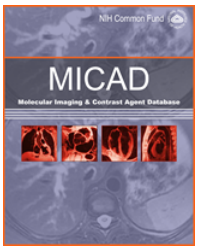
U.S. National Library of Medicine
National Center for Biotechnology Information

NLM Citation: Chopra A. [^{64}Cu](1-*N*-(4-aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]-eicosane-1,8-diamine)-anti-GD2 monoclonal antibody. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.
Bookshelf URL: https://www.ncbi.nlm.nih.gov/books/



[^{64}Cu](1-*N*-(4-aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]-eicosane-1,8-diamine)-anti-GD2 monoclonal antibody

Arvind Chopra, PhD[1]

Chemical name:	[^{64}Cu](1-*N*-(4-aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]-eicosane-1,8-diamine)-anti-GD2 monoclonal antibody
Abbreviated name:	[^{64}Cu]SarAr-anti-GD2 mAb
Synonym:	[^{64}Cu]SarAr-mAb
Agent Category:	Monoclonal antibody
Target:	Disialogangliosides
Target Category:	Antibody-ligand binding
Method of detection:	Positron emission tomography (PET)
Source of Signal/Contrast:	^{64}Cu
Activation:	No
Studies:	• *In vitro* • Rodents

Structure not available.

Background

[PubMed]

Positron emission tomography (PET) is a common technique used in conjunction with fluorodeoxyglucose labeled with radioactive fluorine ([^{18}F]FDG) to investigate metabolism and to detect and evaluate the efficacy of cancer therapy (1, 2). Although a variety of ^{18}F-labeled small molecule compounds, their derivatives, and antibodies (including those that were bioengineered) have been developed and evaluated in addition to [^{18}F]FDG for PET imaging, the short physical half-life of this isotope (110 min) and the requirement of specialized equipment and personnel for the synthesis of ^{18}F derivatives are major limitations for its use in the clinic (3). Investigators have also evaluated the use of radioactive copper (^{64}Cu) in an effort to develop PET imaging agents that have a longer half-life (12.7 h); however, biodistribution studies in rats have revealed that transchelation of ^{64}Cu to copper binding proteins such as ceruloplasmin and superoxide dismutase in the liver and albumin results in the high background observed with this isotope during imaging (4). In this regard, investigators have been in search of chelating agents that can be used to produce ^{64}Cu complexes with an

Author Affiliation: 1 National Center for Biotechnology Information, NLM, NIH, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

extended stability under *in vivo* conditions, have good labeling properties, and are easily conjugated to molecules or antibodies with specific targets for PET imaging (5, 6).

Disialogangliosides (GD2) are ubiquitous and abundant surface glycolipid antigens found on tumors of neuroectodermal origin, including melanoma and neuroblastoma tumors. Therefore, these antigens are considered ideal targets for the immunotherapy of these diseases (7, 8). The use of an anti-GD2 monoclonal antibody (mAb) is one of the options available for the treatment of these cancers, and several clinical trials have been approved by the United States Food and Drug Administration for the use of this antibody to treat children and adults. The status of some of the clinical trials being conducted with the anit-GD2 antibody has been reviewed by Modak et al. (7).

Voss et al. developed a novel chelating agent, 1-*N*-(4-aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]-eicosane-1,8-diamine (SarAr), that they used to chelate ^{64}Cu to an anti-GD2 mAb. The authors evaluated SarAr under *in vivo* conditions for the imaging of human neuroblastoma and melanoma tumors in athymic mice (6).

Synthesis

[PubMed]

A murine anti-GD2 antibody, 14.G2a, was purchased commercially, and a chimeric derivative of this antibody, ch14.18, was engineered as detailed elsewhere (9). The ch14.18 mAb was used to perform the studies detailed in this chapter. The two antibodies have a similar murine variable region sequence and an identical antigen-binding Fv region, and they were reported to contain distinct constant region sequences (6).

Synthesis of the SarAr chelator was described by Di Bartolo et al. (10). Using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), the SarAr ligand was conjugated through its primary aromatic group to the carboxyl groups of the glutamate and aspartate residues of the 14.G2a and ch14.18 mAbs (6). To achieve this, EDC was added to the mAb in a ratio of 250:1 for SarAr:IgG as described elsewhere using sodium acetate buffer (pH 5.0) at 37°C for 30 min (11). Unbound SarAr was removed from the immunoconjugates with high-performance liquid chromatography (HPLC), and the SarAr-conjugated mAbs were concentrated by use of spin filters for storage at -80°C. Four SarAr molecules were estimated to be bound to each mAb molecule as determined by serial dilution experiments (6).

The SarAr-conjugated antibodies were radiolabeled using [^{64}Cu]copper chloride in sodium acetate buffer (pH 5.0) at 37°C for 30 min (6). The investigators reported the same labeling efficiency could also be obtained at 21°C for 10 min. The labeling efficiency was routinely 99% as determined by HPLC and thin-layer chromatography (TLC); the Rf values for TLC were not provided. Specific activity of [^{64}Cu]SarAr mAb was reported to be 10 μCi/6.6 nmol (370 kBq/6.6 nmol). An investigation of ^{64}Cu-labeled ch14.18 by radioimmunoassay and cell-binding studies showed that the labeled mAb had immunoreactivity similar to the unlabeled mAb (6).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

No publications are currently available.

Animal Studies

Rodents

[PubMed]

Biodistribution studies were performed with athymic *nu/nu* mice bearing xenograft tumors derived from M21 (human melanoma cells that express high levels of GD2 antigen (12)), IMR-6 (human neuroblastoma cells that express moderate levels of GD2 antigen (13)), or PC-3 cells (human prostate carcinoma cells that are negative for the GD2 antigen) (6). Animals were injected with the ^{64}Cu-labeled mAb through the tail vein, and the animals were killed at various time points (n = 4 animals per time point) between 6 and 48 h after administration of the radiotracer. The major organs, including tumors, were then removed and weighed, and incorporated radioactivity was counted. Approximately 15–20% of the radioactivity (injected dose per gram tissue (% ID/g) was determined to be associated with the tumors 24 h after injection of the radiotracer (126.7 ± 0.6% ID/g for the IMR-6 neuroblastoma, 20.5 ± 2.8% ID/g for M21 melanoma cells) (6). Under the same conditions, the GD2-negative xenograft tumors (PC-3) showed a low level of radiotracer incorporation (<5% ID/g). Low levels of radioactivity were observed in the liver of these animals.

PET imaging was performed at 24 and 48 h after the radiotracer injection (6). High accumulation of the label was evident in the GD2-positive tumors with low uptake in the surrounding nontarget tissue, including the liver. These results were in agreement with those obtained from the individual tissue removed from the animals.

Other Non-Primate Mammals

[PubMed]

No publications are currently available.

Non-Human Primates

[PubMed]

No publications are currently available.

Human Studies

[PubMed]

No publications are currently available.

Supplemental Information

[Disclaimers]

References

1. Kumar R., Basu S., Torigian D., Anand V., Zhuang H., Alavi A. Role of modern imaging techniques for diagnosis of infection in the era of 18F-fluorodeoxyglucose positron emission tomography. Clin Microbiol Rev. 2008; **21** (1):209–24. PubMed PMID: 18202443.
2. Juweid M.E. 18F-FDG PET as a routine test for posttherapy assessment of Hodgkin's disease and aggressive non-Hodgkin's lymphoma: where is the evidence? J Nucl Med. 2008; **49** (1):9–12. PubMed PMID: 18077522.
3. Smith S.V. Molecular imaging with copper-64. J Inorg Biochem. 2004; **98** (11):1874–901. PubMed PMID: 15522415.
4. Boswell C.A., Sun X., Niu W., Weisman G.R., Wong E.H., Rheingold A.L., Anderson C.J. Comparative in vivo stability of copper-64-labeled cross-bridged and conventional tetraazamacrocyclic complexes. J Med Chem. 2004; **47** (6):1465–74. PubMed PMID: 14998334.
5. Sprague J.E., Peng Y., Fiamengo A.L., Woodin K.S., Southwick E.A., Weisman G.R., Wong E.H., Golen J.A., Rheingold A.L., Anderson C.J. Synthesis, characterization and in vivo studies of Cu(II)-64-labeled cross-

bridged tetraazamacrocycle-amide complexes as models of peptide conjugate imaging agents. J Med Chem. 2007; **50** (10):2527–35. PubMed PMID: 17458949.

6. Voss S.D., Smith S.V., DiBartolo N., McIntosh L.J., Cyr E.M., Bonab A.A., Dearling J.L., Carter E.A., Fischman A.J., Treves S.T., Gillies S.D., Sargeson A.M., Huston J.S., Packard A.B. Positron emission tomography (PET) imaging of neuroblastoma and melanoma with 64Cu-SarAr immunoconjugates. Proc Natl Acad Sci U S A. 2007; **104** (44):17489–93. PubMed PMID: 17954911.
7. Modak S., Cheung N.K. Disialoganglioside directed immunotherapy of neuroblastoma. Cancer Invest. 2007; **25** (1):67–77. PubMed PMID: 17364560.
8. Riemer A.B., Forster-Waldl E., Bramswig K.H., Pollak A., Zielinski C.C., Pehamberger H., Lode H.N., Scheiner O., Jensen-Jarolim E. Induction of IgG antibodies against the GD2 carbohydrate tumor antigen by vaccination with peptide mimotopes. Eur J Immunol. 2006; **36** (5):1267–74. PubMed PMID: 16568495.
9. Gillies S.D., Lo K.M., Wesolowski J. High-level expression of chimeric antibodies using adapted cDNA variable region cassettes. J Immunol Methods. 1989; **125** (1-2):191–202. PubMed PMID: 2514231.
10. Di Bartolo N.M., Sargenson A.M., Donlevy T.M., Smith S.V. Synthesis of a new cage ligand, SarAr, and its complexation with select transition metal ions for potential use in radioimaging. J Chem Soc Dalton Trans. 2001;(15):2303–2309.
11. Di Bartolo N.M., Sargenson A.M., Smith S.V. New 64Cu PET imaging agents for personalized medicine and drug development using the hexa-aza, SarAr. Organic and Biomolecular Chemistry. 2006; **4** :335–3357.
12. Mueller B.M., Reisfeld R.A., Gillies S.D. Serum half-life and tumor localization of a chimeric antibody deleted of the CH2 domain and directed against the disialoganglioside GD2. Proc Natl Acad Sci U S A. 1990; **87** (15):5702–5. PubMed PMID: 2198570.
13. Cheung N.K., Neely J.E., Landmeier B., Nelson D., Miraldi F. Targeting of ganglioside GD2 monoclonal antibody to neuroblastoma. J Nucl Med. 1987; **28** (10):1577–83. PubMed PMID: 3655911.