CHAPTER 1

Introduction and outline of the thesis
Introduction

Background
Lung cancer carries the highest number of cancer related death in the western world. Despite all effort of developing new chemotherapy regimens and treatment strategies, the 5-year survival rate in lung cancer remains poor and has barely risen from 12% in 1975 to only 18% in 2009. More than half of the lung cancer patients has distant metastasis (stage IV disease) at diagnosis [1]. These patients are mostly incurable and treatment with systemic therapy is often the only useful option in an attempt to prolong survival and to reduce cancer-related symptoms. Treatment selection for chemotherapy is based on histology (Fig. 1).

Figure 1. Histology of lung cancer [2].

Lung cancer is divided in small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) which in turn is divided in adeno-, squamous cell- and large cell carcinoma (not otherwise specified (NOS)). Because of the poor prognosis of metastatic lung cancer and only limited efficacy of conventional chemotherapy, the urge for better treatment options is high. Therefore, with the discovery of driver mutations, the treatment of cancer is shifted from a one-size-fits-all approach (conventional chemotherapy) towards personalized medicine.
Chapter 1

Biomarkers

Personalized medicine creates a need for reliable biomarkers. A biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention” and is ideally non-invasive, easy to measure and cheap [3]. There are 2 kinds of biomarkers: predictive and prognostic. Predictive biomarkers can predict success of treatment. They can be used to select patients for the appropriate therapy. Overtreatment and delay for a better treatment option can be prevented. Prognostic biomarkers can provide information about probability of survival, independently of response to treatment. E.g. a poor performance score before start of cancer treatment is an indicator of poor survival. This could have impact on the intent of treatment.

Many clinical trials investigate predictive biomarkers based on tumor characteristics, most prominently mutational status. Today’s available targeted treatments are focused on mutations that cause sustaining growth signalling. These mutations are the driver of the malignant degrading, also called ‘driver mutations’ and most frequently found in adenocarcinoma (Fig. 2) [4, 5]. Effective targeted therapy is available for patients with an epidermal growth factor receptor (EGFR) mutation or an anaplastic lymphoma kinase (ALK) translocation [6, 7]. In this thesis, we took the challenges to study new treatment strategies in NSCLC patients with a Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation, the most frequent driver mutation. Further, we study several biomarkers obtained from blood samples and biopsies that were collected during the phase II trials.

KRAS mutation

In NSCLC, a KRAS mutation is the most common mutation (>90%) in the family of RAS consisting of HRAS, NRAS and KRAS. The mutation occurs in the DNA in codon 12, 13 or 61 of the RAS gene. A single amino acid substitution in one of these codons results in a so-called gain of function mutation. There are more than 15 known types of KRAS mutations, of which 4 types most commonly found: G12C, G12V, G12D and G12A. In the cell, KRAS protein transmits growth signals from the cell surface to the cell nucleus. The active and non-active state of KRAS protein is tightly regulated. When mutated, KRAS protein remains in the active state and consequently produces a constant growth stimulus that results in unrestricted growth and degeneration of the cell [9]. The biology of KRAS is discussed in more detail in chapter 2.
**KRAS** mutation is the most common driver mutation in lung cancer and is present in 20-30% of patients with adenocarcinoma or large cell carcinoma (NOS). Until now, conventional chemotherapy is the only treatment option for NSCLC patients with a *KRAS* mutation, while it is believed that this characteristic results in a more aggressive disease. However, the clinical relevance of *KRAS* mutational status is not fully understood. Therefore we evaluated the prognostic value of *KRAS* mutations in the clinical setting of advanced NSCLC and the predictive value by its influence on the response to conventional chemotherapy.

![Figure 2. Driver mutations in adenocarcinoma of the lung [8].](image)

**Sorafenib**

In this thesis, we performed two clinical trials targeting *KRAS* signalling pathways. Two of the most important growth signalling pathways affected by *KRAS* are RAS-RAF-MEK-ERK-MAPK and RAS-PI3K-AKT-mTOR (Fig. 3). Both trials contained treatment with sorafenib, which is developed as a RAF inhibitor. Sorafenib is a tyrosine kinase inhibitor that targets multiple factors involved in tumor growth and angiogenesis. Because of its broad spectrum, sorafenib could be an attractive drug in the treatment of cancer and is registered for treatment of patients with renal cell and hepatocellular carcinoma. In a biomarker driven study, sorafenib
was identified as a possibly potent agent in KRAS mutated NSCLC [10]. Chapter 5 provides a detailed review of the literature on sorafenib in lung cancer.

Sorafenib was studied as monotherapy and in combination with metformin. Metformin is a biguanide and first choice of treatment in patients with type II diabetes mellitus. It is known as a safe drug, with minor adverse events. In the last decade much research has been done on the possible anti-cancer activity of metformin. It has been described that metformin targets tumor growth signalling by inhibition of mTOR [11,12]. Metformin is not been intensively studied in clinical trials.

![Figure 3. Schematic illustration of KRAS signaling and targets of sorafenib and metformin.](image-url)
Introduction

**Outline of this thesis**

The aim of this thesis is to investigate clinical relevance of *KRAS* mutations in NSCLC and to investigate new treatment strategies in this group of patients.

**Chapter 2** reviews the biology of *KRAS* and provides an overview of literature on the clinical relevance of *KRAS* mutational status in NSCLC.

**Chapter 3** studies clinical behaviour of advanced NSCLC harbouring a *KRAS* mutation by means of a retrospective study in NSCLC patients treated with first-line chemotherapy. Previous studies were contradictory.

**Chapter 4** studies the differences of type of *KRAS* mutation in response to chemotherapy treatment. This study gives insight in the preference of *KRAS* mutated NSCLC to certain chemotherapy regimens and points towards a possible difference in tumour biology in *KRAS* subtypes.

**Chapter 5** provides a review of literature on clinical trials with sorafenib in NSCLC and gives an overview on the clinical application of sorafenib in NSCLC and its future perspectives.

**Chapter 6** presents the results of a single-arm phase II study with sorafenib monotherapy in pre-treated NSCLC patients with a *KRAS* mutation. This study was one of the first clinical trials specially designed for NSCLC with a *KRAS* mutation.

In **Chapter 7** we studied a combination of sorafenib and metformin in NSCLC cell lines. This provided the rational for conducting a single-arm phase II study with sorafenib and metformin in advanced NSCLC patients with a *KRAS* mutation that failed previous platinum based chemotherapy. The results of this study are described in **Chapter 8**.

**Chapter 9** discusses a striking case of tumor flare after start of sorafenib treatment and we propose a mechanism that may explain this sudden flare of the cancer.
Chapter 1

References


