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Monoclonal antibody–fluorescent probe conjugates for *in vivo* target-specific cancer imaging: toward clinical translation

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Abstract

"...fluorescence imaging has the advantages of low cost, portability, no radiation exposure, high resolution and real-time image acquisition, showing promise in intraoperative and endoscopic cancer diagnosis."

Keywords

activatable probe; cancer imaging; molecular imaging; monoclonal antibody; theranostics

Fluorescence imaging, including endogenous fluorophores and exogenous fluorescent probes, has been used in clinical practice for many years. However, clinical applications in cancer remain limited and this seems to be a missed opportunity for improving patient care. Compared with current clinical imaging modalities, such as computed tomography, ultrasound, MRI, single-photon emission computed tomography and positron emission tomography, fluorescence imaging has the advantages of low cost, portability, no radiation exposure, high-resolution and real-time image acquisition, demonstrating promise in intraoperative and endoscopic cancer diagnosis [1–5].

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Molecularly targeted drugs such as monoclonal antibodies (mAbs) and tyrosine kinase inhibitors have become keystones of cancer therapy. Thus, there is a plethora of approved targeted agents that can be harnessed for other purposes. For instance, mAbs can be conjugated with imaging 'beacons' that enable targeted imaging. Radiolabeled mAbs accumulate preferentially within lesions expressing the appropriate antigen; however, a major limitation is that there is substantial background signal owing to the long circulation times of unbound radiolabeled mAb, thus lowering target-to-background ratios. Additionally, even within the targeted tumor tissue, the microdistribution of radiolabeled mAbs is often heterogeneous due to poor penetration of radiolabeled mAbs into the center of tumors. Nevertheless, the US FDA has approved several types of radiolabeled mAb, including:

- ^{111}In-murine anti-TAG-72 mAb (satumomab pendetide; OncoScint®);
- ^{99m}Tc-murine anti-EGP-1 Fab' antibody (nofetumomab merpentan; Verluma®);
- ^{99m}Tc-murine anti-carcinoembryonic antigen Fab' antibody (arcitumomab; carcinoembryonic antigen-Scan®);
- ^{111}In-murine anti-prostate-specific membrane antigen mAb (capromab pendetide; Prosta-Scint®);
- ^{90}Y-murine anti-CD20 IgG + rituximab (Ibritumomab tiuxetan; Zevalin®);
- ^{131}I-murine anti-CD20 IgG + unlabeled tositumomab (^{131}I anti-B1 antibody; Bexxar®).

These radiolabeled mAbs are used for immuno-scintigraphy and/or radioimmunotherapy. These agents have, however, met with mixed financial success and some are no longer commercially available [6].

An alternative to radiolabeling is fluorescent labeling. In theory, fluorescently labeled mAbs should be subject to the same limitations as radiolabeled mAbs, that is, high background signal-hampering specific detection of target tumors. However, fluorescent probes differ in one important respect from radioactivity. Fluorescence is a potentially 'switchable' or 'activatable' property; fluorescent probes can change from the quenched state (no light emission) to the unquenched state (fluorescent) after binding to the target antigen on the cell surface. This occurs because binding is followed by chemical or biological processing that dequenches the fluorophore. This has the singular advantage of providing extremely high target-to-background ratios mostly by suppressing background signals [7–9].

There are multiple strategies in the design of activatable fluorescently labeled mAb-based imaging probes. Fluorescence signals can be selfquenched, when multiple fluorophores of the same type are conjugated to a mAb molecule, resulting in homo-fluorescence resonance energy transfer. For example, seven Alexa Fluor® 680 molecules were conjugated to trastuzumab, an FDA-approved humanized mAb that recognizes HER2. This agent was initially quenched under physiological conditions, but became strongly fluorescent after binding and lysosomal degradation within HER2-expressing cells both *in vitro* and *in vivo* [10]. Another activating mechanism is the use of quencher—fluorophore pairs conjugated to the mAb, resulting in hetero-fluorescence resonance energy transfer quenching. Enzymatic

cleavage of the quencher—fluorophore pair results in separation of the quencher from the fluorophore, thus activating the fluorescent signal. For example, a TAMRA (fluorophore)—QSY7(quencher) pair conjugated to trastuzumab was quenched under physiological conditions. Binding to HER2-positive cells resulted in highly activated fluorescence with low background signal both *in vitro* and *in vivo* [11]. Using yet another mechanism, fluorescence can be quenched by H-dimer formation, wherein fluorescent probes form autoquenching dimers when conjugated to proteins. For instance, indocyanine green (ICG; Cardiogreen®) is a cyanine dye that is fluorescent when unconjugated or bound to albumin. However, when ICG is conjugated to proteins other than albumin, fluorescence is generally quenched. When ICG dissociates from the conjugate protein it becomes fluorescent. The same properties are exhibited when ICG is conjugated to a mAb. Examples include ICG-labeled daclizumab, trastuzumab or panitumumab. These conjugates demonstrated minimal fluorescence in solution, but rapidly became activated in target-expressing cells after binding and lysosomal processing in targeted cells [12,13].

Due to their very high target-to-background ratios, these activatable fluorescently labeled mAb imaging probes are capable of visualizing even tiny lesions on the surface of organs, such as the colon, that would otherwise be invisible to the naked eye. Of course, this requires the use of an endoscopic camera adapted for fluorescence of the proper wavelength for the fluorophore. These alterations to conventional endoscopes are relatively minor and major manufacturers have already designed clinically translatable devices. However, the companies are understandably waiting for the approval of fluorescent probe conjugates for clinical use. Probably the most viable of the candidates described are mAb—ICG conjugates, which are comprised of two already-approved elements, potentially making the regulatory approval process easier. ICG has a maximum absorption at 780 nm and emits in the near-IR (NIR) fluorescence at approximately 820 nm, which is desirable for optical imaging as it permits deeper penetration into tissue while reducing background signal from autofluorescence, which is reduced in the NIR. NIR fluorescent probes can be excited with a broad range of visible light, and, therefore, white light imaging is not compromised [14].

> “...fluorescently labeled mAb imaging probes are capable of visualizing even tiny lesions on the surface of organs, such as the colon, that would otherwise be invisible to the naked eye “

Another promising way to develop a clinically translatable mAb—fluorescent conjugate is to develop agents that are not only diagnostic, but also therapeutic. Recently, a NIR phthalocyanine probe, IRDye 700DX (IR700), was conjugated to trastuzumab or anti-EGF receptor mAb, panitumumab. Target-specific fluorescence was achieved both *in vitro* and *in vivo,* and in addition, cell death was induced at higher excitation energies. Interestingly, only mAb—IR700-bound cancer cells were killed by relatively low levels of NIR light and cells not expressing the antigen were unharmed. Tumor shrinkage was achieved without significant side effects [15–19]. This has been termed photoimmunotherapy.

While a number of promising mAb-fluores- cent probe conjugates have been developed and have been successful in *in vivo* preclinical animal models, there are many hurdles before such agents can be translated to the clinic. In addition, the pharmacokinetics of fluorescent probes are likely to be altered by conjugation to mAbs. Therefore, careful chemical design

and pharmacological analysis of mAb conjugates is needed. Conjugates must have a good stability, low toxicity, be easy to administer and be produced and sold at a modest cost, thereby assuring sustainability. We are close to achieving these goals and seeing these agents in the clinic in the near future.

Biography

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