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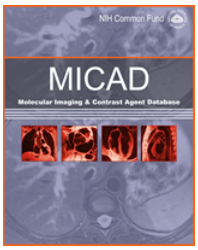


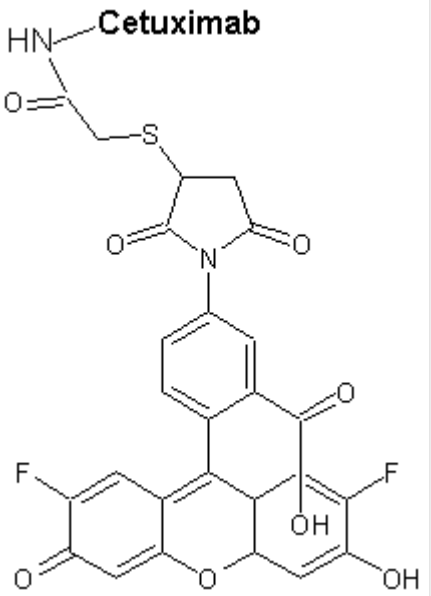
Cetuximab-Oregon Green 488

Cetuximab-OG

The MICAD Research Team

Created: August 22, 2007; Updated: September 24, 2007.

Chemical name:	Cetuximab-Oregon Green 488
Abbreviated name:	Cetuximab-OG
Synonym:	
Agent Category:	Monoclonal antibody
Target:	Epidermal growth factor receptor (EGFR)
Target Category:	Receptor-antibody binding
Method of detection:	Fluorescent imaging
Source of signal:	Oregon Green 488
Activation:	Not required
Studies:	• *In vitro* • Rodents



Structure of Cetuximab-OG

Background

[PubMed]

The epidermal growth factor receptor (EGFR) is a 170-kDa transmembrane protein that promotes cell proliferation by the specific binding of the autocrine epidermal growth factor (EGF) and transforming growth factor α (TGFα). The activity of these factors is believed to contribute to the progression of cancers. The EGFR operates through a receptor-associated tyrosine kinase–mediated signal transduction pathway that triggers cell proliferation. Overexpression of the EGFR is a characteristic feature of a variety of cancers such as squamous cell lung carcinoma and breast, ovarian, head and neck, bladder, and colon cancers (1). Much evidence indicates, in addition to overexpression, that a mutated EGFR gene may also play a role in development of these cancers (2). The most common mutation results in the development of a truncated receptor gene (EGFRvIII) that is deficient in exons 2–7 of the coding sequence. The mutant gene produces a receptor that lacks a part of the extracellular ligand-binding domain that is not activated by ligand binding. However, the intracellular tyrosine kinase portion of the receptor is active constitutively and autophosphorylates the tyrosine residue that initiates the signaling pathway for cell proliferation. In an effort to develop cancer therapies, a variety of EGFR inhibitors have been

developed that either inhibit activation of the receptor kinase or the receptor binding of EGF and TGFα, e.g., monoclonal antibodies (MAb) to the receptor (3, 4).

Cetuximab is a human chimeric anti-EGFR MAb that has been successfully used by itself and in combination with chemo- and radiation therapy for the treatment of solid tumors or metastasized cancers (5, 6). Cetuximab is available commercially in the United States and has been approved by the US Food and Drug Administration for the treatment of metastasized colorectal cancer and advanced squamous cell carcinoma of the head and neck. Currently it is being evaluated in several clinical trials for the treatment of a variety of cancers. Because of EGFR heterogeneity in cancers, the imaging of cetuximab binding could assist in the selection and treatment of patients who are likely to respond to treatment and enhance the possibility of a positive outcome of the therapy. Radiolabeled cetuximab has been used to visualize and monitor EGFR tumors in animals and humans (7-10). In an effort to develop a non-radiolabeled imaging agent for EGFR, Aerts et al. developed a fluorescent dye, Oregon Green 488 (OG), and labeled cetuximab with this dye (cetuximab-OG) for the *in vitro* and *in vivo* visualization of EGFR in tumor tissue (11).

Synthesis

[PubMed]

The synthesis of cetuximab-OG was described by Aerts et al. (11). Cetuximab was purchased commercially as a solution and dialyzed against Hepes-buffered saline (pH 7.4). The protein concentration of the dialysate was determined, and the solution was incubated with succinimidyl-acetylacetate to obtain cetuximab-(Lysine)-acetylthioacetate (cetuximab-(Lys)-ATA). The duration of this incubation and yield of the product was not mentioned in the publication. For de-acetylation, cetuximab-(Lys)-ATA was treated with hydroxylamine and ethylenediamine tetraacetic acid (pH 7.4) for 1 h to obtain cetuximab-(Lys)-SH (yield not provided in the publication). This compound was incubated with OG 488–maleimide for 2 h, which resulted in 1:1 labeling (on average) of cetuximab with OG 488. All intermediates and the final product generated by the synthesis were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy. The final yield of cetuximab-OG was not provided in the publication.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

In vitro binding of cetuximab-OG was investigated in A431 (of human squamous cell carcinoma origin) and T47D (of human breast tumor origin) cells that have high and low levels of EGFR expression, respectively (11). The cells were incubated with cetuximab-OG for 3 h using an increasing concentration range of the labeled MAb (5.2 fmol to 5.2 μmol). Binding of the labeled MAb was observed under a fluorescent microscope, and only the A431 cells showed clear cetuximab-OG binding with a saturation point of 5.2 nM. The T47D cells had only background levels of cetuximab-OG binding at all concentrations. Subsequently, the T47D cell signals were used as the negative control for all experiments. On average, cetuximab-OG binding in A431 cells was 50-fold higher than the negative controls.

In another study, U-373 cells (of human glioblastoma-astrocytoma origin) were transfected with a phβAc vector (not described in the publication) containing the EGFRvIII gene (11). A stable clone with the highest expression of EGFRvIII, as determined by immunofluorescence, was selected for further experiments. It was denoted as U373-vIII(+). Another clone that expressed no EGFRvIII was also selected for use as a control and it was denoted as U373-vIII(-). Compared to the U373-vIII(-) cells, the U373-vIII(+) cells had a 40-fold higher binding of cetuximab-OG. This indicated that the labeled MAb could also bind to, and detect, the mutant EGFR.

No competing studies, to determine specificity of the labeled MAb, were reported in the publication (11).

Animal Studies

Rodents

[PubMed]

The use of cetuximab-OG as an imaging agent was investigated in mice bearing HT-29 cell tumors (of colorectal origin) (11). Compared to the A431 and the T47D cells, the HT-29 cells express an intermediate level of the EGFR. The labeled MAb was injected into the mice through the tail vein and the animals were euthanized after 5 days. The tumors were excised and prepared for immunohistochemistry. A heterogeneous localization of cetuximab-OG was observed in the tumor sections. EGFR expression was also assessed using a polyclonal antibody against EGFR. The polyclonal antibody also showed a heterogeneous expression pattern of the receptor. Merging images obtained with the two antibodies showed a partial mismatch in cellular accumulation of the two tracers. The extent of mismatch was calculated with a difference map of sections from six representative tumors, where intensity within 20% of the maximum was considered an overlap (a match) and a larger difference was considered a mismatch. This resulted in an overlap of 83.4 ± 5.9%, a mismatch of cetuximab-OG–positive/EGFR-negative of 7.9 ± 5.3%, and a mismatch of cetuximab-OG–negative/EGFR-positive of 8.7 ± 2.1%. According to the investigators, poor vasculature along with perfusion of the tumor, dynamic tumor growth, and necrosis may have limited the uptake of cetuximab-OG. This could have contributed to the *in vivo* mismatch observed with the two antibodies. The investigators concluded that cetuximab-OG is a promising imaging agent that could be used to visualize both EGFR and EGFRvIII. However, it was indicated for an *in vivo* study, a mismatch between cell expression of EGFR and cetuximab uptake can be expected.

Other Non-Primate Mammals

[PubMed]

No publications are currently available.

Non-Human Primates

[PubMed]

No publications are currently available.

Human Studies

[PubMed]

No publications are currently available.

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