Summary and future perspectives
Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations are amongst the most prevalent mutations encountered in NSCLC. This thesis highlights important aspects of the clinical relevance of KRAS mutations in NSCLC and new options for targeted therapy are studied, as conventional chemotherapy is the only therapeutic option at this time. The studies described in this thesis have added the following aspects to the current knowledge:

Patients with a KRAS mutation have the same response to conventional chemotherapy and same overall survival as patients with wild type KRAS. Reflecting that there is no evidence that a KRAS mutation is a poor prognostic or predictive marker in stage IV NSCLC. In stage IV KRAS mutated NSCLC patients treated with first-line platinum-based chemotherapy, taxane containing chemotherapy showed a significant improved overall response rate compared to gemcitabine or pemetrexed. There are differences in response to chemotherapy in different types of KRAS mutations, this suggests differences in tumor biology. Targeting (downstream of) KRAS is challenging, combination therapy might be the key to success.

In chapter 2 KRAS mutated non-small cell lung cancer (NSCLC) as a distinct disease entity is discussed by a review of the literature. A KRAS mutation is thought to be the primary driver of cancer in these particular cases, because of its crucial role in growth signaling, also KRAS mutation is found early in tumorigenesis. Early studies report that KRAS mutated NSCLC patients had a poorer response to chemotherapy and worse survival as compared to NSCLC patients without a KRAS mutation. The presence of a KRAS mutation is therefore thought to be a marker of more aggressive disease. However, chapter 2 points out that more recent studies are equivocal about the predictive and prognostic value of KRAS mutation. Due to heterogeneity in patient selection, histology, chemotherapy regimens used and stage of disease a direct comparison is difficult. Therefore we designed a retrospective analysis in 160 advanced nonsquamous NSCLC patients with known KRAS mutational status that were treated with platinum based chemotherapy in first-line. The results of this study are presented in chapter 3. We demonstrated that patients with a KRAS mutation (33% of all patients) had similar response to treatment and survival rates compared to patients with KRAS wild type. With this study we challenge the stigma of KRAS mutation as a poor predictive and poor prognostic biomarker.

In vitro studies suggested that response to treatment may be different per type of mutation. In chapter 4 a retrospective analysis was performed in 464 KRAS mutated NSCLC patients treated with first-line platinum-based chemotherapy. Three regimens were regularly
combined with cisplatin or carboplatin: pemetrexed, gemcitabine and taxanes. Taxane, especially when combined with bevacizumab, showed highest overall response rate (ORR) compared to patients treated with pemetrexed or gemcitabine platinum combinations. In our cohort, the 3 most common types of \textit{KRAS} mutation were G12C, G12V and G12D. These types account for approximately 80\% of all \textit{KRAS} mutated NSCLC patients. Patients with a G12V mutation treated with taxane-containing chemotherapy showed a significant better ORR, but not progression free survival (PFS) and overall survival (OS) compared to pemetrexed or gemcitabine. In patients with G12C or G12D mutation all 3 combinations had similar treatment outcomes. However, the preference of \textit{KRAS} mutated NSCLC for taxanes and the possible difference in outcome between types of \textit{KRAS} mutation should be confirmed in prospective setting.

Sorafenib is an agent designed to target RAF kinase, downstream of RAS, which makes sorafenib an attractive drug for NSCLC patients with a \textit{KRAS} mutation. \textbf{Chapter 5} gives a review of the literature of sorafenib in NSCLC patients. In this review, clinical trials were discussed including a large placebo controlled phase III trial in unselected NSCLC patients receiving sorafenib in 3rd or 4th line of treatment. This trial showed no OS benefit of sorafenib compared to placebo and recommended, despite significant improved ORR and PFS, against further use of sorafenib in NSCLC patients. However, in \textbf{chapter 6} a single-arm phase II trial with sorafenib monotherapy in pretreated \textit{KRAS} mutated patients showed efficacy of sorafenib in terms of DCR, but PFS and OS remained unsatisfactory.

To further improve outcome of treatment with sorafenib in \textit{KRAS} mutated NSCLC patients, combination therapy with another agent targeting downstream of \textit{KRAS} might be the key to success. As discussed in the introduction \textit{KRAS} protein stimulates several growth pathways of which the PI3K-AKT-mTOR pathway is an important one. Combination therapy of sorafenib with an available drug, for instance everolimus (an mTOR inhibitor) would be a logical choice. However, increased toxicity could be a problem. We found an attractive alternative for these kinds of drugs with a mild toxicity profile, which is metformin. Metformin is a widely used drug in type II diabetes and recently it has been reported that metformin has potency as a mTOR inhibitor. \textbf{Chapter 7} demonstrates synergism between sorafenib and metformin in \textit{KRAS} mutated NSCLC cell lines. This provided the rationale for \textbf{chapter 8} which describes a single-arm phase II clinical trial that recruited 55 NSCLC patients with a \textit{KRAS} mutation. This combination showed insufficient activity according to pre-specified efficacy threshold. Approximately one-third of the patients progressed within a short period of time of 3 weeks. In \textbf{Chapter 9} a patient was discussed with striking
progressive disease shortly after start of treatment with sorafenib and metformin. This so-called ‘tumor flare’ may be explained by involvement of an autoregulatory negative feedback loop of RAF protein that is inhibited by sorafenib. The inhibition of that negative feedback results in increased stimulation of the growth signaling pathway. This mechanism demonstrates the complexity of targeting downstream signaling pathways of \textit{KRAS}.

\textbf{Future perspectives}

What we have learned in this thesis is that there might be a future for personalized treatment in NSCLC patients with a \textit{KRAS} mutation. Encouraging data discussed in chapter 4 of this thesis led to designing a randomized phase III study comparing standard choice of first-line treatment (cisplatin/pemetrexed) with carboplatin/paclitaxel/bevacizumab in advanced NSCLC patients and a \textit{KRAS} mutation (NVALT 22). Primary endpoint of this study is PFS and it is hypothesized that PFS will be superior in patients treated with carboplatin/paclitaxel/bevacizumab compared to patients treated with cisplatin/pemetrexed. The finding that response to chemotherapy may different per type of mutation is hypothesis generating and will be a secondary endpoint in the upcoming phase III trial. This new insight also questions our understanding of the biology of \textit{KRAS} and more study is needed.

New advances in the study on \textit{KRAS} mutated cancer has given hope for better treatment options. For many years, numerous attempts to directly target mutated \textit{KRAS} failed and \textit{KRAS} was believed to be an undruggable target. Therefore focus shifted to targeting downstream pathways. Unfortunately, targeting these pathways appeared to be more difficult than expected. As previously discussed and demonstrated in chapter 9, feedback loops and unknown interactions are to be dealt with. This complex problem is poorly understood and the biology behind these mechanisms should be elucidated to attack these pathways effectively. Treatment combining targeted treatment with chemotherapy had some promising results [1]. Also dual inhibition of the same pathway could be promising in \textit{KRAS} mutated cancer [2]. Recently encouraging data is published on successful selective inhibition of \textit{KRAS}-G12C variant [3]. This compound did not affect other types of \textit{KRAS} mutation or \textit{KRAS} wt. The designed small molecule irreversibly binds to G12C and changes the protein structure in such a way that \textit{KRAS} has a preference to the GDP bound (inactive) state and impairs binding to RAF. This leads to shutdown of mutated KRAS protein. This is an important step towards targeted treatment of \textit{KRAS} mutated cancer after years of failed attempts.
To conclude, new advances lead to better understanding and progress in the treatment of KRAS mutated NSCLC, but also raises new questions and challenges. As Dr. Gandara, a renowned investigator on the study of lung cancer, stated: “The KRAS mutation story has evolved from what we thought we knew, to the controversies about what we don’t know” (ASCO post 2014, volume 5, issue 14).

References