Cy5.5-Conjugated anti-epidermal growth factor receptor monoclonal antibody C225

C225-Cy5.5

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Chemical name:	Cy5.5-Conjugated anti-epidermal growth factor receptor monoclonal antibody C225	
Abbreviated name:	C225-Cy5.5	
Synonym:		
Agent Category:	Antibody	
Target:	Epidermal growth factor receptor	
Target Category:	Receptor	
Method of detection:	Near-infrared fluorescence (NIR) imaging	
Source of signal / contrast:	Cy5.5	
Activation:	No	
Studies:	• *In vitro* • Non-primate non-rodent mammals	Click here for the protein and nucleotide sequence of rat EGFR.

Background

[PubMed]

The epidermal growth factor receptor (EGFR) is often overexpressed or constitutively activated in several different cancer types, and these phenotypes are linked to an elevated metastatic potential, which indicates a poor prognosis for the patient (1-3). Because of its role in the development of cancer phenotypes, several anti-EGFR monoclonal antibodies (MAb), which are commercially available or under development and evaluation in clinical trials, have been approved by the United States Food and Drug Administration for the diagnosis and treatment of various cancers (4). It has also been shown that under

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preclinical (5, 6) and clinical (7) conditions, radioisotope-labeled anti-EGFR MAbs can be used for the diagnosis and treatment of cancers. In addition, as an alternative to the radiolabeled MAbs, anti-EGFR antibodies labeled with infrared fluorescent dyes have also been developed and evaluated in preclinical tumor xenograft models for the detection of cancers overexpressing EGFR, including oral cancer (8-11).

According to the Oral Cancer Foundation, >34,000 Americans will be diagnosed with oral or pharyngeal cancers this year, and among them ~8,000 will die from this condition. Oral cancer, ~90% of which is of the squamous cell carcinoma (SCC) type, is usually discovered at a late stage of development because it produces no pain and shows no symptoms during its early stages. Therefore, early detection of this cancer can result in the development of an appropriate, more aggressive, treatment regimen and increase the chances of a good prognosis. In an effort to produce a dual-purpose agent that could be used for early detection of oral cancer and also to determine the efficacy of an anti-cancer drug, Soukos et al. conjugated C225, an anti-EGFR MAb, to an infrared fluorescent dye, *N,N'*-di-carboxypentyl-indodicarbocyanine-5,5'-disulfonic acid (Cy5.5), and evaluated the conjugate for early detection of oral cancer in a hamster cheek pouch carcinogen evaluation model (11). The Cy5.5-conjugated MAb (C225-Cy5.5) was also used to monitor treatment of the cancer with chlorin$_{e6}$ (C_{e6}), a photosensitizer, conjugated to C225 (C225-C_{e6}), which was used for photodynamic therapy of oral cancer.

Synthesis

[PubMed]

The synthesis of C225-Cy5.5 was described by Soukos et al. (11). Both C225 and Cy5.5 were obtained from commercial sources for studies detailed in this chapter. The MAb (formulation not described) was dissolved in 0.1 M bicarbonate buffer (pH 9.3), and a solution of Cy5.5 in the same buffer was added to it. The solution mixture was incubated in the dark for 3 h at room temperature, followed by dialysis three times against excess phosphate buffer (pH 7.4) to remove any unbound dye. For use as controls, bovine serum albumin (BSA)-Cy5.5 and mouse IgG (IgG)-Cy5.5 conjugates were also prepared following the method detailed above. The C225-Cy5.5 and the BSA- and IgG-Cy5.5 conjugates were reported to have 2.1 and 1.6 molecules of Cy5.5, respectively, attached to each protein as determined with a spectroscopic method. The purity, yield, and stability of the various conjugates was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The *in vitro* binding activity of C225-Cy5.5 was studied with the use of A431 cells, a human epidermoid SCC cell line, and HCPC-1 cells, a cell line derived from an experimentally induced epidermoid carcinoma of the hamster buccal pouch (11). The respective cell lines were grown on glass coverslips and exposed to C225-Cy5.5, BSA-Cy5.5, or mouse IgG-Cy5.5 for 3 h at 37°C. The coverslips were then washed thoroughly

with phosphate-buffered saline (pH not reported) and examined under a fluorescence microscope. Both cell lines were reported to show fluorescence only with the C225-Cy5.5 conjugate, indicating that the MAb-Cy5.5 conjugate could be used to detect cell types expressing EGFR under *in vitro* conditions. No blocking studies with unconjugated C225 were reported.

Animal Studies

Rodents

[PubMed]

No references are currently available.

Other Non-Primate Mammals

[PubMed]

To evaluate the use of C225-Cy5.5 for imaging precancerous lesions in the Syrian golden hamster cheek pouch model, the characteristic premalignancy epithelial changes were induced in the right cheek buccal pouch tissue by painting them with a 5% solution of 7,12-dimethylbenz(*a*)anthracene (DMBA, a highly potent carcinogen that produces premalignancy epithelial changes with increased EGFR expression) in mineral oil 3 times a week for 6 weeks (11). For fluorescent imaging of the premalignant lesions, the hamsters were injected (route of administration not described) with 670 µg of C225-Cy5.5 (n = 9 animals), or 610 µg of IgG-Cy5.5 (n = 6 animals), or 390 µg of BSA-Cy5.5 (n = 6 animals), and imaged from 1 day up to 8 days after the treatment as described by Soukos et al. (11). Although no significant difference in fluorescence intensity ratios (FIR) (fluorescence obtained from DMBA-treated cheek pouch/fluorescence obtained from normal cheek pouch) was noted on day 1 between the animals treated with C225-Cy5.5 (1.17 ± 0.25) and the animals treated with IgG-Cy5.5 (1.01 ± 0.12) or BSA-Cy5.5 (0.95 ± 0.18). By day 6, a significant ($P < 0.005$) difference was apparent between the MAb-Cy5.5 (FIR was 1.54 ± 0.46) and control animals (FIR was 0.96 ± 0.09 to 0.99 ± 0.01, respectively, for the two groups in the order given above). This indicated that an elevated expression of EGFR in the premalignant tissue resulted in an increased binding of C225-Cy5.5 in the cancerous lesions. No blocking studies were reported.

In an another study, Soukos et al. investigated the use of C225-Cy5.5 to monitor photoimmunotherapy for the treatment of premalignant oral cancer lesions on the hamster cheek pouch with a C225-C_{e6} conjugate, a photosensitizer (11). The premalignant lesions were generated as described above (n = 11 animals), and the cheek pouches (DMBA-treated and normal) of the animals were divided into two equal parts with the outer part designated for exposure to light and the inner part designated for protection from light with a black cloth. The animals were injected with the C225-C_{e6} conjugate, and 2 days later they were exposed to light as described by Soukos et al. (11). To determine efficacy of the photosensitizer conjugate treatment 5 days later, the animals

were injected with the C225-Cy5.5 conjugate and fluorescence imaging was performed 3 days after the treatment. A significant difference between the fluorescence of the non–light-exposed areas of the normal and the DMBA-treated cheek pouches was reported (110 ± 21 *versus* 160 ± 29, $P < 0.0005$). Under the same experimental conditions, little difference in the fluorescence was observed for the normal cheek pouches with or without light treatment (112 ± 22 *versus* 110 ± 21). A significant ($P < 0.01$) difference in the fluorescence of the respective light-exposed (124.2 ± 26) and non–light-exposed DMBA-treated (160 ± 29) cheek areas of the C225-Cy5.5 conjugate injected animals was reported, indicating that the increased expression of EGFR was reduced to normal levels after DMBA treatment because of the photodynamic therapy after injection of the C225-Cy5.5 conjugate. A histological examination of the DMBA-treated cheek pouches confirmed the presence of precancerous lesions in all animals used for the study.

From these studies the investigators concluded that an infrared-emitting fluorophore could be used as an alternative to radioisotope-labeled agents for the detection of premalignant oral cancer lesions. They also concluded that C225-Cy5.5 could possibly be used to monitor oral cancer progression in a preclinical setting.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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