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SIGNAL TRANSDUCTION–DIRECTED CANCER TREATMENTS*

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■ **Abstract** The pathogenic mechanisms giving rise to cancer frequently involve altered signal transduction pathways. Therefore therapeutic agents that directly address signal transduction molecules are being explored as cancer treatments. Inhibitors of protein tyrosine and threonine kinases including STI-571, ZD-1839, OSI-774, and flavopiridol are ATP-site antagonists that have completed initial phase I and phase II evaluations. Herceptin and C225 are monoclonal antibodies also directed against signaling targets. Numerous other kinase antagonists are in clinical evaluation, including UCN-01 and PD184352. Alternative strategies to downmodulate kinase-driven signaling include 17-allyl-amino-17-demethoxygeldanamycin and rapamycin derivatives, and phospholipase-directed signaling may be modulated by alkylphospholipids. Farnesyltransferase inhibitors were originally developed as inhibitors of *ras*-driven signals but may have activity by affecting other or additional targets. Signal transduction will remain a fertile basis for suggesting cancer treatments of the future, the evaluation of which should include monitoring effects of the drugs on their intended target signaling molecules in preclinical and early clinical studies.

INTRODUCTION

An emerging understanding of the molecular basis of neoplastic cell behavior recognizes that cancer is a signaling disease. Many oncogenes are altered forms of cellular proto-oncogenes, whose expressed proteins normally participate in signal transduction pathways. Negatively acting tumor suppressor genes frequently act to directly modify signaling pathways or are actual substrates for the action of signaling pathways. Thus, it is intellectually satisfying as well as mechanistically well founded that therapeutic interventions taking account of this biology might

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have as primary targets the respective signal transduction molecules contributing to the pathogenesis of cancer.

Numerous drugs have been selected to act as potential signal transduction modulators. This article focuses on those drugs that have already advanced to the clinic and have completed at least one phase I evaluation. Reference is made only in passing to biological functions of the signal transduction pathways affected, as these have been addressed in reviews elsewhere. Figure 1 illustrates an overview of the relationships between signaling systems activated in cancer cells and illustrates where many of the agents to be discussed here have been posited to act.

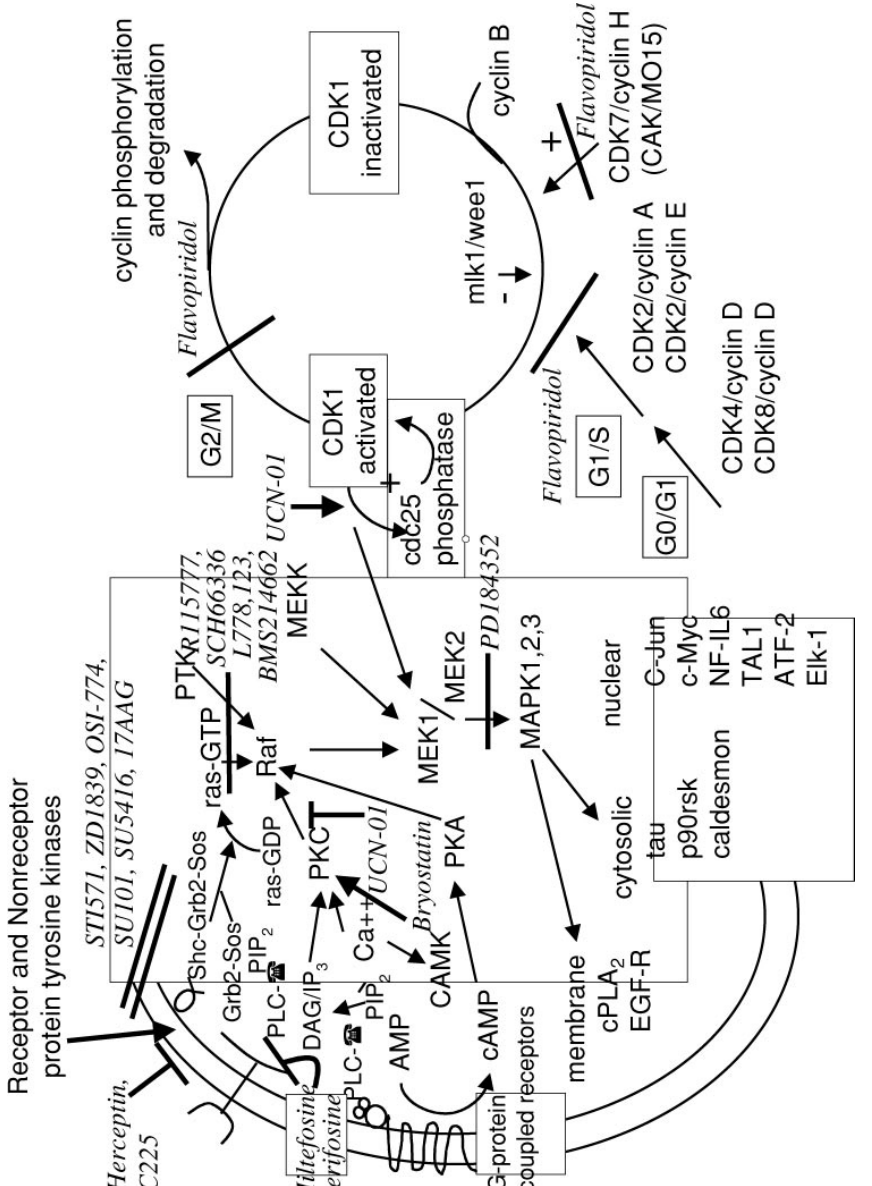
TYROSINE KINASE INHIBITORS

STI-571 (CGP57148B, GLEEVEC®, Imatinib Mesylate)

STI-571 was selected for platelet-derived growth factor (PDGFR) inhibition and later optimized as a potent and specific inhibitor of Abl tyrosine kinase from a series of phenylaminopyrimidines (1). The presence of constitutively active Bcr-Abl tyrosine kinase in chronic myelogenous leukemia (CML) patients and its importance in the pathogenesis of CML provided a convincing rationale to support continued development of the compound. STI-571 inhibited substrate phosphorylation and cellular tyrosine phosphorylation in vitro (at 0.025 μM and 0.25 μM, respectively) (2). Abl (all forms), PDGFR, and c-kit tyrosine kinases are the only kinases known so far to be potently inhibited by STI-571 (3, 4). STI-571 differentially inhibited growth of p210 $^{\text{Bcr Abl}}$CML and p185 $^{\text{Bcr Abl}}$ containing acute lymphoblastic leukemia cells (2), without effect on normal marrow cells. Optimal inhibition of tumor growth in animals required continuous exposure (5).

The efficacy of STI-571 was validated in a remarkable series of clinical trials. A mean maximal concentration of 4.6 μM at steady state and a 24-h trough concentration of $\sim$1.5 μM were achieved after a 400-mg once daily oral dose of STI-571 (6), with a terminal half-life of 16.2 $\pm$ 4.4 h (7, 8). Ninety-eight percent of interferon-refractory chronic phase CML patients who were treated with $\geq$300 mg/day achieved hematologic response; complete cytogenetic responses were seen in 13% and major cytogenetic responses in 31%. Fifty-five percent of patients with

Figure 1 Schematic of signal transduction inhibitor action. Growth factors or matrix components stimulate the action of protein kinase receptors or guanine nucleotide binding protein–coupled receptors, respectively. Activation of ras-related signaling pathways occurs through guanine-nucleotide exchange factor regulators such as Sos, or after calcium release and phospholipase activation. Either one can activate Raf, which stimulates MAP kinase pathways through MEK. Ultimately, transcription of factors leads to entry into the cell cycle and activation of cyclin dependent kinases. Drugs discussed in this review indicate where in this sequence of events there is evidence of their action.


Receptor and Nonreceptor protein tyrosine kinases
Herceptin, C225
STI571, ZD1839, OSI-774, SU101, SU5416, 17AAG
Shc-Grb2-Sos
Grb2-Sos
PLC-γ
PIP2
DAG/IP3
Miltefosine Perifosine
PLC-β
IP3
AMP
cAMP
G-protein coupled receptors
ras-GTP
ras-GDP
Raf
PKC
Ca++
UCN-01
Bryostatin
CAMK
PKA
PTK
R115777, SCH66336, L778,123, BMS214662
MEKK
MEK1
MEK2
PD184352
MAPK1,2,3
cytosolic
nuclear
membrane
cPLA2
EGF-R
tau
p90rsk
caldesmon
c-Jun
c-Myc
NF-IL6
TAL1
ATF-2
Elk-1
cyclin phosphorylation and degradation
Flavopiridol
G2/M
CDK1 activated
CDK1 inactivated
cdc25 phosphatase
mlk1/wee1
cyclin B
Flavopiridol
CDK7/cyclin H (CAK/MO15)
G1/S
CDK2/cyclin A
CDK2/cyclin E
Flavopiridol
G0/G1
CDK4/cyclin D
CDK8/cyclin D

myeloid blast crisis responded to STI-571 treatment, and although 70% of patients with lymphoid phenotype, Philadelphia chromosome-positive ALL, or lymphoid blast crisis responded initially, the majority have relapsed since then (8). Similarly remarkable responses were noted in phase II studies of single agent STI-571 in resistant or refractory chronic phase CML (9), accelerated phase CML (10), and patients in myeloid crisis (11).

The effect of STI-571 on c-kit (CD117) positive tumors has generated much excitement owing to the paucity of interventions for these chemoresistant tumors. A significant response of heavily pretreated patients with rapidly progressive gastrointestinal stromal tumors (GIST) to 400 mg of STI-571 (12) and other GIST and soft-tissue sarcomas (13) has led to large phase II studies in c-kit positive GIST (14) and other tumors, with resulting FDA approval as safe and effective treatment for GIST as well as CML.

ZD 1839 (Iressa)

Inhibition of the epidermal growth factor receptor (EGFR) has been of interest owing to the autocrine activation of EGFR and several downstream pathways, such as ras/MAP kinase and STAT-3 transcription factors, in many tumors. The EGFR pathway induces entry into the cell cycle, inhibition of apoptosis, and activation of angiogenesis and motility. EGFR is overexpressed in an extensive range of human cancers including non-small cell lung (NSCLC), colorectal, head and neck, bladder, brain, pancreas, breast, ovary, prostate, and gastric cancers (15, 16). Overexpression of EGFR has been associated with invasiveness, resistance to treatment, and poor outcome in several tumor types (17, 18).

ZD1839 [4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy) quinazoline] is a synthetic anilinoquinazoline compound selected as a specific potent inhibitor of EGFR. ZD1839 inhibits EGFR through competitive binding to the ATP-binding site. ZD1839 inhibits EGFR autophosphorylation with an IC_{50} of 0.023–0.079 μM (19). ZD1839 produced supra-additive and enhanced antitumor effects of cisplatin, carboplatin, oxaliplatin, paclitaxel, docetaxel, etoposide, topotecan, ralitrexed, and doxorubicin in several tumor types, which resulted in complete regression in some xenograft tumors (20, 21). Similar responses are seen in combination with radiation (22). An important finding in several studies is the apparent effectiveness of ZD1839 regardless of the levels of EGFR protein (21) or gene expression (23). This raised the possibility of other EGFR-related receptors or additional targets for the drug.

Several phase I and II studies have been completed in humans (24–26). Daily oral administration with doses ranging from 50 mg to 700 mg on 14 of 28 consecutive days or continuous administration for 28 days was examined. With intermittent repeated daily dosing, a median half-life was estimated at 46–49 h allowing for once daily dosing (24, 25), with a mean C_{max} of 0.1–2.2 ng/ml (24). In combination with paclitaxel and carboplatin (27), 500 mg per day of ZD1839 was found to be safe with no change in the pharmacokinetics of either. ZD1839 resulted in partial responses in NSCLC and prostate cancer, and stable disease ($\geq$4 months) in several patients with various tumor types (24–26). Five of 23 Japanese patients

achieved partial response (25). Side effects have been relatively mild and have included secretory diarrhea and rash.

OSI-774 (Erlotinib, Tarceva™)

OSI-774 [6,7-bis(2-methoxy-ethoxy)-quinazolin-4-yl]-(3-ethynylphenyl)amine hydrochloride is another orally active and potent quinazoline derivative inhibitor of EGFR with an IC_{50} of 2 nM (purified EGFR inhibition in biochemical assays) or IC_{50} of 20 nM (EGFR autophosphorylation in intact cells). It reversibly inhibits EGFR-TK through competitive binding to the ATP site. Inhibition of EGFR and its downstream P13/MAPK signal transduction pathways by OSI-774 results in accumulation of $p27^{KIP1}$, cell-cycle arrest at G_1 phase and induction of apoptosis (28); EGFR is more than 1000-fold more sensitive to OSI-774 compared with other tyrosine kinases. Evaluation of pre- and posttreatment biopsy specimens from mice bearing xenograft models treated with OSI-774 revealed marked reduction of phosphorylated EGFR. Combination of OSI-774 with cisplatin produced augmented effects (29).

Hidalgo et al. (30) tested the safety and feasibility of protracted administration of OSI-774 in a phase I study. At the maximum tolerated dose (MTD) for continuous administration (150 mg/day), $C_{max} = \sim 1.2\ \mu$g/ml, without evidence of drug accumulation over 28 days and an elimination half-life of 24.4 ± 14.6 h. Diarrhea was dose-limiting in this study, observed in 86% of patients receiving OSI-774 at 150 mg/day. Other notable toxicities included an acneiform eruption. This study demonstrated clinical benefits, with partial responses in patients with renal cell carcinoma, colorectal cancer, and $\geq$5 months stabilization in colon, prostate, cervical, NSCLC, and head and neck cancers. In a phase II study of OSI-774 in patients with EGFR-positive, platinum-refractory NSCLC, 11% of patients achieved partial response and 34% had stable disease (31). These responses did not correlate with higher percent or intense EGFR staining.

SU101

SU101 (N-[4-(trifluoromethyl)phenyl] 5-methylisoxazole-4-carboxamide) (32, 33) was identified as a potent inhibitor of PDGFR. Activity against a rat C6 glioma model engineered to overexpress PDGFR showed diminished p185 phosphotyrosine content ($IC_{50} = 50$–60 uM) upon PDGF stimulation. Inhibition of PDGF-stimulated events such as ligand-induced DNA synthesis and G1/S cell-cycle progression was observed. More potent growth inhibition was observed in cell lines with detectable PDGFRβ relative to cells not expressing PDGFR (IC_{50} 0.2 to 40 uM versus $>$100 uM, respectively).

Eckhardt et al. reported the initial SU101 clinical experience in 26 patients with advanced solid malignancies and variable PDGF expression (34). Treatment cycles consisted of weekly 24-h infusions for four consecutive weeks followed by a two-week rest. Toxicities were mainly grade 1 and 2 nausea/vomiting, fever, and phlebitis, but two patients with prior exposure to myelosuppresive therapies developed grade 3 neutropenia. A maximum-feasible dose of 443 mg/m^2, and

not a MTD, was defined. This endpoint was chosen because this dose required a 2.6 liter infusion volume, and the next 33% higher dose would have required a 3.3 liter volume infusion. SU101 is metabolized to its primary metabolite SU0020 (A77 1726; N-[4-(trifluoromethyl)phenyl] 2-cyano-3-hydroxyl-2-butenamide) rapidly in human plasma at 37C° via an intramolecular rearrangement. This open-ringed metabolite is antiproliferative through inhibition of dihydro-orotate dehydrogenase (IC_{50} 80 to 200 nM), which is a mitochondrial enzyme crucial to pyrimidine biosynthesis. The relative contribution of the parent and the SU0020 metabolite and the actual mechanism of cell growth inhibition vary with the cell system studied. In the initial phase I trial, the half-lives of SU101 (1.8 h) and SU0020 (19 days), as well as the slow clearance rate of SU0020 (0.42 L/day/m^2), suggested that a minimal fraction of the circulating drug was in the SU101 form. In addition, the preponderance of the SU0020 form confounded identification of PDGF pathway inhibition-specific effects. These pharmaceutic challenges have rendered further development of this drug problematic, but interestingly a partial response was reported in a patient with anaplastic astrocytoma stabilized for $>$9 months.

SU5416

This ATP site antagonist of the vascular endothelial growth factor (VEGF) (Flk1/KDR) receptor was designed following crystallographic studies of the indolin-2-one pharmacophore and the fibroblast growth factor (FGF) receptor tyrosine kinase domain. Lineweaver-Burk analysis revealed SU5416 to be a competitive inhibitor with ATP for the Flk1/KDR and PDGF receptors (Ki 0.16 μM and 0.32 μM, respectively) (35, 36).

The first SU5416 clinical trial enrolled 63 patients and administered the drug intravenously on a twice-weekly schedule (37). At the higher dose levels, nausea, projectile vomiting, headache, and increased liver enzymes were prominent toxicities. Stable disease of greater than 6 months duration, but no objective responses, was seen in patients with a variety of advanced diseases (colorectal, lung, renal, and Kaposi's sarcoma). Pharmacokinetic studies revealed that SU5416 had a large volume of distribution (Vd 22 L/M^2), dose-independent clearance (52 L/h), and a half-life of 50 minutes. Peak plasma levels of 17 μM 1 h postinfusion and detectable levels above 5 μM 2 h postinfusion were reported. In an effort to identify surrogate markers of drug levels and antitumor effects, dynamic contrast MRI was performed to assess vascular permeability, and levels of VEGF, tumor necrosis factor alpha (TNF-α), tissue plasminogen activator (tPA), and coagulation parameters were measured (38, 39). A reduction was observed in vascular permeability of up to 37% after repeated doses in stable disease patients, while patients with disease progression had detectable increases in vascularity. Alternative schedules are being explored, although the occurrence of vascular complications, including thrombotic events, raises the possibility that broad application of this drug may be problematic (40).

17-Allyl-amino-17-Demethoxygeldanamycin (17AAG)

The preceding compounds inhibit tyrosine kinase action by competing with ATP. An alternative strategy to block tyrosine kinase signaling is to decrease the expression of relevant kinases. The benzoquinoid ansamycins herbimycin and geldanamycin were found in the 1980s to reverse the transformed phenotype of cells driven by a number of *src*-related tyrosine kinases (41). Detailed studies revealed that herbimycin and geldanamycin did not directly inhibit *src* family kinases but actually increased the rate of degradation of *src*, *lck*, *erbB*1, and *erbB*2 (42–45). An insightful series of experiments by Whitesell & Neckers elucidated a unifying mechanism (46) by showing that derivatized geldanamycin analogs could bind to the ubiquitously expressed cellular chaperone molecule *hsp*90 (heat shock protein 90).

*Hsp*90 acts to catalyze the proper folding and maturation of many proteins including tyrosine kinases (47). Improperly folded kinases are ubiquitinated and then degraded by the proteosome. Herbimycin and geldanamycin, by binding to *hsp*90, cause the displacement and degradation of the client proteins (48, 49). Structural studies (50) have confirmed that the benzoquinoid ansamycins bind to the amino-terminal domain of *hsp*90, and these studies provided a basis for the observation that derivatives in the 17 position, including 17-allyl-amino-17-demethoxy-geldanamycin (17AAG) retained the ability to modulate *hsp*90 client proteins, while fortunately possessing an improved toxicology profile in comparison to geldanamycin. As numerous proteins in addition to tyrosine kinases are affected, 17AAG might be considered a first generation or prototypic compound. Nonetheless, Rosen and colleagues have demonstrated enhanced susceptibility of cell types expressing *erbB*2 (51), and Vande Woude and colleagues have emphasized the susceptibility of *met*-related signals important for metastasis and invasion to geldanamycin congeners (52). These findings raise the possibility that tumors uniquely driven by some *hsp*90 client proteins, including, interestingly, many tyrosine kinase-driven cell types, would actually be selectively sensitive to the agent. Initial phase I trials on a variety of schedules are ongoing.

Herceptin

Yet another distinct strategy to affect tyrosine kinase signaling emerged through identification of antibodies that can bind to the extracellular domain of signaling molecules and alter their function. The HER2/*neu* gene is a Type I receptor tyrosine kinase encoding a 185-kD surface membrane receptor protein. Activation of HER2/*neu* results in an increase in its kinase activity, thus initiating signal transduction leading to proliferation and/or differentiation. Somatic gene amplification of HER2/*neu* occurs in 25%–30% of human breast cancers, to as many as 50 to 100 gene copies per cell (53, 54). Amplification leads to increased transcription and protein levels. HER2 overexpression is an independent prognostic factor predicting poor clinical outcome in breast cancer (55).

The murine monoclonal antibody Ab4D5 directed against human epidermal growth factor receptor 2 $p185^{HER2}$ specifically inhibited proliferation of human

tumor cells overexpressing $p185^{HER2}$, while having no effect on cells expressing physiologic levels of HER2 (56). Development of human antimurine antibodies (HAMA) that neutralize murine antibodies limited the efficacy of the murine monoclonal antibody. A "humanized" antibody, humAb4D5-1, was constructed containing only the antigen binding loops from Ab4D5, with human variable region framework residues plus a human IgG1 constant domain. Trastuzumab, the engineered recombinant, humanized monoclonal antibody directed against HER2 is now known as Herceptin® (57).

Phase I trials revealed that the dose of trastuzumab (i.v. 10–500 mg single dose or once weekly) could be increased without toxicity and that pharmacokinetics were dose dependent (58). A phase II, single agent trial was conducted in 46 HER2-positive [defined as >25% of cells showing membrane staining on immunohistochemistry (IHC)] metastatic breast cancer patients who had failed prior cytotoxic chemotherapy (59). Objective responses were seen in 5 of 43 assessable patients, including 1 complete remission and 4 partial remissions (overall response rate, 11.6%; 95% confidence interval, 4.36 to 25.9). Duration of response ranged from 1 to >60 months. A second phase II trial (60) combined trastuzumab with cisplatin in 39 HER2-positive (defined as light to strong complete membrane staining on IHC using antibody 4D5) metastatic breast cancer patients who had failed prior cytotoxic chemotherapy. Patients received trastuzumab i.v. on day 1 as a 250-mg initial dose followed by weekly doses of 100 mg for 9 weeks. Cisplatin was administered i.v. at a dose of 75 mg/m^2 on days 1, 29, and 57. Of 37 patients assessable for response, 9 achieved a partial response and 9 had a minor response or stable disease. The median duration of response was 5.3 months. The toxicity profile reflected that expected from cisplatin alone. Mean pharmacokinetic parameters of trastuzumab were unaltered by coadministration of cisplatin. This was a key study that clearly suggested a major role of the antibody in augmenting response to chemotherapy agents.

A randomized, placebo-controlled phase III trial was performed to determine efficacy and safety of adding trastuzumab to chemotherapy in breast cancer. Patients received doxorubicin (60 mg/m^2) or epirubicin (75 mg/m^2) plus cyclophosphamide (600 mg/m^2) as first-line therapy, or if they had received prior adjuvant anthracycline therapy, paclitaxel (175 mg/m^2) (61). The dose of trastuzumab added was initially 4 mg/kg followed by 2 mg/kg weekly. Twenty-eight percent of patients treated with chemotherapy and trastuzumab were free of disease progression at 12 months, compared with 9% of the patients treated with chemotherapy alone. The addition of trastuzumab to chemotherapy was associated with a longer time to disease progression (median 7.4 versus 4.6 months), a higher rate of objective response (50% versus 32%), a longer duration of response (median 9.1 versus 6.1 months), and a longer survival (median survival 25.1 versus 20.3 months). The most significant adverse event observed in studies of trastuzumab was cardiac dysfunction. Thirty-eight out of 143 patients receiving anthracycline plus trastuzumab and 11 out of 91 patients that received paclitaxel and trastuzumab had cardiac dysfunction, whereas 1 out of 95 patients that received paclitaxel alone and 10 out 135 patients that received anthracycline alone had cardiac dysfunction.

These clinical trials led to the approval by the FDA of trastuzumab for use in women with metastatic breast cancer with HER2-positive tumors. The treatment is indicated as a single agent for patients having failed earlier therapy and as first-line treatment for metastatic disease when used in combination with paclitaxel. A key issue in considering how to apply the strategy utilized for approval of Herceptin is understanding whether the effect of the antibody in antibody plus chemotherapy regimens actually derives from signaling, as compared to immunological mechanisms. To date this is an open question.

Cetuximab (C225)

Another antibody-based approach to interdicting tyrosine kinase signaling is represented by cetuximab, a humanized monoclonal antibody directed against the EGFR. MAb225, a murine monoclonal antibody that specifically binds to EGFR, specifically competes with signal transduction initiated by TGF-α (62). Cetuximab (also known as C225) is the human–mouse chimeric version of Mab225, which specifically binds to the EGFR with high affinity, preventing the ligand from interacting with the receptor. Preclinical studies have shown that cetuximab results in cell-cycle arrest as well as apoptosis in different contexts (63, 64). A synergistic effect of cetuximab with cytotoxic chemotherapy has been seen with cisplatin, doxorubicin (65), gemcitabine (66), docetaxel (67), and paclitaxel (68).

Early phase I trials demonstrated that cetuximab displays nonlinear, dose-dependent pharmacokinetics that are not altered by coadministration of cisplatin (69). There were three initial, multi-institutional clinical studies with cetuximab; the first was a single-dose trial, and the two subsequent studies administered the antibody on a weekly basis, either alone or in combination with cisplatin. These studies were conducted in patients with tumors overexpressing EGFR. The single dose trial had 13 patients receiving cetuximab in dose range from 5 to 100 mg/m^2. The weekly dose trials had 17 patients in the single agent arm and 22 patients in the arm with cisplatin. There were only 5 episodes of severe C225-related toxicities among the 52 patients. The most frequent adverse events were fever and chills, asthenia, transaminase elevation, nausea, and skin toxicities. Acneiform rash was seen in 6 patients at doses $\geq$100 mg/m^2. The study was completed through the planned dose levels without reaching a MTD. Two patients with head and neck tumors who received cetuximab at doses of 200 mg/m^2 and 400 mg/m^2 with cisplatin exhibited a partial response. In light of these results, the clinical development of cetuximab is continuing with a number of phase II and III studies.

SERINE/THREONINE KINASE ANTAGONISTS

Flavopiridol

Flavopiridol (L86-8275 or HMR 1275) is derived from rohitukine, an alkaloid isolated from *Dysoxylum binectariferum* (a plant indigenous to India). Flavopiridol

has potent antiproliferative activity and modulates several cell signaling pathways in vitro. In the National Cancer Institute (NCI) 60 cell line anticancer drug screen, flavopiridol exhibits significant in vitro activity against all 60 human tumor cell lines (average $IC_{50} = 66$ nM) (70).

Flavopiridol causes cell-cycle arrest at G_1/S phase transition and G_2/M phase transitions and also slows the progression of the cell cycle through the S phase (71). These findings prompted an evaluation of cyclin-dependent kinases, recognized as responsible for governing the orderly transition from G_2 to M phase (CDK1) and G_1 to S phase (CDK4 or 6 with CDK2). Indeed, flavopiridol inhibits all CDKs known so far ($IC_{50} \sim 100$ nM), inhibiting CDK1, CDK2, and CDK4 with a similar potency (72). Studies with purified starfish CDK1 revealed that flavopiridol competitively inhibits ATP with respect to CDK1 (*K*i 41 nM) and noncompetitively with respect to the substrate peptide (73). Cocrystallization studies confirmed binding of deschloroflavopiridol to the ATP binding pocket of CDK2 with its benzopyran ring occupying the same region as the purine ring of ATP (74). Flavopiridol causes loss of regulatory tyrosine and threonine phosphorylation of CDKs (75), and the loss of threonine 161 phosphate inactivates the kinase.

A broadening of the roles for CDKs in cellular regulation recently emerged from the elucidation that certain CDK family members (including CDKs 7, 8, and 9) also have roles in regulating normal transcription. For example, CDK7 is a member of the transcription factor TFIIH complex, and CDK9 corresponds with cyclin T to the transcription factor pTEFb. Flavopiridol can inhibit CDK9 activity potently and in a mechanistically unique way (76, 77), consistent with its formation of a "tightly bound" drug-enzyme complex. This may be the basis for the observation that at certain concentrations, flavopiridol can potently affect the levels of rapidly turning over mRNAs (78) and can serve as a basis for magnifying its capacity for cell-cycle arrest by direct transcription repression of cyclin D1 mRNA (79). This possibility is noteworthy because in mantle cell lymphoma, which overexpresses cyclin D1 in 95% of cases, flavopiridol was able to produce significant delay of disease progression in 84% of patients (80).

Preclinical studies of flavopiridol revealed wide differences in growth inhibition between cell types depending on the duration of exposure and concentration of the drug. Significant cytostasis is observed when flavopiridol is administered in protracted fashion to colorectal (colo 205) and prostate (LnCap/DU145) carcinoma xenograft models (70, 81). Shorter "bolus" administration of flavopiridol to a lymphoma/leukemia (HL60) cell line had a higher degree of apoptosis and cytotoxicity (82).

Two phase I clinical trials have tested the regimen of 72-h continuous infusion every two weeks in humans (83, 84). Seventy-six patients were treated in the NCI phase I trial. The dose-limiting toxicity (DLT) was secretory diarrhea with a maximally tolerated dose (MTD) of 50 mg/m^2/day for 3 days. Flavopiridol was later found to induce chloride ion secretion in intestinal epithelial cells (85) and to have an enterohepatic circulation that may play a role in potentiating this toxicity (86). With antidiarrheal prophylaxis a higher MTD is reachable at 78 mg/m^2/day for 3 days with orthostatic hypotension as the DLT. At the MTD in this study,

the mean steady state concentration (C_{ss}) was 271 nmol/L, well within the range needed to inhibit CDKs and cell proliferation.

Bolus schedules of flavopiridol administration over 1 h were employed to reach higher peak concentrations (87). This study reached an MTD of 37.5 mg/m^2/day for 5 days every three weeks. Mean C_{max} achieved was $\sim$2 μM (1.31–4.2 μM). Although diarrhea and proinflammatory syndrome remain common, bone marrow suppression and neutropenia emerged as a DLT. In some studies other toxicities included thrombosis. Although some cases of partial responses and stable disease have been reported in various phase I studies, several phase II studies revealed few conventionally defined responses in several tumor types, with the possible exception of mantle cell lymphoma (80, 88–90). A more promising role for flavopiridol is envisioned in combination with other agents, including taxol (91), irinotecan (92), and gemcitabine (93), as well as other signal transduction modulators (94–96).

UCN-01 (7-OH Staurosporine)

Staurosporine, a natural product isolated from *Streptomyces staurosporeus*, is a relatively broad, nonspecific protein kinase antagonist, originally isolated in an effort to define inhibitors of protein kinase C (PKC). 7-OH staurosporine (UCN-01) was defined as a more selective, but not specific, PKC antagonist. UCN-01 inhibits "classic" lipid and calcium-dependent PKCs α, β, and γ ($IC_{50} =$ 4–30 nM), Ca^{2+}-independent PKCs * and less potently (IC 50 approximately 500 nM), and is without effect on the atypical PKCs, e.g., PKC ζ (97).

Two prominent effects of UCN-01 have emerged in preclinical studies in vitro: induction of cell-cycle arrest, and abrogation of the checkpoint to cell-cycle progression induced by DNA damaging agents. UCN-01 inhibited cell growth in several in vitro and in vivo human tumor preclinical models (98); however, antiproliferative activity on the part of UCN-01 cannot be explained solely by inhibition of PKC. First, in cell-cycle analyses UCN-01 inhibits Rb^+ cells at G1/S phase of the cell cycle (99). In addition, cells treated with various concentrations of UCN-01 showed decreased pRb phosphorylation in a dose-dependent manner (100). These results suggest that CDK2- or CDK4-regulated steps are targets for UCN-01-induced cell-cycle arrest. As immunoprecipitated CDK1 and CDK2 activity are only moderately susceptible to inhibition by UCN-01 ($IC_{50} =$ 300–600 nM) (101), UCN-01 potentially acts on targets "upstream" of CDKs 1 and 2 rather than directly on the CDKs.

UCN-01 abrogates the DNA damage-induced checkpoints to cell-cycle progression in G2 (102, 103) and in S phase (104). It is noteworthy that these effects were apparent at drug concentrations that appeared to have little direct effect on cell proliferation or that caused enhanced cytotoxicity by clonogenic or proliferation assays. In addition, they provided a mechanistic framework for prior observations that DNA-damaging agents such as mitomycin (105) could greatly potentiate UCN-01 action. Subsequent studies documented that numerous DNA-damaging agents including radiation (103), 5-fluorouracil (5-FU) (106), camptothecin congeners

(104), and temozolamide (107) appeared to act supra-additively with UCN-01 in effecting cytotoxicity. Notably, microtubule-directed agents did not appear to have toxicity enhanced by UCN-01 (108).

A biochemical basis for G2 checkpoint abrogation emerged from the early observation that UCN-01-mediated loss of G2 checkpoint function was accompanied by its ability to decrease CDK1-associated Tyr phosphorylation (101). This phosphorylation is governed by the *wee*1 and *mik*1 kinases, in conjunction with cdc25C phosphatase. Yu et al. (109) demonstrated that UCN-01 mediated G2 checkpoint abrogation requires functional CDK1 and identified that UCN-01 action results both in inhibition of *wee*1 kinase and activation of cdc25C phosphatase. Graves et al. (110) extended these studies by providing evidence to support the idea that cdc25C phosphatase activation resulted from direct, potent inhibition of *chk*1, whose physiologic function is to regulate cdc25C Serine 216 phosphorylation and consequent suitability for 14-3-3 sequestration in the cytoplasm. At the same time, Sarkaria et al. (111) and Busby et al. (111a) also demonstrated potent inhibition of *chk*1 by UCN-01.

Following completion of safety testing in animals, and with demonstration of antitumor activity in its own right reviewed elsewhere (112), UCN-01 entered into initial human phase I clinical trials, administered as a 72-h continuous intravenous infusion every two weeks (113, 114). Surprisingly, and in contrast to studies in animals, UCN-01 displayed avid binding to human plasma proteins, apparently to the α1-acid glycoprotein (AAG). This resulted in a very long half-life and required adjustment of the administration schedule so that the drug was administered once per month, with second and subsequent courses of therapy consisting of 50% of the dose given during the first course. On this schedule, a maximal tolerated and recommended phase II dose of 42.5 mg/m^2/day for 3 days was elucidated. DLTs observed at higher doses included hyperglycemia, acidosis, and pulmonary adverse events, without neutropenia or thrombocytopenia. One partial response occurred in a patient with melanoma, and a protracted (>4 year) period of stabilization of minimal residual disease was observed in a patient with *alk*(+) anaplastic large cell lymphoma. Significantly, despite protein binding, salivary levels of drug (saliva contains low concentrations of AAG) consistent with ability to modulate *chk*1 were achieved.

BRYOSTATIN

The bryostatins represent a large family of secondary metabolites produced in extremely small amounts by the marine invertebrate, *Bugula neritina* of the phylum Ectoprocta (115). The various bryostatins are distinguished by varying side chains off the macrocyclic lactone ring structure. Despite this close structural relationship, these nontumor-promoting PKC activators have different biologic activities and spectrum of toxicity (116, 117). Bryostatin 1 (Bryo 1) is the prototype of this 17-member family and the most extensively studied in humans. Initial isolation of Bryo 1 was based on its antineoplastic activity against the murine P388 lymphocytic

leukemia. Bryo 1 is a potent and rapid activator of PKC; however, unlike other PKC activators, including phorbol myristate acetate (PMA), Bryo 1 lacks tumor-promoting capabilities.

The first two published phase I trials evaluated Bryo 1 administered as a 1 h intravenous infusion (118, 119). Bryo 1 was given every 2 weeks for a maximum of 3 cycles; doses were escalated in steps from 5 to 65 μg/m^2 in successive groups of patients. The DLT was myalgia, occurring approximately 48 h after treatment and lasting up to several weeks at the highest dose levels (65 μg/m^2/dose). The MTD was 50 μg/m^2, and the recommended dose for phase II trials was 35 to 50 μg/m^2 every two weeks. The other trial (119) evaluated three different regimens of Bryo 1 and also concluded that the DLT was myalgia, and the recommended dose for phase II is 25 μg/m^2 weekly for 3 weeks, repeated every 4 weeks. In this phase I study, partial responses were observed in two patients with malignant melanoma, which lasted 6 months and 10 months. Plasma levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) increased 2 h and 24 h after treatment, respectively, and were dose related.

RAPAMYCIN CONGENERS

Rapamycin (Sirolimus, Rapamune) is a macrolide fungicide that binds intracellularly to the immunophilin FKBP12, and the resultant complex inhibits the activity of a 290-kDa kinase known as mammalian target of rapamycin (mTOR). Rapamycin is isolated from the bacteria *Streptomyces hygroscopicus* and is found to have potent antimicrobial and immunosuppressive properties (120). Sirolimus was approved by the FDA for prevention of allograft rejection after organ transplantation (121). Further studies with rapamycin revealed significant antitumor activity (122). This is understandable given the importance of mTOR in mitogenic cell signaling. mTOR is a kinase member of PI3K-related kinase family that is activated in response to growth signaling through the PI3K/Akt pathway. Activation of mTOR results in increased translation of several critical cell-cycle regulatory mRNAs through two downstream effector kinases, p70S6K and 4E-BP1/PHAS (123, 124). Rapamycin causes G_1 cell-cycle arrest by increasing the turnover of cyclin D1 (125), preventing upregulation of cyclins D3 and E (126), upregulating p27^{KIP1}, and inhibiting cyclin A-dependent kinase activity (127). Blockage of mTOR function results in inhibition of PI3K/Akt-mediated proliferative signals and cell-cycle arrest. Mutation of PTEN has also been implicated in chemoresistance of these tumors. Rapamycin treatment of PTEN-deficient PC-3 prostate cancer cells reverses this resistance to doxorubicin (128). Several analogs of rapamycin have been selected for further development as anticancer agents.

CCI-779, an ester of rapamycin, has significant antiproliferative effect and favorable toxicology profile and is being studied in several phase I clinical trials in human cancer (129, 130). In these phase I studies, CCI-779 was administered on a weekly or daily schedule for 5 days every two weeks. Toxicities observed

with CCI-779 treatment included hypocalcemia, neutropenia, thrombocytopenia, mucositis, hypertriglyceridemia, rash, reversible decrease in testosterone levels in men, and allergic reactions. A median half-life of 17.3 h was documented (130). Several partial responses have been documented in renal cell carcinoma, NSCLC, neuroendocrine tumors, and breast cancer, in addition to minor responses or stable disease in several tumor types (129, 130). RAD001, an orally bioavailable hydroxyethyl ether derivative of rapamycin, also has potent activity against various animal xenograft models of human tumors; an antiangiogenic effect may account in part for its antiproliferative properties (131).

MEK Inhibitor PD 184352

The stimulation of Ras-mediated signal pathways results in a cascade of downstream kinase activation including Raf, which phosphorylates two distinct serine residues on the dual-specificity kinase MEK (MAP kinase kinase) (132). MEK, in turn, activates and exclusively phosphorylates two subsequent kinases, ERK1 and ERK2 (MAPK), on specific tyrosine and threonine residues within each kinase. These kinases phosphorylate a variety of substrates including transcription factors critical to cell proliferation and tumor invasion (e.g., 133). Constitutive activation of MEK leads to cellular transformation and may be involved in apoptosis, differentiation, and angiogenesis. The substrate selectivity exhibited by MEK distinguishes the kinase as a potentially important pharmacologic target.

Screening for small molecule MEK inhibitors was implemented with an in vitro assay in which MEK activation of MAPK resulted in a quantifiable phosphorylation of myelin basic protein (134). This assay identified PD184352 as a MEK inhibitor with an IC_{50} of 17 nM. Biochemical analyses supported inhibition by an allosteric mechanism without interference with the ATP binding site or the MAPK site on MEK. A dose-dependent G_1 cell-cycle arrest and reversal of transformed morphology (from rounded to flattened appearances) were subsequently demonstrated with PD184352 exposure (135). Interestingly, in cytotoxicity studies, correlation between sensitivity to PD184352 and increased activated MAPK levels was observed in some cells—in particular, colon cancer cells. Higher levels of MAPK activation were observed in colon tumor tissue versus normal mucosa as this event occurs late in colon carcinogenesis (135). In mice with colon 26 xenograft model treated with PD184352, excision and assay of tumor cells revealed diminished phospho-MAPK levels. After drug withdrawal, a return to baseline levels was observed reflecting the cytostatic nature of the inhibition. The pharmacodynamic measurement of activated MAPK in tumor tissue may be used as a biological marker of drug activity as antibodies specific for phosphorylated MAPK are available.

Miltefosine and Perifosine

Certain alkylphospholipids (ALP), for example, Rac-1-O-octadecyl-2-O-methyl-glycero-3-phosphocholine (ET-18-OCH_3, edelfosine) when given to mice prior

to transplantation of Ehrlich ascites carcinoma cells, effectively prevent growth of this tumor (136). Enhancement of immune defense against tumor cells was initially considered a plausible mechanism and has been demonstrated on multiple occasions by a number of ALP analogs. For example, maturation and activation of macrophages have been seen in in vitro and in vivo experiments (137).

Edelfosine is also able to induce apoptosis in HL60 leukemic cells, even in low concentrations and after short incubation times. In U937 leukemic cells, the compound induced apoptosis rapidly, whereas in epithelial HeLa tumor cells this induction required prolonged times of treatment (138). Octadecyl-(1,1-dimethyl-4-piperidino-4-yl) phosphate, perifosine, induced intranucleosomal DNA fragmentation in human squamous cell carcinoma KB cells and induced activation of caspase 3–like protease; pretreatment with the caspase inhibitor Z-Asp-CH2-DCB inhibited perifosine-induced apoptosis and activation of caspase 3–like protease. However, the mechanism of activation of caspase 3–like protease is unclear; it is speculated to occur through the interference with molecules working in intracellular signal transduction pathways to activate caspases and cause apoptosis.

Initial experiments suggested that protein kinase C (PKC) was the target for ALP analogs (139). However, some ALP analogs that are equally potent antiproliferative agents to miltifosine do not affect PKC in cell-free extracts. Thus, a direct inhibition of PKC does not appear as a prerequisite for antitumor activity of these agents. However, all ALP analogs studied so far cause an indirect inhibition of PKC, most likely as a result of the reduced formation of diacylglycerol through inhibition of phospholipase C (139, 140). Additional antiproliferative mechanisms could involve altered growth factor receptor function, as well as recent evidence of p21 induction by an as yet undefined pathway (142) irrespective of p53 function.

Several phase I and phase II studies were initiated using oral formulations of miltefosine, but gastrointestinal intolerance was observed. Because of the hemolytic tendency of alkylphosphocholine analogs, intravenous application of miltefosine was not possible. In view of the high in vitro cytotoxic activity of miltefosine and its low toxicity against normal cells, development as a cutaneous preparation was pursued. Eight phase I–II studies, consisting of 443 patients using topically applied miltefosine 2%–8% for skin metastases in patients with breast cancer, showed a median response rate of 38% (range 12%–50%) (143–145). The response rate was found to be higher in patients with multiple small nodular or purely superficial infiltrations (rate: 41%) than in patients with predominantly large tumorous nodes that infiltrate the skin (rate: 15%). Evidence from the trials led to the approval of miltefosine, licensed as Miltex©, in Germany for the treatment of cutaneous breast cancer and cutaneous lymphomas.

The heterocyclic alkylphosphocholine derivative octadecyl-(1,1-dimethyl-piperidino-4-yl) phosphate (D-21266; perifosine) was developed and selected for improved gastrointestinal tolerability in animal experiments. A number of phase I studies are presently ongoing in Europe and the United States; early evidence

points to better tolerability and less gastrointestinal toxicity when given as a loading dose followed by a low maintenance dose (146).

Proteosome Inhibitor PS-341

The proteasome, a multicatalytic protease responsible for degradation of most proteins with the cell, has emerged as a new target for anticancer drug development. The 20S proteasome is involved in the degradation of several cell-cycle regulatory proteins such as cyclins (A, B, D, E), cyclin-dependent kinase inhibitors ($p21^{WAF1/CIP1}$ and p27), oncogenes (c-fos/c-jun, c-myc, N-myc), and p53 and regulatory proteins (IκB, p130) (147). Inhibition of the 20S proteasome pathway, therefore, aims at altering the cell cycle to promote apoptosis (148). Although the proteasome is present in all cells, transformed and dividing cells are most sensitive to its inhibition (149).

PS-341 is the first proteasome inhibitor to enter human trials. It is a boronic acid dipeptide that specifically inhibits the 20S proteasome presumably through the stability of a boron-threonine bond that forms at the active site of the proteasome. It was found to have substantial cytotoxicity against a wide range of human tumor cells in the NCI 60 cell line anticancer drug screen (150). The antitumor activity of a series of PS-341 analogs positively correlates with their respective ability to cause proteasome inhibition. PS-341 causes accumulation of cyclin A, cyclin B, $p21^{WAF1/CIP1}$, and wild-type p53 and arrests the cells at the S and G_2/M phases followed by nuclear fragmentation and apoptosis. PS-341 significantly inhibited NF-κB DNA binding and functional reporter activity (151).

More than 90% of PS-341 is rapidly removed from the vascular compartment within 15 min of IV administration (152), and therefore the degree of 20S proteasome inhibition in whole blood was adopted as a surrogate marker for the drug activity (153). Animal studies demonstrated significant toxicities when the proteasome is greater than 80% inhibited. Proteasome activity returned to baseline level within 48 to 72 h of treatment.

Several phase I studies evaluated various schedules of PS-341 administration. At the MTD recommended for phase II studies (1.25 mg/m^2–1.3 mg/m^2), a 65%–72% inhibition of 20S proteasome was achieved (154, 155). An average 54% inhibition of proteasome was achieved in patients' tumors (156). In these phase I studies several patients achieved partial responses and disease stabilization including a bronchoalveolar NSCLC, melanoma, sarcoma, lung adenocarcinoma, and malignant fibrous histiocytoma. The major toxicities observed with PS-341 treatments were painful neuropathy, diarrhea, fatigue, orthostatic hypotension, nausea, vomiting, fever, and thrombocytopenia. Patients usually had more toxicity with the second cycle of treatment. Currently several phase II clinical trials are evaluating PS-341 as a single agent in hematologic malignancies, neuroendocrine, renal cell, melanoma, breast, brain, pediatric tumors, and several other solid tumors. Significant antitumor effects were documented in a phase II study of PS-341 in refractory multiple myeloma (157).

FARNESYL TRANSFERASE INHIBITORS

Ras genes are mutated in 30% of all human cancers with K-Ras being the most common. This family of genes encodes GTP binding proteins important in malignant transformation, cell growth, and intracellular signal transduction. Normal ras binds GTP and in the GTP-bound state interacts with numerous effectors including the *raf* proto-oncogene kinase and phosphatidyl-inositol 3-kinase. Its intrinsic GTPase activity terminates the signal. Three isoforms, Harvey(Ha), Kirsten(K), and N-isoforms have been described, with mutation of the GTPase of the K isoform resulting in a persisting signaling capacity in approximately 20% of human epithelial tumors. N-ras is mutated in a smaller proportion of malignancies, predominantly leukemias. Ras function requires lipophilic anchorage to the cell membrane by lipid prenylation. This requires posttranslational modification or covalent thioether bond formation between a farnesyl group (C15) and a cysteine residue at the ras carboxy terminus. The cysteine lies within a four-amino-acid consensus sequence or CAAX motif (where C = cysteine, A = any aliphatic amino acid, X = serine or methionine), and this prenylation reaction is mediated by the enzyme farnesyl protein transferase (FT). Another important enzyme in protein prenylation is geranylgeranyl transferase (GTT), which can catalyze addition of geranylgeranyl (C20) group to the CAAX motif. The two enzymes exhibit substrate selectivity with FT preferring CAAX-containing proteins ending with Ser, Met, or Gln, whereas GTT targets proteins ending with Leu. The lack of substrate specificity is evident as compensatory geranylgeranyl prenylation of K-Ras and N-Ras by GTT occurs after FT inhibition. This "GTT shunt pathway" maintains K-Ras in an active prenylated, membrane-bound form and explains in part the requirements for higher farnasyl transferase inhibitor (FTI) dose or cotreatment with a GTT inhibitor for significant growth inhibition in K-Ras models (158). Several classes of FTIs have been developed in an initial effort to define inhibitors of Ras function and, in general, compete with the enzyme substrates, the CAAX tetrapeptide, and farnesyl pyrophosphate (FFP). The CAAX competitors are generally peptidomimetic agents that mimic the carboxy terminal portion of the Ras protein. "Bisubstrate" inhibitors have also been evaluated (159).

L-778,123

L-778,123 is a competitive peptidomimetic CAAX analog with a Ki of 1.6 nM for H-ras and 0.3 nM for N-Ras. As with other FTIs, it is capable of reversing anchorage-independent growth of ras-transformed Rat1 fibroblasts. Activity against various cell lines has been reported including K-Ras harboring cells with an IC_{50} of 2 to 5 μM. In available preclinical studies, toxicities included myelosuppression and an increase in the QT interval (QTc).

The initial experience in humans was reported in a study of 25 patients. Patients with history of significant cardiac dysrhythmias or retinal disease were excluded. Concomitant use of medications capable of causing dysrhythmias or CYP3A

induction was not allowed. The drug was administered as a 24-h continuous infusion daily for 7 days every 3 weeks. DLT was observed at 1120 mg/m^2/day with one patient experiencing a 30% QTc prolongation whereas another had grade 4 thrombocytopenia. Grade 3 fatigue was noted in two patients treated at this level. Eight patients treated at 560 mg/m^2/day experienced no DLTs, and this dose level was defined as the phase II recommended dose. Pharmacokinetic studies revealed that at this dose a biologically relevant steady state concentration of 8 μM was attained and exceeded the IC_{50} values for the human cell lines evaluated in cell culture studies. No objective antitumor effects were observed, but evidence of an effect on the prenylation status of a marker protein (HDJ2) was obtained. At the recommended phase II 520 mg/m^2/day dose, the percentage of unprenylated HDJ2 increased from 1% to 30% by day 4 and remained at that level through day 8. One week after the infusion was stopped, pretreatment levels of unprenylated HDJ2 were restored (160, 161).

BMS-214662

BMS-214662 is an imidazole-containing tetrahydrobenzodiazepine, which lacks a thiol moiety or a peptide backbone (162). It is a competitive FT inhibitor and was identified by in vitro screening assay using purified recombinant FT. The degree of enzyme inhibition depended on whether H-Ras or K-Ras was used as the farnesyl substrate as fivefold-less potent inhibition was observed with H-Ras versus K-Ras (IC_{50} 1.3 nM and 8.4 nM, respectively) (163). In contrast, inhibition of GTT required 1000-fold-higher concentrations (IC_{50} H-Ras 1.3 uM and K-Ras 2.3 μM). BMS-214662 also blocks activated ras-mediated events in cell-based studies leading to morphologic reversion to flat monolayer cells and inhibition of anchorage-independent growth. Despite inhibiting soft agarose growth of both H-Ras and K-Ras transformed cells (IC_{50} 0.0025 uM versus 0.3 μM), the compound did not induce the reversion of K-Ras-dependent cells. In a panel of murine and human cell lines from a variety of tumor types, BMS-214662 exhibited extensive activity with a mean IC_{50} of 0.20 μM. Importantly, a lack of correlation between Ras mutational status and cytotoxicity was noted with two of four sensitive cell lines (A431 and OVCAR-3) not expressing Ras mutations. BMS-214662 exhibits nonspecific cytotoxicity at doses higher than 2 μM (164).

The initial clinical reports with BMS-214662 have used various schedules of administration. The drug was initially given intravenously every three weeks and as a single oral dose during cycle 2 (165). Thirty-eight patients were evaluated, and a 225 mg/m^2 intravenous dose and 168 mg/m^2 oral dose were reached. Elevation of transaminases, nausea, vomiting, and diarrhea were observed requiring expansion of the 225 mg/m^2 dose level. Linear pharmacokinetics with rapid systemic clearance, a half-life of 2 to 4 h and 36% oral bioavailability, were reported (166). FT inhibition in peripheral blood mononuclear cells (PBMCs) was measured 1 and 6 h post treatment and returned to baseline by 24 h. At the 225 mg/m^2 dose level, greater than 90% FT inhibition was observed. The only objective response was in a

NSCLC patient with a 40% reduction in undefined measurable disease. A weekly schedule has also been evaluated and an MTD of 245 mg/m^2 defined (167). Thirty patients were treated for a median of 6 weeks, and grade 3 DLTs included vomiting, diarrhea, dehydration, and transient elevations in creatinine and transaminases. As with the previous schedule, 80% FT inhibition in PBMCs was noted at the higher dose levels (168). In addition, FT inhibition by a mean of 80% was observed in 14 posttreatment tumor samples at 2 h and persisted at 30% inhibition at 24 h. In some tumor samples, assessment of apoptosis by a DNA break labeling assay revealed induction of apoptosis after drug exposure, for example, a refractory breast cancer patient with a 5-month minor response. Two other schedules (169, 170), weekly for 4 weeks followed by a 2-week rest and oral twice a day for 14 days every 21 days, have been evaluated with no objective responses reported.

SCH66336

SCH66336 is a novel oral agent derived from a class of nonpeptide, nonthiol-containing, CAAX mimetic FTIs (171). The pyridobenzocycloheptene class of competitive inhibitors contains a common tricyclic nucleus, and SCH66336 is an 11-piperidinyl trihalogenated compound. The drug inhibits in vitro FT activity with an IC_{50} of 1.9 nM for H-ras, 2.8 nM for N-ras, and 5.2 nM for K-ras. Inhibition of cells with activated ras and anchorage-independent growth was noted with IC_{50} 75 nM in H-ras versus 400 nM with K-ras-driven cells (172). The compound also exhibited an IC_{50} of less than 500 nM against a panel of human ras-activated tumor cell lines. The observed growth inhibition of tumor cells in soft agar and in xenografts was independent of ras mutational status because even wild-type ras cells were sensitive (173).

The phase I experience with SCH66336 involved 20 patients using a twice a day schedule over 7 days every 21 days. A recommended phase II dose of 350 mg was determined, with DLT consisting of severe fatigue. Eight patients had stable disease, and treatment for up to 10 cycles was possible in a few patients. Antitumor activity was reported in one patient with advanced NSCLC who had a greater than 50% reduction in an adrenal metastasis and received treatment for 14 months (174).

R115777

R115777 is a substituted quinolone and competitive inhibitor of the CAAX peptide binding site of FT (175). The compound inhibits in vitro K-Ras farnesylation (IC_{50} 7.9 nM) and exerts antiproliferative effects in cell lines such as H-Ras-transformed fibroblasts (IC_{50} 1.7 nM) and K-Ras-driven colon and pancreatic cells lines (at roughly IC_{50} 20 nM) (176). Activity is also reported in cell lines with wild-type ras. In mouse xenograft studies with these sets of cells, growth inhibition is also observed, which further confirms the antitumor activity of R115777. The initial clinical experience with R115777 in 27 patients was reported by Zujewski et al.

(177) using a twice a day schedule for 5 days by oral administration. Patients were retreated after at least a 7-day rest period, intrapatient dose escalation was allowed in this trial, and a DLT (grade 3 peripheral neuropathy in a patient with a history of taxol-induced neuropathy) was observed in 1 of 6 patients at the level of 1300 mg twice a day. A true MTD was not determined, and the recommended phase II dose was 500 mg twice a day for 5 days every 2 weeks. Other observed toxicities included clinically significant fatigue and transient serum creatinine elevations reflecting acute tubular injury. Minimal ophthalmologic and hematologic toxicity was observed, although antiemetic therapy was necessary in 75% of patients. Of the 27 patients treated, 8 had stable disease after 3 treatment cycles, and 4 patients continued treatment with the longest reaching 5 months. A patient with metastatic colon cancer had symptomatic improvement and a 50% reduction in carcinoembryonic antigen (CEA) levels.

Pharmacokinetic analysis revealed R115777 to be rapidly absorbed with peak plasma concentrations being reached by 3 to 4 h and steady state levels after 3 days. The degree of bioavailability and the steady state levels attained were sufficient pharmacologically for antitumor effects as predicted by preclinical experiments. The drug exhibits biphasic elimination with half-lives of 4 h and 16 h.

In another phase I study with R115777 given orally twice a day for 21 consecutive days every 28 days, an estimated MTD of 240 mg/m^2 was established (178). DLTs included grade 3/4 neutropenia and thrombocytopenia in addition to grade 3 fatigue, confusion, and bilirubin elevation. Two patients with stable disease exceeding 6 months were reported. Another chronic dosing trial recommended a dose of 300 mg twice a day with similar toxicities as reported above. A partial response in a NSCLC patient lasting 4 months was reported (179). Finally, 3 advanced breast cancer patients treated continuously at 300 mg twice a day attained confirmed partial responses whereas another 9 patients had stable disease of at least 3 months duration (180).

A most interesting outcome was obtained in patients with myelodysplastic syndrome or relapsed or poor prognosis leukemias, where a phase I dose escalation study revealed DLT at 1200 mg twice per day, consisting of neurotoxicity, with non-DLTs including renal insufficiency and myelosuppression. There was clear evidence of downmodulation of erk kinase activity, along with the farnesylation status of lamin A and HDJ-2. Clinical responses occurred in 29% of 34 evaluable patients, including 2 complete responses (181). Though there were no mutations in N-Ras detected in this patient population, this study did suggest that in addition to clinical activity there was some evidence of downmodulation of signaling as well as farnesylation-directed activities.

SUMMARY AND CONCLUSION

The interim results presented in this review convey both the promise and the difficulties encountered in developing signal transduction inhibitors for cancer treatment. These molecules represent a marked departure from prior therapeutic

approaches based on cytotoxic activity in tumor models without reference to underlying mechanism. The fact that responses have been seen at all, on one hand, reaffirms the relevance of tumor cell biology in charting the further course of cancer developmental therapeutics. On the other hand, the initial experiences raise a number of issues that should be considered as the field moves forward.

First, with a number of agents the actual magnitude of conventionally described responses, though real, is lower than would usually be associated with clinical value. The implication of this finding is that more accurate means of diagnosing the dependence of a tumor on a particular signaling pathway or target must be defined. Microarray and proteomic approaches offer this promise, but these must be integrated into the clinical trials process. Alternatively, intelligent ways of efficiently combining inhibitors of multiple pathways must be discerned and implemented. For example, a logical evolution dictated by the biology of known pathways would consider combinations of signal transduction inhibitors in blast phase CML, which in some forms is only transiently responsive to STI-571. Logical combinations might, for example, include a *bcr-abl*-directed therapy plus PI-3 kinase/*akt*-pathway-directed inhibitors.

A second issue concerns the place of target assessment, or biomarkers, in early clinical trials. Some agents have entered initial clinical trials with extensive efforts to document target-based effects in conjunction with pharmacology and clinical toxicity evaluations; others have not, and in those latter instances one is left at the end of the phase I with little sense that, lacking clear evidence of clinical response, one can confidently move forward to later phase development. Likewise, intelligent design of combinations with standard cytotoxic agents also remains a challenge. Preclinical models of synergistic effect with signaling agents often proceed from empiricism without understanding a mechanistic basis that would guide clinical implementation. These circumstances call for renewed efforts to define robust assays of target effect in the preclinical phase of a drug's development that can be translated to the clinical arena. An additional issue is how one might integrate evidence of effect on the target into dose definition or regimen design. In this regard, studies with signaling molecules might profitably seek to define the biologically effective dose rather than a maximum tolerated dose.

A final point of concern is that many of the agents in both model systems and in initial clinical observations in patients might be associated with protracted periods of cytostasis or disease stability, rather than overt cytotoxic effects that might be attributed to the initiation of an apoptotic response. Though such stable disease can be readily observed in animal tumor models, it is uncertain whether it can be meaningfully and efficiently captured in clinical populations of patients with advanced malignancy. It is important to develop decision-making steps that would aid in the use of the drugs in patients with earlier stage or indeed adjuvant or prevention strategies, and to develop clinical study algorithms that address this biologically relevant possibility in a way that does not compromise safety.

Despite these issues, it is clear that this generation of molecules has marked a turning point in cancer therapeutics and defined a path for future progress. We look forward to the day when cytotoxic strategies will be employed circumspectly and in

coordination with rationally based signaling strategies that address the molecular disorder of cancer as a basis for benefit to patients.

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