Targeted Therapies for Non-Small Cell Lung Cancer

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Abstract: Conventional therapy for non-small cell lung cancer (NSCLC) has reached a plateau in increasing patient survival and overall prognosis still remains dismal. Advances in the knowledge of molecular events governing oncogenesis has led to a number of novel agents targeting specific pathways critical for tumour growth and survival. In the present paper we have thoroughly reviewed the existing evidence of novel agents currently studied in clinical trials, focusing on epidermal growth factor receptor family inhibitors, angiogenesis inhibitors, cyclooxygenase-2 inhibitors, Bcl-2 targeted agents, protein kinase C inhibitors, proteasome inhibitors, farnesyl transferase inhibitors and retinoids. Although erlotinib monotherapy in the second or third line setting and bevacizumab combined with conventional chemotherapy as a frontline therapy manage to prolong the life of patients with NSCLC, there is still much to be learned about the proper design of clinical trials and the selection of patient population enrolled in them. Multi-targeted therapy still remains the most attractive avenue for future treatment strategies.

Key Words: NSCLC, targeted therapy, EGFR inhibitors, angiogenesis.

INTRODUCTION

More than 80% of lung carcinomas are non-small cell lung cancers (NSCLCs) and approximately 60% of the patients have advanced disease at the time of diagnosis [1,2]. Systemic chemotherapy alone or in combination with radiotherapy failed to improve patients overall survival and their prognosis still remains dismal with a 5 year survival rate being below 5% in stage IIIB/IV [3]. At the same time both toxicities and drug resistance limit the use of conventional therapy. Under these circumstances the development of new treatment strategies was obligatory.

We are now living in an exciting new era where the understanding of tumour biology and oncogenesis has begun to evolve. Molecular techniques have allowed insight into specific biological pathways involved in tumourigenesis and these same techniques have developed a cascade of novel therapies directed towards molecular factors critical to the pathogenesis of cancer growth and survival. Due to the targeted mechanism of action, these therapies are more specific, more sensitive and less toxic to noncancerous cells.

In the present paper, we have reviewed the existing evidence of novel agents now studied in clinical trials emphasizing on epidermal growth factor receptor family inhibitors, angiogenesis inhibitors, cyclooxygenase-2 inhibitors, Bcl-2 targeted agents, protein kinase C inhibitors, proteasome inhibitors, farnesyl transferase inhibitors and retinoids.

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) FAMILY INHIBITORS

The EGF family of receptors consists of four structurally similar members including EGFR- (ErbB1/HER-1), HER-2/neu (erbB2), HER3 (erbB3) and HER4 (erbB4) [4]. EGFR is the first receptor discovered which has two predominant ligands, the EGF and the transforming growth factor-a (TGF-a) [5]. This receptor crosses the cell membrane and has two portions; an extracellular portion that is the ligand-binding side and an intracellular portion that has a tyrosine kinase activity [6,7]. Activation of the receptor by the ligand leads to an autophosphorylation of the tyrosine residues in the intracellular portion. This, in turn, leads to a sequential activation of molecules that mediate the transduction of a signal downstream towards the nucleus and activate gene transcription and consequently, oncogenesis. Three major pathways are involved in the HER-1 signal transduction: (1) The mitogen-activated protein kinase (MAPK) which is strongly connected with the transduction of proliferation signals, (2) The phosphatidylinositol –3-OH kinase (PI3K/Akt) which is a major antiapoptotic pathway and (3) The signal transducer and activator of transcription (STAT) - mediated pathways (Fig. **1**). These multi-complex signal cascades involve the interaction or dimerization with other erbB-receptor types, the interactions with signals from heterologous pathways such as stress inducers, hormones and lymphokines, and the complex activation and inhibition of all intermediate molecules.
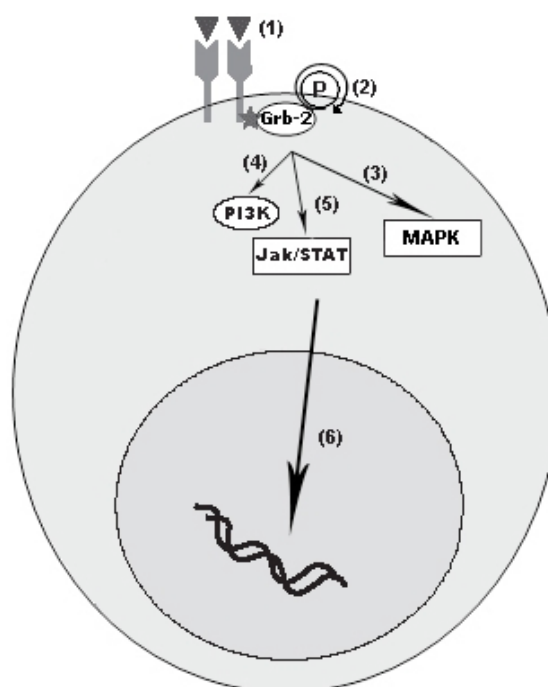

Fig. (1). The major signaling pathway following EGFR activation.

Ligand binding to EGFR (1) leads to autophosphorylation in the intracellular portion (2). Three pathways are subsequently involved: the MAP kinase pathway (3), the PI3K/Akt pathway (4) and the STAT mediated pathway (5). The transduction of the signals to the nucleus leads in upregulation of genes that are involved in oncogenesis (6).

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It is suggested that there is both an overexpression and an abnormal activation of EGFR in many solid tumours, which enhance their growth and survival, leading to a poorer outcome [8,9]. Overexpression of EGFR has been observed in 43-83% of NSCLC [10,11]. The lack of a gold standard method in detecting EGFR expression can explain the variety of levels reported [12]. Most of the available data are based on immunohistochemistry (IHC) in which direct evaluation of the target can be achieved on resected surgical specimens. In addition, EGFR promotes invasiveness through activation or downregulation of matrix metalloproteinases (MMPs) and interacts with the integrin pathway, which is crucial for the tumour to invade local tissues [13,14]. On the other hand, HER-2/neu has a modest expression in NSCLC with expression levels in squamous cell histology of less than 5% [15].

Anti-EGFR Monoclonal Antibodies

Monoclonal antibodies directed against the extracellular portion of EGFR, block ligand binding and inhibit the activation of the EGFR pathway [16]. Cetuximab (IMC-C225, ERBITUX®, Merck KGaA, Darmstadt, Germany) is a monoclonal chimeric human-mouse IgG1 monoclonal antibody that has a higher binding affinity to HER-1 than other ligands (EGF, TGF-a). Cetuximab competes with ligands for receptor binding causing internalization of the receptor and promoting inhibition of angiogenesis and enhancement of apoptosis [17]. An interesting antitumour activity was suggested when this antibody was studied in preclinical models causing EGFR antagonism and tumour regression [18]. Synergistic tumour growth inhibition was also demonstrated when cetuximab was combined with chemotherapy and radiotherapy in preclinical models [19,20].

Two phase I pharmacokinetic studies in patients with solid tumours supported a weekly regimen between 250 and 400 mg/m^2 [21,22]. Most of the patients developed grade 1 or 2 skin rash. In another multicentre phase I trial in patients with EGFR-expressing tumours, cetuximab was examined with or without cisplatin [23]. There was no pharmacokinetic alteration of cetuximab from cisplatin and only one of the 52 patients developed antibodies to cetuximab. Grade 3 toxicities of the above combination were dyspnea, diarrhea, epiglottitis, and anaphylaxis. In a phase I/II study, cetuximab was administered with paclitaxel and carboplatin in 31 untreated patients with stage IV NSCLC [24]. The most common toxicity observed was rash in 84% of the patients (grade 3 in 13%). Objective response (OR) was seen in 8 patients (26%), median time to progression was 5 months, median overall survival was 11 months and the first- and second- year survival rates were 40% and 16%, respectively.

In another phase I/IIa study, cetuximab was combined with carboplatin and gemcitabine in 35 chemotherapy-naïve patients with stage IV NSCLC [25]. The major toxicities related to cetuximab were mild to moderate, presenting either as an acne-like rash (88.6%), skin disorders, dry skin, asthenia, mucositis, fever, chills, or nausea and vomiting. Partial responses (PRs) were observed in 28.6% of the patients. In addition, 60% of the patients had stable disease (SD). The median time to progression was 165 days and the median overall survival was 310 days.

A randomized phase II trial studied the combination of cisplatin and vinorelbine with and without cetuximab in the first-line setting of patients with advanced NSCLC [26]. Forty-three patients were randomized in each arm and 90% of the patients had EGFR-expressing tumours. There was a higher incidence of acneiform rash, diarrhea, asthenia, leukopenia, thrombocytopenia, and infection in the cetuximab arm. The response rate (RR) and median survival time were 31.7% versus 20% and 8.3 months versus 7.0 months in favour of cetuximab, with no statistical significance reported. Patients younger than 60 with normal lactate dehydrogenase (LDH) level and adenocarcinoma histology had a better benefit from the addition of cetuximab to cisplatin/vinorelbine.

The combination of cetuximab with docetaxel in EGFR-positive patients with recurrent NSCLC was examined in another phase II study [27]. The most common grade 3 toxicities were rash, infection, and fatigue. The RR was 28% and in one patient, a complete response (CR) was achieved. Nearly 66% of the patients had SD with this combination regimen.

Finally, in another single-arm phase II trial, cetuximab was examined in 66 patients with relapsed NSCLC who had failed at least 1 regimen [28]. The RR was 3.3% and 28% had SD. The median survival was 8.1 months and median time to progression was 2.3 months. Cetuximab was well tolerated with rash being the most common adverse effect reported (77%, 6.1% with grade 3). The cetuximab-related acneiform rash, which is the commonest adverse effect observed so far, possibly predicts response and prolonged survival [29]. The dosing regimen of cetuximab in clinical studies is a loading dose of 400 mg/m² followed by a maintenance dose of 250 mg/m²/weekly. The role of cetuximab combination with radiotherapy for local disease control is being studied in the RTOG phase II trial 0324.

Panitumumab (ABX-EGF, Abgenix and Immunex, Philadelphia, USA) is a fully humanized IgG2 monoclonal antibody, which has a higher affinity for EGFR than cetuximab [30]. Activity of panitumumab, in combination with cytotoxic agents or as a single agent, has been documented for a wide range of human cancer cell lines *in vivo* and *in vitro* [31]. Its action in xenograft tumour models is quite promising but without any effect on EGFR-negative tumours [32]. In a phase I study, ABX-EGF was well tolerated and its biologic activity, evidenced by a dose dependent acneiform reversible rash, was observed at a dose level of 1.0 mg/kg [33]. The most common adverse effect, which occurred in another phase I trial, also involved skin toxicity [34]. Among the 43 patients of this study, 2 PRs and one minor response (MR) were demonstrated. From the 4 patients who experienced SD, 2 had NSCLC.

The combination of ABX-EGF with paclitaxel and carboplatin in 19 EGFR-overexpressing patients with advanced NSCLC is currently under study in a phase II trial and until now, 1 CR and 4 PRs have been observed [35]. Skin rash was the most common toxicity reported, which did not appear to be dose-related. There was no pharmacokinetic interaction between chemotherapy and this novel agent.

EGFR oral Tyrosine Kinase Inhibitors (EGFR-TKIs)

Two agents are mainly available for clinical trials in this class of tyrosine kinase inhibitors (TKIs), gefitinib (ZD1839, Iressa^{TM}, AstraZeneka, London, UK) and erlotinib (OSI-774, Tarceva^{TM}, Roche, Basil, Switzerland). Both agents belong to the quinazolamine class and inhibit the EGFR by directly blocking the phosphorylation of the intracellular receptor tyrosine kinase, preventing its activity and subsequently, decreasing cellular proliferation, increasing apoptosis and inhibiting angiogenesis. After failure of chemotherapy, gefitinib and erlotinib are able to induce responses in approximately 10% of Caucasian patients and 25-30% of Japanese patients (gefitinib only) with NSCLC tumours [36].

Gefitinib

When gefitinib was studied in a wide range of human tumour xenografts, an antitumour activity was postulated [37]. In phase I trials, gefitinib was well-tolerated achieving objective antitumour responses when used as a single agent in refractory NSCLC patients [38-40]. An acneiform skin rash, diarrhea, asthenia, nausea and vomiting were the most common toxicities. Dose limiting toxicities (DLTs) including reversible rash and diarrhea occurred at daily doses of 700 to 800 mg. In one of these trials, 10% of the 100 NSCLC patients achieved PRs and 13% had disease stabilization [39]. Antitumour activity was reported in all dose levels, without a clear dose-response relationship, and 28% of the patients remained on gefitinib for at least 3 months and 20% for at least 6 months.

The role of gefitinib, as a monotherapy, in preventing brain metastasis was also investigated and showed promising anticancer activity [41]. These encouraging results led to phase II trials in patients with advanced disease who exhibited progression with standard platinum chemotherapy. In the IDEAL1 and IDEAL2 trials, two dosages (250mg and 500mg daily) of gefitinib were administered. Response rates were 18,4% and 11,8% in the low dose group respectively, with an overall symptomatic improvement in approximately 40% of the patients [42,43]. Side effects were mild and included rash, pruritus, and diarrhea which were more commonly seen in the higher dose group. Given the similar response rates of the two dose level groups, the recommended dosage for gefitinib was established at 250 mg/daily.

Based on the results of phase II trials, two phase III clinical trials (INTACT1 and INTACT2) were launched to study the combination of gefitinib with chemotherapy. In the INTACT 1 study, 1,093 chemotherapy-naïve patients with advanced NSCLC were randomized to receive gefitinib in combination with cisplatin and gemcitabine, or cisplatin/gemcitabine with placebo [44]. All patients received up to 6 cycles of chemotherapy with either 250mg gefitinib, 500mg gefitinib, or a placebo. No significant toxicity was reported. Median survival times were identical between the two gefitinib groups (9.9 and 9.9 months) and even slightly better in the placebo group (10.9 months). Median times to progression were also insignificantly different (5.8 months for the 250mg/d dosing, 5.5 for the 500 mg/d dosing, and 6.0 months for the placebo). Response rates varied slightly at 50.3%, 49.7%, and 44.8%, respectively.

In the INTACT 2 study, 1,037 patients with advanced NSCLC were randomized to receive paclitaxel and carboplatin plus either gefitinib 250 mg/d, or gefitinib 500 mg/d, or a placebo [45]. Again, there was also no difference in overall survival (median 9.8, 8.7, and 9.9 months, respectively). In gefitinib patients, dose-related diarrhea and skin toxicity were observed. A trend towards improved survival in patients with adenocarcinoma histology was also demonstrated. Gefitinib was better tolerated in both trials at a dose regimen of 250 mg/d with fewer dose reductions and treatment interruptions.

The possible role of gefitinib as a second- or third-line treatment for patients with advanced NSCLC who were refractory or intolerant to their latest chemotherapy regimen was examined in a phase III clinical trial (ISEL trial) [46]. In this study, 1692 patients were randomized to receive either gefitinib (n=1129) or a placebo (n=563). After a median follow-up of 7 months, the investigators reported no difference in overall survival (5.6 months for gefitinib and 5.1 months for placebo). Longer survival on the gefitinib arm was observed in patients with Asian ethnicity (n=342, hazard ratio [HR]=0.66, p=0.01) or who were nonsmokers (n=375, HR=0.67, p=0.01).

The use of gefitinib as a maintenance therapy after systemic therapy has been completed in the phase III trial SWOG (South-West Oncology Group) 0023 [47]. This study involved patients with stage IIIA/B NSCLC who were randomized to receive either gefitinib or placebo after completing the SWOG 9504 phase II study [48]. In the SWOG 9504 regimen, patients received cisplatin plus etoposide with concurrent thoracic radiation therapy followed by docetaxel in stage IIIB patients. The study was designed to demonstrate a 33% increase in median survival time in the gefitinib group over the estimated 21-month survival of the control group. SWOG 0023 was closed early, after the results of the ISEL trial, with 575 patients of the planned 840 who registered. An interim analysis of 263 patients suggested that gefitinib maintenance could not have possibly improved survival (p=0.0015) even if this trial was completed to accrual. Grade 3 or 4 pneumonitis was more common during docetaxel treatment (8% of the patients) as well as in the gefitinib arm (3% of the patients compared with 0% in the placebo arm).

A randomized trial of gefitinib *versus* observation only in surgically resected patients stage I-III is now ongoing by the NCI-Canada (National Cancer Institute) joined by EORTC and North American cooperative groups. A potential role of TKIs as radiation sensitizers in treating stage III NSCLC is under study.

Erlotinib

Erlotinib showed *in vivo* and *in vitro* activity when tested in preclinical trials in several human cancer cell lines, including NSCLC [49,50]. In two phase I trials, an interesting antitumour activity was demonstrated and erlotinib was evaluated using different doses and schedules [51,52]. The regimen that was selected for further evaluation was daily administration of 150mg. Higher doses resulted in a dose-limiting acneiform rash and diarrhea [52]. Other adverse effects reported were mild to moderate nausea and vomiting, headaches, mucositis, and elevation in bilirubin.

At the same time, encouraging results were observed in an erlotinib phase II trial [53]. The overall response rate was 12.54% (26.3% had an additional SD), the median survival was 9 months and the one-year survival rate was 40%, with EGFR expression being a requirement for entry into the study, unlike the gefitinib studies. Response to erlotinib was not associated with EGFR expression level. Erlotinib was generally well tolerated and the most common toxicity was an acneiform rash (78% of the patients). In another phase II trial of erlotinib in patients with bronchoalveolar carcinoma (BAC), 26% of the patients experienced an objective response [54]. The presence of rash in erlotinib patients may indicate response and improved survival, like cetuximab patients [55,56].

Based on the results of the above studies, two phase III clinical trials (TALENT and TRIBUTE) studied the possible role of erlotinib in combination with chemotherapy. In the TALENT study, 1172 previously untreated patients with advanced NSCLC patients were treated with six cycles of cisplatin/gemcitabine and either erlotinib or placebo [57]. Erlotinib was continued until disease progression. Unfortunately, the combination of erlotinib with platinum-based chemotherapy did not significantly prolong overall survival. In the parallel study, TRIBUTE, 1059 patients were randomized to receive either erlotinib or placebo combined with carboplatin and paclitaxel followed by maintenance monotherapy with erlotinib [58]. Median survival was 10.6 versus 10.5 months for the erlotinib and placebo group, respectively (p=0.95). There was also no difference in OR and median time to progression. Skin toxicity and diarrhea were the main toxicities in the erlotinib group.

An interesting observation from the TRIBUTE study was that never smokers in the erlotinib group had a median survival twice as long as those on chemotherapy alone [59]. Several possible explanations have been postulated so as to interpret these negative results of the concurrent combination of both TKIs with conventional chemotherapy: (a) The concurrent use of TKIs with chemotherapy may result in an antagonistic effect, (b) TKIs and chemotherapy may be targeting the same cell population, (c) Neoplastic cells may use alternative pathways as escape mechanisms, or (d) Clinical trials are made in an unselected population [60-62].

Of vital importance is the result of a phase III randomized placebo-control trial (BR.21 study) in which erlotinib was administered to patients with advanced NSCLC (stage III/B and IV) after failing one or two lines of chemotherapy [63]. In this study, 731 patients were randomly assigned in a 2:1 ratio for erlotinib, to receive either oral erlotinib or placebo. The overall response rate to erlotinib was 8.9% and less than 1% in the placebo group (p<0.001) with a median duration of response 7.9 months and 3.7 months, respectively. Overall survival was 6.7 months for the erlotinib group versus 4.7 months in the placebo group (p<0.001). The most common adverse effects in the erlotinib group were skin toxicity, diarrhea, and infections. Twenty-six patients (5%) discontinued erlotinib due to drug-related toxic effects compared to 4 patients

(2%) who received placebo. In a multivariate analysis, objective response was significantly associated with never smoking status, adenocarcinoma histology and expression of EGFR by IHC. This is the first study demonstrating that EGFR-TKIs may prolong survival after chemotherapy in NSCLC.

Other EGFR-TKIs

CI-1033 (Pfizer Global Research and Development, Ann Arbor, MI) is an irreversible pan-EGFR inhibitor having synergistic action with both chemotherapy and radiotherapy in phase I trials [64,65]. A phase II randomized trial comparing different schedules and doses of CI-1033 in NSCLC patients entering the second-line is ongoing with one-year survival as the primary efficacy parameter.

PKI 166 (Novartis Pharma, Basel, Switzerland) is a selective inhibitor of the tyrosine kinase activities of the EGFR and HER-2/neu which has been recently, evaluated in phase I trials [66,67].

GW 572016 (GlaxoSmitheKline, London, UK) is also a TKI with molecular action on HER-1 and HER-2 having an acceptable toxicity profile as described in phase I trials [68,69].

An essential breakthrough in the field of EGFR-targeted therapy is the identification of somatic mutations of the EGFR gene, which are associated with clinical response to gefitinib and erlotinib in patients with NSCLC [70-72]. These genetic alterations are mainly frame deletions or point mutations in exons 18-24 which encode the kinase domain of the protein. The most common alterations reported are point mutations in the exon 21 and small deletions in the exon 19 that eliminate amino acids 747-750 (Leu-Arg-Glu-Ala). These mutations are most frequently detected in a subpopulation of patients with NSCLC with characteristics associated with an improved outcome: female sex, never smokers, East Asian ethnicity and adenocarcinoma histology and in particular, BAC histology [73-75]. In a recent analysis from IDEAL 1, IDEAL 2, and INTACT studies, a RR was seen in 46% of patients with an EGFR mutation versus 10% of those with wild-type EGFR, and in 29% having a high copy number versus 15% with a low copy number [76]. Recent analysis from BR.21 study has suggested that there was an unusually high EGFR mutation rate (23% of the 177 patients for whom tumour tissue was adequate for the study), with a low positive predictive value (16%) and a higher response rate (20% versus 2.4%) in patients with a high versus a low EGFR copy number, respectively [77]. Dr. Paul Gumerlock has also reported a response rate in 23% of patients with an EGFR mutation versus 13% of those with wild-type EGFR, and in 26% of those with a high copy number versus 11% of those with a low one, with k-ras mutations being a possible resistance marker for response [78]. At the same time, higher results (53% to 100%) were reported by other groups regarding the positive predictive role of an EGFR mutation [79-81]. K-ras gene mutations, which are detected in 30% of NSCLC patients, lead to a resistant form of cancer cells to TKIs and are associated with a lack of response [73,82]. In the TRIBUTE study, k-ras mutations detected in 21% of the tumours, were associated with decreased time to progression and overall survival in patients treated with erlotinib combined with chemotherapy [83]. An acquired resistance to EGFR-TKIs has been suggested to be associated with the prevalence of an additional EGFR mutation [84]. Members of the erbB family of receptors, such as HER-2, have been studied as possible predictive markers of EGFR-TKI therapy. The presence of mutations in the HER-2 kinase domain has been reported in NSCLC patients, although the response to gefitinib seems to be independent of HER-2 levels [85,86]. These mutations are more frequent in patients with clinical characteristics similar to the group of patients bearing EGFR mutations.

ErbB2-(HER) Inhibitors

Trastuzumab (Herceptin[TM] Roche, Bazel, Switzerland) is a chimeric monoclonal antibody that targets HER-2 receptor with activity in metastatic breast cancer. In lung cancer cell lines, anti-

tumour activity was demonstrated in HER-2/neu positive cells with a synergistic activity when combined with cytotoxic agents [87,88]. Unfortunately, all phase II trials of trastuzumab when combined with chemotherapy failed to demonstrate any additional therapeutic benefit [89-92]. In the *largest* trial performed to date, 619 patients screened to select 103 eligible patients with HER-2 positive NSCLC who were randomized to receive gemcitabine and cisplatin with or without trastuzumab [92]. No differences were reported in the RR (36% in the antibody arm and 41% in the control arm), median time to progression, and progression-free survival.

ANGIOGENESIS INHIBITORS

Angiogenesis is unquestionably an essential component in the ability of tumours to grow, invade locally, and metastasize from the primary tumour site [93,94]. An avascular tumour has a modest growth of 2-3 mm and only when it becomes vascularized, it has a rapid expansion [95]. Pro-angiogenic stimuli may be released not only from tumours but also from stromal and inflammatory cells and may trigger the angiogenic switch enhancing the formation of microvessels from the surrounding host vasculature [96]. Once tumours switch to the angiogenic phenotype, they recruit their own blood supply. The neoplastic capillaries often have irregular membranes giving the opportunity for cancer cells to leak into the circulation and increase the metastatic potential [97,98]. The intratumural microvessel density (IMD) has been associated with a poorer prognosis and an increased risk of relapse in patients with operable NSCLC [99,100]. In order to stop this important mechanism of tumourigenesis, several biological agents were studied in clinical trials, mainly vascular endothelial growth factor (VEGF) targeted agents, matrix metalloproteinase inhibitors (MMPIs), endogenous inhibitors of angiogenesis, thalidomide, squalamine and a novel agent ZD 6126 (AstraZeneca, London, UK).

VEGF-Targeted Agents

VEGF was originally discovered in the 1980s and is the major and most potent regulator of the neovascularization process promoting angiogenesis in response to hypoxia [101,102]. This heparin-binding glycoprotein exists in several isoforms and functions as an endothelial-specific cell mitogen [103]. High levels of VEGF have been reported in patients with NSCLC and they may be connected with tumour stage progression [104,105]. Except for the promotion of vascularization and growth of the primary tumour, VEGF may have an essential role in the early stages of establishing new metastatic foci [106]. VEGF may increase vascular permeability and enhance the metastatic tumour potential [107]. By inducing the expression of the survival gene bcl-2, it can also inhibit the endothelial cell apoptosis [108]. VEGF also has a critical role in determining tumour radiation response [109]. In a study examining the importance of VEGF isoforms in NSCLC, it was suggested that VEGF-189 was connected with high IMD, short survival, and early postoperative relapse, whereas VEGF-121 was associated with short survival and relapse [110]. The overexpression of this glycoprotein is associated with poor prognosis in patients with NSCLC [111-113]. VEGF binds to its receptors (VEGFRs), mostly VEGFR-1 and VEGFR-2, causing receptor dimerization and subsequent activation of the TK domains promoting the angiogenesis signaling pathway. VEGF antibodies and VEGFR-TKIs are mostly used in order to stop this important mechanism of tumourigenesis (Fig. **2**).

Monoclonal Antibodies Against VEGF

Bevacizumab (Avastin[TM], Roche, Basel, Switzerland) is a recombinant humanized monoclonal antibody against all VEGF isoforms [114]. VEGF production appears to be involved with mediating tumour resistance to chemotherapy and radiotherapy. Bevacizumab enhances the antitumour activity of both therapeutic modalities [115]. When bevacizumab was examined in murine xenograft models, an interesting antitumour activity was observed [115,116].
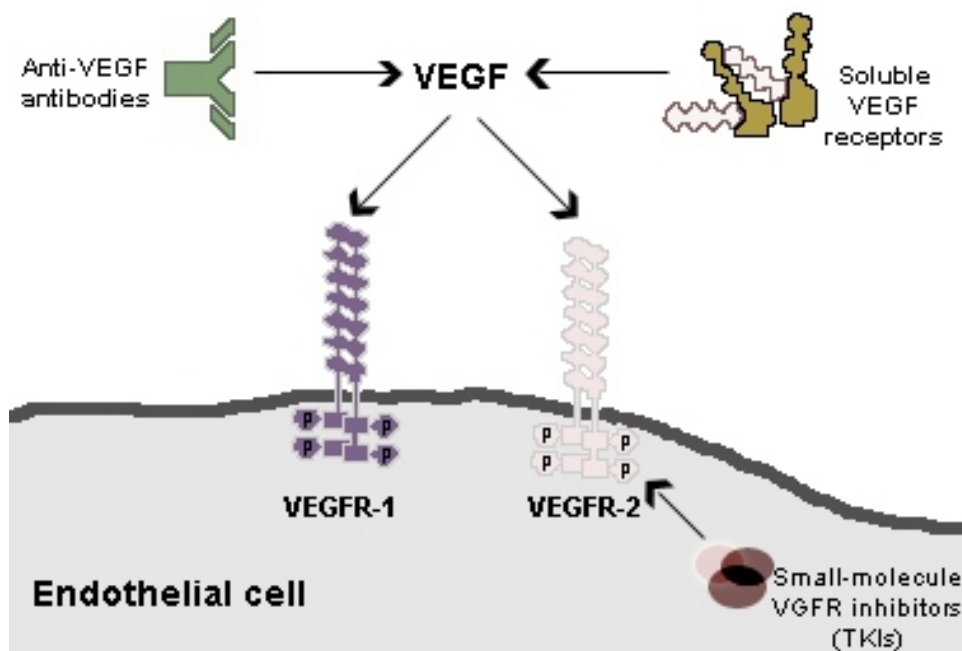
Fig. (2). Various strategies to inhibit VEGF signalling.

Inhibition of VEGF signaling includes monoclonal antibodies targeting VEGF and a variety of small-molecule VEGFR TK inhibitors that inhibit ligand-dependent receptor autophosphorylation of VEGFR-1 and VEGFR-2.

Administration of bevacizumab reversed the protective effect of VEGF against the antiangiogenic effects of docetaxel in endothelial cells *in vitro* and *in vivo* [117]. In a number of phase I studies, bevacizumab produced a significant reduction in VEGF concentration in patients' serum, without causing any pharmacological interactions when combined with a variety of chemotherapeutic agents [118,119]. The most common adverse effects observed included hypertension, epistaxis, thrombosis, and proteinuria.

The above data led to a phase II randomized trial of 99 patients with NSCLC stage IIIB with pleural effusion and stage IV [120]. A combination of carboplatin and paclitaxel (control arm) were compared to carboplatin and paclitaxel alone with either a low dose of bevacizumab (7.5 mg/kg/ every 3 weeks) or a high dose (15mg/kg/ every 3 weeks). Patients in all three arms received an average of 6 cycles of carboplatin plus paclitaxel. When the control group was compared to patients on the higher dose of bevacizumab, there was a higher response rate (31.5% versus 18.8%), a significantly extended time to progression (7.4 months versus 4.2 months) and a longer median survival time (17.7 months versus 14.9 months) in favour of the antibody group. On disease progression, 19 controlled patients received a single agent of bevacizumab and 5 experienced disease stabilization. The addition of bevacizumab to chemotherapy resulted in modest changes to the expected toxicity profile of chemotherapy alone with a slightly greater incidence of diarrhea and leukopenia. Grade 3 hypertension was observed in 2 patients in the high-dose arm. However, no patient discontinued bevacizumab because of hypertension. The most important toxicity was an increased risk of bleeding seen as mucocutaneous or as a major hemoptysis. The most common mucocutaneous bleeding was grade 1 or 2 epistaxis, which was observed in 31% of the low-dose bevacizumab patients and 44% of the high-dose patients compared to 6% of control patients. Life threatening hemoptysis was demonstrated in 6 patients and resulted in 4 deaths. All 6 patients had centrally-located tumours close to major blood vessels, 4 of the severe hemorrhages were observed in patients with squamous histology and 5 occurred in the low-dose bevacizumab arm. In 5 of the 6 cases, patients had at the start of the therapy with bevacizumab, cavitation or necrosis of the tumours or had developed them along the way.

Based on the results of this phase II trial, where the established dose of 15mg/kg for bevacizumab was decided for the treatment of NSCLC stage IIIB and stage IV, a phase III trial (ECOG 4599) comparing the same chemotherapeutic combination with or without bevacizumab in a dose of 15mg/kg/every 3 weeks was organized in 878 chemonaïve patients with advanced NSCLC [121]. Interim analyses of this study showed an improvement in response (10% versus 27%), progression-free survival (4.5 versus 6.5 months) and median survival time (10.2 versus 12.5 months) with the bevacizumab group. Given the information from the phase II trial, patients with squamous cell disease, central nervous system metastases and a history of hemoptysis, coagulopathy, or thrombosis were excluded. Unfortunately, the bevacizumab arm was correlated with higher rates of toxicity, including a larger number of treatment-related deaths (9 versus 2). Although fatal hemoptysis was seen in the bevacizumab arm (5 of the 9 deaths), the difference was not statistically significant between the two arms. In addition, even though an observation of prolonged response rate and time to progression was seen with the women in the bevacizumab arm, an increase in overall survival was not achieved.

These results are quite promising for the role of bevacizumab in addition to the conventional double chemotherapy in patients with advanced NSCLC. Furthermore, the possible initiation of radiation therapy before or concurrent with bevacizumab therapy may have a role in reducing bleeding events. An ongoing European trial of gemcitabine-cisplatin with and without bevacizumab will further confirm the possible beneficial role this agent may play in inhibiting angiogenesis in NSCLC.

The bevacizumab and chemotherapy for operable NSCLC (BEACON) trial involves stage IB-IIIA patients with resectable NSCLC. Patients with nonsquamous peripheral tumours receive two cycles of docetaxel (75 mg/m^2) plus cisplatin (75mg/m^2) plus the addition of bevacizumab (15 mg/kg) every 3 weeks, and patients with squamous or central tumours receive the same chemotherapeutic combination without bevacizumab. Patients who exhibit a 10% or greater reduction in tumor size will receive a further two cycles and the remainder will be removed from the study.

Patients who continue in the trial undergo surgical resection 4 weeks after the last cycle of chemotherapy. Postoperatively, all patients receive a maintenance therapy of bevacizumab (15/mg/kg every 3 weeks) for 1 year. After surgical removal of the primary lesion, patients with squamous cell histology may benefit from the use of this monoclonal antibody to destroy microscopic residual disease after induction chemotherapy and surgery have been performed.

VEGFR-Tyrosine Kinase Inhibitors (TKIs)

There are three isoforms of VEGFR: 1) VEGFR-1 which has a modest TK activity despite its high affinity with VEGF, 2) VEGFR-2 which is mostly connected with cell growth and chemotaxis, and 3) VEGFR-3 which is associated with lymphangiogenesis [93,122]. VEGFR-1 and VEGFR-2 are the two high affinity receptors for VEGF and are exclusively expressed and function on endothelial cells with their expression temporarily regulated in times of angiogenesis and upregulated in response to hypoxia and hypoglycemia [123,124]. Preclinical data suggest an important role for VEGFR in angiogenesis and their inhibitors show a promising new class of antiangiogenic agents [125]. However, SU5416 (Sugen, Inc., South San Francisco, CA) and SU6668 (Sugen, Inc., South San Francisco, CA), which are VEGFR TKIs, will not be developed further because of their unacceptable toxicity profile.

ZD 6474 (Astra Zeneca, Macclesfield, UK) is a novel TKI of VEGFR-2 with activity against EGFR, VEGFR-3 and RET kinases [126,127]. An interesting antitumour activity of this agent was demonstrated both *in vivo* and *in vitro* preclinical models [126,128]. A possible role of ZD 6474 as an adjuvant to radiotherapy was suggested when this agent was studied in combination with and without radiotherapy in a NSCLC tumour model [129]. In a phase I dose escalation study, the most important toxicities observed were grade 3 thrombocytopenia, diarrhea, and rash. Asymptomatic prolongation of QT interval was also demonstrated in 7 of the 49 patients at various dosages [130]. A phase I study in 18 Japanese patients with refractory solid tumours (9 with NSCLC), ZD 6474 was administered in a dose regimen of 100-400 mg daily [131]. ZD 6474 was well tolerated at doses of 300 mg/daily and the most common adverse effects in all of these groups were rash (probably due to its anti-EGFR activity), diarrhea, hypertension, proteinuria and asymptomatic QTc prolongation. DLTs (dose limiting toxicities) were observed in two of the three patients receiving 400 mg/d which was observed as grade 3 hypertension and grade 3 alanine aminotransferase elevations. This dosage is considered to exceed the maximum tolerated dose (MTD). Tumour regression was observed in 4 patients with NSCLC at 200 and 300 mg/d and this effect was maintained despite dose modification in two of these patients.

In a phase II study, ZD6474 was studied in combination with and without docetaxel in 127 previously treated patients with NSCLC [132]. Forty-two of these patients allocated to docetaxel alone, 41 to ZD6474 alone and 44 received docetaxel plus ZD6474. A 57% increase in the time to progression with docetaxel/ZD6474 versus docetaxel alone (18.7 weeks versus 12 weeks) was demonstrated. Currently, NCI-Canada is conducting a trial of adjuvant ZD6474 versus placebo in patients with small cell lung cancer (SCLC).

CP-547,632 is an ATP-competitive kinase inhibitor which blocks VEGFR-2 kinase autophosphorylation and VEGF-induced VEGFR-2 phosphorylation in VEGFR-2 transfected endothelial cells. It also targets EGFR, platelet-derived growth factor (PDGF) and other tyrosine kinases. Phase I trials suggest modest toxicity and interesting pharmacokinetic parameters [133,134]. In a phase I/II trial, CP-547,632 when combined with carboplatin and paclitaxel as a first-line therapy for patients with advanced NSCLC (stage IIIB and IV), objective response was seen in 20% of the pa-

tients. Doses of CP-547,632 ranged from 100-200 mg/daily and the most common adverse effects were diarrhea and rash.

ZD 2171 is also a novel inhibitor of VEGFR VEGFR-1, VEGFR-2 and VEGFR-3 without any interactions with EGFR-TK which is under study.

Matrix Metalloproteinase Inhibitors (MMPIs)

Matrix metalloproteinases (MMPs) belong to a family of endopeptidases and it is suggested that their activity favors tumour growth, invasion and metastasis [135]. It is well known that the extracellular matrix plays an important role in maintaining tissue compartments and is a great obstacle for tumour cells to face to invade locally and to achieve metastasis. These zinc-depended proteases degrade the extracellular matrix, which comprises collagens, laminins, fibronectins, elastins and the protein core of proteoglycans, and in turn, enhance the invasive behaviour of malignant tumours [136]. About 30 MMPs have been identified and are classified in 4 major classes: gelatinases, collagenases, stromelysines and membrane-type MMPs [137]. The majority of MMPs is secreted in a latent form and only a few of these MMPs have a transmembrane domain and are attached to the cell membrane. It is also suggested that MMPs stimulate the release and activation of VEGF, insulin-like growth factor (IL-GF) and basic fibroblast growth factor (bFGF) affecting tumour growth and metastasis [138]. MMP-2 levels have been associated with an increased frequency of local and distant metastases whereas MMP-9 overexpression has been associated with a poor outcome in patients with NSCLC [139].

Marimastat (British Biotech Inc, Oxford, UK) is a broad spectrum MMPI with inhibitory activity against MMPs 1, 2, 3, 7 and 9 [140]. In preclinical models, marimastat demonstrated antitumour activity and was not cytotoxic [141,142]. When marimastat was studied in a randomized double-blind phase III trial in SCLC patients, who had responded to first-line therapy, there was no survival benefit but an important musculoskeletal toxicity was reported which caused a discontinuation of the therapy in 20% of the patients [143]. Phase III trials of marimastat were halted due to a lack of any obvious efficacy [144].

BAY 12-9566 (Bayer Corp, West Haven, CT) is an inhibitor of MMP-2, MMP-3 and MMP-9. When this novel agent was tested in patients with SCLC, who achieved complete or near-complete remission after chemotherapy and radiotherapy, there was also no evident survival benefit [145]. Phase III trials were also halted, as with marimastat, because of the lack of any effective therapeutic evidence [146].

Neovastat (Les Larotatories AEterna, Quebec, Canada) is an MMPI with an additional activity towards VEGF and endothelial cell apoptosis pathways [147]. In a phase I/II trial of neovastat monotherapy in patients with advanced NSCLC, no tumour responses were observed. However, 26% of the patients had SD in the high-dose group compared with 14% in the low one [148]. A phase III trial of this agent combined with conventional chemotherapy and radiation therapy in patients with stage III NSCLC who are not amenable to surgery is ongoing.

Prinomastat (Agouron Pharmaceuticals, Inc, a Phizer Company, La Jolla, CA) is another MMPI with activity specifically directed against MMP-2 and MMP-9. Prinomastat was studied in an orthotopic lung cancer model with and without carboplatin [149]. When both of these agents were administered separately, neither drug increased survival over compared with control animals. The combination of the lower dose of carboplatin and prinomastat significantly enhanced survival in comparison to the control animals as well as the animals treated with carboplatin alone. Two randomized phase III clinical trials of prinomastat in combination with a two-drug conventional chemotherapy regimen for patients with advanced NSCLC did not show any benefit in overall survival, having a toxicity profile not acceptable for chronic use [150,151]. In one of

these trials, 362 chemotherapy-naive patients with advanced NSCLC were randomized to receive either prinomastat or placebo twice daily orally in combination with cisplatin and gemcitabine [151]. There were similar overall response rates for the two treatment arms (27% for prinomastat and 26% for placebo, p=0.81). The median overall survival times were 11.5 months and 10.8 months (p=0.82) and the one-year survival rates were 43% and 38% (p=0.45) for prinomastat and placebo group, respectively. The most important toxicities observed in prinomastat arm were stiffness, arthralgia, and joint swelling. Treatment interruption was necessary in 38% of prinomastat patients and 12% of placebo patients.

BMS-275291 (Bristol-Myers Squibb Co., New Brunswick, NJ) is broad spectrum MMPI that contains a novel mercaptoacyl zinc-binding compound. BMS-275291 minimally affects other metalloproteases including the sheddases, which may be responsible for the polyarthritis seen with marimastat [152]. When BMS-275291 was studied in a phase I trial of 44 patients with advanced cancer, mostly NSCLC and colorectal cancer, the DLTs were rash, transaminitis and shortness of breath and were observed in 2 patients [153]. A randomized phase III trial (BR.18 study) examined the possible role of this novel agent when combined with paclitaxel and carboplatin in 774 chemo-naïve patients with advanced NSCLC [154]. An interim safety analysis not only showed no survival benefit but also an increased toxicity in the BMS-275291 arm and study treatment was therefore terminated. The most important toxicities observed were flu-like symptoms, hypersensitivity reactions, rash, and febrile neutropenia.

Endogenous Inhibitors of Angiogenesis

Endostatin and angiostatin are potent inhibitors of angiogenesis. It is suggested that they are secreted from primary tumours so as to inhibit the growth of their metastatic lesions, creating a great anticipation among oncologists [155]. Increased antiangiogenic activity has been reported for the combination of angiostatin with endostatin [156].

In murine Lewis lung cancer models, when endostatin was administered at doses of 20 mg/kg per day, almost complete regression of the established tumours was demonstrated [157]. Two phase I trials using recombinant human endostatin (rHE) in patients with advanced solid tumours showed modest tumour responses with minimal side effects [158,159]. The combination of endostatin with chemotherapeutic agents suggested an enhanced antiangiogenic effect [160].

Recent studies suggested that application of angiostatin may suppress the growth of a variety of mouse tumours [161,162]. When angiostatin was examined in 143 primary NSCLC tumours with immunohistochemistry, 24% of the cases stained positively [163]. Patients with angiostatin positive tumours survived longer (146 weeks) than patients with angiostatin negative tumours (77 weeks). In a phase II study, recombinant human angiostatin (rh Angiostatin) was combined with carboplatin and paclitaxel in chemotherapy-naïve patients with stage IIIB (with pleural infusion) or IV NSCLC [164]. Patients without disease progression after completing at least 4 cycles continued rh angiostatin as a maintenance therapy until progression. The most important toxicities observed were neutropenia, fatigue, dyspnea, and infection. Overall response rate was 39.1%, and 39.1% of the patients had disease stabilization. Median time to progression was 144 days and the one-year survival was 45.8%.

Thalidomide

Thalidomide (Celgene Corporation, Warren, NJ) has both an antiangiogenic and an immunomodulatory action and is believed to suppress VEGF, tumour necrosis factor alpha (TNF-a), COX-2, interleukin-6, and to modify components of the extracellular matrix [165]. In a dose escalation pilot study of thalidomide combined with carboplatin/paclitaxel in advanced NSCLC, the average toler-

ated dose of thalidomide was 600 mg/d [166]. The most important toxicities were fatigue, nausea, and vomiting. Grade 3 or 4 hematological toxicity occurred in 5 patients who have been previously treated with chemotherapy. The median time to progression was 118 days. ECOG is currently conducting a phase III trial of stage IIIB NSCLC patients implementing chemoradiation with thalidomide or a placebo. The primary end point of this study is survival.

Squalamine

Squalamine [Magainin Pharmaceuticals, Inc. (currently Genaeva Corporation)] is a natural aminosterol, originally isolated from the liver of the dogfish shark, which has been shown to inhibit angiogenesis through a mechanism, which consists of inhibition of mitogen stimulation of endothelial cells through modulation of cellular pH [167]. It seems that the greatest effect of this agent is seen on newly emerging vessels with no apparent effect on unstimulated endothelial cells [168]. Squalamine showed an interesting antitumour activity in xenograft lung models and has also shown to reduce lung metastases [169,170]. Three phase I trials have shown that squalamine is well tolerated in the treatment of NSCLC [171-173]. In a phase I/IIA study, 45 chemotherapy-naïve patients with advanced NSCLC received squalamine (100-400 mg/m^2/day) in a continuous 5-day (days 1-5) infusion in combination with paclitaxel and carboplatin administered on day 1 of every 21 days [174]. Two of three patients who received squalamine at 400mg/m^2/day in phase I experienced DLT that included grade 3 or 4 myalgia, arthralgia, and neutropenia. Reduction of the dosage to 300mg/m^2/day was then used in the phase II portion. PR was observed in 18% of the patients and 19% had SD as their best response. The median survival was 10 months and the 1-year survival was 40%.

ZD6126

ZD6126 (Astra Zeneca, London, UK) is an agent with a potent antiangiogenic activity, which directly causes damage to existing cancer cells [175]. This agent binds to the tubulin of the tumour cells' cytoskeleton, causing vessel occlusion and necrosis. When ZD6126 was studied in a human NSCLC xenograft model with ZD1839 (Astra Zeneca, Macclesfied, United kingdom) (an EGFR-TKI) and radiation, an enhanced activity was observed [176]. Gefitinib has also been shown to enhance its activity in NSCLC xenograft models and in colorectal cancer [177]. In a dose escalation study of ZD6126, the main toxicities were anorexia, dyspnea, constipation, fatigue, headache, nausea, vomiting, and pain [178].

CYCLOOXYGENASE (COX)-2 INHIBITORS

It has been postulated that arachidonic acid and its metabolic pathways play an essential role in tumour pathogenesis. There are mainly three metabolic pathways: the cyclooxygenase (COX) pathway, the lipoxygenase (LOX) pathway and the cytochrome p-450 monooxygenase pathway. In general, the COX pathway has been most extensively studied.

The COX pathway consists of two COX enzyme isoforms, COX-1 and COX-2 [179]. COX-1 is responsible for physiological functions, particularly in the gastrointestinal tract [179]. COX-2, however, is overexpressed in many solid tumours, including lung, colon, and breast. These higher levels of COX-2 result in decreased apoptosis, increased angiogenesis, tissue invasion and a worse prognosis [180-182]. Increased COX-2 expression has been also demonstrated in lung cancer metastatic lymph nodes [183]. It is very common in well-differentiated adenocarcinomas and minimal in squamous subtype [183,184]. COX-2 dependent expression of survinin and the respective COX-2 stabilization in NSCLC, changes the balance between the pro- and anti-apoptotic proteins, facilitating the prosurvival type leading to increased resistance to apoptosis [185,186]. It is also suggested that COX-2 inhibitors may inhibit tumour pathogenesis by a COX independent mechanism [187]. The most important COX-2 inhibitors studied are celecoxib (Celebrex;

Pfizer, New York, NY) and rofecoxib (Vioxx®; Merck & Co., Inc., Whitehouse Station, NJ).

Celecoxib is the first COX-2 inhibitor approved for the treatment of adult arthritis. It inhibits the growth of colon and breast cancer xenograft tumours in nude mice [188,189]. It has also antitumour activity in tumour cells and tissues that lack the COX-2 enzyme [190]. When this COX-2 inhibitor was investigated in human NSCLC cell lines, a decreased cell survival, an increased DNA fragmentation and activation of caspase cascades were observed [191]. In a phase I escalation dose study, celecoxib was administered concurrently with radiation therapy in 47 patients with unfavorable performance status, inoperable/unresectable NSCLC [192]. The main toxicities were grade 1 and 2 nausea and esophagitis, which were independent of the dose of celecoxib or radiotherapy schedule. Celecoxib toxicity developed in three patients, an uncontrolled hypertension in one patient receiving the maximum dose of 800 mg/d and two hemorrhagic episodes in patients on 200 mg of celecoxib who were on warfarin therapy for another reason. Of the 37 patients evaluable for tumour response, 14 had CR and 13 had PRs. The acturial local progression-free survival was 66% at 1 year and 42.2% at 2 years following initiation of radiotherapy.

Two phase II trials of the docetaxel and celecoxib combination in patients with advanced NSCLC suggested that this COX-2 inhibitor may enhance docetaxel activity as monotherapy [193,194]. In these two studies, docetaxel 75 mg/m^2 every 3 weeks was combined with celecoxib 400mg twice daily. In the first trial, 41 patients with advanced NSCLC who had progressed after one or more platinum-based chemotherapy were enrolled. Thirty-nine were deemed eligible and received at least one course of docetaxel [193]. Grade 3 or 4 neutropenia was reported in 25.6% of the patients which included one death due to neutropenic sepsis. Major hepatic or renal toxicity was not observed. The RR for all the eligibly treated patients was 10.2% and the overall survival was 11.3 months with a progression-free survival 19.6 weeks. In the second study, a total of 56 patients with advanced disease were enrolled who had received at least one prior chemotherapy regimen [194]. Celecoxib was administered 5-10 days prior to initiating chemotherapy and urine PGE-M, the major metabolite of prostaglandin E$_2$, was calculated so as to ascertain COX-2 action. The median survival was 6 months (as with docetaxel alone) with a RR of 11%. An interesting observation was that patients with a greatest proportional decline in urinary PGE-M had a longer survival compared to those with no change or an increase in PGE-M (14.8 versus 6.3 versus 5.0 months, respectively). When the above combination was studied in the elderly or in patients with a performance scale 2 with advanced NSCLC, the treatment was well-tolerated and responses were seen in 22% of the patients [195]. COX-2 expression was tested in 10 patients and responses were observed in 3 out of the 4 patients who were COX-2 positive.

In an other phase II trial of celecoxib combined with weekly paclitaxel in 58 patients with platinum-refractory NSCLC, a 24.1% of responses was seen (41.3% had stable disease) [196]. Median time to progression was 5 months and median overall survival 11 months. The one-year survival was 42.5% and the main toxicity was neuropathy (4% had grade 3). Preliminary results suggest that the clinical response is associated with a decrease in serum VEGF levels.

A phase II neoadjuvant trial studying the combination of celecoxib with carboplatin/paclitaxel was carried out by Altorki *et al* [197]. The results were comparable to the results of the neoadjuvant 'BLOT' study employing carboplatin/paclitaxel alone [198]. Increased respectability, as well as slightly higher objective response rates were seen in COX-2 treated patients.

When rofecoxib was studied in NSCLC xenograft models, an inhibition of tumour growth was observed as well as a reduction of the recurrence rate after surgical debulking [199].

BCL-2 TARGETED AGENTS

Bcl-2 is a major antiapoptotic protein and it is overexpressed in a number of solid tumours, including the majority of SCLCs and approximately 40% of NSCLCs, conferring tumour cell chemoresistance and radioresistance *in vitro* leading to a poor prognosis [200-202]. The bcl-2 protein also plays an important role in regulating response to hormonal therapy and monoclonal antibodies and promotes tumour invasion and spread by inducing MMP-2 gene expression through posttranslational and transcriptional mechanisms [203-205]. This ''antideath'' protein causes inhibition of cytochrome C release in the mitochondrial pathway blocking mitochondrial distraction and natural cell death [206]. In xenograft models, noncancerous cells can become highly tumourigenic by transfection with the bcl-2 gene [207-208]. Recent data also suggest that bcl-2 overexpression may indicate taxane sensitivity in NSCLC patients *in vitro* [209]. Although a lot of studies have examined the prognostic role of bcl-2 expression in lung cancer, the impact of bcl-2 expression on outcome is not as important as that of p53 expression. It is believed that bcl-2 overexpression will impact a survival benefit to cells in the face of treatment [210]. At the same time, downregulation of bcl-2 expression may increase sensitivity to chemotherapy and radiation [211].

Oblimersen (G3139, Genasense; Genta Inc. Berkeley Heights, NJ) is an antisense oligonucleotide that blocks production of bcl-2 protein, thereby restoring the principal pathway of cell death. This agent binds to the first six codons of human bcl-2 messenger RNA (mRNA) resulting in degradation of bcl-2 mRNA and a subsequent decrease in bcl-2 protein translation and intracellular concentration [212] (Fig. **3**). When oblimersen was tested in xenograft models, an enhanced antitumour activity was demonstrated when combined with standard chemotherapy in several cancers including breast cancer, gastric cancer, and NSCLC [213]. It also potentiates the effect of radiation and monoclonal antibodies [214]. The combination of oblimersen with vinorelbine in a NSCLC xenograft model showed an enhanced antitumour activity versus that with oblimersen alone [215]. When oblimersen was examined in two phase I/II trials, the most common toxicities observed were fatigue and low grade fever which was usually self-limiting [216-217]. In one of these studies, 12 chemorefractory SCLC patients received paclitaxel plus oblimersen after failing treatment with etoposide plus a platinum regimen [217]. Oblimersen was administered on days 1-8 as a continuous infusion of 3 mg/kg/day and paclitaxel 150 mg/m^2 was administered on day 6 of every 21-day cycle. Four patients (33%) achieved disease stabilization after two treatment cycles and one of them remained free of progression for over 1 year. This patient interestingly had consistently high plasma oblimersen levels. A potential activity of oblimersen when combined with carboplatin/etoposide in 16 previously untreated SCLC patients was suggested in a phase I clinical trial [218]. Of the 14 evaluable patients, 12 patients experienced PR and two patients experienced SD. A phase II randomized trial of docetaxel with or without oblimersen as second-line therapy in relapsed or refractory advanced NSCLC is no longer recruiting patients. In this multicentre trial the primary endpoint will be survival, with tumour response and time to progression as secondary endpoints.

PROTEIN KINASE C INHIBITORS

The protein kinase C (PKC) belongs to a family of serine-threonine kinases and contains more than 12 isoenzymes involved in signal transduction pathways that regulate cell growth, proliferation and death [219,220]. PKC isoenzymes play an important role in downregulating the G$_1$-S and G$_2$-M cell cycle check points as well as apoptosis, angiogenesis, invasion, senescence, drug efflux, and may have a role as a secondary messenger in the mitogenic signaling pathway of PDGF and VEGF [221-223]. It is essential that PKC isoforms regulate the phosphatidylinositol 3'-kinase/Akt
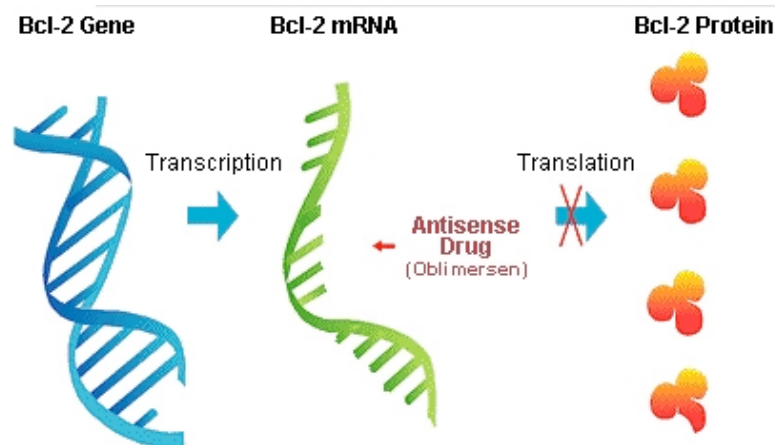
Fig. (3). Effects of Oblimersen on bcl-2 gene expression.

Oblimersen binds to the first six codons of bcl-2 mRNA and inhibits its translation. As a result there is a decrease in total Bcl-2 protein concentration.

and MEK/extracellular signal-regulated kinase pathways which are commonly activated in the majority of NSCLC cell lines [224-225].

UCN-01 (7-hydroxy-staurosporine) (UCN-01 bulk drug was supplied by Kyowa Hakko Kogyo Co, Ltd, Tokyo, Japan. The clinical product was manufactured and provided by the National Cancer Institute) and PKC412 (N-benzoyl-staurosporine) (Novartis, Basel, Switzerland) are two indolocarbazole staurosporine analogues which compete for the binding at the ATP site on PKCs and have been investigated as single agents in patients with advanced malignancies [226-227]. PKC412 was examined in a phase I combined with gemcitabine and cisplatin in 23 patients with advanced NSCLC including 17 chemotherapy-naive and radiotherapy-naive patients [228]. The recommended dose for phase II trials was 50 mg/day. Among 33 cycles in 8 patients, the major adverse effects observed were diarrhea and asthenia. One patient experienced grade 3 headaches at this level. Three patients experienced PR.

The expression of PKC alpha has been known to increase in tumours compared with normal tissue and has been implicated in malignant transformation and proliferation [229,230]. Inhibition of PKC-a may arrest the growth of prostate cancer, hepatoma, and medulloblastoma cell lines [231-233]. A selective inhibitor of PKC-a expression is affinitak (LY900003, ISIS 3521, aprinocarsen, ISIS Pharmaceuticals Inc., Carlsbad, CA). This agent is a 20-base antisense phosphorothioate oligonucleotide that binds PKC-a mRNA, blocking its expression, and reducing its cellular concentration. The phosphorothioate structure of LY900003 (a sulfur substitution of a nonbridging oxygen on the backbone) leads to a resistant form of endonuclease and exonuclease mediated degradation, resulting in a solid heteroduplex with its target mRNA [234]. Inhibition of PKC-a expression by this novel agent *in vivo* and *in vitro* was demonstrated [224,235]. The antitumour activity of LY900003 as a single agent or combined with cisplatin and paclitaxel in xenograft models was also shown [230].

In a phase I study, ISIS 3521 was given in 21 patients with advanced cancer with a minimum of two prior chemotherapy regimens received [236]. Doses were increased from 0.5 to 3.0 mg/kg/day and patients continued on the study until evidence of progression or unacceptable toxicity observed. The MTD was 2.0 mg/kg/day. The DLTs were fatigue and thrombocytopenia at a dose of 3.0 mg/kg/day. Tumour response was observed in 3 of 4 patients with ovarian cancer. In another phase I trial of this agent, 14 patients with refractory solid tumours in a 24-hour weekly infusion schedule, exhibited grade 3 toxicities which included neutropenia, nausea, and chills [237]. Mean prothrombin times and activated

partial thromboplastin times (APTT) increased by 10% and 29%, respectively, from their baseline.

In a phase I/II trial, ISIS 3521 was given in conjunction with carboplatin/paclitaxel in patients with NSCLC [238]. In the phase I part, there was no DLT of the ISIS compound. A phase II trial then followed in 53 previously untreated patients with advanced NSCLC. The most common grade 3/4 toxicities were thrombocytopenia (21%/11%) and neutropenia (26%/43%). The overall response rate was 46% with 21 patients experiencing PRs and one patient experiencing a CR (SD in an additional 35%). The overall median survival was 15.9 months, median time to progression was 6.3 months, and 1-year survival exceeded 50%.

Despite these encouraging results, a phase III clinical trial of the above combination did not demonstrate an advantage in overall survival over chemotherapy alone [239]. In this phase III trial, 616 patients were randomised to receive either paclitaxel 175 mg/m^2 plus carboplatin AUC5 every 3 weeks alone on day 1 or in combination with LY900003 2 mg/m^2 as a continuous infusion on days 1-14. There was an increased incidence of grade 3 thrombocytopenia in the LY900003 arm (44% versus 15%) as well as catheter-related infections (8.2% versus 0.3%). There was no difference with respect to response rate (37% versus 36%), time to progression (4.5 versus 4.7 months), and median survival time (9.7 months versus 10 months).

In another phase I/II trial, LY900003 was studied with gemcitabine and cisplatin in patients with advanced NSCLC [240]. In the phase one portion, 7 patients received the combination regimen with one patient experiencing grade 3 fatigue. Antitumour activity was observed in two patients (one PR in a patient with stage IIIB NSCLC and one MR (minor response) in a patient with metastatic pancreatic carcinoma). In the phase II portion, 55 NSCLC patients received the combination at two gemcitabine doses [1.000 mg/m^2, n=44 (original cohort); 1.250 mg/m^2, n=11 (expanded cohort)]. Fourteen of 39 evaluable patients in the original part 2 cohort had a major response (1 CR and 13 PRs) for a RR of 36%. In the expanded cohort, 2 of the 9 evaluable patients experienced PRs, for a combined part 2 response rate of 33%. The median time to progression for the entire group was 3.9 months and the median survival time 8.9 months.

In a different phase II trial 18 patients with advanced NSCLC were randomized to receive either gemcitabine/cisplatin (control arm) or this chemotherapeutic combination with aprinocarsen (experimental arm) [241]. The major toxicities seen were hematological with a higher incidence of thrombocytopenia in the experimen-

tal arm (87.5% versus 33.3%). Unfortunately, the response rate was 16.7% in the experimental arm and 44.4% in the control arm. Further enrolment was terminated in March 2003 because of the results of a phase III trial suggesting no additional survival benefit of aprinocarsen when combined with cisplatin and gemcitabine in patients with advanced NSCLC [242].

PROTEASOME INHIBITORS

The ubiquitin proteasome pathway has a significant role in regulating the intracellular concentration of specific proteins, protecting cellular homeostasis. A variety of proteins that govern cell growth, regulation, signaling and transcription are substrates that are temporally degraded by this pathway. The strict control of the degradation of these proteins is essential for normal cell functioning and normal protein homeostasis. The first step of degradation is the marking of the protein substrates with ubiquitin, a smaller cytosolic protein. When this procedure is completed, these proteins are presented to the 26s proteasome complex were degradation takes place. This multi-subunit protein consists of 19S proteasome, 20S proteasome and multiple peptidases and ATPases. Tumour cells can often escape the procedure of protein homeostasis and alter the ratio of pro- and anti-apoptotic proteins or pro-survival growth factors, providing a survival advantage for the transformed cells [243]. Cyclins, cyclin-dependent kinases, cyclin-dependent kinase inhibitors as well as oncogenes, such as c-myc and n-myc and tumour suppressor genes are regulated in this way [244]. The proteasome pathway is also necessary for activation of the nuclear factor kB, NF-[kappa] B, by degradation of its inhibitory protein, IkB. NF-[kappa] B is a major antiapoptotic protein which maintains cell viability through the transcription of inhibitors of apoptosis, in response to cytotoxic agents or environmental stress [245,246]. Stabilization of IkB protein and inhibition of NF-[kappa] B activity has been demonstrated to make cells more susceptible to apoptosis [245,247]. It is also suggested that NF-[kappa] B controls the cell surface expression of adhesion molecules such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and E-selectin [248]. The association of factor NF-[kappa] B with NSCLC resistance to therapeutic agents when activated has been suggested [249,250]. Several studies suggested that inhibition of tumour cell proteasome sensitizes tumour cells to conventional therapeutic agents and radiation therapy [251,252]. Proteasome inhibitors may also overcome bcl-2 mediated protection from apoptosis [253].

Bortezomib (Velcade, Millennium Pharmaceuticals, Inc., Cambridge, MA) is a reversible inhibitor of the chymotrypsin-like activity of the 26s proteasome [254]. This novel agent increases the levels of cyclin-dependent kinase inhibitors p21 and causes G2-M cell cycle arrest promoting cancer cell apoptosis [255]. Bortezomib also suppresses the secretion of VEGF in the bone marrow, inhibits VEGF-mediated caveolin phosphorylation, and decreases caveolin expression [256]. Caveolin-1 regulates interleukin-6, insulin-like growth factor-I dependent growth and VEGF-dependent migration of multiple myeloma cells [257]. It is also suggested that the generation of reactive oxygen species plays a significant role in the initiation of the bortezomib-induced apoptotic cascade by mediating a disruption of the mitochondrial membrane potential and by releasing cytochrome C from mitochondria [258].

Bortezomib activity in human NSCLC cell lines was studied including those with p53 wild type, p53 mutant, and p53 null phenotypes [259]. Cell cycle arrest in the G2-M phase was observed without affecting microtubule polymerization or depolymerisation. Activation of cyclin B and cyclin A kinases as well as activation of caspase-3 were also demonstrated. Bortezomib also produced inhibition of the human squamous carcinoma cell lines which was associated with inhibition of NF-[kappa] B activation and increased caspase-induced apoptosis [260]. In all studies, bortezomib was administered twice a week- given that the duration of proteasome inhibition is 48-72 hours. Based on the results of two phase II trials

bortezomib recently received FDA approval for the treatment of relapsed myeloma [261,262].

In a phase I trial, 43 heavily pretreated patients with solid tumours received a total of 89 cycles of bortezomib at doses ranging from 0.13 to 1.56 mg/m^2/dose [263]. DLTs on this schedule were sensory neurotoxicity and diarrhea. There was no dose-limiting hematological toxicity. A dose-related inhibition of 20s proteasome activity with increasing dose of the drug was observed. There was one major response in a patient with refractory NSCLC. In another phase I trial in patients with advanced tumours, the most common toxicities observed were fatigue, anorexia, nausea, electrolyte disturbances, and thrombocytopenia [264].

When bortezomib was combined with gemcitabine in patients with advanced solid tumours, the MTDs were 1.0 mg/m^2 for bortezomib and 1.000 mg/m^2 for gemcitabine. The DLTs seen at these doses were grade 3 thrombocytopenia and leukopenia [265]. One PR was demonstrated in a patient with NSCLC. When the combination of bortezomib with carboplatin/ gemcitabine in a phase I trial was studied, four patients of the ten evaluable patients had PR and five patients had disease stabilization [266]. The combination of bortezomib with irinotecan was also safe without any pharmacokinetic interactions or additive toxicities [267].

In a phase II pharmacodynamic trial, 23 minimally pretreated patients with NSCLC received bortezomib in 3-weekly cycles [268]. The most important grade toxicities observed were nausea, constipation, sensory neuropathy, thrombocytopenia and rash. Four hours after administration, the effects on NF-[kappa] B were had reached a maximum, with recovery beginning after 24 hours. PR was demonstrated in one patient and nine had SD. In a recent randomized phase II study, pretreated NSCLC patients received either bortezomib in a dose of 1.5 mg/m^2 or bortezomib in a dose of 1.3 mg/m^2 with 75 mg/m^2 of docetaxel [269]. Patients receiving the combination schedule experienced fatigue and neutropenia more frequently than the single-agent arm. An interim analysis showed partial response rates of 10.3% in the bortezomib arm and 16% in the combination arm (n=31). In addition, more patients were found to have SD in the combination arm (45% versus 17%), suggesting the possible beneficial role of this novel agent when added to conventional chemotherapy.

FARNESYL TRANSFERASE INHIBITORS (FTIS)

The ras family of proto-oncogenes encodes proteins that play an essential role in critical cellular process such as proliferation, differentiation and apoptosis. Mammalian cells contain 3 functional ras genes (H-ras, N-ras and K-ras) that encode 4 oncoproteins (H-Ras, N-Ras, K-Ras4A and K-Ras4B). K-Ras4A and K-Ras4B are the result of two alternatively spliced K-ras gene products. Mutations in the Ras proteins are found in about 30 % of all human cancers and 40 % of NSCLC indicating poor prognosis [270,271]. Activating mutations in these proteins lead to constitutive signaling, inhibition of apoptosis and promotion of cellular proliferation [272]. When a ras protein is being generated a nonpolar farnesyl group is attached to the COOH-terminal, a reaction catalyzed by the enzyme farnesyl tranferase. This reaction enables the ras protein to move from the cytoplasm to the cell membrane, as it becomes more hydrophobic, where it cycles from the inactive ras-GDP to activated ras-GTP in response to cellular binding of growth promoting factors to its tyrosine kinase receptor. Geranylgeranyl tranferases I (GGTase-I) and II (GGTase-II) can alternatively catalyze the farnesylation of K-Ras and other proteins. FTIs block this procedure and its cellular consequences (Fig. **4**). Other proteins, which may have a role in mediating the antitumour activity of FTIs, are the centromeric (CENP-E and CENP-F) proteins, RhoB, and members of the phosphatidilinositol 3'-kinase/Akt pathway [273-275]. FTIs have been shown to inhibit the growth of a broad spectrum of tumour cell lines *in vitro* and *in vivo* and it is suggested that tumour cells bearing mutated K-ras may be less sensitive to FTIs than those with
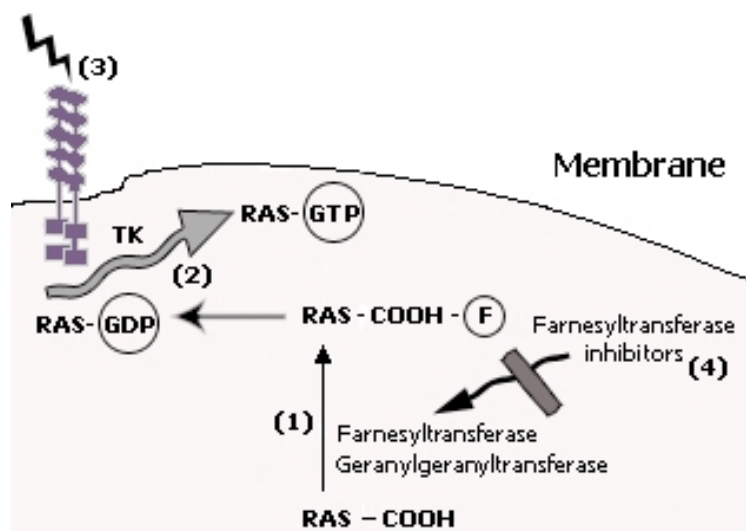
Fig. (4). Effect of FTIs on Ras pathway.

The attachment of the farnesyl group (F) is necessary for Ras-oncoprotein activation. The reaction is catalyzed by farnesyl transferase or alternatively by geranylgeranyl transferase (1). Subsequently the Ras protein becomes more hydrophobic and moves to the cellular membrane, where it is available for phosphorylation (2) in response to ligand binding to its tyrosine kinase receptor (3). FTIs block the above procedure by inactivating farnesyl transferase (4). F: Farnesyl group.

wild-type ras or mutated 11-ras genes [276,277]. The combination of FTIs with irradiation and paclitaxel may increase cell cytotoxicity and death [278,279]. A possible suppression of the angiogenic factors expression such as VEGF from FTIs antitumour action is also suggested [280].

L-778,123 (Merck & Co., Inc., Whitehouse Station, NJ) tipifarnib (R115777 ZarnestraTM; Ortho Biotech Products, L.P., Bridgewater, NJ), lonafarnib (SCH66336 SarasarTM; Schering-Plough Corporation, Kenilworth, NJ)) and BMS-214662 (Bristol-Myers Squibb, Princeton, NJ) are FTIs currently being studied in clinical trials.

L-778,123 was studied in a phase II trial in 23 NSCLC patients with stage IIIB (with pleural effusion) or IV, with a performance status (PS) of 0-1 who have not received chemotherapy before [281]. The DLTs of this agent, when examined in a phase I trial, was fatigue, myelosuppression and neurotoxicity and the recommended dose was 560 mg/m^2 i.v. daily [282]. Throm-boembolic toxicity was observed in patients with a central venous access. Three patients developed a pulmonary embolism and one patient had a superior vena cava thrombosis. Nineteen twenty there of the patients completed at least one cycle without any objective response observed. The development of this compound was then discontinued.

Tipifarnib (R115777) is an oral methylquinolone analogue of the imidazoles which inhibits the growth of tumours with N-ras, H-ras, and wild- type mutations being less active against wild-type ras mutations. When tipifarnib was investigated in a phase I trial, one patient with platinum-refractory NSCLC who received continuous dosing had a partial remission [283]. The major toxicities observed were hematological with peripheral neuropathy. Fatigue and skin toxicities were also reported. A phase II study of tibifarnib as a single agent therapy in advanced NSCLC was organized in 44 patients with performance status 0-2 who have not received either chemotherapy or radiotherapy to more than 25% of their bone marrow [284]. The regimen schedule was 300 mg orally twice daily for 21 days of a 28-day cycle. The most common grade 3 and 4 toxicities were neutropenia, anemia, and anorexia. There were no objective responses, although 7 patients had SD for more than six months. Evidence of farnesyl transferase inhibition was documented in 83% of the patients. Median survival was 7.7 months,

median time to progression was 2.7 months and 70% of the patients were able to continue with conventional chemotherapy after completing tibifarnib.

Lonafarnib (SCH 66336) is an oral tricyclic halogenated competitive FTI which is active in tumours having all ras mutations as well as wild type ras mutations. In a phase I trial, one patient with advanced NSCLC had an objective PR and 8 patients demonstrated disease stabilization for up to 10 cycles of the drug [285]. In another phase I trial, lonafarnib was given in 24 patients with solid tumour in combination with paclitaxel every 3 weeks [286]. Partial remission was demonstrated in 15 patients, including 2 with taxane resistant advanced NSCLC. The DLTs observed were neutropenia, peripheral neuropathy, diarrhea, and hyperbilirubinemia. A phase II trial of lonafarnib combined with paclitaxel in 33 taxane-refractory advanced NSCLC patients was then organized [287]. Toxicities were minimal and included febrile neutropenia, diarrhea and fatigue. Five patients (15%) achieved PR, 4 patients MR and 10 patients sustained disease stabilization. These promising results led to a phase trial III of its combination with carboplatin/paclitaxel against carboplatin/paclitaxel/placebo in 675 good performance status patients with advanced NSCLC [288]. There was no statistically significant improvement in time to progression (137 versus 152 days) and overall survival (144 versus 168 days) for patients receiving lonafarnib with the placebo arm having numerically superior outcomes.

BMS-214662 is an imidazole-containing tetrahydrobenzodiazepine with cytotoxic and apoptotic properties that differentiate it from other FTIs [289]. Because of its unacceptable gastrointestinal toxicity profile, intravenous administration is preferred. In a phase I trial of 44 patients with advanced solid tumour malignancies, the dose-limiting toxicities observed after the first cycle of therapy were nausea, vomiting, and diarrhea [290]. Four patients developed reversible transaminitis, which was not dose limiting. When BMS-214662 was combined with cisplatin in 29 patients with advanced solid tumour malignancies transaminitis, nausea, vomiting, diarrhea and renal failure were the DLTs with 15 patients having disease stabilization as their best response [291]. In another phase I trial, 30 patients with advanced solid tumour malignancies received escalating doses of this novel agent, followed by the combination of carboplatin/paclitaxel on the first day of a 21-day cycle

[292]. Three patients had PR and eight had SD with neutropenia, thrombocytopenia, nausea and vomiting as the most important DLTs.

RETINOIDS

Retinoids (vitamin A and its analogues) play an essential role in a variety of biologic functions especially in epithelial differentiation [293]. The majority of their biologic effects is mediated through retinoic acids, the active metabolites of retinol, mainly all-trans-retinoic acid and 9- cis-retinoic acid [294]. Retinoic acids bind to specific nuclear receptors that regulate a variety of genes, which are connected with cell growth and differentiation, as well as induction of apoptosis that suppresses tumourigenesis and tumour growth [295]. The two families of receptors known are the retinoic acid receptor (RAR) family and the retinoid X receptor (RXR) family, each one of them having three subtypes (α, β, and γ) and for each subtype, there are multiple isoforms [296,297]. These receptors belong to the steroid hormone receptor superfamily and act as ligand-activated transcription factors. The RXR/RAR heterodimers are the functional units that activate transcription by binding to retinoic acid response elements located in the promoter region of retinoic acid target genes [298]. One of the most important genes is the RARβ2 gene. The expression of RARβ2 mRNA is found to be decreased in a lot of solid tumours including lung cancer, breast carcinoma, and squamous cell carcinoma of the head and neck which suggests a possible escape mechanism from normal cellular homeostasis [299,300]. The expressions of all three RAR subtypes have a strong affinity for both all-trans and 9-cis isomers of retinoic acid while the RXR family is activated by 9-cis-retinoic acid only [294]. 13-cis-retinoic acid is converted to all-trans-retinoic acid and exerts its activity through RARs. The mechanisms through which retinoids manage to suppress oncogenesis are multicomplex. The number of genes involved in tumour cell growth, which have a retinoic acid response element in their promoter region, is huge [301]. In addition, retinoids regulate the expression of matrix metalloproteinases (MMPs), transforming growth factor-β and cell cycle regulator proteins such as cyclin-dependent kinase I, p21 and p16, which suppress tumour growth and invasion [302-304]. Normalization of the increased expression of the TGF-a and EGFR in head and neck cancer cell lines with retinoic acid was also reported [305].

Synthetic retinoids that exert their activity by binding to RARs [13-cis-retinoic acid, TAC-101 (Taiho Pharmaceutical Co, Ltd, Tokyo, Japan), Tazarotene (Allergan Inc; Irvine, CA)] have demonstrated a remarkable toxicity profile when studied in phase I trials. Cheilitis, headache, dry skin, mucosal dryness, hypertriglyceridemia, and hypercalcemia are the most common adverse effects reported and are associated to traditional retinoid therapy. Synthetic retinoids bound to RXRs (rexinoids) have an improved toxicity profile compared to those bound to RARs. Given the fact that RXRs have a central role in nuclear receptor signaling, rexinoids create an attractive avenue for targeted therapy. Bexarotene (Targretin®, Ligand Pharmaceuticals; San Diego, CA) is a novel rexinoid which affects RAR-responsive genes only in higher dose levels [306]. The possible role of bexarotene in preventing paclitaxel resistance was studied in human NSCLC cells [307]. Repeated exposure to paclitaxel alone resulted in paclitaxel resistance with cross-resistance to multi-drug resistant P-glycoprotein substrates. The bexarotene/paclitaxel combination, however, prevented the development of drug resistance and the cells remain chemosensitive. When the resistant cells were treated with the combination regimen, paclitaxel resistance was overcome.

The safety and tolerability of bexarotene was tested in two phase I trials. Fifty-two patients, including 20 with NSCLC, were treated at 14 variable dose levels of this novel agent with a dosage regimen from 5 to 500 mg/m^2/day [308]. The maximum tolerated dose was 400 mg/m^2/day. Grade 3 toxicities were reported in seven patients (14%) including neutropenia, hypercalcemia, hypertriglyceridemia and elevated aspartate transaminase (AST). Only 41% of the patients experienced mucus membrane dryness and dry skin compared to 90% of the patients that were treated with the non-RXR-selective retinoids 13-cis-retinoic acid and all-trans-retinoic acid. Eight of the 20 NSCLC patients completed 3 months or more of therapy and one patient had SD for 9 months. In another phase I trial, 60 patients with advanced cancer were treated with bexarotene, all with solid tumours except for one [309]. Leukopenia, skin toxicity, elevated bilirubin, diarrhea and elevated transaminase were the grade 3 toxicities reported in 10% of the patients. No dose-limiting toxicities were observed at dose levels less than 500 mg/m^2/day, which was the recommended dose for the phase II studies. A long term, follow-up analysis of 36 NSCLC patients from these two phase I trials showed a 39% disease stabilization [310]. The Kaplan-Meier estimate of median survival was 11.1 months and the 1-year and 2-year estimated survival rates were 42% and 15%, respectively.

A phase I/II trial investigated the combination of these agents with cisplatin/vinorelbine in 43 patients with stage IIIB (with pleural effusion)/ IV NSCLC who also had not receive any prior therapy [311]. During the phase I portion of the trial, 21 patients received bexarotene 1 week before they started chemotherapy. Bexarotene was administered daily except on the day after each dose of cisplatin. The daily MTD of bexarotene was determined to be 400 mg/m^2. In the phase II portion, 28 patients were treated with the MTD (six from the phase I portion who received the same dose level). Seven patients (25%) had PR and one nearly obtained CR. Disease stabilization was observed in 14 (50%) patients after two completed cycles. The median survival time was 14 months and nine (32%) of the 28 patients were still alive at a minimum follow-up of 2 years. One-year and projected 3-year survival rates were 61% and 30%, respectively. The most common grade 3 and 4 toxicities were nausea, vomiting, dyspnea, asthenia, pneumonia, leukopenia, anemia and hyperlipidemia. There were two cases of pancreatitis which were associated with increased triglyceride levels.

In another phase I/II trial of bexarotene combined with carboplatin/paclitaxel, patients with advanced NSCLC had a 40% objective response, including 5 PRs and one CR, in 15 evaluable patients [312]. All patients received antilipid therapy before or on the same day that they received bexarotene and grade 3 hypertriglyceridemia was reported in only one patient. In a recent phase II trial, bexarotene was combined with carboplatin and gemcitabine in 48 patients with advanced NSCLC [313]. All patients received atrovastatin (Lipitor; Phizer, New York) 10 mg orally before starting bexarotene. The overall median survival was 12.7 months and the median time to progression was 6.7 months. There was a 25% RR and a 1-year survival rate of 53%.

Based upon all the above findings, two multicentre phase III trials explored the possible role of this novel agent when combined with chemotherapy for the treatment of patients with advanced NSCLC in the front- line setting. In the first trial (SPIRIT I), chemotherapy-naïve patients received cisplatin 100 mg/m^2 on day 1 of a 4-week cycle by i.v infusion and weekly vinorelbine 25 mg/m^2 by i.v infusion with or without oral bexarotene 400 mg/m^2/day [314]. In the second trial (SPIRIT II), patients received paclitaxel 200 mg/m^2 on day 1 every 3 weeks by i.v infusion and carboplatin AUC equal to 6 every 3 weeks by i.v infusion with or without oral bexarotene 400 mg/m^2/day [315]. All patients from the two trials received antilipid therapy before starting bexarotene or on the day the study began. Unfortunately, both trials showed no difference between the intervention or placebo arms with respect to median or overall survival time. Subgroup analysis from both trials suggested a potential benefit for patients who developed hypertriglyceridemia of grade 3 or higher.

The possible role of bexarotene as a maintenance therapy in patients with advanced NSCLC, who had responded to first-line

chemotherapy, was also examined in a phase II/III maintenance therapy study [316]. Fifty-two patients who had an OR or SD, most of them treated with paclitaxel/carboplatin, were randomized to receive bexarotene 300 mg/m^2/day, bexarotene 600 mg/m^2/day or placebo. A median TTP of 8 weeks was observed in patients treated with placebo. An increased median TTP of 11.7 months was observed in patients treated with the low dose of bexarotene and 18.3 weeks in patients treated with the high dose. Although the trial was prematurely terminated because of slow accrual and its insufficiency to detect statistically important differences between the treatment groups, a possible survival benefit of this agent when given as a maintenance therapy schedule is suggested. The possible role of bexarotene in the second line setting was examined with or without docetaxel in a randomized phase II trial of 155 pretreated patients with advanced NSCLC [317]. The most common toxicities observed were nausea, vomiting and dehydration.

The most common adverse effects anticipated from bexarotene use are mild to moderate skin toxicity, central hypothyroidism, and hyperlipidemia (both triglycerides and cholesterol) with atrovastatin and fenofibrate, being the main antilipid therapy used. Gemfibrozil interacts with bexarotene and therefore is not the preferred choice in controlling bexarotene's hyperlipidemic reactions [318]. Lipid levels and hepatic biochemistry must be monitor carefully. Central hypothyroidism should be treated with levothyroxine, starting at 0.05 to 0.075 mg/day, so as to maintain total T_4 levels within normal limits. Normal thyroid function will return after the discontinuation of this novel agent [319].

SPECIFITY OF TARGETED THERAPIES

Although novel agents focus on cell signaling and block pathological pathways critical for tumor growth and survival, they also have an effect on normal tissues that share similar growth factor receptors, proteins or enzymes. The ErbB family of receptors is widely expressed in mesenchymal, epithelial and neuronal tissues and inactivation of the EGFR gene was shown to cause impaired epithelial development in many organs including gastrointestinal tract and lung [320,321].

EGFR targeted therapies, such as erlotinib, gefitinib and cetuximab, inhibit ligand-induced activation of EGFR and MAPK in human keratinocytes which may be the key molecular event for the dermatological toxicity observed [322]. ErbB2 was also shown to be required for the maintenance of normal heart function in adults, as HER-2/neu signaling is essential for myocyte survival [323,324]. In a retrospective analysis of seven phase II/III trials for metastatic breast cancer, older age and the combination of trastuzumab with anthracyclines was shown to be associated with a higher risk for cardiotoxicity [325].

Bevacizumab has been associated with poor wound healing and bleeding disorders in clinical trials, while the mechanisms of its unique toxicities have not yet been fully characterized. VEGF is an important proangiogenic factor that optimizes healing in damaged tissues and a defect in VEGF regulation might be associated with wound healing disorders [326,327]. It has also been recently reported that the autocrine expression of VEGF from endothelial cells may present a protective mechanism against injury from cytotoxic agents [328].

MMPs are also expressed in the macrophages, fibroblasts, chondrocytes and synovial lining cells and may play a part in the turnover of bone and cartilage [329]. All trials have shown an important toxicity, especially musculoskeletal, towards the MMPI arm that had a negative impact on quality of life, even in the trial of BMS-275291, which was expected to have less muskuloskeletal toxicity than marimastat or prinomastat.

CONCLUSIONS

Although several targeted agents studied on clinical trials demonstrated an acceptable toxicity profile and efficacy, their influence

on the natural history of the disease was below our expectations. The majority of phase III clinical trials demonstrated negative results (Table **1**).

Table 1.   Results of Phase III Clinical Trials of Targeted Therapies in NSCLC Treatment

Ref.	Target	Agent/ Study Name	Added Benefit in Survival
[44]	EGFR-TK	Gefitinib/ INTACT 1	No
[45]	EGFR-TK	Gefitinib/ INTACT 2	No
[46]	EGFR-TK	Gefitinib/ ISEL	No
[57]	EGFR-TK	Erlotinib/ TALENT	No
[58]	EGFR-TK	Erlotinib/ TRIBUTE	No
[63]	EGFR-TK	Erlotinib/BR.21	2 months
[121]	VEGF	Bevacizumab/ ECOG 4599	2 months
[150]	MMPs	Prinomastat	No
[151]	MMPs	Prinomastat	No
[154]	MMPs	BMS-275291/ BR.18	No
[239]	PKC-a	ISIS 3521	No
[242]	PKC-a	ISIS 3521	No
[288]	FT	SCH 66336	No
[314]	Retinoids	Bexarotene/ SPIRIT I	No
[315]	Retinoids	Bexarotene/ SPIRIT II	No

EGFR-TK: epidermal growth factor receptor-tyrosine kinase; VEGF: vascular endothelial growth factor; MMPs: matrix metalloproteinases; PKC: protein kinase C; FT: Farnesyl Transferase.

We have certainly learned that erlotinib can prolong the life of patients with NSCLC after failing frontline therapy (2 months or 41% comparing to the placebo arm and that the combination carboplatin/paclitaxel and bevacizumab is superior to carboplatin/paclitaxel alone in prolonging life as frontline therapy (2 months or 20% compared to the chemotherapy arm) Nevertheless, all of this information is just the beginning of clinical research in this field and useful conclusions must be extracted without any pessimism. Some important indications that must be taken into consideration are:

a) For HER-1/EGFR targeted agents, a reliable predictive marker of response has not been established yet, as the overexpression of HER-2 for trastuzumab. Current data suggest that a patient's phenotype as well as mutations in the genes regulating oncogenic pathways may have a predictive role for these agents and thereby selecting patients according to the tumours characteristics and by gathering tumour tissue samples consistently, must all be seriously considered when a clinical trial is designed. A variety of results reported about the positive predictive role of an EGFR mutation may be the consequence of differences in the methodology used in several studies. However, it is still unclear whether or not the presence of an EGFR mutation translates into a survival benefit.

b) Blocking only one molecular pathway each time may permit others to act as escape mechanisms for tumour cells therefore targeting multiple pathways simultaneously may have a better chance of shutting down the oncogenetic process and improving the outcome. A phase II study exploring the combination of erlotinib and bevacizumab in patients with advanced NSCLC with one or more chemotherapeutic failures has demonstrated partial response in 20% of the patients (SD an additional 65%) [330]. Gefitinib can be also administered safely with celecoxib and rofecoxib [331,332]. Appealing preclinical data also sug-

gest a synergistic activity when several targeted agents are combined [333,334]. Examining this information in clinical trials is warranted.

c) Novel agents differ considerably from cytotoxic drugs and may have a cytostatic rather than a tumouricidal action [335,336]. This point of view resulted in the initiation of large clinical trials based only in positive *in vitro* studies and phase I trials instead of previous phase II trials, searching for responses. This is an important reason for the disappointing results of most of the large randomized trials. We strongly believe that large phase II clinical trials with an appropriate control arm should be conducted obligatorily after a positive phase I testing. Large randomized phase III trials must be organized only if there is enough positive evidence from phase II studies, as it was done with bevacizumab.

Finally, it is crucially anticipated that by continuing to analyze extensively and to integrate fully all of the pertinent information collected from first generation clinical trials, second generation studies will successfully continue to broaden our knowledge in the direction that will, someday, deliver hope to the patient with NSCLC. Only the future can reveal whether or not molecular technologies will soon unveil critical breakthroughs in this field and whether we are at all aiming in the right direction.

REFERENCES

[1]   Socinski MA, Morris DE, Masters GA, Lilenbaum R; American College of Chest Physicians. Chemotherapeutic management of stage IV non-small cell lung cancer. Chest 2003; 123(1 Suppl): 226S-43S.

[2]   Reck M, Gatzemeier U. Chemotherapy in stage-IV NSCLC. Lung Cancer 2004; 45 (Suppl 2): S217-22.

[3]   Ginsberg RJ, Vokes EE, Rosenzweig K. Non-small cell lung cancer. In: De Vita VT Jr, Hellman S, Rosenberg SA, eds. Cancer: Principles and Practice of Oncology, 6th Edition. Philadelphia: Lippincott, Williams and Wilkins; 2001; 925-83.

[4]   Wells A. EGF receptor. Int J Biochem Cell Biol 1999; 31: 637-43.

[5]   Carpenter G, King L Jr, Cohen S. Epidermal growth factor stimulates phosphorylation in membrane preparations in vitro. Nature 1978; 276: 409-10.

[6]   Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2000; 103: 211-25.

[7]   Yarden Y, Sliwkowski MX. Untangling the ErbB signaling network. Nat Rev Mol Cell Biol 2001; 2: 127-37.

[8]   Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. Eur J Cancer 2001; 37: 9-15.

[9]   Selvaggi G, Novello S, Torri V, Leonardo E, De Giuli P, Borasio P, *et al*. Epidermal growth factor receptor overexpression correlates with a poor prognosis in completely resected non-small-cell lung cancer. Ann Oncol 2004; 15(1): 28-32.

[10]   Volm M, Rittgen W, Drings P. Prognostic value of ERBB-1, VEGF, cyclin A, FOS, JUN and MYC in patients with squamous cell lung carcinomas. Br J Cancer 1998; 77: 663-9.

[11]   Ohsaki Y, Tanno S, Fujita Y, Toyoshima E, Fujiuchi S, Nishigaki Y, *et al*. Epidermal growth factor expression correlates with poor prognosis in non-small cell lung cancer patients with p53 overexpression. Oncol Rep 2000; 7: 603-7.

[12]   Hortobagyi GN, Sauter G. Challenges and opportunities for Erlotinib (Tarceva): what does the future hold? Semin Oncol 2003; 30: 47-53.

[13]   Ellerbroek SM, Halbleib JM, Benavidez M, Warmka JK, Wattenberg EV, Stack MS, *et al*. Phosphatidylinositol 3-kinase activity in epidermal growth factor-stimulated matrix metalloproteinase-9 production and cell surface association. Cancer Res 2001; 61: 1855-61.

[14]   Hazan R.B, Norton L. The epidermal growth factor receptor modulates the interaction of E-cadherin with the actin cytoskeleton. J Biol Chem 1998; 273: 9078-84.

[15]   Hirsch FR, Varella-Garcia M, Franklin WA, Veve R, Chen L, Helfrich B, *et al*. Evaluation of HER-2/neu gene amplification and protein expression in non-small cell lung carcinomas. Br J Cancer 2002; 86: 1449-56.

[16]   Kawamoto T, Sato JD, Le A, Polikoff J, Sato GH, Mendelshon J. Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. Proc Natl Acad Sci USA 1983; 80:1337-41.

[17]   Herbst RS, Langer CJ. Epidermal growth factor receptors as a target for cancer treatment: The emerging role of IMC-C225 in the treatment of lung and head and neck cancers. Semin Oncol 2002; 29: 27-36.

[18]   Sato JD, Kawamoto T, Le AD, Mendelshon J, Polikoff J, Sato GH. Biological effects *in vitro* of monoclonal antibodies to human epidermal growth factor receptors. Mol Biol Med 1983; 1:511-29.

[19]   Milas L, Mason K, Hunter N, Petersen S, Yamakawa M, Ang K, *et al*. *In vivo* enhancement of tumour radioresponse by C225 antiepidermal growth factor receptor antibody. Clin Cancer Res 2000; 6: 701-8.

[20]   Fan Z, Baselga J, Masui H, Mendelshon J. Antitumour effect of anti-epidermal growth factor receptor monoclonal antibodies plus cis-deammine-dichloroplatinum on well established A431 cell xenografts. Cancer Res 1993; 53: 4637-42.

[21]   Fracasso PM, Garrett C, Burris HA. A study to assess the pharmacokinetics (PK) and pharmacodynamics (PD) of a single infusion of cetuximab (IMC-C225) [abstract]. Proc Am Soc Clin Oncol 2003; 22:196a.

[22]   Tan AR, Moore DF, Hidalgo M, Doroshow JH, Poplin EA, Goodin S, *et al*. Pharmacokinetics of cetuximab after administration of escalating single dosing and weekly fixed dosing in patients with solid tumors. Clin Cancer Res 2006; 12 (21): 6517-22.

[23]   Baselga J, Pfister D, Cooper MR, Cohen R, Burtness B, Bos M, *et al*. Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. J Clin Oncol 2000; 18: 904-14.

[24]   Thienelt CD, Bunn PA Jr, Hanna N, Rosenberg A, Needle MN, Long ME, *et al*. Multi-centered phase I/II study of cetuximab with paclitaxel and carboplatin in untreated patients with stage IV non-small cell lung cancer. J Clin Oncol 2005; 23(34): 8786-93.

[25]   Robert F, Blumenschein G, Herbst RS, Fossella FV, Tseng J, Saleh MN, *et al*. Phase I/IIa study of cetuximab with gemcitabine plus carboplatin in patients with chemotherapy-naive advanced non-small cell lung cancer. J Clin Oncol 2005; 23(36): 9089-96.

[26]   Rosell R, Daniel C, Ramlau R. Randomized phase II study of cetuximab in combination with cisplatin (C) and vinorelbine (V) vs. CV alone in the first-line treatment of patients (pts) with epidermal growth factor receptor (EGFR)-expressing advanced non-small-cell lung cancer (NSCLC) [abstract]. Proc Am Soc Clin Oncol 2004; 23: 618a.

[27]   Kim E, Mauer A, Tran H. A phase II study of cetuximab, an epidermal growth factor receptor (EGFR) blocking antibody, in combination with docetaxel in chemotherapy refractory/resistant patients with advanced non-small cell lung cancer: Final report [abstract]. Proc Am Soc Clin Oncol 2003; 22: 642.

[28]   Lilenbaum R, Bonomi P, Ansari R. A phase II trial of cetuximab as therapy for recurrent non-small cell lung cancer (NSCLC): Final results [abstract 7036]. Proc Am Soc Clin Oncol 2005; 23 (Suppl): 629s.

[29]   Saltz L, Kies M, Abbruzzese J. The presence and intensity of the cetuximab-induced acne-like rash predicts increased survival in studies across multiple malignancies [abstract 817]. Proc Am Soc Clin Oncol 2003; 22: 204a.

[30]   Shridhar SS, Seymour L, Shepherd FA. Inhibitors of epidermal-growth-factor-receptors: a review of clinical research with a focus on non-small cell lung cancer. Lancet Oncol 2003; 4(7): 397-406.

[31]   Lynch DH, Yang XD. Therapeutic potential of ABX-EGF: a fully human anti-epidermal growth factor receptor monoclonal antibody for cancer treatment. Semin Oncol 2002; 29: 47-50.

[32]   Yang XD, Jia XC, Corvalan JR, Wang P, Davis CG. Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody for cancer therapy. Crit Rev Oncol Hematol 2001; 38: 17-23.

[33]   Figlin RA, Belldegrun AS, Crawford J. ABX-EGF, a fully human anti-epidermal growth factor receptor (EGFR) monoclonal antibody (mAb) in patients with advanced cancer: phase 1 clinical results [abstract 35]. Proc Am Soc Clin Oncol 2002; 21: 10a.

[34]   Foon KA, Yang XT, Weiner LM, Belldegrun AS, Figlin RA, Crawford J, *et al*. Preclinical and clinical evaluations of ABX-EGF,

a fully human anti-epidermal growth factor receptor antibody. Int J Radiat Oncol Biol Phys 2004; 58: 984-90.

[35] Crawford J, Sandler A, Hammond J. ABX-EGF in combination with paclitaxel and carboplatin for advanced non-small cell lung cancer (NSCLC) [abstract 7083]. Proc Am Soc Clin Oncol 2004; 22 (Suppl 14): 21a.

[36] Giaccone G. Epidermal growth factor receptor inhibitors in the treatment of non-small-cell lung cancer. J Clin Oncol 2005; 23: 3235-42.

[37] Sirotnak FM, Zakowski MF, Miller VA, Scher HI, Kris MG. Efficacy of cytotoxic agents against human tumour xenografts is markedly enhanced by coadministration of ZD1839 (Iressa™), an inhibitor of EGFR tyrosine kinase. Clin Cancer Res 2000; 6: 4885-92.

[38] Herbst RS, Maddox AM, Rothenberg ML, Small EJ, Rubin EH, Baselga J, et al. Selective oral epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 is generally well-tolerated and has activity in non-small-cell lung cancer and other solid tumours: results of a phase I trial. J Clin Oncol 2002; 20: 3815-25.

[39] Herbst RS, Kies MS. ZD1839 (Iressa™) in non-small cell lung cancer. Oncologist 2002; 7 (Suppl 4): 9-15.

[40] Ranson M, Hammond LA, Ferry D, Kris M, Tullo A, Murray PI, et al. ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumours: results of a Phase I trial. J Clin Oncol 2002; 20: 2240-50.

[41] Cappuzo F, Ardizzoni A, Soto-Parra H, Gridelli C, Maione P, Tiseo M, et al. Epidermal growth factor receptor targeted therapy by ZD 1839 (Iressa™) in patients with brain metastases from non-small cell lung cancer. Lung Cancer 2003; 41: 227-31.

[42] Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, et al: Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. J Clin Oncol 2003; 21: 2237-46.

[43] Kris MG, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: A randomized trial. JAMA 2003; 290: 2149-58.

[44] Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rossel R, Miller V, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: A phase III trial (INTACT 1). J Clin Oncol 2004; 22: 777-84

[45] Herbst RS, Giaccone G, Schiller JH, Natale RB, Miller V, Manegold C, et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: A phase III trial (INTACT 2). J Clin Oncol 2004; 22: 785-94.

[46] Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomized, placebo-controlled, multicentre study (Iressa™ Survival Evaluation in Lung Cancer). Lancet 2005; 366(9496): 1527-37.

[47] Kelly K, Gaspar LE, Chansky K. Low incidence of pneumonitis on SWOG 0023: a preliminary analysis of an ongoing phase III trial of concurrent chemoradiotherapy followed by consolidation docetaxel and Iressa™/placebo maintenance in patients with inoperable stage III non-small cell lung cancer [abstract 7058]. Proc Am Soc Clin Oncol 2005; 23 (Suppl): 634s.

[48] Gandara DR, Chansky K, Albain KS, Leigh BR, Gaspar LE, Lara PN Jr, et al. Southwest Oncology Group Study S9504. Consolidation docetaxel after concurrent chemoradiotherapy in stage IIIB non-small-cell lung cancer: phase II. J Clin Oncol 2003; 21: 2004-10.

[49] Pollack VA, Savage DM, Baker DA, Tsaparikos KE, Sloan DE, Moyer JD, et al. Inhibition of epidermal growth factor receptor-associated tyrosine phosphorylation in human carcinomas with CP-358,774: dynamics of receptor inhibition in situ and antitumour effects in athymic mice. J Pharmacol Exp Ther 1999; 291: 739-48.

[50] Moyer JD, Barbacci EG, Iwata KK, Arnold L, Boman B, Cunningham A, et al. Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. Cancer Res 1997; 57: 4838-48.

[51] Hidalgo M, Siu LL, Nemunaitis J, Rizzo J, Hammond LA, Takimoto C, et al. Phase I and pharmacology study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor in patients with advanced solid malignancies. J Clin Oncol 2001; 119:3267-79.

[52] Karp D, Ferrante KJ, Tensfeldt TG. A phase I dose escalation study of epidermal growth factor receptor (EGFR) tyrosine kinase (TK) inhibitor CP-358,774 in patients (pts) with advanced solid tumours [abstract 208]. Lung Cancer 2000; 29:72.

[53] Perez-Soler R, Chachoua A, Huberman M. A phase II trial of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (OSI-774) following platinum-based chemotherapy in patients with advanced EGFR-expressing non small cell lung cancer (NCSLC) [abstract 1235]. Proc Am Soc Clin Oncol 2001; 20:210a.

[54] Patel JD, Miller VA, Kris MG. Encouraging activity and durable responses demonstrated by the EGFR tyrosine kinase inhibitor erlotinib (Tarceva™, OSI-774) in patients with advanced bronchioalveolar (BAC) cell carcinoma. Lung Cancer 2003; 41(Suppl): S56.

[55] Perez-Soler R, Chachoua A, Hammond LA, Rowinsky EK, Huberman M, Karp D, et al. Determinants of tumour response and survival with erlotinib in patients with NSCLC. J Clin Oncol 2004; 22: 3238-3247

[56] Clark GM, Perez-Soler R, Siu L, Gordon A, Santabarbara P. Rash severity is predictive of increased survival with erlotinib Hclas. Proc Am Soc Clin Oncol 2003; 22: 196a.

[57] Gatzemeier U, Pluzanska A, Szczesna A. Results of a phase III trial of erlotinib (OSI-774) combined with cisplatin and gemcitabine (GC) chemotherapy in advanced non-small cell lung cancer [abstract]. Proc Am Soc Clin Oncol 2004; 22: 7010a.

[58] Herbst RS, Prager D, Hermann R, Fehrenbacher L, Johnson BE, Sandler A, et al. TRIBUTE-A phase III trial of erlotinib HCI (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small cell lung cancer. J Clin Oncol 2005; 23(25): 5892-9.

[59] Miller VA, Herbst R, Prager D. Long survival of never smoking non-small cell lung cancer (NSCLC) patients treated with erlotinib HCI (OSI-774) and chemotherapy: subgroup analysis of TRIBUTE. ASCO Annual Meeting Proceedings (Post-Meeting Edition); J Clin Oncol 2004; 22 (14 Suppl): 7061.

[60] Johnson DH. Gefitinib (Iressa™) trials in non-small cell lung cancer. Lung Cancer 2003; 41 (Suppl 1): S23-S28.

[61] Baselga J. Combining the anti-EGFR agent gefitinib with chemotherapy in non-small-cell lung cancer: how do we go from INTACT to impact? J Clin Oncol 2004; 22: 759-61.

[62] Dancey JE, Freidlin B. Targeting epidermal growth factor receptor-are we missing the mark? Lancet 2003; 362: 62-4.

[63] Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small cell lung cancer. N Engl J Med 2005; 353(2): 123-32.

[64] Garrison MA, Tolcher A, McGreery H. A phase I pharmacokinetic study of CI-1033, a pan-erbB tyrosine kinase inhibitor, given orally on days 1,8 and 15 every 28 days to patients with solid tumours [abstract]. Proc Am Soc Clin Oncol 2001; 21:72a.

[65] Shin DM, Nemunaitis J, Zinner RJ. A phase I clinical and biomarker study of CI-1033, a novel pan-erbB tyrosine kinase inhibitor in patients with solid tumours [abstract]. Proc Am Soc Clin Oncol 2001; 20: 82a.

[66] Dumez H, Hoekstra R, Eskens F. A phase I pharmacologic study of PKI 166 and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, administered orally 3 times a week to patients with advanced cancer [abstract]. Proc Am Soc Clin Oncol 2002; 21: 86a.

[67] Murren JTR, Papadimitrakopoulou VA, Sizer KC. A phase I, dose-escalating study to evaluate the biological activity and pharmacokinetics of PKI166, a novel tyrosine kinase inhibitor, in patients with advanced cancers [abstract 377]. Program and abstracts of the 38th Annual Meeting of the American Society of Clinical Oncology; 2002 May 18-21; Orlando, Florida.

[68] De Simone PA, Bence AK, Anderson EB. A phase I study to investigate the safety, tolerability and pharmacokinetics of single oral escalating doses of GW572016 in healthy volunteers [abstract]. Proc Am Soc Clin Oncol 2002; 21: 94a.

[69] Minami H, Nakagawa K, Kawada K. A phase I study of GW572016 in patients with solid tumours; ASCO Annual Meeting Proceedings (Post-Meeting Edition). J Clin Oncol 2004; 22: 3048.

[70]   Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, *et al*. Activating mutations in the epidermal growth factor receptor underlying responsiveness for non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350: 2129-39.

[71]   Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, *et al*. EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 2004; 304: 497-500.

[72]   Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, *et al*. EGF receptor gene mutations are common in lung cancers from 'never smokers' and are associated with sensitivity of tumours to gefitinib and erlotinib. Proc Natl Acad Sci USA 2004; 101: 13306-11.

[73]   Pao W, Miller VA. Epidermal growth factor receptor mutations, small-molecule kinase inhibitors and non-small-cell lung cancer: current knowledge and future directions. J Clin Oncol 2005; 23: 2256-2658.

[74]   Janne PA. Epidermal growth factor receptor mutations in non-small-cell lung cancer: implications for treatment and tumour biology. J Clin Oncol 2005; 23: 3227-34.

[75]   Shah NT, Miller VA, Kris MG. Bronchioalveolar histology and smoking history predict response to gefitinib. Proc Am Soc Clin Oncol 2003; 22: 628a.

[76]   Lynch TJ, Bell DW, Harber DA. Correlation of molecular markers including mutations with clinical outcomes in advanced non small cell lung cancer (NSCLC) patients treated with gefitinib, chemotherapy or chemotherapy and gefitinib in IDEAL and INTACT clinical trials [abstract 7006]. Proc Am Soc Clin Oncol 2005; 23:622s.

[77]   Tsao MS, Sakurada A, Lorimer J. National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) trial BR.21. Molecular analysis of the epidermal growth factor receptor (EGFR) gene and protein expression in patients treated with erlotinib [abstract 7007]. Proc Am Soc Clin Oncol 2005; 23 (Suppl): 622s.

[78]   Gumerlock PH, Holland WS, Chen H. Mutational analysis of K-RAS and EGFR implicates K-RAS as a resistance marker in the Southwest Oncology Group (SWOG) trial S0126 of bronchioalveolar carcinoma (BAC) patients treated with gefitinib [abstract 7008]. Proc Am Soc Clin Oncol 2005; 23 (Suppl): 623s.

[79]   Han SW, Kim TY, Hwang PG, Jeong S, Kim J, Choi IS, *et al*. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. J Clin Oncol 2005; 23: 2493-501.

[80]   Mitsudomi T, Kosaka T, Endoh H, Horio Y, Hida T, Mori S, *et al*. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small cell lung cancer with postoperative recurrence. J Clin Oncol 2005; 23: 2513-20.

[81]   Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, *et al*. Epidermal growth factor receptor gene and protein and gefitinib sensivity in non-small-cell lung cancer. J Natl Cancer Inst 2005; 97: 643-55.

[82]   Rodenhuis S, Slebos RJ, Boot AJ, Evers SG, Mooi WJ, Wagenaar SS, *et al*. Incidence and possible clinical significance of K-ras oncogene activation in adenocarcinoma of the human lung. Cancer Res 1998; 48: 5738-41.

[83]   Eberhard DA, Johnson BE, Amler LC, Goddard AD, Heldens SL, Herbst RS, *et al*. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol 2005; 23(25): 5900-9.

[84]   Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, *et al*. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 2005; 352: 786-92.

[85]   Shigematsu H, Takahashi T, Nomura M, Majmudar K, Suzuki M, Lee H, *et al*. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res 2005, 65: 1642-46.

[86]   Cappuzzo F, Gregorc V, Rossi E, Cancellieri A, Magrini E, Paties CT, *et al*. Gefitinib in pretreated non-small cell lung cancer (NSCLC): analysis of efficacy and correlation with HER2 and epidermal growth factor receptor expression in locally advanced or metastatic NSCLC. J Clin Oncol 2003; 21: 2658-63.

[87]   Bunn PA Jr, Helfrich B, Soriano AF, Franklin WA, Varella-Garcia M, Hirsch FR, *et al*. Expression of HER-2/neu in human lung can-

[88]   cer cell lines by immunohistochemistry and fluorescence in situ hybridization and its relationship to *in vitro* cytotoxicity by trastuzumab and chemotherapeutic agents. Clin Cancer Res 2001; 7: 3239-50.

[88]   Lewis GD, Figari I, Fendly B, Wong WL, Carter P, Gorman C, *et al*. Differential responses of human tumour cell lines to anti-p185HER2 monoclonal antibodies. Cancer Immunol Immunother 1993; 37: 255-63.

[89]   Krug LM, Miller VA, Crapanzano J. Randomized phase II trial of trastuzumab (Tras) plus either weekly docetaxel (Dopc) or paclitaxel (Pac) in previously untreated advanced non-small cell lung cancer [abstract]. Proc Am Soc Clin Oncol 2001; 20: 333a.

[90]   Langer CJ, Adak S, Thor A, Vangel M, Johnson D. Phase II Eastern Cooperative Oncology Group (ECOG) pilot study of paclitaxel (P), carboplatin (C) and trastuzumab (T) in HER-2/neu (+) advanced non-small cell lung cancer [abstract]. Proc Am Soc Clin Oncol 2001; 20: 315a.

[91]   Zinner GR, Glisson BS, Pisters KM. Cisplatin and gemcitabine combined with herceptin in patients with HER2 overexpressing, untreated, advanced, non-small cell lung cancer (NSCLC): a phase II trial [abstract]. Proc Am Soc Clin Oncol 2001; 20: 328a.

[92]   Gatzemeier U, Groth G, Butts C, Van Zandwijk N, Shepherd F, Ardizzoni A, *et al*. Randomized phase II trial of gemcitabine-cisplatin with or without trastuzumab in HER-2-positive non-small cell lung cancer. Ann Oncol 2004; 125: 19-27.

[93]   Folkman J. Tumour angiogenesis: Therapeutic implications. N Engl J Med 1971; 285: 1182-86.

[94]   Bergers G, Benjamin LE. Tumourigenesis and the angiogenic switch. Cancer Nat Rev 2003; 3: 401-10.

[95]   Folkman J. Clinical application of research on angiogenesis. N Engl J Med 1995; 333: 1757-63.

[96]   Hanahan D, Folkman J. Patterns and emerging mechanisms of angiogenetic switch during tumourigenesis. Cell 1996; 86: 353-64.

[97]   Hobson B, Denekamp J. Endothelial proliferation in tumours and normal tissues: continuous labeling studies. Br J Cancer 1984; 49(4): 405-13.

[98]   Cox G, Jones JL, Walker RA, Steward WP, O'Byrne KJ. Angiogenesis and non-small cell lung cancer. Lung Cancer 2000; 27(2): 81-100.

[99]   Dazzi C, Cariello A, Maioli P, Solaini L, Scarpi E, Rosti G, *et al*. Prognostic and predictive value of intratumoural microvessels density in operable non-small-cell lung cancer. Lung Cancer 1999; 24: 81-8.

[100]   Macchiarini P, Fontanini G, Hardin MG, Squartini F, Angeletti CA. Relation of neovascularization to metastasis of non-small cell lung cancer. Lancet 1992; 340: 145-6.

[101]   Senger Dr, Galli Sj, Drovak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumour cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983; 219: 983-5.

[102]   Keshet E, Ben-Sasson SA. Anticancer drug targets: approaching angiogenesis. J Clin Invest 1999; 104: 1497-550.

[103]   Ferrara N, Gerber HP, Lecouter J. The biology of VEGF and its receptors. Nat Med 2003; 9: 669-76.

[104]   Tamura M, Ohta Y, Kajita T, Kimura K, Go T, Oda M, *et al*. Plasma VEGF concentration can predict the tumour angiogenic capacity in non-small cell lung cancer. Oncol Rep 2001; 8: 1097-102.

[105]   Matsuyama W, Hashiguchi T, Mizoguchi A, Iwami F, Kawabata M, Arimura K, *et al*. Serum levels of vascular endothelial growth factor dependent on the stage progression of lung cancer. Chest 2000; 118: 948-51.

[106]   Li CY, Shan S, Huang Q, Braun RD, Lanzen J, Hu K, *et al*. Initial stages of tumour cell-induced angiogenesis: evaluation *via* skin window chambers in rodent models. J Natl Cancer Inst 2000; 92: 143-7.

[107]   Drovak HF, Brown LF, Detmar M, Drovak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995; 146(5): 1029-39.

[108]   Nor JE, Christensen J, Mooney DJ, Polverini PJ. Vascular endothelial growth factor (VEGF)-mediated angiogenesis is associated with enhanced endothelial cell survival and induction of Bcl-2 expression. Am J Pathol 1999; 154(2): 375-84.

[109] Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A, *et al*. Tumour response to radiotherapy regulated by endothelial cell apoptosis. Science 2003; 300: 1155-9.

[110] Yuan A, Yu CJ, Kuo SH, Chen WJ, Lin FY, Luh KT, *et al*. Vascular endothelial growth factor 189 mRNA isoform expression specifically correlates with tumour angiogenesis, patient survival, and postoperative relapse in non-smallcell lung cancer. J Clin Oncol 2001 19: 432-41.

[111] Yano T, Tanikawa S, Fujie T, Masutani M, Horie T. Vascular endothelial growth factor expression and neovascularisation in non-small cell lung cancer. Eur J Cancer 2000; 36: 601-9.

[112] Han H, Silverman JF, Santucci TS, Macherey RS, d'Amato TA, Tung MY, *et al*. Vascular endothelial growth factor expression in stage I non-small cell lung cancer correlates with neoangiogenesis and a poor prognosis. Ann Surg Oncol 2001; 8: 72-9.

[113] Minami K, Saito Y, Imamura H, Okamura A. Prognostic significance of p53, Ki-67, VEGF, and Glut-1 in resected stage I adenocarcinoma of the lung. Lung Cancer 2002; 38: 51-7.

[114] Presta LG, Chen H, O'Connor SJ, Chisholm V, Meng YG, Krummen L, *et al*. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumours and other disorders. Cancer Res 1997; 57: 4593-9.

[115] Kabbinavar FF, Wong JT, Ayala RE. The effect of antibody to vascular endothelial growth factor and cisplatin on the growth of lung tumours in nude mice [abstract]. Proc Am Assoc Cancer Res 1995; 36: 488a.

[116] Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, *et al*. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature 1993; 362: 841-4.

[117] Sweeney CJ, Miller KD, Sissons SE, Nozaki S, Heilman DK, Shen J, *et al*. The antiangiogenic property of docetaxel is synergistic with a recombinant humanized monoclonal antibody against vascular endothelial growth factor or 2-methoxyestradiol but antagonized by endothelial growth factors. Cancer Res 2001; 61: 3369-72.

[118] Margolin K, Gordon MS, Holmgren E, Gaudreault J, Novotny W, Fyfe G, *et al*. Phase Ib trial of intravenous recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer. Pharmacologic and long-term safety data. J Clin Oncol 2001; 19: 851-56.

[119] Gordon MS, Margolin K, Talpaz M, Sledge GW Jr, Holmgren E, Benjamin R, *et al*. Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer. J Clin Oncol 2001; 19: 843-50.

[120] Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, *et al*. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced metastatic non-small cell lung cancer. J Clin Oncol 2004; 22: 2184-91.

[121] Sandler AB, Gray R, Brahmer J. Randomized phase II/III trial of paclitaxel (P) plus carboplatin (C) with or without bevacizumab (NSC # 704865) in patients with advanced non-squamous non-small cell lung cancer (NSCLC): an Eastern Cooperative Oncology Group (ECOG) Trial - E4599 [abstract LBA4]. Proc Am Soc Clin Oncol 2005; 23(Suppl): 2s.

[122] Stacker SA, Achen MG, Jussila L, Baldwin MA, Alitalo K. Lymphangiogenesis and cancer metastasis. Nat Rev Cancer 2002; 2: 573-83.

[123] Yamaguchi TP, Dumont DJ, Conlon RA, Breitman ML, Rossant J. flk-1, an flt-related receptor kinase is an early marker for endothelial cell precursors. Development 1993; 118: 489-98.

[124] Fong GH, Klingensmith J, Wood CR, Rossant J, Breitman ML. Regulation of flt-1 expression during mouse embryogenesis suggests a role in the establishment of vascular endothelium. Dev Dyn 1996; 207: 1-10.

[125] Toi M, Matsumoto T, Bando H. Vascular endothelial growth factor: its prognostic, predictive, and therapeutic implications. Lancet 2001; 2: 667-73.

[126] Wedge SR, Ogilvie DJ, Dukes M, Kendrew J, Chester R, Jackson JA, *et al*. ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumour growth following oral administration. Cancer Res 2002; 62: 4645-55.

[127] Carlomagno F, Vitagliano D, Guida T, Ciardiello F, Tortora G, Vecchio G, *et al*. ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. Cancer Res 2002; 62: 7284-90.

[128] Wedge SR, Ogilvie DJ, Dukes M. Chronic and acute effects of ZD6474, a VEGF receptor tyrosine kinase inhibitor, on established human tumour xenografts. Proc Am Assoc Cancer Res 2000; 41: 3610.

[129] Williams KJ, Telfer BA, Brave S, Kendrew J, Whittaker L, Stratford IJ, *et al*. ZD6474, a potent inhibitor of vascular endothelial growth factor signaling, combined with radiotherapy: schedule-dependent enhancement of antitumour activity. Clin Cancer Res 2004; 10 (24): 8587-93.

[130] Hurwitz H, Halden SN, Eckhardt S. Clinical evaluation of ZD6474, an orally active inhibitor of VEGF signaling in patients with solid tumours [abstract 325]. Proc Am Soc Clin Oncol 2002; 21a.

[131] Minami H, Ebi H, Tahara M. Phase I study of an oral VEGF receptor tyrosine kinase inhibitor ZD6474, in Japanese patients with solid tumours [abstract]. Proc Am Soc Clin Oncol 2003; 21: 194.

[132] Herbst R, Johnson B, Rowbottom J. ZD6474 plus docetaxel in patients with previously treated NSCLC: results of a randomized, placebo controlled phase II trial [abstract O-100]. Lung Cancer 2005; 49(Suppl 2): S35.

[133] Tolcher AW, O'Leary J, De Bono JS. A phase I and biologic correlative study of an oral endothelial vascular growth factor receptor-2 (VEGFR-2) tyrosine kinase inhibitor, CP-547,632 in patients with advanced solid tumours [abstract 334]. Proc Am Soc Clin Oncol 2002; 21: 84a.

[134] Roberts WG, Jani J, Beebe J. Preclinical development of CP-547,632, a novel VEGFR-2 inhibitor for cancer therapy [abstract 473]. Proc Am Soc Clin Oncol 2002; 21: 119a.

[135] Hidalgo M, Eckhardt SG. Development of matrix metalloproteinase inhibitors in cancer therapy. J Natl Cancer Inst 2001; 93: 178-93.

[136] Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000; 18: 1135-49.

[137] Fortunato SJ, Menon R. Screening of novel matrix matalloproteinases (MMPs) in human fetal membranes. J Assist Reprod Genet 2002; 19 (10): 483-6.

[138] Noel A, Gilles C, Bajou K, Devy L, Kebers F, Lewalle JM, *et al*. Emerging roles for proteinases in cancer. Invasion Metastasis 1997; 17: 221-39.

[139] Laack E, Kohler A, Kugler C, Dierlamm T, Knuffmann C, Vohwinkel G, *et al*. Pretreatment serum levels of matrix metalloproteinase-9 and vasculae endothelial growth factor in non-small-cell lung cancer. Ann Oncol 2002; 13: 1550-7.

[140] Wojtowicz-Praga SM, Dickson RB, Hawkins MJ. Matrix metalloproteinase inhibitors. Invest New Drugs 1997; 15(1): 61-75.

[141] Brown PD, Giavazzi R. Matrix metalloproteinase inhibition: a review of anti-tumour activity. Ann Oncol 1995; 6(10): 967-74.

[142] Steward WP. Marimastat (BB2516): current status of development. Cancer Chemother Pharmacol 1999; 43 (Suppl): S56-60.

[143] Shepherd FA, Giaccone G, Seymour L, Debruyne C, Bezjak A, Hirsh V, *et al*. Prospective, randomized, double-blind, placebo-contolled trial of marimastat after response to first-line chemotherapy in patients with small-cell lung cancer: a trial of the national cancer institute of Canada-clinical trials group and the European organization for research and treatment of cancer. J Clin Oncol 2002; 20(22): 4434-9.

[144] British Biotech Plc. Marimastat update [news release]. Available from: http://www.britishbiotech.co.uk/news/173,183 British Biotech Plc. www.britishbiotech.com.

[145] Erlichman C, Adjei AA, Alberts SR, Sloan JA, Goldberg RM, Pitot HC, *et al*. Phase I study of the matrix metalloproteinase inhibitor, BAY 12-9566. Ann Oncol 2001; 12(3): 389-395.

[146] Rigas JR, Denham CA, Rinaldi A. Randomized placebo-controlled trials of the matrix metalloproteinase inhibitor (MMPI), BAY 12-9566 as adjuvant therapy for patients with small cell and non-small cell lung cancer. Proc Am Soc Clin Oncol 2003; 22: 628a.

[147] Falardeau P, Champagne P, Poyet P, Hariton C, Dupont E. Neovastat, a naturally occurring multifunctional antiangiogenic drug, in phase III clinical trials. Semin Oncol 2001; 28: 620-5.

[148] Latreille J, Batist G, Laberge F, Champagne P, Croteau D, Falardeau P, *et al*. Phase I/II trial of the safety and efficacy of AE-941 (Neovastat) in the treatment of non-small-cell lung cancer. Clin Lung Cancer 2003; 4: 231-6.

[149] Liu J, Tsao MS, Pagura M, Shalinsky DR, Khoka R, Fata J, *et al*. Early combined treatment with carboplatin and the MMP inhibitor, prinomastat, prolongs survival and reduces systemic metastasis in an aggressive orthotopic lung cancer model. Lung Cancer 2003; 42(3): 335-44.

[150] Smylie M, Mercier R, Aboulafia D. Phase III study of the matrix metalloprotease (MMP) inhibitor prinomastat in patients having advanced non-small cell lung cancer [abstract]. Proc Am Soc Clin Oncol 2001; 20: 307a.

[151] Bissett D, O'Byrne KJ, von Pawel J, Gatzemeier U, Price A, Nicolson M, *et al*. Phase III study of matrix metalloproteinase inhibitor prinomastat in non-small cell lung cancer. J Clin Oncol 2005; 23(4): 842-9.

[152] Naglich JG, Jure-Kunkel M, Gupta E, Fargnoli J, Henderson AJ, Lewin AC, *et al*. Inhibition of angiogenesis and metastasis in two murine models by the matrix metalloproteinase inhibitor, BMS-275291. Cancer Res 2001; 61(23): 8480-5.

[153] Rizvi NA, Humphrey JS, Ness EA, Johnson MD, Gupta E, Williams K, *et al*. A phase I study of oral BMS-275291, a novel non-hydroxamate sheddase-sparing matrix metalloproteinase inhibitor, in patients with advanced or metastatic cancer. Clin Cancer Res 2004; 10:1963-70.

[154] Leighl NB, Paz-Ares L, Douillard JY, Peschel C, Arnold A, Depierre A, *et al*. Randomized phase III study of matrix metalloproteinase inhibitor BMS-275291 in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: National Cancer Institute of Canada-Clinical Trials group Study BR.18. J Clin Oncol 2005; 23(12): 2831-9.

[155] Harris AL. Are angiostatin and endostatin cures for cancer? Lancet 1998; 351: 1598-9.

[156] Yokoyama Y, Dhanabal M, Griffioen AW, Sukhatme VP, Ramakrishnan S. Synergy between angiostatin and endostatin: inhibition of ovarian cancer growth. Cancer Res 2000; 60: 2190-6.

[157] O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, *et al*. Endostatin: an endogenous inhibitor of angiogenesis and tumour growth. Cell 1997; 88(2): 277-85.

[158] Eder JP Jr, Supko JG, Clark JW, Puchalski TA, Garsia-Carbonero R, Ryan DP, *et al*. Phase I clinical trial of recombinant human endostatin administered as a short intravenous infusion repeated daily. J Clin Oncol 2002; 20: 3772-84.

[159] Kisker O, Becker CM, Prox D, Fannon M, D'Amato R, Flynn E, *et al*. Continuous administration of endostatin by intraperitoneally implanted osmotic pump improves the efficacy and potency of therapy in a mouse xenograft tumour model. Cancer Res 2001; 61: 7669-74.

[160] te Velde EA, Vogten JM, Gebbink MF, van Gorp JM, Voest EE, Borel Rinkes IH. Enhanced antitumour efficacy by combining conventional chemotherapy with angiostatin or endostatin in a liver meatstasis model. Br J Surg 2002; 89: 1302-9.

[161] O'Reilly MS, Holmgren L, Chen C, Folkman J. Angiostatin induces and sustains dormacy of human primary tumours in mice. Nat Med 1996; 2: 689-92.

[162] Kirsch M, Strasser J, Allende R, Bello L, Zhang J, Black PM. Angiostatin suppresses malignant glioma growth in vivo. Cancer Res 1998; 58: 4654-9.

[163] Volm M, Mattern J, Koomagi R. Angiostatin expression in non-small cell lung cancer. Clin Cancer Res 2000; 6(8): 3236-40.

[164] Kurup A, Lin CW, Murry DJ, Dobrolecki L, Estes D, Yiannoutsos CT, *et al*. Recombinant human angiostatin (rh Angiostatin) in combination with paclitaxel and carboplatin in patients with advanced non-small-cell lung cancer: a phase II study from Indiana University. Ann Oncol 2006; 17(1): 97-103.

[165] D' Amato RJ, Loughan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci USA 1994; 91(9): 4082-5.

[166] Merchant JJ, Kim K, Mehta MP, Ripple GH, Larson ML, Brophy DJ, *et al*. Pilot and safety trial of carboplatin, paclitaxel and thalidomide in advanced non-small cell lung cancer [discussion 53-54]. Clin Lung Cancer 2000; 2: 48-52.

[167] Sills AK Jr, Williams JI, Tyler BM, Epstein BS, Sipos EP, Davis JD, *et al*. Squalamine inhibits angiogenesis and solid tumour growth *in vivo* and perturbs embryonic vasculature. Cancer Res 1998; 58: 2784-92.

[168] Akhter S, Nath SK, Tse CM, Williams J, Zasloff M, Donowitz M. Squalamine, a novel cationic steroid, specifically inhibits the brush-border Na+/H+ exchanger isoform NHE3. Am J Physiol 1999; 276: C136-C144.

[169] Teicher BA, Williams JI, Takeuchi H, Ara G, Herbst RS, Buxton D. Potential of the aminosterol, squalamine, in combination therapy in the rat 13,762 mammary carcinoma and the murine Lewis lung carcinoma. Anticancer Res 1998; 18: 2567-74.

[170] Schiller JH, Bittner G. Potentiation of platinum antitumour effects in human lung tumour xenografts by the angiogenesis inhibitor squalamine: effects on tumour neovascularization. Clin Cancer Res 1999; 5: 4287-94.

[171] Patnaik A, Rowinsky E, Hammond L. A phase I and pharmacokinetic study of the unique angiogenesis inhibitor squalamine lactate. Proc Am Soc Clin Oncol 1999; 18: 623a.

[172] Bhargava PTN, Marshall J. A phase I safety, tolerance, and pharmacokinetic study of rising dose, rising duration continuous infusion MSI-1256F (squalamine lactate) in patients with advanced malignancies. Proc Am Soc Clin Oncol 1999; 18: 625a.

[173] Kalidas M, Hammond L, Patnaik A. A phase I and pharmacokinetic study of the angiogenesis inhibitor, squalamine lactate (MSI 1256-F) [abstract 698]. Proc Am Soc Clin Oncol 2000; 19: 180a.

[174] Herbst RS, Hammond LA, Carbone DP, Tran HT, Holroyd KJ, Desai A, *et al*. A phase I/IIA trial of continuous five-day infusion of squalamine lactate (MSI-1256F) plus carboplatin and paclitaxel in patients with advanced non-small cell lung cancer. Clin Cancer Res 2003; 9(11): 4108-15.

[175] Goto H, Yano S, Zhang H, Matsumori Y, Ogawa H, Blakey DC, *et al*. Activity of a new vascular targeting agent, ZD6126, in pulmonary metastases by human lung adenacarcinoma in nude mice. Cancer Res 2002; 62:3711-15.

[176] Raben D, Bianco C, Damiano V, Bianco R, Melisi D, Mignogna C, *et al*. Antitumour activity of ZD6126, a novel vascular-targeting agent, is enhanced when combined with ZD1839, an epidermal growth factor receptor tyrosine kinase inhibitor, and potentiates the effects of radiation in a human non-small cell lung cancer xenograft model. Mol Cancer Ther 2004; 3(8): 977-83.

[177] Guy SP, Ashton SE, Hughes G. Gefitinib (Iressa™, ZD1839) enhances the activity of the novel vascular-targeting agent ZD6126 in human colorectal cancer and non-small cell lung cancer (NSCLC) xenograft models [abstract B13]. Clin Cancer Res 2003; 9: 6142s.

[178] Gadgeel SM, LoRusso PM, Wozniak AJ. A dose escalation study of the novel vascular-targeting agent, ZD6126 in patient with solid tumours [abstract 438]. Proc Am Soc Clin Oncol 2002; 21: 110a.

[179] Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, *et al* Cyclooxygenase in biology and disease. FASEB J 1998; 12:1063-73.

[180] Koki A, Khan NK, Woerner BM, Dannenberg AJ, Olson L, Seibert K, *et al*. Cyclooxygenase-2 in human pathological disease. Adv Exp Med Biol 2002; 507: 177-84.

[181] Brown JR, DuBois RN. Cyclooxygenase as a target in lung cancer. Clin Cancer Res 2004; 10: 4266s-9s.

[182] Achiwa H, Yatabe Y, Hida T, Kuroishi T, Kozaki K, Nakamura S, *et al*. Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinomas. Clin Cancer Res 1999; 5: 1001-5.

[183] Hida T, Yatabe Y, Achiwa H, Muramatsu H, Kozaki K, Nakamura S, *et al*. Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. Cancer Res 1998; 58: 3761-4.

[184] Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, Ristimaki A. Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res 1998; 58: 4997-01.

[185] Krysan K, Merchant FH, Zhu L, Dohadwala M, Luo J, Lin Y, *et al*. COX-2-dependent stabilization of survivin in non-small cell lung cancer. FASEB J 2004; 18: 206-8.

[186] Krysan K, Dalwadi H, Sharma S, Pold M, Dubinett S. Cyclooxygenase-2 dependent expression of survinin is critical for apoptosis resistance in non-small cell lung cancer. Cancer Res 2004; 64(18): 6359-62.

[187]   Sanchez-Alcazar JA, Bradbury DA, Pang L, Knox AJ. Cyclooxygenase (COX) inhibitors induced apoptosis in non-small cell lung cancer through cyclooxygenase independent pathways. Lung Cancer 2003; 40: 33-44.

[188]   Grosch S, Tegeder I, Niederberger E, Brautigam L, GeisslingerG. COX-2 independent induction of cell cycle arrest and apoptosis in colon cancer cells by the selective COX-2 inhibitor celecoxib. FASEB J 2001; 15: 2742-4.

[189]   Blumenthal RD, Waskewich C, Goldenberg DM, Lew W, Fleflech C, Burton J. Chronotherapy and chronotoxicity of the cyclooxygenase-2 inhibitor, celecoxib, in athymic mice bearing human breast cancer xenografts. Clin Cancer Res 2001; 7: 3178-85.

[190]   Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, Chen CS. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. J Biol Chem 2000; 275: 11397-03.

[191]   Liu X, Yue P, Zhou Z, Khuri FR, Sun SY. Death receptor regulation and celecoxib-induced apoptosis in human lung cancer cells. J Natl Cancer Inst 2004; 96 (23): 1769-80.

[192]   Liao Z, Komaki R, Milas L, Yuan C, Kies M, Chang JY, et al. A phase I clinical trial of thoracic radiotherapy and concurrent celecoxib for patients with unfavorable performance status inoperable/unresectable non-small cell lung cancer. Clin Cancer Res 2005; 11(9): 3342-8.

[193]   Nugent FW, Mertens WC, Graziano S, Levitan N, Collea R, Gajra A, et al. Docetaxel and cyclooxygenase-2 inhibition with celecoxib for advanced non-small cell lung cancer progressing after platinum-based chemotherapy: a multicenter phase II trial. Lung Cancer 2005; 48(2): 267-73.

[194]   Csiki I, Morrow JD, Sandler A, Shyr Y, Oates J, Williams MK, et al. Targeting cyclooxygenase-2 in recurrent non-small cell lung cancer: A phase II trial of celecoxib and docetaxel. Clin Cancer Res 2005; 11(18): 6634-40.

[195]   Gadgeel SM, Shehadeh N, Kraut MJ. Preliminary results of a phase II study of celecoxib and weekly docetaxel in elderly (>70 yrs) or PS2 patients with advanced non-small cell lung cancer (NSCLC) [abstract 0-110]. Lung Cancer 2003; 41(Suppl 2): S35.

[196]   Gasparini G, Meo S, Comella G, Stani SC, Mariani L, Gamucci T, et al. The combination of the selective cyclooxygenase-2 inhibitor celecoxib with weekly paclitaxel is a safe and active second-line therapy for non-small cell lung cancer: a phase II study with biological correlates. Cancer J 2005; 11(3): 209-16.

[197]   Altorki NK, Keresztes RS, Port JL. Celecoxib (Celebrex), a selective COX-2 inhibitor, enhances the response to preoperative paclitaxel/carboplatin in early stage non-small cell lung cancer [abstract 101]. Proc Am Soc Clin Oncol 2002; 21:26a.

[198]   Pisters KM, Ginsberg RJ, Giroux DJ, Putnam JB Jr, Kris MG, Johnson DH, et al. Bimodality Lung Oncology Team. Induction chemotherapy before surgery for early-stage lung cancer: a novel approach. J Thorac Cardiovasc Surg 2000; 119: 429-39.

[199]   Tanaka T, Delong PA, Amin K, Henry A, Kruklitis R, Kapoor V, et al. Treatment of lung cancer using clinically relevant oral doses of the cyclooxygenase-2 inhibitor rofecoxib: potential value as adjuvant therapy after surgery. Ann Surg 2005; 241(1): 168-78.

[200]   Rudin CM, Kozloff M, Edelman MJ, Hoffman PC, Vokes EE. Bcl-2 as a target in lung cancer [abstract E-64]. Lung Cancer 2003; 41(Suppl 3): S80.

[201]   Reed JC. Mechanisms of apoptosis avoidance in cancer. Curr Opin Oncol 1999; 11: 68-75.

[202]   Berardo MD, Elledge RM, Moor C, Clark GM, Osborne CK, Allred DC. Bcl-2 and apoptosis in lymph node positive breast carcinoma. Cancer 1998; 82:1296-02.

[203]   Miyashita T, Reed JC. Bcl-2 gene transfer increases relative resistance of S49.1 and WEH17.2 lymphoid cells to cell death and DNA fragmentation induced by glucocorticoids and multiple chemotherapeutic drugs. Cancer Res 1992; 52: 5407-11.

[204]   Schmitt CA, Rosenthal CT, Lowe SW. Genetic analysis of chemoresistance in primary murine lymphomas. Nat Med 2000; 6: 1029-35.

[205]   Choi J, Choi K, Benveniste EN, Rho SB, Hong YS, Lee JH, et al. Bcl-2 promotes invasion and lung metastasis by inducing matrix metalloproteinase-2. Cancer Res 2005; 65(13): 5554-60.

[206]   Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. Genes Dev 1999; 13: 1899-11.

[207]   Ben-Ezra JM, Kornstein MJ, Grimes MM, Krystal G. Small cell carcinomas of the lung express the bcl-2 protein. Am J Pathol 1994; 145: 1036-40.

[208]   Koty PP, Zhang H, Levitt ML. Antisense bcl-2 treatment increases programmed cell death in non-small cell lung cancer cell lines. Lung Cancer 1999; 23: 115-27.

[209]   Inoue Y, Gika M, Abiko T, Oyama T, Saitoh Y, Yamazaki H, et al. Bcl-2 overexpression enhances in vitro sensitivity against docetaxel in non-small cell lung cancer. Oncol Rep 2005; 13(2): 259-64.

[210]   Laudanski J, Niklinska W, Burzykowski T, Chyczewski L, Niklinski J. Prognostic significance of p53 and bcl-2 abnormalities in operable nonsmall cell lung cancer. Eur Respir J 2001; 17: 660-6.

[211]   Koty PP, Tyurina YY, Tyurin VA, Li SX, Kagan VE. Depletion of bcl-2 by an antisense oligonucleotide induces apoptosis accompanied by oxidation and externalization of phosphatidylserine in NCI-H226 lung carcinoma cells. Mol Cell Biochem 2002; 234-235:125-33.

[212]   Dias N, Stein CA. Antisense oligonucleotides: basic concepts and mechanisms. Mol Cancer Ther 2002; 1: 347-55.

[213]   Klasa RJ, Gillum AM, Klem RE, Frankel SR. Oblimersen bcl-2 antisense: facilitating apoptosis in anticancer treatment. Antisense Nucleic Acid Drug Dev 2002; 12: 193-213.

[214]   Miayake H, Tolcher A, Gleave ME. Chemosensitization and delayed androgen-independent recurrence of prostate cancer with the use of antisense Bcl-2 oligodeoxynucleotides. J Natl Cancer Inst 2000; 92:34-41.

[215]   Hu Y, Bebb G, Tan S, Ng R, Yan H, Sartor JR, et al. Antitumour efficacy of oblimersen Bcl-2 antisense oligonucleotide alone and in combination with vinorelbine in xenograft models of uman non-small cell lung cancer. Clin Cancer Res 2004; 10(22): 7662-70.

[216]   Jansen B, Wacheck V, Heere-Ress E. Clinical, pharmacologic, and pharmacodynamic study of Genasense (G3139, Bcl-2 antisense oligonucleotide) and dacarbazine (DTIC) in patients with malignant melanoma [abstract]. Proc Am Soc Clin Oncol 2001; 14: 1429a.

[217]   Rudin CM, Otterson GA, Mauer AM, Villalona-Calero AM, Tomek R, Prange B, et al. A pilot trial of G3139, a bcl-2 antisense oligonucleotide, and paclitaxel in patients with chemorefractory small-cell lung cancer. Ann Oncol 2002; 13: 539-45.

[218]   Rudin CM, Kozloff M, Hoffman PC, Edelman MJ, Karnauskas R, Tomek R, et al. Phase I study of G3139, a bcl-2 antisense oligonucleotide, combined with carboplatin and etoposide in patients with small-cell lung cancer. J Clin Oncol 2004; 22:1110-7.

[219]   Swannie HC, Kaye SB. Protein kinase C inhibitors. Curr Oncol Rep 2002; 4: 37-46.

[220]   Blobe GC, Obeid LM, Hannum YA. Regulation of protein kinase C and role in cancer biology. Cancer Metastasis Rev 1994; 13: 411-31.

[221]   Hofmann J. Modulation of protein kinase C in antitumour treatment. Rev Physiol Biochem 2006; 142: 1-96.

[222]   Jones SM, Kazlauskas A. Connecting signaling and cell cycle progression in growth factor-stimulated cells. Oncogene 2000; 19: 5558-67.

[223]   Buchner K. The role of protein kinase C in the regulation of cell growth and in signalling to the cell nucleus. J Cancer Res Clin Oncol 2000; 126: 1-11.

[224]   Dean NM, McKay R. Inhibition of protein kinase C-alpha expression in mice after systemic administration of phosphorothioate antisense oligodeoxynucleotides. Proc Natl Acad Sci USA 1994; 91: 11762-6.

[225]   Clark A, West K, Blumberg P, Dennis P. Altered protein kinase C (PKC) isoforms in non-small cell lung cancer cells; PKCδ promotes cellular survival and chemotherapeutic resistance. Cancer Res 2003; 63: 780-6.

[226]   Sausville EA, Arbuck SG, Messmann R, Headlee D, Bauer KS, Lush RM, et al. Phase I trial of 72-hour continuous infusion UCN-01 in patients with refractory neoplasms. J Clin Oncol 2001; 19: 2319-33.

[227]   Propper DJ, McDonald AC, Man A, Thavasu P, Balkwill F, Braybrooke JP, et al. Phase I and pharmacokinetic study of PKC412, an inhibitor of protein kinase C. J Clin Oncol 2001; 19: 1485-92.

[228]   Monnerat C, Henriksson R, Le Chevalier T, Novello S, Berthaud P, Faivre S, et al. Phase I study of PKC412 (N-benzoyl-staurosporine), a novel oral protein kinase C inhibitor, combined

with gemcitabine and cisplatin in patients with non-small-cell lung cancer. Ann Oncol 2004; 15(2): 316-23.

[229] Nishizuka Y. The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature 1988; 334: 661-5.

[230] Basu A. The potential of protein kinase C as a target for anticancer treatment. Pharmacol Ther 1993; 59: 257-80.

[231] Henttu P, Vihko P. The protein kinase C activator, phorbol ester, elicits disparate functional responses in androgen-sensitive and androgen-independent human prostatic cancer cells. Biochem Biophys Res Commun 1998; 244: 167-71.

[232] Perletti GP, Smeraldi C, Porro D, Piccinini F. Involvement of the alpha isoenzyme of protein kinase C in the growth inhibition induced by phorbol esters in MH1C1 hepatoma cells. Biochem Biophys Res Commun 1994; 205: 1589-94.

[233] Adesina AM, Dooley N, Yong VW, Nalbantoglu J. Differential role for protein kinase C-mediated signaling in the proliferation of medulloblastoma cell lines. Int J Oncol 1998; 12: 759-68.

[234] Hoke GD, Draper K, Freier SM, Gonzalez C, Driver VB, Zounes MC, *et al*. Effects of phosphorothioate capping on antisense oligonucleotide stability, hybridization and antiviral efficacy versus herpes simplex virus infection. Nucleic Acids Res 1991; 19: 5743-8.

[235] Dean NM, McKay R, Condon TP, Bennett CF. Inhibition of protein kinase C-alpha expression in human A549 cells by antisense oligonucleotides inhibits induction of intercellular adhesion molecule 1 (ICAM-1) mRNA by phorbol esters. J Biol Chem 1994; 269:16416-24.

[236] Yuen AR, Halsey J, Fisher GA, Holmlund JT, Geary RS, Kwoh TJ, *et al*. Phase I study of an antisense oligonucleotide to protein kinase C-alpha (ISIS 3521/CGP 64128A) in patients with cancer. Clin Cancer Res 1999; 5: 3357-63.

[237] Advani R, Lum BL, Fisher GA, Halsey J, Geary RS, Holmlund JT, *et al*. A phase I trial of aprinocarsen (ISIS 3521/LY900003), an antisense inhibitor of protein kinase C-alpha administered as a 24-hour weekly infusion schedule in patients with advanced cancer. Invest New Drugs 2005; 23(5): 467-77.

[238] Yuen A, Halsey J, Fisher G. Phase I/II trial of ISIS 3521, an antisense inhibitor of PKC-alpha with carboplatin and paclitaxel in non-small cell lung cancer [abstract 1234]. Proc Am Soc Clin Oncol 2001; 20: 309a.

[239] Lynch TJ, Raju R, Lind M. A randomized Phase III trial of chemotherapy and antisense oligonucleotide LY900003 (ISIS3521) in patients with advanced NSCLC. Initial report [abstract]. Proc Am Soc Clin Oncol 2003; 22:623.

[240] Villalona-Calero MA, Ritch P, Figueroa JA, Otterson GA, Belt R, Dow E, *et al*. A phase I/II study of LY900003, an antisense inhibitor of protein kinase C-alpha, in combination with cisplatin and gemcitabine in patients with advanced non-small cell lung cancer. Clin Cancer Res 2004; 10(18 Pt 1): 6086-93.

[241] Vansteenkiste J, Canon JL, Riska H, Pirker R, Peterson P, John W, *et al*. Randomized phase II trial of aprinocarsen in combination with gemcitabine and cisplatin for patients with advanced/metastatic non-small cell lung cancer. Invest New Drugs 2005; 23(3): 263-9.

[242] Gandara D, Douillard J, Koralewski P. Phase III trial of the protein kinase C alpha antisense oligonucleotide Aprinocarsen plus gemcitabine/cisplatin versus gemcitabine/cisplatin alone in advanced stage nonsmall cell lung cancer: updated results of a randomized phase III trial [abstract O-085]. Lung Cancer 2005; 49 (Suppl 2): S31.

[243] Orlowski RZ. The role of the ubiquitin-proteasome pathway in apoptosis. Cell Death Differ 1999; 6: 303-13.

[244] Goldberg AL, Stein R, Adams J. New insights into proteasome function: from Archaebacteria to drug development. Chem Biol 1995; 2: 503-508.

[245] Beg AA, Baltimore D. An essential role for NF-κB in preventing TNF-α-induced cell death. Science 1996; 274: 782-4.

[246] Chu ZL, McKinsey TA, Liu L, Gentry JJ, Malin MA, Ballard DN. Suppression of tumour necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-κB control. Proc Natl Acad Sci USA 1997; 94: 10057-62.

[247] Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM. Suppression of TNF-α-induced apoptosis by NF-κB. Science 1996; 274: 787-9.

[248] Read MA, Neish AS, Luscinskas FW, Palombella VJ, Maniat ST, Collin T. The proteosome pathway is required for cytokine-induced endothelial-leukocyte adhesion molecule expression. Immunity 1995; 2: 493-06.

[249] Lin ZP, Boller YC, Amer SM, Russell RL, Pacelli KA, Patierno SR, *et al*. Prevention of brefeldin A-induced resistance to teniposide by the proteasome inhibitor MG-132: involment of NF-kappaB activation in drug resistance. Cancer Res 1998; 58: 3059-65.

[250] Wang CY, Cusack JC Jr, Liu R, Baldwin AS Jr. Control of inducible chemoresistance: enhanced antitumour therapy through increased apoptosis by inhibition of NF-kappaB. Nat Med 1999; 5: 412-7.

[251] Mitchel, BS. The proteasome-An emerging therapeutic target in cancer. N Engl J Med 2003; 348(26): 2597-8.

[252] Voorhees PM, Dees EC, O'Neil B, Orlowski RZ. The proteasome as a target for cancer therapy. Clin Cancer Res 2003; 9(17): 16-25.

[253] An B, Goldfarb RH, Siman R. Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin-dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. Cell Death Differ 1998; 5: 1062-75.

[254] Adams J. The proteasome: a suitable antineoplastic target. Nat Rev Cancer 2004; 4: 349-60.

[255] Adams J, Palombella VJ, Sausville EA, Johnson J, Destree A, Lazarus DD, *et al*. Proteasome inhibitors: a novel class of potent and effective antitumour agents. Cancer Res 1999; 59: 2615-22.

[256] Podar K, Shringarpure R, Tai YT, Simoncini M, Sattler M, Ishitsuka K, *et al*. Caveolin-1 is required for vascular endothelial growth factor-triggered multiple myeloma cell migration and is targeted by bortezomib. Cancer Res 2004; 64: 7500-6.

[257] Dubey S, Schiller JH. Three emerging new drugs for NSCLC: pemetrexed, bertezomib, and cetuximab. Oncologist 2005; 10(4): 282-91.

[258] Ling YH, Liebes L, Zou Y, Perez -Soler R. Reactive oxygen species generation and mitochondrial dysfunction in the apoptotic response to Bortezomib, a novel proteasome inhibitor, in human H460 non-small cell lung cancer cells. J Biol Chem 2003; 278: 33714-23.

[259] Ling YH, Liebes L, Ng B, Buckley M, Elliott PJ, Adams J, *et al* Mechanisms of proteasome inhibitor PS-341-induced G(2)-M-phase arrest and apoptosis in human non-small cell lung cancer cell lines. Clin Cancer Res 2003; 9: 1145-54.

[260] Sunwoo JB, Chen Z, Dong G, Yeh N, Crowl Bancroft C, Sausville E, *et al* Novel proteasome inhibitor PS-341 inhibits activation of nuclear factor-kappa B, cell survival, tumour growth, and angiogenesis in squamous cell carcinoma. Clin Cancer Res 2001; 7: 1419-28.

[261] Richardson PG, Barlogie B, Berenson J, Singhal S, Jagannath S, Irwin D, *et al*. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med 2003; 348: 2609-17.

[262] Jagannath S, Barlogie B, Berebson J. A phase II multicenter randomized study of the proteasome inhibitor bortezomib (VELCADE, formerly PS-341) in multiple myeloma patients relapsed after front line therapy. Blood 2002; 100: 3027a.

[263] Aghajanian C, Soignet S, Dizon D. A phase I trial of the novel proteasome inhibitor PS341 in advanced solid tumour malignancies. Proc Am Soc Clin Oncol 2001; 20: 85a.

[264] Erlichman C, Adjei AA, Thomas JPT. A phase I trial of the proteasome inhibitor PS-341 in patients with advanced cancer. Proc Am Soc Clin Oncol 2001; 20: 85a.

[265] Appleman LJ, Ryan DP, Clark JW. Phase I dose escalation study of bortezomib and gemcitabine safety and tolerability in patients with advanced solid tumours. Proc Am Soc Clin Oncol 2003;22: 209a.

[266] Davies AM, Lara PN, Lau DH. The proteasome inhibitor, bortezomib, in combination with gemcitabine (Gem) and carboplatin (Carbo) in advanced non-small cell lung cancer (NSCLC): final results of a phase I California Cancer Consortium study; ASCO Annual Meeting Proceedings (Post-Meeting Edition). J Clin Oncol 2004; 22 (14 Suppl): 7106.

[267] Ryan DP, O'Neil B, Lima CR. Phase I dose-escalation study of the proteasome inhibitor, bortezomib, plus irinotecan in patients with advanced solid tumours. Proc Am Soc Clin Oncol 2003; 22: 228a.

[268]   Stevenson JP, Nho CW, Johnson SW. Effects of bortezomib (PS-341) on NF-κB activation in peripheral blood mononuclear cells (PBMCs) of advanced non-small cell lung cancer (NSCLC) patients: a phase II/pharmacodynamic trial. Proc Am Soc Clin Oncol 2004; 23: 649a.

[269]   Fanucchi MP, Belt RJ, Fossella FV. Phase 2 study of bortezomib ± docetaxel in previously treated patients (pts) with advanced NSCLC: preliminary results; ASCO Annual Meeting Proceedings (Post-Meeting Edition) J Clin Oncol 2004; 22(14 Suppl): 7107.

[270]   Adjei AA. Blocking oncogenic ras signaling for cancer therapy. J Natl Cancer Inst 2001; 93: 1062-74.

[271]   Huncharek M, Muscat J, Geschwind JF. K-ras oncogene mutation as a prognostic marker in non-small cell lung cancer. A combined analysis of 881 cases. Carcinogenesis 1999; 20: 1507-10.

[272]   Downward J. Ras signaling and apoptosis. Curr Opin Genet Dev 1998; 8: 9-54.

[273]   Liu M, Du W, Liu JP, Jessell TM, Prendergast GC. RhoB alteration is necessary for apoptotic and antineoplastic responses to farnesyl-transferase inhibitors. Mol Cell Biol 2000; 20(16): 6105-13.

[274]   Ashar HR, James L, Gray K, Carr D, Black S, Armstrong L, et al. Farnesyl transferase inhibitors block the farnesylation of CENP-E and CENP-F and alter the association of CENP-E with the microtubules. J Biol Chem 2000; 275(39): 30451-7.

[275]   Jiang K, Coppola D, Crespo NC, Nicosia SV, Hamilton AD, Sebti SM, et al. The phosphoinisitide 3-OH kinase/AKT2 pathway as a critical target for farnesyltransferase inhibitor-induced apoptosis. Mol Cell Biol 2000; 20(1): 139-48.

[276]   End DW, Smets G, Todd AV, ApplegateTL, Fuery CJ, Angibaud P, et al. Characterization of the antitumour effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro. Cancer Res 2001; 61(1): 131-7.

[277]   Nagasu T, Yoshimatsu K, Rowell C, Lewis MD, Garcia AM. Inhibition of human tumour xenograft growth by treatment with the farnesyl transferase inhibitor B956. Cancer Res 1995; 55(22): 5310-4.

[278]   Bernhard EJ, McKenna WG, Hamilton AD, Sebti SM, Qian Y, Wu JM, et al. Inhibiting Ras prenylation increases the radiosensivity of human tumour cell lines with activating mutations of ras oncogenes. Cancer Res 1998; 58:1754-61.

[279]   Moasser MM, Sepp-Lorenzino L, Kohl NE, Oliff A, Balog A, Su DS, et al. Farnesyl transferase inhibitors cause enhanced mitotic sensivity to Taxol and epothilones. Proc Natl Acad Sci USA 1998; 95: 1369-74.

[280]   Rak J, Mitsuhashi Y, Bayko L, Filmus J, Shirasawa S, Sasazuki T, et al. Mutant ras oncogenes upregulate VEG/vPF expression: Implications for induction and and inhibition of tumour angiogenesis. Cancer Res 1995; 55:4574-80.

[281]   Evans TL, Fidias P, Scarin A. A phase II study of efficacy and tolerability of the farnesyl-protein transferase inhibitor L-778,123 as first-line therapy in patients with advanced non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 2002; 2: 13a.

[282]   Britten CD, Rowinski EK, Soignet S, Patnaik A, Yao SL, Deutsch P, et al. A phase I and pharmacological study of the farnesyl-protein transferase inhibitor L-778,123 in patients with solid malignancies. Clin Cancer Res 2001; 7(12): 3894-903.

[283]   Schellens JHM, de Klerk G, Swart M. Phase I and pharmacologic study of the novel farnesyltransferase inhibitor (FTI) R115777 [abstract 715]. Program and abstracts of the 36th Annual Meeting of the American Society of Clinical Oncology; 2000 May 20-23; New Orleans, Louisiana.

[284]   Adjei AA, Mauer A, Bruzek L, Marks RS, Hillman S, Geyer S, et al. Phase II study of the farnesyl transferase inhibitor R115777 in patients with advanced non-small cell lung cancer. J Clin Oncol 2003; 21:1760-6.

[285]   Adjei AA, Erlichman C, Davis JN, Cutler DL, Sloan JA, Marks RS, et al. A phase I trial of the farnesyl transferase inhibitor SCH66336: evidence for biological and clinical activity. Cancer Res 2000; 60: 1171-7.

[286]   Khuri FR, Glisson BS, Kim ES, Statkevich P, Thall PF, Meyers ML, et al. Phase I study of the farnesyl transferase inhibitor lonafarnib with paclitaxel in solid tumours. Clin Cancer Res 2004; 10: 2968-76.

[287]   Kim ES, Kies MS, Fossella FV. A phase I/II study of the farnesyl transferase inhibitor (FTI) SCH66336 (lonafarnib) with paclitaxel in taxane-refractory/resistant patients with non-small cell lung cancer (NSCLC): final report [abstract]. Proc Ann Meet Assoc Cancer Res 2002; 43: 2735.

[288]   BlumenscheinG, Ludwig C, Thomas G. A randomized phase III trial comparing ionafarnib/carboplatin/paclitaxel versus carboplatin/paclitaxel in chemotherapy naive patients with advanced or metastatic non small cell lung cancer [abstract O-082]. Lung Cancer 2005; 49(Suppl 2): S30.

[289]   Rose WC, Lee FY, Fairchild CR, Lynch M, Monticello T, Kramer RA, et al. Preclinical anitumour activity of BMS-214662, a highly apoptotic and novel farnesyltransferase inhibitor. Cancer Res 2001; 61: 7507-17.

[290]   Ryan DP, Eder JP Jr, Puchlaski T, Seiden MV, Lynch TJ, Fuchs CS, et al. Phase I clinical trial of the farnesyltranferase inhibitor BMS-214662 given as a 1-hour intravenous infusion in patients with advanced solid tumours. Clin Cancer Res 2004; 10: 2222-30.

[291]   Mackay HJ, Hoekstra R, Eskens FA, Loos WJ, Crawford D, Voi M, et al. A phase I pharmacokinetic and pharmacodynamic study of the farnesyl transferase inhibitor BMS-214662 in combination with cisplatin in patients with advanced solid tumours. Clin Cancer Res 2004; 10: 2636-44.

[292]   Dy GK, Bruzek LM, Croghan GA, Mandrekar S, Erlichman C, Peethambaram P, et al. A phase I trial of a novel farnesyl protein transferase inhibitor, BMS-214662, in combination with paclitaxel and carboplatin in patients with advanced cancer. Clin Cancer Res 2005; 11:1877-1883.

[293]   De Luca LM, Darwiche N, Celli G, Kosa K, Jones C, Ross S, et al. Vitamin A in epithelial differentiation and skin carcinogenesis. Nutr Rev 1994; 52: S45-S52.

[294]   Mangelsdorf DJ, Umesono K, Evans RM. The retinoid receptors. In: Sporn MB, Roberts AB, Goodman DS, eds. The retinoids: biology, chemistry, and medicine, 2nd edition, New York: Raven Press; 1994. p. 319-50.

[295]   Oridate N, Lotan D, Xu XC, Hong WK, Lotan R. Differential induction of apoptosis by all-trans-retinoic acid and N-(4-hydroxyphenyl) retinamide in human head and neck squamous cell carcinoma cell lines. Clin Cancer Res 1996; 2: 855-63.

[296]   Petkovich M, Brand NJ, Chambon P. A human retinoic acid receptor which belongs to the family of nuclear receptors. Nature 1987; 330: 444-50.

[297]   Mangelsdorf DJ, Ong ES, Dyck JA, Evans RM. Nuclear receptor that identifies a novel retinoic acid response pathways. Nature 1990; 345: 224-9.

[298]   Chambon P. A decade of molecular biology of retinoic acid receptors. FASEB J 1996; 10: 940-54.

[299]   Lotan R, Xu XC, Lippman SM, Ro JY, Lee JS, Lee JJ, et al. Suppression of retinoic acid receptor-β in premalignant oral lesions and its up-regulation by isotretinoin. N Engl J Med 1995; 332: 1405-10.

[300]   Gebert JF, Moghal N, Frangioni JV, Sugarbaker DJ, Neel BG. High frequency of retinoic acid receptor β abnormalities in human lung cancer. Oncogene 1991; 6: 1859-68

[301]   Leid N, Kastner P, Chambon P. Multiplicity generates diversity in the retinoic acid signalling pathways. Trends Biochem Sci 1992; 17: 427-33.

[302]   Guerin E, Ludwig MG, Basset P, Anglard P. Stromelysin-3 induction and interstitial collagenase repression by retinoic acid. Therapeutical implication of receptor-selective retinoids dissociating transactivation and AP-1-mediated transrepression. J Biol Chem 1997; 272: 11088-95.

[303]   Han GR, Dohi DF, Lee HY, Rajah R, Walsh GL, Hong WK, et al. All transretinoic acid increases transforming growth factor-ß2 and insulin-like growth factor binding protein-3 expression through a retinoic acid receptor-α-dependent signaling pathway. J Biol Chem 1997; 272: 13711-6.

[304]   Liu M, Iavarone A, Freedman LP. Transcriptional activation of the human p21 (WAF1/CIP1) gene by retinoic acid receptor. Correlation with retinoid induction of U937 cell differentiation. J Biol Chem 1996; 271: 31723-8.

[305]   Rubin Grandis JR, Zeng Q, Tweardy DJ. Retinoic acid normalizes the increased gene transcription rate of TGF-alpha and EGFR in head and neck cancer cell lines. Nat Med 1996; 2: 237-40.

[306]   Boehm MF, Zhang L, Badea BA, White SK, Mais DE, Berger E, et al. Synthesis and structure-activity relationships of novel retinoid X receptor-selective retinoids. J Med Chem 1994; 37: 2930-41.

[307]   Yen WC, Corpus MR, Prudente RY, Cooke TA, Bissonnette RP, Negro-Vilar A, *et al*. A selective retinoid X receptor agonist bexarotene (Targetin) prevents and overcomes acquired paclitaxel (Taxol) resistance in human non-small cell lung cancer. Clin Cancer Res 2004; 10(24): 8656-64.

[308]   Miller VA, Benedetti FM, Rigas JR, Verr*et AL*, Pfister DG, Straus D, *et al*. Initial clinical trial of a selective retinoid X receptor ligand, LGD1069. J Clin Oncol 1997; 15: 790-5.

[309]   Rizvi NA, Marshall JL, Dahut W, Ness E, Truglia JA, Loewen G, *et al*. A phase I study of LGD1069 in adults with advanced cancer. Clin Cancer Res 1999; 5:1658-64.

[310]   Yocum RC, Miller VA, Warrel RP. Long-term follow-up of patients with advanced non-small cell lung cancer treated with oral bexarotene. Proc Am Soc Clin Oncol 2001; 20: 267b.

[311]   Khuri FR, Rigas JR, Figlin RA, Gralla RJ, Shin DM, Munden R, *et al*. Multi-institutional phase I/II trial of oral bexarotene in combination with cisplatin and vinorelbine in previously untreated patients with advanced non-small-cell lung cancer. J Clin Oncol 2001; 19:2626-37.

[312]   Rizvi NA, Miller W Jr, Frank H. Phase I/II safety evaluation of bexarotene in combination with carboplatin and paclitaxel in patients with chemotherapy-naïve advanced non small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 2002; 21: 334a.

[313]   Edelman MJ, Smith R, Hausner P, Doyle LA, Kalra K, Kendall J, *et al*. Phase II trial of the novel retinoid, bexarotene, and gemcitabine plus carboplatin in advanced non-small-cell lung cancer. J Clin Oncol 2005; 23(24): 5774-8.

[314]   Jassem J, Zatloukai P, Ramlau R. A randomized phase III trial comparing bexarotene/cisplatin/vinorelbine versus cisplatin/vinorelbine in chemotherapy-naïve patients with advanced or metastatic non-small cell lung cancer (NSCLC) [abstract LBA7024]. Proc Am Soc Clin Oncol. 2005; 23: 627s.

[315]   Blumenschein GR, Khuri F, Gatzemeier U. A randomized phase III trial comparing bexarotene/carboplatin/paclitaxel versus carboplatin/paclitaxel in chemotherapy-naïve patients with advanced or metastatic non-small cell lung cancer (NSCLC) [abstract LBA700]. Proc Am Soc Clin Oncol 2005; 23: 621s.

[316]   Rizvi N, Hawkins MJ, Eisenberg PD, Yocum RC, Reich SD; Ligand L 1069-20 Working group. Placebo-controlled trial of bexarotene, a retinoid X receptor agonist, as maintenance therapy for patients treated with chemotherapy for advanced non-small cell lung cancer. Clin Lung Cancer 2001; 2: 210-5.

[317]   Fanucchi MP, Fossella F, Fidias P. Bortezomib/docetaxel in previously treated patients with advanced non-small cell lung cancer (NSCLC): a phase 2 study [abstract 7034]. Proc Am Soc Clin Oncol 2005; 23:629s.

[318]   Talpur R, Ward S, Apisarnthanarax N, Breuer-Mcham J, Duvic M. Optimizing bexarotene therapy for cutaneous T-cell lymphoma. J Am Acad Dermatol 2002; 47: 672-84.

[319]   Sherman SI, Gopal J, Haugen BR, Chiu AC, Whaley K, Nowlakha P, *et al*. Central hypothyroidism associated with retinoid X receptor-selective ligands. N Engl J Med 1999; 340:1075-9.

[320]   Erickson SL, O'Shea KS, Ghaboosi N, Loverro L, Frantz G, Bauer M, *et al*. ErbB3 is required for normal cerebellar and cardiac development: a comparison with ErbB2-and heregulin-deficient mice. Develoment 1997; 124: 4999-5011.

[321]   Miettinen PJ, Berger JE, Meneses J, Phung Y, Pedersen RA, Werb Z, *et al*. Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. Nature 1995; 376: 337-341.

[322]   Laux I, Jain A, Sinqh S, Agus DB. Epidermal growth factor receptor dimerization status determines skin toxicity to HER-kinase targeted therapies. Br J Cancer 2006; 94(1): 85-92.

[323]   Crone SA, ZhaoYY, Fan L, Gu Y, Minamisawa S, Liu Y, *et al*. ErbB2 is essential in the prevention of dilated cardiomyopathy. Nat Med 2002; 8: 459-465.

[324]   Chien KR. Stress pathways and heart failure. Cell 1999; 98: 555-558.

[325]   Seidman A, Hudis C, Pierri MK, Shak S, Paton V, Ashby M, *et al*. Cardiac dysfunction in the trastuzumab clinical trials experience. J Clin Oncol 2002; 20: 1215-1221.

[326]   Nissen NN, Polverini PJ, Koch AE, Volin MV, Gamelli RL, DiPietro LA. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. Am J Pathol 1998; 152(6): 1445-52.

[327]   Altavilla D, Saitta A, Cucinotta D, Galeano M, Deodato B, Colonna M, *et al*. Inhibition of lipid peroxidation restores impaired vascular endothelial growth factor expression and stimulates wound healing and angiogenesis in the genetically diabetic mouse. Diabetes 2001; 50(3): 667-74.

[328]   Castilla MA, Caramelo C, Gazapo RM, Martin O, Gonzalez-Pacheco FR, Tejedor A, *et al*. Role of vascular endothelial growth factor (VEGF) in endothelial cell protection against cytotoxic agents. Life Sci 2000; 67(9): 1003-13.

[329]   Konttinen YT, Ainola M, Valleala H, Ma J, Ida H, Mandelin J, *et al*. Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. Ann Rheum Dis. 1999; 58: 691-697.

[330]   Herbst RS, Johnson DH, Mininberg E, Carbone DP, Henderson T, Kim ES, *et al*. Phase I/II trial evaluating the anti-vascular endothelial growth factor monoclonal antibody bevacizumab in combination with the HER-1/epidermal growth factor receptor tyrosine kinase inhibitor erlotinib for patients with recurrent non-small-cell lung cancer. J Clin Oncol 2005; 23: 2544-55.

[331]   Gadgeel S, Shehadeh N, Ruckdeschel J. Gefitinib and celecoxib in patients with platinum refractory non-small cell lung cancer (NSCLC). ASCO Annual Meeting Proceedings (Post-Meeting Edition) J Clin Oncol 2004; 22 (14 Suppl): 7094.

[332]   O'Byrne KJ, Clarke L, Dunlop D, Ranson M, Danson S, Botwood N. Clinical evaluation of gefitinib (Iressa,ZD1839) in combination with rofecoxib in cisplatin pre-treated relapsed non-small cell lung cancer [abstract O-113]. Lung Cancer 2003; 41(Suppl 2): S36.

[333]   Matar P, Rojo F, Cassia R, Moreno-Bueno G, Di Cosimo S, Tabernero J, *et al*. Combined epidermal growth factor receptor targeting with the tyrosine kinase inhibitor gefitinib (ZD1839) and the monoclononal antibody cetuximab (IMC-C225): superiority over single-agent receptor targeting. Clin Cancer Res 2004; 10: 6487-501.

[334]   Tortora G, Caputo R, Damiano V, Melisi D, Bianco R, Fontanini G, *et al*. Combination of a selective cyclooxygenase-2 inhibitor with epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 and protein kinase A antisense causes cooperative antitumour and antiangiogenic effect. Clin Cancer Res 2003; 9: 1566-72.

[335]   Natale RB, Skarin AT, Maddox AM. Improvement in symptoms and quality of life for advanced non-small-cell lung cancer patients receiving ZD1839 (Iressa^{TM}) in IDEAL 2 [abstract 1167]. Proc Am Soc Clin Oncol 2002; 21: 292a.

[336]   Braun AH, Dirsch O, Hilger RA. Preclinical evaluation of the combination of epidermal growth factor inhibitor ZD1839 (Iressa^{TM}) and irinotecan (SN-38) in human colon cancer cells [abstract 329]. Proc Am Soc Clin Oncol 2002; 21: 83a.