Chapter 1

INTRODUCTION
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Cancer is the disease with the second highest mortality worldwide, causing nearly 1 in 6 deaths globally (1). In an early stage curative treatment is often possible, however many patients present with advanced stage disease limiting treatment options to palliative therapy. To improve cancer specific survival, efforts are made on detecting cancer at an earlier stage and developing new treatment options. Over the recent years many new drugs have been developed and approved for clinical use improving cancer survival for a large number of tumor types (2). In addition, many new promising compounds are currently under development and hopefully further decrease the global cancer burden and improve survival.

The development of novel drugs is characterized by a long process of trial and error and demands a large financial investment. Most of the costs linked to clinical trials required for market authorization are made in the phase 2 and 3 trials (3). Eliminating ineffective drugs in an early phase would therefore markedly reduce development costs. As uncontrolled proliferation of tumor cells is a central hallmark of any form of cancer (4), this would be an ideal candidate to assess of drug efficacy in an early phase. Therefore, several pharmaceutical companies requested for the development of a non-invasive tool to assess the antiproliferative effects of novel drugs in an early phase. Moreover, with the availability of novel (targeted) treatment options, antitumor therapy will become more and more personalized. Yet, to aid clinical decision-making and enable personalized therapy prognostic and predictive tools for response are required. Quantitative imaging biomarkers have potential to fulfill both needs.

IMAGING BIOMARKERS

Biomarkers are defined as objectively measured parameters that can be used as an indicator of a normal biological process, pathogenic processes, or pharmacologic response to a therapeutic intervention (5). Within the field of oncology, biomarkers are widely incorporated and aid clinical decision-making as well as the development and evaluation of novel drugs. Biomarkers can be divided into biospecimen-derived and imaging biomarkers. Quantitative imaging biomarkers have the advantage of being able to be measured in a non-invasive manner and provide a more complete picture of the disease process. They can be used to monitor the response to therapy, guide treatment decisions, and predict outcome. In recent years, imaging biomarkers have become an increasingly important tool in oncology research and clinical practice.
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biomarkers (e.g. tissue, blood) and non-biospecimen-derived biomarkers (6). Imaging biomarkers (e.g. positron emission tomography [PET], computed tomography [CT] or magnetic resonance imaging [MRI]) fall within the non-biospecimen-derived group and can serve as diagnostic, predictive or prognostic biomarkers within the oncological setting. Especially in the case of drug development and response evaluation, imaging biomarkers may be of added value to biospecimen-derived biomarkers. Firstly, it enables repeated measurements of individual tumor lesions, also in case of extensive disease. Secondly, inter- and intralesional heterogeneity can be assessed and lastly it is minimally-invasive, especially when compared to invasive methods such as biopsies. On the contrary, biospecimen-derived biomarkers have the advantage of micro resolution compared to imaging biomarkers.

To date, mainly conventional imaging techniques, such as CT or MRI, are used in daily clinical practice to evaluate tumor response by assessing morphological changes after initiation of treatment (7). It may however take up to several weeks before any changes are seen in morphological features (e.g. tumor size) (8, 9). If the tumor does not respond to the initiated therapy, this delay can significantly postpone the start of effective therapy in non-responders. Therefore a pressing need exists for biomarkers predicting early response to treatment. The validation of such biomarkers could markedly reduce unnecessary toxicity and costs by minimizing exposure to non-effective therapies. Moreover, validated imaging biomarkers can also be used for drug development and to assess efficacy of new drugs at an early stage. This could lead to a faster, more efficient new drug development process.

Treatment-induced morphological changes are often preceded by changes in tumor metabolism, such as glucose consumption, proliferation rate or cell death (8). PET/CT imaging provides a way to visualize metabolic processes and drug targeting, and enables in vivo evaluation of several hallmarks of cancer through a large variety of PET radiotracers. This technique harbors a range of promising imaging biomarkers for early response assessment in the oncological setting. However, before any of these radiotracers can be used in clinical practice or for drug development, several technical, biological and clinical validation “hurdles” have to be taken (Figure 1.1).
Introduction

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Figure 1.1: An overview of the “hurdles” that have to be taken as part of the technical, and biological and clinical validation of quantitative imaging biomarkers.

RESPONSE EVALUATION USING PET/CT

PET/CT imaging enables evaluation of radiolabeled compounds (radiotracers). All PET tracers are labeled using a positron-emitting isotope. The emitted positron will annihilate with an electron in the tissue, producing two 511 keV photons which are moving in the opposite direction and can be detected by the PET camera (10). The most
commonly used PET tracer is $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG), although many other tracers are also available (11).

After administration, tracer pharmacokinetic characteristics will determine its biodistribution. PET/CT provides a combination of functional and anatomical imaging of tracer uptake and localization throughout the body. Evaluation of PET/CT images can be performed visually or (semi)quantitatively. Visual assessment is the most frequently used method to evaluate the amount of tracer uptake in daily clinical practice, yet is susceptible to interobserver variability (12). Quantification of the PET signal increases objectivity and limits observer variability, which is essential in case of response evaluation. However, before quantitative PET imaging can be used as imaging biomarker for response evaluation technical validation has to be performed. This requires assessment of feasibility, quantitative accuracy and precision (repeatability and reproducibility) of the biomarker (6).

The reference standard for quantification of tracer uptake is pharmacokinetic compartmental modelling of the dynamic PET signal (13). Based on tracer kinetics this method estimates several kinetic rate constants of tracer uptake, binding and clearance in the region of interest. Although pharmacokinetic compartmental modelling is quantitatively accurate, it is a complex method requiring arterial blood sampling, long scan durations and has limited anatomical coverage (14), which all limit feasibility in daily clinical practice. Particularly in case of metastatic disease this small field of view impairs its usability. Therefore, simplified methods enabling whole body PET/CT scans are preferred.

The use of simplified methods increases feasibility of PET/CT as an imaging biomarker as these methods are also applicable in combination whole body imaging procedures and can be performed in all PET facilities. There are several simplified uptake metrics, although standardized uptake values (SUV) are by far the most used semi-quantitative assessment of tracer uptake (11). SUV is calculated as the activity concentration in a region of interest normalized to total injected dose and patient weight. This method enables whole body PET/CT scanning and does not require any blood sampling, which makes it suitable for incorporation in clinical practice. Nonetheless, simplified methods do not take tracer kinetics into account and therefore
have to be validated against pharmacokinetic compartmental modelling to assess their quantitative accuracy for all new tracers (15).

When the optimal quantitative PET/CT uptake metrics have been established, the repeatability and reproducibility of these measurements has to be determined. Here, repeatability is defined as repeated measurements done in one patient using the same measurement procedure, same operators, same measuring system, same operating conditions and same physical location over a short period of time. Reproducibility, on the other hand, indicates replicate measurements on the same or similar subjects under a set of conditions that include different locations, operators and measuring systems (16). Knowledge about these two components of precision is used as a basis for the biological and clinical validation studies. These studies correlate quantitative PET/CT uptake measures to tumor biology and clinical outcome measures such as treatment response, progression free and overall survival.

**LUNG CANCER**

Lung cancer is the most common cancer in the world and the number one cause of death with 1.6 million deaths annually, responsible for nearly one in five cancer related deaths worldwide (17). There are two main histological types of lung cancer: non-small cell lung cancer (NSCLC) and small cell lung cancer (18). The majority of the lung cancer cases is classified as NSCLC and can be treated with curative surgery or radiotherapy if diagnosed in an early stage. Yet, patients are often diagnosed at an advanced stage, thereby limiting treatment options to systemic therapy. Treatment can still be very effective, although most regimens fail to do so in a substantial number of patients. Early response evaluation could enable the treating physician to stop the treatment in the non-responder cohort timely and potentially limit side effects and avoid treatment delay of subsequent lines of anticancer therapy.

In general first line systemic treatment in NSCLC contains a platinum-based doublet chemotherapy (19). Furthermore, several targeted therapies for the treatment of advanced stage NSCLC have been developed and introduced as well as immunotherapy more recently (20-25). Currently, evaluation of tumor response to
these drugs is still mainly performed using RECIST, which does not allow for early response assessment due to slow changes in morphological features or even pseudoproggression (9, 26). Despite the ambiguous morphological changes, these systemic treatments are likely to affect glucose consumption and proliferation rate in an early phase (27). Therefore $^{18}$F-FDG and $^{18}$F-fluorothymidine ($^{18}$F-FLT) PET/CT are potentially valuable imaging biomarkers for early response evaluation in NSCLC (Figure 1.2).

$^{18}$F-fluorodeoxyglucose

As a glucose analogue $^{18}$F-FDG PET/CT enables in vivo visualization of glycolysis throughout the body. $^{18}$F-FDG is transported into the cell and phosphorylated similar to normal glucose, but is unable to undergo further metabolization due to the labeled $^{18}$F and is therefore retained within the cell. Combined with the increased aerobic glycolysis in cancer cells, this causes accumulation of $^{18}$F-FDG. $^{18}$F-FDG PET/CT is therefore widely used as a diagnostic tool in oncology, but its role as response biomarker to antitumor therapy is less well established.

In previous studies SUV has been validated as a quantitatively accurate measure of $^{18}$F-FDG uptake in tumor lesions (28). The PET Response Criteria in Solid Tumors 1.0 (PERCIST) proposed the use of SUV metrics for quantitative response assessment in oncology and suggested a 30% threshold to discriminate between intrinsic variability of $^{18}$F-FDG uptake metrics and true response or progression (9). PERCIST served as a starting point for structured use of $^{18}$F-FDG PET/CT in response evaluation setting. Consequently several issues should be further optimized and validated for use in clinical practice, including a comprehensive study to evaluate the precision of various uptake metrics and the influence of uptake interval, plasma glucose levels and lesion selection strategies.
Introduction

21

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Figure 1.2: An $^{18}$F-FDG PET/CT scan (A) and $^{18}$F-FLT PET/CT scan (C) of a NSCLC patient to illustrate differences in biodistribution of both tracers.

$^{18}$F-fluorothymidine

Increased proliferation is a central hallmark of tumor genesis and observed in the majority of the malignancies. Thymidine is one of the compounds necessary for DNA replication; a crucial step in the process of cell proliferation (29). Therefore, tissues with high proliferation rates have increased thymidine demands. $^{18}$F labeled thymidine may thus serve as a noninvasive method for measurement of tumor proliferation. After injection $^{18}$F-FLT follows the endogenous thymidine pathway, but in contrast to endogenous thymidine it is not incorporated into the deoxyribonucleic acid but retained in the cytoplasm (DNA) (30). Measuring proliferation using $^{18}$F-FLT is less sensitive, yet more specific for malignancies compared to measured glycolysis as increased glucose metabolism might also be induced by other causes. Especially (therapy-induced) inflammation may hamper the interpretation of $^{18}$F-FDG uptake when assessing metabolic response. Therefore $^{18}$F-FLT PET/CT could be a potential promising imaging biomarker for response assessment (27). $^{18}$F-FLT uptake has been validated against the immunohistochemistry proliferation marker Ki67 for several tumor types (31-33). In addition, the use of SUV and tissue-to-blood ratios has been validated, enhancing the feasibility of $^{18}$F-FLT PET/CT (34). As a next step, technical validation studies need to provide solid information on precision and provide thresholds for assessment of tumor response to therapy and antiproliferative effects of
investigational drugs using $^{18}$F-FLT uptake metrics in clinical validation and drug development studies, respectively.

**PROSTATE CANCER**

Prostate cancer is the most frequent diagnosed type of cancer in developed world and the third most common cause of mortality in the US, with an estimated mortality of 27,000 in 2016 ($17, 35$). The androgen receptor (AR) plays a central role in the early as well as the later stages of prostate cancer ($36$). If patients are progressive on first-line androgen deprivation therapy a castration resistant state is reached. Metastasized castration resistant prostate cancer (mCRPC) is the lethal form of the disease, responsible for substantial suffering, due to symptoms caused by prostate cancer metastasis. Despite castrate levels of serum testosterone ($\leq 1.7$ nmol/L), persistent AR axis signaling continues to drive prostate cancer progression in mCRPC ($36$). Based on this mechanism several AR-directed therapies, such as abiraterone and enzalutamide, have been developed and introduced in clinical practice ($37-40$). These drugs are administered orally, well-tolerated and highly effective as first line therapy in the majority of mCPRC patients. Unfortunately, the median time to progression is less than two years after which treatment with a second AR-directed therapy is only effective in a very limited group ($41$).

Enzalutamide and abiraterone have become part of the standard of care in mCRPC patients. Yet, there is still a pressing need for biomarkers that are able to biologically characterize tumors, monitor pharmacologic targeting and predict the outcome and response to these drugs. Current candidate biomarkers for response to AR-targeted therapy mainly include serum biomarkers such as circulating tumor cells to assess AR splice variance and tissue specimens for genomic profiling ($42-45$). A significant limitation of these biomarkers is that only a small part of the disease is evaluated as this may not represent the biology of the total cancer burden. Molecular imaging enables evaluation of the entire disease burden using $^{18}$F-fluorodihydrotestosterone ($^{18}$F-FDHT) and could therefore potentially serve as a prognostic or even predictive imaging biomarker for response to AR-directed therapy in mCRPC.
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**PROSTATE CANCER**

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**18F-fluorodihydrotestosterone**

18F-FDHT is an 18F labelled analogue of dihydrotestosterone, the primary ligand

... of the AR. This tracer therefore potentially offers an innovative approach to directly

... imaging the driving force of mCRPC. Previous studies in mCRPC patients have
demonstrated the feasibility of 18F-FDHT PET/CT (Figure 1.3) (46-48). 18F-FDHT binds
irreversibly to the AR and has a competitive binding with endogenous androgens.

Therefore, all patients need to have castrate levels of serum testosterone prior to

... injection and need to remain on androgen deprivation therapy, if not surgically

castrated. After administration 18F-FDHT undergoes rapid metabolization by the liver

(> 90% in 30 min) and is excreted via the bile and urine. Circulating metabolites cause

... high blood pool activity, however do not bind to the AR (48).

In contrast to many other imaging modalities used for evaluation of prostate

... cancer, 18F-FDHT PET/CT primarily focuses at phenotyping disease rather than
detecting malignant lesions per se. Consequently, it is possible to have 18F-FDHT
positive as well as negative lesions and thus other imaging modalities are essential for

... adequate interpretation of the scan. This is also shown by Vargas et al. (47) who

... assessed the feasibility of 18F-FDHT PET/CT at identifying tumor localizations and

... extent of 18F-FDHT avid metastatic lesions by comparing them to CT and 18F-FDG

... PET/CT. Most studies on 18F-FDHT in mCPRC to date only qualitatively evaluated 18F-

![Figure 1.3: A bone scans (A), 18F-FDHT PET/CT scan (B) and 18F-FDG PET/CT scan (C) of a mCRPC patient.](image-url)
FDHT uptake. Before quantitative uptake metrics can reliably be used to assess AR, a pharmacokinetic modeling study has to be performed, simplified methods have to be validated and precision has to be determined.

In order to investigate the above-presented questions and knowledge gaps considering the technical validation of $^{18}$F-FDG, $^{18}$F-FLT and $^{18}$F-FDHT PET/CT, a series of clinical studies will be presented in this thesis.

**THESIS OUTLINE**

In part one of this thesis the aim is to investigate several technical and methodological aspects of $^{18}$F-FLT and $^{18}$F-FDG PET/CT and validate: 1) $^{18}$F-FLT and $^{18}$F-FDG as early response biomarkers in non-small cell lung cancer patients; 2) $^{18}$F-FLT as tool for assessment of proliferation in drug development studies. In the second part the aim was to technically validate $^{18}$F-FDHT PET/CT as potential tool for response evaluation of AR-directed therapies in castrate resistant prostate cancer patients.

In **chapter two** we perform a systematic review to investigate the clinical value of $^{18}$F-FLT PET imaging for prediction of response to treatment. In **chapter three** we comprehensively evaluate repeatability of $^{18}$F-FLT uptake metrics by conducting an individual patient data meta-analysis on all available $^{18}$F-FLT repeatability data from previously published reports. We also investigate the repeatability of several whole-body $^{18}$F-FDG uptake metrics in **chapter four** and assess the influence of tracer uptake interval and lesion selection strategies. Furthermore, we evaluate the performance of two methods for correction of differences in uptake time. In **chapter five** we study the accuracy and performance of several parametric methods to assess whether these methods could be used for assessment of intratumoral heterogeneity and detection of therapy resistant sub-volumes within lesions at baseline as well as during treatment. In the second part of this thesis we assess the accuracy of simplified methods for quantification of $^{18}$F-FDHT uptake in **chapter six**. In **chapter seven** we evaluate the repeatability of simplified whole-body $^{18}$F-FDHT uptake metrics in multi-center setting.
to provide a threshold for further clinical trials. In chapter eight we will discuss and summarize the results and touch on the future perspectives.
Chapter 1

REFERENCES


Chapter 1


Introduction


A systematic review on FLT-PET uptake as a measure of treatment response in cancer patients


* G.M. Kramer and V.R. Bollineni contributed equally to this work.