Activatable Alexa Fluor680-conjugated panitumumab and indocyanine green-conjugated trastuzumab cocktail

Pan-Alexa680 and Tra-ICG cocktail

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Chemical name:	Activatable Alexa Fluor680-conjugated panitumumab and indocyanine green-conjugated trastuzumab cocktail	
Abbreviated name:	Pan-Alexa680 and Tra-ICG cocktail	
Synonym:		
Agent Category:	Antibodies	
Target:	Human epidermal growth factor receptor type 1 (EGFR, HER1) and type 2 (HER2)	
Target Category:	Receptors	
Method of detection:	Optical imaging	
Source of signal / contrast:	Indocyanine green (ICG) and Alexa Fluor680	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	No structures are available.

Background

[PubMed]

The cocktail of activatable Alexa Fluor680 (Alexa680)-conjugated panitumumab (Pan) and indocyanine green (ICG)-conjugated trastuzumab (Tra) is a mixture of two self-quenched (SQ), activatable monoclonal antibody (mAb) probes (abbreviated as Pan-

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Alexa680 and Tra-ICG, respectively) that was developed by Sano et al. for multicolor optical imaging of breast cancer (1).

mAbs are widely used to develop optical imaging probes because of their high specificity and affinity to target antigens. However, use of intact mAbs as imaging probes has some disadvantages, such as slow clearance from body, which results in high background and slow penetration of solid tumors (2, 3). To overcome this issue, activatable or "smart" fluorescent probes have been engineered to be silent (fluorescence quenching) but emit signal when or after binding with antigens in the tissue of interest (4, 5).

Several photochemical mechanisms of fluorescence quenching and activation have been proposed in the literature, such as homo- (SQ) and hetero-Förster resonance energy transfer (FRET), autoquenching, dimer formation, and photon-induced electron transfer (PeT) (2, 6, 7). Self-quenching of the fluorescence occurs when two excited fluorophores of the same molecule are close enough (<10 nm) to enable them to absorb energy from each other. Hetero-FRET refers to the quenching that occurs between two fluorophores from different molecules (a fluorophore and a quencher molecule). Autoquenching has been observed for some compounds, which can spontaneously induce a quenched state when conjugated with proteins. Autoquenching appears to be induced by the interactions between fluorophores and aromatic rings of amino acids. ICG is a dye that can be fully autoquenched when it is covalently conjugated with mAbs *via* the side chain of lysine, even at a low conjugation ratio. Some compounds can form H- or J-homodimers at high concentrations (~mM) in aqueous solutions and quench the emission fluorescence signal. PeT is used for the fluorescence quenching that happens within a single fluorophore molecule that is engineered to contain two parts, with one part acting as the PeT donor and another part acting as the fluorophore. Electron transfer from the PeT donor to the excited fluorophore diminishes the fluorescence signal, and cleavage of the PeT donor leads to full activation of the fluorophore. In reality, some activatable probes are designed on the basis of two or more mechanisms of fluorescent quenching and activation (8).

The activatable probes published to date can be classified into two types based on the location of fluorophore activation (2). One type involves enzymatic activation of the probes through cleavage by the extracellular or cell surface enzymes. This type of probe produces fluorescent signal primarily in the extracellular space. Another type of probe, known as a target cell–specific activatable probe, is quenched until activated through the lysosomal processing within targeted cells. Therefore, this type of probe generates fluorescent signal within the target cells. Regardless of the types, an important parameter that must be optimized for many activatable probes is the number of conjugated fluorophores per single mAb molecule. The number of conjugated fluorophores not only determines whether the fluorescence is "always on" or "silent," but it also influences the binding specificity and *in vivo* pharmacokinetics (1, 2).

Sano et al. labeled two different antibodies, Pan and Tra, with activatable near-infrared Alexa680 and ICG, respectively (1). Pan is a fully human IgG2 mAb against the extracellular domain of human epidermal growth factor receptor (EGFR, HER1), and Tra

is a recombinant humanized mAb against the human EGFR type 2 (HER2). The two fluorescent dyes emit light at different wavelengths (650/702 nm and 780/820 nm for Alexa680 and ICG, respectively). The investigators administered the two activatable antibodies as a cocktail to mice bearing tumor xenografts and demonstrated the feasibility of multicolor target-specific fluorescence imaging with reduced background noise (1).

Related Resource Links:

EGFR- and HER2-targeted imaging agents in MICAD

Nucleotide and protein sequences of EGFR

Nucleotide and protein sequences of HER2

EGFR-related clinical trials in ClinicalTrials.gov

HER2-related clinical trials in ClinicalTrials.gov

Bioassays of EGFR and HER2 in PubChem

Synthesis

[PubMed]

Pan and Tra are commercially available. Fluorescent labeling was achieved by incubating Pan and Tra with Alexa680-NHS ester and ICG, respectively (1). The concentrations of Alexa680 and ICG were calculated by measuring the absorption with a UV-Vis system to confirm the number of fluorophore molecules conjugated to each mAb molecule. The numbers of Alexa680 and ICG molecules per mAb were adjusted to ~4.0–4.5 and 0.7–1.0, respectively, for SQ, activatable Pan-Alexa680(SQ) and Tra-ICG. As a comparison, Pan conjugated with ~1 Alexa680 molecule (always-on (ON) type; Pan-Alexa680(ON)) was also synthesized.

The quenching ability of each conjugate was evaluated by denaturing them with 1% sodium dodecyl sulfate in phosphate-buffered saline (PBS) for 15 min at room temperature (1). The results showed that the quenching capacities were 50-, 5.2-, and 1.2-fold for Tra-ICG, Pan-Alexa680(SQ), and Pan-Alexa680(ON), respectively.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

In vitro characterization of the probe fluorescence was performed with EGFR+/HER2– breast cancer cell line MDA-MB-468 and EGFR–/HER2+ cell line NIH/3T3 (3T3/HER2) after incubation of the cells with each probe (10 μg) for 1 h and 8 h (1).

Under microscopy, the fluorescent signal from both Pan-Alexa680(ON) and Pan-Alexa680(SQ) was observed on the surface of MDA-MB-468 cells at 1 h and within the cells at 8 h after incubation. The fluorescent signal was stronger for Pan-Alexa680(SQ)

than for Pan-Alexa680(ON). The signal could be completely blocked by addition of excess Pan (100 μg). For Tra-ICG, the fluorescence signal was observed within the 3T3/HER2 cells. The fluorescent signal was minimal at 1 h (due to the greater quenching magnitude of Tra-ICG than Pan-Alexa680) and became stronger over time after incubation. The signal of Tra-ICG could be blocked by addition of excess Tra (100 μg).

Flow cytometry studies also showed fluorescent signal from Pan-Alexa680(ON), Pan-Alexa680(SQ), and Tra-ICG in MDA-MB-468 and 3T3/HER2 cells, respectively. Pan-Alexa680(SQ) and Tra-ICG showed progressively brighter signal over time compared with Pan-Alexa680(ON), which showed lower and constant signal.

Animal Studies

Rodents

[PubMed]

In vivo two-color imaging was performed in mice bearing tumor xenografts of MDA-MB-468 and 3T3/HER2 (n = 4 mice/group per time point). A cocktail of Tra-ICG and Pan-Alexa680(SQ) or Pan-Alexa680(ON) (50 μg each) was injected *via* the tail vein (1). Images were obtained with a fluorescence camera with two filter sets for Alexa680 and ICG, respectively.

Images showed accumulation of Pan-Alexa680(ON) in the targeted MDA-MB-468 tumors (EGFR+), with high background and non-specific uptake in the 3T3/HER2 tumors on day 1 through day 3 after injection. On day 4, relatively selective accumulation in MDA-MB-468 tumors was obtained. In contrast, images obtained with Pan-Alexa680(SQ) clearly showed MDA-MB-468 tumors with high tumor/background ratios from day 2, although weak signal was detected in the 3T3/HER2 tumors and bladder on day 1. Tumor/background (non-targeted tumors, liver, and neck) ratios for Pan-Alexa680(SQ) were significantly higher than the ratios for Pan-Alexa680(ON) for all 4 days ($P < 0.01$). The tumor/non-targeted tumor, tumor/liver, and tumor/neck ratios on day 4 after injection were 8.1, 10.3, and 8.8 for Pan-Alexa680(SQ), respectively, and 2.7, 3.9, and 4.5 for Pan-Alexa680(ON), respectively. Tra-ICG specifically accumulated in the 3T3/HER2 tumors with very weak fluorescent signal in the liver and intestine. The tumor/background ratios were not significantly different between the mice given Tra-ICG + Pan-Alexa680(SQ) and the mice given Tra-ICG + Pan-Alexa680(ON).

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.

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