^{177}Lu-CHX-A''-DTPA-ABD-Affibody $(Z_{HER2:342})_2$

^{177}Lu-ABD-$(Z_{HER2:342})_2$

Huiming Zhang, PhD[1]

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Chemical name:	^{177}Lu-CHX-A''-DTPA-ABD-Affibody $(Z_{HER2:342})_2$	
Abbreviated name:	^{177}Lu-ABD-$(Z_{HER2:342})_2$	
Synonym:		
Agent category:	Peptide (macromolecule)	
Target:	Human epidermal growth factor receptor (HER2)	
Target category:	Receptor	
Method of detection:	Single-photon emission computed tomography (SPECT)	
Source of signal/contrast:	^{177}Lu	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Human epidermal growth factor receptor (HER2, erbB2, neu) is a 185-kDa transmembrane glycoprotein and is a member of the superfamily of epidermal growth factor receptor (EGFR)-type receptor tyrosine kinases (RTKs) (1, 2). HER2 consists of an extracellular domain with four subdomains, a single transmembrane span, a cytoplasmic juxtamembrane linker region, a tyrosine kinase component, and a carboxyl-terminal tail (2). HER2 forms homo-oligomers of itself and heteroligomers with other HER receptors to trigger a complex network of multilayered signal transduction (1), which involves more than 30 ligands and their related adaptor proteins, cascaded enzymes, second messengers,

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

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and transcription factors (3). Activation of the network regulates cell growth, cell differentiation, and cell survival. Overexpression of HER2 has been found in a variety of malignant tumors, such as breast cancers (~30% of patients), ovarian cancers, and urinary bladder cancers (4). No ligand that directly binds to HER2 has been clearly identified to date (4). Radionuclide imaging of breast cancers largely relies on radiolabeling of monoclonal antibodies directed against HER2, such as trastuzumab and pertuzumab (5). These antibodies are normally large in size (molecular weight ~150 kDa) and have slow blood clearance and slow tumor penetration, which lead to low contrast in images (6).

Affibody molecules are scaffold proteins that bind to targets with high affinity and specificity (7). Although Affibody molecules are able to bind to the same targets as immunoglobulins, Affibody molecules have no relation to the molecular structures or amino acid sequences of the immunoglobulin family. For example, $Z_{HER2:342}$ (molecular weight ~7 kDa) is an Affibody molecule that specifically targets HER2, which consists of three-helix bundle Z-domains, each formed by 58 cysteine-free amino acids (8). The construction of $Z_{HER2:342}$ is started with a three-helix bundle derived from the immunoglobulin-binding domain (B-domain) of staphylococcal protein A. Then the amino acids on the binding surface are replaced and randomized to remove the original binding affinity and create a completely new binding affinity. The randomization produces a library containing ~10^9 members, from which $Z_{HER2:342}$ is identified as a ligand with high affinity to HER2 (22 pM). The helix bundle in $Z_{HER2:342}$ provides structural rigidity and conformational stability for efficient binding to the target (9). Affibody molecules can be fused with other proteins/molecules to further modify their affinity/avidity, modulate *in vivo* kinetics, or introduce a peptide/protein effector function (6).

^{177}Lu-CHX-A"-DTPA-ABD-Affibody $(Z_{HER2:342})_2$ (^{177}Lu-ABD-$(Z_{HER2:342})_2$, molecular weight ~19 kDa) is used with single-photon emission computed tomography (SPECT) imaging of HER2 (5). ^{177}Lu-ABD-$(Z_{HER2:342})_2$ consists of a $Z_{HER2:342}$ dimer, an albumin-binding domain (ABD), and a complex of ^{177}Lu-[(*R*)-2-amino-3-(4-isothiocyanatophenyl)propyl]-trans-(*S*,*S*)-cyclohexane-1,2-diamine-pentaacetic acid) (^{177}Lu-CHX-A"-DTPA). The use of dimeric $Z_{HER2:342}$ allows for quick extravasation and tumor penetration and stabilizes $Z_{HER2:342}$ binding to HER2 in the presence of ABD. The fused ABD (molecular weight ~5 kDa) is a stable, three-helix bundle of 46 amino acids derived from the monovalent variant of albumin-binding motif in streptococcal protein G (10). ABD binds to albumin in blood reversibly with a dissociation constant of 4 nM (10) to generate a complex of ~87 kDa, leading to a prolonged plasma half-life and reduced uptake in kidneys (5). ^{177}Lu is a radionuclide belonging to the group of rare earth radionuclides, and it is produced by neutron bombardment of purified target material in reactors (11). With a half-life of 6.71 days for β^- emission at 498 keV and 78% branch fraction, ^{177}Lu has been a very promising radionuclide in radiotherapy for effectively destroying small tumors and metastasis (optimal size 1.2–3.0 mm) while sparing normal tissue (12). ^{177}Lu also emits low-energy gamma rays at 208 and 113 keV with 10% and 6% abundance, respectively, which allows for direct monitoring of the activity distribution with SPECT and subsequent dosimetry (12).

Synthesis

[PubMed]

Tolmachev et al. reported the synthesis of ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$ in several steps (5). First, a DNA fragment encoding $Z_{HER2:342}$ was PCR-amplified followed by subcloning in Pseudomonas exotoxin (pET)-derived expression vector pAY540, which contained a gene to encode ABD. Using the restriction enzyme *Acc*I, the molecule was further subcloned in dimeric form to produce a pAy770 vector that encoded a fused ABD ($Z_{HER2:342}$)$_2$. pAy770 vector was transformed to chemocompetent *Escherichia coli* strain BL21(ED3) to express ABD-($Z_{HER2:342}$)$_2$. Then the obtained protein (ABD-($Z_{HER2:342}$)$_2$) with purity >98% was conjugated with the commercial chelator CHX-A"-DTPA at a protein/chelator molar ratio of 1:1 in sodium borate buffer (pH 9.2). Finally, the produced CHX-A"-DTPA-ABD-Affibody ($Z_{HER2:342}$)$_2$ was reacted with ^{177}Lu salt in acetate buffer (pH 5.5) to produce ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$ with labeling efficiency of 88.6%. As determined with liquid chromatographic/mass spectrometry (LC/MS) measurement, ~0.91 chelator was found per ABD-($Z_{HER2:342}$)$_2$ molecule. The dissociation constant of the obtained ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$ as measured with the surface plasmon resonance method was 31 nM and 8.2 nM when binding to murine and human albumin, respectively, and 18 pM when binding to the extracellular domain of HER2.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Tolmachev et al. used the surface plasmon resonance method to examine the binding capacity of ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$*in vitro* (5). ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$ was added to HER2-expresssing SKOV-3 cells at a ratio of one labeled Affibody dimer per HER2 receptor. After 72 h incubation, the cell radioactivity was measured to evaluate the retention of ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$ in the cells. The tissues absorbed ~80% and ~68% of the radioactivity at 8 h and 24 h, respectively, after interrupted incubation. To examine the binding specificity of ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$, a 1,000-fold excess of unlabeled Affibody dimer ($Z_{HER2:342}$)$_2$ or pertuzumab was added into SKOV-3 cells to saturate HER2 receptors. Then ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$ was added 10 min later. Presaturation with ($Z_{HER2:342}$)$_2$ almost completely blocked the binding of ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$, whereas presaturation with pertuzumab did not reduce the binding at all. These results suggested that ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$ and pertuzumab bound to different epitopes.

Animal Studies

Rodents

[PubMed]

The biodistribution of ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$ was measured in mice (5). After subcutaneous injection of 1 µg ^{177}Lu-ABD-($Z_{HER2:342}$)$_2$ (110 kBq), mice were

euthanized at 4, 8, 24, 48, and 72 h (n = 4 for each time point). Blood and tissues were collected for radioactive measurement. At 4 h, the radioactivity (expressed as percent injected activity per gram (% IA/g)) was found to be 5 ± 1 in blood, 1.5 ± 0.4 in lung, 1.0 ± 0.3 in liver, 5.8 ± 0.6 in kidney, and 1 ± 1 in skin. At 24 h, the radioactivity (% IA/g) was 7 ± 0.8 in blood, 3.4 ± 0.4 in lung, 4.1 ± 0.1 in liver, 11 ± 1 in kidney, and 5 ± 3 in skin. At 72 h, the radioactivity (% IA/g) was 3.2 ± 0.3 in blood, 2.2 ± 0.2 in lung, 5 ± 1 in liver, 9 ± 1 in kidney, and 2.6 ± 0.8 in skin. The radioactivity in the blood and kidneys (highest among healthy tissues) peaked at 12 h and 24 h after injection, respectively. The high radioactivity in skin was consistent with the presence of abundant albumin due to relatively large fractional interstitial volume. Compared to the non–albumin-fused analog ^{177}Lu-$(Z_{HER2:342})_2$, the peak radioactivity of ^{177}Lu-ABD-$(Z_{HER2:342})_2$ in kidneys was reduced 25-fold; its half-life in the blood also increased from 0.64 ± 0.2 min to 35.8 ± 0.0 min.

The tumor uptake of ^{177}Lu-ABD-$(Z_{HER2:342})_2$ was examined in nude mice bearing SKOV-3 tumors (5). After subcutaneous injection of 1 μg ^{177}Lu-ABD-$(Z_{HER2:342})_2$ (110 MBq), mice were euthanized at 1, 4, 12, 48, 168, and 332 h (n = 4 for each time point). The tumor radioactivity (% IA/g) values in the tumor at these time points were 0.13 ± 0.04, 1.9 ± 0.4, 7 ± 4, 19 ± 7, 26 ± 4, 21 ± 6, 9 ± 1, and 1.8 ± 0.2, respectively. The tumor radioactivity concentration of ^{177}Lu-ABD-$(Z_{HER2:342})_2$ exceeded that in blood and kidney and peaked at 48 h after injection. As a control to examine binding specificity, a group of mice (n = 4) was injected with 1 μg non–HER2-specific Affibody molecule ^{177}Lu-ABD-$(Z_{abeta})_2$ (110 kBq). The mice were euthanized 48 h after injection; a two-fold reduction of radioactivity was found in blood, and a 9.6-fold reduction was found in the tumor. This suggested that accumulation of ^{177}Lu-$(Z_{HER2:342})_2$ in tumor primarily depended on its interaction with HER2 but not on unspecific trapping of proteins in tumor interstitium due to higher fractional interstitial volume in tumor tissue. The higher ^{177}Lu-ABD-$(Z_{HER2:342})_2$ radioactivity found in blood could result from its dissociated portion, which drained from the tumors and then re-entered the blood circulation, whereas such a depot did not exist for ^{177}Lu-ABD-$(Z_{abeta})_2$. The tumor uptake of ^{177}Lu-ABD-$(Z_{HER2:342})_2$ was further confirmed with *in vivo* gamma-camera imaging. At 52 h after injection, prominent accumulation of radioactivity was found in the tumor and in the abdominal area.

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.

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