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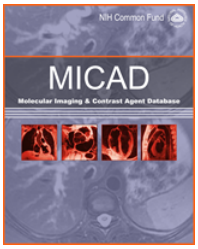


# $^{124}$I-Labeled residulizing ligand IMP-R4 conjugated chimeric monoclonal antibody ch806 targeting the epidermal growth factor receptor deletion variant de2-7 (EGFRvIII)

## [$^{124}$I]-IMP-R4-ch806

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| **Chemical name:** | $^{124}$I-Labeled residulizing ligand IMP-R4 conjugated chimeric monoclonal antibody, ch806, targeting the epidermal growth factor receptor deletion variant de2-7 (EGFRvIII) | |
| **Abbreviated name:** | [$^{124}$I]-IMP-R4-ch806 | |
| **Synonym:** | | |
| **Agent Category:** | Antibody | |
| **Target:** | Epidermal growth factor receptor deletion variant de2-7 (EGFRvIII) | |
| **Target Category:** | Receptor | |
| **Method of detection:** | Positron emission tomography | |
| **Source of signal / contrast:** | $^{124}$I | |
| **Activation:** | No | |
| **Studies:** | • *In vitro*<br>• Rodents | Structure not available in PubChem. |

# Background

[PubMed]

The biological characteristics, activating ligands, and functioning of the different members of the transmembrane epidermal growth factor receptor (EGFR) family are described elsewhere (1-3). These receptors 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 (1, 2). Overexpression of the EGFR in the tumors has been attributed to gene amplification, and this phenomenon is believed to introduce mutations in the receptor (2, 4). Also, overexpression of the EGFR usually indicates a poor clinical prognosis for the patient (4). The most common mutation observed in the

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receptor is the deletion of a segment of the EGFR extracellular domain, including the ligand-binding region, which results in the generation of a variant known as the de2-7 EGFR or EGFRvIII (2, 4). The generation, structure, functions, and role of EGFRvIII in tumor malignancy have been reviewed by Gan et al. (5). Although EGFRvIII is nonresponsive to the ligand, 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 the cancerous state of cells, several antibodies that inhibit the receptor activity and small molecules that block the downstream TK signaling pathway have been developed and have been approved by the United States Food and Drug Administration (FDA) for the treatment of certain cancers (2). The antibodies are directed toward the extracellular domain of the receptor, block ligand binding, and inhibit activation of the TK signal transduction pathway, which ultimately results in downregulation of the EGFR on the cell surface. However, because the EGFRvIII lacks the ligand-binding region on the extracellular domain, these antibodies cannot obstruct the constitutive mutant receptor activity (2). As a consequence, the monoclonal antibody (mAb) 806, which specifically targets the EGFRvIII, was generated and characterized in preclinical studies (7, 8). Subsequently, a chimeric form of the mAb (chAb), designated as ch806, was developed and evaluated in a phase I clinical trial with patients having cancerous tumors overexpressing the EGFRvIII (4). Results obtained from this trial indicated that ch806 could be a good biotherapeutic agent for the treatment of cancers expressing the ch806 antigen (4). In addition, several other clinical trials approved by the FDA are in progress to evaluate the targeting of EGFRvIII as a treatment against various cancers.

The internalization, intracellular trafficking, and biodistribution (in nude mice bearing xenograft human epidermoid carcinoma cell tumors) of mAb806 labeled with $^{125}$I and $^{111}$In, respectively, are described in separate chapters (9, 10) in MICAD (www.micad.nih.gov). The characterization and biodistribution (in nude mice bearing xenograft human glioblastoma cell tumors) of chb806 labeled with $^{125}$I or $^{111}$In are also described in separate chapters (11, 12) in MICAD. This chapter describes the evaluation of $^{124}$I-labeled ch806 ([$^{124}$I]-ch806) for the detection of EGFRvIII-expressing xenograft human glioblastoma tumors in nude mice using an immuno–positron emission tomography (PET) technique as reported by Lee et al. (6). The biodistribution of the radiolabeled chimeric antibody was also studied in the tumor-bearing animals.

## Other sources of information

Human EGFR Gene (Gene ID: 1956)

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

Related chapters in MICAD

# Synthesis

[PubMed]

The synthesis of [$^{124}$I]-ch806 was described by Lee et al. (6). Briefly, labeling of ch806 with $^{124}$I was performed after conjugation of the chAb to IMP-R4, a residualizing ligand, to obtain [$^{124}$I]-IMP-R4-ch806. IMP-R4 is MCC-Lys(MCC)-Lys(X)-D-Tyr-D-Lys(X)-OH, where MCC is 4-(*N*-maleimidomethyl)-cyclohexane-1-carbonyl and X is 1-[(4-thiocarbonylamino)benzyl]-diethylenetriamine pentaacetic acid. The labeling efficiency of the

reaction was reported to be 51.2%, and 96.6% of the radioactivity was bound to the protein as determined with thin-layer chromatography. The radiochemical purity of the labeled chAb was not reported. The specific activity of [$^{124}$I]-IMP-R4-ch806 was reported to be 1.961 MBq/6.6 pmol (~ 8,030 Ci/mmol). The number of IMP-R4 moieties bound per molecule of ch806, and the final formulation and storage conditions of the labeled chAb were not reported.

For use as controls, ch806 and another mAb, huA33 (a humanized mAb that targets the A33 antigen), were also labeled with $^{124}$I (6). The characteristics and cell-binding properties of these labeled antibodies are described by Lee et al. (6).

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

[PubMed]

With the use of the Lindmo assay, the immunoreactivity of [$^{124}$I]-IMP-R4-ch806 to U97MG.de2-7 cells, which express the EGFRvIII receptor, was reported to be 78.3% (6). The apparent association constant of [$^{124}$I]-IMP-R4-ch806 (determined in the presence of unlabeled ch806 using a Scatchard plot) and the number of chAb molecules bound per cell were reported to be $5.6 \times 10^8$ $M^{-1}$ and $1.12 \times 10^6$, respectively (6).

The stability of [$^{124}$I]-IMP-R4-ch806 on day 3 and day 7 after labeling was 52.1% and 34.3%, respectively, as determined with an immunoreactive assay (described above) and high-performance liquid chromatography analysis of the samples (6).

## Animal Studies

### Rodents

[PubMed]

The biodistribution of [$^{124}$I]-IMP-R4-ch806 was studied in BALB/c nude mice bearing xenograft U97MG.de2-7 cell tumors (6). The animals ($n$ = 5 animals/time point) were injected with the labeled antibody through the tail vein, and the mice were euthanized at different time points for the collection of all major organs, including the tumors (6). The tumors had a maximum accumulation of radioactivity of 30.95 ± 6.01% injected dose per gram tissue (% ID/g) at 48 h post-injection (p.i.), which decreased to 14.64 ± 2.52% ID/g by 120 h p.i. [$^{111}$In]-chAb806 peaked at ~31.0% ID/g at 48 h.The tumor/blood ratios were reported to increase from 0.45 ± 0.07% ID/g at 4 h p.i. to 5.25 ± 2.45% ID/g at 120 h p.i., and a similar trend in radioactivity uptake was noted in the normal tissues. Compared to [$^{124}$I]-IMP-R4-ch806, low levels of radioactivity (ranging from ~5% to 7.00% ID/g) were detected in the tumors with the control $^{124}$I-labeled antibodies (6). No blocking studies using unlabeled ch806 were reported.

PET/computed tomography (CT) imaging was performed at 4, 24, 48, and 168 h p.i. ($n$ = 2 mice/time point) (6). The tumor uptake of radioactivity was clearly evident at 24 h p.i. and was reported to increase with time, remaining visible up to 168 h p.i. The pattern of radioactivity uptake by the normal tissues observed with PET/CT imaging was similar to that observed during the biodistribution studies. No blocking studies were performed.

From these studies, the investigators concluded that immuno-PET with [$^{124}$I]-IMP-R4-ch806 can be used for the detection of tumors overexpressing EGFRvIII in mice (6).

### 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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