Radioiodinated anti-TAG-72 humanized C_H2 domain-deleted antibody

Radioiodinated HuCC49ΔC_H2 Ab

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Chemical name:	Radioiodinated anti-TAG-72 humanized C_H2 domain-deleted antibody	
Abbreviated name:	Radioiodinated HuCC49ΔC_H2 Ab	
Synonym:	^{125}I-HuCC49ΔC_H2 Ab, ^{125}I-CC49 Ab, ^{131}I-HuCC49ΔC_H2 Ab, ^{131}I-CC49 Ab	
Agent Category:	Humanized C_H2 domain-deleted antibody variant	
Target:	(Sialyl-Tn) TAG-72	
Target Category:	Antibody to antigen binding	
Method of detection:	Single-photon emission computed tomography (SPECT), planar gamma imaging	
Source of signal:	^{125}I, ^{131}I	
Activation:	No	
Studies:	• *In vitro* • Rodents • Humans	Click on protein, nucleotide (RefSeq), and gene for more information about TAG-72

Background

[PubMed]

Radioiodinated anti-TAG-72 humanized C_H2 domain-deleted antibody (radioiodinated HuCC49ΔC_H2 Ab), which is formed by the conjugation of radioactive iodine (^{125}I or ^{131}I) with a bioengineered anti–tumor-associated glycoprotein 72 (TAG-72) antibody

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construct, has been developed for gamma imaging of cancers that express TAG-72 (1-4). ^{125}I has a physical half-life ($t_{½}$) of 60 days, and ^{131}I has a $t_{½}$ of 8 days.

The TAG-72 antigen was isolated from the LS-174T human colon cancer xenograft as a high molecular weight glycoprotein (molecular mass of 10^6 Da) with mucin-like characteristics (3, 5-7). It 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 was initially generated by immunization of mice with a membrane-enriched fraction of a human breast carcinoma (9). With use of affinity-purified TAG-72 from LS-174T as an immunogen, CC49 and other anti–TAG-2 MAbs with higher affinity constants (K_a) have been produced and characterized (5, 6, 9, 10). To optimize the pharmacokinetics of CC49 MAb, Hutzell et al. (11) generated a recombinant chimeric B72.3 (cCC49) with a human γ_1 constant region. Whittle et al. (12) also reported the preparation of a cCC49 Ab with a human γ_4 constant region. Faster plasma clearance of cCC49 Ab was seen in mice, but similar results were not observed in monkeys and humans.

Radiolabeled MAbs have been developed for both the diagnosis and treatment of tumors (13). Radiolabeled B72.3 and CC49 (intact IgGs) have shown excellent tumor localization capabilities with potential diagnostic and therapeutic applications in the clinical setting (14, 15). Because of their relatively large size, radiolabeled intact MAbs tend to have unfavorable imaging kinetics, poor tumor penetration, and high potential for human anti-mouse antibody response (10, 16-18). The removal of the C_H2 domain of IgG appears to result in a significantly faster blood clearance and more rapid tumor uptake (19-21). Slavin-Chiorini et al. (1) bioengineered a complementary determining region-grafted humanized CC49 MAb (HuCC49) with a C_H2 domain deletion (ΔC_H2). The light chain constant domain was a human Cκ, and the human γ_1 chain was genetically modified to produce a heavy chain composed of a C_H1 domain followed by a partial IgG_1 hinge region tethered to the C_H3 heavy chain domain by addition of a flexible 10-amino-acid GGGSSGGGSG spacer (22). The structure of HuCC49ΔC_H2 was investigated by Larson et al. (23), and they reported that the antigen combining site of HuCC49ΔC_H2 was very similar, but not identical, in topology and charge distribution to that of B72.3. Radioiodinated (^{125}I or ^{131}I) HuCC49ΔC_H2 constructs have demonstrated faster blood clearance in mice (1, 21, 24). Clinical studies in patients showed potential applications of radioiodinated HuCC49ΔC_H2 Ab (2-4).

Synthesis

[PubMed]

Slavin-Chiorini et al. (1) first reported the preparation and radiolabeling of ^{131}I-HuCC49ΔC_H2 Ab. The HuCC49 Ab was prepared by grafting the CC49 MAb hypervariable regions onto the variable light and variable heavy frameworks of the human MAbs LEN and 21/28' CL, respectively. The HuCC49ΔC_H2 heavy and light chain

expression constructs were introduced by sequential electroporation into the SP2/0-Ag14 cell line. HuCC49ΔC$_H$2 was then purified by filtration and dialysis from the bioreactor supernatant. Purified HuCC49ΔC$_H$2 was labeled with either Na^{125}I or Na^{131}I using the standard 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (IodoGen) method. The labeling conditions, radiochemical yield, and radiochemical purity were not reported. The specific activity was 74–185 kBq/μg (2–5 μCi/μg) or 11.3–28.3 kBq/pmol (418.7–1046.6 kBq/pmol) based on a molecular mass of 153 kDa. The average immunoreactivity was reported to be 95%–98%. The molecular mass profile of ^{125}I/^{131}I-HuCC49ΔC$_H$2 was evaluated by autoradiography after SDS-PAGE under non-reducing and reducing conditions. Under non-reducing conditions, the HuCC49ΔC$_H$2 migrated as two bands, a major band of 153 kDa and a band of 73 kDa. The band of 153 kDa was consistent with the molecular mass of an IgG with a C$_H$2 domain deletion. Size-exclusion chromatography under non-denaturing conditions yielded only one major peak, which corresponded to the C$_H$2-deleted HuCC49. Under reducing conditions, the HuCC49ΔC$_H$2 was resolved into two bands with molecular masses of 43 and 27 kDa. This was consistent with the molecular masses of an IgG heavy chain with a C$_H$2 domain deletion and an intact light chain, respectively.

Liu et al. (25) reported biosynthesis of HuCC49ΔC$_H$2 in mammalian Chinese hamster ovary cells produced two homodimeric isoforms (form A and form B) in ~50:50 mixture. Form A was found to be more stable and is the preferred molecule for clinical development. On the basis of this observation, Glaser et al. (22) developed a panel of hinge-engineered HuCC49ΔC$_H$2 Abs to produce >98% form A isoform. In human studies with radioiodinated HuCC49ΔC$_H$2, the unbound radioactive iodine was removed by ion exchange cartridge or high-performance liquid chromatography (HPLC). The final product was sterilized by filtration. Quality control tests including purity, immunoreactivity, apyrogenicity, and sterility in accordance with the United States Food and Drug Administration regulations were performed before patient administration (2-4). Forero et al. (2) reported a radiochemical efficiency and purity >99.0% by HPLC and an immunoreactivity of 67%–69%.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Slavin-Chiorini et al. (1) conducted *in vitro* solid-phase competition immunoassays of ^{125}I-HuCC49ΔC$_H$2 Ab with bovine submaxillary mucin (BSM) as the TAG-72 antigen source. The 50% inhibitory concentration value (IC$_{50}$) was ~1.5–3.5 nM. The apparent affinity constant (K_a) of unlabeled HuCC49ΔC$_H$2 Ab was 5.1 × 10–^{9}.

Animal Studies

Rodents

[PubMed]

Pharmacokinetics studies of radioiodinated-HuCC49ΔC$_H$2 Ab were performed in groups of five normal nude mice (1). No thyroid blockage was reported. Each mouse received a mixture of 122.1 kBq (3.3 μCi) of ^{131}I-HuCC49ΔC$_H$2 Ab and 118.4 kBq (3.2 μCi) ^{125}I-HuCC49 Ab by i.v. or i.p. administration. In the i.v. injection group, starting at 0.5 h after administration, ^{131}I-HuCC49ΔC$_H$2 Ab showed a significantly faster plasma clearance than the intact ^{125}I-HuCC49 Ab. About 74% and 99% of ^{131}I-HuCC49ΔC$_H$2 had cleared the plasma at 6 h and 14 h, respectively. In comparison, 58% and 74% of ^{125}I-HuCC49 were cleared at 6 h and 24 h, respectively. In the i.p. injection group, the percentage injected dose per g (% ID/g) radioactivity of ^{131}I-HuCC49ΔC$_H$2 that moved out of the abdominal cavity and into serum peaked at 2 h while the radioactivity of ^{125}I-HuCC49 peaked between 2h and 4 h. Less than 1% of ^{131}I-HuCC49ΔC$_H$2 radioactivity remained in the plasma at 24 h. In comparison, 3% of ^{125}I-HuCC49 radioactivity remained in the plasma at 120 h. Even more pronounced differences in the pharmacokinetic profiles between ^{131}I-HuCC49ΔC$_H$2 and ^{125}I-HuCC49 were found in severely compromised immunodeficient mice by either i.v. or i.p. injection of the radioiodinated Abs.

Slavin-Chiorini et al. (1) performed biodistribution studies of ^{131}I-HuCC49ΔC$_H$2 in nude mice bearing TAG-72 tumors. No thyroid blockage was reported. Each mouse received a mixture of 122.1 kBq (3.3 μCi) ^{131}I-HuCC49ΔC$_H$2 and 118.4 kBq (3.2 μCi) intact ^{125}I-HuCC49 by i.v. or i.p. administration. In the i.v. injection group (n = 5), the average ^{131}I-HuCC49ΔC$_H$2 tumor radioactivity levels (% ID/g) were 11.1 (24 h), 10.6 (48 h), 10.3 (72 h), and 5.6 (120 h). The ^{125}I-HuCC49 tumor radioactivity levels (% ID/g) were 9.0 (24 h), 11.0 (48 h), 14.2 (72 h), and 18.6 (120 h). The average radiolocalization indices (RI = tumor % ID/g divided by normal tissue % ID/g) of ^{131}I-HuCC49ΔC$_H$2 for the tumor/blood ratio were 7.4 (24 h), 102.0 (48 h), 226.1 (72 h), and 264.4 (120 h). The tumor/blood RI values for ^{125}I-HuCC49 were 1.4 (24 h), 4.9 (48 h), 6.1 (72 h), and 10.6 (120 h). At 24 h, the radioactivity levels (% ID/g) of ^{131}I-HuCC49ΔC$_H$2 in other major organs were 2.0 (blood), 6.4 (liver), 8.0 (spleen), 1.5 (kidney), and 1.5 (lung). At 120 h, these values changed to 0.0 (blood), 0.1 (liver), 0.3 (spleen), 0.0 (kidney), and 0.0 (lung). In the i.p. injection group, the average ^{131}I-HuCC49ΔC$_H$2 tumor radioactivity levels (% ID/g) were 5.4 (24 h), 5.6 (48 h), 3.9 (72 h), and 2.8 (120 h). The average tumor/blood ratio RI values were 7.0 (24 h), 51.6 (48 h), 146.5 (72 h), and 409.7 (120 h). In comparison, the average ^{125}I-HuCC49 tumor radioactivity levels (% ID/g) were 5.8 (24 h), 10.6 (48 h), 11.0 (72 h), and 18.8 (120 h). The average tumor/blood ratio RI values were 1.2 (24 h), 3.0 (48 h), 4.5 (72 h), and 12.9 (120 h). At 24 h, the radioactivity levels (% ID/g) of ^{131}I-HuCC49ΔC$_H$2 in other major organs were 0.8 (blood), 2.9 (liver), 2.0 (spleen), 0.6 (kidney), and 0.6 (lung). At 120 h, these values changed to 0.0 (blood), 0.0 (liver), 0.2 (spleen), 0.0 (kidney), and 0.0 (lung).

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

Agnese et al. (4) conducted a pilot study of ^{125}I-HuCC49ΔC$_H$2 in 20 patients with recurrent colorectal cancer. After thyroid blockage, each patient received 74 MBq/mg (2 mCi/mg) or 74 MBq/6.54 nmol (2 mCi/6.54 nmol based on a molecular mass of 153 kDa) of ^{125}I-HuCC49ΔC$_H$2. Surgical operations were performed 3–24 days after injection. During the operation, a radioimmunoguided surgical (RIGS) exploration with a handheld gamma-detecting probe was performed immediately after the traditional exploration. The final study group comprised five patients who underwent exploration when distinction of truly positive tissue from background counts was possible. In the traditional exploration, 9 out of 10 tumor sites (90%) were confirmed by conventional histological methods. In the RIGS exploration, 14 out of 15 tumor sites (93%) were confirmed by pathological studies. There were 5 pathologically confirmed tumor sites identified by RIGS but not identified by traditional exploration. Only 1 pathologically confirmed tumor site was identified by traditional exploration but not by RIGS. None of the patients expressed significant human anti-mouse antibody (HAMA) response after exposure to ^{125}I-HuCC49ΔC$_H$2.

Forero et al. (2) obtained HuCC49ΔC$_H$2 from the National Cancer Institute under its Investigational New Drug Application No. 9260 for study in four patients with TAG-72–positive metastatic colorectal carcinoma. HuCC49ΔC$_H$2 was labeled with ^{131}I at 370 MBq/mg (10 mCi/mg) or 56.6 kBq/pmol (1.52 μCi/pmol) based on a molecular mass of 153 kDa. With thyroid blockage, unlabeled HuCC49ΔC$_H$2 Ab was infused in sufficient amounts to bring the total dose of antibody protein to 20 mg prior to the injection of the ^{131}I-HuCC49ΔC$_H$2. Each patient received 370 MBq (10 mCi) ^{131}I-HuCC49ΔC$_H$2 by i.v. infusion. The mean pharmacokinetic parameter values of these 4 patients were 20 ± 2.6 h (serum $t_{½}$), 3.5 ± 0.5 μCi/ml (peak concentration), 10 ± 2 h/μCi/ml (area under the curve), 1.5 ± 0.1 ml/h/kg (clearance), and 43 ± 6 ml/kg (volume of distribution). In comparison, the serum $t_{½}$ of intact ^{131}I-murine CC49 was 50 ± 11 h. Gamma imaging of ^{131}I-HuCC49ΔC$_H$2 showed at least one known tumor site in each patient but fewer tumor sites were consistently detected than those found with conventional x-ray computed tomography scan. Dosimetry studies showed that the mean absorbed doses were 0.135 mGy/MBq (0.5 rad/mCi) for whole body, 0.270 mGy/MBq (rad/mCi) for marrow, 0.621 mGy/MBq (rad/mCi) for liver, 1.081 mGy/MBq (rad/mCi) for kidney, 4.351 mGy/MBq (16.1 rad/mCi) for thyroid, and 2 mGy/MBq (7.4 rad/mCi) for tumor. Immunogenicity studies showed that there was no evidence of antibody response to HuCC49ΔC$_H$2 in three patients over a 6-week period, and one patient had a minimal

response by week 6. No acute toxicity, bone marrow suppression, or other nonhematologic toxicities were reported in these four patients.

Xiao et al. (3) studied ^{125}I-HuCC49ΔC_H2 in 21 patients with colorectal carcinoma by giving 74 MBq/mg (2 mCi/mg) of the radiolabeled Ab by i.v. bolus. Thyroid blockage was instituted, and each patient underwent exploratory laparotomy 3–20 days after injection. Using precordial detection of radioactivity with a handheld gamma-detecting probe, the ^{125}I-HuCC49ΔC_H2 circulation appeared to fit into a two-compartment open model with first-order elimination from the central compartment. The $t_{½\alpha}$ (day 1 to day 10) and $t_{½\beta}$ (day 10 to day 20) were ~1.34 and 12.8 days, respectively. Urinary excretion accounted for 63.6 ± 20.3% of the total dose. There appeared to be a linear relationship between the tumor radioactivity level and the circulating radioactivity level. The tumors showed five- to ten-fold higher radioactivity levels above the background (blood and normal organs) over 3–21 days. After 20 days, the radioactivity levels in blood and normal organs decreased to a minimal level. Intestinal primary tumors and metastatic liver tumors had the highest radioactivity levels, whereas the abdominal wall tumors had the lowest levels. No patients showed a HAMA response.

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