University of Groningen

Fluorescently labelled monoclonal antibodies for real-time molecular imaging

Linssen, Matthijs

DOI:
10.33612/diss.566494449

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2023

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Linssen, M. (2023). *Fluorescently labelled monoclonal antibodies for real-time molecular imaging: pharmaceutical development of near-infrared tracers and their application in clinical settings*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. https://doi.org/10.33612/diss.566494449

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

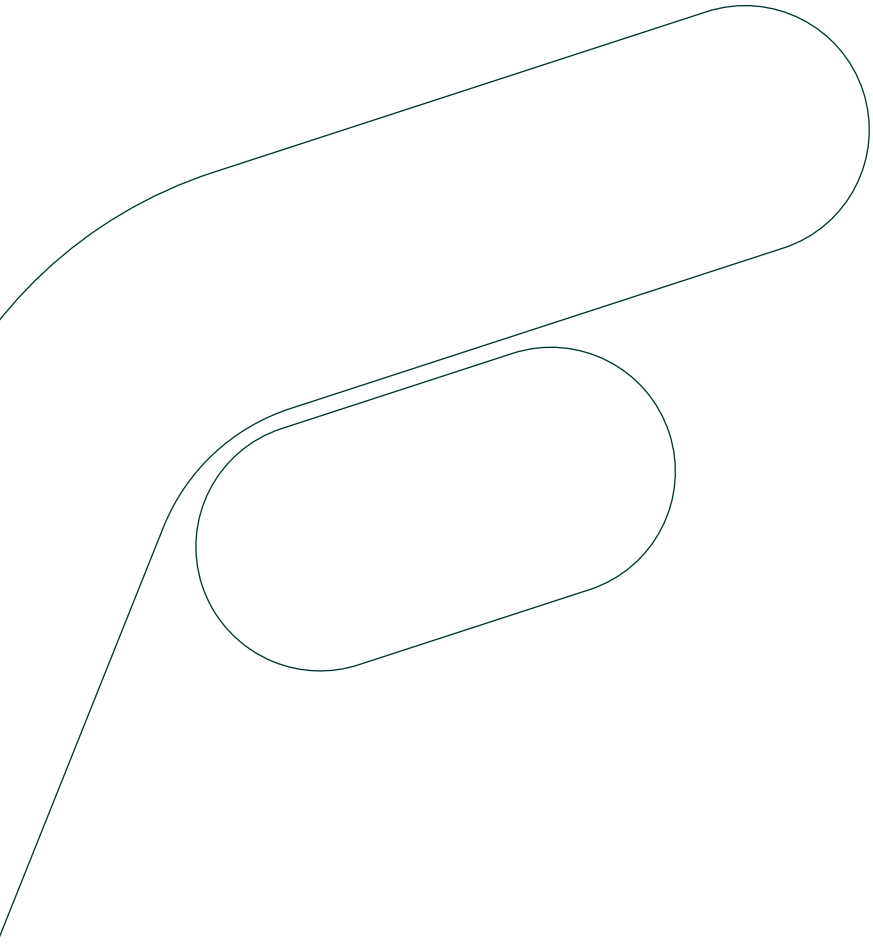
Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 14-11-2024

CHAPTER 1

General introduction & outline of the thesis



Molecular imaging is the *in vivo* assessment and measurement of biological processes at the molecular and cellular level. (1) This distinguishes it from anatomical imaging, commonly performed with X-ray computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound (US). Anatomical imaging is based on morphologically perceivable changes in shape and size within tissue. (2) Molecular imaging instead relies on changes in protein expression or cellular behaviour, thereby allowing it to image disease before anatomical integrity is disrupted. (3) Until recently, molecular imaging has primarily been applied in combination with radionuclide probes. Both Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) are commonly used to image diseases like cancer and rheumatoid arthritis. (4) Radionuclide imaging makes it possible to perform whole-body scans and allows determination of the location and size of multiple lesions in a single image, but lacks spatial resolution. Image acquisition and interpretation is a time-consuming process, and there is a radiation burden for both the patient and any hospital staff involved with the imaging. Radioactive isotopes that are required for this modality have a limited half-life, which can present several logistical problems during manufacturing and administration due to time constraints. When surgical intervention is required, nuclear imaging and anatomical imaging are typically performed days to weeks in advance. The images can be accessed during the intervention, but options for molecular imaging within an operating theatre or endoscopy suite are limited. (5) The availability of an imaging modality in these situations could allow for more precise resections, reductions in miss rates and improved sparing of healthy tissue. Therefore, an alternative option for molecular imaging during invasive procedures is desired.

Optical imaging is a novel form of molecular imaging that may fill this niche. This technique uses fluorescent light for signal detection instead of ionizing radiation. By using light, optical imaging allows for high-resolution and real-time imaging. (6) Aspecific optical imaging, where a dye is injected to serve as a visual contrast or as a fluorescent marker has already been in use for several decades. Dyes like methylene blue (MB) or indocyanine green (ICG) can show tissue perfusion, and have therefore been shown to be useful in many different indications. These can range from ureter visualization, to tumour location, to hepatic or cardiac function. (7,8) More recent developments in optical imaging have seen the transition from aspecific dyes to targeted tracers. Based on the molecular properties of a disease, a tracer can be developed that targets and delivers its fluorescent dye to specific tissues only, similar to molecular tracers used for PET. (9) This enables specific tissue highlighting based on molecular characteristics, and was shown to be feasible for real-time evaluation of cancer nodules during surgery, using a folate tracer conjugated to the fluorophore FITC. (10) Building on this proof of concept, new tracer concepts were introduced in rapid succession. In order to improve the capacity for *in vivo* imaging, research groups have investigated new tracers that used alternatives to MB and ICG for visualization,

and moieties emitting near-infrared fluorescence have become a popular choice. These dyes emit their peak fluorescent signal between 650 nm to 1000 nm, a range in which absorbance, scattering and reflection due to tissue properties is limited. This results in a fluorescent signal that is brighter than dyes with other spectra and can penetrate tissue up to several millimetres (11,12). Optical dyes are easily conjugated to different molecules like antibodies, nanobodies and small peptides, which allows for a versatile application of the technique to image different aspects of disease.

However, while conjugation of optical dyes to a targeting scaffold is relatively simple, probes made in the lab generally are not fit for clinical application. Novel tracer molecules must go through a process referred to as clinical translation, which involves more in-depth development and characterization of the probe characteristics as well as revision of the production process to be in line with the requirements of Good Manufacturing Practice guidelines (GMP) (13). Figure 1 gives an overview of some key activities in the development and application of tracers, and the position of clinical translation in this process. However clinical translation is complex, time-consuming and resource intensive, making performing clinical translation therefore not always feasible for research groups (14). This thesis aims to provide an overview of the different phases of fluorescent optical tracer development, translation and application for use in oncology and gastroenterology. A focal point of the thesis will be the steps required to transfer a laboratory-grade fluorescent probe to an investigational medicinal product fit for use in humans in the context of phase I clinical trials, and the application of multiple antibody-based tracers in clinical trials after they completed clinical translation.

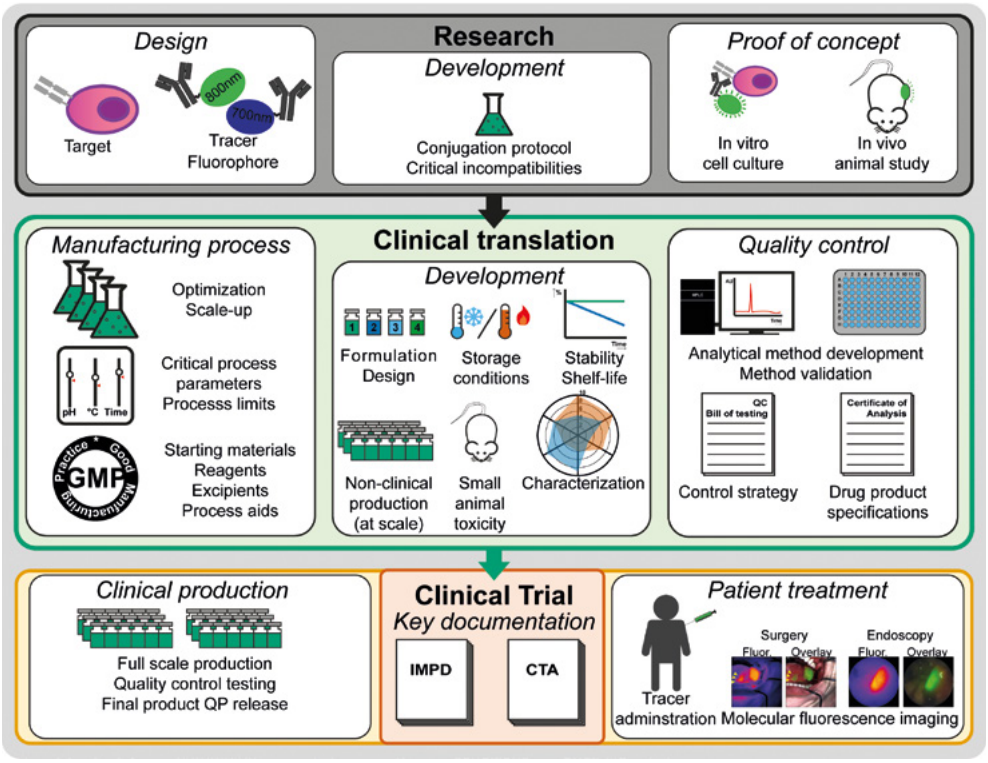


Figure 1: Overview of research, development and production phases for clinical tracer studies. Three main phases of the tracer development process can be identified. During research, the basic design, including antibody and dye compounds used, and conjugation protocol for the tracer is devised, and the concept is tested in a lab setting on for instance cell lines or a small animal model. The product at this point is not fit for human application, and will undergo clinical translation in order to be usable as an investigational medicinal product. During clinical translation, the production process is optimized and scaled up, and adapted where required for compliance with Good Manfacturing Practice guidelines. The product development is also continued, investigating ways to store the product and the associated product characteristics and shelf-life. Product from an optimized, at-scale batch can be used for toxicity studies, and for developing and validating analytical methods and setting parameters for release. The completed process is described in an IMPD, and the trial design is described in a CTA. The IMPD describes the product that is produced according to the development results in a large scale for human trial application, and the CTA describes the use of the product. After the approval of the trial application, fluorescent molecular imaging can be performed in the described patient group. Abbreviations: 800CW: IRDye 800CW; 680LT: IRDye 680LT, CTA: Clinical trial application; Fluor.: Fluorescence channel image; GMP: Good Manufacturing Practice; HPLC: High performance liquid chromatography, IMPD: investigational Medicinal Product Dossier; Overlay: Fluorescence channel overlayed on a full colour channel; QC: Quality control; QP: Qualified person.

OUTLINE OF THE THESIS

Development and translation of tracers goes through several phases, at different scales of production. As a starting point, the conjugation of the antibody to the dye, and the target reactivity and basic effectivity should be shown on small scale, in vitro. In **Chapter 2**, small scale tracer conjugation is performed for cetuximab and trastuzumab, both antibodies that bind targets in the epidermal growth factor receptor family of proteins (ErbB/EGFR and HER2, respectively). The antibodies are conjugated to IRDye 700DX, a photodynamic dye which emits fluorescence for imaging but also has a phototoxic effect that is able to selectively treat malignant cells after binding to them, referred to as near-infrared targeted photodynamic therapy (NIR-tPDT). This chapter describes in-vitro experiments to investigate the primary mode of action for these tracers, consisting of cell binding and killing the bound cells when exposed to light that excites the dye. The tracers are tested on four different cell lines. In addition it is demonstrated that the effectivity of NIR-tPDT can be increased by upregulation of the targeted receptors by tyrosine kinase inhibitor treatment of the cells. Data from laboratory experiments form the basis for the rational design of a probe for use in pre-clinical and clinical studies.

After the small-scale experiments done during the research phase have provided the basic conjugation process, further characterization and technology transfer has to be performed to confirm the safety and suitability of the tracer for clinical use. **Chapter 3** describes the development of bevacizumab-800CW, a tracer targeting vascular endothelial growth factor (VEGF). The initial laboratory scale development of this tracer had resulted in a production process for small batches of tracer which were made for each patient individually and used immediately (analogous to the production of PET tracers). However, the planned application in the clinical setting called for an improvement to the process in order to increase the yield and allow a single batch of product to be used for multiple patients and to be stably stored for longer periods of time. To achieve this, optimization experiments followed by a formulation and stability study were performed. This chapter discusses the initial probe development, the changes made during the additional development, extended characterization, the design and selection of a stable formulation for the product, and animal toxicity testing, to eventually deliver a product that complies with GMP, can be stored for extended periods of time, retains binding capacity to VEGF and is safe to administer during phase I clinical trials.

The methodology applied for the development, translation and clinical application of optical tracers was further expanded upon in **Chapter 4**. This chapter describes a standardized approach for performing experiments on and collecting data about tracer development in order to support the Investigational Medicinal Product Dossier (IMPD) and the clinical trial application (CTA). The approach was consolidated into a roadmap for development, and its application was illustrated with the development results of two new tracers: cetuximab-800CW

and trastuzumab-800CW. Both antibodies were conjugated, the process was optimized and the formulation tested, and a long-term stability study was performed.

Once development and translation are completed, the tracer can be produced at full scale, performed according to Good Manufacturing Practice guidelines. The resulting product is fit for human application and can be tested in clinical trials. **Chapters 5 and 6** describe the application of bevacizumab-800CW in clinical trials both during surgery and during endoscopy. First, **Chapter 5** investigates the use of bevacizumab-800CW in patients with breast cancer who are scheduled to undergo breast-conserving surgery. In this dose-finding study, bevacizumab-800CW is administered in escalating doses (4.5 mg, 10 mg, 25 mg and 50 mg), in order to determine the optimal dose for intra-operative imaging. In addition, this chapter describes a methodology framework for data analysis in imaging trials, in order to support comparability between studies and the formation of consensus within the field for optical imaging trials.

A second clinical trial with bevacizumab-800CW is described in **Chapter 6**. This chapter describes a study in patients with locally advanced rectal carcinoma in whom quantitative fluorescent endoscopy (QFE) was performed to detect residual tumour after neoadjuvant chemoradiotherapy (nCRT). For this purpose, the intravenously administered tracer was imaged during an endoscopy performed on the day of surgical intervention after nCRT treatment, and was used to determine the extent of the response to nCRT. Fibres for fluorescent imaging and for multi-diameter single fiber reflectance single fiber fluorescence (MDSFR/SFF) spectroscopy measurements could be inserted (separately) into the working channel of a standard-of-care high definition white light endoscope to investigate the fluorescence.

The final 3 chapters of this thesis apply the approach to tracer development and translation displayed in the previous sections to two new fields within optical imaging. First, fluorescent optical tracers for multispectral imaging was investigated. Since fluorescent dyes emit light in a narrow and dye-specific bandwidth, it is possible to image multiple wavelengths of fluorescent light simultaneously within the same image. This setup would involve administering a pair of complementary tracers that can be imaged separately. By imaging both wavelengths, it is possible to determine where and to what extent interaction between the two targeted molecules takes place. A field in which this could be useful is the anti-cancer therapy with immune checkpoint inhibition. This treatment is based on the stimulation of the patient's immune system against tumour cells, and several antibodies have been developed targeting receptors with a function in immune checkpoint inhibition, on both the tumour cells and on the immune cells involved. (15) **Chapter 7** displays the development of a clinical tracer pair with complementary dyes, fluorescent around 700nm and 800nm. These antibodies are targeted against programmed death-ligand 1 (PD-L1; durvalumab-680LT)

and the programmed cell death protein 1 (PD-1 nivolumab-800CW) and would allow for simultaneous imaging of checkpoint-inhibitor sensitive tumour cells, as well as the presence of tumour-infiltrating lymphocytes.

Second, we investigated the options for broadening the field of optical imaging research by applying the tracer development method to inflammatory diseases of the gastro-intestinal tract. In gastro-enterology, endoscopy has a key role for diagnosis, disease staging, biopsy and tissue resection. Several monoclonal antibodies have been licenced for the treatment of inflammatory bowel disease (IBD). These agents are used as third-line treatment for patients with moderately or severely active disease. However, approximately 50%-60% of patients that receive biological treatment for IBD either show no response or lose response over time. (16) Optical imaging with antibodies used for treatment of IBD could predict response to these treatments and additionally help to elucidate the *in vivo* biodistribution, and mechanism of action of these agents. In **chapter 8** the antibodies infliximab, adalimumab, vedolizumab and ustekinumab, all licenced for treatment of IBD, are conjugated to 4 different dyes and the performance of each tracer candidate is evaluated, in order to screen their feasibility as a tracer for clinical use. One of the antibodies tested in this panel, vedolizumab-800CW, was then further developed into a clinical product according to the standard approach discussed previously. **Chapter 9** describes a phase I clinical trial that evaluates vedolizumab-based fluorescent molecular endoscopy, and shows the preliminary results of the first in-human application of vedolizumab-800CW. Patients who had an established diagnosis of IBD, but were naïve to vedolizumab were included in a dose-finding trial. Patients received either 4.5 mg vedolizumab-800CW, 15.0 mg vedolizumab-800CW or 15.0 mg vedolizumab-800CW preceded by 75.0 mg unlabelled vedolizumab, and underwent a fluorescent molecular endoscopy procedure. The feasibility of performing *in vivo* optical imaging in the mucosa of IBD patients and on the biopsy material was investigated.

A final overview of the development of the products discussed in this thesis, and their clinical applications, is provided in **Chapter 10**, along with overall considerations and conclusions on the subject matter. A summary in Dutch is provided in **Chapter 11**.

REFERENCES

1. Weissleder R, Mahmood U. Molecular Imaging. Radiology. 2001 May;219(2):316–33.
2. Willmann JK, van Bruggen N, Dinkelborg LM, Gambhir SS. Molecular imaging in drug development. Nat Rev Drug Discov. 2008 Jul;7(7):591–607.
3. Son H, Jang K, Lee H, Kim SE, Kang KW, Lee H. Use of Molecular Imaging in Clinical Drug Development: a Systematic Review. Nucl Med Mol Imaging (2010). 2019;53(3):208–15.
4. Kubota K, Yamashita H, Mimori A. Clinical Value of FDG-PET/CT for the Evaluation of Rheumatic Diseases: Rheumatoid Arthritis, Polymyalgia Rheumatica, and Relapsing Polychondritis. Semin Nucl Med. 2017;47(4):408–24.
5. de Boer E, Harlaar NJ, Taruttis A, Nagengast WB, Rosenthal EL, Ntziachristos V, et al. Optical innovations in surgery. Br J Surg. 2015 Jan 27;102(2):e56–72.
6. Alam IS, Steinberg I, Vermesh O, van den Berg NS, Rosenthal EL, van Dam GM, et al. Emerging Intraoperative Imaging Modalities to Improve Surgical Precision. Mol Imaging Biol. 2018;20(5):705–15.
7. Cwalinski T, Polom W, Marano L, Roviello G, D'Angelo A, Cwalina N, et al. Methylene Blue—Current Knowledge, Fluorescent Properties, and Its Future Use. J Clin Med. 2020;9(11):3538.
8. Reinhart MB, Huntington CR, Blair LJ, Heniford BT, Augenstein VA. Indocyanine Green :Historical Context, Current Applications, and Future Considerations. Surg Innov. 2016;23(2):166–75.
9. Terwisscha van Scheltinga AGT, van Dam GM, Nagengast WB, Ntziachristos V, Hollema H, Herek JL, et al. Intraoperative Near-Infrared Fluorescence Tumor Imaging with Vascular Endothelial Growth Factor and Human Epidermal Growth Factor Receptor 2 Targeting Antibodies. J Nucl Med. 2011 Nov 1;52(11):1778–85.
10. van Dam GM, Themelis G, Crane LMA, Harlaar NJ, Pleijhuis RG, Kelder W, et al. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-α targeting: first in-human results. Nat Med. 2011 Oct 18;17(10):1315–9.
11. Kobayashi H, Ogawa M, Alford R, Choyke PL, Urano Y. New strategies for fluorescent probe design in medical diagnostic imaging. Chem Rev. 2010;110(5):2620–40.
12. Cao J, Zhu B, Zheng K, He S, Meng L, Song J, et al. Recent Progress in NIR-II Contrast Agent for Biological Imaging. Front Bioeng Biotechnol. 2020;7(January):1–21.
13. European Commission. Eudralex Volume 4 - EU Guidelines for Good Manufacturing Practices (GMP) of Medicinal Products for Human and Veterinary Use.
14. Tummers WS, Warram JM, Tipirneni KE, Fengler J, Jacobs P, Shankar L, et al. Regulatory aspects of optical methods and exogenous targets for cancer detection. Cancer Res. 2017 May 1;77(9):2197–206.
15. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med. 2018 Dec 13;50(12):1–11.
16. Barré A, Colombel JF, Ungaro R. Review article: predictors of response to vedolizumab and ustekinumab in inflammatory bowel disease. Aliment Pharmacol Ther. 2018;47(7):896–905.