CHAPTER 12

Summary and discussion

The majority of patients presenting with lung tumours have tumours histologically classified as non-small cell lung cancer (NSCLC), and they also present with advanced stage disease. Until recently, platinum-based doublet chemotherapy regimens were the standard treatment for patients with a good performance status. However, only approximately 30% of patients respond to chemotherapy and the results obtained remain disappointing, with median survivals ranging from 8 to 12 months and 1-year survival rates varying from 33% to 46%.[1-5] New insights into the molecular biology of cancer have identified key biological processes, providing potential targets for anti-cancer treatment.[6] The development of new "targeted therapies" is yielding promising results, with the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib and the angiogenesis inhibitor bevacizumab having already been granted regulatory approval for the treatment of advanced NSCLC. Simultaneous inhibition of multiple pathways involved in tumourigenesis may be even more effective. Indeed, early clinical trials combining erlotinib and bevacizumab reported encouraging although modest results in NSCLC.[7;8] However, as is the case with chemotherapy, not all patients will respond to these new treatments, and all responding patients will ultimately develop disease progression due to the emergence of resistance. A major challenge is to identify biomarkers that predict which patients benefit from which drugs so as to enable individualised treatment. The work presented in this thesis reflects research aimed at improving and personalising treatment of patients with advanced/metastatic NSCLC conducted at the VU University Medical Centre. It is subdivided into two sections, namely **Part 1,** in which new treatment approaches were investigated, and **Part 2,** in which new biomarkers for patient selection and treatment monitoring were explored.

Part 1: Novel treatment approaches

In ***Chapter* 2**, we reviewed 167 patients who were treated at our centre for a newly diagnosed NSCLC and synchronous brain metastases (BM). The aim was to identify patient groups for whom radical treatment of the primary site may be appropriate. Of 86 patients who underwent surgery or radio-surgery for BM, the 30% who subsequently underwent a radical treatment strategy of the primary tumour site had a significantly longer median OS (28.4 months) than those undergoing only chemotherapy (12.1 months) or supportive therapy (5.6 months). The stage of intra-thoracic disease also significantly influenced survival, with patients with stage I thoracic disease having a median OS of 18.5 months, those with stage III 9.4 months and those with stage IV 2.7 months. These data suggest that radical thoracic treatments may be justified in patients who are eligible to undergo surgery/radio-surgery for synchronous BM from NSCLC, even when stage III intra-thoracic disease is present. The prolonged survival seen in our patients undergoing radical treatment of both the primary tumour and BM is consistent with published data.[9-14]

Emerging data has demonstrated efficacy of EGFR TKIs in patients with BM from a NSCLC.[15-20] In addition, EGFR inhibition has been shown to have a radiation-enhancement effect, at least in head and neck tumours.[21] We therefore hypothesised that combining EGFR inhibitors with WBRT may improve the prognosis of patients with BM from NSCLC who are ineligible for surgery/radio-surgery. A dose-escalation phase I trial was performed to assess the safety and tolerability of combining WBRT with concurrent and maintenance erlotinib. As described in ***Chapter 3***, all 11 patients who were enrolled completed WBRT (30 Gy in 10 fractions) and received erlotinib (100mg/day in cohort 1 and 150mg/day in cohort 2), which was started 1 week prior to, and continued throughout WBRT, followed by maintenance erlotinib (150mg/day). WBRT with concurrent erlotinib was well tolerated and safe, with no observed treatment-related neurotoxicity and only 2 grade 3 toxicities (1 acneiform rash and 1 fatigue). Furthermore, only 1 patient developed intracranial progression and of the 7 patients with follow-up neuro-imaging at 3 months, 5 had an intracranial partial response and 2 stable disease. This encouragingly high intracranial disease control rate was also seen in a subsequently reported single arm phase II study of erlotinib with concurrent WBRT for patients with BMs from NSCLC. With a median follow-up of 21 months, the median survival time of 10.9 months compares favourably to historical controls.[22] These and our results justifies further study of this combination in a larger, randomised phase II study looking also at the influence of *EGFR* mutation status. However, 2 of our patients developed erlotinib-related interstitial lung disease contributing to death during maintenance therapy and 6 patients developed extracranial progression, suggesting that maintenance erlotinib monotherapy after WBRT has both limited safety and efficacy in unselected Western populations.

Angiogenesis is the multi-step process whereby new blood vessels are formed from the existing vasculature. The key player in tumour angiogenesis is the vascular endothelial growth factor (VEGF) pathway.[23] ***Chapter 4*** reviews the various inhibitors of angiogenesis that are currently undergoing clinical investigation in NSCLC. The VEGF pathway can be inhibited in two main ways: 1. direct VEGF inhibition by monoclonal antibodies like bevacizumab or 2. VEGFR inhibition by tyrosine kinase inhibitors such as sorafenib, sunitinib, montasenib and cediranib. Currently bevacizumab, in combination with platinum-based chemotherapy, is the only angiogenesis inhibitor that has been approved for the treatment of advanced (non-squamous) NSCLC.[24;25] Trials evaluating other angiogenesis inhibitors have shown modest results and further research is needed. There is interplay between the EGFR and VEGF pathways with preclinical and early clinical studies demonstrating an additive anti-tumour effect after their simultaneous inhibition.[26] In ***Chapter 5***, we reported the results of the first phase II study evaluating

the efficacy and safety of erlotinib (150 mg/day) plus sorafenib (800 mg/day) as first-line treatment for advanced/metastatic NSCLC. In the 50 patients who commenced therapy, the non-progression rate after 6 weeks of treatment was 74%, and the overall objective response rate (ORR) was 28%. The median time to disease progression (TTP) was 5.0 months, and median survival was 10.9 months. This promising clinical activity was achieved despite lowered, sub-therapeutic plasma erlotinib levels, which were possibly mediated by sorafenib (CYP3A4 polymorphism-associated). The safety profile was acceptable with only one possible treatment-related death. Exploratory analysis showed that patients with an activating *EGFR* mutation in either exon 19 or exon 21 had a higher response rate (71%) and tended towards a longer time to progression and survival. However, these results are comparable to the phase III IPASS study (gefitinib versus carboplatin/paclitaxel as first-line treatment) which reports an ORR of 71% and prolonged progression-free survival (PFS) in gefitinib-treated *EGFR* mutation positive patients.[27] Our data, therefore, do not suggest that the combination of sorafenib and erlotinib is superior to EGFR TKI monotherapy as first-line treatment in *EGFR* mutation positive patients and thus do not support further study of the combination in this setting. Clinical outcomes in our *EGFR* mutation negative patients were not sufficient to merit further study of the combination of sorafenib and erlotinib in the first-line setting in this subgroup of patients. However, the ORR of 19% is higher than that reported in previous trials evaluating EGFR TKI monotherapy in *EGFR* mutation negative patients. Further evaluation of sorafenib plus erlotinib as salvage therapy in *EGFR* mutation negative patients, therefore, seems justified. Indeed, more recently the results of a randomised, double-blind, placebo-controlled, phase II trial of sorafenib and erlotinib versus erlotinib alone in previously treated advanced NSCLC have been published.[28] Subset analyses in EGFR WT and EGFR FISH-negative patients suggest a benefit for the combination of erlotinib/sorafenib compared with single-agent erlotinib with respect to PFS and OS.

Part 2: Optimising patient selection and treatment monitoring

Although new targeted therapies are considered generally safe, data is emerging on novel and sometimes fatal adverse effects. In ***Chapter 6*** we reported 2 cases of histologically confirmed, fatal erlotinib-associated interstitial lung disease (ILD). After starting erlotinib treatment both patients developed clinical and radiological signs of ILD resulting in respiratory failure and died as a consequence of autopsy-confirmed diffuse alveolar damage. In ***Chapter 7*** we described the results of a phase I trial of chemotherapy, followed by thoracic radiotherapy with concurrent bevacizumab for inoperable stage III NSCLC, which was terminated early when 4 out of the 6 enrolled patients developed grade ≥2 radiation pneumonitis. In view of the increasing use of EGFR-TKIs and angiogenesis inhibitors, physicians should be aware of these serious and potentially

fatal complications. Such toxicity highlights the importance of both selecting only those patients who are most likely to benefit from treatment, and the need to monitor responses after treatment initiation.

Activating mutations in the tumour *EGFR* gene, most commonly exon 19 deletions and exon 21 point mutations, are associated with sensitivity to EGFR TKI therapy, with reported response rates up to 71% and a significantly longer progression-free and overall survival.[29-33] In ***Chapter 8***, we described a case of dramatic tumour response to only a third of the standard dose, and subtherapeutic plasma level, of erlotinib in a patient with an *EGFR* (exon 21) mutated tumour who developed a severe rash on full dose erlotinib. Our case suggests that in patients with *EGFR* mutation positive NSCLC who develop a high-grade rash, erlotinib dose reductions to "subtherapeutic" levels remain effective while reducing the severity of toxicity.

However, not all patients with an activating *EGFR* mutation respond to EGFR TKI therapy and, conversely, some *EGFR* mutation negative patients do respond. Other biomarkers for predicting treatment efficacy need to be identified. In ***Chapter 9***, we reported the results of a study measuring the changes in circulating endothelial cells (CECs) and $CD133^+$ haematopoietic progenitor cells (HPCs) in 25 patients enrolled on the sorafenib plus erlotinib phase II trial. The latter were compared to changes in a control group of 18 patients receiving bevacizumab plus erlotinib, and in 10 patients receiving erlotinib monotherapy. Our study revealed that sorafenib plus erlotinib treated patients showed a three-fold increase in CECs ($p< 0.0001$) at day 7. This was comparable to bevacizumab/erlotinib treated patients ($p<0.01$), but was not seen with erlotinib monotherapy ($p=0.8$), suggesting the increase may be anti-angiogenic specific. At day 7, $CD133^+$/HPCs decreased with sorafenib/erlotinib treatment ($p<0.0001$) but did not change with either bevacizumab/erlotinib or erlotinib. More importantly, however, in sorafenib/erlotinib treated patients pre-treatment $CD133^+$/HPCs were significantly lower in objective responders ($p=0.01$) and pre-treatment $CD133^+$/HPC numbers less than the median correlated with a longer TTP ($p=0.04$). Our results suggest that pre-treatment $CD133^+$/HPCs merits further study as a candidate biomarker for identifying patients who might benefit from sorafenib plus erlotinib treatment.

The use of functional imaging techniques, such as positron emission tomography (PET) and dynamic contrast-enhanced computed tomography (DCE-CT), for predicting and monitoring treatment effect are increasingly being investigated in oncology. There was no such data available on patients with NSCLC treated with sorafenib or erlotinib. In a subgroup of patients enrolled on the sorafenib plus erlotinib trial, we investigated for the first time the feasibility of serial DCE-CT to measure tumour blood flow (BF) in NSCLC

patients, and correlated tumour BF with treatment outcome (RECIST/Crabb response and PFS). As reported in ***Chapter 10,*** we found serial DCE-CTs to be feasible, with tumour BF successfully analysed at baseline and after 3 and/or 6 weeks of treatment in 23 out of the 34 enrolled patients. We demonstrated a significant reduction in tumour BF after 3 weeks ($p<0.001$) and 6 weeks ($p<0.001$) of treatment, which provides support for the anti-angiogenic action of sorafenib. Moreover, early changes in tumour BF correlated with objective anatomic (RECIST/Crabb) response, with a lower tumour BF at weeks 3 ($p=0.03$) and 6 ($p=0.04$) in anatomic responders versus non-responders, and a trend with longer PFS ($p=0.06$). Although a number of important limitations of DCE-CT prevent its routine use, our results are promising and further study of the predictive value of DCE-CT in response monitoring is warranted.

Osteoblastic bone flare or response as a manifestation of treatment response has been reported in only a few patients with NSCLC.[34-37] In ***Chapter 11,*** we reported the first cases of osteoblastic lesions developing during treatment with erlotinib for NSCLC, indicating major response. With the increasing use of EGFR-TKIs in NSCLC, osteoblastic responses are likely to be increasingly seen. Awareness of this phenomenon is important as misinterpretation as progressive disease results in premature cessation of effective therapy.

Future prospects

The introduction of targeted therapies has clearly led to some progress in the treatment of advanced NSCLC. Combining bevacizumab with chemotherapy as first-line treatment demonstrated a survival prolongation beyond the historical bench mark of 12 months. EGFR TKIs have proven superior to standard chemotherapy in patients with activating *EGFR* mutations, and these are now the recommended first-line therapy for such patients. However, despite the progress made, several important issues in the clinical development of these targeted agents remain unresolved. There is a need to identify when and how these targeted agents should be safely and effectively integrated with conventional therapies so as to maximise clinical benefit and minimise toxicity. An example of a new strategy being studied at our centre is maintenance targeted therapy after initial treatment. The recently published phase III SATURN trial showed that maintenance therapy with erlotinib for patients with NSCLC who do not progress after chemotherapy significantly prolongs PFS compared with placebo.[38]

With limited therapeutic benefit achieved so far, it is clear that successful drug development will depend on early identification of patient subgroups in whom the agent(s) is/are effective. This is achieved on the one hand by pretreatment patient and tumour characteristics that predict for clinical benefit or primary (*de novo*) resistance,

and thus guide patient selection. An example of a recently validated biomarker for efficacy of EGFR TKIs is the presence of an activating *EGFR* mutation, as discussed above. Biomarkers of primary resistance to EGFR TKI include insertion mutations in exon 18, mutations in *KRAS* (occurring in 20-30% of NSCLC) and the recently identified *EML4-ALK* translocations.[39] *EGFR* mutations, *KRAS* mutations and *ALK* fusion genes are mutually exclusive and prospective genotyping for these genetic mutations may lead to the identification of distinct and non-overlapping molecular subsets of NSCLC patients. However, mutation testing is currently not universally available, is invasive and requires adequate tumour tissue specimens. With the advent of improved tests and the potential to test on blood samples, the use of EGFR TKIs in *EGFR* mutation positive patients will hopefully increase in the near future.[40-42]

Another promising field of ongoing research in predictive biomarkers for targeted therapies is proteomics. A matrix-assisted laser desorption ionisation (MALDI) mass spectrometry (MS) classifier (VeriStrat) on pretreatment serum has been identified which predicts for PFS and OS in NSCLC patients treated with EGFR TKIs, cetuximab and erlotinib plus bevacizumab.[43-46] Further validation of this classifier in large, randomised prospective trials is needed but these results are encouraging. The advantages of a MS serum based classifier are that it uses easily obtainable samples, results are fully automated and quickly available, assay failure rates are small and the cost is low. We are currently performing mass spectrometry on pretreatment, week 1 and week 3 serum samples of patients enrolled in our erlotinib-WBRT and erlotinib-sorafenib trials. Results should be available soon.

On the other hand, almost all patients who initially respond eventually become refractory to treatment. Such acquired resistance is achieved by the development of new *EGFR* mutations or the development of EGFR-independent pathway(s). The most frequently identified underlying molecular mechanism for acquired resistance to reversible EGFR TKI therapy is a T790M *EGFR* mutation, found in 50% of patients who develop resistance.[47] A new class of dual, irreversible EGFR/HER2 inhibitors has shown preclinical efficacy against T790M and resistance emerges less frequently than with reversible EGFR TKI such as erlotinib and gefitinib.[48;49] Several ongoing clinical trials, including at our institute, are currently evaluating these newer agents in patients with advanced NSCLC including those who have progressed after gefitinib or erlotinib.

Another example of acquired resistance to reversible EGFR TKIs is MET amplification, accounting for 20% of acquired resistance.[39] Small molecule MET inhibitors are undergoing clinical evaluation in the treatment of NSCLC and the monoclonal antibody MetMab is currently undergoing III trial investigation.[39;50]

The detection of *KRAS* mutations, *ALK* translocations, *T790M* mutations and *MET* amplification before and/or during treatment with reversible EGFR TKI may prove valuable for tailoring NSCLC treatment regimens in the near future. However, our current understanding of primary and acquired drug resistance remains limited. Studies aimed at further characterising resistance mechanisms should provide new insight into how to potentially overcome this problem. Such knowledge is the cornerstone of more effective personalised therapy.

Monitoring for early treatment failure enables early initiation of salvage therapy and minimises unnecessary toxicity. The optimal monitoring strategy is yet unknown. Promising imaging techniques include PET and DCE-CT scans as mentioned above. A number of ongoing and futures studies at our centre will hopefully clarify the potential role of these techniques in response monitoring. However, the major current limitations are the lack of universal availability and lack of standardised protocols.

Despite the emergence of new targeted therapies, a large proportion of patients do not benefit from the currently available molecular targeted agents. For example only 10-40 % of patients with NSCLC have activating *EGFR* mutations. Further elucidation of other driver mutations that lead to oncogene addiction will identify new potentially "drugable" targets and is thus essential in the pursuit to improve the treatment and prognosis of these patients. Recently, a number of novel driver mutations in NSCLC have been identified and drugs targeting these mutations have shown encouraging clinical results. *EML4-ALK* fusion genes, present in 3-7% of patients with NSCLC, lead to constitutively active tyrosine kinase.[50] Such tumours are sensitive to pharmacological inhibition of *ALK*. A recently updated phase I study of crizotinib, an ALK and MET tyrosine kinase inhibitor, in previously treated *ALK* positive NSCLC showed an objective response rate of 56% and PFS of 9.2 months.[51;52] Based on these promising results crizotinib is now being evaluated in phase III trials of *ALK* positive patients. Other recently identified clinically relevant driver mutations include *HER2* mutations (2% of NSCLC; drugs: lapatinib (phase II), afatinib (phase III), trastuzumab (phase III)), *PIK3CA* mutations (2% of NSCLC; drugs: phase II), *AKT1* mutations (1% of NSCLC; drugs phase I), *BRAF* mutations (1-3% of NSCLC, drugs phase II), *MAP2K1* mutations (1% of NSCLC, drugs phase II), and *MET* amplifications (discussed above).

To conclude, with the increasing knowledge of tumourigenesis, resistance mechanisms, development of novel treatment strategies and emerging imaging and biochemical techniques we are moving towards a personalised genotype-specific treatment approach for advanced NSCLC. This exciting development should substantially improve the prognosis of this pandemic disease.

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