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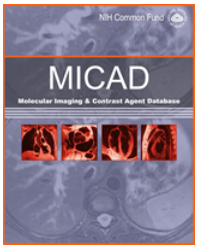

[^{111}In-1,4,7-Triazacyclononane,1-glutaric acid-4,7-acetic acid-10-maleimidoethylacetamide-Cys^{61}]-Affibody $Z_{HER2:2395}$

[^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$

Kam Leung, PhD[1]

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Chemical name:	[^{111}In-1,4,7-Triazacyclononane,1-glutaric acid-4,7-acetic acid-10-maleimidoethylacetamide-Cys^{61}]-Affibody $Z_{HER2:2395}$	
Abbreviated name:	[^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$	
Synonym:		
Agent category:	Antibody fragment, Affibody	
Target:	Epidermal growth factor receptor (EGFR, HER2)	
Target category:	Receptor	
Method of detection:	Single-photon emission computed tomography (SPECT), gamma planar imaging	
Source of signal:	^{111}In	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about HER2.

Background

[PubMed]

Epidermal growth factor (EGF) is a growth factor composed of 53 amino acids (6.2 kDa), and it is secreted by ectodermic cells, monocytes, kidneys, and duodenal glands (1). EGF stimulates growth of epidermal and epithelial cells. EGF and at least seven other growth factors and their transmembrane receptor kinases play important roles in cell proliferation, survival, adhesion, migration, and differentiation. The EGF receptor (EGFR) family consists of four transmembrane receptors: EGFR (HER1/erbB-1), HER2 (erbB-2/neu), HER3 (erbB-3), and HER4 (erbB-4) (2). HER1, HER3, and HER4 comprise three major functional domains: an extracellular ligand-binding domain, a hydrophobic transmembrane domain, and a cytoplasmic tyrosine kinase domain. No ligand has been clearly identified for HER2. However, HER2 can be activated as a result of ligand

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binding to other HER receptors with the formation of receptor homodimers and/or heterodimers (3). HER1 and HER2 are overexpressed on many solid tumor cells such as breast, non-small cell lung, head and neck, and colon cancers (4-6). The high levels of HER1 and HER2 expression on cancer cells are associated with a poor patient prognosis because high levels are related to increased proliferation (7-10).

Trastuzumab is a humanized IgG_1 monoclonal antibody (mAb) against the extracellular domain of recombinant HER2 with an affinity constant (K_d) of 0.1 nM (11). ^{111}In-Trastuzumab, Cy5.5-trastuzumab, and ^{68}Ga-trastuzumab-F(ab')$_2$ have been developed for imaging of human breast cancer (12-16). However, the pharmacokinetics of intact radiolabeled mAbs, with high liver uptake and slow blood elimination, are generally not ideal for imaging. Smaller antibody fragments, such as Fab or F(ab')$_2$, have better imaging pharmacokinetics because they are rapidly excreted by the kidneys. A novel class of recombinant affinity ligands (Affibody molecules) for HER2 was constructed based on the 58-amino-acid *Z*-domain residues from one of the IgG-binding domains of staphylococcal protein A (10). Affibody molecules exhibit high binding affinity to HER2, with K_d values of <100 pM. Various radiolabeled Affibody molecules have been studied in terms of their ability to image HER2 in tumors [PubMed]. A cysteine molecule was introduced at the C-terminus of Affibody molecules for site-specific coupling with the chelator 1,4,7,10-tetraazacyclododecane-1,4,7-tris-acetic acid-10-maleimidoethylacetamide (MMA-DOTA) for ^{111}In labeling. ^{111}In-[MMA-DOTA-Cys^{61}]-$Z_{HER2:2395}$ (^{111}In-DOTA-$Z_{HER2:2395}$) has been evaluated as a single-photon emission computed tomography (SPECT) agent in nude mice bearing human colon adenocarcinoma tumors (17). Altai et al. (18) prepared [^{111}In-1,4,7-triazacyclononane,1-glutaric acid-4,7-acetic acid-10-maleimidoethylacetamide-Cys^{61}]-Affibody $Z_{HER2:2395}$ ([^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$) for SPECT imaging of HER2 expression.

Related Resource Links:

- Chapters in MICAD (HER2)
- Gene information in NCBI (EGF, HER2)
- Articles in Online Mendelian Inheritance in Man (OMIM) (EGF, HER2)
- Clinical trials (Trastuzumab, HER2 imaging)
- Drug information in FDA (Trastuzumab)

Synthesis

[PubMed]

Affibody $Z_{HER2:2395}$ was reduced with dithiothreitol (30 mM) in 0.02 M ascorbic acid for 2 h at 40°C (18). The reduced $Z_{HER2:2395}$ was incubated with MMA-NODAGA in 1:3 molar ratio in 0.2 M ammonium acetate buffer (pH, 6.5) for 1 h at 37°C. [MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ was isolated with high-performance liquid chromatography, with 85% of $Z_{HER2:2395}$ conjugated and >96% purity. There was one MMA-NODAGA moiety per [MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ (7.5 kDa), as confirmed with mass spectroscopy. [MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ (~4 nmol) was mixed with ~30 MBq (0.81 mCi) $^{111}InCl_3$ and incubated for ~30 min at 60°C, with 99% labeling yield. [^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ was isolated with column chromatography. The specific activity was 7 MBq/nmol (0.19 mCi/nmol) at the end of synthesis. [^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ was >99% intact after incubation with 1,000-fold excess EDTA for 60 min. The DOTA compound have similar analytical data (17).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Altai et al. (18) performed binding experiments with $Z_{HER2:2395}$ with the use of a Biacore sensor chip immobilized with extracellular domain of HER2 protein. The K_d values of $Z_{HER2:342}$ and [MMA-NODAGA-

Cys^{61}]-$Z_{HER2:2395}$ were 78 pM and 67 pM, respectively. The K_d value of $Z_{HER2:2395}$ was reported to be 27 pM (17). *In vitro* cellular accumulation of [^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ was determined to be 18% and 7% of incubation dose (ID) in human ovarian carcinoma SKOV-3 and prostate carcinoma DU-145 cells expressing HER2, respectively, for 1 h at 37°C. Co-incubation with excess unlabeled $Z_{HER2:342}$ reduced the accumulation in both cell types to <1% ID. Internalization of [^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ into DU-145 cells was 28% of the cell-associated radioactivity after 8 h of incubation. Internalization of ^{111}In-[MMA-DOTA-Cys^{61}]-Z_{2395}-C into SKOV-3 cells was 21% of the initially bound activity at 24 h (17).

Animal Studies

Rodents

[PubMed]

Altai et al. (18) performed *ex vivo* biodistribution studies with 0.03 MBq (1 μCi) [^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ and [^{111}In-MMA-DOTA-Cys^{61}]-$Z_{HER2:2395}$ in normal nude mice (n = 4/group) at 1, 4, and 24 h after injection. Both tracers exhibited a rapid blood clearance, with 0.45% injected dose/gram (ID/g) for the NODAGA tracer and 1.3% ID/g for the DOTA tracer in the blood at 1 h after injection. At 24 h, the blood levels were 0.03% ID/g and 0.13% ID/g for the NODAGA and DOTA tracers, respectively. In general, the NODAGA tracer exhibited lower accumulation than the DOTA tracer in most organs. The clearance of both tracers was *via* the kidneys, with low radioactivity in the intestines (<2% ID). The kidney accumulation levels at 4 h were 70% ID/g and 203% ID/g for [^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ and [^{111}In-MMA-DOTA-Cys^{61}]-$Z_{HER2:2395}$, respectively. The biodistribution was characterized by quick clearance of radioactivity from the blood and normal organs except the kidneys. No blocking studies were performed.

Altai et al. (18) performed *ex vivo* biodistribution studies with [^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ and [^{111}In-MMA-DOTA-Cys^{61}]-$Z_{HER2:2395}$ in nude mice (n = 4/group) bearing DU-145 xenografts at 4 h after injection. The tumor accumulation of radioactivity was 4.7 ± 0.8% injected dose/gram (ID/g) and 7.5 ± 1.6% ID/g, respectively. The radioactivity level in tumors was higher than in other organs and tissues (the lung, liver, spleen, bone, muscle, and intestines) except the kidneys (60% ID/g and 128% ID/g for the NODAGA and DOTA tracers, respectively). The tumor/blood ratios were 51 and 11 for the NODAGA and DOTA tracers, respectively. In general, [^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ exhibited 1- to 2-fold higher tumor/tissue ratios than the DOTA tracer. Pretreatment with >3,000-fold excess $Z_{HER2:342}$ 60 min before injection of [^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ decreased tumor accumulation by >95% (P = 0.0005) at 1 h after injection. Little reduction was observed in the other organs.

SPECT gamma planar imaging scans were performed in nude mice (n = 3) bearing DU-145 tumors at 4 h after injection of 1 MBq (0.027 mCi) [^{111}In-MMA-NODAGA-Cys^{61}]-$Z_{HER2:2395}$ (18). The tumors were clearly visualized along with the kidneys. The tumor/background ratio was 10, as determined with region of interest analysis. 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.

References

1. Carpenter G., Cohen S. *Epidermal growth factor.* . J Biol Chem. 1990;265(14):7709–12. PubMed PMID: 2186024.
2. Yarden Y. *The EGFR family and its ligands in human cancer: signalling mechanisms and therapeutic opportunities.* . Eur J Cancer. 2001;37 Suppl 4:S3–8. PubMed PMID: 11597398.
3. Rubin I., Yarden Y. *The basic biology of HER2.* . Ann Oncol. 2001;12 Suppl 1:S3–8. PubMed PMID: 11521719.
4. Grunwald V., Hidalgo M. *Developing inhibitors of the epidermal growth factor receptor for cancer treatment.* . J Natl Cancer Inst. 2003;95(12):851–67. PubMed PMID: 12813169.
5. Mendelsohn J. *Anti-epidermal growth factor receptor monoclonal antibodies as potential anti-cancer agents.* . J Steroid Biochem Mol Biol. 1990;37(6):889–92. PubMed PMID: 2285602.
6. Yasui W., Sumiyoshi H., Hata J., Kameda T., Ochiai A., Ito H., Tahara E. *Expression of epidermal growth factor receptor in human gastric and colonic carcinomas.* . Cancer Res. 1988;48(1):137–41. PubMed PMID: 2446740.
7. Ang K.K., Berkey B.A., Tu X., Zhang H.Z., Katz R., Hammond E.H., Fu K.K., Milas L. *Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma.* . Cancer Res. 2002;62(24):7350–6. PubMed PMID: 12499279.
8. Costa S., Stamm H., Almendral A., Ludwig H., Wyss R., Fabbro D., Ernst A., Takahashi A., Eppenberger U. *Predictive value of EGF receptor in breast cancer.* . Lancet. 1988;2(8622):1258. PubMed PMID: 2903994.
9. Ethier S.P. *Growth factor synthesis and human breast cancer progression.* . J Natl Cancer Inst. 1995;87(13):964–73. PubMed PMID: 7629883.
10. Yarden Y. *Biology of HER2 and its importance in breast cancer.* . Oncology. 2001;61 Suppl 2:1–13. PubMed PMID: 11694782.
11. Carter P., Presta L., Gorman C.M., Ridgway J.B., Henner D., Wong W.L., Rowland A.M., Kotts C., Carver M.E., Shepard H.M. *Humanization of an anti-p185HER2 antibody for human cancer therapy.* . Proc Natl Acad Sci U S A. 1992;89(10):4285–9. PubMed PMID: 1350088.
12. Perik P.J., Lub-De Hooge M.N., Gietema J.A., van der Graaf W.T., de Korte M.A., Jonkman S., Kosterink J.G., van Veldhuisen D.J., Sleijfer D.T., Jager P.L., de Vries E.G. *Indium-111-labeled trastuzumab scintigraphy in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer.* . J Clin Oncol. 2006;24(15):2276–82. PubMed PMID: 16710024.
13. Lub-de Hooge M.N., Kosterink J.G., Perik P.J., Nijnuis H., Tran L., Bart J., Suurmeijer A.J., de Jong S., Jager P.L., de Vries E.G. *Preclinical characterisation of 111In-DTPA-trastuzumab.* . Br J Pharmacol. 2004;143(1):99–106. PubMed PMID: 15289297.
14. Garmestani K., Milenic D.E., Plascjak P.S., Brechbiel M.W. *A new and convenient method for purification of 86Y using a Sr(II) selective resin and comparison of biodistribution of 86Y and 111In labeled Herceptin.* . Nucl Med Biol. 2002;29(5):599–606. PubMed PMID: 12088731.
15. Smith-Jones P.M., Solit D., Afroze F., Rosen N., Larson S.M. *Early tumor response to Hsp90 therapy using HER2 PET: comparison with 18F-FDG PET.* . J Nucl Med. 2006;47(5):793–6. PubMed PMID: 16644749.
16. Smith-Jones P.M., Solit D.B., Akhurst T., Afroze F., Rosen N., Larson S.M. *Imaging the pharmacodynamics of HER2 degradation in response to Hsp90 inhibitors.* . Nat Biotechnol. 2004;22(6):701–6. PubMed PMID: 15133471.

17. Ahlgren S., Orlova A., Rosik D., Sandstrom M., Sjoberg A., Baastrup B., Widmark O., Fant G., Feldwisch J., Tolmachev V. *Evaluation of maleimide derivative of DOTA for site-specific labeling of recombinant affibody molecules.* . Bioconjug Chem. 2008;19(1):235–43. PubMed PMID: 18163536.
18. Altai M., Perols A., Karlstrom A.E., Sandstrom M., Boschetti F., Orlova A., Tolmachev V. *Preclinical evaluation of anti-HER2 Affibody molecules site-specifically labeled with (111)In using a maleimido derivative of NODAGA.* . Nucl Med Biol. 2012;39(4):518–29. PubMed PMID: 22172396.