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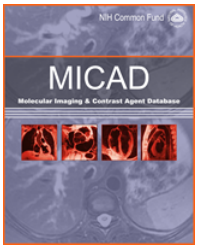

Trastuzumab complexed to near-infrared fluorophore indocyanine green

Tra-ICG

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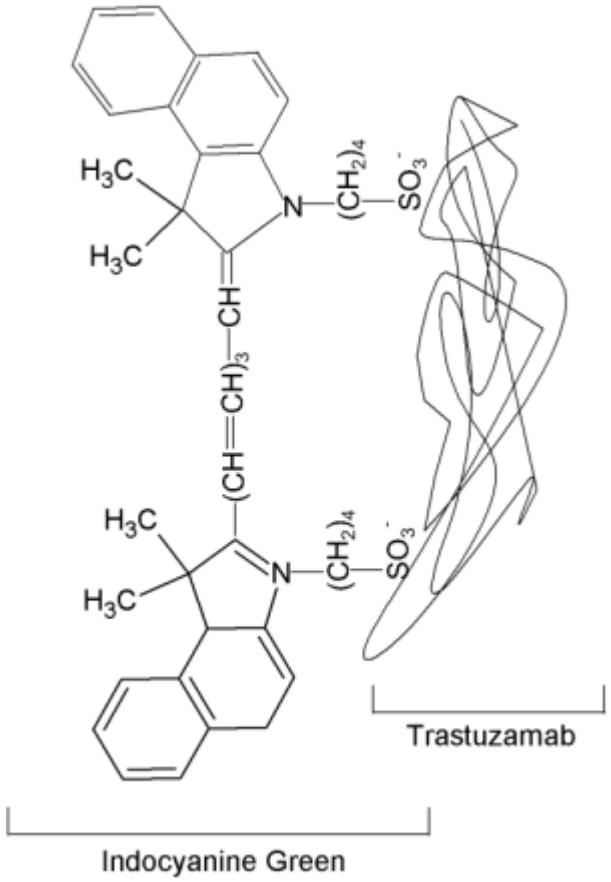
Chemical name:	Trastuzumab complexed to near-infrared fluorophore indocyanine green
Abbreviated name:	Tra-ICG
Synonym:	
Agent Category:	Monoclonal antibody
Target:	Human epidermal growth factor receptor 2 (HER2)
Target Category:	Receptor
Method of detection:	Optical: near-infrared (NIR) fluorescence
Source of signal / contrast:	Indocyanine green
Activation:	Yes
Studies:	• *In vitro* • Rodents



Proposed structure of Trastuzumab-Indocyanine green conjugate..

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Background

[PubMed]

The technique of non-invasive optical imaging is based on the principle that when a dye is exposed to light it absorbs energy and is elevated from the steady state to a high-energy state. To return to its normal, low-energy state, the molecule releases energy in the form of photons with wavelengths that are either in the visible (400–700 nm) or the near infrared (NIR; 700–900 nm) range (1). Although both types of dyes can be used for imaging, in general, NIR agents are favored over those functional in the visible range because fluorescence from NIR probes can be detected in deep tissue, have low autofluorescence, and generate low background signals because tissues do not absorb in the NIR wavelength range (2). Among the various NIR agents, only one, indocyanine green (ICG; also known as IR-125 or Cardiogreen), with absorption at ~780 nm and emission at ~820 nm, is approved by the United States Food and Drug Administration (US FDA) for clinical applications (3) and is under evaluation in several clinical trials for other applications. ICG is a tricarbocyanine dye that is fluorescent only in the free state and easily binds to proteins through non-covalent linkages (such as ionic, hydrophobic, electrostatic, or hydrogen bonds). When bound to proteins ICG is non-fluorescent, but on dissociation from the protein complex it reverts to the fluorescent state and the signal has been shown to be suitable for *in vivo* imaging in small animals (4).

The human epidermal growth factor receptor (HER; it has several isoforms: HER1, HER2, HER3, and HER4) plays an important role in the development and progression of cancers (5-7). The HER mediates its activity through a tyrosine kinase signaling pathway, and on binding to a ligand the receptor signaling pathway is activated, which promotes cell growth and division. Once activated the receptor-ligand complex is internalized by the cell for enzymatic digestion in the lysosome (8). Because of its role in the cancer process, several commercially available humanized monoclonal antibodies (MAb) directed toward HER, including trastuzumab (Tra), are approved by the US FDA to treat cancers. Tra targets the HER2 receptor and is used as the primary agent or as an adjuvant for the treatment of breast cancer in the clinical setting (9). It is also being investigated in several clinical trials to treat other neoplasms.

The characteristic properties of ICG (fluorescence in an unbound state) and the HER (internalization by the cell) prompted Ogawa et al. to develop an imaging probe for cancer by complexing ICG to Tra (Tra-ICG) (4). The investigators used the Tra-ICG complex to image cells that express the HER2 receptor under *in vitro* conditions and showed that the complex could also be used for the imaging of HER2-expressing xenograft tumors in mice.

Synthesis

[PubMed]

The synthesis of Tra-ICG was described by Ogawa et al. (4). ICG-*N*-hydroxysulfosuccinimide ester (ICG-sulfo-OSu) and Tra were obtained from commercial sources. Tra was incubated with ICG-sulfo-OSu (Tra:ICG ratio was either 1:1 or 1:5) in phosphate buffer (pH 8.5) for 30 min at room temperature. The Tra-ICG complex was purified on a PD-10 Sephadex G50 column with phosphate-buffered saline (PBS; pH not reported) as the elution buffer. With the reaction conditions used to generate the MAb-ICG complex, either one or five ICG molecules (Tra-ICG(1:1) or Tra-ICG(1:5), respectively) were reported to be bound to each Tra molecule, respectively. To determine specificity of the Tra-ICG complex, another MAb, panitumumab (Pan), which specifically binds to HER1, was complexed with ICG (Pan-ICG) and purified as described above.

For use as a control, a human polyclonal IgG-Cy5.5 complex was also synthesized in the manner described above. The storage conditions and stability of the MAb-ICG complexes were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Fluorescence imaging with the Tra-ICG complex was investigated in HER2 gene-transfected NIH3T3 (3T3/HER2+) cells (4). The cells were grown on a glass-bottomed tissue culture well and exposed to the Tra-ICG(1:1) or Tra-ICG(1:5) complex for 1 and 8 h at 37^{O}C. The cells were then washed with PBS and examined under a fluorescent microscope. No fluorescence was observed in cells exposed to Tra-ICG(1:1) at either time point. Fluorescence was apparent with both complexes at 8 h, indicating that the signal was obtained only after internalization of the MAb-ICG complex followed by degradation of the MAb and release of the ICG.

Animal Studies

Rodents

[PubMed]

Xenograft tumors were generated with 3T3/HER2+ cells in the left and right flank and the right buttock of three mice for imaging studies (4). The tumors were visible on the mice 14 to 18 days after the cell injection. The Tra-ICG (n = 1 animal) or the Pan-ICG (n = 1 animal) complex was administered intravenously to the animals through the tail vein. Fluorescent imaging was performed on the mice 4 days after the Tra-ICG or Pan-ICG injection. To confirm the presence of tumor histology, an *ex vivo* histological examination of the tumors was performed using the hematoxylin and eosin (H&E) stain of embedded tumor sections. Only animals injected with Tra-ICG generated a fluorescent signal in the 3T3/HER2+ cell tumors and were imaged under these conditions, indicating that the Tra-ICG complex bound specifically to the HER2+ tumors. A histological examination of the tumors was reported to show expected differences between the tumors.

To confirm the specificity of the Tra-ICG complex under *in vivo* conditions, mice bearing the NIH3T3/HER2+ tumors were co-injected with Tra-ICG or the Pan-ICG complex along with the polyclonal IgG-Cy5.5 conjugate (4). Imaging was performed on these animals 4 days after the injection. An *ex vivo* histological examination of the tumors was also performed with the H&E stain as described above. Under these conditions, all tumors could be imaged because the IgG-Cy5.5 complex generated similar fluorescence (red) in all the tumors. Using commercially available software, separation of the respective fluorescent signals obtained from ICG and Cy5.5 showed that the ICG signal was visible only in the HER2+ cell tumors, but the Cy5.5 fluorescence was detected in all tumors in the mice. This again confirmed that the Tra-ICG complex was specific for the HER2+ tumors. No competition studies using animals pretreated with Tra were reported.

With observations from this study, the investigators concluded that the Tra-ICG complex technique could probably be adapted for tumor imaging in humans during surgery. However, they cautioned that the Tra-ICG complex could have a different toxicity compared with individual components of the complex, and extensive clinical testing of Tra-ICG would have to be performed before its application in humans.

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.

References

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