^{64}Cu-Anti-human integrin $\alpha_v\beta_3$ monoclonal antibody

^{64}Cu-DOTA-hLM609-II

Kenneth T. Cheng, PhD[1]

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Chemical name:	^{64}Cu-Anti-human integrin $\alpha_v\beta_3$ monoclonal antibody	
Abbreviated name:	^{64}Cu-DOTA-hLM609-II	
Synonym:	^{64}Cu-Abegrin™, ^{64}Cu-Vitaxin™, ^{64}Cu-MEDI522™, ^{64}Cu-LM609, ^{64}Cu-anti- $\alpha_v\beta_3$ MAb	
Agent Category:	Antibody	
Target:	Integrin $\alpha_v\beta_3$	
Target Category:	Antibody-antigen binding	
Method of detection:	Positron emission tomography (PET)	
Source of signal:	^{64}Cu	
Activation:	No	
Studies:	• *In vitro* • Rodents • Humans	Click on protein, nucleotide (RefSeq), and gene for more information about integrin $\alpha_v\beta_3$.

Background

[PubMed]

^{64}Cu-Anti-human integrin $\alpha_v\beta_3$ monoclonal antibody (^{64}Cu-DOTA-hLM609-II) is a radiolabeled antibody developed for positron emission tomography (PET) imaging of integrin $\alpha_v\beta_3$–positive tumors (1). ^{64}Cu-DOTA-hLM609-II is labeled with ^{64}Cu which is a positron emitter with a half-life ($t_{½}$) of 12.7 h.

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.nih.gov.

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Cellular survival, invasion, and migration control embryonic development, angiogenesis, tumor metastasis, and other physiological processes. Among the molecules that regulate angiogenesis are integrins, a superfamily of cell adhesion proteins that form heterodimeric receptors for extracellular matrix (ECM) molecules (2, 3).These transmembrane glycoproteins consist of two noncovalently associated subunits, α and β (18 α- and 8 β-subunits in mammals), which are assembled into at least 24 α/β pairs. Several integrins, such as integrin $\alpha_v\beta_3$, have affinity for the arginine-glycine-aspartic acid (RGD) tripeptide motif, which is found in many ECM proteins. The integrin $\alpha_v\beta_3$ receptor is generally not found in normal tissue but is strongly expressed in vessels with increased angiogenesis, such as tumor vasculature. It is significantly upregulated in certain types of tumor cells and in almost all tumor vasculature. Increased levels of integrin $\alpha_v\beta_3$ expression are closely associated with increased cell invasion and metastasis. Molecular imaging of a probe that binds to the integrin $\alpha_v\beta_3$ can be used to image tumor vasculature and evaluate angiogenic response to tumor therapy (4, 5).

Radiolabeled monoclonal antibodies (MAbs) have been developed for both the diagnosis and treatment of tumors (6-8). Radiometals with appropriate photon or positron emissions can be used to radiolabel antibodies and in imaging studies (9, 10). Cheresh and Harper (11, 12) first reported the synthesis of LM609 MAb, a murine IgG1 antibody (mLM609), which appeared to react with and recognize $\alpha_v\beta_3$ as one entity. The mLM609 was humanized by grafting the murine complementary-determining regions (CDRs) onto a human framework (13, 14). The humanized LM609 (hLM609) was shown to retain the affinity properties of the murine antibody. This first version of hLM609 (hLM609-I) was used in clinical studies for cancer therapy (15). These studies reported no significant toxicity or immune response from hLM609-I. However, ^{99m}Tc labeling of hLM609-I was unsuccessful due to the *in vivo* instability of the ^{99m}Tc label (16). Another clone (hLM609-II) with >50-fold enhanced affinity was also produced using a phage-expressed libraries and focused mutagenesis strategy in a stepwise fashion (13, 15). hLM609-II is currently in clinical trials for treatment of prostate cancer, psoriasis, melanoma, and rheumatoid arthritis. Cai et al. (1) successfully radiolabeled hLM609-II with ^{64}Cu using 1,4,7,10-tetra-azacyclododecane *N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) as the bifunctional chelator.

Synthesis

[PubMed]

Cheresh et al. (11, 15) used the standard hybridoma technique to produce murine mLM609 MAb against the M21 human melanoma cell adhesion receptor. mLM609 specifically blocked endothelial cell attachment to von Willebrand factor, fibrinogen, vitronectin, and an RGD peptide. mLM609-I was humanized by phage expression technologies and codon-based mutagenesis (13, 14). This hLM609 retained the properties of mLM609 as determined by *in vitro* and *in vivo* characterization. Wu et al. (13) improved the binding characteristics of hLM609-I by constructing phage-expressed libraries of hLM609-I Fab variants and a mutagenesis strategy that mutated every position

of all six CDRs. The expression and screening of a combinatorial library produced 2592 variants. This resulted in obtaining the hLM609-II clone that displayed >50-fold enhanced affinity. Cai et al. (1) used DOTA to conjugate and radiolabel hML609-II. Briefly, commercially obtained DOTA was activated by 1-ethyl-3-[3-(dimethylamino)-propyl] carbodimide (EDC) and *N*-hydroxysulfonosuccinimide (SNHS) at pH 5.5 for 30 min with a molar ratio of 10:5:4 (DOTA:EDC:SNHS). The activated product, DOTA-*N*-hydroxysulfosuccinimidyl, was cooled to 4°C and added to hLM609-II. The mixture was adjusted to pH 8.5 and allowed to incubate for overnight at 4°C. In the radiolabeling, ^{64}Cu chloride ($^{64}CuCl_2$) was diluted in 300 µl of 0.1 mol/liter sodium acetate buffer (pH 6.5) and added to the DOTA-hLM609-II conjugates in a ratio of 25 µg DOTA-hLM609-II per mCi of ^{64}Cu. The reaction mixture was incubated for 1 h at 40°C. ^{64}Cu-DOTA-hLM609-II was purified by PD-10 column. Radiolabeling yields appeared to be dependent on the DOTA/hLM609-II ratios in the reaction mixtures. The % yields (n = 3) were 5.20 ± 3.16 (20:1), 14.05 ± 17.12 (50:1), 25.32 ± 11.50 (100:1), 37.32 ± 25.94 (200:1), and 88.12 ± 6.98 (1000:1). The average numbers of DOTA per hLM609-II molecule were calculated as 1.65 ± 0.32 (20:1), 3.29 ± 2.01 (50:1), 6.38 ± 3.52 (100:1), 9.20 ± 3.16 (200:1), and 38.53 ± 5.71 (1000:1). The specific activities varied from 78.07 ± 47.36 MBq/mg (2.11 ± 1.28 mCi/mg) to 957.93 ± 75.85 MBq/mg (25.89 ± 2.05 mCi/mg).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The immunoreactivity of each ^{64}Cu-DOTA-hLM609-II preparation from different DOTA/hLM609-II ratios was tested by binding assay with integrin $\alpha_v\beta_3$–positive U87MG cells (1). Regardless of the DOTA/hLM609-II ratios and yields, all preparations (n = 3) had similar immunoreactivity which ranged from 59.78 ± 1.33% to 71.13 ± 2.58%.

Animal Studies

Rodents

[PubMed]

Biodistribution studies of ^{64}Cu-DOTA-hLM609-II were conducted in nude mice bearing MDA-MB-435 human breast tumors (1). Each mouse received 0.74 MBq (20 µCi) of ^{64}Cu-DOTA-hLM609-II. All ^{64}Cu-DOTA-hLM609 preparations with different DOTA/hLM609-II ratios showed good tumor radioactivity levels. The 1000:1 DOTA/hLM609-II ratio preparation (n = 3) had the highest tumor radioactivity uptakes expressed in percentage injected dose/g (% ID/g) with 12.50 ± 2.73 (4 h), 39.88 ± 7.05 (25 h), and 49.41 ± 4.54 (71 h). High radioactivity levels were also found in the heart and liver at early time points, which then dropped steadily over time. The radioactivity levels (% ID/g) in the heart were 29.08 ± 5.90 (4 h), 13.89 ± 3.13 (17 h), and 4.38 ± 0.28 (71 h). The radioactivity levels in the liver were 19.56 ± 4.43 (4 h), 11.91 ± 1.68 (17 h), and 8.51 ± 1.31 (71 h). The serum $t_{½}$ was ~12–24 h.

The 1000:1 DOTA/hLM609-II ratio preparation was used in PET imaging of mouse tumor models with different integrin $\alpha_v\beta_3$ cell density levels in the following order: U87MG human glioblastoma > MDA-MB-435 human breast carcinoma > PC-3 human prostate adenocarcinoma ~GL-26 mouse glioblastoma (1). Each mouse received 7.4–11.1 MBq (200-300 μCi) ^{64}Cu-DOTA-hLM609-II radioactivity. All tumors exhibited high tumor activity by 17 h after injection and increased over time. The tumor signal intensity was highest with U87MG, whereas the signals for MDA-MB-435 and PC-3 were similar. The GL-26 mouse glioblastoma signal intensity reached a peak at 24 h and then steadily dropped over time. The authors suggested that this low activity uptake in mouse tumor was because hLM609-II did not cross-reacted with mouse integrin $\alpha_v\beta_3$. Blocking studies with coinjection of 2 mg of unlabeled hLM609-II showed effective blocking of 50-65% ^{64}Cu-DOTA-hLM609-II radioactivity uptake in mice bearing MDA-MB-435 tumors (extrapolated from Figure 5A).

Immunofluorescence staining was performed in tumor sections after PET imaging studies with ^{64}Cu-DOTA-hLM609-II (1). These *ex vivo* experiments confirmed the human integrin $\alpha_v\beta_3$– specific binding of ^{64}Cu-DOTA-hLM609-II.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

Based on PET imaging data from rats, Cai et al. (1) estimated human absorbed doses (n = 3) of ^{64}Cu-DOTA-hLM609-II to normal organs. The rat organ distribution of ^{64}Cu-DOTA-hLM609-II was similar to that of mice with the exception that the liver radioactivity level was more prominent and persistent. The liver appeared to be the critical organ with 0.330 ± 0.020 mGy/MBq (1.220 ± 0.076 rad/mCi). The estimated radiation absorbed doses (mGy/MBq) in other major organs of an adult human were 0.207 ± 0.011 in the spleen (0.766 ± 0.040 rad/mCi), 0.044 ± 0.001 in the gallbladder (0.163 ± 0.004 rad/mCi), 0.060 ± 0.002 in the heart: (0.221 ± 0.009 rad/mCi), 0.036 ± 0.003 in the kidneys (0.132 ± 0.012 rad/mCi), 0.032 ± 0.000 in the adrenals (0.117 ± 0.001 rad/mCi), 0.032 ± 0.000 in the pancreas (0.117 ± 0.001 rad/mCi), and 0.015 ± 0.001 in the brain (0.117 ± 0.001 rad/mCi), The whole-body effective dose was 0.034 ± 0.001 mGy/MBq (0.127 ± 0.003 rad/mCi).

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