radioactivities (%ID/g) for ^{125}I-NP-4 MAb and ^{131}I-NP-4 MAb F(ab´)$_2$ fragment were 2.6 ± 0.94 and 2.0 ± 0.57, respectively. The plasma $t_{½}$ (h) and 48-h urinary excretion rates (%) for ^{131}I-NP-4 MAb F(ab')$_2$ and ^{125}I-NP-4 MAb were 29 ± 3.2 h and 50.2 ± 5%, and 43.2 ± 7.3 h, and 56.7 ± 4.7%, respectively.

In a phase I/II clinical trial of ^{131}I-NP-4 MAb, 57 patients with CEA-expressing tumors (29 colorectal, 9 lung, 7 pancreas, 6 breast, and 4 medullary thyroid cancer) in advanced metastatic stages were studied (1). Patients received 296-1110 MBq (8-30 mCi)/1-3 mg ^{131}I-NP-4 MAb with a specific activity of 444-592 MBq (12-16 mCi)/mg (67.3-89.7 GBq (1.8-2.4 Ci)/μmol) for planar and single-photon emission computed tomography (SPECT) imaging. Thirty-seven patients had already received murine MAbs before the study. Ten patients had HAMA titers >300 units. Above this titer, rapidly increasing plasma and whole-body clearance rates were observed. Colorectal cancer patients had a faster blood $t_{½}$ (21.4 ± 11.1 h) and whole-body $t_{½}$ (61.9 ± 39.9 h) than patients with other types of cancers. In the presence of human anti-mouse antibodies (HAMAs), ^{131}I-NP-4 MAb was increasingly localized in the liver, spleen, and bone marrow as high-molecular-weight fractions at 1 h (determined by size-exclusion chromatography). At 24 h, the majority of ^{131}I-NP-4 MAb was rapidly excreted renally as low-molecular-weight fractions (metabolites). No significant difference in pharmacokinetics was observed between HAMA-negative patients and patients with HAMA titers as long as HAMA titers were <300 units. The red marrow and whole-body radiation absorbed doses in HAMA-negative (<300 units) patients were 2.2 cGy/mCi and 0.73 ± 0.37 cGy/mCi, respectively. The highest absorbed dose (26.06 ± 20.83 cGy/mCi) was to the (blocked) thyroid. The kidney and spleen received the second highest doses of 3.57 ± 0.97 cGy/mCi and 3.57 ± 1.48 cGy/mCi, respectively. In the same clinical trial, the therapeutic efficacy of ^{131}I-NP-4 MAb for treating CEA-expressing tumors was studied, and it was found that the red marrow was the dose-limiting organ. Mittal et al. (18), in a study involving 9 patients, showed that it

was feasible to combine hyperthermia and ^{131}I-NP-4 MAb for treating patients who had colorectal carcinoma with liver metastases.

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