Trastuzumab conjugated to tetramethyl-6-carboxyrhodamine-QSY®7

Traz-TM-Q7

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Chemical name:	Trastuzumab conjugated to tetramethyl-6-carboxyrhodamine-QSY®7	
Abbreviated name:	Traz-TM-Q7	
Synonym:		
Agent Category:	Receptor ligand	
Target:	Human epidermal growth factor 2 (HER2)	
Target Category:	Receptor	
Method of detection:	Optical imaging: fluorescence	
Source of signal / contrast:	Tetramethyl-6-carboxyrhodamine	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	Click here for information regarding human EGFR.

Background

[PubMed]

Early detection of cancer helps in the development of a proper treatment and monitoring regimen that may result in a suitable prognosis for the patient (1-3). Although invasive methods are often used for the detection of cancer, these procedures have limitations because they may detect the neoplasm only in a specific location and cannot determine whether the cancer has metastasized to other parts of the body. In addition, the various imaging techniques and modalities available for the non-invasive detection of cancer have low sensitivity and resolution, which are insufficient to detect and quantify microscopic

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tumors or a small cluster of cancerous cells (4), and some of the contrast agents used for imaging purposes are known to have toxic side effects (5). An attractive alternative to radionuclides or contrast agents is the use of fluorescent optical imaging agents that was used to visualize and manage or treat clinical pathologies, including that of cancer in small animals (6-9). However, fluorescent dyes used for imaging can be toxic or nonspecific, and the signal generated by these agents may be masked by autofluorescence, leading to a low signal/background ratio (10). Investigators have proposed the use of targeted fluorescent probes as a solution to improve the signal/background ratio with optical imaging agents, and it was shown that a fluorescent dye conjugated to avidin or a monoclonal antibody (MAb) such as trastuzumab (Traz) can be used to detect submillimeter disseminated tumors under *in vitro* and *in vivo* conditions (11-13). It is important to mention here that currently fluorescent dyes can be used only to visualize only surface tumors or after exposure of the tumor if it is present in a body cavity (e. g. peritoneum).

The human epidermal growth factor receptor (HER; it has several isoforms: HER1, HER2, HER3, and HER4) plays an important role in the development of various neoplasms (14-16). Once a ligand is bound to HER, a tyrosine kinase signaling pathway is activated, which initiates cell growth and division that can lead to the development and progression of cancer. Soon after the signaling pathway is activated, the receptor-ligand complex is internalized by the cell for inactivation by enzymatic digestion in the lysosome (17). Because of their role in the cancer process, several commercially available humanized MAbs directed toward HER, including Traz, are approved by the United States Food and Drug Administration to treat cancers. Traz specifically targets the HER2 receptor and is used either as a primary agent or as an adjuvant for the treatment of breast cancer in the clinical setting (18). It is also under investigation in several clinical trials for the treatment of other neoplasms.

Ogawa et al. proposed the use of fluorophores that could be activated only after binding to the target to further improve the target/background ratios obtained with the optical imaging agents (13). These investigators developed a targeted and activatable fluorophore-quencher (FQ) probe that could be used for the optical imaging of tumors. The chemical structure of the FQ probe was designed so that, in the native state, the signal from the fluorophore is quenched by a quencher molecule. However, a fluorescence signal would be obtained from the fluorophore only after the FQ probe was bound to, internalized, and activated (by degradation of the targeting molecule, in this case the MAb) in the target cells. The mechanism of quenching and generating fluorescence from the FQ probe is discussed in detail elsewhere (19). Ogawa et al. investigated the use of tetramethyl-6-carboxyrhodamine (TAMRA, fluorophore)-QSY®7 (quencher) pair conjugated to Traz (Traz-TM-Q7) for the detection of cancer cells under *in vitro* conditions and in mice bearing NIH3T3 cell tumors. These cells had previously been transfected with the HER2 gene (3T3/HER2+). Normal NIH3T3 cells (Balb/3T3/Her2-) cells were used as controls for this study (13).

Synthesis

[PubMed]

The synthesis of Traz-TM-Q7 was described by Ogawa et al. (13). Briefly, Traz was incubated with QSY®7-hydroxysuccinimide (NHS) ester (dissolved in dimethylsulfoxide) in 0.1 M sodium phosphate buffer (Na_2HPO_4) (pH 8.5) for 30 min at room temperature. The Traz- QSY®7 conjugate was purified on a Sephadex G50 column, concentrated, and added to a solution of TAMRA-NHS. The mixture was incubated as described above, and the final product, Traz-TM-Q7, was purified on a Sephadex G50 column. Purified Traz-TM-Q7 was stored at 4°C until needed. The stability of Traz-TM-Q7 under these storage conditions was not reported. Approximately one and three molecules of TAMRA and QSY®7, respectively, were reported to be conjugated to each molecule of Traz, as determined with a spectroscopic system after denaturation with 5% sodium dodecylsulfate (SDS). A TAMRA conjugate of Traz (Traz-TM) was also generated by incubating Traz with TAMRA-NHS for 30 min at room temperature. Traz-TM was purified as described above, and the purified product was stored at 4°C. The stability of Traz-TM was not reported.

To generate a control for use in some studies, a TAMRA-QSY®7 conjugate of daclizumab (a MAb that is specific for HER1) was generated (Dac-TM-Q7) using a protocol similar to that described for the Traz conjugate (13). For use as a control, the TAMRA conjugate of Dac lacking QSY-7 (Dac-TM) was also prepared as described above. The purification and stability of Dac-TM-Q7 and Dac-TM were not reported. In addition, the number of TAMRA-QSY-7 or TMARA conjugated to each daclizumab molecule was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The quenching ability of the MAb conjugates was determined by denaturing them with 5% SDS containing 2-mercaptoethanol (2-ME) in phosphate-buffered saline (PBS) for 2 min at 95°C (13). For comparison, the two MAb-TM conjugates were incubated in PBS under the same conditions. Measurement of the fluorescence signal from each denatured sample showed that only the dissociated MAb conjugate samples had an increase in fluorescent signal over background. The Traz-TM-Q7 and the Traz-Tm conjugates exhibited 13- and 3-fold increases in fluorescence, respectively. For the Dac-TM-Q7 and Dac-TM conjugates, 9- and 4-fold increases were reported, respectively. The increase in fluorescence of the Traz-TM and Dac-TM conjugates was attributed to an intrinsic characteristic of rhodamine containing fluorophores that are activated after internalization by the cells (the rhodamine effect) (20).

To demonstrate intracellular activation of Traz-TM-Q7, Traz-TM, Dac-TM-Q7, and the Dac-TM conjugates, 3T3/HER2+ cells were exposed to the respective conjugates at 37°C for either 1 or 8 h to allow internalization and activation of the fluorophore in the lysosomes (13). An examination of the exposed cells under a fluorescence microscope

after 1 h exposure to Traz-TM-Q7 or Traz-TM showed that the fluorescence was visible only on the cell surface. At 8 h after exposure, the fluorescent dots were visible in the lysosomes of the cells, indicating that the Traz-TM-Q7 and Traz-TM conjugates had been internalized by the cells. Fluorescence from cells exposed to Traz-TM-Q7 was reported to be higher than that from cells treated with the Traz-TM conjugate. No fluorescent signal was obtained from cells treated with either Dac-TM-Q7 or Dac-TM because these conjugates did not target HER2 and did not bind to or were not internalized by the 3T3/HER2+ cells. Pre-exposure of the 3T3/HER2+ cells to unlabeled Traz (at a concentration equal to the Traz fluorescent conjugates) was reported to reduce the fluorescence signal obtained from Traz-TM-Q7 treatment of the cells. This indicated that the conjugated MAb had a binding specificity similar to unlabeled Traz.

In another study to investigate the intracellular localization of Traz-TM-Q7, 3T3/HER2+ tumors were removed from the lungs of mice (see below for details) and treated with a commercially available fluorescent lysosomal marker 30 min before exposure to the MAb conjugate. The tumor cells were then observed under a fluorescent microscope. The fluorescent signals from the lysosomal marker and Traz-TM-Q7 were reported to have a similar location in the cells, indicating that the MAb conjugate was internalized by the cells and degraded in the lysosomes.

Animal Studies

Rodents

[PubMed]

The uptake of Traz-TM-Q7, Traz-Tm, Dac-TM-Q7, and Dac-TM was investigated in mice bearing 3T3/HER2+ tumors in the lungs (13). Imaging was performed on the animals (5 mice/MAb conjugate) 1 d after intravenous injection of the respective MAb conjugates through the tail vein. A low background was reported with Traz-TM-Q7, whereas nonspecific fluorescence was obtained with Traz-TM. The tumor/normal lung tissue fluorescence ratios were reported to be significantly higher ($P < 0.01$) for Traz-TM-Q7 (22.4 ± 4.3) than for Traz-TM (5.7 ± 1.8). Pretreatment of the tumor-bearing mice with unlabeled Traz (~13 μmol/animal) reduced the accumulation of both MAb conjugates in the tumors and the surrounding lung tissues (tumor/normal lung tissue ratios were not reported). With Dac-TM-Q7, a very low fluorescent signal was observed in the lungs, but Dac-TM generated a generalized signal in the organs.

To determine the sensitivity and specificity of Traz-TM-Q7, mice with the NIH3T3/HER2+ and NIH3T3/HER2-/ZsGreen cell tumors (these cells constitutively express the green fluorescence protein) were injected with the MAb conjugate and imaged 1 d after the treatment; the number of animals injected with each conjugate was not reported (13). No overlap of the fluorescent signal from TAMRA and ZsGreen was observed in tumors of these animals because the NIH3T3/HER2-/ZsGreen cell tumors did not express any HER2.

On the basis of the information obtained from the various studies performed with Traz-TM-Q7, the investigators concluded that this activatable FQ probe could probably be used to manage cancer with a "see and treat" strategy (13).

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]

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