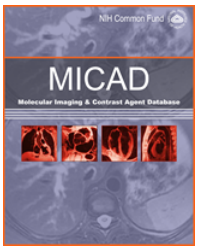
U.S. National Library of Medicine
National Center for Biotechnology Information

NLM Citation: Leung K. Gadolinium-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid monoamide-G2 nanoglobule-CGLIIQKNEC (CLT1)-Cy5. 2013 Apr 21 [Updated 2013 Jun 13]. 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/



Gadolinium-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid monoamide-G2 nanoglobule-CGLIIQKNEC (CLT1)-Cy5

CLT1-G2-(Gd-DOTA-MA)-Cy5

Kam Leung, PhD[1]

Created: April 21, 2013; Updated: June 13, 2013.

Chemical name:	Gadolinium-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid monoamide-G2 nanoglobule-CGLIIQKNEC (CLT1)-Cy5	
Abbreviated name:	CLT1-G2-(Gd-DOTA-MA)-Cy5	
Synonym:		
Agent category:	Peptide	
Target:	Fibrin-fibronectin clot complexes	
Target category:	Acceptor	
Method of detection:	Multimodal: magnetic resonance imaging (MRI); near-infrared (NIR) fluorescence imaging, optical	
Source of signal/contrast:	Gadolinium, Gd; Cy5	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available in PubChem.

Background

[PubMed]

Extracellular matrix adhesion molecules consist of a complex network of fibronectins, collagens, chondroitins, laminins, glycoproteins, heparin sulfate, tenascins, and proteoglycans that surround connective tissue cells, and they are mainly secreted by fibroblasts, chondroblasts, and osteoblasts (1). Cell substrate adhesion molecules are considered essential regulators of cell migration, differentiation, and tissue integrity and remodeling. These molecules play a role in inflammation and atherogenesis, but they also participate in the process of invasion and metastasis of malignant cells in the host tissue (2). A meshwork of clotted plasma protein is present in the tumor

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

Corresponding author.

stroma but not in normal tissues, providing a functional matrix for angiogenesis, cell migration, and tumor cell invasion (3). There are high levels of collagens, fibronectin, and fibrin in the tumor connective tissues.

Gadolinium (Gd), a lanthanide metal ion with seven unpaired electrons, has been shown to be very effective in enhancing proton relaxation because of its high magnetic moment and water coordination (4, 5). Gd-Diethylenetriamine pentaacetic acid (Gd-DTPA) was the first intravenous magnetic resonance imaging (MRI) contrast agent to be used in the clinic, and a number of similar Gd chelates have been developed in an effort to further improve clinical use. However, these low molecular weight Gd chelates have short blood and tissue retention times, which limit their use as imaging agents in the vasculature and tumor tissues (6). Furthermore, these agents are largely nonspecific. CGLIIQKNEC (CLT1), a fibronectin-fibrin–binding cyclic peptide, was identified with phage display screening (3). The peptide was conjugated with Gd-DTPA to form Gd-DTPA-CLT1 for use with imaging of fibronectin-fibrin complexes in tumor tissues, and this agent exhibited specific accumulation in the tumors (7). Tan et al. (8) prepared Gd-DOTA-G3-CLT1, a CLT1-targeted contrast agent with Gd-tetraazacyclododecane-1,4,7,10-tetraacetic acid (GD-DOTA)$_{43}$ monoamide chelates and three CLT1 molecules conjugated to a generation 3 (G3) polylysine dendrimer with a cubic silsesquioxane core. Gd-DOTA-G3-CLT1 was evaluated as a MRI tumor contrast agent in nude mice bearing MDA-MB-231 human breast carcinoma xenografts. Subsequently, Tan et al. (9) prepared CLT1-G2-(Gd-DOTA-MA)-Cy5, a CLT1-targeted G2 nanoglobular Gd-DOTA monoamide and Cy5 conjugate for use with multimodality (MRI and optical) imaging of prostate cancer.

Related Resource Links:

- Chapters in MICAD (CLT1)
- Gene information in NCBI (fibrinogen, fibronectin)
- Articles in Online Mendelian Inheritance in Man (OMIM) (fibrinogen, fibronectin)
- Clinical trials (Gd-DTPA, Gd-DOTA)
- Drug information in FDA (Gd-DTPA, Gd-DOTA)

Synthesis

[PubMed]

The CLT1 conjugate with a polyethylene glycol (PEG)-propargyl group at the N-terminus was synthesized using solid-phase peptide synthesis (9). The G2 nanoglobules were synthesized as previously described (10) and activated with azido-PEG *N*-hydroxysuccinimide for CTL1 conjugation and DOTA-tris(t-Bu) for labeling with Gd (9). The G2 nanoglobule-(DOTA)$_{20}$-(Lys-PEG-azido)$_3$ conjugate was prepared with 70% yield, and its molecular weight was confirmed with mass spectroscopy. The G2 nanoglobule-(DOTA)$_{20}$-(Lys-PEG-azido)$_3$ conjugate (10.8 μmol) was incubated with excess Gd(OAc)$_3$ for 6 d at room temperature. The product, G2 nanoglobule-(Gd-DOTA)$_{20}$-(Lys-PEG-azido)$_3$ conjugate, was purified with column chromatography with 64% yield. The G2 nanoglobule-(Gd-DOTA)$_{20}$-(Lys-PEG-azido)$_3$ conjugate (2 μmol) was incubated with Cy5-NHS ester (7.2 μmol) for 24 h at room temperature to conjugate Cy5 to the ε-amino group of the (Lys-PEG-azido)$_3$ linkers. The G2 nanoglobule-(Gd-DOTA)$_{20}$-(PEG-azido)$_3$-Cy5 conjugate was purified with ultrafiltration with 87% yield. The G2 nanoglobule-(Gd-DOTA)$_{20}$-(PEG-azido)$_3$-Cy5 conjugate (2 μmol), copper(II) sulfate (2 μmol), and CTL1-PEG-propargyl conjugate (16 μmol) were incubated for 40 h at room temperature. The final product, (CTL1)$_3$-G2 nanoglobule-(Gd-DOTA)$_{20}$-Cy5$_1$ (CLT1-G2-(Gd-DOTA-MA)-Cy5) conjugate, was purified with ultrafiltration and dialysis with 33% yield, and its molecular weight (30.3 kDa) was confirmed with mass spectroscopy. The control peptide, KAREC-modified G2 nanoglobule (KAREC-G2-(Gd-DOTA-MA)-Cy5) (29.9 kDa), was similarly prepared. There were twenty Gd, one Cy5, and three peptide moieties per G2 nanoglobule.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

CLT1-G2-(Gd-DOTA-MA)-Cy5 exhibited r_1 and r_2 values of 13.4 $mM^{-1}s^{-1}$ per Gd and 14.6 $mM^{-1}s^{-1}$ per Gd at 1.5 T (9), respectively. Non-targeted KAREC-G2-(Gd-DOTA-MA)-Cy5 exhibited r_1 and r_2 values of 12.2 $mM^{-1}s^{-1}$ per Gd and 13.9 $mM^{-1}s^{-1}$ per Gd at 1.5 T, respectively. Both conjugates had the same fluorescence spectra as Cy5.

Animal Studies

Rodents

[PubMed]

Tan et al. (9) performed dynamic T1-weighted MRI (7 T) studies with intravenous injection of 0.03 mmol Gd/kg CLT1-G2-(Gd-DOTA-MA)-Cy5 or KAREC-G2-(Gd-DOTA-MA)-Cy5 in nude mice (n = 4/group) bearing orthotopic PC3 human prostate tumors. Contrast-enhanced MRI images were obtained before injection and at 1, 5, 10, 15, 20, 25, 30, 35, and 40 min after injection. Enhanced contrast in the tumor tissues was visualized for CLT1-G2-(Gd-DOTA-MA)-Cy5 at 1–40 min after injection. Contrast/noise ratios (CNRs) were 16, 17, 16, 14, and 13 at 1, 5, 10, 30, and 40 min after injection, respectively. The tumor CNRs for non-targeted KAREC-G2-(Gd-DOTA-MA)-Cy5 were significantly lower than those for CLT1-G2-(Gd-DOTA-MA)-Cy5 ($P < 0.05$), ranging from 6 at 1 min to 8 at 40 min. *Ex vivo* fluorescence imaging was performed at 2 h after injection of CLT1-G2-(Gd-DOTA-MA)-Cy5 in nude mice bearing orthotopic PC3 human prostate tumors. The tumor and lung were clearly visualized, and little fluorescence intensity was observed in the other organs. No blocking studies were performed.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

NIH Support

R01 CA097465

References

1. Bosman F.T., Stamenkovic I. *Functional structure and composition of the extracellular matrix.* . J Pathol. 2003;200(4):423–8. PubMed PMID: 12845610.

2. Jiang W.G., Puntis M.C., Hallett M.B. *Molecular and cellular basis of cancer invasion and metastasis: implications for treatment.* . Br J Surg. 1994;81(11):1576–90. PubMed PMID: 7827878.
3. Pilch J., Brown D.M., Komatsu M., Jarvinen T.A., Yang M., Peters D., Hoffman R.M., Ruoslahti E. *Peptides selected for binding to clotted plasma accumulate in tumor stroma and wounds.* . Proc Natl Acad Sci U S A. 2006;103(8):2800–4. PubMed PMID: 16476999.
4. Brasch R.C. *New directions in the development of MR imaging contrast media.* . Radiology. 1992;183(1):1–11. PubMed PMID: 1549653.
5. Runge V.M., Gelblum D.Y. *Future directions in magnetic resonance contrast media.* . Top Magn Reson Imaging. 1991;3(2):85–97. PubMed PMID: 2025435.
6. Laurent S., Elst L.V., Muller R.N. *Comparative study of the physicochemical properties of six clinical low molecular weight gadolinium contrast agents.* . Contrast Media Mol Imaging. 2006;1(3):128–37. PubMed PMID: 17193689.
7. Ye F., Wu X., Jeong E.K., Jia Z., Yang T., Parker D., Lu Z.R. *A peptide targeted contrast agent specific to fibrin-fibronectin complexes for cancer molecular imaging with MRI.* . Bioconjug Chem. 2008;19(12):2300–3. PubMed PMID: 19053180.
8. Tan M., Wu X., Jeong E.K., Chen Q., Lu Z.R. *Peptide-targeted Nanoglobular Gd-DOTA monoamide conjugates for magnetic resonance cancer molecular imaging.* . Biomacromolecules. 2010;11(3):754–61. PubMed PMID: 20131758.
9. Tan, M., Z. Ye, D. Lindner, S.M. Brady-Kalnay, and Z.R. Lu, *Synthesis and Evaluation of a Targeted Nanoglobular Dual-Modal Imaging Agent for MR Imaging and Image-Guided Surgery of Prostate Cancer.* Pharm Res, 2013
10. Kaneshiro T.L., Jeong E.K., Morrell G., Parker D.L., Lu Z.R. *Synthesis and evaluation of globular Gd-DOTA-monoamide conjugates with precisely controlled nanosizes for magnetic resonance angiography.* . Biomacromolecules. 2008;9(10):2742–8. PubMed PMID: 18771313.