Abstract. During recent years, we have seen an increasing awareness among physicians about the possibilities of helping patients stricken by non-small cell lung cancer using active intervention with chemotherapeutics. This has emerged mainly from the development of new chemotherapeutics and novel drug combinations with an improved therapeutic ratio better tolerated by the patients. However, these new combinations of chemotherapeutics have proved to be only marginally better in terms of survival than the earlier used cytotoxic agents. Thus, many clinicians consider the effects of systemic therapy on symptom control and improved quality of life to be at least as important as survival when evaluating new drugs or new combinations. It is also obvious that improvements using traditional cytotoxics are slow and that there is a need for novel approaches. The present review focuses on novel drugs that have recently been introduced, or soon await to be included, in the management of advanced lung cancer and which have a potential value for use in neoadjuvant treatment of patients with non-small cell lung cancer, i.e. pemetrexed, EGFR-inhibiting agents, anti-angiogenesis inhibitors and other small molecules.

Lung cancer, a rare disease until the beginning of the twentieth century, is now a most devastating disease worldwide killing more than 1 million people each year (1). Smoking is the overwhelming cause for the majority of all lung cancer cases and, if smoking could be abolished, an estimated reduction of 90% in the number of lung cancer cases could be achieved (2). Lung cancer is divided into two entities, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC represents 80% of all lung cancer cases and this review will focus entirely on NSCLC. At the time of diagnosis, approximately 20-30% (stage I and 2) are candidates for surgical intervention (3), and these patients with a good performance status usually have a good prognosis after surgical resection. The 5-year survival rate for small tumours less than 4 cm (T1N0 and T2N0 tumours) is around 50-80% (4), however, in patients with localised advanced disease (stage 3) after extended resection an increased morbidity and mortality are observed (5). In order to increase the efficacy of surgical interventions, the role of neoadjuvant chemotherapy, i.e. administration of systemic therapy prior to locoregional therapy (Table I), and the value of adjuvant chemotherapy, i.e. administration of systemic therapy after locoregional therapy (Table II), have been evaluated. The beneficial effects of neoadjuvant therapy are early treatment of micrometastatic disease, down-staging of the primary tumour before surgery, and the possibility to evaluate in vivo the efficacy of the given systemic treatment on the disease (3). To our knowledge, five controlled randomised phase III trials in the neoadjuvant setting (Table I) have been conducted in patients with localised advanced disease (stage IIIA). The results are not consistent. In three of the studies (6-8), a median survival advantage in the preoperative chemotherapy group was seen compared to the surgery only group. However, it should be emphasized, that these studies were closed at interim analysis due to the large statistical advantage in favour of the treatment arms. Thus, the issue was not fully elucidated due to the small sample sizes. Two well conducted randomized phase III studies, which included numbers of stipulated patients, showed diverging results (9, 10). Depierre et al. showed for patients in stage I+II, a statistically significant advantage in terms of survival, however this was not found for patients with stage IIIA. Mattson et al. did, however, not demonstrate a survival advantage in favour of the neoadjuvant chemotherapy arm.

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Key Words: Lung cancer, neoadjuvant chemotherapy, NSCLC, pemetrexed, EGFR-inhibiting agents, anti-angiogenesis inhibitors, cisplatin, gemcitabine, review.
The value of neoadjuvant chemotherapy is, therefore, still controversial, and phase III studies are currently ongoing. Despite recent advances in the treatment modalities regarding NSCLC in total, there is only a modest improvement in overall survival. However, with the use of newer agents, focusing on different aspects of tumour cell molecular biology, hopefully a substantial clinical breakthrough will occur. The treatment paradigm towards the goal of total eradication of tumour cells will perhaps be challenged, as the focus might shift to life-long maintenance therapies resulting in tumour dormancy. The present review deals with the new chemotherapeutic agent pemetrexed disodium (Alimta®), EGFR-inhibiting agents, anti-angiogenesis inhibitors and other small molecules and their implications as potential agents in neoadjuvant treatment for patients with NSCLC.

Pemetrexed disodium (Alimta)

Antimetabolites are compounds that down-regulate or abolish the effects of metabolites needed by the cell for continuous division and exert their effect through interference with DNA synthesis, causing inhibition of purine or pyrimidine nucleoside pathways (11). Antimetabolites have been used extensively, especially in the treatment of malignancies with a high growth rate. However, one of the major problems has been the development of drug resistance, which is attributed to their dependence on a series of enzymatic steps needed for their activation. The newly developed antifolate, pemetrexed disodium (Alimta®), is a multi-targeted antifolate targeting the enzymes thymidylate synthase (TS), glycinamide ribonucleotide formyltransferase (GARFT) and dihydrofolate reductase (DHFR) in the pathways of folate metabolism (12). The multi-targeting approach should theoretically reduce the problem with drug resistance that otherwise is common in the group of antimetabolites (13).

Pemetrexed disodium is incorporated into the cell via the reduced folate carrier and binds to folate receptor-α. Inside the cell it becomes polyglutamated, a necessary process since the polyglutamated form increases the competitive inhibition, especially of the enzymes TS and GARFT (15). Preclinical studies have shown that

### Table I. Randomized phase III trials of neoadjuvant chemotherapy in patients with localized advanced NSCLC.

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of patients</th>
<th>Stage</th>
<th>Treatment</th>
<th>Resection rate</th>
<th>Median survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase III</td>
<td>373</td>
<td>IIIA</td>
<td>Mitomycin + Ifosfamide + Cisplatin or Surgery</td>
<td>NR</td>
<td>37</td>
</tr>
<tr>
<td>Depierre (9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>Mattson (10)</td>
<td>274</td>
<td>IIIA</td>
<td>Docetaxel or Surgery alone</td>
<td>77%</td>
<td>14.8</td>
</tr>
<tr>
<td>Pass (8)</td>
<td>28</td>
<td>IIIA</td>
<td>Cisplatin + Etoposide or Surgery alone</td>
<td>85%</td>
<td>28.7</td>
</tr>
<tr>
<td>Roth (7)</td>
<td>60</td>
<td>IIIA</td>
<td>Cisplatin + Etoposide Cyclophosphamide or Surgery</td>
<td>61%</td>
<td>64</td>
</tr>
<tr>
<td>Rosell (79)</td>
<td>60</td>
<td>IIIA</td>
<td>Cisplatin + Mitomycin Ifosfamide or Surgery</td>
<td>87%</td>
<td>26</td>
</tr>
</tbody>
</table>

### Table II. Randomized phase III trials of adjuvant chemotherapy in patients with localized advanced NSCLC.

<table>
<thead>
<tr>
<th>Author</th>
<th>Treatment</th>
<th>Number of patients</th>
<th>Stage</th>
<th>5-year survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endo et al. (80)</td>
<td>Uracil plus Tegafur (UFT) or Surgery</td>
<td>Tot 221</td>
<td>I-II</td>
<td>79%</td>
</tr>
<tr>
<td>Scagliotti et al. (81)</td>
<td>Mitomycin + Vinodesine + Cisplatin or Surgery</td>
<td>606</td>
<td>I-III</td>
<td>Follow-up at 64 months NS</td>
</tr>
<tr>
<td>LeChevalier et al. (82)</td>
<td>Cisplatin + Etoposide, Vinca-alkaloids or Surgery</td>
<td>935</td>
<td>I-III</td>
<td>44.5%</td>
</tr>
<tr>
<td>Winton et al. (83)</td>
<td>Vinorelbine + Cisplatin or Surgery</td>
<td>Tot 459</td>
<td>Ib+II</td>
<td>69%</td>
</tr>
<tr>
<td>Strauss et al. (84)</td>
<td>Paclitaxel + Carboplatin or Surgery</td>
<td>173</td>
<td>Ib</td>
<td>71% (4-year follow-up) 59% (p=0.028)</td>
</tr>
</tbody>
</table>
pemetrexed disodium exerts activity against a broad spectrum of malignancies (16). Initial phase I studies were done without vitamin supplementation, folic acid and vitamin B12, and suggested clinical efficacy, but with disturbing toxicity (17, 18). Trials initiated later included vitamin supplements and the toxicity profiles became acceptable (19, 20). Pemetrexed has been evaluated as a single agent in phase I studies, combined with paclitaxel and docetaxel (21, 22) and with gemcitabine/cisplatin (23) with acceptable toxicity profiles.

**Single agent studies.** Two phase II studies have been performed in patients with NSCLC using single agent pemetrexed disodium. Rusthoven and coworkers (24) included 33 chemotherapy-naïve patients in the first single agent trial, treating patients with 600 mg/m² intravenously for 10 minutes every 3 weeks. However, the initial dose was reduced to 500 mg/m² due to the fact that the first three patients experienced grade 3 neutropenia, grade 3 dyspnea and mucositis. Toxicity data showed that one patient developed grade 4 thrombocytopenia, 13 patients experienced grade 3 or 4 neutropenia and 4 patients experienced febrile neutropenia. Non-haematological toxicity was dominated by rash (13 patients; grade 3 rash), but in general the non-haematological toxicity was mild. The median follow-up period was 7.9 months. No complete responses were seen, however, 7 patients (23.3%) experienced a conformed partial response and the median duration of response was 3.1 months with a median survival of 8.9 months. No vitamin supplementation was given in this study. In another study, 59 chemotherapy-naive patients were included and treated with pemetrexed disodium at a dose of 600 mg/m² without vitamin supplementation(25). Twenty-five patients experienced grade 3 or grade 4 neutropenia, and 2 patients developed grade 3 infections. Eighteen patients developed grade 3 or 4 cutaneous toxicity, which improved following prophylactic oral dexamethasone administration. No complete responses were seen, however, 9 patients experienced partial responses and the median duration of response was 4.9 months. The median survival was 7.2 months.

**Combination studies.** Pemetrexed disodium has been combined in phase II studies with cisplatin, carboplatin, oxaliplatin, vinorelbine and gemcitabine in NSCLC.

**Cisplatin.** Two phase II trials including advanced disease patients combining pemetrexed (500 mg/m²) with cisplatin (75 mg/m²) every third week have been presented. The first study from Shepherd and coworkers (26) detected, among 31 chemotherapy-naive patients, 7 patients with grade 3 granulocytopenia and grade 4 in 4 patients, whereas one patient developed febrile neutropenia. The dominating non-haematological toxicity was mainly reported as grade 2 and 3 fatigue. Grade 3 nausea and emesis was reported in 2 patients and grade 3 and 4 diarrhoea in 3 patients, while 2 patients experienced grade 3 motor neuropathy. In 13 patients, a partial response was seen with a median duration of 6.1 months, and the median survival was 8.9 months. In the other study (27), 36 chemotherapy-naïve patients were included. Twenty-one patients experienced grade 3 or 4 granulocytopenia, however, no cases of infection or fever were reported. Non-haematological toxicity was dominated by nausea (2 patients). Antitumour activity, with a verified partial response, was seen in 14 patients and a median survival of 10.9 months was demonstrated.

**Carboplatin.** In 50 chemotherapy-naïve patients with advanced NSCLC, 92% stage IV patients, the effects of pemetrexed disodium 500 mg/m², vitamin supplementation, steroid supplementation and carboplatin AUC=6, day 1 every third week for a total of 6 cycles, were evaluated (28). Grade 3/4 neutropenia were seen in 34% of the patients. Thrombocytopenia and anaemia were recorded in 4% and 10%, respectively. Five out of 50 (10%) patients experienced grade 3/4 non-haematological toxicities. A partial response rate was recorded in 28% and stable disease was recorded in 48%. The median time to progression was 4.9 months. The authors estimated the median and 1-year survival to be 13.5 months and 55.3%, respectively. They concluded that this 3-week schedule is convenient and causes minimal non-haematological and haematological toxicities with promising efficacy, comparable with earlier reported pemetrexed/cisplatin data, but better tolerated.

**Carboplatin or oxaliplatin.** Scagliotti and coworkers (29) conducted a randomised phase II study in which patients were randomized to either pemetrexed disodium (500 mg/m² + vitamin supplementation and dexamethasone) + carboplatin (AUC=6) (PC) or pemetrexed disodium (500 mg/m² + vitamin supplementation and dexamethasone) + oxaliplatin 120 mg/m² (PO). Eighty chemotherapy-naïve patients with advanced NSCLC were included. In the PC arm, toxicity data showed that 26% of patients treated had grade 3 or 4 neutropenia, whereas the corresponding data for the PO arm was 7.3%. The dominating non-haematological toxicity in the PC arm was grade 1 and 2 nausea observed in 24 patients (62%), whereas 28 patients experienced grade 1-2 neuropathy in the PO arm. The overall response rate was 32% in the PC arm and 27% in the PO arm. The median survival figures were 4.5 months and 4.9 months, respectively.

**Gemcitabine.** In a multicentre study (30), 60 chemotherapy-naïve patients with advanced NSCLC were given gemcitabine 1250 mg/m² days 1 and 8 and pemetrexed
disodium 500 mg/m², day 8, supplemented with vitamins and steroids. The dominating haematological toxicity was grade 3 (29%) and grade 4 (34%) neutropenia, whereas 15% of the patients developed grade 3 or 4 neutropenic fever. Non-haematological toxicity was evident as grade 3 fatigue in 23% of the patients. Partial responses were seen in 17% with a median duration of response of 3.3 months. The median overall survival was 11.3 months.

In a randomized phase II trial (31) using three different schedules of pemetrexed disodium 500 mg/m² (pem) plus gemcitabine 1250 mg/m² (gem), patients were randomized to: a) pem followed by gem on day 1, gem on day 8 or b) gem followed by pem on day 1, gem on day 8 or c) gem on day 1, pem followed by gem on day 8. Toxicity data revealed a significant difference in the rate of grade 3 febrile neutropenia (a=5%, b=20%, c=5%). The patients treated according to schedule a showed less severe grade 4 toxicity than arm c (a = 40%, c = 50%). All patients included in the study were eligible for response. Patients treated according to schedule a experienced a partial response in 29%, whereas the corresponding values for arm c was 17%.

Second line treatment. In a large randomized phase III study (32), 571 patients with performance status 0-2, with one prior chemotherapy regime, received either pemetrexed disodium 500 mg/m² day 1 (with vitamin B12, folic acid and dexamethasone) every 21 days or docetaxel 75 mg/m² (and dexamethasone) every 21 days. Survival data was similar between the two groups, with a median progression-free survival of 2.9 months as well as a one-year survival rate of 29.7% for both arms. However, toxicity was concluded to be more pronounced in the docetaxel arm, especially concerning grade 3 or 4 neutropenia (40.2% vs. 5.3%) and neutropenia associated febrile episodes (12.7% vs. 1.9%) and infections (3.3% vs. 0%). A summary of studies involving pemetrexed is presented in Table III.

EGFR-targeted therapies

The development of tumour biology has greatly advanced our understanding of how our bodies function at the cellular level and, most importantly, has provided new tools to

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**Table III. All phase II and III studies investigating the role of pemetrexed.**

<table>
<thead>
<tr>
<th>Ref</th>
<th>Drugs</th>
<th>Stage IV</th>
<th>Grade 3/4 Grade 3/4 Dominating RR TTP Median OS</th>
<th>Neutropenia</th>
<th>Neutropenia</th>
<th>Thrombocytopenia</th>
<th>Toxicity</th>
<th>Rash</th>
<th>Skin tox.</th>
<th>Fatigue</th>
<th>Diarrhea</th>
<th>Rash</th>
<th>Skin tox.</th>
<th>Fatigue</th>
<th>Diarrhea</th>
<th>Rash</th>
<th>Skin tox.</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>PEM</td>
<td>76%</td>
<td>39%</td>
<td>13%</td>
<td>3%</td>
<td>Rash 39%</td>
<td>23%</td>
<td>3.1</td>
<td>9.6</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>25</td>
<td>PEM</td>
<td>66%</td>
<td>42%</td>
<td>42%</td>
<td>3%</td>
<td>Skin tox. 31%</td>
<td>16%</td>
<td>4.9</td>
<td>7.2</td>
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<tr>
<td>26</td>
<td>PEM/CIS</td>
<td>84%</td>
<td>23%</td>
<td>3%</td>
<td>3%</td>
<td>Fatigue 87%</td>
<td>45%</td>
<td>6.1</td>
<td>8.9</td>
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<tr>
<td>27</td>
<td>PEM/CIS</td>
<td>50%</td>
<td>59%</td>
<td>0%</td>
<td>17%</td>
<td>Diarrhea 3%</td>
<td>39%</td>
<td>NR</td>
<td>10.9</td>
<td></td>
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<tr>
<td>28</td>
<td>PEM/CARBO</td>
<td>92%</td>
<td>34%</td>
<td>NR</td>
<td>4%</td>
<td>Any 3/4 10%</td>
<td>28%</td>
<td>4.9</td>
<td>EST. 13.5</td>
<td></td>
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<tr>
<td>29</td>
<td>PEM/CARBO vs PEM/OX</td>
<td>64%</td>
<td>26%</td>
<td>3%</td>
<td>18%</td>
<td>Fatigue 8%</td>
<td>33%</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>30</td>
<td>PEM/GEM</td>
<td>87%</td>
<td>62%</td>
<td>17%</td>
<td>5%</td>
<td>Fatigue 23%</td>
<td>16%</td>
<td>3.3</td>
<td>10.1</td>
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<tr>
<td>31</td>
<td>PEM/GEM a*</td>
<td>NR</td>
<td>This arm closed at time of interim-analysis due to toxicity and low response rates.</td>
<td>a&gt;c</td>
<td>a&gt;c</td>
<td>a&gt;c</td>
<td>29%</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>31</td>
<td>PEM/GEM b**</td>
<td>NR</td>
<td></td>
<td>c&lt;a</td>
<td>c&lt;a</td>
<td>c&lt;a</td>
<td>17%</td>
<td>NR</td>
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</tr>
<tr>
<td>32</td>
<td>PEM/GEM c***</td>
<td>NR</td>
<td></td>
<td>c&lt;a</td>
<td>c&lt;a</td>
<td>c&lt;a</td>
<td>17%</td>
<td>NR</td>
<td>NR</td>
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</tr>
<tr>
<td>33</td>
<td>PEM vs DOCETAX</td>
<td>40%</td>
<td>13%</td>
<td>0.4%</td>
<td>2%</td>
<td>Fatigue 5%</td>
<td>2.9</td>
<td>2.9</td>
<td>7.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>DOCETAX</td>
<td>5%</td>
<td>16%</td>
<td>2%</td>
<td>2%</td>
<td>Fatigue 5%</td>
<td>2.9</td>
<td>2.9</td>
<td>8.3</td>
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</table>

**Abbrevations: PEM=pemetrexed (Alimta®), CIS=cisplatin, CARBO=carboplatin, DOCETAX= docetaxel, OX=oxaliplatin, NR=not reported, EST=estimated. ** Gemcitabine was administered before pemetrexed on day 1. ** Pemetrexed was administered before gemcitabine on day 1. *** Pemetrexed was administered before gemcitabine on day 8.
attack cancer. The proliferation and growth of cancer cells are known to be affected by a disturbed, but sophisticated, balance between a multitude of growth-promoting and-inhibiting factors. The malignant cell evades the normal physiological control. One important key driver for the development and progression of a number of solid tumours, including NSCLC, is the epidermal growth factor (EGF) and its receptor (EGFR). In lung cancer, abnormal regulation of EGFR occurs mainly in NSCLC, and EGFR is highly expressed at levels varying from 30 – 80% (33, 34). The over-expression of EGFR occurs independently of mutations in p53, RAS or other oncogenes. Deregulation in signal transduction in various parts of the EGFR pathway is implicated in the growth of tumour cells in NSCLC. Thus, the signal pathway involving EGFR is an attractive target for the treatment of NSCLC. Such therapies reduce the EGFR-signalling and, hopefully, abrogate the downstream pathways with subsequent apoptosis, and inhibition of angiogenesis and spread of tumour cells (34-36). Today there are two major approaches in clinical use that down-regulate EGFR-signalling, i.e. anti-EGFR antibodies and EGFR tyrosine kinase inhibitors (EGFR-TKIs).

**Anti-EGFR monoclonal antibodies.** Cetuximab (Erbitux®), a chimeric antibody, is approved in combination with irinotecan in some countries for the treatment of chemotherapy-resistant colorectal cancer. In order to reduce the immunogenicity, the constant region of the original mouse mAb was replaced with the constant region of a human immunoglobulin. However, severe fatal anaphylactic reactions have occurred due to the formation of human anti-mouse antibodies (35). These problems are mainly associated with the first administration of cetuximab, so that a test dose is used prior to the initial loading dose, which reduces, but does not abolish, the number of patients at risk for hypersensitive reactions. Nevertheless, this risk must be considered when using cetuximab. Other toxicities seen are diarrhoea and skin rash. A fully humanised EGFR monoclonal antibody, panitumumab, is under clinical evaluation.

The experience of using cetuximab in NSCLC is still limited. In a randomised phase II study in chemo-naive patients with EGFR-positive tumours, cetuximab plus cisplatin and vinorelbine displayed an overall response rate of 35%, median survival of 8.3 months and 1-year survival of 32%. The corresponding figures in a parallel treated control group were 28%, 7.0 months and 26%, respectively (37). Ongoing phase II evaluations indicated an overall response rate around 6% and stable disease in approximately 20% of the treated EGFR+ patients in previously chemotherapy-treated patients (38).

**Small-molecule EGFR inhibitors.** In parallel with the development of antibodies against EGFR, a large number of small molecules that interact with EGF signal pathways have been developed and many of these agents are under evaluation in the clinic. Among these, the EGFR-tyrosine kinase inhibitors gefitinib and erlotinib are the most advanced in development. Gefitinib (Iressa®) is now approved in over 30 countries for the treatment of advanced NSCLC following failure of chemotherapy and has been used in the treatment of almost 200,000 patients worldwide.

The important advantages of EGFR-TKI, so far observed in the clinic, are the wide therapeutic margin and low frequency of adverse effects compared to other treatment approaches. Side-effects associated with gefitinib are generally grade 1 or 2, dominated by diarrhoea in approximately 40% and skin rashes in 45% of the treated patients. The incidence of grade 3 and 4 adverse events are low. In two large well controlled phase 2 studies (IDEAL 1 and 2), gefitinib monotherapy was shown to induce tumour responses in the order of 12 – 18% in previously heavily chemotherapy-treated patients (39, 40). A dose of 250 mg daily was found to be an optimal biological dose in lung cancer since this dose had efficacy comparable to higher doses but was better tolerated by the patients. Almost 30% of patients who received gefitinib 250 mg daily were alive 1 year after starting gefitinib treatment in several evaluations worldwide, including treatment in a real-life setting based on more than 21,000 patients treated with a compassionate use basis in the US (41). Efficacy has also been seen in patients with poor performance status, who are often considered unsuitable for treatment with chemotherapy (42). In addition, symptoms associated with lung cancer were significantly improved by this treatment in 40% of the patients (39, 40). Erlotinib (Tarceva®) has, in general, displayed comparable effects to those observed with gefitinib, however, diarrhoea, the dose-limiting toxicity, required loperamide administration (43). The only well performed phase 3 study with EGFR-TKI so far presented showed that erlotinib 150 mg daily was superior to placebo in a subpopulation of 731 patients with advanced NSCLC that had progressed after previous chemotherapy (44). Erlotinib produced a response rate of 9%, and overall survival (median survival 4.7 vs. 6.7 months) and progression-free survival were significantly better. Fatigue and skin rash were the most common adverse effects associated with erlotinib, which occurred in 79% (19% grade 3/4) and 76% (9% grade 3/4), respectively, of the patients. It is obvious from the clinical practice that only a subpopulation of the NSCLC patients responded to EGFR-TKI treatment. The reason why some patients benefit from EGFR-TKI while others do not is thus of major interest in the clinical use of these drugs. Some studies have suggested that female non-smokers with adenocarcinomas are those who benefit. The IDEAL studies indicated that Japanese patients responded better than non-Japanese, but bias of
baseline factors between strata, not ethnicity, is thought to account for the difference (39). However, owing to the paucity of current experience with gefitinib, and of its closely related family member erlotinib, the possibility of differences in toxicity and efficacy due to ethnicity cannot be ruled out. A most interesting explanation to variations in responses for EGFR-TKI was recently reported. Different, but specific, somatic mutations within the TK domain of the EGFR appear to correlate with dramatic tumour responses to both gefitinib and erlotinib among patients with NSCLC (38, 45-47). However, mutations have also been seen in a few patients denoted as non-responders, which might indicate that certain mutations could explain resistance to EGFR-TKIs (45). Moreover, these mutations appeared to be more common in Japanese patients than in non-Japanese. Although these data are very compelling, further studies are needed in a large group of patients, to evaluate whether these mutations are seen in patients who benefit from stable disease or symptomatic improvement following EGFR-TKI treatment. Initial clinical reports indicate that EGFR mutations may not be of importance when using anti-EGFR antibodies (48). Although EGFR mutations are of importance to explain responses to EGFR-TKIs, other factors must be of considered. The signal pathways in the EGFR-mediated stimulation of cancer cells contain a lot of molecules that could interact with the response to EGFR-TKIs. In a recent publication, it was shown that Akt-positive status and EGFR-positive status predicted a higher response rate and time to progression with gefitinib treatment in NSCLC patients (49). The proteomic pattern of tumours also seems to be of great importance to predict a good response. Nevertheless, the real clinical significance of all these potential biomarkers for predicting responses to EGFR-TKIs must undergo meticulous prospective validation in well controlled studies before they can be used in the clinic. Chemotherapy has for a long time been the only treatment option for patients with advanced NSCLC, and currently the only approved cytotoxic drugs for previously treated relapsing NSCLC are docetaxel and pemetrexed. These drugs, given as i.v. injections on an outpatient basis, offer a tumour response around 10% with a median survival of 12.6 months and an overall survival of 12 months) using gefitinib in this study. Another issue of importance to evaluate is the scheduling of EGFR-TKIs in relation to chemotherapy. Preclinical investigations and also some retrospective evaluations of early clinical studies suggest that EGFR-TKIs are able to sensitize for chemotherapy and radiotherapy. Hence, it could be of interest to evaluate pre-chemotherapy treatment with EGFR inhibitors in the early treatment of NSCLC. An early study combining the anti-VEGF antibody bevacuzimab with erlotinib displayed encouraging results with a median survival of 12.6 months and an overall response of 20% in 34 previously treated non-squamous cell NSCLC patients. Further studies are awaited in which different novel approaches have been combined.

**Combination of EGFR inhibitors and other modalities in NSCLC.** Preclinical studies and some early clinical evaluations have shown that EGFR inhibitors can enhance the efficacy of both radiation and chemotherapy, suggesting a potential role for these drugs as an adjunct to the current combined-modality approach for the treatment of localised advanced NSCLC. Therefore, clinical evaluations have recently been initiated to address the potential value of the addition of EGFR inhibitors to the routine management of stage III NSCLC, such as using these drugs in a neoadjuvant setting. The issue of exactly how to combine EGFR-TKIs and chemotherapy is controversial. Four large randomised well controlled phase III studies in previously untreated patients, in which gefitinib or erlotinib were concomitantly given with various platinum-containing doublet chemotherapy regimens, failed to demonstrate any benefit at all for EGFR-TKIs (51-54). There was no obvious explanation for these disappointing and surprising results. Recently, and most interestingly, in one of these negative trials, a higher response rate was shown in those patients with EGFR mutations than those lacking these mutations (45).

Thus, it is of definite clinical interest to evaluate EGFR-TKIs in the first-line setting or as an adjunct to other treatment approaches in patients with EGFR mutations. Such trials are ongoing. Encouraging results have been encountered in a prospective trial of 138 eligible patients with advanced bronchoalveolar carcinoma. The results which demonstrated 19% tumour response (6% CR and an overall survival of 12 months) using gefitinib in this extremely chemotherapy-resistant subtype of NSCLC (55). Female gender, never-smokers, patients developing rash, PS 0 or 1, all had better survival than their counterparts. Almost 50% of mutations in the EGFR gene were found in this study. Another issue of importance to evaluate is the scheduling of EGFR-TKIs in relation to chemotherapy. Preclinical investigations and also some retrospective evaluations of early clinical studies suggest that EGFR-TKIs are able to sensitize for chemotherapy and radiotherapy. Hence, it could be of interest to evaluate pre-chemotherapy treatment with EGFR inhibitors in the early treatment of NSCLC. An early study combining the anti-VEGF antibody bevacuzimab with erlotinib displayed encouraging results with a median survival of 12.6 months and an overall response of 20% in 34 previously treated non-squamous cell NSCLC patients. Further studies are awaited in which different novel approaches have been combined.

Other EGFR interacting drugs. There are also other EGFR-inhibiting drugs in early clinical evaluation. Many of these drugs are various TKIs that interact with both EGFR1 and erB2 and have indicated some promising effects. In addition to the above discussed strategies, other studies are ongoing, using antisense oligonucleotides and toxins...
attached to EGFR ligands or antibodies. However, it is too early to give a realistic view about the value of these approaches in the clinical setting.

**Drugs targeting different levels in the angiogenic process**

Angiogenesis is a process under tight regulatory control and present under normal conditions as, for example, wound healing, the menstruation cycle and ovulation (56). The tight regulatory control of angiogenesis has been postulated to be balanced between angiogenic stimulators and angiogenic inhibitors (57). It is initiated by the release of proteases from activated endothelial cells, leading to degradation of the basement membrane, migration of endothelial cells into the interstitial space, with subsequent endothelial cell proliferation and differentiation into mature blood vessels (58, 59). Each of these processes is tightly regulated through the complex interplay of endogenous factors that promote and inhibit angiogenesis. As discussed earlier, less than half of the patients who have undergone complete resection are cured and the pattern of failure depends on the stage of the disease (3). As a consequence, it is reasonable to hypothesize that the inhibition of endothelial proliferation or sprouting could be a challenging approach to cure, or at least make the disease “dormant” in these patients. Studies published or presented with an anti-angiogenic approach in phase II/III settings are described below. Several agents targeting angiogenesis have been developed and can be grouped into a few categories based on their mechanisms of action.

**Matrix remodelling and tumour invasion.** BAY12-9566 is one of a series of matrix metalloproteinase inhibitors (MMPIs) selected for inhibition of MMP-2, -3 and -9. In the H-23 human lung cancer xenograft model, BAY12-9566 together with cisplatin caused an increased number of complete responses compared to cisplatin alone (60). In two independent multicenter phase III trials, 474 patients with unresectable lung cancer (327 SCLC and 147 NSCLC) were randomised to receive 800 mg of BAY12-9566 orally twice a day or matched placebo at the time of completion of initial therapy. There was no significant difference in survival for patients with SCLC or NSCLC receiving BAY12-9566 or placebo. However, the drug had a different impact on disease progression depending on histology. BAY12-9566 significantly prolonged TTP in NSCLC, while it shortened significantly the disease progression in SCLC. There was also a higher incidence of adverse events in the BAY12-9566 groups and, because of this, the study was stopped prematurely (61).

BMS-275291 is a p.o. bioavailable, sulfhydryl-based potent inhibitor (nM) of the activities of MMP-1, MMP-2, MMP-7, MMP-9 and MMP-14 (62). In a phase III randomized double-blind trial recently presented, 774 chemotherapy-naive patients with Stage IIIB/IV NSCLC received paclitaxel (225 mg/m²) + carboplatin (AUC 6) i.v. q21d (max 8 cycles) +/- BMS-275291, 1200 mg p.o. daily or placebo until disease progression. Since an interim analysis revealed no survival advantage and an increased toxicity in the experimental arm was found, the study treatment was stopped (63).

**Marimastat (BB-2516),** a compound structurally related to batimastat, is an MMP inhibitor which is orally bioavailable. *In vitro* it inhibits a variety of MMPs including collagenase 3 (MMP-13), gelatinases A and B (MMP-2 and -9), metalloelastase (MMP-12) and stromelysin-1 (MMP-3) (64). A phase III randomized, double-blind study compared the effect of marimastat versus placebo, taken twice daily, in patients with Stage III NSCLC who had minimal residual disease following chemotherapy, radiotherapy, and/or surgery had just been stopped. This study might answer the question of whether MMPIs have a role in the minimal residual disease, as implicated in the pre-clinical studies.

**Prinomastat (AG3340),** displays activity mainly against MMP-2 and MMP-9 (65). One phase III study investigated prinomastat in combination with paclitaxel (200 mg/m²) and carboplatin (AUC6) in 686 chemotherapy-naïve patients with NSCLC (66). Data from 677 patients displayed differences among the treatment arms in overall or 1-year survival, progression-free survival (PFS), symptomatic PFS or response rate. Musculoskeletal effects were the only adverse experiences, with time- and dose-relationship to prinomastat. Another randomized phase III study compared gemcitabine (1250 mg/m² i.v. days 1 and 8) and cisplatin (75 mg/m² i.v. day 1) +/- prinomastat. A q3wk schedule was used. The duration of treatment with Gem/Cis and incidence of Gem/Cis-related toxicities were comparable in both arms. Prinomastat-related adverse experiences mostly originated from the musculoskeletal system. The addition of prinomastat to the platinum-doublet added no survival benefit compared with placebo. Nor did the progression-free survival and response rate increase with the addition of prinomastat (67). Thus, it seems that MMPIs are not likely to contribute significantly to the treatment of advanced cancers, though they might be expected to have a more favourable effect in the adjuvant setting. Furthermore, the toxicity of the second generation MMPIs hampers the long-term administration that would be necessary for adjuvant treatment. However, new MMPIs are under investigation and hopefully they will prove to be more useful as adjuvants.

**Effects on endothelial effects.** Bevacizumab (Avastin®) is a recombinant, humanised anti-VEGF MAb, where the amino acid sequence of the monoclonal antibody is 93% of human
origin and 7% murine (68). In a phase II trial, two doses (15 mg/kg compared to 7.5 mg/kg) combined with carboplatin (AUC= 6) and paclitaxel (200 mg/m²) every 3 weeks resulted in a higher response rate, longer median time to progression and a modest increase in survival compared with Carb/Tax alone. Bleeding was the most prominent adverse event and was manifested in two distinct clinical patterns; minor mucocutaneous hemorrhage and major hemoptysis, which in turn was associated with squamous cell histology, tumor necrosis and cavitation, and disease location close to major blood vessels. The authors’ conclusion was that bevacizumab added to carboplatin and paclitaxel schedules improved overall response and time to progression in patients with advanced or recurrent NSCLC. Patients with non-squamous cell histology appeared to be a subpopulation with improved outcome and acceptable safety risks (69). Bevacizumab have also been investigated in combination with the tyrosine kinase inhibitor erlotinib (Tareceva®). In the phase I/II setting, Sandler and coworkers (70) demonstrated that the data did not indicate a pharmacokinetic interaction between the two agents. Seven patients (17.5%) had partial responses, 2 (5%) had minor responses and 14 (35%) had stable disease and the median overall survival and time to progression for patients on the phase II dose level (n=31) was 9.3 and 4.6 months, respectively. Based on the data presented, the authors concluded that the encouraging antitumour activity and favourable safety profile of this combination in advanced NSCLC warrants further investigations in larger randomized studies.

Squalamine, an aminosterol, was originally isolated as a low molecular weight antibiotic factor that exhibited potent bactericidal activity (71). Later, this compound’s anti-angiogenic properties were investigated (72), and squalamine was demonstrated to inhibit angiogenesis and solid tumour growth. Furthermore, the compound blocked the mitogen-induced proliferation and migration of endothelial cells, preventing tumour neovascularisation (72). One randomized phase II study compared a weekly or a three-weekly addition of squalamine to weekly carboplatin (AUC2) and paclitaxel (75 mg/m²), for 12 weeks. They demonstrated that the weekly infusion of squalamine was better tolerated, however this "metronomic" dosing approach did not improve efficacy in the treatment of advanced NSCLC (73).

In the beginning of the era of the angiogenesis field, compounds such as endostatin, angiostatin and kringle-5 emerged as candidates for the treatment of cancer. To our knowledge, none of these compounds have been tested in phase II/III in NSCLC patients. All of the above-discussed drugs are mentioned to be administered continually over months or years, resulting in a substantial increment of drug-related expenses. However, recently a new class of drugs, mechanistically known as vascular-disrupting agents (VDAs), can be delivered in intermittent doses, which seems more interesting in the view of cost-benefit.

This alternative approach involves the use of therapeutic agents to preferentially destroy the established tumour vessel network (74) and currently one drug, combretastatin A4 disodium phosphate (CA4DP), has reached the phase II setting. The most attractive characteristics of this agent are the induction of antivascular and antitumour effects at doses that are less than one-tenth of the maximum tolerated dose and the selective shutdown of blood flow in the tumour, with the effects observed in critical normal tissue being limited or non-existent (75-78). However, further studies regarding lung carcinomas are eagerly awaited and only then will any firm conclusions regarding the impact in NSCLC could be drawn.

Conclusion

In the present review, data concerning pemetrexed, EGFR-TKIs and anti-angiogenic treatment approaches have been reviewed. The use of these compounds in the neoadjuvant setting needs further study so, for the present, the use of these compounds should not be recommended in the neoadjuvant setting, outside clinical study protocols.

References


Received November 2, 2004
Revised February 28, 2005
Accepted March 7, 2005