Summary, conclusions and future perspectives
Summary

Despite advances in medicine and patient care, infections and infectious diseases remain a great threat to human health\(^1\). An infection occurs through the invasion of the host by a microorganism that impairs the normal functioning of the host’s system. Infections are caused by pathogens, such as bacteria, fungi, or viruses.

One important factor that contributes to the prevalence of bacterial infectious diseases is the use of biomaterials, such as osteosynthesis devices and prosthetic joints, which are inherently susceptible to infection. In particular, prosthetic joints are increasingly applied to replace arthritic or dysfunctional joints as a consequence of the increased human life expectancy\(^2\). Moreover, the rapid emergence of microorganisms resistant to antibiotics poses additional challenges to patient care\(^3\). In many infectious diseases, such as fracture-related infections, the suspicion of an infection leads to an initial administration of broad-spectrum antibiotics, while molecular and microbiological diagnostic techniques are performed to identify the causative pathogen\(^4\). This broad-spectrum choice of antibiotics ultimately leads to the selection of microorganisms with increased resistance to antibiotics. In order to curb down the rapid development of multi-drug resistant microorganisms and to ensure a targeted treatment, the development of novel tools to detect the causative organism or organisms of the infection at an early stage is crucial.

The aim of the PhD research described in this thesis was to develop novel tracers for fluorescence imaging of microbial infections, and to evaluate the optical molecular fluorescence imaging technology for the detection of infections at early stages.

Imaging the site of infection has been applied for decades in diagnosis and treatment\(^5\). Molecular imaging, as we know it today, may have started with the use of Gallium-67 citrate (\(^{67}\)Ga). For more than 50 years, molecular imaging has evolved and improved, and from the poor imaging characteristics and the lack of specificity of \(^{67}\)Ga, we have moved to \(^{18}\)Fluorine-fluorodeoxyglucose (\(^{18}\)F-FDG) positron emission tomography with multi-slice helical computed tomography (PET/CT) that is widely used to assess glucose metabolism (i.e. an indicator of disease) in the human lungs, heart and brain\(^5\). Briefly, \(^{18}\)F-FDG (PET/CT) combines PET technology to measure metabolic changes with CT, which provides anatomical information. PET/CT allows an accurate localization of the abnormal uptake of \(^{18}\)F-FDG. However, this abnormal uptake can be related to various causes, such as inflammation, neoplastic processes or vascular diseases. Therefore, \(^{18}\)F-FDG and most of the tracers currently in use, have an important limitation as they reflect the host’s response to an infection, but not the causative microorganism itself. In short, these tracers are non-specific and unable to reliably differentiate between infection and sterile inflammation as a consequence of mechanical trauma, stress or ischemia among others.
Targeted molecular imaging is an emerging technique that has been developed to solve the above-mentioned specificity challenge. This molecular imaging approach enables specific, non-invasive and real-time visualization, characterization and quantification of the causative pathogens. This imaging modality is based on the use of specific tracers, which consist of an imaging agent (e.g. a fluorophore or radionuclide) coupled to a molecule that binds a specific pathogen.

Part I of this thesis focuses on the use of fluorescently labeled antibodies as tracers to specifically detect infections. Chapter 2 describes the production and evaluation in vitro, ex vivo and in vivo of a fully human monoclonal antibody (HumAb) named 7G2 to specifically target infections caused by the bacterium Staphylococcus aureus. S. aureus is considered a leading cause of infections world-wide, being responsible for a broad range of mild to life-threatening diseases. The early detection of an S. aureus infection would allow a rapid and specific treatment, thereby improving the patient’s outcome.

The characterization of HumAb 7G2 was aimed at exploring its potential for development of fluorescent imaging tracers or theragnostic agents that can be applied in antimicrobial photodynamic therapy (aPDT). In vitro immunodetection assays and microscopic imaging results using wild-type and mutant S. aureus isolates showed that 7G2 specifically binds to the staphylococcal Protein A (Spa). Spa is an important virulent factor, which enables S. aureus to evade the human immune system. As previously shown by Kim et al., murine monoclonal antibodies that neutralize Spa can be used to provide protection against S. aureus in murine infection models. In accordance with these observations, the HumAb 7G2 was shown to enhance S. aureus association with neutrophils and complement-mediated bacterial killing. It is thus able to break the protection provided by Spa and to expose S. aureus to the human innate immune defenses. Moreover, the fluorescently labeled 7G2 was shown to maintain its specificity and sensitivity for Spa in vitro, not only in the detection of mono-species S. aureus biofilms, but also of dual-species biofilms, and intracellularly in a HeLa cell S. aureus infection model. In addition, to evaluate the potential of 7G2 coupled with IRDye800CW-NHS ester (7G2-800CW) as a tracer for S. aureus-targeted infections, an in vivo imaging approach was followed. This involved a murine myositis model, where the infection was caused by a genetically engineered bioluminescent strain of S. aureus (Xen29). One day after infection of the murine legs with the bioluminescent S. aureus bacteria, 7G2-800CW was injected intravenously via the tail vein. Fluorescence and bioluminescence macroscopic imaging was performed 24, 48 and 72h post tracer administration. Indeed, the results show a colocalization of the bioluminescence signal of S. aureus Xen29 and the fluorescence signal of 7G2-800CW. Importantly, 7G2-800CW specifically highlighted the site of infection for at least 4 days post tracer injection at which time point the mice were sacrificed.

Due to the specific binding of HumAb 7G2 to Spa, it was important to check whether the
binding of 7G2 is affected by the regular binding of immunoglobulins by Spa. Competition experiments with the previously developed IsaA-specific HumAb 1D9, which also belongs to the IgG1 class and is therefore also bound by Spa\textsuperscript{11}, showed that the specific 7G2 binding to Spa is not outcompeted by 1D9. Most importantly, this experiment demonstrates that it is possible to simultaneously apply different tracers that target different epitopes presented by \textit{S. aureus}. This minimizes the risk that particular \textit{S. aureus} lineages escape detection due to mutation of the targeted epitope.

Lastly, in a proof-of-principle aPDT study, 7G2 conjugated with the light-activatable fluorophore, 700DX (7G2-700DX) was shown to effectively eliminate the vast majority of \textit{S. aureus} bacteria present in all the layers of an \textit{S. aureus} biofilm. This suggest that the HumAb 7G2 has great potential for future clinical applications. For instance, in PJIs, where a fluorescent fiber can be introduced to apply red light \textit{in situ} to activate the IRDye 700DX and thereby disinfect the prosthesis. Altogether, the research presented in this chapter demonstrates that HumAb 7G2 targeting specifically Spa could become an effective weapon in the fight against \textit{S. aureus}.

The studies presented in \textbf{Chapter 3} explored the potential of combining the emerging technique of probe-based confocal laser endomicroscopy (pCLE) with optical fluorescence imaging technologies to visualize infections in the alveolar space. The pCLE system developed by Mauna Kea Technologies (Cellvizio; Mauna Kea Technologies, France) provides a minimally invasive microscopic visualization of the alveolar areas in the distal lung during a bronchoscopy. Briefly, a bronchoscope is inserted down to the smallest reachable area in the lung. To achieve a deeper visualization of the alveolar space, the pCLE mini-probe of 1.4 mm diameter (AlveoFlex, Mauna Kea Technologies, France) is introduced into the working channel of the bronchoscope, allowing inspection of the distal bronchioles. pCLE has previously been used in a variety of clinical specialties, such as, gastroenterology to image the gastrointestinal mucosa at subcellular level, pulmonology and urology. The combination of pCLE with molecular fluorescence imaging has shown promising first-in-human results for the diagnosis of Gram-negative bacterial pneumonia\textsuperscript{14}.

The project described in chapter 3 was specifically aimed at applying the same principles to evaluate the feasibility of pCLE for visualization of \textit{Aspergillus} spp. infections in the alveolar space. In particular, a humanized monoclonal antibody, referred to as hJF5, was evaluated to specifically detect invasive pulmonary aspergillosis (IPA). IPA is an infectious disease primarily caused by the mold \textit{Aspergillus fumigatus}, increasingly observed in patients especially after solid-organ or hematopoietic stem cell transplants. In the latter case, the mortality rate of IPA reaches up to 90\%.\textsuperscript{15} The gold standard for IPA diagnosis is to obtain a biopsy for histopathological identification of invasive fungal hyphae. However, performing a biopsy is not always possible due to the patient’s condition and, unfortunately, it is associated with mortality of up to 20\%.\textsuperscript{16} Therefore,
the development of a bedside tool, which gives immediate feedback to the clinician, without the requirement of a biopsy and time-consuming culture results, has potentially great benefit in optimized specific detection of fungal infection. In this research project, the hJF5 antibody that binds to a mannoprotein antigen secreted during active growth of the pathogen was evaluated as a fluorescence imaging tracer for real-time detection of IPA. Importantly, hJF5 allows the distinction between the inactive spores present in the air and the invasive hyphae present in the infected lung, giving extra information in the differentiation between colonization and infection.

Microscopic (Leica AF6000 fluorescent microscope), and macroscopic (IVIS Lumina II) in vitro analyses demonstrated the specificity of hJF5 for hyphae of Aspergillus spp. Moreover, target specificity of fluorescent hJF5 was verified in vivo using a Galleria mellonella-based Aspergillus infection model. Due to the comparable innate immune cells of Galleria mellonella and vertebrates, the Galleria mellonella larvae represent a suitable alternative in vivo model to perform infection experiments. The results demonstrate the binding of hJF5 to A. fumigatus in vivo based on the fluorescence emission by A. fumigatus-infected Galleria mellonella larvae treated with conjugated hJF5 compared to the relevant controls over a 48 h time period. In addition, the applicability of hJF5 was assessed in an animal lung infection model and resected human lung tissue. The recorded images and videos from these ex vivo lung models showed clear A. fumigatus fluorescence signals when the fluorescently labeled-hJF5 tracer was applied. Fluorescently labeled-hJF5 has thus yielded very promising results in vitro, ex vivo and in vivo as a tracer for the in situ detection of A. fumigatus in the alveolar space. On this basis, it can be concluded that a clinician performing the proposed pCLE would be able to discriminate Aspergillus-infected areas from non-infected areas.

Part II of this thesis focuses on the use of fluorescently labeled antibiotics as tracers to specifically detect infections. Chapter 4 presents a general strategy for the conjugation of near infrared (NIR) dyes (IRDye800CW-NHS ester and IRDye700DX) to small molecules (vancomycin and amphotericin B) for the provision of novel conjugates to detect and treat bacterial and fungal infections. The glycopeptide antibiotic vancomycin binds specifically to the D-alanine moiety present in bacteria cell walls and, therefore, targets Gram-positive bacteria which are the most frequently encountered pathogens responsible for soft tissue and biomaterial-associated infections. Amphotericin B is an antifungal that binds ergosterol, a component of fungal cell membranes, and this drug could be used to detect fungal infections. These two small molecules were conjugated with the NIR dyes, because the NIR wavelengths are preferable for clinical use. The latter relates to the tissue autofluorescence and limited light penetration in the visible spectrum. To achieve the conjugation of small molecules with IRDye800CW, effective use was made of the IRdye800CW-NHS ester (LI-COR Inc., USA), which has been approved for clinical implementation and has been broadly applied in recent years.
The successful conjugation and evaluation of vancomycin-IRDye800CW (vanco-800CW) was previously described\textsuperscript{22} and, accordingly, vanco-800CW was used as a reference compound. Unfortunately, following the published procedure for the conjugation of vanco-800CW, it was initially not possible to obtain this compound due to hydrolysis. This problem was solved by screening different coupling reagents that would facilitate the formation of the desired amide bond from the acid liberated upon hydrolysis. Fortunately, the application of 1- [Bis (dimethylamino) methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium3-oxide hexafluorophosphate (HATU) or 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) resulted in efficient formation of the desired vanco-800CW product, probably due to their solvent-drying effects. Lastly, purification of the synthesized vanco-800CW was achieved by (semi-)preparative reversed-phase HPLC, and the applicability as a fluorescent tracer to detect Gram-positive bacteria was verified microscopically (Leica AF6000, Germany).

Based on the successful production of vanco-800CW, the devised conjugation protocol was applied to conjugate other small molecules with the same NIR dye (IRDye800CW-NHS ester) or to another NIR dye (IRDye700DX NHS ester, LI-COR Inc., USA). This allowed the successful synthesis of amphotericin B-800CW, a double conjugated vanco-FL-800, and vanco-700DX. Importantly, conjugation of small molecule targeting agents with the photosensitizer IRDye700DX broadens the use of the newly created tracers from infection diagnosis to potential therapeutic interventions through antimicrobial photodynamic therapy (aPDT)\textsuperscript{23-25}.

The research in Chapters 5 and 6 evaluated the feasibility for clinical implementation of targeted-fluorescence imaging in orthopaedic- and trauma surgery using fluorescently labeled-vancomycin. Chapter 5 presents a proof-of-principle study in which the potential of fluorescence-guided arthroscopy with vanco-800CW to accurately detect bacterial biofilms on prosthetic joints is evaluated. Nowadays, the replacement of the total joints to enhance the patients’ quality of life is commonly performed. However, approximately 2% of the patients experience device failure due to the formation of bacterial biofilms on the prosthesis\textsuperscript{2,26}. Such prosthetic joint infections (PJI) are severe complications that are challenging to diagnose and treat. This is due to the matrix produced by the bacteria growing in a biofilm, which acts as a barrier against the human immune system and antibiotic treatment. Currently, to diagnose PJI, blood tests, inspection of synovial fluid, culturing, biopsies and non-specific imaging tests are performed. However, these diagnostic modalities are time-consuming and cannot accurately diagnose PJI, leading to an unclear diagnosis\textsuperscript{27-29}. As a first approach to evaluate the use of vanco-800CW for improved diagnosis of PJI, a representative panel of clinical bacterial isolates was evaluated \textit{in vitro} in biofilms grown on cobalt chrome discs. This demonstrated the potential of vanco-800CW to detect biofilms caused by clinically relevant PJI-causing bacteria. Subsequently, a human post-mortem prosthetic
knee infection model was used to assess the clinical feasibility of detecting Gram-positive bacterial biofilms by combined arthroscopy and fluorescence imaging. Briefly, *S. epidermidis* was grown on one side of a knee prosthesis for 4 days. Afterwards, the prosthesis containing a mature *S. epidermidis* biofilm was implanted on the distal femur. To perform the imaging, an arthroscope (Arthrex, Naples, FL, USA) connected to a saline irrigation pressure pump was placed inside the knee joint cavity. Fluorescence imaging was performed using a fluorescence fiber (Schoelly Fiberoptic GmbH, Germany) attached to a white-light and NIR light camera (SurgVision Explorer Custom, Netherlands). The resulting post-mortem observations showed a clear fluorescence signal coming from the side of the prosthesis containing the biofilm, whereas no fluorescence was observed on the non-biofilm coated side of the prosthesis. Altogether, the *in vitro* and *ex vivo* results show that the combined arthroscopic detection and real-time imaging of Gram-positive bacterial biofilms on a human knee prosthesis is feasible with vanco-800CW.

Chapter 6 describes an evaluation of the use of vanco-800CW for enhanced detection of fracture related infection (FRI) of osteosynthesis devices as a step-up towards real-time image-guided trauma surgery. FRI is a severe complication after bone fracture treatment, resulting in severe patient morbidity and loss of quality of life. Treatment of FRI usually consists of revision surgery, including debridement and removal of osteosynthesis devices (e.g. plates, nails and screws), combined with long-term antibiotic therapy\(^{30,31}\). During this revision surgery, surgeons rely on tactile and visual information to establish whether and to what extent wound areas are affected by the suspected infection. However, there is no adequate tool during this revision surgery to help the surgeon to accurately distinguish infected devices from sterile devices, which can lead to the unnecessary removal of the osteosynthesis device and a prolonged antibiotic treatment. In this project, the applicability of vanco-800CW to detect Gram-positive bacterial biofilms in osteosynthesis devices extracted during revision surgery was assessed. In particular, osteosynthesis devices extracted from 12 trauma patients were included in the study. Upon extraction and incubation with vanco-800CW, the incubated devices were washed twice with PBS to remove unbound vanco-800CW and imaged in the near-infrared range with an IVIS Lumina II imaging system (PerkinElmer Inc., USA) and an intra-operative Explorer Air camera coupled to a closed-field imaging box (Vault; SurgVision B.V. Groningen, NL). This *ex vivo* imaging study represents the first clinical example of successful bacteria-targeted fluorescence imaging with vanco-800CW in a clinical setting in patients with FRI. Moreover, the sensitivity and specificity of vanco-800CW was supported by the case of a patient with fracture-related low-grade infection showing no clinical symptoms. Even for the respective low-grade infection, a clear fluorescence signal was observed after incubation of the extracted osteosynthesis device with vanco-800CW. This clinical investigation therefore shows that detection of Gram-positive bacterial biofilms on osteosynthesis devices with vanco-800CW is highly
specific, sensitive, fast and feasible in the clinical setting.

Chapter 7 goes beyond the use of molecular imaging to detect and diagnose infections in real time, to explore the potential use of aPDT to treat infections. This concept was briefly introduced in chapter 2 and is the focus of the present chapter. In the era of multi-drug resistant bacteria, the study presented in this chapter was motivated to fight against these emerging better-adapted bacteria by developing bacteria-targeted aPDT agents. aPDT is based on the production of ROS by a photosensitizer after its photoactivation with light of the appropriate wavelength. These ROS can kill pathogens by causing oxidative damage to different cellular components and, importantly, no microbial resistance has been reported yet\textsuperscript{32-34}. In this study the potential of the previously synthesized photosensitizer vanco-700DX (chapter 4) was evaluated to help in the fight against PJIs and FRIs. In particular aPDT with vanco-700DX was tested in planktonic cells, co-cultures and in biofilms. The present study showed that targeted aPDT with vanco-700DX is very effective in destroying the outer layers of Gram-positive bacterial biofilms leading to the formation of “cracks”. Moreover, it was demonstrated that antibiotic treatment applied after aPDT could improve the eradication of the biofilm by allowing the antibiotic to reach deeper layers in the biofilm community. These findings were also supported by a cobalt-chrome disc biofilm model, in which vanco-700DX aPDT substantially destroyed the MRSA biofilm, demonstrating the potential of this small targeting photo-conjugate to treat Gram-positive biofilms grown on the surface of prosthetic devices.

Part III of this thesis focuses on the use of smart-activatable tracers for the fast and accurate detection of infections. Smart-activatable tracers, in contrast to the above-mentioned tracers, have the unique feature of being switched on only under specific conditions. For instance, a smart-activatable fluorescence tracer, in the normal state, upon excitation with the appropriate wavelength, will not emit any detectable fluorescence signal since its emission is quenched. Such activatable tracers release their signal only upon encountering an appropriate target molecule. This characteristic offers important advantages over the non-activatable tracers in terms of target-to-background signal ratio, since the tracer is “silent” if it is not interacting with the targeted molecule (e.g. a specific pathogen or a feature produced by the pathogen). Chapter 8, explores the advantages of a smart-activatable fluorescent tracer, named P2&3TT, for early detection of \textit{S. aureus} bacteremia (SAB) in blood cultures. Despite the availability of antibiotics, the mortality rates of SAB have remained high with around 2 to 10 deaths per 100,000 population\textsuperscript{35,36}. Currently, the diagnosis of bacteremia still relies on culture-based methods that take days and the initial treatment of patients with suspected bacteremia is empiric with broad-spectrum antibiotics, which leads to poor outcomes. In contrast, the study presented in chapter 8 shows that the nuclease-activatable P2&3TT tracer allows the diagnosis of SAB in less than 2 hours. Activation of P2&3TT
relies on the enzymatic activity of the micrococcal nuclease (MN), which is specifically secreted by all clinical *S. aureus* isolates. Consequently, P2&3TT will only emit a fluorescent signal upon cleavage by MN and can be used to detect infections caused by *S. aureus*. Importantly, all *S. aureus*-positive blood cultures included in the study yielded clear fluorescent signals upon incubation with P2&3TT. Based on its time-to-result, it can be concluded that the P2&3TT tracer-based detection of *S. aureus* provides the means to expedite identification of *S. aureus* bacteremia by at least several hours versus the current methods in common use. Of note, the P2&3TT assay is specific for *S. aureus* and, therefore, it will not replace current culturing methods which detect pathogens regardless of their species. Instead, this diagnostic assay may be used as a complementary tool to the current methods (e.g. mass spectrometry- and PCR-based identification of culture isolates), providing a more rapid identification of the most impactful bacterial bloodstream pathogen, *S. aureus*.

**Conclusions and Future perspectives**

Altogether, the present thesis showcases the great promise for clinical application of tracers based on pathogen-specific monoclonal antibodies (chapters 2-3) and antibiotics (chapters 5-6) as targeting molecules and NIR fluorescent dyes as signaling molecules for the localization of different bacterial or fungal infections. The translation of such tracers for optical molecular fluorescence infection imaging towards day-to-day clinical practice would greatly improve clinical decision-making and the successful management of infections. However, there are still a few hurdles that need to be taken before the actual clinical implementation can be realized.

First of all, any tracer to be tested for molecular imaging of infection in patients will require synthesis according to good manufacturing practice (GMP) and toxicity testing, and its formulation will have to comply with the current standards and regulations prior clinical implementation. This still needs to be done for all tracers evaluated in the present PhD research. However, in this respect it is noteworthy that all targeting molecules, be it antibodies or antibiotics, can be considered safe for use in humans, and the same applies to the IRDye-800CW fluorophore. Also, as implied by the name ‘tracer’, the tracers to track down infections will be applied in very small doses compared to the doses of antibodies or antibiotics that are administered in therapeutic interventions, thereby limiting the risk of possible side-effects.

Furthermore, a better appreciation of the microorganism’s biology in the context of the human body is needed. For instance, each pathogen displays different condition-dependent metabolic features, and resistances to antibiotics. Perhaps even more importantly, the numbers of accumulating microorganisms at sites of infection will differ from case to case. Therefore, in order to select appropriate targeting molecules for
infection imaging, in vitro experiments must include MDR microorganisms, clinical isolates, and microorganisms in different metabolic states. Based on the specific biology of the particular type of infection one wants to image, it can be decided which targeting molecule will be most appropriate (i.e. antibiotics, antibodies or metabolic tracers). Moreover, for successful imaging of an infection, appropriate tissue contrast needs to be achieved, which will vary depending on the infected tissue and the volume of microorganisms at the infection site. In the particular case of fluorescence imaging, one of the most important limitations is the light penetration of tissue. Clearly, it is critical to understand the inherent limitations of the different molecular imaging technologies and to select and apply them appropriately for the detection of particular infections or infectious diseases. Thus, before clinical trials to test the feasibility of molecular imaging approaches can be initiated, it is essential to define the appropriate patient groups.

Another aspect that needs to be considered concerns the fact that, depending on the infection, available clinical samples, such as blood and urine, may yield insufficient information while, in other situations, obtaining adequate samples or biopsies is impractical, technically complicated or even risky for the patient. Different infections and infectious diseases will, therefore, require the implementation of different tracers and molecular imaging modalities. For instance, molecular fluorescence imaging could help to address clinical questions concerning infections of the skin or subcutaneous infections, but it may also be applicable for orthopedic and trauma surgery as exemplified in chapter 6 of this thesis. The distinctive visualization of infected areas will allow a surgeon to decide whether there is a need for osteosynthesis device removal, or whether in situ ‘cleaning’ by debridement and disinfection may suffice. As illustrated in chapters 3 and 5, fluorescence molecular imaging may be also applied in those cases in which a fiber can be inserted into the body, such as a bronchoscope or an arthroscope. In such cases, the light excitation and subsequent emission are, respectively, applied and imaged in situ to overcome the limits of light penetration imposed by human tissue. On the other hand, if an infection is seated deep in the body, or the infectious disease affects organs, PET/CT will provide functional and anatomical information that could not be obtained by fluorescence imaging. PET is considered the most sensitive imaging technique, giving more detailed information than SPECT, photoacoustic imaging, MRI and ultrasound. In PET, the imaging is based on the decay of a radionuclide and, therefore, it is possible to image infections deep in the body. However, due to the decay of the radionuclide, the time between tracer injection and imaging is inherently limited. Other important barriers for the use of PET are imposed by the perceived risk of radiation due to the use of a radionuclides, as well as the high costs incurred for purchasing the appropriate equipment and the rapid incorporation of the short-lived radionuclides into the actual tracers.
In conclusion, the beauty of molecular imaging is mirrored by the fact that different imaging technologies and approaches can be applied depending on the particular question that needs to be answered for treating patients suffering from infection. Importantly, these technologies are not mutually exclusive, but they can be performed simultaneously in multimodal molecular imaging approaches. For instance, tracers can be synthesized that encompass one targeting molecule and two signaling molecules (i.e. a radionuclide and a fluorophore). This was showcased by Zoller et al., who demonstrated the applicability of an antibody-based tracer with two conjugated signaling molecules, namely $^{89}\text{Zr}$-zirconium and the fluorescent NIR-680 dye. With this particular tracer, it is possible to first perform PET imaging to diagnose an infection and, afterwards, to precisely remove the infected tissue or implant through fluorescence-guided surgery. In addition, it is even conceivable that NIR light-activatable fluorophores are used for this purpose, which generate ROS upon illumination thereby killing the microorganisms that are responsible for the infection, as exemplified in chapter 7. Multi-purpose tracers of this type will open up a new window of so-called ‘theragnostic’ applications, where the diagnosis of infection and the subsequent treatment are performed with one and the same molecule. Such approaches will accelerate the time-to-result between diagnosis and treatment in an unprecedented manner, which is crucial for successful infection management.
Summary, conclusions and future perspectives

Figure 1. Example of a molecular imaging decision workflow. Molecular imaging can be useful in daily clinical decision-making, for example helping to diagnose infections, to identify the specific pathogen and to follow the response to treatment. (The image includes elements from BioRender.com).
References


