The use of monoclonal antibodies in near-infrared fluorescence (NIRF) tumour targeted (intraoperative) imaging

By Thea van den Bosch

Summary

The development of monoclonal antibodies has been very important in applied immunology. They have found a broad variety of applications. One such application lies within (intraoperative) imaging of cancer. Specifically, antibody targeted near infrared fluorescence (NIRF) intraoperative imaging of cancer is an important and very promising novel approach with respect to cancer treatment.
In this literature study, it is outlined what investigations have currently been performed using antibody targeted NIRF (intraoperative) imaging of cancer. Other approaches to NIRF labelling in tumour targeted (optical) imaging are also discussed and compared to the antibody targeted technique. Finally, it is discussed which approach will have the better prospects of clinical application for (intraoperative) imaging in cancer therapy.
The study concludes that targeted imaging of cancer using NIRF antibodies has the important advantage over non targeted NIRF cancer imaging techniques of specifity for the tumour. However, whether the technique will actually aid in cancer therapy still remains to be seen. Many further (clinical) studies will be required in order to determine whether the technique can truly assist in cancer treatment.
Furtermore, important developments have been achieved in ‘smart activatable probes’. These labeling agents are specifically activated by tumour enzymes (upon cleavage). These ‘smart activatable probes’ are not necessarily antibody targeted but may also be peptide based.

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Introduction

Magic Bullets

It was Paul Ehrlich who first introduced the concept of a 'magic bullet'; he reasoned that a chemical substance could be created that would specifically bind to a bacterium or another pathogen within the human body and destroy it. [Winau *et al*, 2004]

All vertebrates actually possess such magic bullets. These are known as antibodies, proteins which have an extremely important function within the immune system of vertebrates. Normally, an antibody binds an antigen; it recognizes a surface molecule displayed by a pathogen in a very specific manner (this results in a specifity for that pathogen). Its aim is then to neutralize this pathogen, as well as to further induce the immune response against this pathogen. Vertebrates possess an incredibly large variety of antibodies, each of which have a unique specifity and are produced by a unique B lymphocyte. [Abbas]

With the onset of monoclonal antibody production, the magic bullet concept was truly realized. This technique exploits the fact that each B lymphocyte produces an antibody of a unique specifity. In order to obtain an antibody against a certain antigen, a mouse or rat will fist have to be challenged with this antigen. B lymphocytes will then be isolated from the spleen of the animal. The B lymphocytes producing the antibody of the desired specifity will then be fused with myeloma cells (cancerous B lymphocytes) in order to provide for the ability to divide indefinitely. The resulting immortalized cell line(s) will then produce the desired antibody. These monoclonal antibodies will have an identical specifty, and can be produced in unlimited quantities. Nowadays, monoclonal antibodies of basically any specifity can be produced. It should be noted, however, that when use in humans is required, monoclonal antibodies produced as such must be humanized. [Abbas]

Monoclonal antibodies are extremely useful and have various applications. Some of these include identification of phenotypic markers unique to particular cell types, immunodiagnosis, various kinds of therapies, functional analysis of cell surface and secreted molecules, and tumour imaging and diagnosis. [Abbas] In this literature study, however, the application of tumour imaging will be important.

The use of monoclonal antibodies in tumour imaging

Monoclonal antibodies can be used in tumour imaging. For this reason, they have a very promising and important application in (guided) oncological surgery. In (guided) oncological surgery, it is important to visualize boundaries between healthy and cancerous tissues. Currently, incomplete removal of tumours is common, due to inadequate techniques of imaging such tumours and their boundaries. Intuitively, when a tumour is inadequately removed, this may contribute to recurrence of the disease. [Mieog *et al*, 2010] Visualizing techniques for tumours are therefore very important to investigate into. Such techniques using antibodies are very promising, as these molecules may relatively easily be labelled and have the ability of very specifically binding a certain antigen, as explained earlier.

Antibodies that are used in tumour imaging specifically bind to tumour antigens. The antigens displayed will vary between tumours, and therefore, unique target(s) will need to be identified for individual tumour types. As a side note, generally, requirements for such tumour antigens (or targets present on tumours) for imaging should include the following [van Oosten *et al*]:

1. The target must have an extracellular location in order to allow for binding to it.
2. It must have an evenly distributed expression pattern throughout the tumour.

3. Its expression should be minimal in healthy tissue
4. It should be overexpressed in tumours in a high percentage of the patients
5. Enzymatic activity of the target would be advantagous in order to allow for the use of smart activatable probes (see the heading “target specific activation of fluorescence: smart activatable probes”)
6. Internalization of the target after the probe has bound would also be advantageous, as the imaging agent would then accumulate within the tumour cells. This would result in a higher signal to noise ratio.

When antibodies are used in imaging, they can be labelled in various ways.
One example of tumour imaging using monoclonal antibodies constitutes a technique called RIGS (radio-immuno-guided surgery), which has been described in a number of articles, including a number of clinical trials. RIGS uses monoclonal antibodies against the tumour antigen TAG-72, labelled with radioactive ^{125}I. A hand held gamma probe may be used during surgery to determine the sites that the TAG-72 antibody has bound. This technique has proven to be successful in identification of tumours and was able to significantly increase survival chances in patients. However, as RIGS uses ^{125}I, it would be impractical to actually be used in a clinical setting. The technique would involve exposure to harmful radiation, for both patient and physician and ^{125}I has a very long halflife. [Zou *et al*, 2009, Sun *et al*, 2007]
A more suitable approach in tumour imaging, however, involves fluorescently labelling antibodies, also rendering very effective ‘targeting probes’. Fluorescently labelling the antibodies provides the important advantage over radioactively labelling, as in RIGS, that it does not involve exposure to harmful radiation, making it much more suitable for clinical use.
Targeting fluorescent dyes to tumours with the purpose of imaging was first attempted in two sequential studies by the direct conjugation of fluorescent dyes to monoclonal antibodies directed against tumour-associated antigens. These studies showed specific tumour targeting first using an anti-carcinoembryonic antigen monoclonal antibody coupled with fluorescein (anti-CEA mAb–fluorescein) for endoscopic diagnosis of human colon carcinoma, first in mice [Pelegrin *et al*, 1991], and then in human colon carcinoma patients [Folli *et al*, 1992].
However, in particular, the development of near infrared fluorescence (NIRF) labelling techniques for antibodies is interesting, as will be explained shortly. Studies using NIRF have been very promising. The applications of NIRF labelled antibodies in cancer imaging will be important in this literature study. Other approaches to NIRF labelling in tumour targeted (optical) imaging will also be discussed and compared to the antibody targeted technique. Finally, the aim will be to determine which approach will have the better prospects of clinical application for (intraoperative) imaging in cancer therapy. For clarity, it should be noted that from hereon, only NIRF labels will be discussed (no other types of fluorescence).

Near infrared fluorescence (NIRF) labelling
Of course, there are many aspects to be considered with respect to the use of a certain fluorescent dye in imaging studies. First and foremost is the question of its toxicity. Furthermore, it must have an appropriate size that will allow for sufficient transportation to the tumour. Also, importantly, the fluorescent signal of the probe should be sufficiently strong to penetrate through tissue. The dye must therefore have a sufficient quantum efficiency (brightness). [Crane, 2010] Fluorescent dyes that emit within the near infrared region of the electromagnetic spectrum are most promising due to their increased tissue penetration capacity, as will be discussed below.

The near infrared region generally refers to those waves having a wavelength of around 700 – 1000 nm, although the boundaries between the near, mid and far infrared regions are not agreed upon and can vary. The region is invisible to the human eye [Staninger, 2004].
NIRF labelling provides advantages over 'usual' fluorescence labelling within the visible and UV regions of the spectrum. Human tissue absorbs and scatters visible and UV light, limiting its penetration to only a few millimetres, whereas tumour imaging requires deep tissue penetration. The absorption coefficient of human tissue for near-infrared light is much lower, and therefore, there will less autofluoresence and less scattering of the signal. Hence, there will be less background signal, and no need for spectral unmixing. Also, the NIRF signal will be able to penetrate to depths of several centimeters. This aspect will increase the sensitivity of the signal. These combined advantages assist in detection of early stages of disease, and even metastasis [Amoit *et al*, 2008].

Literature overview

NIRF dye coupled antibodies – studies so far

NIRF dyes can be linked to antibodies relatively easily, and are frequently used in studies involving tumour imaging. Therefore, these will comprise the most important type of NIRF material discussed in this literature study. Currently, only one such NIRF dye has been approved of for clinical use by the FDA (indocyanine green). [Ogawa *et al*, 2009, Cancer Research] The cyanine dyes have been used in a large number of (clinical) studies. An important development in the use of NIRF dye coupled antibodies are the smart activatable probes, as is discussed later on under a separate heading. Here, however, a number of other studies will be discussed.
The first study to use a NIRF dye conjugated to a monoclonal antibody was performed by Folli *et al*. It was coupled to an indocyanine fluorophore (Cy5) and targeted against squamous cell carcinomas in mice. In the study, Folli *et al*. tested the serum lifetime of radioactively labeled and Cy5-labeled conjugates and found that Cy5 conjugates to have a lifetime essentially equal to that of iodinated antibodies (as in RIGS). The labels colocalized, with a constant ratio of the two labels among different tumour masses and normal tissues in the same animal. Ordinary fluorescein conjugates, on the other hand, had a shorter half-life and were less sensitive with respect to tumour location. [Folli *et al*, 1994].
Ballou *et al*. then produced fluorophore conjugates of monoclonal antibodies to a number of NIRF cyanine dyes Cy3, Cy5 and Cy5.5 with increasingly red-shifted excitation and emission spectra. The monoclonal antibodies were anti-SSEA-1, which localizes tumours expressing the SSEA-1 antigen and antibody 9.2.27, which is directed to a human melanoma antigen. These were two well investigated and characterized antibodies. The study demonstrated the Cy5 and Cy5.5 conjugates to be more effective in visualizing the tumours, especially in deeper tissue, due to their fluorescence excitation and emission spectra at longer wavelengths compared to those of Cy3 and 'ordinary' fluorescein. [Ballou *et al*, 1995]
From this point on, numerous studies have been done with NIRF labelled antibodies targeted against a number of different tumour antigens. Examples include the following:

- A Cy7 dye coupled to antibody fragments targeted against oncofetal fibronectin, which allowed for visualization of angiogenic vessels expressing this type of fibronectin associated with an aggressive teratoma tumour in mice. [Neri, 1997]

- A Cy5.5 dye coupled to an anti-epidermal growth factor receptor monoclonal antibody in a hamster cheek pouch carcinogenesis model [Soukos *et al*, 2001]. Premalignant leasions, characterized by upregulation of the receptor, after treating the cheek pouch with a carcinogen, could be imaged.
- A Cy5.5 dye coupled to a anti-HER2 antibody (herceptin), allowing for visualization of cancerous breast carcinoma cells overexpressing the HER2 protein in mice [Hilger *et al*, 2004]
- A cyanine dye (DY-676) coupled to a specific antibody fragment directed against carcinoembryonic (CEA) antigen (Arcitumomab), allowing for visualization of CEA expressing human colonic adenocarcinoma cells in mice [Lisy *et al*, 2008]
- A Cy7 dye coupled to a anti-tumour associated glycoprotein 2 antibody, used for human colon adenocarcenoma imaging in mice [Zou *et al*, 2009]
- An Alexa Fluor 750 dye coupled to a anti-HER2 affibody, allowing for visualization of HER2 protein (over)expression in tumours [Chernomordik *et al*, 2010]

Target specific activation of fluorescence: smart activatable probes

A difficulty in using antibodies in tumour imaging may arise from the fact that antibodies are subject to an extensive circulation time. From a therapeutic point of view, this may be advantageous as it will result in continuous drug delivery to the tumour. However, in imaging of cancer, this effect is usually disadvantageous as it will render a large amount of background signal arising from unbound, circulating antibody. As delayed imaging (which would involve imaging after the unbound antibody has cleared from the circulation) would be impractical, researchers have attempted to remove circulating antibodies in various ways. An example includes a study in which secondary antibody was targeted to a circulating primary radiolabelled (note: not NIRF) fluorescently labeled, however, the example still applies) antibody, administered after a sufficient amount of the primary antibody had bound. This secondary antibody would clear the primary antibody inactive upon binding. The method was found to reduce the level of blood radioactivity and to enhance tumour/nontumour ratios. [Sharkey *et al*, 1984]

More recently, however, researchers have been focusing on the more sensible and very useful approach of target specific activation of fluorescence. Weissleder *et al* published an important article in this respect in 1994. In this study, the idea of specific cleavage of quenched dyes by tumour proteases was presented, which lead on to the idea of smart activatable probes (the technique is currently also available for antibodies, as will be discussed below). The Weissleder study demonstrated the feasibility of such an approach. The researchers loaded a fluorophore (Cy5.5) onto a long circulating polymer consisting of poly-L-lysine and methoxypolyethylene glycol succinate. When conjugated to the polymer backbone in high concentration, the fluorophore was self-quenched (in fluorescence labelling, quenching refers to a general loss in fluorescent signal. Self quenching, then, occurs when a fluorescent agent is able to quench its own signal due to molecular interactions between the individual dyes). However, as the polymer backbone was subjected to cleavage through lysosomal proteases (which had enhanced activity within cancer cells), the fluorochromes were released and emitted signal, specifically from human tumours implanted in mice [Weissleder *et al*, 1994].

A recent example of such an approach using NIRF conjugated antibodies would be the study that employed the activatable probe described in fig. 1. [Ogawa *et al*, 2009,

Molecular Cancer Therapy] This particular probe consists of Trastuzamab antibody, which specifically binds to HER2, coupled to multiple Alexa Fluor 680 self quenching fluorophores. In the study by Ogawa *et al*, this probe is described to successfully and specifically visualize HER2+ tumours. Its mechanism of action is as follows. The probe will be inactive in its quenched form. Upon binding to the HER2 receptor, however, the probe will be internalized and cleaved within lysosomes. The fluorescent agents will no longer be quenched at this point, and their fluorescent activity will be triggered within target cell lysosomes only.

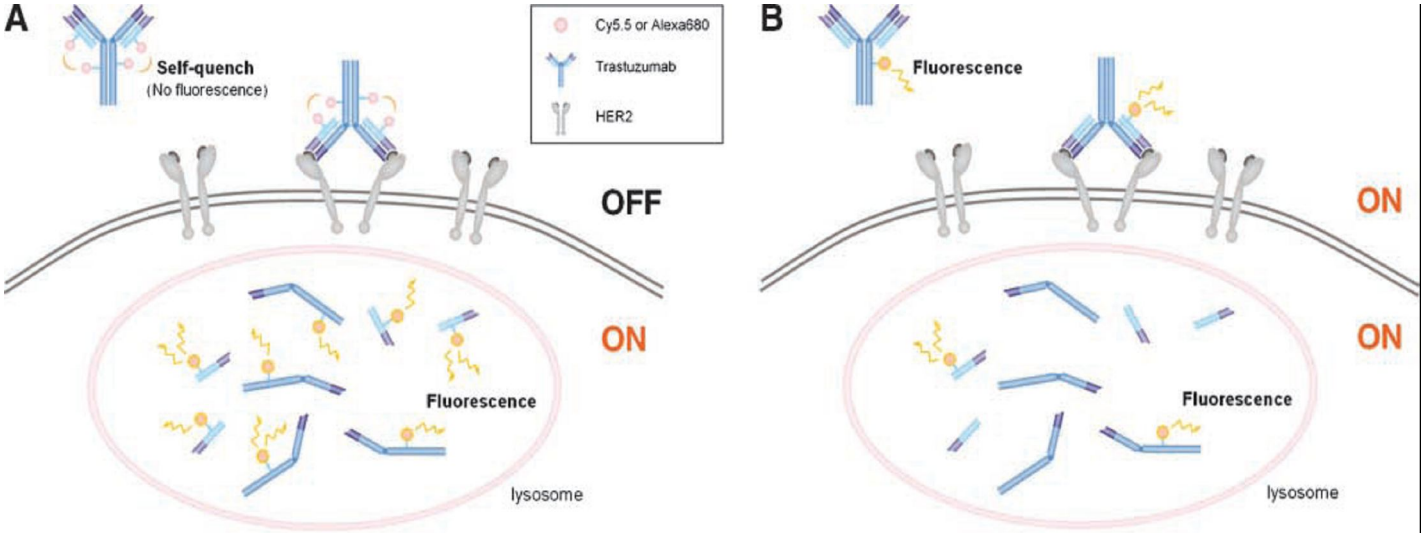


Figure 1. Adapted from [Ogawa *et al* 2009, Molecular Cancer Therapy]. A)The activatable probe used in the study of Ogawa *et al*. B) conventional fluorescent probe.

Finally, recent significant contributions to research into smart activatable probes have been made by Professor R.Y. Tsien (who shared in a nobel prize for the discovery and development of green fluorescent protein) and coworkers. His research group has been working on activatable Cy5 labelled cell penetrating peptides (ACPP). These are targeting agents comprised of a cell penetrating peptide (CPP) component, which is cationic, and a neutralizing anion component. The two components are attached to one another through a cleavable linker. Adsorption and uptake into cells will occur only upon cleavage of the linker, which also releases the Cy5 signal. In one of their studies, it was shown that ACPPs are effective targeting agents in various tumour xenografts. Furthermore, the researchers demonstrated that the ACPP's were being cleaved by matrixmetalloproteinases (MMPs) in these tumour models. The probes can therefore image MMP activity, and therefore certain tumours which show enhanced MMP activity [Olson *et al*, 2009]. In a later study, the researcher group demonstrated that the method improves cancer surgery in rats [Nguyen *et al*, 2010].
There are a number of advantages to the use of ACPPs. First of all, the particles are small in size, allowing for improved penetration into tumours as compared to antibodies. Furthermore, there is usually a good spatial resolution due to the closely packed distribution of MMP activity in tumours. Finally, inert macromolecules of 30–50 KDa may be added to the polyanionic inhibitory domain, reducing background uptake of ACPPs into healthy tissue [Olson *et al*, 2009].

'Passive' tumour targeting
Techniques of 'passive' tumour targeting do not rely on the specific targeting that antibodies provide. In stead, these probes can 'target' the tumour through the

‘enhanced permeability and retention’ effect (EPR effect) of the tumour microvasculatures. Practically, this implies that the probes will accumulate within the tumour, due to the particular characteristics of the vascular environment of the tumour (this is the case for all macromolecules). [Lyer *et al*, 2006] An example of such passive tumour targeting involves peptide dye conjugates.
Among the first of examples of this technique is the following. Inspired by the Weissleder article (as discussed above), Becker *et al* decided to also develop a protease activated probe, however, without using antibodies for targeting. The researchers aimed to circumvent the long circulation time that antibodies are subject to, as well as the immunogenicity that they are accompanied by. When introduced into mice, the cyanine dye labeled octreotate (a somatostatin analog) peptide accumulated in tumour tissue which could thus be imaged [Becker *et al*, 2001]. Of note, the administration of only a NIRF dye which is not conjugated to any other substance for tumour imaging is also possible. The limitation of all such ‘passive’ targeting techniques, however, is their lack of tumour specifity.

NIRF nanoparticle based probes
Another category of passive tumour imaging may comprise the studies using NIRF nanoparticle based probes. These are not necessarily linked to antibodies (this is possible, however, which is why they are placed under a separate heading), but may also rely on the EPR effect for tumour targeting. These nanoparticle based materials have been thoroughly studied for applications in intraoperative cancer imaging, including in animal models, and will briefly be discussed below.
NIRF nanoparticle based probes that have been used in such studies include the following:

- NIRF dye containing nanoparticles. Such nanoparticles are composed of polymers or inorganic matrices. They contain NIRF dyes within them. Advantages of these probes include their high quantum yield (brightness), and the protection they provide to the dyes from the external environment. This results in high sensitivity and photostability. However, these nanoparticles induce inflammatory reactions and will therefore most likely not be suitable for a clinical application.
- Carbon Nanotubes. These are carbon cylinders consisting of benzene rings. Some types of carbon nanotubes are naturally NIR fluorescent, and have a high signal to noise ratio. The main issue with carbon nanotubes is their insolubility, rendering them toxic. There are currently SWCNT with non-toxic cores. Such carbon nanotubes, however, are still too toxic for clinical application.
- Quantum dots. Quantum dots are semiconductor crystals with narrow but tunable emission bands (this is dependent upon the size and properties of the material) and broad exicitation spectra. They also have a high quantum yield and are resistant to photobleaching. Unfortunately, however, these are also too toxic to be applied in the clinic.

Research is still being conducted into NIRF nanoparticle based probes, both in the Netherlands and abroad, however, the toxicity that is associated with them is likely to impair their introduction into the clinic.
For the NIRF nanoparticle based probes, as mentioned above, antibodies specifically recognizing receptor(s) at a tumour surface may be linked to linked to the nanoparticles in order to realize active tumour targeting. The carrying of several of such antibodies might enhance binding and specificity for the tumour, resulting in a

lower amount of toxicity of the probes to the normal tissues. The toxicity of the nanoparticles remains a large issue, however. [He *et al* 2010; Cho *et al*, 2008; Dr. G.M. van Dam, personal communication]

Camera systems

As NIRF is not visible to the naked eye, special camera systems are required in order to first excite the fluorophore present in the targeting probe at the appropriate wavelength, and to then make the fluorescent signal that is being emitted from the targeting probe visible to the surgeon. Three dimensional images are the most useful, as they will provide for information on the depth of the signal. A number of such camera systems exsist for *in vivo* imaging studies. They will be described below.
First of all, Fluorescence Molecular Tomography (FMT) is an important technique that can be used for calculation of the three dimensional position and distribution of the NIRF signal within tissue. In tomograpy, after imaging numerous slices of the tissue, a three dimensional integrated image can be created by virtue of mathematical models. Two imaging systems that exploit such techniques are the following:

- Ivis Spectrum imaging system from Caliper LifeSciences. The Ivis Spectrum (fig. 2A) is an advanced imaging system with excitation and emission filters from across the blue to the NIR region. It allows for three dimensional visualization of fluorescent probes in small animals such as mice and rats [Caliper LifeSciences].
- FMT2500 imaging system from PerkinElmer. The FMT2500 (fig. 2B) allows for fluorescence imaging within the range of 650-850 nm in small animals such as mice and rats, and can therefore be used to visualize NIRF. The system has four different channels with specific excitation and emission filters within this range [PerkinElmer].

For intraoperative NIRF imaging, however, only prototypes exsist. Currently, only one has succesfully been used for imaging of cancer in human patients [Crane 2010].
Furthermore, a number of researchers have attempted to introduce more creative imaging systems. The most notable example is perhaps the fluorescence surgical goggle (fig. 2C), described by Ruogu Qin [Qin, 2009]. The idea of such a system might seem useful, however, there are significant limitations associated with it. These include its heavy weight, its excessive heat production, and finally, in this system, the fluorescence is not projected over the field of interest. Due to these limitations, the fluorescence surgical goggle will not be suitable for surgeons to use in the clinic [Dr. G.M. van Dam, personal communication].
Finally, another example involves a handheld intraoperative camera system [Mieog *et al*, 2010]. A drawback associated with this type of camera system, however, might be that it would render the simultaneous removal of fluorescent tissue difficult.

A. 

B. 

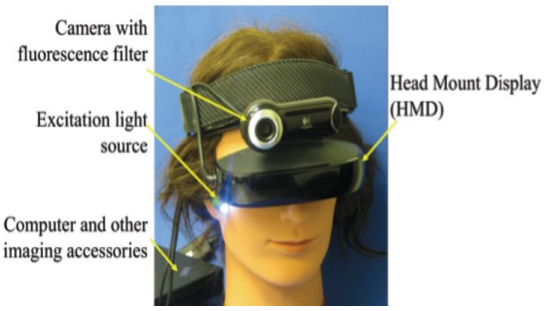
C. 

Figure 2. A) the Ivis spectrum from Caliper LifeSciences. B) the FMT2500 from PerkinElmer. C). the intraoperative fluorescent surgial goggle described by Ruogu Qin.

Research at the University Medical Center Groningen

In order to outline what research into (intraoperative) NIRF imaging of cancer is being conducted at the University Medical Center Groningen (UMCG), Dr. G.M. van Dam was consulted. Dr. G.M. van Dam is the head of the intraoperative imaging research group (research leader). Dr. van Dam coordinates all the investigations that take place within the research group. He is also a surgeon. The aim of this research group is to develop and apply optical techniques in surgery. The researchers aim at using such techniques in treatment of bacterial infections, cardiovascular disease and cancer. There is also a bio-optical imaging centre that was set up in the animal research facility at UMCG. The surgery department was responsible for its initiation. This is a facility that provides researchers with camera systems that are able to measure optical signals *in vivo*, such as bioluminescence and fluorescence signals. Importantly, there is an extensive amount of collaboration between the intraoperative imaging research group and the oncology department. Dr. van Dam is especially involved in the oncological research that is being performed in collaboration with the intraoperative imaging group.

The fluorescent dye that is mostly used within the research group is CW800. This infrared dye is 50 times brighter than indocyanine green. It is a fluorophore that currently has no approval for clinical use of the FDA, however, the research group is aiming at getting it approved for clinical use at the UMCG. The group is currently working on this.

Dr. van Dam estimates that the research that is being conducted at the UMCG is more advanced compared to research that is being conducted elsewhere in the Netherlands or abroad. Presently, the UMCG researchers are 1 to 2 years ahead and more studies are being conducted this year. The more advanced state is due to the UMCG's more

advanced camera systems, its experienced surgeons who are experienced with handling this equipment, good contrast dyes, expertise in preclinical systems and due to the UMCG's own GMP facility. This is a unique combination of factors that has greatly accelerated research at the UMCG.
Dr. van Dam is of the opinion that NIR fluorophores, but not the nanoparticle based probes (due to the toxicty related issues involved in the use of such probes), have the most potential for a clinical application. Such fluorophores may be introduced into the clinic in three years. However, presently, it remains unclear precisely how much they will contribute to cancer treatment. A large amount of further studies will be required in order to determine this.
Important developments in the field have been small animal studies, FMT systems allowing for 3D quantifications and the first trials that have been conducted in patients [Crane, 2010]. Further developments that we may expect within the coming five years, according to Dr. van Dam, are improvements of already existing systems, more broad applications, more substances being tested for clinical application (NIRF dyes for example), and, finally, studies on larger groups of patients. [Dr. G.M. van dam, personal communication].

Conclusions and future perspectives

The development of monoclonal antibodies has been very important in applied immunology. They have found a broad variety of applications. One such application lies within (intraoperative) imaging of cancer. Specifically, antibody targeted NIRF (intraoperative) imaging of cancer is an important and very promising novel approach with respect to cancer treatment. Currently, incomplete removal of tumours, due to inadequate techniques of visualizing these tumours and their boundaries, is a common phenomenon in cancer surgery which contributes to recurrence of the disease [Mieog *et al*, 2010]. NIRF imaging of such tumours has great potential in guiding surgeons in accomplishing complete removal, which would greatly improve clinical outcome in patients. It is therefore a very important technique to investigate into, and many researchers are doing so.
In this literature study, it was outlined what investigations have currently been performed using antibody targeted NIRF (intraoperative) imaging of cancer. Furthermore, other approaches to (intraoperative) NIRF labeling of cancer have also been discussed. These included 'passive' targeting techniques using peptide NIRF dye conjugates, and NIRF nanoparticle based probes. Also, the 'smart activatable probes', which are not necessarily antibody targeted, have been discussed. Specifically, the aim of this literature study was to outline the role of monoclonal antibodies in (intraoperative) NIRF imaging of cancer. The focus of this section will be a comparison of the antibody targeted technique to the other approaches that have been described, in order to determine which approach will have the better prospects of clinical application for (intraoperative) imaging in cancer therapy.
As has been described, promising results have been accomplished using NIRF dye antibody conjugates. Succesful tumour imaging was achieved in animal models for various types of cancer, mostly using cyanine NIRF dyes. Targeted imaging of cancer using NIRF antibodies has the important advantage over non targeted NIRF cancer imaging techniques (such as the peptide dye conjugates) of specifity for the tumour. Furthermore, the fact that many antibodies are already food and drug administration (FDA) approved, will benefit the introduction of the technique into the clinic. However, of course, whether the technique will actually aid in cancer therapy still

remains to be seen. Many further (clinical) studies will be required in order to determine whether the technique can truly assist in cancer treatment.
The nanoparticle based NIRF probes have attractive advantages (such as a high quantum yield, signal to noise ratio and resistance to photobleaching). Antibodies may be conjugated to them, inferring the advantage of tumour specifity upon them. However, the toxicity associated with the nanoparticle based probes impairs their introduction to the clinic, and this is likely to remain so.
The production of 'smart activatable probes' has been an important contribution to the field and seems very promising. These are not necessarily antibody targeted; promising results using peptide based 'smart activatable probes' have also been achieved. Whether antibody targeting will provide advantages in this respect remains to be seen. This is not necessarily so, however, improving the specifity for the tumour target might also be of benefit here.
Finally, when looking at NIRF dyes only (non targeted, simply used as a constrast agent), these might be introduced into the clinic for intraoperative cancer imaging within three to five years. This is then likely to increase the chances of introduction of the NIRF antibody targeted techniques into the clinic, and to speed up this process.

References

Abbas AK , Lichtman AH, Pillai S.. Elsevier, Cellular and Molecular Immunology. Philadelphia. 6th edition, 2007. (1st edition published in 1991)

Dr. G.M. van Dam.
University of Groningen, University Medical Center Groningen, Division of Abdominal and Surgical Oncology, Department of Surgery, BioOptical Imaging Center Groningen, Groningen, The Netherlands

Crane, L.M.A. "Intraoperative fluorescence imaging in cancer" Dissertation University of Groningen, 2010.

PerkinElmer website [online: 14-01-'2011] URL:
http://las.perkinelmer.com/Catalog/ProductInfoPage.htm?ProductID=FMT2500

Caliper LifeSciences [online: 14-01-'2011] URL: http://www.caliperls.com/products/ivis-spectrum.htm

Qin, Ruogu [online: 14-01-'2011] URL:
https://kb.osu.edu/dspace/bitstream/1811/38492/6/Intraoperative_Fluorescence_Surgical_Goggle.pdf

Articles:
Amiot *et al* (2008) "Near-Infrared Fluorescent Materials for Sensing of Biological Targets" Sensors, vol. 8: 3082-3105

Ballou Byron, Fisher Gregory W., Waggoner Alan S., Farkas Daniel L., Reiland Jean M., Jaffe Ronald, Mujumdar Ratnarkar B., Mujumdar Swati R., Hakala Thomas R. (1995) "Tumour labeling in vivo using cyanine-conjugated monoclonal antibodies" Cancer Immunol lmmunother; 41: 257-263

Becker A, Hessenius C, Licha K, Ebert B, Sukowski U, Semmler W, Wiedenmann B, Grotzinger C (2001) "Receptor-targeted optical imaging of tumours with near-infrared fluorescent ligands." Nat Biotechnol; 19:327-331.

Chernomordik Victor, Hassan Moinuddin, Lee Sang Bong, Zielinski Rafal, Gandjbakhche Amir, and Capala Jacek (2010) "Quantitative Analysis of HER2 Receptor Expression *In vivo* by Near-Infrared Optical Imaging" Molecular Imaging; 9, 4: 192-200

Cho Kwangjae, Wang Xu, Nie Shuming, Chen Zhuo (Georgia) and Shin Dong M. (2008) “Therapeutic Nanoparticles for Drug Delivery in Cancer” Clinical Cancer Research; 14, 5: 1310-1316

Folli S, Wagnieres G, Pelegrin A, Calmes J-M, Braichotte D, Buchegger F, Chalandon Y, Hardman N, Heusser CH, Givel J-C *et al.* (1992) “Immunophotodiagnosis of colon carcinomas in patients injected with fluoresceinated chimeric antibodies against carcinoembryonic antigen” PNAS USA; 89:7973-7977.

Folli S, Westermann P, Braichotte D, Pelegrin A, Wagnieres G, van de Bergh H, Mach JP (1994) “Antibody–indocyanin conjugates for immunophotodetection of human squamous cell carcinoma in nude mice” Cancer Res; 54:2643-2649.

He Xiaoxiao, Wang Kemin and Zhen Cheng (2010) “*In vivo* near-infrared fluorescence imaging of cancer with nanoparticle-based probes” WIREs Nanomedicine and Nanobiotechnology; 2: 349–366

Hilger Ingrid, Leistner Yvonne, Berndt Alexander, Fritsche Christine, Haas Karl Michael, Kosmehl Hartwig, Kaiser Werner A.(2004) “Near-infrared fluorescence imaging of HER-2protein over-expression in tumour cells” Eur Radiol; 14: 1124-1129

Lisy Marcus-René, Goermar Annika, Thomas Claudia, Pauli Jutta, Resch-Genger Ute, Kaiser Werner A., Hilger Ingrid (2008) “*In vivo* Near-infrared Fluorescence Imaging of Carcinoembryonic Antigen–expressing Tumour Cells in Mice” Radiology;, 247, 3, 779-787

Lyer Arun K., Khaled Greish, Fang Jun and Maeda Hiroshi (2006) “Exploiting the enhanced permeability and retention effect for tumour targeting” Drug Discovery Today; 11, 17/18: 812-818

Mieog J. Sven D., Vahrmeijer Alexander L., Hutteman Merlijn, van der Vorst Joost R., Drijfhout van Hooff Maurits, Dijkstra Jouke, Kuppen Peter J.K., Keijzer Rob, Kaijzel Eric L., Que Ivo, van de Velde Cornelis J.H., and Löwik Clemens W.G.M. (2010) “Novel Intraoperative Near-Infrared Fluorescence Camera System for Optical Image-Guided Cancer Surgery” Molecular Imaging; 9, 4: 223-231

Neri D (1997) “Targeting by affinity-matured recombinant antibody fragments of an angiogenesis-associated fibronectin isoform.” Nat Biotechnol; 15:1271-1275.

Nguyen, Q.T., Olson, E.S., Aguilera, T.A., Jiang, T., Scadeng, M., Ellies, L.G., Tsien, R.Y. (2010) “Surgery with molecular fluorescence imaging using activatable cell-penetrating peptides decreases residual cancer and improves survival” PNAS; 107, 9: 4317-22.

Ogawa *et al* (2009) “*In vivo* Molecular Imaging of Cancer with a Quenching Near-Infrared Fluorescent Probe Using Conjugates of Monoclonal Antibodies and Indocyanine Green” Cancer Research; 69, 4: 1268-1272

Ogawa *et al* (2009) “*In vivo* target-specific activatable near-infrared optical labeling of humanized monoclonal antibodies” Molecular Cancer Therapy; vol. 8, no. 1: 232-239

Olson Emilia S., Aguilera Todd A., Jiang Tao, Ellies Lesley G., Nguyen Quyen T., Wong Edmund, Gross Larry, and Tsien Roger Y. (2009) “*In vivo* characterization of activatable cell penetrating peptides for targeting protease activity in cancer” Integrative Biology; 1, 5-6:382-393

Pelegrin A, Folli S, Buchegger F, Mach J-P, Wagnieres G, van den Bergh H (1991) “Antibody-fluorescein conjugates for photoimmunodiagnosis of human colon carcinoma in nude mice” Cancer; 67:2529-2537.

Sevick-Muraca Eva M, Houston Jessica P and Gurfinkel Michael (2002) “Fluorescence-enhanced, near infrared diagnostic imaging with contrast agents” Current Opinion in Chemical Biology; 6:642–650

Sharkey, R. M.; Primus, F. J.; Goldenberg, D. M. (1984) “Second antibody clearance of radiolabeled antibody in cancer radioimmunodetection.” PNAS U.S.A.; 81: 2843-2846.

Soukos NS, Hamblin MR, Keel S, Fabian RL, Deutsch TF, Hasan T (2001) “Epidermal growth factor receptor–targeted immunophotodiagnosis and photoimmunotherapy of oral precancer *in vivo*.” Cancer Res; 61:4490-4496.

Staninger, Hildegarde. (2004) “What is Far-Infrared?” Healthy Life USA Magazine, volume 2.

Sun *et al* (2007) “Radioimmunoguided Surgery (RIGS), PET/CT Image-Guided Surgery, and Fluorescence Image-Guided Surgery: Past, Present, and Future” Journal of Surgical Oncology; vol. 96, no 4: 297–308

van Oosten Marleen, Crane Lucia M.A., Bart Joost, van Leeuwen Fijs W, van Dam Gooitzen M. “Selecting potential targetable biomarkers for imaging purposes in colorectal cancer using target selection criteria (TASC): a novel target identification tool” Accepted for publication in Translational Oncology

Weissleder R, Tung C-H, Mahmood U, Bogdanov A (1999) “*In vivo* imaging of tumours with protease-activated near-infrared fluorescent probes” Nat Biotechnol; 17:375-378.

Winau *et al* (2004) “Paul Ehrlich - in search of the magic bullet” Microbes and Infection; vol. 6: 786–789

Zou *et al* (2009) “Near-Infrared Fluorescence Labeled Anti-TAG-72 Monoclonal Antibodies for Tumour Imaging in Colorectal Cancer Xenograft Mice” Molecular Pharmaceutics; vol. 6, no. 2: 428–440