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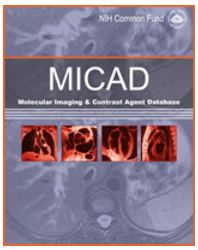

[^{177}Lu]-Labeled [(*R*)-2-amino-3-(4-isothiocyanatophenyl)propyl]-trans-(*S*,*S*)-cyclohexane-1,2-diamine-pentaacetic acid (CHX-A″-DTPA) conjugated monoclonal antibody L8A4 against epidermal growth factor receptor variant III (EGFRvIII)

[^{177}Lu]CHX-A''-DTPA-L8A4

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Chemical name:	[^{177}Lu]-Labeled [(*R*)-2-amino-3-(4-isothiocyanatophenyl)propyl]-trans-(*S*,*S*)-cyclohexane-1,2-diamine-pentaacetic acid (CHX-A″-DTPA) conjugated monoclonal antibody L8A4 against epidermal growth factor receptor variant III (EGFRvIII)	
Abbreviated name:	[^{177}Lu]CHX-A''-DTPA-L8A4	
Synonym:		
Agent Category:	Monoclonal antibody	
Target:	Epidermal growth factor receptor variant III (EGFRvIII)	
Target Category:	Receptor	
Method of detection:	Single-photon emission computed tomography; gamma planar imaging	
Source of signal / contrast:	^{177}Lu	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available in PubChem.

Background

[PubMed]

The biological characteristics, activating ligands, functions, and signal transduction pathways of the various transmembrane epidermal growth factor receptors (EGFRs) are described elsewhere (1-3). The EGFRs are known to regulate the growth, survival, differentiation, and migration of cells through the activation of an associated intracellular tyrosine kinase (TK) signaling pathway, and they are overexpressed in many malignant epithelial tumors (2, 3). Overexpression of the EGFR in tumors has been attributed to cellular amplification of

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the receptor gene, and this phenomenon may result in the production of a mutated receptor in the cell (2, 4). In addition, overexpression of the EGFR in tumors usually indicates a poor clinical prognosis for a cancer patient (4). The most common mutation observed in the receptor is the deletion of an extracellular domain segment of the EGFR, including the ligand-binding region, and this generates a variant known as EGFRvIII or de2-7 EGFR (2, 4). The generation, structure, functions, and role of EGFRvIII in tumor malignancy was reviewed by Gan et al. (5). Although EGFRvIII is nonresponsive to a ligand due to the absence of a ligand-binding site, it is constitutively active with a constantly operating downstream TK signal transduction pathway that appears to promote the development of a neoplastic phenotype, particularly for glioblastoma and to some extent for other cancers such as those of the prostate and the breast (2, 6).

Because the EGFR promotes and helps maintain transformed cells, several anti-EGFR antibodies that inhibit the activity of this receptor and small molecule drugs that block the downstream TK signaling pathway were developed and have been approved by the United States Food and Drug Administration for the treatment of certain cancers (2). The antibodies are designed to target the extracellular domain of the receptor, block ligand binding, and inhibit activation of the TK signal transduction pathway, which leads to downregulation of the EGFR on the cell surface. However, because EGFRvIII lacks the ligand-binding region on the extracellular domain, these antibodies cannot obstruct the constitutive mutant receptor activity (2). Hence, a monoclonal antibody (1), designated mAb806 and specifically targets the EGFRvIII, was generated and has been characterized in preclinical studies (7, 8). Subsequently, a chimeric form of the mAb (chAb), designated ch806, was developed and evaluated in a phase I clinical trial involving patients with cancerous tumors that overexpressed the EGFRvIII. Results from this trial indicated that ch806 can be a suitable biotherapeutic agent to treat cancers (4). Various studies performed with mAb806 or ch806 are described in separate chapters of MICAD.

Studies are also in progress with another anti-EGFRvIII mAb, L8A4, to develop a radioimmunotherapeutic (RIT) agent for the treatment of cancer (9, 10). In a recent study, the use of acyclic ([(*R*)-2-amino-3-(4-isothiocyanatophenyl)propyl]-*trans*-(*S*,*S*)-cyclohexane-1,2-diamine-pentaacetic acid (CHX-A''-DTPA) and 2-(4-isothiocyanatobenzyl)-6-methyldiethylene-triaminepentaacetic acid (1B4M-DTPA) as well as the macrocyclic ligands (*S*)-2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-tetraacetic acid (C-DOTA) and α-(5-isothiocyanato-2-methoxyphenyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (MeO-DOTA) was evaluated for the labeling of L8A4 with ^{177}Lu, which was considered suitable to generate an RIT agent against cancer (10). The characteristics of the various ^{177}Lu-labeled conjugates of L8A4 ([^{177}Lu]-CHX-A''-DTPA-L8A4, [^{177}Lu]-1B4M-DTPA-L8A4, [^{177}Lu]-C-DOTA-L8A4, and [^{177}Lu]-MeO-DOTA-L8A4) were compared to the characteristics of L8A4 labeled with *N*-succinimidyl 4-guanidinomethyl-3-[^{125}I]iodobenzoate ([^{125}I]SGMIB-L8A4) under *in vitro* (9) and *in vivo* (10) conditions.

This chapter describes results obtained from the *in vitro* and the biodistribution studies performed with [^{177}Lu]-CHX-A''-DTPA-L8A4 in athymic mice bearing subcutaneous U87MG.ΔEGFR cell glioma xenograft tumors. Results obtained with [^{125}I]SGMIB-L8A4, [^{177}Lu]-1B4M-DTPA-L8A4, [^{177}Lu]-C-DOTA-L8A4, and [^{177}Lu]-MeO-DOTA-L8A4 are presented in separate chapters of MICAD (www.micad.nih.gov) (11-14).

Other Sources of Information

Protein and mRNA sequence of human EGFR variant 1

EGFR in OMIM (Online Mendelian Inheritance in Man)

EGFR signaling pathways (NCI-Nature Pathways Interaction Database)

Anti-EGFR antibodies in PubMed

EGFR tyrosine kinase inhibitors in PubMed

Chapters on anti-EGFR antibodies in MICAD

Other chapters on anti-EGFRvIII antibodies in MICAD

Clinical trials with EGFRvIII as a target

Synthesis

[PubMed]

The preparation, purification, characterization, conjugation with CHX-A"-DTPA, and radiolabeling of CHX-A"-DTPA-L8A4 with ^{177}Lu are discussed by Hens et al. (10). The production of [^{125}I]SGMIB-L8A4 (labeled with ^{125}I using *N*-succinimidyl 4-guanidinomethyl-3-[^{125}I]iodobenzoate ([^{125}I]SGMIB)) is also described by Hens et al. (10). The radiochemical yield (RCY) of [^{177}Lu]-CHX-A"-DTPA-L8A4 was 76%. The RCY of [^{125}I]SGMIB-L8A4 and the purity of the two labeled mAbs was not reported. The specific activity of [^{177}Lu]-CHX-A"-DTPA-L8A4 was reported to be 0.092 MBq/6.6 pmol (2.5 μCi/6.6 pmol) and that of [^{125}I]SGMIB-L8A4 varied from 0.111–0.185 MBq/6.6 pmol (3–5 μCi/6.6 pmol) to 0.026–0.118 MBq/6.6 pmol (0.7–3.2 μCi/6.6 pmol). The two labeled mAbs were shown to elute as a single peak with L8A4 and had a similar retention time as the native mAb when analyzed with a size-exclusion column using high performance liquid chromatography. Each mAb molecule was determined to bear 0.3 moieties of CHX-A"-DTPA (9, 10).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Using a magnetic bead assay, the immunoreactive fraction of [^{177}Lu]-CHX-A"-DTPA-L8A4 was determined to be 60% compared to between 60% to 71% for [^{125}I]SGMIB-L8A4 (9). A cell internalization assay with U87MG.ΔEGFR cells showed that internalization of [^{177}Lu]-CHX-A"-DTPA-L8A4 continued to increase for at least 24 h (was ~35% at this time point). With the ^{125}I-labeled mAb the internalization of radioactivity peaked to ~10% of the original cell-bound radioactivity at 1 h after exposure and was reduced to ~1% of the original value by 24 h after exposure.

Animal Studies

Rodents

[PubMed]

To investigate the biodistribution of [^{177}Lu]-CHX-A"-DTPA-L8A4 and compared it to that of [^{125}I]SGMIB-L8A4, athymic mice (n = 5 animals/group) bearing subcutaneous U87MG.ΔEGFR cell glioma xenograft tumors were injected with a mixture of the two labeled mAbs as described by Hens et al. (10). The rodents were euthanized at 1, 4, and 8 days postinjection (p.i.) of the labeled mAbs and all major organs were harvested from the animals. Radioactivity incorporated in the various organs was counted using a dual-channel automated gamma counter and the data was presented as percent of injected dose per gram tissue (% ID/g). With [^{177}Lu]-CHX-A"-DTPA-L8A4 the amount of radioactivity in the blood was 15.18 ± 3.30% ID/g at 1 day p.i. and this was reduced to 0.99 ± 0.71% ID/g by 8 days p.i. At 1 day p.i., the liver, kidneys, spleen, lungs and bone exhibited accumulation of between 8.53 ± 1.86% ID/g (liver) and 5.98 ± 1.86% ID/g (spleen) and the by 8 days p.i. amount of label in all these organs, except the bone, decreased to between 0.56 ± 0.23% ID/g (lungs) and 4.45 ± 0.87% ID/g (Liver). During this period the incorporation of radioactivity in the bone increased from 5.95 ± 1.27% ID/g at 1 day p.i. to 7.56 ± 1.88% ID/g at 8 days p.i. This phenomenon was attributed to the bone seeking characteristic of unchelated ^{177}Lu indicating that [^{177}Lu]-CHX-A"-DTPA-L8A4 may not be suitable detection agent for EGFRvIII expressing tumors. The amount of tracer accumulated in the muscles was 1.30 ± 0.44% Id/g

at 1 day p.i. and was reduced to 0.19 ± 0.05% ID/g by 8 days p.i. The amount of radioactivity accumulated in the tumor was 36.28 ± 6.60% ID/g at 1 day p.i. and decreased to 8.67 ± 3.42% ID/g by 8 days p.i. The tumor/normal tissue ratios for liver, kidneys, spleen, brain, and bone were reported to be approximately 4, 6, 4, 95, and 6, respectively, at 1 day p.i. and approximately 2, 5, 2, 200 and 2, respectively at 8 days p.i.

With [^{125}I]SGMIB-L8A4 the amount of radioactivity in the blood at 1 day p.i. was 18.78 ± 2.24% ID/g and decreased to 1.29 ± 0.87% ID/g by 8 days p.i. At 1 day p.i., the liver, kidneys, spleen, and lungs exhibited accumulation between 5% and 7% ID/g and the amount of label in all these organs decreased to ~0.5% ID/g by 8 days p.i. The amount of tracer accumulated in the muscles and bones was ~1.5 and ~3.5% ID/g, respectively, at 1 day p.i.; by 8 days p.i., accumulation decreased to ~0.15% ID/g and ~0.33% ID/g, respectively, in these tissues. The tumor had a radioactivity accumulation of 21.64 ± 4.53% ID/g at 1 day p.i., which decreased to 2.86 ± 0.98% ID/g by 8 days p.i. The tumor/normal tissue ratios for liver, kidneys, spleen, brain, and bone were reported to be approximately 6, 8, 6, 60, and 10, respectively, at 1 day p.i. and approximately 7, 8, 7, 60 and 6, respectively, at 8 days p.i.

Table 1: Summary of tumor/normal tissue radioactivity uptake ratios for various organs obtained with [^{125} I]SGMIB-L8A4 and [^{177} Lu]CHX-A"-DTPA-L8A4. Data obtained from Fig. 3 in Hens et. al. (10).

Conjugated mAb	Liver		Kidney		Spleen		Brain		Bone	
	Time after labeled mAb injection									
	1 day	8 days	1 day	8 days	1 day	8 days	1 day	8 days	1 day	8 days
	Tumor-to-normal tissue ratio									
[^{125} I]SGMIB-L8A4	6	7	8	8	6	7	60	60	10	6
[^{177} Lu]CHX-A"-DTPA-L8A4	4	2	6	5	4	2	95	200	6	2

Based on the results presented in Table 1 [^{125}I]SGMIB-L8A4 appeared to be superior to [^{177}Lu]-CHX-A"-DTPA-L8A4 for the detection of EGFRvIII overexpressing tumors in rodents because it had higher tumor-to-normal tissue ratios compared to the ^{177}Lu-labeled mAb during the entire duration of the experiment (10).

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.

Supplemental Information

[Disclaimers]

No information is currently available.

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References

1. Hasselbalch B., Lassen U., Poulsen H.S., Stockhausen M.T. *Cetuximab insufficiently inhibits glioma cell growth due to persistent EGFR downstream signaling.* . Cancer Invest. 2010;28(8):775–87. PubMed PMID: 20504227.
2. Pines G., Kostler W.J., Yarden Y. *Oncogenic mutant forms of EGFR: lessons in signal transduction and targets for cancer therapy.* . FEBS Lett. 2010;584(12):2699–706. PubMed PMID: 20388509.
3. Spector N.L., Blackwell K.L. *Understanding the mechanisms behind trastuzumab therapy for human epidermal growth factor receptor 2-positive breast cancer.* . J Clin Oncol. 2009;27(34):5838–47. PubMed PMID: 19884552.
4. Scott A.M., Lee F.T., Tebbutt N., Herbertson R., Gill S.S., Liu Z., Skrinos E., Murone C., Saunder T.H., Chappell B., Papenfuss A.T., Poon A.M., Hopkins W., Smyth F.E., MacGregor D., Cher L.M., Jungbluth A.A., Ritter G., Brechbiel M.W., Murphy R., Burgess A.W., Hoffman E.W., Johns T.G., Old L.J. *A phase I clinical trial with monoclonal antibody ch806 targeting transitional state and mutant epidermal growth factor receptors.* . Proc Natl Acad Sci U S A. 2007;104(10):4071–6. PubMed PMID: 17360479.
5. Gan H.K., Kaye A.H., Luwor R.B. *The EGFRvIII variant in glioblastoma multiforme.* . J Clin Neurosci. 2009;16(6):748–54. PubMed PMID: 19324552.
6. Lee F.T., O'Keefe G.J., Gan H.K., Mountain A.J., Jones G.R., Saunder T.H., Sagona J., Rigopoulos A., Smyth F.E., Johns T.G., Govindan S.V., Goldenberg D.M., Old L.J., Scott A.M. *Immuno-PET quantitation of de2-7 epidermal growth factor receptor expression in glioma using 124I-IMP-R4-labeled antibody ch806.* . J Nucl Med. 2010;51(6):967–72. PubMed PMID: 20484439.
7. Jungbluth A.A., Stockert E., Huang H.J., Collins V.P., Coplan K., Iversen K., Kolb D., Johns T.J., Scott A.M., Gullick W.J., Ritter G., Cohen L., Scanlan M.J., Cavenee W.K., Old L.J. *A monoclonal antibody recognizing human cancers with amplification/overexpression of the human epidermal growth factor receptor.* . Proc Natl Acad Sci U S A. 2003;100(2):639–44. PubMed PMID: 12515857.
8. Perera R.M., Zoncu R., Johns T.G., Pypaert M., Lee F.T., Mellman I., Old L.J., Toomre D.K., Scott A.M. *Internalization, intracellular trafficking, and biodistribution of monoclonal antibody 806: a novel anti-epidermal growth factor receptor antibody.* . Neoplasia. 2007;9(12):1099–110. PubMed PMID: 18084617.
9. Hens M., Vaidyanathan G., Welsh P., Zalutsky M.R. *Labeling internalizing anti-epidermal growth factor receptor variant III monoclonal antibody with (177)Lu: in vitro comparison of acyclic and macrocyclic ligands.* . Nucl Med Biol. 2009;36(2):117–28. PubMed PMID: 19217523.
10. Hens M., Vaidyanathan G., Zhao X.G., Bigner D.D., Zalutsky M.R. *Anti-EGFRvIII monoclonal antibody armed with 177Lu: in vivo comparison of macrocyclic and acyclic ligands.* . Nucl Med Biol. 2010;37(7):741–50. PubMed PMID: 20870149.
11. Chopra, A., *[125I]-Labeled monoclonal antibody L8A4 against epidermal growth factor receptor variant III (EGFRvIII).* Molecular Imaging and Contrast agent Database (MICAD) [database online]. National Library of Medicine, NCBI, Bethesda, MD, USA. Available from www.micad.nih.gov, 2004 -to current.
12. Chopra, A., *177Lu-Labeled [(R)-2-amino-3-(4-isothiocyanatophenyl)propyl]-trans-(S,S)-cyclohexane-1,2-diamine-pentaacetic acid (CHX-A″-DTPA) conjugated monoclonal antibody L8A4 against epidermal growth factor receptor variant III (EGFRvIII).* Molecular Imaging and Contrast agent Database (MICAD) [database online]. National Library of Medicine, NCBI, Bethesda, MD, USA. Available from www.micad.nih.gov, 2004 -to current.
13. Chopra, A., *177Lu-Labeled (S-2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-tetraacetic acid (C-DOTA) conjugated monoclonal antibody L8A4 against epidermal growth factor receptor variant III*

(EGFRvIII). Molecular Imaging and Contrast agent Database (MICAD) [database online]. National Library of Medicine, NCBI, Bethesda, MD, USA. Available from www.micad.nih.gov, 2004 -to current.

14. Chopra, A., *177Lu-Labeled α-(5-isothiocyanato-2-methoxyphenyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (MeO-DOTA) conjugated monoclonal antibody L8A4 against epidermal growth factor receptor variant III (EGFRvIII).* Molecular Imaging and Contrast agent Database (MICAD) [database online]. National Library of Medicine, NCBI, Bethesda, MD, USA. Available from www.micad.nih.gov, 2004 -to current.