IRDye 700DX-Labeled annexin V

NIR700-Annexin V

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Chemical name:	IRDye 700DX-Labeled annexin V	
Abbreviated name:	NIR700-Annexin V	
Synonym:		
Agent Category:	Ligand	
Target:	Phosphatidylserine	
Target Category:	Phospholipid	
Method of detection:	Near-infrared (NIR) imaging	
Source of signal / contrast:	IRDye 700DX	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

The epidermal growth factor (EGF) receptor (EGFR) that mediates its activity (cell development, proliferation and migration etc.) through an intracellular kinase (TK) is overexpressed in several different types of cancers, including that of the colorectal area. Because of its cancer-promoting activity, the EGFR has been targeted to treat the malignancies with monoclonal antibodies (mAbs) directed toward its extracellular domain, with inhibitors against the TK, or with a combination of the two treatment methods (1-3). Although the United States Food and Drug Administration has approved these therapies for cancers, the response rate to a single agent treatment, particularly for colorectal cancer (CRC), is low, and it is difficult to predict which patient is likely to respond to the therapy (4, 5). Often biopsies have to be performed on the patients to

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predict possible cancer treatment outcomes, with mAbs targeting the EGFR or with drugs directed toward the intracellular TK, and no noninvasive biomarkers are available to determine the prognosis or to monitor therapy response (5). Biopsies, however, carry a risk of infection for the patient, the tissue must be examined microscopically by a specialist for diagnosis, and sampling of a small fraction of the tumor can result in false negative observations.

Manning et al. developed IRDye 700DX-labeled annexin V (NIR700DX-Annexin V), an apoptosis biomarker, in an effort to develop a noninvasive, near-infrared optical imaging technique to determine the efficacy of cetuximab in mice bearing human CRC cell line xenograft tumors (4). The investigators also used IRDye 800CW-labeled EGF (NIR800-EGF) to assess the inhibition of cetuximab signaling in the tumor cells, and they used 3'-[^{18}F]fluoro-3'-deoxythymidine ([^{18}F]FLT) with positron emission tomography (PET) to determine cell proliferation in the tumors.

Other sources of information

Cetuximab and annexin chapters in MICAD.

Drug information regarding cetuximab.

Clinical trials related to annexin.

Clinical trials related to EGFR.

Annexin in OMIM.

EGFR in OMIM.

Drug and other information regarding annexin V – FDA.

Drug and other information regarding EGFR – FDA.

Synthesis

[PubMed]

The synthesis of NIR700DX-Annexin V has been discussed in detail by Manning et al. (4). A similar method was used to obtain NIR800-EGF (4). Purity of the two conjugates was assessed with a chromatographic method (not specified), and the dye/protein ratio was routinely 1:1 as determined with spectrophotometry. The storage conditions and stability of the labeled proteins were not reported.

The synthesis of [^{18}F]FLT was described by Manning et al. (4). The radiochemical identity, purity, and specific activity of the tracer were determined with analytical high-performance liquid chromatography. The average radiochemical purity of [^{18}F]FLT was reported to be 98.3% with a specific activity of 128.88 TBq/mmol (3,480 Ci/mmol). The storage conditions and stability of the labeled compound were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The human colorectal cell line DiFi (6), which expresses high levels of EGFR, was used to characterize the *in vitro* properties of NIR800-EGF and NIR700DX-Annexin V (4). The DiFi cells were shown with fluorescence microscopy to bind and internalize NIR800-EGF. These observations were confirmed using A431 cells that also express high levels of the EGFR. The binding of NIR800-EGF to both cell types was reduced nearly to background levels in the presence of unlabeled EGF, indicating that the probe bound specifically to the EGFR. Treatment of the DiFi cells with cetuximab was shown to induce apoptosis (as measured by caspase-3/7 activity) and reduce cell proliferation (as determined with cell cycle analysis using propidium iodide (Ki) and flow cytometry) in a concentration-dependent manner.

In another study, DiFi cells treated with increasing concentrations of cetuximab were exposed to NIR700DX-annexin V, and uptake of the probe was shown to correlate with the amount of mAb used to treat the cells (4).

With results from these studies, the investigators concluded that NIR800-EGF and NIR700DX-annexin V could be used as reporter probes to determine the binding of cetuximab to the EGFR (because the mAb would compete with EGF for binding to the EGFR) and to determine the degree of apoptosis induced by cetuximab in the cells under *in vitro* conditions (4).

Animal Studies

Rodents

[PubMed]

Using nude mice respectively bearing xenograft tumors derived from HCT-116 (cetuximab-resistant cells with moderate EGFR expression; $n = 16$ animals), DiFi (high EGFR expression; $n = 14$ animals), and SW620 (EGFR-negative controls; $n = 9$ animals) cells, NIR800-EGF imaging was shown to quantify the level of EGFR expressed on the various tumor cells (4). This quantification was confirmed with EGFR immunoreactivity in the tumor tissues.

To investigate the *in vivo* uptake and clearance properties of NIR700DX-Annexin V, a group of mice ($n = 8$) bearing DiFi xenograft tumors was treated with cetuximab (three treatments of 40 mg/kg every 3 days) followed by intravenous administration of the annexin probe by retro-orbital injection (4). Imaging was performed on the mice over a period of 40 h after injection of the probe. Control animals received the probe injection without cetuximab pretreatment. Minimal uptake of NIR700DX-Annexin V was observed in the tumors of control animals, although some accumulation of the probe was observed in the kidneys. A significantly ($P < 0.0001$) higher retention of the annexin probe was

reported in tumors of the cetuximab-treated animals, indicating that the probe was useful in the quantification of apoptosis/necrosis. Apoptosis of tumor cells from the cetuximab-treated animals was confirmed with a histological analysis of the caspase-3 activity.

The cetuximab inhibition of TK signaling (initiated by the activation of EGFR) and induction of apoptosis in the tumors was assessed by imaging the uptake of NIR800-EGF and NIR700DX-Annexin V, respectively, in mice bearing DiFi (n = 26 animals) or HCT-116 (n = 16 animals) cell xenograft tumors (4). The proliferation of tumor cells in the two groups of animals was determined with PET imaging using [^{18}F]FLT (4). Control animals were not pretreated with cetuximab. Compared with controls, tumors of the cetuximab-treated animals showed a significantly ($P < 0.0001$) reduced accumulation of NIR800-EGF with a corresponding increase ($P = 0.0006$) in the signal from NIR700DX-Annexin V. However, little difference was noted in the tumor uptake of [^{18}F]FLT in the cetuximab-treated or untreated animals, indicating that cetuximab did not reduce cell proliferation in these tissues. The induction of apoptosis in the cetuximab-treated tumor cells was confirmed by significantly elevated caspase-3 histological staining compared with controls, confirming the observations made with NIR700-Annexin V imaging that cetuximab induces apoptosis in tumor cells. In addition, Ki-67 staining of the cetuximab-treated and untreated tumor cells as a measure of proliferation showed there was little difference in staining between the two groups, corroborating the observation made earlier with [^{18}F]FLT PET imaging. Compared with the DiFi cell tumors of the cetuximab-treated animals, the HCT-116 cell xenograft tumors showed little or no change in uptake of the NIR800-EGF, NIR700DX-annexin, or the PET imaging probes, and these observations were verified by histological methods mentioned above. These observations indicated that concomitant use of the NIR800-EGF, NIR700DX-Annexin V, and [^{18}F]FLT probes could distinguish cetuximab-sensitive tumors from cetuximab-insensitive tumors.

From these results the investigators concluded that the imaging methods performed in this study to determine the efficacy of EGFR-targeted therapies (e.g., the use of mAbs against the receptor) could be useful for the preclinical evaluation of new cancer treatment regimens in mice (4).

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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