REVIEW

Therapeutic integration of signal transduction targeting agents and conventional anti-cancer treatments

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Abstract

The currently available treatment of cancer patients is based on the use of cytotoxic drugs and/or of ionizing radiations, which have potent antitumor activity, but also cause clinically relevant side effects, since they affect cellular targets that are common to both cancer cells and normal proliferating cells. In the past 20 years, the discoveries on the molecular mechanisms of cancer development and progression have prompted the search for agents which are more selective for cancer cell molecular targets. The possibility of combining conventional cytotoxic drugs with novel agents that specifically interfere with key pathways controlling cancer cell survival, proliferation, invasion and/or metastatic spreading has generated a wide interest. This could be a promising therapeutic approach for several reasons. First, since the cellular targets for these agents and their mechanism(s) of action are different from those of cytotoxic drugs, it is possible for their combination with chemotherapy without cross-resistance. Second, alterations in the expression and/or the activity of genes that regulate mitogenic signals not only can directly cause perturbation of cell growth, but also may affect the sensitivity of cancer cells to conventional chemotherapy and radiotherapy. In this review, we will discuss the biologic bases of the combination of molecular targeted drugs with conventional medical cancer treatments and the available results of the first series of clinical trials in cancer patients.

Endocrine-Related Cancer (2004) 11 51–68

Introduction

Current cancer therapies are mainly based on the use of cytotoxic drugs or of ionizing radiations that have a potent antitumor activity, but a relatively low therapeutic index with significant side effects, since they affect cellular targets that are common to both cancer and normal cells. Furthermore, several cancer types are intrinsically not sensitive or become resistant to treatment with cytotoxic drugs or radiotherapy. This situation determines treatment failure in a majority of advanced cancer patients because of therapy-resistant disease relapse or of progression despite therapy. For these reasons, there is a need for the development of novel effective anticancer agents that are more specific for cancer cells and less toxic for normal cells. In this respect, the discoveries about the mechanisms of neoplastic development at the molecular level have opened new avenues for the design of drugs that selectively interfere with key cancer cell targets and that could be used in combination with conventional therapies possibly to overcome drug resistance in cancer patients.

The possibility of combining conventional cytotoxic drugs with novel agents that specifically interfere with key pathways controlling cancer cell survival, proliferation, invasion and/or metastatic spreading has generated wide interest. This could be a promising therapeutic approach for several reasons. First, since the cellular targets for these agents and their mechanism(s) of action are different from those of cytotoxic drugs, it is possible that they may be used in combination with chemotherapy without cross-resistance. Secondly, alterations in the expression and/or the activity of genes that regulate mitogenic signals can not only directly cause perturbation of cell growth, but
may also affect the sensitivity of cancer cells to conventional chemotherapy and radiotherapy (Mendelsohn & Fan 1997, Harari & Huang 2000, Ryan & Chabner 2000).

**Epidermal growth factor receptor (EGFR) targeted agents**

Growth factors are peptide hormones that are involved through autocrine and paracrine mechanisms in regulating cell proliferation, differentiation and survival. These proteins bind to and activate specific receptors that are localized on the surface of target cells. Ligand-induced growth factor receptor activation triggers a cascade of intracellular signal transduction pathways that regulate different cellular functions.

Epidermal growth factor (EGF) is the prototype of a large family of closely related growth factors, which bind to and activate receptors of the ErbB family that includes four members: the EGFR (also known as ErbB1/HER1), ErbB-2/Neu/HER2, ErbB-3/HER3 and ErbB-4/HER4 (Salomon et al. 1995). The EGFR exists as inactive monomers, which dimerize following ligand activation. Following ligand binding, the tyrosine kinase intracellular domain of the receptor is activated, with autophosphorylation of the intracellular domain, which initiates a cascade of intracellular events (Salomon et al. 1995). The signaling pathway involves activation of ras and mitogen activated protein kinase (MAPK), which activates several nuclear proteins required for cell cycle progression from G1 to S phase. EGFR activation is not only critical for cell proliferation. Several studies have demonstrated that EGFR-mediated signals also contribute to other processes that are crucial to cancer progression, including angiogenesis, invasion, metastasis and inhibition of apoptosis (Salomon & Gullick 2001).

Activation of the transforming growth factor α (TGFα)–EGFR autocrine growth pathway is a common mechanism for autonomous, dysregulated cancer cell growth in most human epithelial cancers. TGFα and/or EGFR are overexpressed in the majority of different human cancer types, including non-small-cell lung cancer (NSCLC), breast, head and neck, gastric, prostate, bladder, ovarian, colorectal carcinomas, and glioblastomas, in which there is an association with advanced disease and poor prognosis (Salomon et al. 2001). Overexpression of EGFR has also been associated with the development of resistance to anticancer treatments such as chemotherapy and radiotherapy and to hormone-therapy in hormone-dependent tumors such as breast carcinomas (Wosikowski et al. 1997, Akimoto et al. 1999, McClelland et al. 2001).

Two anti-EGFR therapeutic approaches have shown promising clinical activity: monoclonal antibodies (MAbs) and small molecule inhibitors of the EGFR tyrosine kinase enzymatic activity (Table 1). MAbs are raised against the extracellular domain of the EGFR to block ligand binding and receptor activation. Tyrosine kinase inhibitors (TKIs) prevent the autophosphorylation of the EGFR intracellular tyrosine kinase domain. These molecules are generally reversible competitors of ATP for binding to the intracellular catalytic domain of the EGFR tyrosine kinase. The blockade of EGFR signaling in

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<td>IMC-225 (Cetuximab) (ImClone; Merck AG) EMD 72000 (Merck AG) ABX-EGF (Abgenix) HR3 (York Medical BioSciences) Gefitinib (Iressa) (AstraZeneca) Erlotinib (Tarceva) (OSI Pharmaceuticals; Genentech) CI-1033 (Pfizer) PKI-166 (Novartis) GW2016 (GlaxoSmithKline)</td>
<td>Human-mouse Chimeric MAb Humanized MAb Fully human MAb Humanized MAb Reversible TKI Reversible TKI Reversible TKI Reversible TKI</td>
<td>Selective for EGFR Selective for EGFR Selective for EGFR Selective for EGFR Selective for EGFR Selective for EGFR Selective for EGFR</td>
<td>Phase II–III Phase I Phase I Phase I Phase II–III Phase II–III Phase I Phase I</td>
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MAb, monoclonal antibody; TKI, tyrosine kinase inhibitor.
cancer cells determines not only inhibition of cell proliferation but also other effects that could be relevant in the clinical setting, such as anti-angiogenetic effects by inhibition of tumor cell production of pro-angiogenic growth factors and by, possibly, direct cytotoxicity on endothelial cells in tumor vessels and such as anti-invasive and anti-metastatic effects. These studies have also provided a strong scientific rationale for the development of combined treatments with anti-EGFR targeted agents and conventional anti-cancer therapies such as chemotherapy, hormonotherapy and radiotherapy.

Anti-EGFR monoclonal antibodies

Cetuximab

Mendelsohn’s laboratory has developed several blocking anti-EGFR MAbs which inhibit the in vitro and in vivo growth of human cancer cell lines that express TGFα and EGFR (Mendelsohn 2000). Among these, the anti-EGFR blocking mouse MAb 528 and 225 were able to significantly enhance the antitumor activity of doxorubicin and cisplatin against well-established, palpable, subcutaneous xenografts of human squamous A431 and breast MDA-MB-468 cancer cell lines (Baselga et al. 1993, Fan et al. 1994). Eradication of well-established A431 cancer xenografts and a significantly prolonged, six months tumor-free survival were observed in the majority of mice treated with a combination of cisplatin and MAb 528 (Fan et al. 1992).

A chimeric human–mouse MAb 225 (IMC-C225, Cetuximab) that contains the human IgG1 constant region has subsequently been developed and purified for clinical use (Goldstein et al. 1995). Cetuximab binds the EGFR with a greater affinity than mouse MAb 225 and blocks EGF-induced autophosphorylation of the EGFR in cancer cell lines in vitro (Goldstein et al. 1995, Prewett et al. 1996); in vitro studies demonstrated that Cetuximab also induces dimerization and internalization of the EGFR (Fan et al. 1994, Prewett et al. 1996). Cetuximab treatment blocks cell cycle progression by inducing a G1 arrest through an increase in the protein levels of the p27<sup>Kip1</sup> inhibitor of cyclin-dependent kinases (Peng et al. 1996, Wu et al. 1996). Cetuximab inhibits the growth of human epidermoid, prostate, colon and renal cell carcinoma xenografts in vivo (Fan et al. 1994, Prewett et al. 1998, Ciardiello et al. 1999). Studies using human cancer xenografts in nude mice have demonstrated that Cetuximab inhibits tumor-induced angiogenesis (Perrotte et al. 1999, Bruns et al. 2000, Ciardiello et al. 2000, Inoue et al. 2000). This is probably due to reduced tumor production of several angiogenic factors, including TGFα, vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), and basic fibroblast growth factor (bFGF) (Perrotte et al. 1999, Bruns et al. 2000, Ciardiello et al. 2000a, Inoue et al. 2000).

An additive increase in growth inhibition has been observed when both in vitro cancer cells and human pancreatic, colon or bladder carcinoma xenografts in nude mice have been treated with Cetuximab plus various cytotoxic agents including doxorubicin, cisplatin, paclitaxel, gemcitabine and topotecan (Mendelsohn & Fan 1997, Ciardiello et al. 1999, Bruns et al. 2000, Inoue et al. 2000).

Cetuximab is in advanced clinical development and has been the first anti-EGFR targeted agent to start phase II and III evaluation in cancer patients. Three phase I clinical trials have evaluated Cetuximab as a single intravenous (i.v.) infusion, as weekly i.v. infusions for 4 weeks, and as weekly i.v. infusions in combination with cisplatin. Overall, 52 patients were included in these studies. Antibodies against Cetuximab were detected in only one of 52 patients. Cetuximab toxicity was mild. The most frequent IMC-C225-related adverse events were skin toxicities (21%), fever and chills (13.5%), asthenia (13.5%), transient transaminase elevations (11.5%) and nausea (11.5%). Skin toxicities were mainly flushing, acneform rashes and folliculitis. All these toxic effects were reversible upon cessation of treatment. Single-agent Cetuximab treatment has resulted in disease stabilization after 4 weeks in 18/28 patients. Two of 13 patients with head and neck cancer (HNC) treated with Cetuximab in combination with cisplatin had a partial response (Baselga et al. 2000).

A clinical pilot study evaluating Cetuximab in combination with radiotherapy showed that, out of 15 patients with locally advanced unresectable HNC, 13 patients experienced a complete response and the other two patients had a partial response when treated with Cetuximab and radiotherapy, a response rate significantly higher than the expected 30–45% with radiotherapy alone (Bonner et al. 2000). The median duration of response in this study was 17 months, with a range of 1–32+ months.

A phase Ib clinical trial with Cetuximab and cisplatin in 12 patients with advanced HNC has been reported. Inhibition of EGFR tyrosine kinase activity following IMC-225 treatment within the tumor mass was demonstrated in some patients. Six of nine evaluable patients achieved a major response including two complete responses (Shin et al. 2001).

In a small series of patients with EGFR-positive tumors refractory to or in relapse from previous therapeutic regimens including surgery, radiotherapy or chemotherapy, Cetuximab in combination with cisplatin has demonstrated antitumor activity (Rubin M. et al. 2000). One patient with HNC and one patient with
colorectal cancer had a complete response. In addition, four patients with HNC had partial responses.

In a phase II study of Cetuximab monotherapy in 54 patients with metastatic renal cell carcinoma, one partial response and disease stabilization for >6 months in >25% of treated patients were reported (Gunnett et al. 1999).

Results of a phase II study of Cetuximab in combination with gemcitabine in 41 advanced pancreatic cancer patients have been reported (Abbruzzese et al. 2001). Antitumor activity has been observed in 5 (12%) patients with a partial response and in 21 (53%) patients with a stable disease. The one-year progression-free survival was 17.5% and the one-year overall survival was 32.5%.

Results from two phase II clinical trials of therapy with IMC-225 in combination with CPT-11 or cisplatin in patients with diseases refractory to these cytotoxic drugs have also recently been reported. Hong et al. (2001) have treated with Cetuximab plus cisplatin advanced HNC patients that had stable disease (41 patients) or progressive disease (27 patients) after two cycles of a cisplatin-based combination chemotherapy. In the first group of patients, the authors observed one complete response, 9 partial responses and 25 disease stabilizations, with a median duration of the response of 24 weeks. In the second group, 5 patients experienced a partial response and 6 patients had a disease stabilization for at least 12 weeks. Saltz et al. (2001) have shown that treatment with IMC-225 plus CPT-11 in advanced colorectal cancer patients that have failed a previous treatment with CPT-11 gave partial responses in 27/120 patients (22.5%) with a median duration of response of 186 days and disease stabilization for more than 12 weeks in another 9 patients (7%). In this study, Cetuximab treatment was well tolerated and did not increase the toxicity due to CPT-11.

Recently, the initial results of a randomized multicenter phase II study evaluating the antitumor activity of Cetuximab monotherapy or of the combination of Cetuximab and irinotecan in EGFR-positive, irinotecan-refractory metastatic colorectal cancer patients (BOND trial) were reported. In this heavily pretreated population of patients (approximately 60–65% patients were also pretreated with an oxaliplatin-containing regimen and approximately 75–80% patients received two or more lines of chemotherapy), partial responses were observed in 22.9% of patients treated in the combination arm and in 10.8% of patients treated with Cetuximab alone (P = 0.0074) (Cunningham et al. 2003).

In a recent update of four clinical trials with Cetuximab alone or in combination with cisplatin, irinotecan or gemcitabine, in colorectal, head and neck, or pancreatic cancer patients, the presence and intensity of Cetuximab-induced acne-like rash correlated with increased response rates and survival (Saltz et al. 2003).

Phase II and III clinical trials of Cetuximab in combination with different cytotoxic drugs and/or with radiotherapy in patients with head and neck or colorectal cancers are currently in progress. In this respect, a large multicenter randomized phase III study evaluating radiotherapy with or without weekly Cetuximab i.v. treatment as first line therapy in locally advanced HNC has recently completed patient accrual.

Small molecule EGFR tyrosine kinase inhibitors

**Gefitinib**

Gefitinib (4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy) quinazoline) (Iressa) is a low molecular weight (447 Da), synthetic anilino-quinazoline. Gefitinib is an orally active, selective reversible inhibitor of EGFR tyrosine kinase. A cytostatic growth inhibitory effect of Gefitinib has been demonstrated in human cancer cell lines that express functional EGFRs, including prostate, breast, ovarian, colon, epidermoid cancers and NSCLC (Budillon et al. 2000, Cullinan et al. 2000, Ohmori et al. 2000, Sirotnak et al. 2000, Chan et al. 2001, Lichtner et al. 2001). At higher doses, Gefitinib treatment can also cause an increase in apoptosis in vitro (Chan et al. 2001). Gefitinib, like IMC-225, has been shown to induce G1 arrest in human HNC cell lines, with upregulation of p27kip1 cyclin-dependent kinase inhibitor (Ohmori et al. 2000); in vitro studies have suggested that Gefitinib as well as other quinazoline-derived EGFR-TKIs may block EGFR activation by also causing the formation of functionally inactive EGFR dimers (Ciardiello et al. 2000b). Daily oral administration of single-agent Gefitinib (12.5–200 mg/kg/dose) to athymic nude mice caused marked reductions in tumor growth in a variety of human cancer xenografts, including hormone-resistant prostate, ovarian, ductal carcinoma in situ of the breast, colon, vulval, and NSCLC (Budillon et al. 2000, Cullinan et al. 2000, Chan et al. 2001). Gefitinib demonstrates similar antitumor activity in xenograft models with differing levels of EGFR expression (Budillon et al. 2000), suggesting that factors other than simply the number of EGFR per cell may influence cancer cell sensitivity to EGFR-targeted therapies.

We performed a series of experiments to evaluate the combined antiproliferative effect of treatment with Gefitinib and a wide variety of cytotoxic drugs with different mechanism(s) of action that are currently used in the treatment of human epithelial cancers (Ciardiello et al. 2000b). A supra-additive growth inhibitory effect was observed with all doses of Gefitinib and each cytotoxic
drug tested in all cancer cell lines. The cooperative growth inhibitory effect of cytotoxic drugs and Gefitinib involves induction of programmed cell death in cancer cells. In fact, treatment with Gefitinib potentiated cytotoxic drug-induced apoptosis by approximately 2- to 3.5-fold. The increase in cytotoxicity obtained by the blockade of EGFR activation with Gefitinib treatment seems to be independent of the mechanism(s) of action of the chemotherapeutic agents used in combination. In fact, an enhanced effect was found with structurally and functionally different drugs. We also demonstrated a cooperative antitumor activity of Gefitinib and cytotoxic drugs (paclitaxel, topotecan, raltitrexed) in nude mice bearing established human colon cancer xenografts. Gefitinib was given for four weeks in combination with four weekly administration of paclitaxel, topotecan, or raltitrexed to nude mice bearing established GEO tumors. A cooperative antitumor effect was observed when Gefitinib was used in combination with each cytotoxic drug with a significant suppression of tumor growth. This was accompanied by a prolonged life span of the mice. This effect was more pronounced with the paclitaxel plus Gefitinib in combination. Combined treatments with Gefitinib and each cytotoxic drug at the dose and schedule tested were well tolerated by mice, with no weight loss or signs of acute or delayed toxicity. Similar results have recently been reported by Sirotnak et al. (2000). In fact, coadministration of Gefitinib significantly enhances the efficacy of paclitaxel, docetaxel, cisplatin, carboplatinum, doxorubicin, or edatrexate in nude mice bearing established GEO tumors. Gefitinib treatment has an additive or frankly synergistic interaction with colorectal, ovarian, NSCL, head and neck, renal and hormone-resistant prostate cancers. In particular, Gefitinib treatment prevents c-erbB-2 signaling in human breast cancer cell lines that overexpress c-erbB-2 and that express functional EGFRs, possibly by preventing EGFR/c-erbB-2 heterodimerization (Anido et al. 2001, Moasser et al. 2001, Moulder et al. 2001, Normanno et al. 2001). Gefitinib growth inhibition is accompanied in these cells by inhibition of the AKT survival pathway and by induction of apoptosis. Furthermore, a cooperative antitumor activity in vitro and in vivo in these breast cancer models has been demonstrated by treatment with Gefitinib in combination with the anti-ErbB2 MAb trastuzumab (Herceptin) (Anido et al. 2001, Moasser et al. 2001, Moulder et al. 2001, Normanno et al. 2001).

Five phase I trials have been conducted to assess either intermittent Gefitinib (14 days treatment followed by 14 days observation) (Ferry et al. 2000, Kris et al. 2000, Tamura et al. 2000) or continuous Gefitinib (28 consecutive days treatment) administration (Baselga et al. 2000, Kris et al. 2000, Goss et al. 2001). Gefitinib was given as a once-daily, oral dose (50–1000 mg/day). Nearly all of the 254 patients in these phase I trials were heavily pre-treated advanced cancer patients. Both intermittent and continuous dosing of Gefitinib were well tolerated. The most frequently reported adverse events were diarrhea and an acneform skin rash. These side effects were reversible upon discontinuation of treatment and the skin rash resolved without scarring. Grade 3 diarrhea was dose limiting at the 700 mg dose level after intermittent dosing and at the 1000 mg level after continuous dosing. Pharmacokinetic assessments confirm that Gefitinib is suitable for once-daily dosing with a mean elimination half-life of 46 h. Steady-state plasma concentrations were reached by day 7. Exposure to Gefitinib increased approximately linearly with dose, although exposure varied within each dose group. An elegant pharmacodynamic study of the effects of Gefitinib treatment on skin biopsies obtained from 65 patients enrolled in phase I studies has recently been reported (Albanell et al. 2002). Immunohistochemical evaluation of normal keratinocytes following oral treatment with Gefitinib revealed suppression of EGFR phosphorylation, inhibition of MAPK activation and reduced proliferation index. Furthermore, an increase in p27kip1 expression and enhanced apoptosis were detected. This study is the first clinical evidence that Gefitinib treatment at tolerable doses inhibits EGFR activation and affects downstream receptor-dependent processes in vivo in cancer patients. In the phase I clinical trials, antitumor activity has been observed in patients with colorectal, ovarian, NSCL, head and neck, renal and hormone-resistant prostate cancers. In particular,
encouraging results have been reported in patients with NSCLC. Of 100 patients with NSCLC, ten patients had a partial response lasting from 1 to 16 months, and 2 patients had regression of non-measurable evaluable disease. In addition, approximately one-third of patients have had long-lasting stable disease for 3 or more months.

Recently, the final results of a multicenter randomized, double-blind, phase II trial of Gefitinib as second or third line single agent therapy in patients pretreated with a platinum-containing regimen with advanced NSCLC (IDEAL 1 trial) have been presented (Fukuoka et al. 2003). Two hundred and ten advanced NSCLC patients that were not selected for EGFR status received randomly either 250 mg or 500 mg oral daily treatment. Objective response rates were 18.4% and 19% for the two doses respectively. Median overall survival times were 7.6 and 8.0 months respectively. A parallel study in a more heavily pretreated population of NSCLC (at least two previous chemotherapy regimens containing platinum and taxanes) was also reported (IDEAL 2 trial) (Kris et al. 2002). Objective response rates were 11.8% and 8.8% for the two doses respectively. Median overall survival times were 6.5 and 5.9 months respectively. In both IDEAL studies, Gefitinib at the 250 mg dose was equally active as compared with the 500 mg dose. However, the tolerability profile was significantly better with the 250 mg daily dose. Based on these studies Gefitinib, 250 mg tablets, has been registered in Japan in July 2002 and, more recently, in May 2003 in Australia and the USA for the treatment of chemorefractory NSCLC patients that have progressed following platinum and docetaxel based chemotherapy.

A pilot study has shown that Gefitinib in combination with carboplatin/paclitaxel appears feasible and well tolerated in previously untreated patients with advanced NSCLC (Miller et al. 2003). Pharmacokinetic data suggest that co-administration of Gefitinib does not affect the clearance of either carboplatin or paclitaxel. Of 24 patients affected by advanced NSCLC and treated with carboplatin/paclitaxel plus Gefitinib, 1 patient had a complete response (8+ months), 5 patients experienced partial responses (range, 3.1 to 12.4 months) and 8 patients obtained disease stabilization (Miller et al. 2003). The combination of another platinum-based chemotherapy regimen (cisplatin/gemcitabine) with oral Gefitinib (250 or 500 mg daily dose) was evaluated in 18 advanced cancer patients as first-line treatment (Giaccone et al. 2001). Preliminary data from this phase I study show that the combination was tolerable, with no increase in chemotherapy-related toxicity and no interference on the pharmacokinetics of these drugs. Recently, preliminary results of a phase I study of oral Gefitinib in combination with 5-fluorouracil and leucovorin in patients with advanced colorectal cancer have been reported (Hammond et al. 2001). This study included 26 patients that were treated with Gefitinib doses from 250 to 500 mg/day. No significant increase in the frequency and the severity of diarrhea or skin toxicity beyond that expected with this chemotherapy alone was observed in this trial. Finally, the role of Gefitinib in combination with standard platinum-based chemotherapy in advanced NSCLC is currently under investigation. Two large multicenter three arm, placebo-controlled phase III studies (more than 1000 patients in each study) of Gefitinib (250 mg or 500 mg daily) in combination with cytotoxic agents (carboplatin/paclitaxel or cisplatin/gemcitabine) (INTACT 1 and 2 studies) as first-line treatment in non-operable stage IIIB and stage IV NSCLC patients have been completed. In these trials, patients were enrolled without selection for EGFR expressing tumors. No differences in response rates, time to progression and overall survival between the three groups were observed in both trials (Giaccone et al. 2001). However, a subset analysis of patients in the INTACT 2 trial (carboplatin plus paclitaxel chemotherapy) showed a trend to an improved survival in those NSCLC patients with adenocarcinoma that were treated for at least 90 days with chemotherapy plus Gefitinib, 250 mg, as compared with chemotherapy plus placebo (P = 0.05) (Herbst et al. 2003).

Erlotinib
Erlotinib (6,7-bis(2-methoxy-ethoxy)-quinazolin-4-yl)-(3-ethynyl-phenyl)amine, formerly known as CP358,774) is a different quinazoline derivative which selectively and reversibly inhibits the kinase activity of EGFR. The proliferation of DiFi human colon tumor cells is inhibited by Erlotinib at submicromolar concentrations (Moyer et al. 1997, Pollack et al. 1999). Erlotinib also blocks the cell cycle in G1, resulting in significant accumulation of the cell cycle inhibitor p27Kip1 (Moyer et al. 1997, Pollack et al. 1999). Erlotinib induces apoptosis in vitro and has activity against various EGFR-expressing human tumor xenografts in vivo (Moyer et al. 1997, Moasser et al. 2001). Erlotinib in combination with cisplatin produced substantial growth inhibition of human cancer xenografts with no major toxicity in nude mice (Moyer et al. 1997, Pollack et al. 1999).

Erlotinib has been evaluated in two phase I dose-escalation pharmacokinetic trials in patients with advanced solid tumors (Karp et al. 1999, Siu et al. 1999, Hidalgo et al. 2001). Oral Erlotinib (100–1600 mg) was administered once weekly every 3 out of 4 weeks (n = 28), on three consecutive days for 3 weeks, or every day for 3 weeks (n = 27). All these treatment schedules were followed by a week of rest. All the patients had prior treatment for advanced solid tumors. The maximum tolerated dose (MTD) was not reached in the once-weekly
schedule and 1600 mg weekly was a well-tolerated dose. Like Gefitinib, dose-limiting toxicity (DLT) was diarrhea (at dose levels above 150 mg daily dose) in the continuous once-daily dose schedule. Half of the patients in the continuous dose schedule have reported grade 1–2 acne-form skin rashes. Pharmacokinetic analysis showed large intra- and interpatient variability, but dose-proportional increases in exposure. The recommended dose for an orally administered, continuous schedule is 150 mg/day. Of 28 patients receiving Erlotinib weekly, 12 patients remained alive (9–22 months), including 5/11 with lung cancer and 3/5 with HNC. Erlotinib is currently in phase II development in advanced HNC, NSCLC, and ovarian cancer. Preliminary results of a phase II study of Erlotinib in patients with pretreated, advanced HNC have recently been presented (Senzer et al. 2001). One hundred and twenty-four patients (98 patients with EGFR overexpression cancer as detected by immunohistochemistry) received Erlotinib, 150 mg daily. Major toxicities were skin rash in 74.2% patients (11.3%, grade 3) and diarrhea (3.2%, grade 3). Partial responses were observed in 7 patients (5.6%) and stable disease occurred in 39 patients (33.9%). The median survival was 5.8 months, with a one-year survival of 24% patients. A phase II study in ovarian cancer patients has also recently been reported (Finkler et al. 2001). Thirty-four patients with advanced, heavily pretreated, ovarian cancer received oral Erlotinib, 150 mg daily. Major toxicities were skin rash (88% patients, 9%, grade 3) and diarrhea (35% patients, 6%, grade 3). Two patients had partial responses and 16 experienced disease stabilization. The median survival for the patients in this study was 242 days (Finkler et al. 2001). Finally, evidence of antitumor activity of Erlotinib in patients with advanced NSCLC that had failed a platinum-based therapy was recently reported (Perez-Soler et al. 2001). A complete response occurred in 1 of 57 patients and partial responses were recorded in 6 patients; an additional 17 patients had stable disease.

RAS and farnesyl transferase inhibitor (FTI)

The signal transduction cascade downstream from various extracellular signals from the cell surface, including growth factors that activate cell-surface receptors (e.g. EGFR and HER-2), cytokines (e.g. interleukin (IL)2, IL3, granulocyte macrophage colony-stimulating factor), and hormones (e.g. insulin, insulin-like growth factor) (McCormack 1994), involves the 21 kDa guanine-nucleotide-binding proteins encoded by the ras proto-oncogene. Once in its GTP-bound form, ras activates several downstream effector pathways that mediate cell proliferation and suppression of apoptosis. Point mutations in the ras gene are oncogenic or transforming because they result in a permanently active GTP-bound form of ras which continuously activates the downstream pathways in the absence of any upstream growth-factor stimulation.

Furthermore, in the absence of any mutations in the ras gene itself, continuous activation of ras protein can still occur in cells as a result of permanent upstream growth-factor activation. Thus, a strategy of targeting ras function in cancer need not be limited to tumors with proven oncogenic ras mutation. For the ras protein to function in the signal transduction function of the ras gene in cancer. The detailed understanding of the farnesyl transferase reaction and the substrate specificity for the enzyme led to the rational design of several different FTIs. One of the first reports of FTI-induced tumor regression in an in vivo model was obtained by treatment with L744,123, the ester prodrug of a peptidomimetic compound based on the C-terminal sequence of ras as the determinant for enzyme recognition (Reiss et al. 1990). L778,123 inhibited the growth of spontaneous mammary tumors in H-RAS transgenic mice without any systemic toxicities (Kohl et al. 1995). Screening of natural products or compound libraries led to the discovery of two unrelated compounds, SCH66336 and R115777. SCH66336 is a tricycle compound, a potent FTI, which in in vivo human xenograft studies showed dose-dependent growth inhibition of various tumors, including those of colon, bladder, lung, prostate and pancreas (Bishop et al. 1995). R115777 is an imidazole-containing heterocyclic agent (Skrzat et al. 1998), which inhibits the growth of several wild-type or mutant ras gene tumor cell lines (Todd et al. 1998). In vitro studies have suggested that FTIs may be combined with some conventional cytotoxic drugs, and that the anticancer effects may be additive or synergistic in some systems. This hypothesis has been supported by preclinical studies with both SCH66336 and R115777 in combination with paclitaxel (Ranganathan et al. 1999, Shi et al. 1999, Skrzat et al. 1999). The finding that SCH66336 prevents farnesylation of centromere-binding proteins (Ashar et al. 2000) raises the possibility of a direct FTI effect on microtubule formation during the G2/M phase of the cell cycle, which may lead to increased sensitivity to the microtubule-stabilizing action of the taxanes.
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<th>Drug</th>
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<td>L778,123</td>
<td>35–560 mg/m² daily</td>
<td>7-day continuous intravenous infusion (q21)</td>
<td>16</td>
<td>Nausea, thrombocytopenia</td>
<td>Dose-related inhibition of farnesylation of marker protein (hDJ2) in peripheral-blood leucocyte samples</td>
<td>Britten et al. (1999)</td>
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<td>140–840 mg/m² daily</td>
<td>14 or 28-day continuous intravenous infusion (q21 or 35)</td>
<td>24</td>
<td>Neutropenia, QTc prolongation on ECG</td>
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<td>Rubin E et al. (2000)</td>
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<td>BMS214662</td>
<td>36–225 mg/m² daily</td>
<td>1-h infusion (q21)</td>
<td>38</td>
<td>Nausea, vomiting, diarrhea</td>
<td>Clinical activity seen in 3 patients (NSCLC, colorectal and breast)</td>
<td>Ryan DP et al. (2000)</td>
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<td>36–168 mg/m² daily</td>
<td>Single oral dose</td>
<td>13</td>
<td>Nausea, vomiting, diarrhea</td>
<td>Dose-related inhibition of farnesylation in peripheral-blood leucocyte samples</td>
<td>Sonnichsen et al. (2000)</td>
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<tr>
<td>SCH66336</td>
<td>25–400 mg/m² twice daily</td>
<td>7-days oral (q21)</td>
<td>20</td>
<td>Nausea, vomiting, diarrhea</td>
<td>Partial response in 1 patient with NSCLC; 8 patients stable for 5–20 cycles</td>
<td>Adjei et al. (2000)</td>
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<td></td>
<td>25–300 mg/m² twice daily</td>
<td>14-days oral (q21)</td>
<td>21</td>
<td>Nausea, diarrhea, malaise</td>
<td>2 patients with colon cancer stable for 4 months; mixed response in 1 patient with pancreatic cancer</td>
<td>Hurwitz et al. (1999)</td>
</tr>
<tr>
<td></td>
<td>25–300 mg/m² twice daily</td>
<td>Continuous oral</td>
<td>24</td>
<td>Neutropenia, thrombocytopenia, vomiting, confusion</td>
<td>Stable disease lasting &gt; 9 months in 2 patients (thyroid cancer, pseudomyxoma peritonei)</td>
<td>Eskens et al. (1999)</td>
</tr>
<tr>
<td>R115777</td>
<td>25–1300 mg/m² twice daily</td>
<td>5-days oral (q14)</td>
<td>27</td>
<td>Peripheral neuropathy, myelosuppression, nausea, fatigue</td>
<td>Clinical and marker response in 1 patient with colon cancer</td>
<td>Zujewski et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>60–420 mg/m² twice daily</td>
<td>21-days oral (q28)</td>
<td>12</td>
<td>Neutropenia, fatigue</td>
<td>2 patients (prostate cancer, parotid tumor) with stable disease for &gt; 6 months</td>
<td>Hude et al. (1999)</td>
</tr>
<tr>
<td></td>
<td>50–500 mg/m² twice daily</td>
<td>Continuous oral</td>
<td>18</td>
<td>Peripheral neuropathy, neutropenia, thrombocytopenia</td>
<td>1 partial response in NSCLC; 1 patient with pancreatic cancer stable disease (5 months); marker reduction in 2 patients with colon cancer</td>
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</tr>
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</table>
At least five FTIs are in clinical development and the key findings from these studies are summarized in Table 2. In terms of efficacy in patients with solid tumors, the results from these phase I trials are encouraging. Clinical activity has been reported in various types of tumors, in addition to stable disease in several patients who had been heavily pretreated. In phase I studies of combination with chemotherapy agents, L778,123 and SCH66363 have been combined with paclitaxel (Khuri et al. 2000, Sharma et al. 2000), R115777 and SCH66336 with gemcitabine (Hurtitz et al. 2000, Patnik et al. 2000) and R115777 with fluorouracil plus leucovorin (Peeters et al. 1999). Toxic effects seem predictable and manageable and there is evidence of activity in heavily pretreated patients, some of whom had been resistant to the cytotoxic given as a single agent. In a phase II trial, SCH66336 has been administered as a single agent at a dose of 200 mg given continuously in patients with metastatic colon cancer refractory to 5-fluorouracil and irinotecan (Sharma et al. 2002). Treatment has been associated with moderate to severe toxicity in a majority of patients and no responses were observed, although three patients had stable disease for 4–6 months.

In preclinical models, FTIs have shown radiation-sensitizing properties. Therefore, a clinical trial has been conducted combining a continuous infusion of L778,123 with radiotherapy in NSCLC and HNC (Hahn et al. 2000). L-778,123 was given by continuous i.v. infusion and was dose escalated in conjunction with standard radiotherapy. Local responses have been observed in four NSCLC patients without a clear increase in radiotherapy-associated toxicities.

**Bcl-2 and G3139**

Perturbed regulation of apoptosis, the cell-death programme that is mediated by proteases called caspases, underlies many diseases including cancer. Neoplastic cells with a defective suicide program have various selective advantages: the cells can persist in hostile environments, escape death induced by other oncogenic changes and evolve into more aggressive derivatives. Furthermore, defective apoptosis facilitates metastasis, because the cells can ignore restraining signals from their milieu and survive detached from the extracellular matrix.

The link between selective cell suicide and cancer emerged when Bcl-2 (B-cell lymphoma 2), the gene that is linked to an immunoglobulin locus by chromosome translocation in follicular lymphoma, was found to inhibit cell death (Vaux et al. 1988). Bcl-2 and its closest homologs, Bel-xl and Bel-w, potently inhibit apoptosis in response to many cytotoxic insults. Bcl-2 and other pro-survival proteins associate with the mitochondrial outer membrane and the endoplasmic reticulum/nuclear membrane, maintain their integrity and can prevent cytochrome c release, and hence caspase-9 activation (Cory & Adams 2002). Bcl-2 and its relatives have been implicated not only in the pathogenesis of cancer but also in resistance to cancer treatment. Overexpression of Bel-2 renders tumor cells refractory to diverse therapeutic drugs and radiation, in vitro and in vivo (Strasser et al. 1994, Schmitt et al. 2000), and selection for drug resistance in cancer cells is often accompanied by upregulation of Bel-2 (Sartorious & Krammer 2002).

For these reasons, the prospect of directly switching on the apoptotic machinery is gaining widespread interest (Nicholson 2000). One promising approach is to target Bel-2 with an antisense oligonucleotide (ASO). ASOs are chemically synthesized, highly purified sequences of single-stranded DNA that are complementary to specific coding regions of mRNA and can inhibit gene expression. The antisense drug, augmerosen (Genasense, Genta Inc., Berkeley Heights, NJ, USA; code G3139), an 18-mer phosphorothioate oligonucleotide complementary to the first six codons of Bel-2 mRNA, hybridizes to the respective RNA bases and leads to selective decreases in concentration of the RNA and protein product. The first results of a clinical study evaluating G3139 was reported in patients with non-Hodgkin’s lymphoma (NHL) (Webb et al. 1997, Waters et al. 2000). In this phase I dose escalation trial conducted in a total of 21 patients, pharmacokinetics, toxicity and therapeutic activity were assessed after subcutaneous infusion of the ASO as a single agent. All patients had skin inflammation at the infusion site. Dose-limiting toxicities were thrombocytopenia, hypotension, fever and asthenia. The maximum tolerated dose was 147.2 mg/m² daily. By standard criteria, there was one complete response, two minor responses, nine patients with stable disease and nine with progressive disease. However, the mean inhibition of Bel-2 expression was moderate (24%) and the biological importance of this relatively small decline is uncertain (Gurtitz 2000). A phase II study with G3139 in combination with standard chemotherapy for patients with relapsed, chemoresistant NHL is underway.

Preclinical studies have shown that G3139 improves chemosensitivity to dacarbazine (DTIC) in models of human melanoma xenografts in nude mice (Jansen et al. 1998). For these reasons, in a phase I/II trial the combination of G3139 and DTIC was tested in patients with advanced malignant melanoma (Jansen et al. 2000). G3139, 0.6–6.5 mg/kg, was given intravenously or subcutaneously along with standard DTIC treatment (total doses up to 1000 mg/m² per cycle) to 14 patients with progressive metastatic disease on entry after failure of standard treatments. The combination regimen was well tolerated as a single agent at a dose of 200 mg given continuously in patients with metastatic colon cancer refractory to 5-fluorouracil and irinotecan (Sharma et al. 2002). Treatment has been associated with moderate to severe toxicity in a majority of patients and no responses were observed, although three patients had stable disease for 4–6 months. In preclinical models, FTIs have shown radiation-sensitizing properties. Therefore, a clinical trial has been conducted combining a continuous infusion of L778,123 with radiotherapy in NSCLC and HNC (Hahn et al. 2000). L-778,123 was given by continuous i.v. infusion and was dose escalated in conjunction with standard radiotherapy. Local responses have been observed in four NSCLC patients without a clear increase in radiotherapy-associated toxicities.

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tolerated with non dose-limiting toxicity. Hematological abnormalities were mild to moderate. Lymphopenia was common, but no febrile neutropenia was observed. High doses of G3139 were associated with transient fever. Six of 14 patients showed antitumor responses (one complete, two partial, three minor). The estimated median survival of all patients was more than 12 months, which compares favourably with survival of stage IV malignant melanoma patients (usually 6–9 months with and without treatment). Based on the results of this trial, the combination DTIC and G3139 therapy in patients with malignant melanoma is in a phase III multicenter study. Phase I and II studies (Chen et al. 2000, Tolcher et al. 2000) are in progress to test G3139 in combination with docetaxel in patients with advanced breast cancer, and in hormone-refractory prostate cancer in combination with mitoxantrone, and in small-cell lung cancer in combination with paclitaxel (Rudin et al. 2002). Furthermore, a phase I/II study of G3139 and irinotecan has been initiated in patients with metastatic or recurrent colorectal cancer.

### Protein kinase C (PKC) and ISIS 3521

PKC is an attractive target in cancer therapy. PKC belongs to a class of serine–threonine kinases involved in a myriad of intracellular responses arising from G-protein-coupled receptors, receptors with tyrosine kinase activity and non-receptor tyrosine kinases (Newton 1997), and perturbations of its expression have been implicated in the growth and progression of some human tumors (O’Brian et al. 1989, Morotomi et al. 1990, Kopp et al. 1991). PKC exists as a family of at least 12 closely related isozymes (Asaoka et al. 1992, Nishizuka 1992, Dekker & Parker 1994). Because of their similar structural characteristics, the development of selective PKC isozyme inhibitors has been difficult and their inability to discriminate between the various forms may result in unacceptable toxicity to normal tissues (Basu 1993, O’Brian & Kuo 1994). An alternative approach is the use of ASOs (Dolnik 1991, Crooke 1995). Because the specificity of ASOs is derived from selective hybridization to a target mRNA sequence, it is possible to design oligonucleotide inhibitors of members of a closely related gene family such as PKC (Dean & McKay 1994, McKay et al. 1996).

ISIS 3521 is a 20-mer phosphorothioate oligodeoxynucleotide (ODN) that hybridizes to the 3′-untranslated region of human PKCα mRNA, resulting in its degradation by RNase H. Preclinical evidence of antitumor activity and PKCα inhibition by an antisense mechanism was demonstrated in a variety of in vitro and in vivo experiments (Dean et al. 1994). The ODN was found to inhibit the growth of three different subcutaneously grown human tumor cell lines, the T-24 bladder, human NSCLC A549 and Colo 205 colon carcinoma (Dean et al. 1996), as well as the growth of s.c. and orthotopically transplanted U87 glioblastoma cells (Yazaki et al. 1996). Based on the biological evidence implicating PKC in the pathogenesis of certain solid tumor types, and the broad spectrum of antitumor activity of ISIS 3521, human clinical trials have been initiated with this compound for the treatment of cancer. The phase I trials of ISIS 3521 were dose-escalation studies in patients with treatment resistant solid tumors. In one of these phase I studies (CS02), ISIS 3521 was administered to 21 patients with end-stage malignancies for three weeks via continuous i.v. infusion followed by a 1-week treatment-free period (Yuen et al. 1999). The maximum tolerated dose was 2.0 mg/kg/day, while dose-limiting thrombocytopenia and fatigue were observed at the dose of 3 mg/kg/day. Antitumor activity was observed in three of four patients with ovarian cancer. Based on single-agent experience, a phase I/II trial of combination therapy was initiated. In the first phase I/II combination therapy trial (CS3/3N) ISIS 3521, 2 mg/kg/day, was administered by continuous i.v. infusion on days 0–14, while carboplatin (AUC 6) and paclitaxel, 175 mg/m², were administered on day 4, in patients with stage IIIB or IV NSCLC. In 53 patients, 83% in stage IV, 258 cycles of therapy were administered, with a median of 6 cycles and a range of 1–9 cycles. Thirteen patients had received one previous therapeutic regimen, while 12 patients had already received at least 2 former regimens. Toxicity consisted mostly of neutropenia and thrombocytopenia, which were responsible for the delay for treatment in 12 cycles (6 patients) in a total of 19 cycles delayed for more than 1 week. In the 48 evaluable patients, the response rate was 48%, with 2% (1 patient) obtaining a complete response and 46% (22 patients) a partial response, while a stable disease was observed in 35% (17 patients). The median time to progression and the median survival were 6.3 months and 15.9 months respectively. Thus, the combination of ISIS 3521, carboplatin and paclitaxel was well tolerated, and showed promising activity in NSCLC (Yuen et al. 2001). In a second phase I/II trial (CS15) ISIS 3521 at 2 mg/kg/day was administered by continuous i.v. infusion on days 0–14, while cisplatin, 80 mg/m², and gemcitabine, 1000 mg/m², were administered i.v. on day 0, with gemcitabine administration also on day 7. This schedule was evaluated in a cohort of advanced NSCLC patients, with 93% of patients with stage IV disease. The combination was well tolerated, with manageable neutropenia and thrombocytopenia as the main toxicities. No pharmacokinetic interactions were observed. In the phase II portion of the study, 44 evaluable, chemonaive, advanced NSCLC patients received a median of 3 cycles.
of treatment. Toxicity was moderate with thrombocytopenia, neutropenia, anemia, fatigue, dehydration, sepsis, and neutropenic fever. In the updated analysis of the trial, the response rate was 37%, including 1 complete remission and 11 partial remissions, while 50% of patients obtained a stable disease. ISIS 3521 combined with cisplatin and gemcitabine is well tolerated. Survival data are still pending (Ritch et al. 2002). A third phase II trial was conducted in patients with advanced NSCLC to evaluate the safety and efficacy of ISIS 3521 administered by continuous i.v. infusion for 14 days plus docetaxel 75 mg/m² on day 3 of each cycle. In the 53 patients treated, toxicity was moderate and consisted of thrombocytopenia, neutropenia, neutropenic fever and fatigue. In the 36 evaluable patients, 5 partial responses (14%) and 15 stable disease (42%) were observed (Moore et al. 2002).

Collectively, the results of the above studies conducted in NSCLC with ISIS 3521 in combination with different chemotherapies were encouraging. In fact, in three studies with comparable NSCLC patient cohorts receiving chemotherapy alone, the median time to progression and median survival ranged between 4.2–6.9 and 8.1–9.1 months respectively (Cardenal et al. 1999, Sandler et al. 2000, Schiller et al. 2002). On the basis of the above results, two large randomized phase III trials have started as first line treatment in NSCLC patients: a 600-patient study of ISIS 3521 in combination with carboplatin and paclitaxel and a 1000-patient study of ISIS 3521 in combination with cisplatin and gemcitabine (the ALERT study). The initial results of this trial have recently been reported (Lynch et al. 2003). No significant differences were observed between the experimental and the standard treatment in both response rates and survival. The second phase III trial is a 3-arm study which will examine, in a controlled randomized fashion, the optimal timing of ISIS 3521 dosing in relation to chemotherapy. In one arm, patients are treated with a standard regimen of gemcitabine and cisplatin. In a second arm, patients receive a 14-day infusion of ISIS 3521 at 2 mg/kg/day beginning on day 1, followed by cisplatin (80 mg/m²) on day 4 and gemcitabine (1250 mg/m²) on day 4 and day 11. In the third arm, patients initiate all therapy on day 1 and have their second gemcitabine dose on day 8.

Studies with ISIS 3521 in combination with chemotherapy have been undertaken in patients affected by other types of cancer. A phase I study with ISIS 3521 in combination with 5-fluorouracil (5-FU) and leucovorin (LV) was conducted in patients with refractory solid tumors, the majority affected by colorectal cancer. Fifteen patients received ISIS 3521 at either 1.0, 1.5 or 2 mg/kg/day by 21-day continuous i.v. infusion and simultaneously received 5-FU and LV for 5 consecutive days; treatment was repeated every 4–5 weeks. Moderate toxicity was observed except for one grade 3 mucositis and five grade 4 neutropenia. There were no effects on prothrombin time and activated partial thromboplastin time. A clinically defined MTD or classical DLT were not reached. ISIS 3521 and 5-FU pharmacokinetics did not affect each other and were similar to other studies. About 20% tumor responses were observed, ranging from minor reduction in tumor size (4 patients) to partial response (2 patients) (Mani et al. 2002).

### Protein kinase A (PKA) and GEM231

PKA plays a key role in the control of cell growth and differentiation and is present in mammalian cells with two distinct isoforms, defined as PKAI and PKAII, which differ only in their regulatory subunits (termed RI in PKAI and RII in PKAII, respectively). Only during the past decade has experimental evidence established distinct functions for PKAI and PKAII and has demonstrated that their intracellular balanced expression may play a critical role in the control of cell growth and differentiation (Cho-Chung et al. 1995). In fact, preferential expression of PKAII is found in normal nonproliferating tissues and in growth-arrested cells, while PKAI and/or its regulatory subunit RIα are: (a) transiently induced by physiologic stimulation of cell proliferation, favouring the propagation of mitogenic signaling; (b) generally overexpressed in human cancer cell lines and in primary tumors; (c) induced following transformation by certain growth factors and receptors, such as TGFα, EGFR and erbB-2, or oncogenes, such as ras and myc; (d) associated with a worse prognosis in patients affected by different types of cancer; (e) implicated in multidrug resistance (Miller et al. 1993, Bradbury et al. 1994, Cho-Chung et al. 1995, Tortora & Ciardiello 2002).

For all the above reasons, PKAI has been proposed as a potentially relevant target for cancer therapy. In the past 15 years, selective pharmacologic tools able to modulate PKAI expression have been developed, providing a major contribution in the understanding of several PKAI functions, but also revealing themselves as potential anticancer agents. Among the most useful agents, a relevant role is occupied by the ASOs targeting the PKAI subunit R1α, from the early unmodified oligos to the DNA/RNA hybrid mixed backbone oligos (MBOs), such as the MBO AS-PKAI (GEM231, defined hereafter as AS-PKAI), which exhibit significant improvement of pharmacokinetic properties and target interaction in vivo, as compared with first generation phosphorothioate oligonucleotides (Nesterova & Cho-Chung 1997, Cho-Chung 1999, Akhtar & Agrawal 1997).
Several studies have shown that the AS-PKAI is able to cooperate with a variety of anticancer drugs of different classes, following intraperitoneal as well as oral administration of the AS-PKAI. In particular, a synergistic antitumor activity, associated with increased apoptosis, can be obtained with taxanes, topoisomerase I and II inhibitors and platinum derivatives, both in vitro and in nude mice bearing a wide variety of human cancer types (Tortora et al. 1997, 2000, Wang et al. 1999, 2002, Cho & Cho-Chung 2003).

Conclusions
The enhancement of antitumor activity of conventional cytotoxic treatments by interfering with signal transduction pathways may have relevant clinical implications. In this respect, treatment with conventional doses of cytotoxic drugs or with radiotherapy in combination with signal transduction inhibitors could be an effective novel anticancer strategy for increasing the activity of currently available therapies. However, the first series of clinical trials with molecular targeted agents in combination with radiation or chemotherapy have provided mixed results in terms of antitumor activity and of clinical efficacy. Several important issues need to be addressed in clinical studies in this area of therapeutic research, such as which is the best combination of cytotoxic agents and signal transduction inhibitor, and which is the best schedule of treatment combination (i.e. sequential versus concurrent regimens). Preclinical and early clinical trials data suggest that most of the molecular targeted agents could be effectively combined with most chemotherapy drugs regardless of their mechanism(s) of action. In current clinical trials cytotoxic agents are delivered as in standard monotherapy or polychemotherapy schedules with the signal transduction inhibitors chronically administered during the planned number of cycles. In some trials a maintenance period of variable length of time with these drugs following cessation of chemotherapy is planned. Finally, a series of clinical trials are addressing the question of whether the addition of molecular targeted agents, such as anti-EGFR drugs, could overcome the resistance to a previously administered drug. After the first series of phase I, II and III clinical trials, the next generation of clinical studies with signal transduction inhibitory agents in cancer patients should be designed with a strong translational research effort to address several key clinical questions, such as which patients are most likely to have a therapeutic benefit, what are the potential predictive factors of response or resistance that could be useful in a clinical setting and what are the best strategies for their combination with conventional and/or with other molecular targeted anti-cancer treatments?

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