CHAPTER 1

Introduction and outline

"I believe that it is an excellent thing for a physician to practice forecasting. He will carry out the treatment best if he knows beforehand from the present symptoms what will take place later."

From: 'The book of prognostics' by Hippocrates

One of the earliest medical writings originates from Hippocrates in 400 years before Common Era (BCE) [1]. The importance of forecasting and biomarkers was already highlighted in these historic writings. Since then medicine has progressed in many ways, but appearance and symptom recognition and later on combined with more objective and quantitative biomarkers are still used to indicate disease, disease severity, prognosis and to evaluate treatment efficacy. Moreover, forecasting, e.g. accurate prognostic biomarkers, are key elements in current medical healthcare, almost 2,500 years after 'The book of prognostics'. Biomarkers have evolved from subjective measures (e.g. shape of the nose as described by Hippocrates) to more objective measures like quantitative blood tests and medical imaging. This thesis describes the technical and clinical aspects of the measurement of proliferation and glucose metabolism with positron emission tomography (PET) as imaging biomarker in lung cancer patients.

1.1 IMAGING BIOMARKER

A biomarker is a parameter which represents (patho)physiologic processes and is used as indicator to diagnose and treat patients. A biomarker should be objective and quantitative, like blood pressure, body temperature and plasma glucose levels, which are examples of frequently used biomarkers. The quantitative level of a biomarker should correspond to a biological condition which is meaningful for medical decision making. In the evolution of medicine numerous biomarkers have been developed. The definition of biomarkers has been described by the 'Biomarkers definition working group' in 2001 as: 'A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention' [2]. Biomarkers should have a high feasibility, precision and accuracy (Figure 1.1):

1. Feasibility; simple implementation to perform the test in any medical center.
2. Precision; low variability in repeated measures (high repeatability) to detect small changes and to be consistent.
3. Accuracy; valid measurement of the desired (patho)physiology, or in other words, measuring the 'truth'.

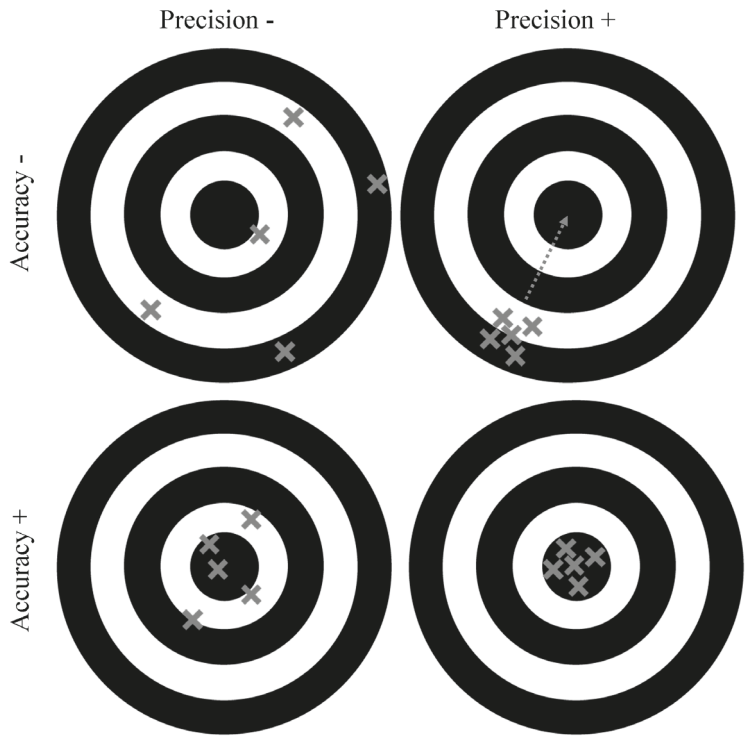

FIGURE 1.1 Characteristics of a biomarker: precision and accuracy (feasibility is not shown, but implies the complexity of performing the measurement, e.g. placing the cross). Upper left, invalid biomarker with no precision and no accuracy; Upper right, biomarker with precision and no accuracy only valid if correction factor is known to correct for bias (represented by the dotted arrow); Lower left, biomarker with low precision and high accuracy, which could only detect large differences; Lower right, ideal biomarker with high precision and high accuracy.

First, a technical validation study should determine the feasibility and precision of a measurement. This can be done by performing a test-retest study to assess both repeatability and feasibility at the same time. Based on such studies, the optimal method to measure a certain biomarker can be proposed. After this technical validation phase, biological validation studies must be performed to investigate the accuracy of a measurement. This is usually done by correlating the experimental measurement with the gold standard. If this correlation contains an offset or known bias, a correction or calibration factor could be determined (Figure 1.1). If the correlation is poor, a technical valid measurement remains, which is biologically invalid in that specific

setting.
Molecular functional imaging quantifies biological processes non-invasively and is therefore an attractive candidate as clinical imaging biomarker. In this thesis the performance of quantification of tracer uptake with PET as imaging biomarker for proliferation and glucose metabolism is investigated in oncologic patients. We used 3'-deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]FLT) as proliferation biomarker and [^{18}F]-fluorodeoxyglucose ([^{18}F]FDG) as glucose metabolism biomarker. We focused on the feasibility and precision of quantification of tracer uptake and herewith we would like to provide a foundation for future biological validation studies to investigate their accuracy and clinical utility.

1.2 POSITRON EMISSION TOMOGRAPHY

PET is a non-invasive imaging technique based on the use of biologically relevant compounds labelled with positron-emitting radionuclides such as carbon-11, nitrogen-13, oxygen-15 and fluorine-18. In clinical applications, a very small amount of labelled compound (radiotracer) is introduced into the patient by intravenous injection and tracer concentration in tissue is measured using the PET system. During its decay process, the radionuclide emits a positron which, after travelling a short distance (1-2 mm), encounters an electron from the surrounding environment. The two particles combine and 'annihilate' with each other, resulting in the emission of two γ-rays of 511 keV each in opposite directions. The image acquisition is based on the coincidence (simultaneous) detection of the emitted γ-rays. The lines connecting these coincident detections are called lines of response and are used in the image reconstruction process (Figure 1.2). Besides true coincidences, a PET system suffers from random, scatter and multiple coincidences and dead time of the detectors. More detailed description of the principles of PET can be found in [3].

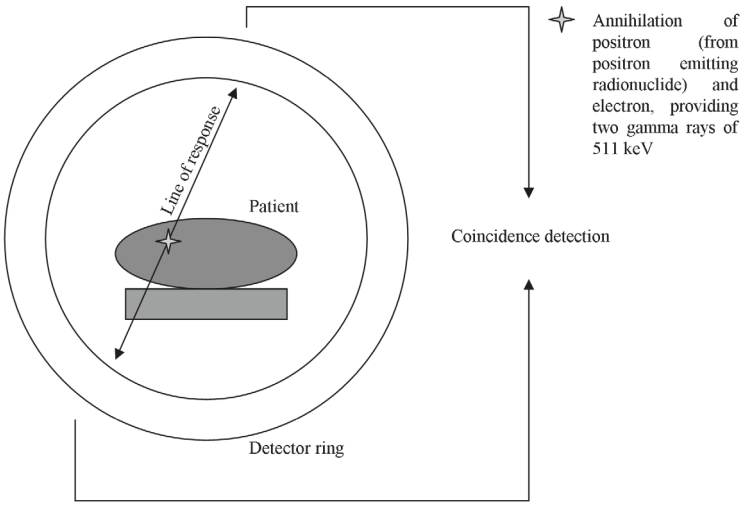

FIGURE 1.2 Principles of PET.

1.3 [^{18}F]-FLUORODEOXYGLUCOSE

[^{18}F]FDG is the most widely used PET tracer. [^{18}F]FDG is an analogue of glucose and measures glycolysis. Based on the Warburg effect, there is increased aerobic glycolysis in tumor tissue, resulting in increased radiotracer uptake. Therefore, [^{18}F]FDG can detect malignant lesions and stage oncology patients. However, because increased glycolysis is also seen in inflammation, [^{18}F]FDG is not specific and pathological confirmation is needed to diagnose cancer. Standardized uptake value (SUV) is a simplified parameter to quantify [^{18}F]FDG uptake [4-6]. [^{18}F]FDG PET acquisition is standardized within the guidelines of the European Association of Research Ltd (EARL) [7]. However, for SUV still various SUV parameters and different tumor delineation methods to determine [^{18}F]FDG uptake within the tumor are used [6, 8, 9]. Technical validation studies to provide an optimal methodology for tumor delineation and SUV measurement are therefore needed to provide a strategy for biological validation studies which should be standardized between centers.

1.4 3'-DEOXY-3'-[^{18}F]FLUOROTHYMIDINE

[^{18}F]FLT is a radiotracer which provides a noninvasive measure of proliferation. It is based on the increased uptake of thymidine together with [^{18}F]FLT during the S-phase of the cell cycle. Tissue with an increased proliferation rate has a higher demand of thymidine and therefore also increased [^{18}F]FLT uptake. [^{18}F]FLT transport into the cell is facilitated by ENT1, whereafter [^{18}F]FLT can be posphorylated by thymidine kinase (TK) and dephosphorylated by deoxynucleotidase (dNT). Unlike endogenous thymidine, [^{18}F]FLT is not incorporated in the deoxyribonucleic acid (DNA). [^{18}F]FLT is metabolized in the liver to [^{18}F]FLT-glucuronide and thereafter subjected to renal clearance. Physiologic [^{18}F]FLT uptake is seen in red bone marrow due to haematopoeisis, in liver due to the breakdown of [^{18}F]FLT into [^{18}F]FLT-glucuroinde and in bladder and kidneys due to renal clearance. Pathologic [^{18}F]FLT uptake is seen in malignant tumors and is correlated with immunohistochemistry for proliferation, ki-67, in lung, breast and brain malignancies [10], indicating accuracy, e.g. measuring the 'truth'. Challenges for [^{18}F]FLT PET are quantification in tissue with high uptake, such as red bone marrow and liver, and the use of simplified methods as replacement of complex pharmacokinetic compartmental modeling.

1.5 QUANTIFICATION IN PET

The gold standard for tracer uptake quantification is pharmacokinetic compartmental modeling (Figure 1.3) [11]. However, this is a complex procedure and simplified methods like SUV are preferred in daily clinical practice to augment feasibility. In addition, volume measurements, called metabolically active tumor volume (MATV), can be derived from PET in several ways, with the simplest method being a fixed relative threshold of the voxel with the maximum intensity within the tumor [12]. Manual tumor delineation is also feasible, although this can be time-consuming and introduces interobserver variability [13, 14] since tumor borders are often hard to delineate if tumor to background ratio is low. Therefore, reproducible and (semi) automatic MATV delineation methods are needed.

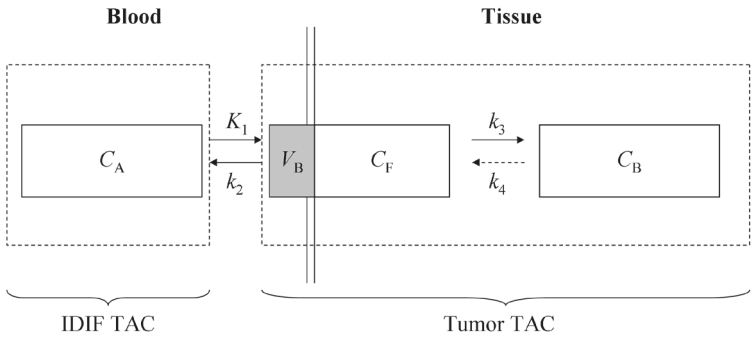

FIGURE 1.3 Two-tissue-compartment model with arterial tracer concentration in plasma C_A, free tracer concentration in tissue C_F and bound tracer concentration in tissue C_B and blood volume fraction (V_B). The kinetic rate constants (K_1, k_2, k_3, k_4) are estimated with full kinetic modelling of the image derived input function (IDIF) and tumour time activity curve (TAC) with non-linear regression. Illustration is adapted from the course manual of the PET pharmacokinetic course.

Subsequently, tracer uptake quantification within these MATV are preferably as simple as possible, as in agreement with the quote of the famous scientist Albert Einstein in 1930s: 'Everything should be made as simple as possible, but no simpler'. Thus, imaging biomarkers should be assessed in the simplest manner without loosing too much precision or accuracy. Simplified parameters should therefore be validated against pharmacokinetic compartmental modeling (gold standard) and this procedure should be repeated for every radiotracer and every simplified method [4].

1.6 NON-SMALL CELL LUNG CANCER

Lung cancer is the most common malignancy in the world, with an estimate of 1.8 million new lung cancer cases in 2012 [15]. The majority of lung cancer cases are non-small cell lung cancer (NSCLC) and patients often present at a late stage when treatment options are limited. First line treatment of stage IV NSCLC consists of platinum based combination chemotherapy [16]. The last decade, new drugs have been developed which are targeted to specific receptors or kinases which are involved in oncogenic pathways. Tyrosine kinase inhibitors (TKI) are an example of this and have shown great benefit in progression free survival for patients with an activating

epidermal growth factor receptor (EGFR) mutation [17-19]. These mutations are most common in NSCLC with subtype adenocarcinoma, female patients, non-smoking patients, and Asian patients [20]. Response assessment of original and new treatment regimens is preferably performed before (patient stratification) or early after the start of treatment. In this way, ineffective treatment can be stopped at an early stage to provide an effective treatment regimen, prevent unnecessary toxicity and prevent unnecessary costs. Response evaluation criteria in solid tumors (RECIST) are currently the gold standard to evaluate treatment efficacy based on computed tomography (CT) [21]. In lung cancer, response assessment with CT is usually performed after the 2nd cycle of chemotherapy (~6 weeks after the start of treatment). However, anatomical changes on CT are preceded by metabolic changes which could be visualized and quantified with PET at an earlier time point. In 2009, Wahl et al. [22], described the potential of PET imaging for response assessment in oncology and recommended PET response criteria in solid tumors (PERCIST). These response criteria should be further optimized and validated based on technical and biological PET validation studies as described above to establish these criteria for clinical decision making in treatment regimens.

1.7 OUTLINE OF THIS THESIS

The aim of this thesis is to investigate proliferation and glucose metabolism imaging biomarkers using [^{18}F]FLT and [^{18}F]FDG PET in lung cancer patients. The methodological aspects of quantification of metabolically active tumor volume and the amount of tracer uptake are evaluated.

First the optimal input function for kinetic modeling for [^{18}F]FLT PET is described in **chapter two**. In **chapter three** we evaluate [^{18}F]FLT PET as an early read out of treatment effect in NSCLC patients treated with pemetrexed in a pilot study. Simplified measures as replacement for pharmacokinetic modeling of [^{18}F]FLT uptake are evaluated in **chapter four** in NSCLC patients treated with tyrosine kinase inhibitors. In **chapter five and six** repeatability of PET derived parameters are determined for both [^{18}F]FLT and [^{18}F]FDG to provide limits of agreement for interpretation of repeated measures in future clinical trials. In **chapter seven** results are discussed and summarized, including the future perspectives.

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