Self-quenching Alexa fluor 680 conjugated to trastuzumab

Tra-Alexa680(SQ)

Arvind Chopra, PhD[1]

Created: March 5, 2009; Updated: April 6, 2009.

Chemical name:	Self-quenching Alexa fluor 680 conjugated to trastuzumab	
Abbreviated name:	Tra-Alexa680(SQ)	
Synonym:		
Agent Category:	Humanized monoclonal antibody	
Target:	Epidermal growth factor receptor 2 (HER2)	
Target Category:	Receptor	
Method of detection:	Optical imaging: fluorescence	
Source of signal / contrast:	Alexa fluor 680	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	Click here for information regarding human EGFR.

Background

[PubMed]

Humanized monoclonal antibodies (MAbs) conjugated to radionuclides used for the detection and treatment of cancers have limited efficacy because they are large glycoprotein molecules (~150 kD) that cannot penetrate deep into cancerous tumors (1). In general, radioimmunoconjugates, including those used for imaging purposes, have a prolonged blood circulation and, if used for radioimmunotherapy (high-energy β-emitters such as yttrium or rhenium, etc., or α-emitters such as bismuth or astatine, etc.), can impair the functioning of normal cells and may be myelotoxic (1). The use and

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

NLM Citation: Chopra A. Self-quenching Alexa fluor 680 conjugated to trastuzumab. 2009 Mar 5 [Updated 2009 Apr 6]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

limitations of radioimmunoconjugates as imaging and therapeutic agents were discussed in detail by Sharkey and Goldenberg (1). As an alternative to using radionuclides in conjunction with MAbs for the detection of cancer, investigators have explored the use of fluorescent agent-MAbs and other molecule conjugates for the preclinical imaging of cancerous tumors (2-6). However, the fluorescent dye-MAb probes, although cheap to generate, have blood circulation and tumor penetration drawbacks similar to the radioimmunoconjugates and also exhibit a high background because of autofluorescence generated in the tissue (6, 7). To circumvent problems observed with the optical probes, investigators have generated self-quenching, targeted fluorescent dye-MAb conjugates that are non-fluorescent in the native state but generate a signal only when activated by degradation of the targeting MAb or molecule by a specific cellular process or organelle (8). Accumulation of the fluorescent dye within a target cell would reduce the non-specific background and decrease the blood pool signal.

The chemical structure of self-quenching probes is such that a quencher molecule is closely associated in alignment with the fluorophore. Enzymatic cleavage of the structure results in separation of the quencher from the fluorophore and in the generation of a fluorescent signal (8). Near-infrared (NIR) probes such as Alexa fluor and Cy5.5 are favored over other optical agents because they exhibit superior tissue penetration (hemoglobin and water have low absorption of the NIR signal at the operating wavelengths of these probes), and when two or more NIR probe molecules are conjugated, the fluorescent signal quenches itself. Cleavage of the conjugation bonds results in fluorescence dequenching and generation of a signal that is suitable for imaging (9-11). However, it is pertinent to mention that only a specific number of the NIR probe molecules can be conjugated to a macromolecule without changing the structural and pharmacokinetic properties of the macromolecule. Ogawa et al. conjugated multiple copies of Alexa fluor 680 (Alexa680) to trastuzumab (Tra), a humanized MAb that specifically targets the human epidermal growth factor receptor 2 (HER2), to synthesize a self-quenching, targeted NIR probe (Tra-Alexa680(SQ)) (12). Tra is approved by the United States Food and Drug Administration for use as a single agent or an adjuvant to treat certain types of breast cancer and is being tested in clinical trials for the treatment of other neoplasms in humans. A characteristic feature of HER2 is that, upon binding a ligand, including the MAb directed against it, the receptor-ligand complex is internalized by the cell for enzymatic digestion in the lysosomes (13, 14). Proteolytic digestion of the receptor and the bound MAb in the lysosome releases the quencher from the fluorophore, resulting in generation of a signal. Therefore, a fluorophore that is "off" in the extracellular environment is turned "on" in an intracellular location. Ogawa et al. investigated the receptor specificity of Tra-Alexa680(SQ) under *in vitro* conditions using NIH-3T3 cells transfected with the HER2 gene (3T3/HER2$^+$ cells), and to study the *in vivo* characteristics of the fluorophore-MAb conjugate the investigators used nude mice bearing xenograft and orthotropic tumors generated with 3T3/HER2$^+$ or BALB/3T3/HER2$^-$ cells (12).

Synthesis

[PubMed]

Synthesis of the self-quenching Tra-Alexa680(SQ) conjugate was detailed by Ogawa et al. (12). Alexa680 (as a N-hydroxysuccinimide ester) and Tra were obtained from commercial sources. Briefly, the Tra-Alexa680(SQ) conjugate was synthesized by incubating Tra with Alexa680 in phosphate buffer (pH 8.5) for 30 min at room temperature. The conjugate was purified on a Sephadex G50 column and stored at 4°C as a stock solution. Approximately seven Alexa680 molecules conjugated to each MAb molecule, as determined with absorption on a UV-Vis spectrophotometric system.

A control "always on" conjugate, Tra-Alexa680(ON), was also synthesized, purified, and stored as above. The Tra-Alexa680(ON) conjugate was reported to have approximately one Alexa680 molecule per Tra molecule, as determined above (12).

The yield, purity, and stability of either conjugate, after purification, was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Ogawa et al. used a fluorescence spectrometer to investigate the fluorescence quenching ability of the Tra-Alexa680 conjugates by exposing them to 5% sodium dodecyl sulfate and 1% 2-mercaptoethanol in phosphate-buffered saline for 2 min at 95°C (12). The quenching capacities of Tra-Alexa680(SQ) and Tra-Alexa680(ON) were reported to be 8- and 2-fold, respectively compared to the native (undenatured),self quenching, molecules (12).

The *in vitro* binding specificity of the Tra-Alexa680 conjugates was studied with the use of 3T3/HER2$^+$ and the control BALB/3T3(HER2$^-$) cells as described by Ogawa et al. (12). The two cell types were exposed to Tra-Alexa680 conjugates and viewed under a fluorescent microscope after 1 and 8 h. At 1 h, cell surface fluorescence was observed only on the 3T3/HER2$^+$ cells, and at 8 h the fluorescence was emitted by fluorescent dots located inside the cells. A higher fluorescent signal was reported for cells treated with Tra-Alexa680(SQ) than for those treated with Tra-Alexa680(ON). A fluorescent signal due to Tra-Alexa680(ON) binding or internalization was not observed with the BALB/3T3/ZsGreen/HER2$^-$ cells (these cells were transfected with the green fluorescent protein gene) when exposed respectively to either conjugate. This indicated that the two Tra-Alexa680 conjugates were bound to, and internalized by, only the HER2$^+$ cells.

Animal Studies

Rodents

[PubMed]

In vivo and *ex vivo* imaging studies performed with nude mice bearing 3T3/HER2$^+$ and BALB/3T3/ZsGreen/HER2$^-$ cell tumors were described by Ogawa et al. (12). The mice were injected with either Tra-Alexa680(SQ) or Tra-Alexa680(ON), respectively (number of animals used per conjugate was not reported), and imaging was performed 2 d after administration of the conjugates. Whole-body imaging of the animals revealed that the 3T3/HER2$^+$ tumor images were much more enhanced compared to those generated with the BALB/3T3/ZsGreen/HER2$^-$ cells. Also, with Tra-Alexa680(ON), the fluorescent signal was obtained not only from the HER2$^+$ cells but also from the HER2$^-$ cells, although the signal generated from the latter cells was considerably lower. *Ex vivo* imaging of the tumors yielded results similar to those obtained during the whole-body imaging studies (12). In addition, the fluorescent signal obtained from the blood pool was reported to be low with Tra-Alexa680(SQ) compared with Tra-Alexa680(ON). Similar results were also reported with animals bearing 3T3/HER2$^+$ and BALB/3T3/ZsGreen/HER2$^-$ cell orthotropic tumors (12).

With results obtained from this study, the investigators concluded that it is possible to apply this technology to other humanized MAbs of the immunoglobulin G_1 class for the development of targeted imaging agents (12).

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]

NIH Support

Some studies in this chapter were supported by grants from the National Institutes of Health Intramural Research Program, the National Cancer Institute, and the Center for Cancer Research.

References

1. Sharkey R.M., Goldenberg D.M. *Novel radioimmunopharmaceuticals for cancer imaging and therapy.* Curr Opin Investig Drugs. 2008;9(12):1302–16. PubMed PMID: 19037837.
2. Amoh Y., Katsuoka K., Hoffman R.M. *Color-coded fluorescent protein imaging of angiogenesis: the AngioMouse models.* Curr Pharm Des. 2008;14(36):3810–9. PubMed PMID: 19128234.
3. Barrett T., Koyama Y., Hama Y., Ravizzini G., Shin I.S., Jang B.S., Paik C.H., Urano Y., Choyke P.L., Kobayashi H. *In vivo diagnosis of epidermal growth factor receptor expression using molecular imaging with a cocktail of optically labeled monoclonal antibodies.* Clin Cancer Res. 2007;13(22 Pt 1):6639–48. PubMed PMID: 17982120.
4. Koyama Y., Hama Y., Urano Y., Nguyen D.M., Choyke P.L., Kobayashi H. *Spectral fluorescence molecular imaging of lung metastases targeting HER2/neu.* Clin Cancer Res. 2007;13(10):2936–45. PubMed PMID: 17504994.
5. Sampath L., Wang W., Sevick-Muraca E.M. *Near infrared fluorescent optical imaging for nodal staging.* J Biomed Opt. 2008;13(4):041312. PubMed PMID: 19021320.
6. Pierce M.C., Javier D.J., Richards-Kortum R. *Optical contrast agents and imaging systems for detection and diagnosis of cancer.* Int J Cancer. 2008;123(9):1979–90. PubMed PMID: 18712733.
7. Cai W., Chen X. *Multimodality imaging of vascular endothelial growth factor and vascular endothelial growth factor receptor expression.* Front Biosci. 2007;12:4267–79. PubMed PMID: 17485373.
8. Lee S., Park K., Kim K., Choi K., Kwon I.C. *Activatable imaging probes with amplified fluorescent signals.* Chem Commun (Camb). 2008;(36):4250–60. PubMed PMID: 18802536.
9. Bremer C., Ntziachristos V., Weissleder R. *Optical-based molecular imaging: contrast agents and potential medical applications.* Eur Radiol. 2003;13(2):231–43. PubMed PMID: 12598985.
10. Ntziachristos V., Bremer C., Weissleder R. *Fluorescence imaging with near-infrared light: new technological advances that enable in vivo molecular imaging.* Eur Radiol. 2003;13(1):195–208. PubMed PMID: 12541130.
11. Wunderbaldinger P., Turetschek K., Bremer C. *Near-infrared fluorescence imaging of lymph nodes using a new enzyme sensing activatable macromolecular optical probe.* Eur Radiol. 2003;13(9):2206–11. PubMed PMID: 12802615.
12. Ogawa M., Regino C.A., Choyke P.L., Kobayashi H. *In vivo target-specific activatable near-infrared optical labeling of humanized monoclonal antibodies.* Mol Cancer Ther. 2009;8(1):232–9. PubMed PMID: 19139133.
13. Ogawa M., Kosaka N., Choyke P.L., Kobayashi H. *In vivo molecular imaging of cancer with a quenching near-infrared fluorescent probe using conjugates of monoclonal antibodies and indocyanine green.* Cancer Res. 2009;69(4):1268–72. PubMed PMID: 19176373.
14. Palyi-Krekk Z., Barok M., Kovacs T., Saya H., Nagano O., Szollosi J., Nagy P. *EGFR and ErbB2 are functionally coupled to CD44 and regulate shedding, internalization and*

motogenic effect of CD44. Cancer Lett. 2008;263(2):231–42. PubMed PMID: 18276068.