Selected Novel Anticancer Treatments Targeting Cell Signaling Proteins

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ABSTRACT

Empirical approaches to discovery of anticancer drugs and cancer treatment have made limited progress in the cure of cancer in the last several decades. Recent advances in technology and expanded knowledge of the molecular basis of tumorigenesis and metastasis have provided unique opportunities to design novel compounds that rationally target the abnormal molecular and biochemical signals leading to cancer. Several such novel agents have completed advanced stages in clinical development. The excellent clinical results achieved by some of these compounds are creating new paradigms in management of patients with neoplastic diseases. Clinical development of these agents also raises challenges to the traditional methods of drug evaluation and measurement of efficacy. The Oncologist 2001;6:517-537

INTRODUCTION

Recent advances in molecular medicine have identified several molecular targets within cancer cell cycle regulation as a basis for anticancer treatments. Dysregulation of cell cycle control is a hallmark of human cancers, causing lack of differentiation and aberrant growth [1-3]. The cell cycle is a very complex and tightly regulated process that can result in cell division, differentiation, or growth, or contribute to programmed cell death through apoptosis. Proper regulation of this process involves environmental signals ultimately leading to the activation of cyclin-dependent serine/threonine kinases (CDK), regulated by activating cyclins and cyclin-dependent kinase inhibitors (CKI). Key steps regulated by CDKs are the DNA integrity control checkpoints, mediated by the retinoblastoma susceptibility tumor suppressor gene product (pRB), p53 tumor suppressor gene and E2F transcriptional factors family [4, 5]. To date, nine CDKs (cdk1-9) and at least 15 preferentially binding cyclins have been identified [6-8]. CDKs are typically small proteins of 300 amino acids in length and 33-40 kDa molecular weight. CDKs are activated through a 1:1 noncovalent binding with specific cyclins and trigger and coordinate the transition between the different phases of the cell cycle. Cyclin/CDK complex formation is usually transient and is affected by ubiquitin-mediated degradation of cyclins as a basis for regulating CDK activity, with rapid degradation of cyclins causing loss of CDK function. CDKs are also negatively regulated by small inhibitory molecules called endogenous CKIs. Two families of these inhibitors have been identified to date. The p21 gene family that includes p21^WAF1/CIP1/SD1, p27^KIP1, and p57, and the p16 gene family that includes p16^INK4A, p15^INK4B, p18^INK4C, and p19^INK4D [9, 10]. Members of p21 gene family interact with both cyclins and CDK subunits while members of p16 gene family interact only with CDKs [7].

Cell Cycle

Figure 1 is an overview of the cell cycle. The regulation of cell growth and division generally occurs in a precise and predictable manner dictated by tightly controlled successive waves of CDK/cyclin accumulation and rapid degradation of cyclins. The nondividing quiescent G0 cell enters the G1 phase in response to external mitogenic stimuli such as growth factor action or internal demands. Cyclin D is then expressed and binds to and activates CDK4 and/or CDK6 depending on the particular cell type. The cyclin-D/CDK4
is committed to cell cycle progression and thus becomes independent of growth factors. Cyclins A and E bind and activate CDK2 and this allows the cell to traverse the S phase. Cyclin-A/CDK1 then facilitates the transition from S to G2 phase. Cyclin B/CDK1 complex accumulates in late G2 phase, and is required for progression of the cell through the M phase [13]. Following completion of anaphase, cyclin B is degraded, thus returning the cell to a G1 state, which, in the presence of maintained growth factor stimulation, proceeds to successive rounds of cell division. The integrity of the synthesized DNA is examined and the repair of damaged DNA or apoptosis occurs at G1 and G2 checkpoints, an example of the checkpoint control operating to assure the fidelity of the replicated genome [14, 15]. CDKs play an important role in regulating these checkpoints. For example in response to various stress signals, p53, a transcriptional factor, is activated and causes transcriptional induction of p21 and establishment of the G1 checkpoint [10]. The length of the individual phases of the cell cycle can vary depending on cell type and particular conditions. The CDK activities during the cell cycle are controlled at multiple levels including association with activating cyclins, transient expression and rapid degradation of these cyclins, post-translational modifications by kinases and phosphatases, interactions with CKIs, and intracellular translocations [9].

**Cell Cycle Regulation in Cancer**

Oncogenic alterations of cyclins, CDKs, CKIs, and other components of the pRB pathway have been reported in more than 90% of human neoplasms and are summarized in Table 1 [1, 2, 16-41]. G1 phase cyclin and CDK abnormalities are the most frequent. One or more mechanisms may be responsible for these alterations including chromosomal translocation, gene overexpression, deletion, insertion, point mutation, missense mutation, frame shift mutation, splicing, or methylation. These alterations usually result in tumorigenesis by oncogene activation and silencing of tumor suppressor genes. CDK dysregulation through its inappropriate activation is essential in maintaining malignant transformation. The cyclin D1 gene is induced by various oncogenic signals including activating mutation ras, src, and mitogen-activated protein kinases (MAPK) [42, 43]. Cyclin D1 promotes transformation and malignancy [44, 45], and in transgenic mice it facilitates development of breast adenocarcinoma [46] and lymphoma [47]. Cyclin D is also associated with higher incidence of recurrence in head and neck cancers [48], and tumors that overexpress cyclin D1 generally have a poor prognosis [48, 49]. Cyclin E dysregulation is associated with hyperproliferation and malignant transformation [24]. Overexpression of cyclin E correlated well with breast tumor aggressiveness and independently

or 6 complexes phosphorylate pRB (Fig. 2). Cyclin E is also expressed and binds to activate CDK2, resulting in phosphorylation of pRB on distinct sites. E2F transcriptional factors (E2F 1-5) then dissociate from the hyperphosphorylated pRB in order to activate S-phase promoting gene transcription including thymidylate synthase, dihydrofolate reductase, DNA polymerase, and others [11, 12]. By this point the cell has passed the “restriction point” beyond which the cell
predicted the risk of distant visceral relapse [50]. Inactivation of the p16 or p21 family of endogenous inhibitors by mutation, deletion, or p53-mediated inactivation might result in aberrant activity of CDKs, and in turn phosphorylation with inactivation of pRB. The loss of p16INK4A, p27KIP1, and p21WAF1 was a predictor of poor outcome in several tumor types [51]. Protein tyrosine kinases (PTK) are an integral part of the cell regulatory mechanisms, acting to propel cells through G1 and past restriction points. Overexpression, amplification, or constitutive activation of several PTKs such as epidermal growth factor receptor tyrosine kinase (EGFR-TK), HER2/neu, c-kit kinase, PDGF, Ax1, src, and others have been associated with inappropriate entry into the cell cycle, cellular proliferation, and neoplastic changes. These changes are mediated through alteration in growth factor signaling pathways, nuclear oncogene activities, and cell cycle control. Certain translocation products, chimeric proteins, reflecting in one molecule sequence or both sides of a DNA translocation breakpoint also promote aberrant entry into the cell cycle. For example bcr-abl with its constitutively active bcr-abl tyrosine kinase is recognized as the sole initial oncogenic event in chronic myelogenous leukemia (CML) that drives the malignant transformation.

### Cell Cycle Modulators in Cancer Treatment

Although the conventional and empirically discovered chemotherapeutic agents have saved thousands of lives over the years, they are very toxic, nonselective in targeting tumors, and have efficacy far less than desired. Recently the emphasis in cancer drug discovery has shifted toward novel compounds that target the molecules resulting from genetic changes associated with the cancerous state. In the last decade extensive research has focused on restoring the control of the cancer cell cycle by targeting the molecular basis of cancer cell dysregulation. This has a potential of defining selective antineoplastic agents. Several potential mechanisms have been proposed including direct catalytic CDK inhibition, downregulation of cyclins, upregulation of endogenous CDK inhibitors, altering the critical activating phosphorylation of CDKs, interruption of cyclin and CDK interaction, altered proteolysis, degradation of cyclins, and specific inhibition of tyrosine kinases that lead to cell cycle activation. Several compounds that modulate the cell cycle through one or more of these mechanisms have been identified (Table 2). The response of tumors to cell cycle modifiers varies from simple cytostasis to cell death depending on the achievable concentrations and the downstream responses to cell cycle arrest. Cytostasis with clinically “stable disease”

### Table 1. Abnormalities of cyclins, cyclin-dependent kinases, and cyclin-dependent kinase inhibitors in various human cancers

<table>
<thead>
<tr>
<th>Cyclin/CDK/CKI</th>
<th>Phase/activity</th>
<th>Tumor types</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclin D1</td>
<td>G₁</td>
<td>Lymphoma, mantle cell lymphoma 95%, breast 35%-81%, esophagus 40%, lung 10%-20%, parathyroid, myeloma, head and neck 40%, sarcoma 33%, hepatocellular 10%, bladder 15%</td>
<td></td>
</tr>
<tr>
<td>Cyclin D2</td>
<td>G₁</td>
<td>Colorectal, testicular, CLL</td>
<td></td>
</tr>
<tr>
<td>Cyclin D3</td>
<td>G₁</td>
<td>Lymphoma 50%, ALL 50%, retinoblastoma</td>
<td></td>
</tr>
<tr>
<td>Cyclin K (D-like)</td>
<td>G₁</td>
<td>Kaposi sarcoma</td>
<td></td>
</tr>
<tr>
<td>Cyclin E</td>
<td>late G₁, early S</td>
<td>Colorectal, breast, prostate, ovarian, gastric, lung, CLL, renal, pancreatic</td>
<td></td>
</tr>
<tr>
<td>Cyclin E2</td>
<td>G₁/S</td>
<td>Breast, small cell lung, cervical</td>
<td></td>
</tr>
<tr>
<td>Cyclin B1</td>
<td>G₂/M</td>
<td>Colorectal, breast</td>
<td></td>
</tr>
<tr>
<td>Cyclin A</td>
<td>mid S/G₂</td>
<td>Hepatocellular, breast</td>
<td></td>
</tr>
<tr>
<td>CDK2</td>
<td>G₁/S</td>
<td>Colorectal</td>
<td></td>
</tr>
<tr>
<td>CDK4</td>
<td>G₁/S</td>
<td>Sarcoma 8%-36%, glioma 10%, melanoma, Colorectal, breast</td>
<td></td>
</tr>
<tr>
<td>CDK6</td>
<td>G₁/S</td>
<td>Glioma</td>
<td></td>
</tr>
<tr>
<td>p16INK4</td>
<td>inhibits CDK4/6</td>
<td>Melanoma, ALL 30%, bladder 30%, head and neck 10%, lung 30%, breast, ovary 20%, esophagus 30%, pancreas 40%, glioma 50%, mesothelioma 50%, nasopharyngeal 40%, sarcoma 10%, biliary tract 50%</td>
<td></td>
</tr>
<tr>
<td>p15INK4</td>
<td>inhibits CDK4/6</td>
<td>Melanoma, T-cell ALL, lung, head and neck</td>
<td></td>
</tr>
<tr>
<td>p21^{WAF1/CIP1}</td>
<td>inhibits all CDKs</td>
<td>Brain, colorectal, leukemia, melanoma</td>
<td></td>
</tr>
<tr>
<td>p27^{kip}</td>
<td>inhibits all CDKs</td>
<td>Breast, colon, melanoma</td>
<td></td>
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</tbody>
</table>
may not be the preferable response to treatment, however, sustained cytostasis might allow the cell to restore functional control and proceed toward apoptosis. Inhibition of CDKs is particularly attractive from the perspective of anticancer drug design given their pivotal role in the cell cycle. High-throughput screening and structure-based drug design have produced several novel compounds that inhibit CDK and PTK activity with reasonable specificity [8, 52-57]. To date all potent CDK and PTK inhibitors compete with ATP binding in the kinase domain, but others may be defined that act indirectly by affecting the presence or activation state of the kinase.

<table>
<thead>
<tr>
<th>Table 2. Current investigational cell cycle modulators and their possible molecular targets</th>
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<tbody>
<tr>
<td><strong>Investigational agent</strong></td>
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<tr>
<td>-----------------------------</td>
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<tr>
<td>I) Direct inhibitors of cyclin-dependent kinases</td>
</tr>
<tr>
<td>Flavopiridol</td>
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<tr>
<td>UCN-01/staurosporine</td>
</tr>
<tr>
<td>Butyrolactone</td>
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<tr>
<td>Purines and purine-like analogs</td>
</tr>
<tr>
<td>Olomucine</td>
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<tr>
<td>Roscovitine</td>
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<tr>
<td>6-dimethylamine purine</td>
</tr>
<tr>
<td>Isopentenyldenine</td>
</tr>
<tr>
<td>CVT-313</td>
</tr>
<tr>
<td>Purvalanol B</td>
</tr>
<tr>
<td>9-hydroxyleptilicetine</td>
</tr>
<tr>
<td>Toyocamycin</td>
</tr>
<tr>
<td>Suramin</td>
</tr>
<tr>
<td>Paullones</td>
</tr>
<tr>
<td>Indigoids (Indirubin)</td>
</tr>
<tr>
<td>p16, p21 peptides</td>
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<tr>
<td>II) Indirect inhibitors of cyclin-dependent kinases</td>
</tr>
<tr>
<td>A) Tyrosine kinase inhibitors</td>
</tr>
<tr>
<td>STI571</td>
</tr>
<tr>
<td>Cetuximab (C-225)</td>
</tr>
<tr>
<td>Iressa (ZD 1839)</td>
</tr>
<tr>
<td>OSI-774</td>
</tr>
<tr>
<td>Rapamycin (CCI-779)</td>
</tr>
<tr>
<td>Geldanamycin derivatives and conjugates</td>
</tr>
<tr>
<td>B) Increasing endogenous CDK inhibition</td>
</tr>
<tr>
<td>Proteasome inhibitor PS-341</td>
</tr>
<tr>
<td>Histone deacetylase inhibitors</td>
</tr>
<tr>
<td>MS-275</td>
</tr>
<tr>
<td>Depsipeptide (FR-901228)</td>
</tr>
<tr>
<td>Trichostatin A</td>
</tr>
<tr>
<td>Butyrate</td>
</tr>
<tr>
<td>C) Altering cyclin levels</td>
</tr>
<tr>
<td>Flavopiridol</td>
</tr>
<tr>
<td>Rapamycin (CCI-779)</td>
</tr>
<tr>
<td>III) Altering checkpoint control</td>
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<tr>
<td>UCN-01</td>
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</table>

**CELL CYCLE MODULATORS IN CLINICAL DEVELOPMENT**

**Flavopiridol**

Flavopiridol (NSC 649890; L86-8275) (Fig. 3A) was identified by National Cancer Institute (NCI) screening as a flavonoid with potent antiproliferative activity against all 60 human cell lines in the NCI anticancer screen panel [58]. Flavopiridol is a semisynthetic flavone derived from rohitukine, an alkaloid isolated from a plant indigenous to India, Dysoxylum binectarieferum. In vitro cell culture and in vivo animal xenograft studies revealed that flavopiridol causes significant inhibition of various tumor cell lines including breast, lung, colon COLO205, prostate DU145, lymphoma HL60 and SUDHL4, and head and neck HN-12 [58-62]. The inhibitory capacity of flavopiridol varies from cytostatic to cytotoxic depending on its concentration, type of tumor cells, and duration of exposure. Certain cell lines including colon cancer (COLO205) and lymphoma cell lines (HL60, SUDHL4) are more sensitive to growth inhibition by flavopiridol with a lower 50% inhibitory concentration (IC50) while other cell lines require longer incubation periods. Intravenous “bolus” administration of flavopiridol was cytotoxic with induction of apoptosis in leukemia and lymphoma models [60] in contrast to the cytostatic effect observed with infusional administration [58]. At higher concentrations, flavopiridol induces death in both cycling and noncycling lung carcinoma cells [63]. Flavopiridol arrests cells at the transition of G2/M phases and from G1 to S phase, and also slows the progression of cell cycle through the S phase [59]. Flavopiridol is competitive with ATP when assayed with purified cyclin/CDK complexes, and it inhibits CDK1, 2, and 4 with similar potency in the 10⁻⁹-10⁻⁸ molar range [64]. Flavopiridol also causes loss of regulatory tyrosine and threonine phosphorylation of CDKs [65, 66]. Other noted effects of flavopiridol include less potent inhibition of protein kinase A (IC50 = 122 µM), protein kinase C (IC50 = 65 µM), and EGFR-TK (IC50 = 21 µM) [8], and flavopiridol decreases the levels of cyclin D1 and D3 in living cells as a result of a decline in cyclin D promoter activity [67]. Flavopiridol has also been shown to have an antiangiogenic effect by downregulating vascular endothelial growth factor (VEGF) mRNA [68] and limiting blood vessel formation in a mouse matrigel model [69]. At high concentrations it can bind to duplex DNA [70], although the physiologic significance of this is unclear. Schedule-dependent synergy was found when flavopiridol followed treatment with paclitaxel, topotecan, doxorubicin, and etoposide. Initial treatment with flavopiridol for 24 hours followed by 5-fluorouracil also has a synergistic effect [71]. The demonstration of synergy with cisplatin remains controversial at present [71, 72].
Pharmacology

The best antitumor activity in animal models was associated with frequent or prolonged exposure to the drug and as such several phase I and II clinical trials have employed a 72-hour continuous infusion schedule every 2 weeks [73-76]. The maximal tolerated dose (MTD) was identified as 50 mg/m²/day for 3 days on this schedule, and the dose-limiting toxicity (DLT) was secretory diarrhea [73]. Prophylactic use of anti-diarrheal regimens, a combination of loperamide and cholestyramine, allowed escalation to a higher MTD of 78 mg/m²/day for 3 days where the DLT was reversible hypotension. The mean steady state concentration (Css) at the recommended phase II dose of 50 mg/m²/day for 3 days is 271 nm/l. The half-life for the terminal phase is 11.6 hours (range 1.3 to 29.1 hours), and the total clearance is 17.23 l/h/m² (range 11.5 to 27.3 l/h/m²). The pharmacology of flavopiridol is predictable in the majority of patients, however, a 30% interpatient variability was observed in this study. Polymorphic metabolism has been suggested for flavopiridol glucuronidation in the liver and poor glucuronidation has been associated with diarrhea [77]. Since activity in hematopoietic and squamous cell cancer preclinical in vivo models appeared to be superior with bolus administration of flavopiridol [60, 78], a trial administering flavopiridol as a 1-hour infusion daily for 5 days every 3 weeks has recently been completed [79]. The recommended phase II dose of

![Chemical structures of selected protein kinase inhibitors currently in clinical development.](image-url)
37.5 mg/m²/day for 5 consecutive days was identified. At this dose level an area under the concentration curve (AUC) of 25.8 ± 0.9 and a maximum concentration (Cₘₐₓ) of 1.622 ± 0.4 μM were achieved.

**Clinical Response**

The NCI phase I study of 72-hour infusional flavopiridol [73] treated 76 patients and documented one partial response (≥50% shrinkage) in renal cancer, three minor responses (<50% shrinkage) in lymphoma, colon, and renal cancers, and several episodes of stable disease for ≥6 months in prostate, adenocystic, and renal cancers. Five patients were able to receive flavopiridol for more than a year and one patient for more than 2 years. Tumor site inflammation and necrosis have been reported with flavopiridol treatment [73, 75]. One patient with gastric carcinoma achieved complete remission in a phase I study [80]. Tested outside the phase I trial context, however, 72-hour infusional flavopiridol as a single agent had no significant objective response in subsequent phase II studies in gastric [75], non-small cell lung (NSCLC) [76], colorectal [81], or renal cell cancers [74]. Stable disease for more than 6 months has been documented with bolus administration of flavopiridol in colon cancer, melanoma, NSCLC, and lymphoma [79]. The combination of flavopiridol with paclitaxel and cisplatin resulted in clinical responses in patients with esophageal and lung cancer [82]. Some of those esophageal cancer patients had failed treatment with paclitaxel or paclitaxel and cisplatin combination before responding to flavopiridol plus the same cytotoxic agent. This raises the possibility that modulation of standard agent activity may be an equally interesting strategy to pursue.

**Toxicity**

In several clinical trials the DLT of flavopiridol at 50 mg/m²/day was identified as secretory diarrhea with no evidence of mucosal damage [73, 74]. This was attributed to a local effect of flavopiridol and its glucuronidated metabolites in the intestine causing chloride ion secretion [77, 83, 84]. Diarrhea was treatable and preventable with loperamide and cholestyramine, however, in some patients this approach was unsuccessful and required dose de-escalation or stopping the drug. A unique constellation of dose-related pro-inflammatory toxicities comprised of fever, malaise, myalgia, arthralgias, and tumor pain accompanied by an increase in serum levels of acute phase reactants such as C-reactive protein, haptoglobin, and fibrinogen, and a decrease in albumin was experienced by almost all patients in most studies [73-75]. The near universal chronic fatigue was debilitating in some patients. Recent studies have shown a dose-dependent parallel increase in interleukin-6 levels following flavopiridol infusion [Senderowicz, unpublished data].

Flavopiridol treatment has also been associated with variable incidences of thrombosis in different clinical trials. This occurrence varied by tumor type from none in chronic lymphocytic leukemia (CLL) and lymphomas to 26% in renal cell cancer [74] and 33% in gastric cancer [75]. Although the majority of these thrombotic events were central venous catheter-related, several cases of myocardial ischemia, transient ischemic attacks, strokes, and pulmonary embolisms have been reported [73, 74]. Other toxicities observed with flavopiridol treatment included nausea, vomiting, reversible hypotension that constituted a DLT at the higher dose levels, reversible lymphocytopenia, rash, isolated hyperbilirubinemia, and hyperglycemia more pronounced in diabetic patients [73-75]. No significant myelosuppression was seen, however, mild to moderate decreases in hemoglobin and platelets were seen in some studies [74, 75]. A protocol employing administration of flavopiridol as a 1-hour bolus has a somewhat different toxicity profile such as dose-limiting nausea, vomiting, and neutropenia [79]. Grade 3 and 4 neutropenia have been reported in patients who received the combination of flavopiridol, paclitaxel, and cisplatin [82].

**Current Clinical Trials**

Several problems have been identified with flavopiridol as an anticancer treatment including its relatively nonselective action (pan CDK inhibitor) and existence of metabolic pathways that contribute to its toxicity profile, and thus far low antitumor activity as a single agent. The significantly increased apoptosis and synergistic effect when flavopiridol is combined with other cytotoxic drugs is promising as a basis for combination therapy. Evidence of this was clear from phase I combination studies with paclitaxel and cisplatin [82] and also from the curative responses seen in colon cancer xenografts when flavopiridol was combined with CPT-11 [85]. At present several clinical trials using flavopiridol as a single agent or in combination are under way in CLL, breast cancer, NSCLC, non-Hodgkin’s lymphoma, colon, prostate, and other solid tumors [86]. Different administration schedules in relation to other agents are being explored to identify the optimal antitumor combinations.

**UCN-01**

The parent compound of UCN-01, staurosporine, was originally isolated from *Streptomyces* species and found to be a nonspecific inhibitor of many kinases causing cell cycle arrest in G₁ and G₂ phases in different cell types. It has significant toxicities, which precluded its clinical development. UCN-01, 17-hydroxystaurosporine (Fig. 3B) is a naturally occurring derivative of staurosporine. UCN-01 is relatively more selective for PKC (IC₅₀ = 30 nM) [87], although many kinases are also affected. It possesses sig-
significant antiproliferative activity in several human tumor cell lines [88, 89] and a tolerable toxicity profile. UCN-01 has demonstrated significant cytotoxic effects against human xenografts in vivo [90].

The effects of UCN-01 on cell growth are complex. In Rb-competent cells, the drug causes arrest in G1 with loss of CDK activity [89]. In cells released from nocodazole synchrony at M phase, arrest in G1 and S phases can occur [91]. In lymphoblasts, an early effect of the drug was actual loss of G2/M fraction, and induction of apoptosis [92]. UCN-01-induced apoptosis of lymphoblasts can be related to inappropriate activation of CDK1 and CDK2 resulting in inappropriate cell entry into M phase and induction of apoptosis [92]. A potential mechanism for this effect has been provided by the demonstration of its capacity to inhibit checkpoint kinase 1, an inhibitor of cdc25 phosphatase, at a low drug concentration [93]. This could lead to enhanced cell death after DNA damaging-agents including radiation [94], 5-fluorouracil (5-FU) [95], cisplatin [96], and mitomycin C. Increased cytotoxicity to UCN-01 is also seen in cells containing the mutated p53 gene [97]. UCN-01 was recently found to inhibit E2F expression by a ubiquitin-proteasome-dependent process [98].

**Pharmacology**

The cytotoxic effect of UCN-01 is dependent on exposure duration, and the 72-hour infusion was found to yield the maximal benefit both in vitro and in vivo [90, 99-101]. A surprising difference between UCN-01 pharmacology in humans and in rodents emerged when it was documented that UCN-01 binds avidly to α-acidic glycoprotein (AAG) resulting in a prolonged half-life of 600-1,000 hours in humans [100-102]. Eighty-eight to 98% plasma protein binding was also seen with other staurosporine derivatives [103]. Owing to these properties a 4-weekly dosing schedule was found to be appropriate, with retreatments with 50% of the original dose.

In a recently published phase I study [101], 47 patients were treated and the recommended phase II dose was identified at 42.5 mg/m²/day for 3 days every 4 weeks. A mean total plasma concentration of 36.4 μM (400 nM free UCN-01) was achieved at this dose level with half-life ranging from 447 to 1,176 hours. A median salivary concentration of UCN-01 was 111 nM at the MTD, and the drug was detectable in saliva for several weeks following infusion.

No alteration in UCN-01 pharmacology was seen when it was combined with 5-FU [104]. One-hour bolus administration of UCN-01 had similar pharmacokinetics and low clearance [105].

**Clinical Response**

In the phase I study described above [101], one patient with metastatic melanoma achieved a partial response for more than 6 months, and a heavily treated patient with progressive anaplastic lymphoma kinase-positive large cell lymphoma had an initial response followed by stable disease for more than 3.5 years. Nineteen other patients had a stable disease for a median of 5 months duration. UCN-01 also may have enhanced the sensitivity of chemotherapy-resistant plasmacytoid lymphoma to EPOCH chemotherapy resulting in complete response in a 68-year-old man who had progressive lymphoma in spite of prior high-dose EPOCH therapy [106]. Partial response and stable disease were reported in two cases of progressive cholangiocarcinoma treated with staurosporine-derivative PKC412 [103]. Similar responses were reported in CLL patients treated with UCN-01 and fludarabine [107].

**Toxicity**

In the NCI phase I study, the DLTs were hyperglycemia, nausea, vomiting, and pulmonary dysfunction at 53 mg/m²/day. Hyperglycemia was a consistent toxicity at all dose levels tested, with its severity dose dependent. Some patients developed evidence of metabolic acidosis. Although hyperglycemia was more common in patients with existing glucose intolerance, it also occurred in patients with no prior abnormality in glucose metabolism. UCN-01-induced hyperglycemia was accompanied by increase in serum insulin and immunoreactive C-peptide levels [101] indicating a normal islet cell response to increase in glucose stress and is suggestive of increased peripheral tissue resistance to insulin, although still somewhat responsive to exogenous insulin administration. Staurosporine has been demonstrated to inhibit insulin-stimulated translocation of GLUT1 and GLUT4 glucose transporters [108]. Other adverse events observed with UCN-01 included pulmonary toxicity characterized by hypoxemia with small bilateral pleural effusions or no significant radiological findings, headache at the higher dose levels, asymptomatic hypotension, fever, fatigue, rash, and myalgia [101]. Hypotension emerged as a significant DLT when UCN-01 was administered as a 1-hour bolus infusion at 95 mg/m² [105]. Single agent UCN-01 treatment was not myelosuppressive and no significant adverse effect was noted on renal or hepatic functions. Myelosuppression became significant when UCN-01 was combined with other chemotherapeutic agents such as fludarabine [107].

**Current Clinical Trials**

Several clinical trials are ongoing to explore the utility of UCN-01 as a single agent or in combination with radiation and other chemotherapeutic agents. Approaches to decrease the binding of UCN-01 to AAG, such as the use of displacing agents or structural modification of UCN-01 to bind less avidly with AAG, are being investigated. Studies combining
UCN-01 with fludarabine in CLL and indolent lymphoma [107] and with 5-FU [104] are under way.

**STI571 (Gleevec, CGP 57148b)**

STI571 (imatinib mesylate), a 2-phenylaminopyrimidine derivative (Fig. 3C), emerged from an effort by Novartis Pharmaceuticals to inhibit PDGF-R, and was also found to inhibit the abl-related tyrosine kinases including p210*abl*, p185*abl*, v-abl, and c-abl in addition to c-kit tyrosine kinase [109]. STI571 has been proposed to bind to a distinctive “inactive” conformation of the centrally located activation loop of abl kinase, preventing its catalytic utilization of ATP, and therefore activation [110]. Release of cytochrome c from mitochondria to cytosol, activation of caspase-9 and caspase-3, DNA fragmentation, and apoptosis follow inhibition of p210*abl* kinase by STI571 [111]. Continuous exposure is required to maintain the inhibition of bcr-abl kinase activity [112]. In addition to its direct inhibitory effects, STI571 also plays a role in modulating resistance pathways in tumors expressing PDGF-R and c-kit kinases. STI571 has also been shown to inhibit the growth of glioblastoma (perhaps by PDGF-R effects) [113] and small cell lung cancer (SCLC) known to express c-kit [114]. In Philadelphia-chromosome positive (Ph+) leukemia cell lines, STI571 produced synergistic effects with α-interferon and vincristine, and an additive effect in combination with cyclophosphamide, hydroxyurea, cytarabine, doxorubicin, and etoposide. Methotrexate exerted an antagonistic effect when combined with STI571 [115].

Several mechanisms of resistance to STI571 have been proposed including a bcr-abl amplification, reduction of STI571 uptake, and overexpression of multidrug resistant P-glycoproteins among others. Studies have documented the effect of STI571 in reversing chemotherapy resistance [116], and a synergistic effect has been seen with other chemotherapy agents including interferon-α (IFN-α), daunorubicin, cytarabine, and etoposide. Methotrexate exerted an antagonistic effect when combined with STI571 [115].

**Pharmacology**

STI571 is well absorbed orally and once daily administration of 400 mg achieved a steady-state mean maximal concentration of 2.3 µg/ml and a 24-hour trough concentration of 0.72 µg/ml [118]. These values are well above that required for inhibition of bcr-abl kinase. The drug has a half-life of 13 to 16 hours. STI571 is metabolized in the liver primarily through CYP3A4 enzyme, and its plasma levels may be affected by other drugs that induce this enzyme [118].

**Clinical Response**

Recently published studies in patients with chronic phase CML [118], blast crisis, and Ph+ acute lymphocytic leukemia (ALL) [119] demonstrated significant antileukemic effects. Ninety-eight percent of patients with chronic phase CML who received a dose of 300 mg/day or more achieved complete hematologic response and 31% achieved complete or major cytogenetic response. The hematologic response was apparent as early as 3 weeks after initiation of treatment and was maintained for a median of 265 days in 96% of patients. Complete cytogenetic responses (28%) occurring within 3 to 9 months were reported in a phase II trial of chronic-phase CML [120]. Response rates of 44% and 62% were reported in phase II studies of accelerated phase and myeloid blast crisis, respectively. Fifty-five percent of patients with myeloid and 70% with lymphoid blast crisis achieved either a complete hematologic response or a marrow response with ≤15% blasts [119], however, the majority of patients with lymphoid phenotype later relapsed [120].

An excellent clinical response to 400 mg of daily STI571 was also noted in one patient with metastatic gastrointestinal stromal tumor (GIST) expressing c-kit who failed several prior chemotherapy regimens [121]. c-kit is implicated in the pathogenesis of several human tumors including mast cell leukemia, SCLC, GIST, germ cell tumors, ovarian cancer, melanoma, breast cancer, and neuroblastoma. A subsequent European Organization for the Research and Treatment of Cancer phase I study in GIST and other soft tissue sarcoma documented partial responses in four patients, and stable diseases and clinical improvement in 8 of 20 treated patients [122]. Positron-emission tomography (PET) proved to be a valuable method for evaluating metabolic response to this agent. Dramatic reduction in 18F-DG uptake was seen as early as day 8 of treatment [122]. The reduction in the metabolic activity of tumors correlated well with decrease in tumor size on magnetic resonance imaging [121].

**Toxicity**

Treatment with STI571 was associated with a tolerable toxicity profile. In the phase I study [118], patients were treated with a dose range of 25-1,000 mg without identification of MTD. Toxicities were minimal at the lower dose levels, and no grade 3 or 4 toxicity was reported at the 300 mg daily dose. In both published studies [118, 119] the most common toxicity observed was nausea in 43%-55% of patients. Myalgias were reported in 21%-41% of patients, edema in 39%-41%, diarrhea in 17%-25%, fatigue in 10%-20%, rash in 17%-19%, vomiting in 18%-41%, anorexia in 10%, and arthralgias in 13%. Other infrequent side effects included grade I/II anemia, elevation of liver enzymes, exfoliative dermatitis, gastric hemorrhage, renal failure, and congestive heart failure with possible relation to STI571. Cases of solid tumor bleeding were also reported and related to rapid tumor lysis [122]. A more frequent grade 3 or 4 eleva-
and OSI-74 have reached advanced clinical development. Pounds that act intracellularly to inhibit EGFR-TK, ZD 1839 phase III clinical trials [127]. Of the small molecule compromising results in phase I/II studies and is now entering is a human-chimeric monoclonal antibody that has shown antibodies and small molecule inhibitors. Cetuximab (C-225) human diseases are being explored including monoclonal EGFR is associated with poor outcome in several tumor types neck tumors overexpress this protein. Overexpression of EGFR in squamous cell carcinomas, and 90% of head and cancers [123, 124]. There is a high level of expression of human cancers including NSCLC, colorectal, head and neck, tumors. EGFR is overexpressed in an extensive range of signal transduction events are critical for the growth of many as ras/MAP kinase and STAT-3 transcription factors. These autophosphorylation of the tyrosine residues within the intracellular domain and activation of downstream pathways such as ras/MAP kinase and STAT-3 transcription factors. These signal transduction events are critical for the growth of many tumors. EGFR is overexpressed in an extensive range of human cancers including NSCLC, colorectal, head and neck, bladder, brain, pancreas, breast, ovary, prostate, and gastric cancers [123, 124]. There is a high level of expression of EGFR in squamous cell carcinomas, and 90% of head and neck tumors overexpress this protein. Overexpression of EGFR is associated with poor outcome in several tumor types [125, 126]. Several approaches to blocking the EGFR-TK in human diseases are being explored including monoclonal antibodies and small molecule inhibitors. Cetuximab (C-225) is a human-chimeric monoclonal antibody that has shown promising results in phase I/II studies and is now entering phase III clinical trials [127]. Of the small molecule compounds that act intracellularly to inhibit EGFR-TK, ZD 1839 and OSI-74 have reached advanced clinical development.

**Inhibitors of EGFR-TK**

EGFR (erbB-1) is a transmembrane glycoprotein with an external ligand binding domain and an intracellular tyrosine kinase domain. It is a part of the erb-B family of receptors that form homodimers or heterodimers on ligand binding (transforming growth factor-α [TGF-α] or EGFR). This leads to autophosphorylation of the tyrosine residues within the intracellular domain and activation of downstream pathways such as ras/MAP kinase and STAT-3 transcription factors. These EGFR is overexpressed in an extensive range of human cancers including NSCLC, colorectal, head and neck, bladder, brain, pancreas, breast, ovary, prostate, and gastric cancers [123, 124]. There is a high level of expression of EGFR in squamous cell carcinomas, and 90% of head and neck tumors overexpress this protein. Overexpression of EGFR is associated with poor outcome in several tumor types [125, 126]. Several approaches to blocking the EGFR-TK in human diseases are being explored including monoclonal antibodies and small molecule inhibitors. Cetuximab (C-225) is a human-chimeric monoclonal antibody that has shown promising results in phase I/II studies and is now entering phase III clinical trials [127]. Of the small molecule compounds that act intracellularly to inhibit EGFR-TK, ZD 1839 and OSI-74 have reached advanced clinical development.

**ZD 1839 (IRESSA)**

ZD 1839 (Fig. 3D) is an anilinoquinazoline compound developed as a specific potent inhibitor of EGFR-TK. ZD 1839 inhibits EGFR-TK through competitive binding to the ATP-binding site. Specificity for EGFR-TK was demonstrated in cell cultures of KB human tumors with an IC₅₀ of 0.08-0.09 μM and 3.64 μM for EGF-stimulated and non-EGF-stimulated growth respectively, and no significant inhibitory effect on other related PTKs such as erb-B2 kinase, KDR, c-fil kinase, PKC, and MAPK. In vivo animal models revealed significant inhibitory effect on xenograft of several tumor types including A431, A549, HT29, DU145, and MCF-7. Although ZD 1839 has cytostatic effects, cytotoxicity was seen at higher dose levels. Continuous administration is required for maintaining this inhibition.

ZD 1839 produced supra-additive and enhanced antitumor effects of cisplatin, carboplatin, oxaliplatin, paclitaxel, docetaxel, etoposide, topotecan, raltrexed, and doxorubicin in several tumor types resulting in complete regression in some xenograft tumors [128, 129]. Similar responses were seen in combination with radiation [130]. No additive effects were seen with gemcitabine [128]. Schedule dependency seems to exist between ZD 1839 and some of these drugs, such as cisplatin. The cytotoxicity of cisplatin is greater with pre-exposure to ZD 1839 for 48 hours rather than 1 hour [131]. Other studies have demonstrated that pretreatment with low doses of cisplatin enhanced the sensitivity of NSCL carcinoma cells to ZD 1839 [132]. An important finding in several studies is the apparent effectiveness of ZD 1839 regardless of the levels of EGFR protein [128] or gene expression [133]. Thus, additional targets for the drug action may exist and would be of interest to define.

**Pharmacology**

Preclinical studies documented a 50% bioavailability of orally administered ZD 1839 and several phase I studies evaluated its pharmacology in humans [134-136]. Daily oral administration with doses ranging from 50 to 700 mg in an intermittent schedule of 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 hours [134, 135]. Mean Cmax and AUC0-24hr were 113-2,255 ng/ml and 1.8-38.5 μg/ml, respectively [134].

Continuously administered ZD 1839 reaches a steady-state plasma concentration by day 7 of treatment. Lower doses of ZD 1839 were required when it was combined with other chemotherapeutic agents, and this was independent of the levels of EGFR expression [128]. Five hundred mg per day of ZD 1839 was found to be safe when administered in combination with paclitaxel and carboplatin [137]. No changes in the exposure to paclitaxel or carboplatin were observed during this combination.

**Clinical Response**

ZD 1839, given either intermittently or continuously, resulted in partial responses in NSCLC and prostate cancer, and stable disease lasting more than 4 months in several
patients with various tumor types [134-136]. Clinical trials conducted by Baselga et al. [136] documented an improvement in quality of life in patients treated with ZD 1839. Quality of life was determined by scores on the functional assessment of cancer therapy-lung (FACT-L) patient’s questionnaire and disease-related symptoms. Of 23 Japanese patients with NSCLC treated with an intermittent schedule ZD 1839, five patients with adenocarcinoma achieved partial response [135].

In an ongoing phase II combination study of ZD 1839, carboplatin, and paclitaxel in chemonaive advanced NSCLC patients, 28% achieved partial response and 40% had stable disease at day 56 of treatment [137].

Toxicity

On the schedule of daily administration for 14 days, diarrhea and elevated transaminases were identified as the DLTs at 700 mg [134, 135]. Grade 3 diarrhea was reported in three patients on the continuous daily schedule [136]. Interestingly, no DLT was identified in the 28-consecutive-day schedule up to an 800 mg/day dose level [136]. Another frequent toxicity was an acneiform rash that has been documented in more than 50% of treated patients. Other toxicities included nausea, vomiting, and anorexia. These toxicities were reversible, and the rash resolved in some patients even with continued treatment.

Current Clinical Trials

Combining ZD 1839 with cytotoxic drugs produced greater antiproliferative effects even in tumors (e.g., LX-1) with minimally detectable EGFR expression [128], and the mechanism for this phenomenon is not clear. For these reasons, ZD 1839 has a promising role in enhancing the therapeutic benefit of existing standard agents, and several studies evaluating these combinations are ongoing. Phase III studies in lung cancer are evaluating ZD 1839 in combination with gemcitabine/cisplatin or paclitaxel/cisplatin and also its combination with paclitaxel and carboplatin. Another study is evaluating ZD 1839 in head and neck cancer.

Recent preclinical studies demonstrated efficacy of ZD 1839 in Herceptin-resistant cell lines with enhanced induction of apoptosis when ZD 1839 was combined with Herceptin [138]. A role for ZD 1839 is also anticipated in breast cancer patients who become resistant to tamoxifen [139]. The impact of EGFR overexpression on the efficacy of treatment is being correlated with clinical outcome in several studies, and this may provide important information on optimal candidates for such therapy.

OSI-774 (CP-358, 774)

OSI-774 (Fig. 3E) is another orally active and potent inhibitor of EGFR-TK with an IC₅₀ of 2 nM in biochemical assays. It reversibly inhibits EGFR-TK through competitive binding to the ATP site. Inhibition of EGFR-TK and its downstream P13/MAPK signal transduction pathways by OSI-774 results in accumulation of p27KIP₁, cell cycle arrest at G₁ phase, and induction of apoptosis [137]. EGFR-TK was shown to have more than a 1,000-fold sensitivity to OSI-774 inhibition compared with other tyrosine kinases [140]. A substantial inhibitory activity against EGFR-overexpressing tumor cells and inhibition of tumor growth were demonstrated in human tumor xenograft models (50% effective dose = 10 mg/kg daily for 20 days) [141]. Evaluation of pre- and 3-day-post-treatment biopsy specimens from patients with squamous cell carcinoma of the head and neck treated with OSI-774 revealed a 60% reduction in phosphorylated EGFR and complete abolition of phosphorylated Akt [142]. Significantly enhanced cytotoxicity was also demonstrated when combined with cisplatin [141], doxorubicin, gemcitabine, and other agents.

Pharmacology

Several phase I studies have evaluated the dose and scheduling of OSI-774 as a once-weekly treatment for 3 of 4 weeks, or continuous daily dosing for consecutive 21 of 28 days [143, 144]. Patients who received 100 mg or greater daily doses of OSI-774 achieved the projected target average plasma concentration for clinical efficacy (Cavg) of 500 ng/ml [143, 145]. Large inter- and intrapatient variability and evidence of dose-related accumulation in exposure were observed with oral OSI-774 treatment [144, 145]. In another phase I study evaluating several administration schedules, an average half-life of 24.4 ± 14.6 hours, Vₐ, of 136.4 ± 93.1 l, and Css of 1.20 ± 0.62 µg/ml at the 150 mg/day dose level were documented [143]. Liver metabolism of OSI-571 is cytochrome P-450-dependent and involves the CYP-3A4 enzyme system.

Clinical Response

Partial responses were observed in patients with renal and colon cancers, and stable disease for more than 5 months was documented in patients with head and neck, NSCL, prostate, and cervical cancers [143]. Partial and minimal responses in head and neck cancers in addition to significant reduction in metabolic activity assessed by PET scan were reported also in other phase I studies [146]. 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 [147]. These responses did not correlate with higher percentages or intense EGFR staining. A similar 13% partial response and 29% rate of stable disease were also reported in patients with advanced squamous cell carcinoma of the head and neck [148]. Advanced refractory ovarian carcinoma had a similar pattern of response [149].
Toxicity

On continuous daily dosing, diarrhea became a DLT at 200 mg/day. No DLT was identified in the weekly schedule up to 1,600 mg/day dose level. Other frequent toxicities were an acneiform rash, fatigue, headache, mucositis, nausea, and transient rise in bilirubin and transaminases [144]. The maculopapular rash was similar to that seen with ZD 1839 and occurred in up to 78% of patients affecting mainly the face and upper part of the body [147]. Biopsies of the rash revealed subepidermal neutrophilic infiltration and epidermal hyperproliferation.

Current Clinical Trials

In addition to its single-agent activity, OSI-571 is also promising in combination with chemotherapy, and its evaluation is under way or planned in several human tumors.

Proteasome Inhibitor PS-341

The proteasome is an exciting new target for cancer treatment. The ubiquitin-proteasome pathway plays an important role in cell cycle regulation through precisely programmed degradation of intracellular proteins resulting in either activation or blockage of certain signal transduction pathways. The 26S proteasome degrades proteins that have been marked for elimination and conjugated to multiple units of polypeptide ubiquitin [150] (Fig. 4). Examples of proteins that are degraded through the ubiquitin-proteasome are the transcription regulator IκB. Degradation of IκB results in activation of NF-κB, which plays a significant role in cell survival, adhesion, metastasis, and angiogenesis [151]. Cell cycle arrest can result from increased 20S proteasome-mediated cyclin degradation and loss of CDK activity. On the other hand, inhibition of 20S proteasome activity can lead to accumulation of CKIs and cell arrest with or without apoptosis [152]. Several inhibitors of the proteasome pathway have been identified including lactacystin, peptide aldehydes, and dipeptide boronate derivatives.

PS-341 (Fig. 3F) 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 at the NCI in vitro testing [152]. It is cytotoxic to breast cancer cells in vitro with IC₅₀ = 0.05 μM [153] and to PC-3 tumor xenografts [152]. The antitumor activity of a series of PS-341 analogs positively correlates with the degree of proteasome inhibition. PS-341 causes accumulation of cyclins A and B and arrests the cells at the S and G₂/M phases [154]. Cytotoxicity of PS-341 is associated with accumulation of p21WAF1/CIP1 and wild type p53 followed by nuclear fragmentation and apoptosis [155, 156]. Increase in ubiquinated proteins in peripheral blood mononuclear cells was seen following PS-341 treatment [156]. PS-341 significantly inhibited NF-κB DNA binding and functional reporter activity in addition to proangiogenic cytokines such as GRO-α and VEGF [157]. Response with a decline in prostate-specific antigen in prostate cancer patients treated with PS-341 was accompanied by a parallel decline

Figure 4. The ubiquitin-proteasome pathway. Ubiquitin is activated by ubiquitin-activating enzymes (E1) and then transferred via transacylation to ubiquitin-carrier/conjugating enzymes (E2). The ubiquitin ligase (E3) helps the activated ubiquitin bind to a lysine residue of a substrate/protein and generates a polyubiquitin chain. The polyubiquitinated substrates then bind to the ubiquitin receptor subunit of 19S complex and are then degraded to small peptides. This process is ATP-dependent. The ubiquitin is recycled. The multi-subunit 26S proteasome is assembled from two 19S regulatory complexes attached at each side of the cylinder-shaped 20S core catalytic unit.
in interleukin-6, offering a possible valid marker for NF-κB inhibition [158]. PS-341 has demonstrated an additive and synergistic cytotoxic effect when combined with radiation, cyclophosphamide, cisplatin, 5-FU, gemcitabine, CPT-11, and adriamycin [153, 159]. Contradicting results of PS-341 interaction with taxol have been reported with increasing evidence of interference with taxol-induced cell arrest and apoptosis in some studies [153, 159].

**Pharmacology**

PS-341 is rapidly removed from the vascular compartment and is widely distributed with a half-life of 10 minutes [152]. The degree of 20S proteasome inhibition in whole blood was adopted as a surrogate marker for the drug activity [160]. Several phase I studies evaluated various schedules of PS-341 administration, such as twice weekly injections every 2 weeks followed by 1 week off treatment, and twice or once weekly for 4 of 6 weeks. At the MTD recommended for phase II studies (1.25-1.3 mg/m²), a 65%-72% inhibition of 20S proteasome was achieved [156, 161]. An average 54% inhibition of proteasome was achieved in patients’ tumors [162].

**Clinical Response**

Phase I studies documented a partial response in an extensively treated patient with a bronchoalveolar NSCLC [161], and 50% reduction in lung metastatic lesions and stable cutaneous lesions in two patients with melanoma [162]. Stable disease was seen in other patients with sarcoma, lung adenocarcinoma, and malignant fibrous histiocytoma [162].

**Toxicity**

PS-341 has a dose- and schedule-dependent toxicity profile with severe toxicity expected when the proteasome inhibition exceeds 80%. In phase I dose-escalation trials, DLTs were reported as painful neuropathy, diarrhea, fatigue, and orthostatic hypotension [161]. Other frequent toxicities noted with PS-341 treatment were nausea, vomiting, fever, and thrombocytopenia. Patients usually had more toxicity with the second cycle of treatment.

**Current Clinical Trials**

Several phase II clinical trials evaluating PS-341 as a single agent in hematologic malignancies, breast, brain, and several other solid tumors, as well as in pediatric patients are ongoing. In addition, PS-341 in combination with 5-FU, doxorubicin, CPT-11, gemcitabine, and etoposide is also being evaluated in a phase II trial [163]. The interaction of PS-341 with paclitaxel and docetaxel is being evaluated in a phase I study. Since PS-341 has a radiosensitizer effect probably mediated through NF-κB inhibition, its combination with concurrent radiation in squamous cell carcinoma of head and neck is also being evaluated in a phase I study [163].

**Histone Deacetylase Inhibitors**

Histone acetylation by histone acetyltransferases is important for promoting the action of several transcription factors. Acetylation facilitates binding of transcription factors to specific target DNA sequences by destabilizing nucleosomes bound to the promoter region of the target genes [164]. On the other hand, histone deacetylases (HDAC) facilitate suppression of the transcriptional activity [165]. The transcriptional repression activity of HDACs was documented for pRB/E2F [166]. Disruption of histone acetylation is associated with cancer development in several human neoplasms. Inhibition of HDAC activity was found to be cytostatic and to arrest cells in G1 and G2/M phases. Therefore, inhibition of HDAC activity is being pursued as a novel approach to cancer treatment, and several classes of HDAC inhibitors (HDACI) have recently been identified including butyrate, trichostatin A, oxamflatin, depsipeptide, and benzamide compounds (MS-275). Depsipeptide and MS-275 have exhibited significant preclinical antitumor activities and are being evaluated in phase I and II clinical trials.

**Depsipeptide (FR901228)**

Depsipeptide (Fig. 3G) is a fermentation product from *Chromobacterium violaceum* that demonstrated potent cytotoxic activity against several human cell lines and xenograft models of human tumors in nanomolar concentration [167]. Depsipeptide causes downregulation of cyclin D1, upregulation of cyclin E, and p21-dependent cell cycle arrest at G1 and G2 [168]. Exposure to depsipeptide in vitro induced proapoptotic changes in B-cell CLL including a decrease in bcl-2:bax ratio and p27 expression [169].

In a phase I study conducted at the NCI [170], depsipeptide was administered intravenously over a 4-hour period on days 1 and 5 of a 21-day cycle, to 27 patients in eight dose levels. MTD was established at 17.8 mg/m². Several toxicities were experienced with depsipeptide treatment including neutropenia, thrombocytopenia, hypocalcemia, fatigue, nausea, vomiting, and T-wave inversions on electrocardiogram not associated with elevation in cardiac enzymes. This study documented an elimination half-life of 12 hours and volume of distribution of 14 L. Partial responses occurred in three patients with cutaneous T-cell lymphoma and a complete response in a patient with peripheral T-cell lymphoma [171]. Hyperacetylation of histones was demonstrated in Sézary cells after treatment with depsipeptide in this study. A phase II study of depsipeptide in T-cell lymphoma is now open for accrual at the NCI.
MS-275

MS-275 (Fig. 3H) is a benzamide-derivative that induces p53-independent accumulation of p21\(^{WAF/CIP1}\) and cell cycle arrest [172]. MS-275 also induced TGF-β type II receptor expression in human breast cancer [173], and this may contribute to its induction of p21\(^{WAF/CIP1}\) expression. MS-275 has significant growth inhibitory effects on several human tumor cell lines including myeloma, pro-myelocytic leukemia, colon, lung, and ovarian tumors. This effect is time dependent. Compared with 5-FU, MS-275 has a comparable or superior antitumor effect in xenograft models of several human tumors including St-4 gastric, KB-3-1 epidermoid, Ca-pan-1 pancreatic, HT-29 colon, A2780 ovarian, and Calu-3 lung tumors [172]. MS-275 is absorbed orally with 28%-55% bioavailability. Its antiproliferative effect requires prolonged exposure. In animal models the main toxicities were gastrointestinal and bone marrow suppression. A phase I study evaluating orally administered MS-275 in lymphomas and solid tumors has just started at the NCI.

Geldanamycin Derivatives and Immunconjugates

Geldanamycin is a highly cytotoxic benzoquinoid ansamycin antibiotic produced by the actinomycete Streptomyces hygroscopicus. Ansamycin antibiotics were found to inhibit several PTKs, reverse the morpholgy of cells transformed by PTK oncogenes such as src, yes, fps, c-myc, enhance expression of hyperphosphorylated pRB, and induce G\(_s\)/M cell cycle arrest [174, 175]. Geldanamycin and its derivatives bind with high affinity to and interfere with the chaperone function of the cytosolic heat-shock-protein-90 (Hsp90), and enhance the proteasomal degradation of several cell cycle regulatory proteins including receptor tyrosine kinases, steroid receptors, RAF, and CDKs [176-178]. Preclinical data demonstrated in vitro and in vivo antitumor activities in several tumor types including melanoma, prostate, ovary, and breast cancers.

Since the parent compound geldanamycin is not suitable for clinical use due to significant hepatic toxicity, other approaches have been identified to utilize its cytotoxic properties. A structurally modified derivative, 17-(Allylamino)-17-Demethoxygeldanamycin (17-AAG) has a better toxicity profile and exerts similar antitumor effects. Two phase I clinical studies [179, 180] presented this year documented the tolerability and pharmacology of 17-AAG in humans. 17-AAG was administered as a 1-hour daily infusion for 5 consecutive days and repeated every 3 weeks. An MTD of 40 mg/m\(^2\) was identified in the NCI study [179]. DLT in this study was reversible hepatotoxicity. Other adverse events observed with 17-AAG treatment were fever, emesis, fatigue, thrombocytopenia and diarrhea [179, 180]. Terminal half-life of 2.5 ± 0.5 hour and C\(_{max}\) of 1,860 ± 660 nM at the 40 mg/m\(^2\) dose were identified [179]. Plasma drug concentrations exceeded the levels (10-500 nM) required for cell death in vitro and in xenograft models. Both studies suggested evidence of disease stabilization.

Another promising approach is utilization of geldanamycin in immunconjugates. Recent studies have demonstrated the feasibility of conjugating derivatives of geldanamycin to internalizing anti-HER2 monoclonal antibodies [181]. These immunconjugates exhibited enhanced antiproliferative activity compared with the native monoclonal antibody. Conjugation to monoclonal antibodies directed at other growth hormones and oncogenic receptors is being explored. This promising approach will likely continue to develop and reach clinical trials in the near future.

Rapamycin Analog (CCI-779)

Rapamycin (Sirolimus, Rapamune) (Fig. 3I) 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). mTOR, also known as FRAP, RAFT1, and RAP1, is a kinase member of phosphoinositide 3 kinase related kinases 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, p70\(^{S6K}\) and 4E-BP1/PHAS [182, 183]. Rapamycin causes G\(_1\) cell cycle arrest by increasing the turnover of cyclin D1 [184], upregulating p27\(^{kip1}\), and inhibiting cyclin A-dependent kinase activity [185]. Blockage of mTOR function results in inhibition of PI3K/Akt-mediated proliferative signals and cell arrest. In addition to its antimicrobial and immunosuppressive properties, rapamycin was found to have significant antiproliferative effects in human tumors [186]. CCI-779, an ester of rapamycin, has a significant antiproliferative effect and favorable toxicity profile and is being studied in several phase I clinical trials in human cancer [187, 188]. In these Phase I studies CCI-779 was administered on a weekly or daily basis for 5 days every 2 weeks schedule. Toxicities observed with CCI-779 treatment included hypocalcaemia, neutropenia, thrombocytopenia, mucositis, hypertriglyceridemia, rash, reversible decrease in testosterone levels in men, and allergic reactions. A median half-life of 17.3 hours was documented [188]. Several partial responses have been documented in renal cell carcinoma, NSCLC, neuroendocrine tumors, and breast cancer in addition to minor responses and stable disease in several tumor types [187, 188]. Several studies evaluating combination of CCI-779 with other chemotherapeutic agents are under way.
CONCLUSIONS AND FUTURE DIRECTIONS

Development of therapeutic anticancer drugs has entered a new era of targeted treatment that promises rational modulation of malignant cell growth with acceptable toxicity. The development of these drugs depends on the identification of suitable targets within the pathophysiologic pathway of carcinogenesis and on designing agents that modulate these targets. Targets are being discovered continuously and this has been made possible by recent advances in technology such as DNA microarray and proteomics. Although many of the agents described in this article are rationally targeted to specific effectors in the neoplastic process, several issues have emerged regarding the development of such agents, their range of toxicities, and how to measure their clinical effect.

Several of the new agents achieve antitumor effects through a wide range of mechanisms including cell cycle arrest, antiangiogenesis, induction of apoptosis, and cytotoxicity. These processes are linked and result from modulation of several survival and death pathways downstream of the drugs’ targets. These sequential and time-dependent changes may result in apoptosis and cell death. A unique feature of most of these new compounds is the reversibility of action and the need for continuous administration to attain significant growth inhibition and allow for the apoptotic process to take place. While newer generations of these agents such as the EGFR-TK inhibitor CI-1033 (PD 183805) have irreversible effects, which may result in a sustained blockage of carcinogenic signals, there is also a potential for greater toxicity.

All inhibitors of signal transduction under clinical development at present exhibit a concentration-dependent inhibitory effect on a wide range of signaling events. It is anticipated that more active and selective chemical inhibitors of CDK and tyrosine kinases will be available in the near future. The clinically successful compounds are anticipated to have very defined and limited targets and also a reasonable selectivity to avoid side effects. It is estimated that there are 2,000 kinases in the human body and therefore the required selectivity of these compounds may be difficult to achieve given that all kinases have an ATP-binding pocket with some high degree of homology. The recently described inhibitory mechanism of STI571 by binding to an inactive form of Abl tyrosine kinase [110] and preventing its activation provides an exciting approach for designing new highly selective inhibitors. Inactive kinases may have more distinctive conformations that allow for better selectivity.

Expression levels of cell surface antigens and receptors associated with a specific PTK may be of theoretical importance to the maximal inhibitory effect of these drugs. However, recent studies have not found a correlation between the degree of EGFR positivity and the clinical response [128, 133]. Unlike monoclonal antibodies such as Herceptin, where the degree of expression may be essential for better response, “small” molecules acting intracellularly may not require a higher expression of targets. This may allow administration of these drugs to specific tumor types without the need for assessing the degree of receptor expression. They also question, however, the specific relevance of the target against which the drugs were designed.

These new agents raise a challenge to the traditional dose-finding approach in cancer drug discovery and the concept that more is better. A new approach that determines the biologically optimal dose may be more appropriate than the traditional MTD. Biological endpoints should determine the recommended dose for certain conditions if that dose correlates with inhibition of target kinase and clinical outcome. The majority of the newly designed drugs achieved a biologically active plasma concentration that is sufficient to inhibit the target kinase at doses well below the MTD.

Assessing the clinical response to these novel agents is an area of extensive discussion. Many of the novel targeted antitumor agents that affect signal transduction may have a cytostatic endpoint rather than the traditional cytotoxic one. Therefore, the traditional methods of assessing disease response may not be valid, and new approaches to examine the changes in rate of tumor growth and perhaps other biological surrogate markers may be useful in determining which of these drugs are most suitable for advanced phase development. Such issues are important if drugs with greater utility in earlier stage disease (i.e., adjuvant application) are not discarded owing to need-to-document cytotoxicity.

Other endpoints that may be useful include time to progression, stable disease as best response, symptomatic benefit, and other quality-of-life measures. Another important issue is that these agents may require a longer treatment period before a sensible reduction or stabilization in the tumor size could be appreciated. The current 2-3 months interval of assessment may not be suitable for these agents.

A unique constellation of toxicities has emerged with these agents. The occurrence of diarrhea, rash, hyperglycemia, hepatic dysfunction, and inflammatory syndromes was common with these agents. This syndrome is most likely related to the blockage of ATP binding and interference with downstream cellular signaling pathways that regulate common features in human physiology. The exact affected pathways resulting in these adverse events have not been identified yet, but the majority of these side effects were easily treatable.
As mentioned above, the combination of various cell signaling inhibitors with conventional agents and radiation is being pursued in several studies based on the promising preclinical data. Schedule dependency has emerged as an important factor to achieve the optimal antiproliferative activity desired and also to minimize toxicity. This raises the question of how to combine different agents to achieve maximal blockage of multiple intracellular pathways leading to cancer development. These targeted therapies are likely to have a wide range of applications in cancer treatment, however, greater roles are envisioned as adjuvant, chronic stabilizing, and preventative modalities.

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