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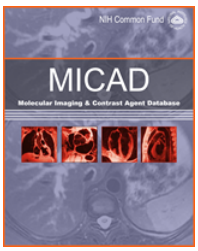


# $^{64}$Cu-Labeled cysteine–tagged epidermal growth factor

## [$^{64}$Cu]Cys-EGF

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| **Chemical name:** | $^{64}$Cu-Labeled cysteine–tagged epidermal growth factor | |
| **Abbreviated name:** | [$^{64}$Cu]Cys-EGF; EGF-PEG-DOTA/Cu | |
| **Synonym:** | | |
| **Agent Category:** | Ligand | |
| **Target:** | Epidermal growth factor receptor (EGFR) | |
| **Target Category:** | Receptor | |
| **Method of detection:** | Positron emission tomography (PET) | |
| **Source of signal / contrast:** | $^{64}$Cu | |
| **Activation:** | No | |
| **Studies:** | • *In vitro*<br>• Rodents | Click here for the protein and nucleotide sequence of human epidermal growth factor. |

# Background

[PubMed]

Growth factors such as the epidermal growth factor (EGF), the vascular endothelial growth factor (VEGF), and the tumor necrosis factor are known to have important roles in the development and progression of cancers (1-3). The EGF mediates its activity through a group of tyrosine kinase (TK)–dependent EGF receptors (EGFR) that promote not only cell proliferation but also cell survival and the motility phenotype that is necessary for the development of an invasive cancer (4). As a consequence, the EGFRs and their associated TKs are the targets of several anti-EGFR antibodies and TK inhibitors that are either approved by the United Stated Food and Drug Administration or are being evaluated in clinical trials for the treatment of various neoplasms. Before treatment, the patients are screened to determine the extent of EGFR expression in the tumors with the use of immunohistochemistry (IHC) or fluorescence *in situ* hybridization (FISH) because these techniques help predict the probable therapeutic response and outcome for the individual (5, 6). However, only 10–25% of the patients who are EGFR-positive according to IHC or FISH analysis respond to the anti-EGFR therapy, which indicates that a poor correlation exists between the EGFR expression analysis and the treatment (7, 8). This is probably because only the active receptors are important to predict efficacy of the anti-EGFR agents for the treatment of cancers, and ICH and FISH do not distinguish between the active and inactive EGFR (9).

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On binding the ligand, a characteristic feature of a functional EGFR is that it is internalized by the cell. Levashova et al. (10) envisioned that perhaps the natural ligand EGF itself could be used as an imaging agent to detect the active EGFRs in tumors. The investigators used an *Escherichia coli* (*E. coli*)–expressed EGF containing an N-terminal cysteine fusion tag of 15 amino acids (Cys-EGF) that can be used to conjugate therapeutic or imaging probes as done earlier with VEGF (11). In addition, because an active EGF/EGFR complex is composed of two molecules each of EGF and EGFR, the investigators also bioengineered an N-terminal cysteine fusion tag containing a dimeric EGF (dEGF) molecule, with the second EGF molecule linked to the first through a linker of 9 amino acids (11). The EGF and the dEGF were then labeled with radioactive copper ($^{64}$Cu) to obtain [$^{64}$Cu]Cys-EGF and [$^{64}$Cu]Cys-dEGF. The biodistribution and imaging characteristics of the two molecules were then investigated in mice bearing xenograft tumors. It is pertinent to mention here that the imaging and biodistribution studies of only [$^{64}$Cu]Cys-EGF are described in this chapter. Investigations performed with [$^{64}$Cu]Cys-dEGF are described separately in MICAD (www.micad.nih.gov).

## Synthesis

[PubMed]

The Cys-EGF was expressed in and purified from *E. coli* as described elsewhere to obtain Cys-tagged single-chain VEGF (Cys-scVEGF) (12). Purified Cys-EGF was labeled with $^{64}$Cu as described elsewhere (12) with a procedure developed to label scVEGF using pegylated-1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid (PEG-DOTA). The EGF-PEG-DOTA complex was purified with reverse-phase high-performance liquid chromatography, and purity of the product was reported to be >90% (10). Unconjugated $^{64}$Cu was removed by the addition of ethylenediamine tetraacetic acid, and the radiolabeled Cys-EGF (EGF-PEG-DOTA/Cu) was purified with size-exclusion chromatography on a NP-5 column (10). The radiochemical purity of EGF-PEG-DOTA/Cu was not reported. Specific activity of EGF-PEG-DOTA/Cu was reported to be ~4.6 MBq/μg (~125 μCi/μg) (10). The stability of EGF-PEG-DOTA/Cu was reported to be at least 3 h at room temperature in phosphate-buffered saline and at least 75 min at 37°C in plasma (10).

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

Using F98/EGFR(F) rat glioma cells that overexpress the human EGFR, the receptor affinity constant for EGF-PEG-DOTA/Cu was determined with Scatchard's analysis to be 3.0 nM (10). In another study, EGF-PEG-DOTA was reported to induce EGFR TK phosphorylation in these cells in a dose-dependent manner (10). EGF-PEG-DOTA/Cu was also reported to compete with an EGF-Shiga-like toxin subunit A conjugate (EGF-SLT) for binding to the EGFR (10). EGF-SLT is extremely toxic to MDA23 1luc human breast cancer cells, and the ability of proteins to compete for binding with the EGFR was measured by counting the number of cells surviving after a 72-h exposure to EGF-SLT (1 nM) in the presence of an increasing concentration of Cys-EGF (0–25 nM). It was reported that cell survival increased with an increasing EGF-PEG-DOTA/Cu concentration under these experimental conditions, which indicates that EGF-PEG-DOTA/Cu protected the cells from EGF-SLT toxicity.

## Animal Studies

### Rodents

[PubMed]

Levashova et al. studied the blood clearance and biodistribution of EGF-PEG-DOTA/Cu in mice ($n$ = 4 animals) bearing F98/EGFR or MDA23 1luc cell tumors (10). The animals were injected with the radiotracer, and blood was collected at preselected time points for up to 60 min. More than 95% of the radioactivity was reported to

clear from blood within 60 min. To determine biodistribution of the labeled protein, the animals were euthanized 3 h after administration of the tracer, all the major organs and tumors were harvested, and incorporated radioactivity in the harvested organs was counted using a gamma counter. The radioactivity was reported to accumulate primarily in the liver (~14.5% injected dose/gram tissue (% ID/g)) and kidneys (~10.5% ID/g). Also, the MDA23 1luc cell tumors were reported to have a higher accumulation of radioactivity than the F98/EGFR cell tumors although the former cells are known to express approximately five-fold less EGFR compared with the latter cells (10). The investigators suggested that this was probably because a higher number of functional receptors were present in the MDA23 1luc cells than in the F98/EGFR cells. No competition studies were reported.

The accumulation of EGF-PEG-DOTA/Cu was investigated in mice bearing orthotopic MDA23 1luc cell tumors (10). The animals ($n$ = 4 mice per model) were injected with the radiolabeled protein and imaged 3 h later with positron emission tomography. The investigators observed selective radioactivity uptake by the tumors in the animals. No competition studies were reported.

## Other Non-Primate Mammals

[PubMed]

No references are currently available.

## Non-Human Primates

[PubMed]

No references are currently available.

# Human Studies

[PubMed]

No references are currently available.

# Supplemental Information

[Disclaimers]

No information is currently available.

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