Novel Agents on the Horizon for Cancer Therapy

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Abstract

Although cancer remains a devastating diagnosis, several decades of preclinical progress in cancer biology and biotechnology have recently led to successful development of several biological agents that substantially improve survival and quality of life for some patients. There is now a rich pipeline of novel anticancer agents in early phase clinical trials. The specific tumor and stromal aberrancies targeted can be conceptualized as membrane-bound receptor kinases (HGF/c-Met, human epidermal growth factor receptor and insulin growth factor receptor pathways), intracellular signaling kinases (Src, PI3k/Akt/mTOR, and mitogen-activated protein kinase pathways), epigenetic abnormalities (DNA methyltransferase and histone deacetylase), protein dynamics (heat shock protein 90, ubiquitin-proteasome system), and tumor vasculature and microenvironment (angiogenesis, HIF, endothelium, integrins). Several technologies are available to target these abnormalities. Of these, monoclonal antibodies and small-molecule inhibitors have been the more successful, and often complementary, approaches so far in clinical settings. The success of this target-based cancer drug development approach is discussed with examples of recently approved agents, such as bevacizumab, erlotinib, trastuzumab, sorafenib, and bortezomib. This review also highlights the pipeline of rationally designed drugs in clinical development that have the potential to impact clinical care in the near future. CA Cancer J Clin 2009;59:111-137. ©2009 American Cancer Society, Inc.

Introduction

Significant advances have been made in cancer therapy during the last decade as our understanding of molecular biology and carcinogenesis has evolved. Cancer cell proliferation, apoptosis, angiogenesis, invasion, and metastasis are regulated by an interconnecting network of cellular signaling pathways involving extracellular ligands, transmembrane receptors, intracellular signaling protein kinases, and transcription factors.¹ These intracellular signaling effectors are modulated by external factors, such as epigenetic changes, oncogenic mutations, molecular chaperones, and ubiquitin-proteasome pathways. Insights into these complicated intracellular processes have exposed many novel cancer targets for which chemotherapeutic agents may be developed.

Most traditional cancer drugs directly interfere with mitosis, DNA synthesis, and repair systems. A new class of agents induces tumor growth retardation (cytostasis) and apoptosis by exploiting aberrant tumor stroma, tumor vasculature, and cellular signaling mechanisms. Their toxicity profile is also significantly different from traditional cancer drugs. Bcr-Abl, epidermal growth factor receptor (EGFR), and angiogenesis are successful examples of targets that may be treated with drugs. Drugs that target these pathways, including imatinib, cetuximab, and bevacizumab, have already entered clinical practice.²⁻⁵ More importantly, these agents are only the vanguard of an exciting pipeline of novel anticancer drugs. This article reviews the various classes of cancer targets and drugs that are under early phase clinical evaluation, focusing on those that are likely to enter clinical practice in the near future.

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DISCLOSURES: Dr. Adjei has received research grants from Eli Lilly and Ardea and honoraria from Array BioPharma. No other conflict of interest relevant to this article was reported.

©2009 American Cancer Society, Inc., doi:10.3322/caac.20003.
Available online at http://cajournal.org and http://cacancerjournal.org
Strategies of Intervention

Cancer targets can be exploited by different strategies. Thus far, the more successful clinical approaches have been with monoclonal antibodies (MoAbs) and small-molecule, protein-kinase inhibitors. These represent the majority of anticancer drugs currently in clinical testing and are the focus of our review of the pipeline of anticancer drugs on the horizon. The application of other technologies, such as cancer vaccines, antisense oligonucleotides, and small-interfering RNAs (siRNAs), to contemporary chemotherapeutic development has recently been reviewed.6-8

Therapeutic use of MoAbs in cancer patients was possible after development of hybridoma technology by Kohler and Milstein in 1975.9 Early murine MoAbs performed poorly in the clinic, partly because of short antibody half-life and immunogenicity of murine antigens in human hosts. Subsequent technological improvements allowed production of chimeric and humanized MoAbs that overcame these disadvantages and that were better suited for clinical development. The MoAb approach is particularly suited for membrane-bound targets. Proposed mechanisms of action include interference of ligand-receptor interaction, antibody-dependent cellular cytotoxicity (ADCC), complement-mediated cytotoxicity (CMC), and immune modulation.10

In comparison, small-molecule protein-kinase inhibitors are efficacious against both membrane-bound and nonmembrane-bound targets. These agents are chemically diverse and can be broadly categorized into ATP analogs, catalytic domain binders, noncatalytic domain binders, natural products, and inactive kinase conformation binding ligands.11 The selection of a lead compound from a vast chemical library is complicated and challenging. This requires complex computational analyses of the structural–functional relation between a candidate kinase and a panel of small molecules. Many of these small-molecule compounds, eg, sorafenib, can inhibit multiple protein kinases because of structural homology within the same class of protein kinases.12 In anticancer therapy, the ability to target multiple kinases and signaling pathways with a single small-molecule inhibitor has attraction. However, this ability can also hamper our attempt to understand these inhibitor’s mechanisms of action in specific tumor types, an understanding that is vital to the development of these compounds.

When researchers develop therapies against a specific cancer target, monoclonal antibody and small-molecule inhibitor technologies are often complementary. Epidermal growth factor receptor (EGFR), a valid target in many epithelial malignancies, is a transmembrane protein with an extracellular ligand-binding domain joined to an intracellular tyrosine kinase domain. Cetuximab is an anti-EGFR MoAb that targets the extracellular domain by interrupting ligand binding, whereas erlotinib is a small-molecule inhibitor that blocks the EGFR intracellular tyrosine kinase activity.13 Both agents have shown antineoplastic activities against colon, pancreatic, and lung cancers in preclinical experiments. However, cetuximab was found to be more efficacious in colon cancer but not in pancreatic cancer, when tested clinically, and vice versa for erlotinib.2-4 Both agents showed activity in lung cancers.14 Cetuximab seems to be the more optimal agent to combine with radiation. This disparity between preclinical discovery and clinical findings is not uncommon during development of chemotherapeutics, highlighting the importance of early phase clinical trials to identify susceptible tumor types.

Cellular Signal Transduction Pathways

Receptor Kinases

**Human Epidermal Growth Factor Receptor Family**

The human epidermal growth factor receptor (HER) family members include EGFR (erbB1), HER2/neu (erbB2), HER3 (erbB3), and HER4 (erbB4) that are structurally related, and all except HER3 contain intracellular tyrosine kinase domain (Fig. 1).13 All of the HER members, except HER2, bind to extracellular ligands. The structure of EGFR was described in the above section. Activation of EGFR and HER2/neu induces a cascade of downstream signaling through several pathways, such as mitogen-activated protein kinase (MAPK) and PI3-kinase/Akt/mTOR, resulting in cellular proliferation, differentiation, survival, motility, adhesion, and repair.15

EGFR and HER2/neu are overexpressed or abnormally activated in several epithelial malignancies. This finding eventually led to the United States Food and Drug Administration’s (FDA) approval of several agents specifically targeting these receptors.
These include monoclonal antibodies such as cetuximab and panitumumab for colorectal cancers, trastuzumab for breast cancers, and small-molecule inhibitors, such as erlotinib, for lung and pancreatic cancers. These anticancer drugs are now readily available to the general oncology community, and reviews of their clinical development have been published. These include monoclonal antibodies such as cetuximab and panitumumab for colorectal cancers, trastuzumab for breast cancers, and small-molecule inhibitors, such as erlotinib, for lung and pancreatic cancers. These anticancer drugs are now readily available to the general oncology community, and reviews of their clinical development have been published.16-19

Research in this area currently focuses on targeting more than one HER-family receptor simultaneously (Table 1). Lapatinib, a small-molecule inhibitor, is such an agent that targets both EGFR and HER2/neu receptors, and was approved by the US FDA for the treatment of breast cancer. Other drugs that target more than one HER-family receptor and that are under clinical development include BMS-599626, PF-00299804, and BMS-690514.

**Hepatocyte Growth Factor and the c-Met Pathway**

C-Met is a membrane-spanning receptor tyrosine kinase involved in several biological activities including motility, proliferation, survival, invasion, and morphogenesis. Hepatocyte growth factor (HGF) is the only known ligand for c-MET (Fig. 1). Upon HGF binding, c-MET autophosphorylates and recruits several downstream effectors including growth factor receptor-bound protein (Grb) Grb2, Gab1, PI3k, phospholipase C-γ, Shc, Src, Shp2, Ship1, and STAT3. Grb2 and Gab1 interact directly with c-MET and are critical in HGF/c-MET signaling. c-MET receptor expression is regulated by the MET proto-oncogene, and oncogenic mutations have been found in gastric carcinoma and hereditary papillary renal carcinoma type 1.21,24 The tumori-
genic nature of MET mutants was confirmed in transgenic and knock-in animal models.\textsuperscript{25} In addition to receptor mutations, dysregulated HGF/c-MET signaling could be a result of gene amplification and/or rearrangement, ligand and/or receptor overexpression, abnormal paracrine stimulation, and autocrine loop formation. The HGF/c-Met axis is implicated in a wide variety of epithelial, mesenchymal, and hematological malignancies, rendering c-MET an attractive cancer target.\textsuperscript{21}

There are currently several HGF/c-MET inhibitors under clinical evaluation (Table 2). AMG-102 is a fully humanized IgG2 MoAb against HGF with antitumor activity in preclinical models.\textsuperscript{26} The interim result of the phase 1 study was reported at the 2007 American Society of Clinical Oncology (ASCO) annual meeting. Two schedules were tested: a 5 days on and 9 days off schedule and a daily fixed-dose schedule.\textsuperscript{28} Fifty-one solid tumor patients were enrolled, and hypertension was universally observed. Dose-limiting toxicities associated with the first schedule were proteinuria and elevated lipase and liver enzymes. The maximum tolerated dose was 3.6 mg/kg. The maximum tolerated dose for the second schedule was not reached at the time of analysis, and common side effects included hypertension and fatigue. Correlative studies showed inhibition of c-MET, RON, Erk, Akt, and increased apoptosis at dose levels less than the maximum tolerated dose. The agent is being evaluated in papillary renal cell carcinoma, gastric, and head and neck cancers.

Thirty-one patients were treated at doses up to 20 mg/kg. Dose-limiting toxicities included dyspnea/hypoxia (at a 0.5 mg/kg dose) and gastrointestinal bleed (at a 1 mg/kg dose). Common treatment-related side effects included fatigue, constipation, anorexia, nausea, and vomiting. Pharmacokinetic (PK) analysis showed a linear relation in the dose range of 0.5 mg/kg to 20 mg/kg, and no anti-AMG-102 antibodies were detected after administration. The 20 mg/kg dose was deemed tolerable and safe, and the agent is being tested in renal cell carcinoma and malignant glioma.

XL-880 is an oral small-molecule inhibitor of c-MET and has activity against VEGFR2, PDGFR-\(\beta\), kit, FLT3, Tie-2, and Ron. The interim result of the phase 1 study was presented at the 2007 ASCO annual meeting. Two schedules were tested: a 5 days on and 9 days off schedule and a daily fixed-dose schedule.\textsuperscript{28} Fifty-one solid tumor patients were enrolled, and hypertension was universally observed. Dose-limiting toxicities associated with the first schedule were proteinuria and elevated lipase and liver enzymes. The maximum tolerated dose was 3.6 mg/kg. The maximum tolerated dose for the second schedule was not reached at the time of analysis, and common side effects included hypertension and fatigue. Correlative studies showed inhibition of c-MET, RON, Erk, Akt, and increased apoptosis at dose levels less than the maximum tolerated dose. The agent is being evaluated in papillary renal cell carcinoma, gastric, and head and neck cancers.

### TABLE 1. Drugs Targeting HER Receptors

| DRUGS                  | TARGET      | TRIAL STAGE          |
|------------------------|-------------|----------------------|
| Monoclonal antibodies  |             |                      |
| Cetuximab (Erbitux)    | EGFR        | FDA approved (colorectal, head and neck) |
| Panitumumab (Vectibix) | EGFR        | FDA approved (colorectal) |
| Trastuzumab (Herceptin)| HER2        | FDA approved (HER2-overexpressing breast) |
| Small-molecule inhibitors |             |                      |
| Erlotinib (Tarceva)    | EGFR        | FDA approved (nonsmall cell lung, pancreatic) |
| Lapatinib (Tykerb)     | EGFR, HER2  | FDA approved (HER2-overexpressing breast) |
| XL-647                 | EGFR, HER2, HER4, VEGFR2, EPHB4 | Phase II (nonsmall cell lung) |
| BIBW-2992              | EGFR, HER2  | Phase II (breast, head and neck, nonsmall cell lung, prostate) |
| BMS-599626             | EGFR, HER2, HER4 | Phase I |
| BMS-690514             | EGFR, HER2, HER4, VEGFR2 | Phase I |
| PF-00299804            | EGFR, HER2, HER4 | Phase I |
| ARRY-334543            | EGFR, HER2  | Phase I |

### TABLE 2. Drugs Targeting the c-MET Receptor

| DRUGS                  | TRIAL STAGE          |
|------------------------|----------------------|
| Monoclonal antibodies  |                      |
| AMG-102                | Phase I              |
| OA-SDS                 | Phase I              |
| Small-molecule inhibitors |                  |
| XL-880                 | Phase I/I (gastric, head and neck, papillary renal cell) |
| ARQ-197                | Phase I              |
| PF-02341066            | Phase I              |
| JNJ-388                | Phase I              |
| MGCD-265               | Preclinical          |
| SU-11274               | Preclinical          |
| PHA-665752             | Preclinical          |
ARQ-197 and PF-02341066 are similar oral small-molecule c-Met inhibitors that are in early phase trials. The recommended phase 2 dose for ARQ-197 was determined to be 120 mg twice daily. Common side effects included fatigue, diarrhea, and constipation. Grade 3 elevated liver enzymes were the more severe toxicity.29 Compounds with activity against the HGF/c-MET axis in the preclinical pipeline include MGCD-265, SU-11274, and MGCD-265.

**Insulin-Like Growth Factor Receptor Pathway**

Similar to the EGFR pathway, the insulin-like growth factor receptor (IGFR) signaling system comprises multiple circulating ligands, such as IGF-I, IGF-II, and insulin, interacting with membrane-bound receptors, such as type I IGF receptor (IGF-1R) and insulin receptor (IR) (Fig. 1).30 Most anti-IGFR pathway agents undergoing clinical development are targeted against the IGF-1R.

The IGF-1R is a heterotetramer of two extracellular ligand-binding α subunits and two β subunits with transmembrane and tyrosine kinase domains.30 The IGF-1R undergoes conformational changes and phosphorylation upon ligand binding and recruits insulin-receptor substrates (IRS) and/or Src homology 2 domain-containing (Shc) proteins. The mitogenic, proliferative and/or antiapoptotic signals are then transmitted downstream through the MAPK and PI3k/Akt/mTOR axes.

In normal physiological states, the IGF-1R plays an important role in fetal development and linear growth of many organs, whereas insulin/IR interaction regulates carbohydrate and lipid metabolism. IGF-1R was implicated in the development and maintenance of malignant phenotypes, and interruption of IGF-1R signaling inhibited cancer cell growth and motility in vitro and in vivo models.31 Aberrant activation of the IGF-1R axis was also associated with worse prognosis in many neoplasms, including multiple myeloma, prostate cancer, nonsmall cell lung cancer, and renal cell cancer. Aside from the IGF-1R, abnormally activated IR by insulin or IGF-II stimulation enhances mitogenesis in cancer cells, thus highlighting their therapeutic value.32 However, IR inhibition may lead to type 2 diabetes mellitus, and IGF-I–deficient states have been associated with osteoporotic fractures and ischemic heart disease. These potential toxicities should be taken into consideration during the clinical development of these agents.

There are several IGF-1R–targeting agents in clinical testing, and none are approved by the FDA for general oncological use yet (Table 3). CP-751,871 is a fully humanized IgG2 MoAb antagonist of IGF-1R with preclinical anticancer activities.33 The MoAb interrupts the binding of IGF-I to IGF-1R, IGF-1R autophosphorylation, and induces down-regulation of IGF-1R in vitro and in tumor xenograft models. CP-751,871 was administered intravenously every 21 days in advanced solid-tumor patients.34 In this phase 1 study, the CP-751,871 was escalated to the maximally feasible dose of 20 mg/kg without reaching the maximum tolerated dose. Correlative studies revealed an increased expression of serum insulin and human growth hormone, supposedly through a negative feedback loop. The most common adverse events were hyperglycemia, anorexia, nausea, elevated liver transaminases, hyperuricemia, and fatigue. Investigators analyzed IGF-1R–expressing circulating cancer cells (CTCs) with an exploratory assay. Three patients with detectable IGF-1R–expressing CTCs at baseline were reported to have a decreased level of CTCs after CP-751,871 administration that rebounded at the end of the 21-day period.35

This agent is being evaluated in many tumor types, including myeloma and sarcoma. In a randomized phase 2 study of advanced treatment-naive nonsmall

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**TABLE 3. Drugs Targeting the IGF-1R Receptor**

| DRUGS                      | TRIAL STAGE                        |
|----------------------------|------------------------------------|
| **Monoclonal antibodies**  |                                    |
| CP-721,871                 | Phase III (nonsmall cell lung cancer) |
| AMG-479                    | Phase II (breast, colorectal, nonsmall cell lung, prostate, Ewing sarcoma) |
| IMC-A12                    | Phase II (breast, lymphoma, ovarian, pancreatic, sarcoma) |
| R1507                      | Phase I                            |
| BII8022                    | Phase I                            |
| **Small-molecule inhibitors** |                                    |
| XL-228                     | Phase I                            |
| OSI-906                    | Phase I                            |
| Nordihydroguareacetic acid (NDGA) | Phase I                       |
cell lung cancer, 178 patients received carboplatin and paclitaxel with CP-751,871 (study arm), and 143 patients received carboplatin and paclitaxel without CP-751,871 (control). Patients who responded or who had stable disease in the study arm continued to receive the agent alone, while patients who progressed in the control arm were eligible to receive CP-751,871. Patients in the study arm had a superior response rate (51% vs 36%; \( P < 0.01 \)) and progression-free survival (hazard ratio [HR], 1.18). The agent seemed to be especially active in squamous cell histology. This encouraging result is waiting to be confirmed in a larger phase 3 trial.

AMG-479 is a fully humanized anti–IGF-1R MoAb with broad preclinical antitumor activity. This agent was a potent inhibitor of PI3k/Akt axis with increased antitumor effect when combined with anti-EGFR therapies in pancreatic cancer xenograft models. The phase 1 results of AMG-479 testing were published in an abstract; 16 patients with advanced solid tumors received escalating doses of the agent intravenously. The final dose level reached 20 mg/kg every 2 weeks, and one patient experienced grade 3 dose-limiting thrombocytopenia at 20 mg/kg. No hyperglycemia greater than grade 2 was observed. The agent is currently being tested in non-Hodgkin lymphoma, Ewing sarcoma, and desmoplastic small round-cell tumors. At the time of this writing, phase 1 studies in combination with gemcitabine or panitumumab were being planned.

Other anti–IGF-1R agents under phase 1 evaluation include MoAbs (IMC-A12, R-1507, and BIIB022), small-molecule inhibitors (XL-288, OSI-906), and nordihydroguaiaretic acid.

**Intracellular Signaling Kinases**

**Src**

c-Src is a nonreceptor tyrosine kinase and was the first proto-oncogene to be described. This protein has several functional domains as follows: an N-terminal membrane-association domain (SH4), a variable “unique” domain, a proline-rich sequence-binding domain (SH3), a phosphotyrosine-binding domain (SH2), a tyrosine kinase domain responsible for the catalytic activity of the molecule (SH1), and a C-terminal Tyr530-containing negative regulatory domain that autoinhibits the kinase activity when phosphorylated. The C-terminal interacts with SH2 and SH3 domains when phosphorylated to inactivate Src, whereas loss of the C-terminal phosphotyrosine activates c-Src.

Accumulating data suggest that Src plays an important role in cancer cell mitosis, adhesion, invasion, motility, and progression. Src mediates the mitogenic signals between growth factor receptors, like EGFR, c-Met, and IGF-1R, and downstream signaling cascades, like focal-adhesion kinase (FAK), MAPK, and PI3k/Akt/mTOR (Fig. 1). Dysregulated Src activity has been implicated in the development and progression of several human cancers, including breast, colorectal, lung, ovarian, and hematological malignancies. As such, much interest exists in developing Src-targeting compounds for cancer therapy.

Dasatinib (BMS-354825) is an orally available dual-specific Src and Abl kinase inhibitor with antiproliferative activity against a broad spectrum of hematological and solid cancer cell lines. The compound has less stringent conformational requirements for Abl kinase inhibition than imatinib; dasatinib is active against many imatinib-resistant Bcr/Abl mutants in preclinical models. Dasatinib was granted accelerated approval by the FDA in 2006 for the treatment of chronic myeloid leukemia (CML) in chronic, accelerated, or blast phase, and regular approval for Philadelphia chromosome-positive (Ph1+) acute lymphoblastic leukemia (ALL) with resistance or intolerance to prior therapy. This followed an analysis of four single-arm studies involving 445 patients treated at a starting dose of 70 mg twice daily. The agent achieved significant cytogenetic and hematologic responses in the study population. Toxicities included fluid retention, as well as constitutional, gastrointestinal, and hematological events. Bleeding was reported in 40% of patients, of which 14% had gastrointestinal bleed. The recommended dosing schedules included 70 mg twice daily and 100 mg once daily. As a multitarget inhibitor, dasatinib is being evaluated in breast, lung, colorectal, and pancreatic cancers.

Bosutinib (SKI-606) is another potent oral Src inhibitor with anti-Abl activities. The compound demonstrated antitumor activities in preclinical models, and clinical development in hematological and solid malignancies is underway. AZD-0530, XL-999, and XL-228 are other Src inhibitors undergoing early phase testing. Most of these small molecules have activities against other kinases as well (Table 4).
PI3k/Akt/mTOR Pathway

The phosphoinositide 3’-kinase (PI3k)/Akt/mam-malian target of rapamycin (mTOR) pathway acts as a cellular sensor for nutrients and growth factors and integrates signals from multiple receptor kinases to regulate cellular growth and metabolism (Fig. 1). Although the pathway is often described in a linear–vertical fashion, the regulation of the pathway is rather complex, with internal feedback loops and horizontal “cross-talks” with parallel signaling axes, including the MAPK pathway.

PI3k is a lipid kinase that generates 3’-phosphoinositides (PIP3) at the cell membrane when activated by receptor kinases.45 This leads to recruitment of phosphoinositide-dependent kinase 1 (PDK1) and Akt to cell membrane. The generation of PIP3 is negatively regulated by phosphatase and tensin homologue (PTEN). Akt is activated fully by several enzymes, including PDK1, mTORC2, and IRS-1, which then inhibit the tuberous sclerosis (TSC) protein 2. Inhibition of the TSC complex leads to mTOR-mediated activation of p70s6k and 4EBP1, which regulate cellular translational and transcriptional mechanisms.

The PI3K/Akt/mTOR pathway is attractive as a cancer target for several reasons. Kinases in the pathway are found to be activated in several cancers, resulting from aberrant events including loss of PTEN function, Akt amplification, activating mutations of TSC complex, or constitutive activation of kinases upstream to the pathway.45 Activation of PI3K/Akt/mTOR axis was associated with early events in carcinogenesis and interruption of the pathway-achieved antiproliferation, antisurvival, antian-angiogenic, and proapoptotic effects. Moreover, activation of the pathway was associated with poor prognosis and contributed to chemoresistance in many cancers. mTOR, Akt, PI3k, and PDK-1 are main foci for most small-molecule inhibitors that target the PI3k/Akt/mTOR pathway (Table 5A).

mTOR Inhibitors

Among agents targeting this axis, mTOR inhibitors are furthest along in development. The mTOR protein is a cytosolic serine/threonine kinase serendipitously discovered in the 1990s when the mechanism of action of rapamycin was investigated.46 Rapamycin (also known as sirolimus; Rapamune) is a macrolide isolated from Streptomyces hygroscopicus, a bacterial species native to Easter Island, and has widely been used as an immunosuppressant in organ transplantation. Rapamycin has been evaluated orally as an anticancer agent in solid tumors and pancreatic can-cer.47 mTOR complexes with raptor (regulatory-associated protein of mTOR) and rictor (rapamycin-insensitive companion of mTOR) to form mTOR Complex-1 (mTORC1) and mTORC2, respectively. mTORC1 is downstream to Akt and is susceptible to inhibition by rapamycin and its analogs, whereas mTORC2 is an upstream regulator of Akt, and the activity is up-regulated in certain circumstances as a compensatory response to mTORC1 inhibition.48 Interestingly, recent evidence refuted the belief that mTORC2 is rapamycin-resistant. It has been demonstrated that mTORC2 can, in fact, be inhibited by rapamycin and its analogs in a time-dependent and cell-line dependent manner.49

Temsirolimus (CCI779) is a water-soluble, syn-thetic, rapamycin ester available in oral and intrave-nous formulations.50 This drug was the first of its class to receive FDA approval, and current indica-tions include the treatment of poor-risk untreated advanced renal cell carcinoma patients. In the pivotal randomized trial, the temsirolimus-alone arm achieved longer overall survival (HR, 0.73) and pro-gression-free survival (3.8 months vs 1.9 months; P<0.001) than the interferon-alone arm, whereas the overall survival of patients in the temsirolimus and interferon combination arm was not significantly dif-ferent from that of patients in the interferon-alone arm.51 The median survival times were 10.9 months, 7.3 months, and 8.4 months in the temsirolimus, interferon, and combination groups, respectively. The most common grade 3 or 4 toxicities were asthenia, anemia, and dyspnea. The recommended

| DRUGS | TRIAL STAGE |
|-------|-------------|
| Dasatinib (BMS-354825; Sprycel) | FDA approved (Ph-positive acute lymphoid leukemia, chronic myeloid leukemia) |
| Bosutinib (SKI-606) | Phase 1/II (breast, chronic myeloid leukemia) |
| XL-999 | Phase 1/II (colorectal, nonsmall cell lung, ovarian) |
| AZD-0530 | Phase I |
| KX010107 | Phase I |
The dose of temsirolimus for this indication is 25 mg weekly by intravenous administration. The drug is currently being tested either alone or in combination therapy in tumor types such as melanoma, myeloma, and renal and gynecological cancers.

Everolimus (RAD001) is an oral mTOR inhibitor with antineoplastic activity similar to other rapalogs. In a phase 1 study in solid tumor patients, the optimal biological dose for everolimus was determined to be 20 mg weekly, which achieved the pharmacokinetic and pharmacodynamic changes correlated with antineoplastic effects in animal models. The toxicities were mild and included anorexia, fatigue, rash, mucositis, headache, hyperlipidemia, and gastrointestinal disturbances. When administered continuously, everolimus was well tolerated at a 10 mg dose in patients with refractory or relapsed hematological malignancies. No dose-limiting toxicities were reported, and activity was seen in patients with myelodysplastic syndrome. The dose of 5 mg/m² was the maximum tolerated dose in pediatric solid-tumor patients, and dose-limiting toxicities included diarrhea, mucositis, and elevation of alanine transaminase. No objective tumor responses were observed.

Everolimus demonstrated antitumor activity in metastatic renal cell carcinoma patients who progressed on sunitinib, sorafenib, or both in a randomized phase 3 trial. The patients who received everolimus at 10 mg once daily achieved a longer median progression-free survival than the placebo group (4.0 months vs 1.9 months; HR, 0.3; P<0.0001). Commonly reported everolimus-related toxicities included stomatitis, rash, and fatigue. The agent is being tested as a therapy for nonsmall cell lung, prostate, colorectal, and breast cancers as either a single agent or in combination.

Deforolimus (AP-23,573) is the other mTOR inhibitor currently under clinical testing. During phase 1 testing, the maximum tolerated dose was 18.75 mg/day and mouth sores was the dose-limiting toxicity. Antitumor activity was seen in nonsmall cell lung cancer, carcinosarcoma, renal cell carcinoma, and Ewing sarcoma. Phase 2 studies in sarcoma are ongoing.

**Akt Inhibitors**

Akt, also known as protein kinase B (PK-B), is a serine/threonine kinase upstream to mTORC1 and is implicated in the formation and maintenance of malignancies. Akt is as attractive a target as...
mTOR, if not more so, because of its role in several important cellular functions, including cell-cycle progression, protein translation and transcription, apoptosis, and cellular metabolism. Given Akt’s key role in the axis, Akt inhibition produces theoretically more severe side effects than mTOR inhibition. So far, the development of this class of agents has been disappointing.

Perifosine, a lipid-based derivative of miltefosine, is perhaps the best characterized Akt inhibitor in human testing so far. This compound inhibits Akt translocation to the cell membrane and exhibits in vitro antiproliferative effects in several cancer cell lines. It should be noted, however, that perifosine is a relatively nonspecific AKT inhibitor since it interrupts cell membrane biology. Perifosine was tested as a daily oral dose on a 3-week cycle in patients with advanced solid tumors. The patients reported dose-dependent gastrointestinal adverse events, such as nausea, diarrhea, and vomiting, which led to early therapy discontinuation in an increasing number of patients who were receiving higher dose levels. The tolerated-dose maximum was determined to be 200 mg/day. An alternative loading/maintenance dosing schedule was tested in patients with advanced solid tumors. The maximum tolerated dose was a loading dose of 150 mg every 6 hours for four doses followed by 100 mg once daily for maintenance. The dose-limiting toxicities during the loading period were nausea, diarrhea, dehydration, and fatigue and were manageable with prophylactic antiemetics. However, the side effects were more difficult to manage during the maintenance period. Despite encouraging evidence in preclinical studies, perifosine failed to demonstrate significant single-agent anticancer activity in sarcoma, melanoma, pancreatic, and head and neck cancers during phase 2 tests. Perifosine continues to be evaluated as a single agent and in combinations. Several lipid-based and peptide-based Akt inhibitors are being evaluated preclinically.

Currently, there is limited clinical experience with inhibitors of PI3k and PDK-1. The PI3k inhibitors that are undergoing phase 1 evaluation include PI-103, BGT-226, BEZ-235, XL-765, and XL-147. Current PDK-1 inhibitors are derivatives of staurosporin and celecoxib. UCN-01 is a staurosporin derivative that inhibits multiple kinases, including PDK-1, and has in vitro proapoptotic activity. The drug is synergistic with cytotoxic agents in preclinical studies, but the proapoptotic activity seems to be from inhibition of Chk1, a cell-cycle checkpoint kinase. UCN-01 can be administered intravenously as an initial 72-hour continuous infusion on a monthly schedule or as a short infusion over 3 hours every 28 days with the second and subsequent doses at 50% of the first dose. However, the clinical activity of UCN-01 was not associated with PI3k/Akt/mTOR-pathway inhibition, and its role as a PDK-1 inhibitor remains ambiguous. OSU-03,012 is a celecoxib derivative that inhibits PDK-1 and induces apoptosis in rhabdomyosarcoma cell lines. This drug is currently under preclinical evaluation.

**Mitogen-activated Protein Kinase Pathway**

Anchorage-independent growth, a hallmark of neoplasm, describes the ability of cells to proliferate in the absence of substratum adhesion. Studies into the phenomenon revealed the mitogen-activated protein kinase (MAPK) pathway to be a major connector between extracellular and intracellular stimuli, such as growth factors, cytokines, and oncogenes, and cellular responses related to adhesion, motility, proliferation, and malignant transformation. Ras is a small GTPase protein that transmits activating signals from growth factors, cytokines, and oncogenes, to Raf and then to MAPK kinase (MEK). MEK then phosphorylates and activates the extracellular signal-regulated kinase (ERK, also known as MAPK). Ras, Raf, and MEK are the main targets in this pathway (Fig. 1, Table 5B).

**RAS Inhibitors**

Ras is implicated in the formation and maintenance of malignant phenotypes, and activating Ras mutations were found in greater than 20% of human cancers. K-Ras is the most frequent mutation and can be found in cancers of the pancreas (90%), colon (50%), lung (30%), and thyroid (50%), and in acute leukemias (5% to 30%) and chronic myelomonocytic leukemias (65%). H-Ras and N-Ras mutations are relatively less common. The observation that post-translational modifications, such as farnesylation, are required for membrane localization, and activation of Ras has led to an interest in developing farnesyltransferase inhibitors (FTIs) as Ras inhibitors.

Tipifarnib (R115777), the best characterized FTI so far, has shown promising antiproliferative, proapoptotic, and antiangiogenic activities in preclinical studies. This finding has led to clinical testing in many tumor types, including leukemias, nonsmall
cell lung, prostate, breast, pancreatic, and colorectal cancers. However, tipifarnib failed to show convincing anticancer effects in phase 2 studies. The failure of tipifarnib to improve survival in a phase 3 pancreatic cancer trial further shed doubts on this approach. Preclinical data indicated that the K-Ras–expressing tumor may be escaping tipifarnib inhibition because the oncogenic K-Ras mutant requires a higher concentration for inhibition than the wild-type Ras and H-Ras mutants. In addition, it seemed that clinical efficacy was not related to Ras mutational status, and multiple other signaling pathways were affected in addition to Ras. Lonafarnib (SCH-66,336) and BMS-214662 are the other FTIs being evaluated clinically. Their role in anticancer therapy as a Ras inhibitor remains to be defined. Thus, the focus of development of a more viable approach to inhibit the MAPK pathway has turned to Raf and MEK.

**Raf Inhibitors**

The Raf protein is an important effector of Ras, belonging to a family of three structurally conserved serine-threonine kinases as follows: A-Raf, B-Raf, and C-Raf (Raf-1). Mutations of Ras can result in a constitutively activated MAPK pathway with resultant malignant properties. Wild-type Raf can also be activated in malignant cells from aberrant stimulation by upstream regulators, such as Ras and growth factor receptors. B-Raf mutations are found in nearly 70% of melanoma and also frequently in other solid tumors, such as colorectal and ovarian cancers.

Sorafenib (BAY43–9006) is an oral, dual inhibitor of Raf and vascular endothelial growth factor receptor (VEGFR). The molecule has demonstrated preclinical antineoplastic activity against a wide spectrum of human cancers. It has potent in vitro inhibitory effects against Raf-1, B-Raf, VEGFR-2, platelet-derived growth factor receptor (PDGFR), and VEGFR-3. The dose-limiting toxicities reported during phase 1 development were diarrhea, fatigue, and skin rash. The recommended dose is 400 mg twice daily on a constant basis. Rash, diarrhea, fatigue, and hand–foot syndrome were the common side effects during phase 2 and 3 studies. Correlative studies showed MAPK pathway inhibition in peripheral lymphocytes with a sorafenib dose above 200 mg, indicating potential usefulness of this pharmacodynamic assay for Raf inhibitor development.

Sorafenib is approved by the FDA for treatment of advanced renal cell and unresectable hepatocellular carcinomas. The agent demonstrated significant disease-stabilization effects in advanced renal cell cancer. In the pivotal phase 3 trial, 903 patients with advanced renal cell carcinoma who progressed after one systemic therapy, who had an Eastern Cooper-

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**TABLE 5B. Drugs Targeting the MAPK Pathway**

| DRUGS            | TRIAL STAGE                                      |
|------------------|--------------------------------------------------|
| FTS inhibitors   |                                                  |
| Tipifarnib (R115777; Zarnestra) | Phase III (leukemia) Phase II (bladder, brain, breast, colorectal, leukemia, lymphoma, melanoma, myeloma, pancreatic, sarcoma) |
| Lonafarnib (SCH-66336; Sarasar) | Phase II (brain, breast, genitourinary, head and neck) |
| BMS-214662       | Phase II (leukemia)                              |
| Raf inhibitors   |                                                  |
| Sorafenib (Nexavar) | FDA approved (renal cell carcinoma, hepatocellular carcinoma) |
| RAF-265(CHIR-265) | Phase I                                         |
| XL-281           |                                                  |
| PLX-4032         |                                                  |
| MEK inhibitors   |                                                  |
| Ci-1040          | Development stopped                              |
| PD-0325901       |                                                  |
| AZD-6244 (ARRY-142886) | Phase II (biliary, colorectal, leukemia, liver, nonsmall cell lung, ovarian, pancreatic, thyroid) |
| RDEA-119         | Phase I                                         |
| XL-518           | Phase I                                         |
ative Oncology Group (ECOG) performance status of 0 or 1, with low to intermediate risk (according to the Memorial Sloan-Kettering Cancer Center prognostic score) were randomized to receive sorafenib 400 mg twice daily (n = 451) or placebo (n = 452). The median progression-free survival time was superior in the sorafenib group compared with the placebo group (5.5 months vs 2.8 months; HR, 0.44; P<0.01). The overall survival of the sorafenib group at first-interim analysis was also superior to that of the placebo group (HR, 0.72), although not statistically significant (P = 0.02). In a supporting phase 2, randomized, discontinuation trial, patients with metastatic renal cell carcinoma who initially received sorafenib 400 mg twice daily for 12 weeks during a run-in period and who had stable disease (tumor bidimensional measurements changes of less than 25% from baseline) were randomized to receive sorafenib or placebo (n = 32 and 33, respectively) for another 12 weeks. Patients who had tumor growth of 25% or greater discontinued treatment, and those with tumor shrinkage of 25% or greater continued sorafenib. Median progression-free survival time from randomization was significantly longer for the sorafenib group than the placebo group (24 weeks vs 6 weeks; P = 0.0087). Patients who progressed on placebo crossed over to receive sorafenib until their disease progressed.

In a phase 3 trial involving 602 patients who had advanced hepatocellular carcinoma, an ECOG performance status of 2 or less, and a Child-Pugh class A liver dysfunction, those who received sorafenib 400 mg twice daily achieved a longer median survival time than those who received a placebo (10.7 months vs 7.9 months, respectively; P<0.001). The median time to radiologic progression was also longer in the sorafenib group than in the placebo group (5.5 months vs 2.8 months, respectively; P<0.001).

Despite these successes, the contribution of Raf inhibition to sorafenib’s clinical efficacy is difficult to assess. The success of bevacizumab and sunitinib in renal cell carcinoma indicates that the drug’s antitumor effects may be related more to its antiangiogenic effects. The real benefit of Raf inhibition in cancer therapy will perhaps be answered only by specific Raf inhibitors. XL-281 and PLX-4032 are oral inhibitors that are, reportedly, highly selective against Raf and are currently in phase 1 testing. RAF-265 (CHIR-265), currently in early phase trial, is another oral inhibitor of Raf and VEGFR.

MEK Inhibitors

The MEK protein family consists of MEK1 and MEK2, which have dual-specificity kinase activity and are involved in the phosphorylation of tyrosine and serine/threonine residues. The MEK kinases are highly specific and are known to phosphorylate only Erk1 and Erk2. Constitutively activated MEK, from enhanced upstream stimuli or activating mutations, is tumorigenic and is implicated in a broad spectrum of human cancers. Most of the known MEK inhibitors do not bind to the ATP-docking pocket of MEK. Instead, the inhibitors stabilize the inactive conformation of the kinase by binding to a unique binding site next to the ATP-binding pocket. This unique interaction is thought to explain the high degree of specificity of MEK inhibitors.

CI-1040 is one of the first MEK inhibitors to be developed clinically. The oral agent demonstrated encouraging preclinical effects on tumor proliferation, survival, invasion, and angiogenesis. A phase 1 study showed that the drug had poor metabolic stability and bioavailability, so high doses had to be administered in phase 2 trials. The encouraging antitumor activity seen in phase 1 development was not seen in phase 2 studies, leading to the termination of the agent’s development. Despite this, correlative studies from a phase 1 trial showed adequate target inhibition with CI-1040, and a subsequent research effort was focused on improving upon CI-1040.

PD0325901 is a second-generation MEK inhibitor that is structurally related to CI-1040. PD0325901 has a higher potency, better bioavailability, and more sustained MEK inhibition than CI-1040. Preclinical studies have shown antitumor activities against a broad spectrum of human cancer cell lines. The dose-limiting toxicities reported during phase 1 development included acneiform rash, syncope, and elevated liver enzymes. Visual disturbances such as halos, spots, and decreased acuity were also reported. Antitumor effects were seen in melanoma and in colon and nonsmall cell lung cancers. Phase 2 trials in melanoma, breast, lung, and colon cancers had ceased enrolling patients at the time of this writing.

AZD-6244 (ARRY-142886) is another second-generation, highly selective MEK inhibitor. The drug inhibited Erk phosphorylation and was associated with growth inhibition in cell lines containing B-Raf and Ras mutations and was associated with tumor regres-
sion in preclinical xenograft models. The dose-limiting toxicities during phase 1 development were hypoxia, rash, and diarrhea, and common adverse events included nausea, fatigue, peripheral edema, altered taste, and blurred vision. The recommended phase 2 dose was determined to be 200 mg twice daily. The best response was stable disease observed in three melanoma patients and one nonsmall cell lung cancer patient. AZD6244 is being tested in phase 2 trials of various cancers, including lung, liver, colorectal, pancreatic, and ovarian. Other MEK inhibitors under phase 1 testing include XL-518 and RDEA-119.

**Tumor Vasculature**

**Angiogenesis Inhibitors**

Angiogenesis describes the formation of new blood vessels (neovascularization) from existing vasculature that is vital for normal physiological processes but dysfunctional in malignancies. Folkman, in the 1970s, observed that tumors are unable to grow beyond 2 mm³ unless they are supported by neovascularization. This observation led to one of the most fascinating success stories in cancer therapy development. Vascular endothelial growth factors and receptors (VEGF, VEGFR) are now well validated targets in cancer therapy (Fig. 2). A plethora of anti-VEGF/VEGFR MoAbs and small-molecule inhibitors (Table 6) are now in clinical development. These have recently been reviewed.

**Hypoxia-inducible Factors**

Hypoxia-inducible factors (HIF)-1 and HIF-2 are recognized as important regulators of tumor angiogenesis and metabolism. These functions are determined by HIF-1α and HIF-2α subunits, respec-

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**FIGURE 2.** The therapeutic targets in tumor vasculature are illustrated. Vascular endothelial growth factor receptor-2 (VEGFR) mediates angiogenic signals from ligands such as vascular endothelial growth factor (VEGF), fibroblast growth factor, and hepatocyte growth factor in the formation of new tumor vasculature. Hypoxia-inducible factor 1α (HIF-1α) expression is regulated by MAPK, PI3K/Akt/mTOR, and epigenetic changes, and overexpression leads to tumor formation and neovascularization. Integrin α5β1 are cell surface adhesion receptors on vascular endothelial cells up-regulated in tumor vasculature. These angiogenic nodes are amenable to intervention. Vascular disrupting agents disrupt endothelium of established tumor vasculature and lead to potentially “catastrophic” downstream tumor necrosis. Agents that target these angiogenic nodes are indicated in boxes.
HIF-1α is rapidly degraded by the ubiquitin–proteasome system under normoxic conditions, and it is up-regulated in a hypoxic tumor microenvironment. Aberrant HIF-1α expression, through MAPK or PI3k/Akt/mTOR stimulation, activation of oncogenes, or loss of tumor suppressors (VHL, p53 and PTEN), can lead to tumor formation, making HIF a rational target for anticancer therapy (Fig. 2). The success of temsirolimus in HIF-driven renal cell carcinoma has validated the mTOR target approach to antiangiogenic therapy. Recent preclinical evidence has demonstrated that histone deacetylases (HDAC) inhibitors possess antiangiogenic properties. The HDAC inhibitors are thought to inhibit angiogenesis by up-regulating antiangiogenic genes (eg, activin A, neurofibromin 2, and thrombospondin 1), down-regulating proangiogenic genes (eg, HIF-1α, VEGF, PDGF, basic fibroblast growth factor [bFGF]), promoting HIF–1 degradation, and repressing the function of HIF–1α–containing transcriptional complexes. As such, mTOR inhibitors and HDAC inhibitors are being developed as antiangiogenic agents either as monotherapy or in combination therapies in clinical trials (see sections on PI3k/Akt/mTOR Pathway and Histone Deacetylase Inhibitors). In addition, antagonists to the integrin family have shown encouraging antiangiogenic activity in preclinical studies and are been tested as antiangiogenic agents clinically (see section on Integrins).

Vascular Disrupting Agents

Vascular disrupting agents (VDAs) target endothelial cells of established tumor vasculature and represent an alternate approach to disrupting tumor blood supply (Fig. 2). VDAs have the theoretical advantage of shutting down the vascular supply, which can result in “catastrophic” downstream tumor necrosis. Compared with targeting tumor cells directly, VDAs can be delivered to the endothelium with few impediments, and drug resistance is a lesser problem given the relative genetic stability of endothelial cells. Preclinical studies have shown that the VDA approach induces central tumor necrosis but leaves behind a viable tumor rim that has potential for regrowth.

### TABLE 6. Examples of Anti-VEGF/VEGFR Agents

| AGENTS | TARGETS | TRIAL STAGE |
|--------|---------|-------------|
| Bevacizumab (Avastin) | VEGF-A | FDA approved (breast cancer, colorectal cancer, nonsmall cell/nonsquamous lung cancer) |
| Aflibercept (VEGF-Trap) | VEGF | Phase II (brain, colorectal, gynecological, leukemia, nonsmall cell lung, lymphoma, melanoma, myelodysplasia, pancreatic, prostate, renal, sarcoma, urothelial) |
| IMC-1C11 | VEGFR-2 | Phase I |
| Small-molecule inhibitor | | |
| Sorafenib (Nexavar) | VEGFR-1, -2, -3, PDGFR, Raf, c-kit | FDA approved (See Table 5B) |
| Sunitinib (Sutent) | VEGFR-1, -2, PDGFR, c-kit, Flt3 | FDA approved (gastrointestinal stromal tumor, renal cell carcinoma) |
| Vatalanib (PTK-87) | VEGFR-1, -2, PDGFR, c-kit, c-fms | Phase III (colorectal) |
| | | Phase II (brain, breast, gynecological, nonsmall cell lung, lymphoma, melanoma, mesothelioma, myelodysplasia, neuroendocrine, pancreatic, prostate) |
| | | Phase II (breast, gastrointestinal stromal tumors, gynecological, neuroendocrine, nonsmall cell lung, thyroid) |
| AMG-706 | VEGFR-1, -2, -3, PDGFR, c-kit, Ret | Phase II (gynecological) |
| CP-547,632 | VEGFR-1, -2, -3, bFGF | Phase II (gynecological) |
| Pazopanib (GW-786034) | VEGFR-1, -2, -3, PDGFR, c-kit | Phase II (brain, breast, cervical, gynecological, liver, nonsmall cell lung, mesothelioma, myeloma, nasopharyngeal, neuroendocrine, ovarian, prostate, renal, sarcoma, thyroid, urothelial) |
| ABT-869 | VEGFR-1, -2, -3, PDGFR, c-kit, Flt3 | Phase II (breast, colorectal, liver, nonsmall cell lung, renal) |
| Cediranib (AXD-2171) | VEGFR-1, -2, -3 | Phase II (brain, breast, colorectal, leukemia, liver, nonsmall cell lung, small cell lung, melanoma, mesothelioma, myelodysplasia, ovarian, prostate, renal) |
Tumor endothelium is characterized by high endothelial-cell proliferation and an abnormal basement membrane. Structurally, the tumor vasculature is tortuous, disorganized, and lacks smooth muscle, pericyte, and nerve support. These lead to increased vascular permeability and high interstitial pressure in tumor microenvironments such that a small decrement in perfusion pressure within the vasculature becomes catastrophic to the tumor. In addition, endothelial cells are highly dependent on the tubulin cytoskeleton for motility, invasion, attachment, alignment, and proliferation. The specificity of VADs in exploiting these distinct characteristics of tumor vasculature can potentially spare normal blood vessels from bystander effects.

Most VDAs disrupt the cytoskeleton and cell-to-cell junction of endothelial cells, which leads to increased interstitial pressure and reduced vessel caliber. As plasma leaks from tumor vasculature, blood flow becomes more viscous and rouleaux begin to form. The coagulation cascade is then activated as platelets come in contact with exposed basement membrane, leading to vascular thrombosis and tumor necrosis. VDAs currently under clinical testing can be divided into two categories, tubulin-destabilizing agents and flavonoids (Table 7).

### Tubulin Destabilizers

Combretastatins, structurally related to colchicine, destabilize the endothelial cytoskeleton by binding to tubulin and induce microtubule depolymerization. In addition, the agent disrupts the VE-cadherin/β-catenin complex, resulting in loss of cell-cell contact. Combretastatin A4 phosphate (CA4P) is a lead compound in its class and has antivascular and antitumor activities in preclinical models. Animal studies have shown that CA4P induces a 100-fold blood flow reduction in the tumor, less than 7-fold in spleen, skeletal muscle, and brain, and no significant decrease in heart, kidney, and intestine. The agent is administered intravenously, and three dosing schedules were tested in phase 1 studies. The dose-limiting toxicities included dyspnea, neurological disturbances (syncope, motor neuropathy, ataxia), and cardiac and intestinal ischemia. Responses were seen in thyroid cancer, sarcoma, and adrenocortical carcinoma. The agent was tested in anaplastic thyroid cancer as a single-agent and in combination with carboplatin/paclitaxel and cisplatin/doxorubicin/radiation in phase 2 studies. CA4P is also being tested with carboplatin, carboplatin/paclitaxel, and bevacizumab in patients with solid tumors. Efforts to develop combretastatin and its derivatives continue clinically.

Soblidotin (TZT-1027) is a synthetic derivative of dolastatin-10 with antitumor and antivascular activity against various human tumor xenografts. Dolastatin-10 was isolated from an Indian Ocean mollusc, Dolabela auricularia. The dose-limiting toxicities during phase 1 trials included fatigue, neutropenia, peripheral neuropathy, constipation, hyponatremia, and pain at the infusion site. The agent was tested in sarcoma and nonsmall cell lung cancer during phase 2 development.

### Flavanoids

5,6-dimethylxanthenone-4-acetic acid (DMXAA, AS-1404) is a flavanoid derivative that damages DNA and induces apoptosis in endothelial cells in preclinical models. The exact mechanism that leads to tumor cell death remains unknown but involves NFκB, serotonin, TNF-α, and nitric oxide. DMXAA is administered intravenously, and two dosing schedules were tested in phase 1 studies, once weekly and once every 3 weeks. The dose-limiting toxicities were anxiety, tremor, slurred speech, urinary incontinence, visual disturbances, and possibly left ventricular failure. DMXAA is being evaluated in combination with docetaxel in second-line treatments for advanced nonsmall cell lung cancer in a

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**TABLE 7. Vascular Disrupting Agents**

| AGENTS                        | TRIAL STAGE                        |
|-------------------------------|------------------------------------|
| Tubulin binders               |                                    |
| Combretastatin-derivatives     |                                    |
| Combretastatin A4 phosphate (CA4P) | Phase II (nonsmall cell lung, thyroid) |
| AVE-8062                      | Phase II (sarcoma)                 |
| ZD-6126                       | Phase II (colorectal, renal)       |
| EPC-2407                      | Phase I                            |
| MN-029                        | Phase I                            |
| Dolastatin-derivatives        |                                    |
| TZT-1027                      | Phase II (nonsmall cell lung, sarcoma) |
| Flavanoids                    |                                    |
| 5,6-dimethylxanthenone-4-acetic acid (DMXAA) | Phase III (nonsmall cell lung) |
phase 3 trial. The efficacy of DMXAA is also being explored in ovarian and hormone-refractory metastatic prostate cancers.

VADs are a promising class of chemotherapeutic agents with unique mechanisms of action. In the near future, careful clinical studies and attention to toxicities will clarify the role of VADs in anticancer therapy.

Epigenetic Modulators

Epigenetics is the study of heritable changes in gene expression that are not due to any alteration in the DNA sequence. This field received much attention as researchers sought to explain varied phenotypic expressions throughout an individual’s lifetime in response to external stressors and aging, when the genetic code is almost always constant. Another puzzle is that, despite having the same DNA sequences, there are differences in phenotypes between monozygotic twins and also between cloned animals.

Epigenetic modification can be viewed as “on” and “off” switches for gene expression, where shutting down tumor-suppressor genes or activating oncogenes can lead to dysregulated cellular proliferation and apoptosis. DNA methylation and histone modification are two areas most studied in the development of anticancer therapy (Fig. 1 and 2).

DNA methylation is the addition of a methyl group to specific stretches of a DNA sequence, called CpG islands, often located in or near promoter regions. Methylation of a CpG island, catalyzed by DNA methyltransferases (DNMTs), can lead to silencing of the downstream gene. DNA strands are wrapped around histones to form nucleosomes, and certain genes are silenced when the packing becomes too tight. The addition of an acetyl group to histones (acetylation) loosens this binding and allows expression of tumor suppressor genes. Conversely, deacetylation can result in tumor formation. The process is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Interestingly, DNA methylation and histone deacetylation are closely related in gene silencing through direct interactions between DNMTs and HDACs, such that simultaneous targeting is possibly synergistic.

Global epigenetic disturbance is thought to contribute to carcinogenesis through oncogene activation, loss of imprinting, genomic instability, X-chromosome inactivation, and harmful expression of inserted viral sequences. Studies have found that malignant tissues harbor more epigenetic aberrancies than healthy tissues of the same type, and the degree of epigenetic abnormalities increases during malignant transformation. This finding has led to an interest in developing DNMT inhibitors and HDAC inhibitors, which have enjoyed early successes in hematological malignancies, and testing continues in solid tumors. Side effects from these agents at epigenetic-modulating doses are relatively mild compared with conventional cytotoxic agents.

DNA Methyltransferase Inhibitors (DNMTIs)

5-azacytidine (azacitidine; Vidaza) and 5-aza-2′-deoxycytidine (decitabine; Dacogen), the two most studied DNMT inhibitors, were developed initially as cytotoxic agents to treat leukemia at much higher doses. Interestingly, the agents are predominantly epigenetic modulating, instead of cytotoxic, at a much lower dose when administered over a longer duration. These nucleoside analogs replace cytosine during DNA replication and are, thus, only active during the S phase. The DNA/nucleoside-analog complex then stoichiometrically binds to and inhibits DNMTs. Azacitidine also binds to RNA and interrupts protein translation, whereas decitabine binds to DNA only.

Azacitidine and decitabine were approved by the FDA for treatment of myelodysplastic syndrome in 2004 and 2006, respectively. In a randomized controlled trial involving 191 poor-risk myelodysplastic syndrome patients, the overall response rate (complete or part normalization of blood cell counts and bone marrow morphology) was 23% in the azacitidine arm versus none in the best supportive care arm (control). Patients who responded became transfusion-independent for the duration of their response. Crossover was allowed in the study, and more than one-half of the patients in the control arm received azacitidine. After controlling for crossover effects, the median survival time in the study arm was 18 months compared with 11 months in the control arm, and the quality of life was superior in those initially randomized to receive azacitidine. Myelosuppression, gastrointestinal disturbances, fevers, rigors, ecchymoses, petechiae, injection site reaction, arthralgia, and dizziness were common azacitidine-related toxicities.
The clinical efficacy of decitabine was demonstrated in a randomized controlled trial involving 170 patients with poor-risk myelodysplastic syndrome. The patients who received the study drug had a significantly higher overall response rate (17% complete response plus partial response) than those receiving best supportive care (0%). The median duration of response was 10.3 months and was associated with transfusion independence. There was a trend toward delay in acute myelogenous leukemia transformation or death for patients who received the study drug. The most common adverse events were hematologic, hepatic (hyperbilirubinemia), or pulmonary (pneumonia), whereas gastrointestinal disturbances were mild and infrequent.

However, azacitidine and 5-aza-2’-deoxycytidine are degraded rapidly in the body. Several more stable cytidine analogs, such as 5,6-dihydro-5-azacitidine and 5-fluoro-2’-deoxycytidine, were tested clinically with mixed results. Zebularine, a novel cytidine analog, is more stable and amenable for oral administration. This molecule is an effective inhibitor of DNMT and cytidine deaminase and targets tumor cells preferentially. Zebularine appears to be an optimal lead compound upon which future improvement can be based. Significant interest in developing non-nucleoside DNMT inhibitors exists for the purpose of avoiding toxicities associated with incorporation of nucleoside analogs into DNA. The candidate compounds include procainamide, procaine, RG-108, and MG-98.

Histone Deacetylases Inhibitors

There are several classes of histone deacetylases (HDAC) with nonoverlapping class-specific actions. Class I and II HDAC share a highly conserved zinc-containing catalytic domain that is crucial for HDAC-inhibitor binding. Class I HDAC are located primarily in cell nuclei, whereas class II HDAC transverse between the nucleus and cytoplasm. Class III HDAC are NAD-dependent and are related to the yeast protein Sirt2. This class of proteins is unaffected by inhibitors of class I and II HDACs.

HDAC inhibitors are structurally heterogeneous (Table 8) but share a common ability to recognize and bind to the catalytic zinc-pocket on class I and II HDAC. HDAC inhibitors can induce in vitro cell cycle arrest and differentiation. Cells restart cell cycling and become less differentiated when the agent is withdrawn. Despite encouraging in vitro activity, early compounds derived from natural products, such as depudecin, trapoxin, and trichostatin, have limited in vivo antineoplastic activity, partly because of poor retention, instability, and toxicity.

In addition, HDAC inhibitors cause hyperacetylation of nonhistone proteins, such as HSP90, Raf, Akt, ErbB2, and Bcr-Abl, thus achieving antitumor effects. Specifically, hyperacetylation of HSP90 by HDAC inhibitors leads to the destabilization of HSP90-client protein complexes and achieves similar effects as HSP90 inhibitors. The transcriptional and nontranscriptional effects of HDAC inhibitors render this class of agents attractive for clinical development as drugs that target multiple pathways.

Vorinostat (suberoylanilide hydroxamic acid, SAHA) is the first in its class to be approved by the FDA for cancer therapy. The pivotal trial supporting the approval for treatment of advanced primary cutaneous T-cell lymphoma (CTCL) was a phase 2, single-arm, multicenter, open-label trial involving 74 patients with stage IB and higher disease refractory to two previous bexarotene-containing systemic therapies. The objective response rate was 30%, which lasted for a median duration of 168 days. The supporting study was a single-center phase 2 trial that enrolled 33 patients of similar characteristics and reported a response rate of 31%. The most common side effects were diarrhea, fatigue, nausea, and anorexia. Vorinostat (Zolinza) is being evaluated in combination with carboplatin and paclitaxel in stages IIIB and IV nonsmall cell lung cancer, and as a single-agent in previously treated mesothelioma patients in phase 3 trials.

In preclinical studies, MS-275 (now called SNDX-275), an oral benzamide, had antitumor activity against a broad spectrum of solid and hematological malignancies. The half-life of MS-275 is between 33 and 80 hours, making it amenable to weekly or biweekly dosing. When administered on a 14-day schedule, the maximum tolerated dose was 10 mg/m², and the dose-limiting toxicities were fatigue, anorexia, and gastrointestinal disturbances. The maximum tolerated dose was 6 mg/m² when administered on a weekly schedule for 4 weeks followed by 2 weeks of rest. The dose-limiting toxicities were hypophosphatasia, hyponatremia, and hypoalbuminemia. MS-275 demonstrated a linear pharmacokinetic relationship and HDAC inhibition was observed in
peripheral blood mononuclear cells at all doses studied. The agent is being developed clinically in several tumor types, including melanoma and nonsmall cell lung cancer. The synergy with retinoic acid and azacitidine is also being studied.

FDA approval of vorinostat validated the concept of HDAC inhibition in cancer therapy. However, there are still many hurdles to overcome, and determining the exact mechanism of action of HDAC inhibitors in tumors is difficult. HDAC deacetylate both histone and nonhistone proteins. The nonhistone proteins include the receptor and nonreceptor signaling pathways mentioned in this review. Gene-expression studies revealed that HDAC inhibitors cause complicated cellular changes that include alteration in cell cycles, apoptosis, angiogenesis, and metabolism. The elucidation of underlying mechanisms and relevant biomarkers in specific tumor types will help development of HDAC inhibitors. Also, HDAC inhibitors may be most effective when used in combination therapies rather than as single agents.

**Integrins: Targeting the Extracellular Matrix**

Integrins are heterodimeric cell-surface–adhesion receptors for extracellular matrix (ECM) comprising an α and a β subunit. Unlike receptor kinases, integrins lack intrinsic enzymatic activity. The proteins transduce proliferative, survival, migratory, and angiogenic signals by clustering together with kinases.
and adaptor proteins to form focal adhesion complexes. Blockade of integrin/ECM-ligand interactions inhibits tumor metastasis and angiogenesis and can be achieved by MoAbs, small-molecule peptides, and peptidomimetics (Table 9). Antagonists of pro-metastatic and proangiogenic integrins, such as α5β1, αvβ3, and αvβ5, are under clinical evaluation.

Integrin α5β1 is expressed mainly on vascular endothelial cells and up-regulated together with fibronectin in tumor neovasculature (Fig. 2). Volociximab is a chimeric human IgG4 against α5β1 that inhibits angiogenesis independent of VEGF/VEGFR and induces apoptosis in proliferating, but not quiescent, endothelial cells in preclinical experiments.120 A multicenter, phase 2 study tested volociximab in 40 previously treated patients who had metastatic clear cell renal cell carcinoma.121 The most frequent adverse events were fatigue, nausea, dyspnea, and arthralgia. Stable disease was reported in 80% of the patients. The median time to progression was 4 months, and 79% of the patients were alive at 6 months. Volociximab is being tested in platinum-resistant advanced ovarian cancer and in combination with gemcitabine in metastatic pancreatic cancer.

Integrins αvβ3 and αvβ5 are involved in angiogenesis and expressed in malignancies such as melanoma, gliomas, and cancers of the breast, prostate, and colon. Cilengitide (EMD-121974) is a synthetic cyclic pentapeptide small-molecule inhibitor of αvβ3 and αvβ5 integrins.122 The peptide has demonstrated antiangiogenic and antitumor activities in vitro and in vivo. In a phase 1 trial in patients with advanced solid tumors, cilengitide was administered twice weekly every 28 days and was well tolerated with no dose-limiting toxicities observed at the tested dose levels.123 The agent is being tested in adult and pediatric patients who have refractory glioma.

### Heat Shock Protein: Molecular Chaperone

Heat shock protein 90 (HSP90) is a chaperone protein that assists in proper folding and functioning of client proteins (Table 10; Fig. 3).124 Physiologically, chaperone proteins protect client proteins from degradation and environmental stress, including heat, hypoxia, free radicals, radiation, and chemotherapy. Elevated HSP90 level permits accumulation of proteins of mutated genes in the cell, which may be evolutionarily advantageous to genetic diversity. However, increased HSP90 activity would have, as hypothesized, also permitted the survival of genetically unstable cancer cells.

HSP90 inhibition has several advantages in cancer therapy. First, HSP90 inhibition minimizes toxicities in normal tissues while it maximizes target-specific damage in tumor tissues. This is due to increased HSP90 expression in tumors and preferential accumulation of HSP90 inhibitors in malignant tissues.125 Second, multiple signaling pathways can be targeted simultaneously with HSP90 inhibition because many of the signaling proteins are HSP90 client proteins. Third, because HSP90 inhibition targets multiple pathways, the likelihood that tumor cells will escape a single-target therapy lessens. This is especially relevant in solid tumors because by the time of diagnosis, most solid tumors harbor multiple genetic abnormalities that could have conferred the ability to overcome a single-target therapy.

### Table 9. Integrin Targeting Agents

| AGENTS            | TARGETS          | TRIAL STAGE     |
|-------------------|------------------|-----------------|
| Monoclonal antibodies |                  |                 |
| Volociximab       | α5β1             | Phase II (nonsmall cell lung, melanoma, pancreatic) |
| Vitaxin           | αvβ3             | Phase II (colorectal, melanoma, renal)          |
| CNT-95            | αv               | Phase I         |
| Peptide inhibitors |                  |                 |
| Cilengitide (EMD-121974) | αvβ3     | Phase II (brain, head and neck, leukemia, melanoma, prostate) |
| E-7820            | α2               | Phase I         |
| Peptidomimetics   |                  |                 |
| S247              | αvβ3             | Preclinical     |

### Table 10. Examples of HSP90 Client Proteins

| Protein | Client Proteins |
|---------|-----------------|
| EGFR    | ErbB2           |
| c-Met   | Bcr-Abl         |
| RET     | CDK4            |
| Androgen receptors | FLT3 |
| B-Raf   | NfκB            |
| C-Raf   | NPM-ALK         |
| PS3     | HIF-1α          |

See http://www.picard.ch/downloads/Hsp90interactors.pdf for an up-to-date list of HSP90 client proteins.
tion, preclinical studies have demonstrated that combined HSP90 and proteosome inhibition leads to the accumulation of unfolded proteins, which are insoluble and toxic to tumor cells.126

17-allylamino-geldanamycin (17-AAG), a geldanamycin analog, is the first of its class to enter clinical trials. 17-AAG inhibits HSP90-dependent conformational folding and promotes degradation of oncoproteins, such as ErbB2, mutant p53, C-Raf, and Bcr-Abl, and has shown antitumor activities in preclinical experiments.127 Various dosing schedules were tested in phase 1 trials, which ranged from daily to weekly administration. Hepatotoxicity seen in preclinical studies was confirmed in these trials. In addition, 17-AAG’s dose-limiting toxicities included gastrointestinal disturbances, anemia, thrombocytopenia, dehydration, and hyperglycemia. Common side effects included fatigue, anorexia, diarrhea, nausea, and vomiting. The agent was tested in renal cell carcinoma in a phase 2 study, and none of the 20 patients responded to the dosing schedule of administering the drug every 3 weeks at 220 mg/m² twice weekly.128 Adverse events included elevated liver enzymes, optic neuritis, dyspnea, fatigue, and gastrointestinal problems. 17-AAG is currently being tested in various solid and hematological malignancies, either as a single agent or in combination therapies. However, clinical trials with 17-AAG failed to show anti-tumor efficacy so far, despite extensive testing. It is felt that second-generation and third-generation inhibitors may be needed to adequately determine whether HSP90 is a valid target for cancer therapy. Other HSP90 inhibitors under clinical evaluation include 17-DMAG, IPI-504, and KOS-953 (Table 11). Disruption of HSP-client protein complex can similarly be achieved by the nonhistone effects of HDAC inhibitors (see discussion of Histone Deacetylation Inhibitors under Epigenetic Modulators).
Ubiquitin–Proteasome System

The ubiquitin–proteasome system (UP-S) is an evolutionarily conserved lysosome-independent cellular protein degradation system. Proper functioning of UP-S is vital to cellular functions, such as cell-cycle regulation, signaling, differentiation, and DNA repair. In addition, the system is involved in degrading tumor-suppressing, proapoptotic, and oncogenic proteins. The process involves the attachment of multiple ubiquitin molecules to target substrates that lead to degradation by the proteasome complex (Fig. 3). The UP-S is a multistep process with multiple enzymes amenable to intervention. Preclinical studies have shown that proteasome disruption inhibits proliferation, induces apoptosis, enhances chemotherapy and radiation, and reverses chemoresistance.

Bortezomib (Velcade, PS-341), a boronic acid derivative, was the first proteasome inhibitor to be developed successfully. It blocks proliferation and induces apoptosis of plasma cells independent of their sensitivity to chemotherapy. The agent also inhibits the proteasomal degradation of IkBα and down-regulates the anti-apoptotic NF-κB, leading to the reversal of chemotherapy resistance or enhancement of chemotherapy sensitivity. Bortezomib was recently approved by the FDA for initial treatment of patients with multiple myeloma. The agent was previously approved for multiple myeloma patients who failed at least one prior therapy. In the pivotal, multicenter, open-label trial, 682 previously untreated multiple myeloma patients, who were ineligible for high-dose therapy plus stem-cell transplantation, were randomized to receive melphalan and prednisone combination alone (control group) or with bortezomib. Time to progression, the primary endpoint, was significantly longer for the bortezomib group (24.0 months vs 16.6 months, respectively; HR, 0.48; P<0.001). The overall survival and response rates were also superior in the bortezomib group. The more common toxicities in the bortezomib group included peripheral sensory neuropathy, gastrointestinal symptoms, and herpes zoster.

The proteasome inhibitor also received FDA approval for treatment of mantle cell lymphoma patients who had received at least one prior therapy. A total of 155 patients who failed one prior therapy received bortezomib in a phase 2 open label study. The overall response rate was 31%, and the median duration of response was 9.3 months. Responses were assessed according to 1999 International Workshop Response Criteria. The toxicity profile was similar to those observed in other bortezomib studies. The most commonly reported adverse events included asthenia, peripheral neuropathy, gastrointestinal complaints, and anorexia.

Other proteasome inhibitors in early phase trials include CEP-18,770, RP-171, and NPI-0052 (Table 12).

Direct Apoptosis Enhancers

Apoptosis, or programmed cell death, is a highly regulated process vital to fetal development and postnatal physiological functions. The ability to evade apoptosis through a gain of antiapoptotic function or loss of proapoptotic signals is a hallmark of cancer. The apoptotic machinery can be categorized into extrinsic and intrinsic pathways, albeit this is an oversimplified dichotomy (Fig. 3). The extrinsic pathway is activated through cell-surface death-mediating receptors, such as tumor necrosis factor (TNF) receptor 1 (TNFR1), TNFR2, death receptors 3 to 6 (DR3–6), and CD95/Fas/Apo1. The intrinsic pathway is activated by intracellular stimuli,
including cytotoxic drugs, cellular stress, cell detachment, hypoxia, and DNA damage. These signals converge on mitochondria, inducing leakage of proteins, such as cytochrome c and Smac/Diablo, from mitochondrial intermembrane space into cytosol. This mitochondria-dependent pathway is tightly regulated by a variety of antiapoptotic factors, such as Bcl2 and Bcl-XL, and proapoptotic proteins, including Bid, Bad, and Bim.

**TNF-related Apoptosis-inducing Ligand (TRAIL)**

TRAIL is a member of the TNF superfamily that induces apoptosis and exerts antitumor activity against a variety of cancer cell lines and tumor xenograft models. The preferential expression of death receptors on cancer cells and the relative inactivity of soluble TRAIL in normal, healthy tissues make the TRAIL-DRs axis an attractive target for highly selective cancer therapy. DR4 and DR5 (or TRAIL-R1 and TRAIL-R2) are the main agonistic receptors for TRAIL. DR1, DR2, and osteoprotegerin are decoy receptors and may theoretically compromise the clinical efficacy of TRAIL-like agents by antagonizing apoptotic signals from TRAIL.

Early development of TNF-α and recombinant Fas ligand as anticancer agents was fraught with severe liver toxicity, although this toxicity was later found to be related to manufacturing artifacts. Several recombinant forms of TRAIL with alternate chemical structures have since been developed, such as Apo2L/TRAIL (Table 13). Several DR4-specific and DR5-specific, agonistic MoAbs are entering clinical testing. With no de novo decoy receptors, they are theoretically more advantageous than TRAIL-like ligands, although liver toxicity remains a concern.

Mapatumumab is a fully humanized IgG1 agonist of DR4 with a tolerable toxicity profile during phase 1 development. Forty-nine solid tumor patients received 158 courses of escalating doses of mapatumumab. The most common adverse events were grade 1 or 2 fatigue, fever, and myalgia. Hematological toxicity was not clinically significant. The most severe toxicity was one grade 4 acute respiratory distress syndrome. Grade 3 elevation in liver enzymes was reported in two patients who had underlying liver disease. The agent is in phase 2 testing. AMG-655, a DR5 MoAb agonist, is another apoptosis inducer currently in clinical development.

### Survivin

Survivin, encoded by *Birc5*, is an inhibitor of apoptosis that is undetectable in normal, fully differentiated tissues but is highly expressed in a broad spectrum of neoplasms. Increased expression of survivin is a risk factor for cancer progression, recurrence, and poor prognosis. In vitro down-regulation of survivin expression by an antisense approach increases spontaneous apoptosis and inhibits cancer cell proliferation. LY2181308 is an antisense molecule currently in phase 1 testing. YM-155 and EM-1421 are small-molecule inhibitors of *Birc5* transcription. The most common treatment-related toxicities for YM-155 in a phase 1 trial were mucosal inflammation and elevated prothrombin time. This compound is currently being tested in melanoma and prostate cancer.

The *Bcl-2* gene is a proto-oncogene first identified in follicular B-cell lymphoma with antiapoptotic properties when transformed. Up-regulation of *Bcl-2* is a common cause of tumorigenesis and chemotherapy resistance in multiple tumor types and correlates with poor survival and disease progression. Reduced *Bcl-2* expression by antisense oligonucleotide in preclinical studies exerts cytotoxic and cytostatic effects on leukemia.

### TABLE 13. Direct Apoptosis Enhancers

| ENHANCER | TRIAL STAGE |
|----------|-------------|
| TRAIL Agonist | |
| Mapatumumab | Phase II (liver, nonsmall cell lung, lymphoma, myeloma) |
| AMG-655 | Phase II (colorectal, nonsmall cell lung, pancreatic, sarcoma) |
| Apo2/TRAIL | Preclinical |
| Survivin Antagonist | |
| YM-155 | Phase II (nonsmall cell lung, lymphoma, melanoma, prostate) |
| LY-2181308* | Phase II (leukemia, prostate) |
| EM-1421 | Phase I |
| ISIS-23722* | Phase I |
| Bcl2 Antagonist | |
| Oblimersen* (Genasense) | Phase III (melanoma, myeloma) |
| | Phase II (breast, colorectal, gastric, gastrointestinal stromal tumors, leukemia, lymphoma, nonsmall cell lung, small cell lung, Merkel cell, prostate, renal) |
| Obatoclax (pan-Bcl) | Phase I/II (small cell lung, lymphoma, myelodysplasia, myeloma) |
| ABT-263 | Phase I |

*Antisense oligonucleotide.
mia and lymphoma cells and is enhanced when combined with other chemotherapeutic agents. Oblimersen, a Bcl-2 antisense oligonucleotide, has demonstrated encouraging preclinical activity against melanoma and breast cancer cell lines but has not achieved statistically significant survival-time improvement during its phase 3 evaluation for treating melanoma and chronic lymphocytic leukemia.144,145

Several approaches exist to target the antiapoptotic Bcl-2. Firstly, members of the antiapoptotic Bcl-2 family (Bcl-2, Bcl-XL, A1, Mcl-1) share several Bcl-2 homology domains (BH1 to BH4).146 Of these, BH-3 has been a target of interest. BH-3 domain-specific peptides are being developed by mimicking endogenous Bcl-2 antagonists. These peptides are thought to mitigate the anti-apoptotic function of Bcl-2 proteins. Secondly, BH-3 mimetic SAHB (stabilized alpha helix of Bcl-2 domains) is also under development, albeit pre-clinical, which overcomes the in vivo instability of endogenous BH-3 targeting peptides.147 It is more difficult to identify small-molecule inhibitors with high affinity for this target because the candidate molecule has to interrupt protein-protein interaction, which is more challenging compared with inhibiting the enzymatic activity of tyrosine and serine/threonine kinases. ABT-737 inhibits Bcl-2, Bcl-XL, and Bcl-W in preclinical studies but suffers from limited bioavailability.136 ABT-263 is a second-generation molecule with preclinical antitumor effects based on ABT-737 and is currently in phase 1 testing.148 Obatoclax (GX015-070) is a pan-Bcl inhibitor with selectivity for Mcl-1 that is currently in early phase clinical testing.149

PARP Inhibitors: Therapeutic Sensitizers

Poly(ADP-ribose) polymerase (PARP) acts as a cellular sensor for DNA breaks and facilitates DNA repair by engaging mechanisms such as base excision repair (BER), homologous recombination repair, and nonhomologous end-joining pathways.150 PARP-1 is the principal isoform involved in the cellular process, whereas PARP-2 and other PARP members constitute the residual activities (=10%) when PARP-1 is inhibited. Enhanced PARP activity results in depletion of NAD+ and energy stores, leading to cell necrosis. Multiple mechanisms exist to counter this homeostatic state.

Increased PARP activity confers tumor-cell resistance to DNA-damaging agents, such as platinums, alkylators, topoisomerase inhibitors, and radiation treatments. In fact, PARP inhibitors potentiate their antitumor effects in in vitro and in vivo models. PARP inhibitors developed so far are competitive inhibitors of NAD+, and early molecules lack specificity and potency, whereas newer generation PARP inhibitors have a more optimal chemical structure.151 PARP inhibitors are speculated to be better suited as single-agent therapies in tumors with specific DNA-repair defects, such as BRCA mutations, and in combination with therapeutics that target DNA-repair pathways in wild-type tumors.152

AG-014699 was the first PARP inhibitor to enter clinical trial. Preclinical studies have shown that AG-014699 causes growth inhibition when it is combined with irinotecan and radiation and causes tumor shrinkage with temozolomide in tumor xenograft models.153 AG-014699 was administered with temozolomide to 27 patients with solid tumors in a phase 1 dose-escalation study, and no dose-limiting toxicity was observed up to a dose of 12 mg/m² AG-014699 and a dose of 200 mg/m² temozolomide.154 This combination at the mentioned dosages was then tested in a phase 2 metastatic malignant melanoma trial.155 However, increased temozolomide-related myelosuppression and one toxic death were observed at this dose level, and the temozolomide dose was reduced to 150 mg/m² in 12 of 40 patients. Partial response was seen in 7 (18%) patients.

PARP inhibitors play a potential role in enhancing temozolomide’s efficacy in the treatment of intracranial neoplasm. Temozolomide is an oral alkylating agent with a high penetration of the central nervous system and is approved by the FDA for treatment of malignant glioma. PARP inhibitors disrupt the BER, an important mediator of temozolomide resistance, and enhance the efficacy of the alkylator. In vivo studies have shown that PARP inhibitors significantly enhance temozolomide’s antitumor effects against intracranial neoplasms, such as glioma, lymphoma and melanoma.156 BSI-201 is being developed clinically with temozolomide and radiation therapy for the treatment of newly diagnosed malignant glioma. Other PARP inhibitors in clinical development include INO-1001, KU-59,436, and ABT-888 (See Table 14).
Mitotic Kinase Inhibitors

Mitosis, a central event in tumor growth, is highly regulated to ensure accurate and equal segregation of genetic materials from parent cells to daughter cells. Main effectors of this process are mitotic spindles and centrosomes. Disruption of the process results in aneuploidy, and genomic instability renders the cellular condition optimal for apoptosis to occur. The rationale of targeting mitosis in cancer therapy is substantiated by the successful clinical development of tubulin-disrupting agents, such as vinca alkaloids and taxanes. These agents interfere with proper formation and function of mitotic spindles and block deregulated mitosis in cancer cells. Novel approaches aimed at interrupting the non-structural components of mitosis, or mitotic kinases, have recently entered clinical testing.

Members of the Aurora kinase family are serine/threonine kinases that are highly conserved throughout eukaryotic evolution and exist in three structurally related homologs in mammalian cells, Aurora A, Aurora B, and Aurora C. These key mitotic regulators have distinct subcellular localization and functions. Aurora A localizes to centrosomes from the S phase to mitosis exit and primarily regulates centrosome function and mitotic spindle formation. Aurora B is part of the chromosomal passenger protein complex, localizing to centrosomes and mitotic spindles during different phases of mitosis. The kinase functions to ensure accurate chromosomal separation and cytokinesis. Aurora C is specifically expressed in testes and is involved in spermatogenesis. Its role in tumorigenesis is unclear.

Aurora A received initial attention as a cancer target after it was found to be amplified or overexpressed in many tumor types and shown to be oncogenic by inducing genomic instability and amplification of centrosomes. Knockdown and mutational studies that disrupt Aurora A function induce mitotic arrest and apoptosis, making Aurora A a prime therapeutic target. Ideally, molecules that inhibit only Aurora A should result in the formation of monopolar mitotic spindles and mitotic arrest followed by rapid induction of apoptosis. Cells treated with inhibitors specific to Aurora B typically go through a cell cycle without dividing (cytokinestasis) and become polyploid, abnormally large, and multinucleated.

However, selection of small-molecule inhibitors specific to Aurora A or B is challenging. Cell-based assays with most of the molecules developed so far have shown formation of bipolar spindles and cells that undergo apoptosis only several days after treatment, which is consistent with the phenotype observed in Aurora B-only inhibition. Later it was shown that Aurora B must be present and active to achieve the expected phenotype of Aurora A inhibition. Thus, dual inhibition of Aurora A and B will result in the phenotype of Aurora B-only inhibition. As one can see, the definition of Aurora A or B inhibitors is complicated and may impair the study of target effects during clinical testing.

AZD-1152, a pyrazoloquinazoline derivative, is a highly potent and selective inhibitor of Aurora B. This molecule induces chromosome misalignment, halts cell division, and induces apoptosis in vitro studies. AZD-1152 inhibits proliferation in leukemic cells, and in colon and lung xenografts. The agent was administered as a 2-hour intravenous infusion at a weekly interval, and dose-limiting toxicity of grade 4 neutropenia occurred at a dose level of 450 mg. The 300-mg dose was declared the tolerable dose. Five of 13 (38%) patients remained on therapy for more than 12 weeks, and clinical development continued.

MK-0457 (VX-680) is a 4,6-diaminopyrimidine that targets the ATP-binding site common to all Aurora kinases, delays in vitro mitotic progression, and inhibits growth in tumor xenograft models. Sixteen patients with refractory solid tumors were enrolled on a phase 1 trial to receive MK-0457 by constant 5-day intravenous infusion every 28 days at 0.5 mg/m² to 12 mg/m² per hour. The dose-limiting toxicity was asymptomatic neutropenia for more than 5 days at 12 mg/m² per hour. Three patients achieved stable disease. Synergism between MK-0457 and other tubulin-interrupting agents, such as docetaxel, is being examined. Other Aurora kinase inhibitors in early phase clinical testing are included in Table 15.

### TABLE 14. PARP Inhibitors

| INHIBITOR | TRIAL STAGE |
|-----------|-------------|
| AG-014699 | Phase II (ovarian) |
| BSI-201  | Phase II (brain) |
| INO-1001 | Phase I |
| KU-59436 | Phase I |
| ABT-888  | Phase I |
| GPI-21016| Preclinical |

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TABLE 15. Aurora Kinase Inhibitors

| INHIBITOR   | TRIAL STAGE                  |
|-------------|------------------------------|
| AZD-1152    | Phase II (leukemia)          |
| PHA-739358  | Phase II (chronic myeloid leukemia, prostate) |
| AT-9283     | Phase III (leukemia)         |
| CYC-116     | Phase I                      |
| Hesperadin  | Phase I                      |
| MK-0457 (VX-680) | Phase I                |
| MLN-8054    | Phase I                      |
| AS-703569 (R-763) | Phase I               |
| PF-03814735 | Phase I                      |
| MLN-8237    | Phase I                      |

**Kinesin Spindle Protein**

Kinesin spindle protein (KSP) (Hs Eg5) is a member of the kinesin superfamily of molecular motors that function to transport cellular organelles, vesicles, and microtubules in an ATP-dependent fashion. Disruption of KSP function causes mitotic arrest and eventual apoptosis. Monastrol is the first KSP-specific inhibitor and causes collapse of pre-existing bipolar spindles and cell-cycle arrest. Subsequently, other more potent KSP inhibitors were developed. Ispinesib (SB-715992) is a potent selective inhibitor of KSP that affects KSP ATPase and causes mitotic arrest and growth inhibition in several human cancer cell lines. In a phase 1 trial, ispinesib was administered intravenously every 21 days at escalating doses to 46 patients with solid tumors. Dose-limiting toxicities occurred at the 21 mg/m² level and included grade 4 neutropenia of more than 5 days duration and neutropenic fever. The 18 mg/m² dose was established as the recommended phase 2 dose. Common drug-related adverse events included fatigue, leucopenia, and anemia. In phase 2 development, ispinesib is being tested in head and neck squamous cell carcinoma and malignant melanoma and in combination with docetaxel.

**Conclusion**

Advances in molecular biology have led to an explosion in the number of potential cancer targets and a rich pipeline of anticancer drugs. It is not our intent to provide an exhaustive account of every cancer drug in early phase trials. Instead, we hope to have provided a broad introduction to the classes of chemotherapeutic agents that are likely to enter clinical practice in the near future. With time, we can expect more drugs against targets that have already been validated clinically, such as HER and VEGF, and the emergence of novel agents against new classes of targets that have been validated preclinically such as Notch, Hedgehog, and JAK-STAT. Novel and innovative clinic trial designs will be needed to efficiently test all of these agents.

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