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ANTICANCER DRUGS AND MARKETS

ANTICANCER AGENTS FROM ANTIBIOTIC SOURCES
PART II — NOVEL AGENTS IN DEVELOPMENT

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New drugs in development derived from antibiotic sources (Exhibit 1) involve novel agents, various formulations of commercially available drugs, and prodrugs and targeted cytotoxics representing creative approaches to enhance the delivery and efficacy of these drugs while at the same time limit their toxicity.

NOVEL ANTICANCER ANTIBIOTICS

Drugs based on naturally occurring antibiotics represent one of the most important classes of anticancer agents. As illustrated in Part I of this series, commercialized cytotoxic antibiotics, based on various Streptomyces species, represent prominent chemotherapeutics in wide use. Currently, microbial products predominate among the agents under development by the Division of Cancer Treatment and Diagnosis (DCTD) of the National Cancer Institute (NCI). Also, academic researchers with financial backing by the government have expanded research in this area to the study of organisms from diverse environments, such as marine ecosystems, and deep terrestrial subsurface layers that have proven to be invaluable sources of novel bioactive antibiotic metabolites.

Discovery of novel antibiotics, and synthesis of naturally occurring compounds, has accelerated in recent years, as new methodologies have dramatically improved drug screening and synthesis of novel analogs with more favorable performance characteristics than their predecessors. In the last decade, there was great progress in identifying, characterizing, and analyzing biosynthetic gene clusters that specify the assembly of complex natural products. Currently, discovery of new metabolites is not limited to bioassay-guided screening of microbial or plant-derived extracts. Rather, combinatorial biosynthesis is used as a powerful approach to create novel chemical entities based on natural-product biosynthetic systems. This approach has been particularly important in regard to polyketide-derived secondary metabolite biosynthesis in a variety of microorganisms. Cloning and characterization of antibiotic biosynthesis gene clusters from various classes of natural antibiotics and genetic engineering of antibiotic biosynthesis has also enabled researchers to create 'unnatural' versions of natural products for drug discovery and development.

There is incredible diversity, not only in the composition of newly discovered antibiotics, but also in the anticancer mechanisms of action of these agents. Each subclass of antibiotics acts by unique mechanisms and, in most cases, exhibits multiple mechanisms of action. Most antibiotics exert their antitumor effects by interfering with the structure and function of DNA. They may be minor groove binders (MGB), or inhibit topoisomerase (topo) I or II, or interfere with the cell cycle. In addition to DNA damage, antibiotics exert some of their cytotoxic effects by direct binding to DNA, inhibiting transcription of certain genes. Such inhibition leads to disruption of various pathways implicated in tumorigenesis and metastasis.

Despite their promise, the road to development of viable drugs form natural antibiotic sources has been fraught with obstacles and strewn with failures. Several novel analogs of commercially available anthracyclines recently failed in the clinic, either because of unacceptable toxicity, or lack of activity, or both. In 2000, Pharmacia discontinued development of methoxymorpholinyl doxorubicin (MMDX; PNU-152243) while in phase I/II in advanced ovarian cancer. Similarly, drugs derived from many other antibiotic sources also failed in the clinic either because of low effectiveness, unacceptable toxicity, or both. Suradista [PNU-145156E NSC 651016, PNU-153429 (formerly FCE-26644)], a sulphonated distamycin A derivate that blocks angiogenesis and neovascularization, also failed in the clinic because of unacceptable toxicity. Nevertheless, the quest to find novel agents continues.

Actinonin Analogs

(S,S,R)-(-)-Actinonin, first isolated by MLH Green and RB Singh from the Malayan strain of Actinomyces, was initially developed as an antibacterial agent, but has since been shown to exhibit cytotoxic properties. In studies performed at Memorial Sloan-Kettering Cancer Center (MSKCC; New York, NY) actinonin exhibited cytotoxicity towards tumor cell lines *in vitro*, inducing G1 arrest, and induced apoptosis in human leukemia and lymphoma cells. It was also efficacious in leukemia in AKR mice, causing minimal toxicity.

To further explore the potential of actinonins as anticancer agents, a rationally designed library of analogs was produced and synthesized by MSKCC researchers, using both solution and solid-phase parallel synthesis. Actinonin was divided retrosynthetically into four sets of diversity.

The biologic activity obtained from this library has been analyzed, and a structure activity relationship (SAR) has emerged. Results show very promising cytotoxicity against human ovarian, prostate, lung and breast cancer cell lines (Borella C, etal, AACR02, Abs. 1032:206, and AACR03, Abs. 3474).

Anthracyclines

Because doxorubicin, and daunorubicin differ from each other only by a single hydroxyl group, researchers have worked hard to find analogs of doxorubicin that are generally less toxic in the acute setting, are not associated with cardiomyopathy, can be administered orally, and/or exhibit different, or greater, antitumor efficacy. However, modification of daunomycin or doxorubicin, generally failed to yield dramatically improved agents, mostly because structural and other physicochemical data had not been incorporated into the design process.

Subsequently, a new generation of bisanthracyclines that form ultratight DNA complexes was created using detailed structural data (Chaires JB, etal, J Med Chem 1997;40: 261-266), accumulated over several decades by many investigators. Because there is a direct relationship between anthracycline activity and DNA affinity, the chemical conversion of monointercalative anthracyclines to bisintercalative forms is expected to dramatically increase DNA affinity and, therefore, activity (Hu GG, etal, Biochemistry 1997;36:5940-5946).

GPX-100 and GPX-150, under development by Gem Pharmaceuticals (Birmingham, AL), in collaboration with Access Oncology (New York, NY), are novel analogs of doxorubicin that, in preclinical studies, maintained the anticancer effects of doxorubicin, with virtually none of its chronic cardiotoxicity. Based on its mechanism of action and preclinical data, GPX-100 may be useful in the treatment of breast cancer, sarcoma and certain hematologic malignancies.

In an open-label, multicenter, dose-escalation, phase I clinical trial (protocol IDs: GEM-97-002, MAYO-IRB-153-98, NCI-V98-1441), initiated in December 1998, GPX-100 was investigated in patients with refractory solid tumors. Trial objectives were to determine the maximum tolerated dose (MDT) of GPX-100, and establish the toxicity and, possibly, effectiveness of this treatment. A maximum of 30 patients were to be accrued for this trial, to be treated with IV GPX-100, once every 3 weeks, with 2 courses, in the absence of disease progression or dose-limiting toxicity (DLT). Treatment may continue for up to 6 courses (4 courses in those exposed to prior doxorubicin-based chemotherapy) in patients with responding or stable disease. This trial was completed in February 2001.

MEN 10755, in development by Menarini (Florence, Italy), is a novel third-generation anthracycline, and the first disaccharide anthracycline. MEN 10755 is a potent topo II inhibitor and, consequently, a strong inductor of DNA breaks and apoptosis in human tumor cells. Its range of activity is wider than that of doxorubicin. Histologic and functional evidence indicates that, unlike doxorubicin, MEN 10755 does not produce a long-term progressive cardiotoxic effect in rats.

MEN 10755 was identified by a screening system for new anthracyclines totally based on human tumor material. In the first step of process, the relative cytotoxicity of all new compounds was compared to that of doxorubicin, in a panel of human tumor cell lines that were well characterized for resistance factors and p53 status. Only a few analogs were selected through this step for further evaluation. The second step investigated the therapeutic efficacy and tolerability of the analog, compared to doxorubicin, in a series of human tumor xenografts selected for presenting natural or acquired (by known mechanisms) resistance to the parent drug. Cardiotoxicity in mice was also studied. Cellular and molecular pharmacology studies were also carried out. MEN 10755, an analog from the series of disaccharide anthracycline analogs screened, was selected for clinical investigation (Pratesi G and Monestiroli SV, Curr Med Chem 2001;8(1):9-13).

In preclinical trials MEN 10755 was superior to currently used anthracyclines as an antitumor agent, with 58% of positive results versus 25% for doxorubicin, and was also associated with lower cardiotoxicity. The evidence that MEN 10755 was also more effective than doxorubicin as an inducer of apoptosis, provided additional insights to better understanding the cellular processes that confer sensitivity to anthracyclines (Perego P, etal, Curr Med Chem 2001;8(1):31-37).

The cellular pharmacokinetics of MEN 10755 substantially differed from that of doxorubicin, exhibiting reduced cellular accumulation, and a different subcellular distribution (higher cytoplasmic/nuclear ratio). In spite of the lower nuclear concentration, MEN 10755 was as potent as doxorubicin in eliciting DNA single- and double-strand breaks, G2/M cell arrest, and apoptosis. MEN 10755 and doxorubicin shared a common DNA cleavage pattern, but the extent of DNA cleavage stimulation induced by MEN 10755 was greater than that produced by doxorubicin. MEN 10755-stimulated DNA cleavage sites were more persistent than those induced by doxorubicin, suggesting a more stable interaction of the drug in the ternary complex. In spite of the apparently unfavorable cellular pharmacokinetics, MEN 10755 was as potent as doxorubicin in inducing topoisomerase-mediated DNA damage. However, MEN 10755 efficacy as apoptosis inducer and antitumor agent could not be adequately explained on the basis of DNA damage mediated by topo II, thus supporting additional cellular effects that may be relevant in cellular response (Bigioni M, etal, Biochem Pharmacol, 1 Jul 2001;62(1):63-70).

In athymic nude mice bearing a human ovarian carcinoma xenograft (A2780), accumulation of MEN 10755 was lower compared with doxorubicin in all tissues investigated (tumor, heart, kidney and liver). This reduction was

more significant in normal than tumor tissues. Moreover, in spite of the reduced drug uptake by tumor tissues, MEN 10755, administered in its optimal regimen, displayed enhanced antitumor efficacy compared with doxorubicin. MEN 10755 effects on tumor growth paralleled a significant activation of apoptosis. The pattern of tissue distribution and the pharmacokinetic behavior of MEN 10755 were consistent with a better tolerability, which allowed a higher cumulative dose to be delivered. The superior therapeutic efficacy of the analog over doxorubicin, in spite of a reduced tumor accumulation, may be attributed to increased tumor selectivity (Gonzalez-Paz O, etal, Eur J Cancer, Feb 2001;37(3):431-7).

Two phase I clinical trials with MEN-10755, coordinated by EORTC, have been completed, and 6 phase II clinical trials were initiated in 2000 to verify therapeutic activity of the compound on different types of solid tumors, including ovarian, breast, prostate, and lung cancer, and sarcoma. MEN-10755 was evaluated in two pharmacokinetic phase I clinical trials with different dosing schedules in adults with solid refractory malignancies. MEN-10755 was studied as a 15-minute IV infusion, administered either once every 3 weeks, or once every week for 3 weeks, followed by 1 week rest. Compared to epirubicin and doxorubicin, the pharmacokinetics of MEN-10755 were characterized by an approximately twofold shorter terminal half-life, a much lower total plasma clearance, and a much smaller volume of distribution (Bos AE, etal, Cancer Chemother Pharmacol, Nov 2001;48(5):361-9).

A phase I clinical trial was performed at the University Hospital (Antwerp, Belgium) with MEN-10755, in patients with solid tumors to determine MTD, DLT, establish a safe dose and assess antitumor activity. According to the protocol, MEN-10755 was administered at a starting dose of 15 mg/m^2/week by a short IV infusion, weekly, for 3 weeks; cycles were repeated every 28 days. All in all, 24 patients were administered 55 cycles at MEN-10755 doses of 15, 30, 40 and 45 mg/m^2. MDT of MEN-10755 was established at 45 mg/m^2, because of neutropenia and a decrease in cardiac function in one patient. Among chemotherapy-naive patients treated at an MEN-10755 dose of 40 mg/m^2/week, there was one DLT (Grade 4 neutropenia). At this dose, 3/6 chemotherapy-pretreated patients developed DLT during their first treatment cycle, one patient developed Grade 4 thrombocytopenia, one Grade 4 neutropenia, and one Grade 3 acute hypersensitivity reaction resulting in treatment discontinuation. At this dose level, one other patient was not treated on day 15 as planned because of Grade 3 neutropenia. There were no responses. The recommended phase II dose of MEN-107555 is 30 mg/m^2 in pretreated patients, and 40 mg/m^2 in chemotherapy-naive patients, weekly, for 3 consecutive weeks, followed by 1-week rest (Schrijvers D, etal, Ann Oncol, Mar 2002;13(3):385-91).

A phase II clinical trial, being conducted in Europe, is investigating the activity and safety profile of MEN 10755 in patients with locally advanced or metastatic ovarian cancer, who failed first-line platinum- and/or taxane-based chemotherapy, or relapsed within 6 months after prior chemotherapy. MEN 10755 at 80 mg/m^2 (dose level 0) was administered IV, every 3 weeks, over 30 minutes. Dose was escalated to 90 mg/m^2 (dose level 1) after the 1st cycle if toxicity was ≤Grade 1. As of May 2002, a total of 54 courses had been administered to 18 patients. Drug-related hematologic toxicity was moderate involving 6 episodes of Grade 3/4 leukopenia, 1 episode of Grade 3 febrile neutropenia, 10 episodes of uncomplicated Grade 3/4 neutropenia, and 2 cases of Grade 3 anemia. Other Grade 3/4 toxicities were fatigue (n=4), and one case each involving stomatitis, general health deterioration, anorexia, nausea, vomiting, abdominal pain, and hyponatremia. There was 1 PR, and disease stabilized in 8, and progressed in 5. Among 3 patients not evaluable for response, 1 died of malignancy immediately after the second course, another had no measurable lesions at re-evaluation, while the third died early, possibly because of treatment-related Grade 4 stomatitis in presence of clinical signs of disease progression (Caponigro F, etal, EORTC-NCI-AACR02, Abs. 42, and Eur J Cancer, Nov 2002;38(Suppl. 7):18).

An ongoing, multicenter, phase II clinical trial (protocol ID: EORTC-16006-30005; MAC-07) of single-agent MEN-10755 in progressive prostate cancer that did responded to hormone therapy, to enroll a total of 18-32 patients at several cancer centers in Belgium, France, Germany, Israel, Spain, and Switzerland, was initiated in December 2001. Walter Fiedler at Universitats-Krankenhaus Eppendorf (Hamburg, Germany) is Study Chair. Beginning within 2 weeks after the last PSA measurement, IV MEN-10755 is administered over 30 minutes on day 1. Treatment is repeated every 3 weeks, for at least 4 courses, in the absence of disease progression or unacceptable toxicity. Patients with CR or PR may be treated with additional courses. Those with stable disease may be treated with >4 courses at the discretion of the investigator. Patients are followed every 6 weeks until disease progression, or initiation of a new therapy. Another single-agent phase II clinical trial of MEN 10755 is ongoing at the University of Mainz in Germany under PI A. Kirsten, MD, and B. Fischer, MD. Patients with small-cell lung cancer (sclc) are treated with IV MEN 10755 (80 mg/m^2) over 15 minutes.

PNU-159548 (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulphonyl-daunorubicin) is the lead compound of a novel class of cytotoxic agents (alkycyclines) with a unique mechanism of action combining DNA intercalation with alkylation of guanines in the DNA major groove.

As of 2002, two phase I clinical trials were completed in Europe that investigated IV PNU-159548, once every 21 days, at dose levels 1, 2, 4, 6, 9, 12, 14 and 16 mg/m^2, administered IV over 10 or 60 minutes to patients with advanced solid tumors. In an interim report, a total of 53 patients with advanced solid tumors were treated with 120 courses; 16 patients were treated at 12 mg/m^2 and 13 patients at 14 mg/m^2. Thrombocytopenia was the main

hematologic toxicity and was the DLT in 7/53 patients, with Grade 3 in 3/16 and 5/13 of first cycles at 12 mg/m^2 and 14 mg/m^2, respectively. Nonhematologic toxicities consisted of mild nausea and vomiting; 7/53 patients experienced a histamine release syndrome during PNU-159548 administration, mostly characterized by chills with/without fever (n=4), erythema (n=3) and dyspnea (n=3). On retreatment patients were premedicated with H1-blokers and steroids. Disease stabilized for 18-30 weeks in 2 patients with nsclc and 1 patient with renal cancer (de Jonge MJ, etal, ASCO00, Abs.782:201a).

In these two phase I clinical trials, a total of 69 patients were treated with 161 courses. MTD was reached at 14 mg/m^2 in heavily pretreated patients (HP), and 16 mg/m^2 in minimally pretreated/nonpretreated (MP) patients, with thrombocytopenia as the DLT. A hypersensitivity reaction was observed in 8 patients across all dose levels, characterized by fever with chills, erythema, facial edema and dyspnea. Pharmacokinetics of PNU-159548, and its active metabolite PNU-169884, were linear over the dose range studied. The recommended dose for phase II studies of PNU-159548 is 12 mg/m^2 and 14 mg/m^2 administered IV over 10 minutes, once every 21 days, in HP and MP patients, respectively (de Jonge MJ, etal, Eur J Cancer, Dec 2002;38(18):2407-15).

A phase II clinical trial in metastatic colorectal cancer was initiated in July 2000 at State University of New York (SUNY) at Stony Brook under PI Stefan Madajewicz, MD. The study was closed in July 2001. A phase II clinical trial (protocol ID:548-ONC-0050-004) of PU-159548 in patients with advanced epithelial ovarian cancer, persistent or recurrent after platinum-based chemotherapy, was initiated in May 2000 at Hospital Universitario Central de Asturias, in Spain, under PI Angel Jiménez Lacave, MD.

WP631, a six-base-pair-binding agent, is a bisintercalating anthracycline antibiotic synthesized in 1997 at the University of Mississippi Medical Center (Jackson, MI), by linking together several small subunits of daunorubicin (Chaives JB, etal, *ibid*). The structure-based rational design of this compound was based upon the geometry of monomeric anthracyclines bound to DNA oligonucleotides observed in high-resolution crystal structures. This combinatorial chemistry approach that links building blocks that can be assembled to produce new compounds with much higher affinity and extended-sequence specificity than any of the parent compounds, creates small molecules that bind targeted DNA sequences, such as gene promoters, with extremely high affinity and sequence specificity.

An *in vitro* assay showed that WP631 was very effective at inhibiting transcription from an adenovirus promoter containing an Sp1 protein-binding site, which the new compound was designed to bind. Sp1 (named according to the original purification scheme that included sephacryl and phosphocellulose columns) is a protein belonging to a family of transcription factors characterized by a highly conserved DNA-binding domain consisting of three zinc fingers. Sp1 binds to GC box promoter elements and selectively activates mRNA synthesis from genes that contain functional recognition sites. For sometime, it was thought that Sp1 was the major factor acting through the GC box and the related GT/CACC box, the most common regulatory elements, which are widely distributed in promoters, enhancers and locus control regions of housekeeping as well as tissue-specific genes. More recent discoveries, however, have shown that Sp1 is one of many transcription factors with at least 16 different mammalian members, binding and acting through these elements.

Although initial studies showed that WP631 was slightly less cytotoxic than doxorubicin in the sensitive cell line, it appeared to overcome MRP-mediated MDR, and was much more cytotoxic than doxorubicin against the MCF-7/VP-16 cell line (Chaires JB, etal, *ibid*).

WP631 may circumvent some kinds of tumor resistance at rather low drug concentrations, inhibit c-myc expression in some cell lines, and exert its antitumor effect by inducing apoptosis (Portugal J, etal, Curr Med Chem, Jan 2001;8(1):1-8). WP631 is highly cytotoxic against Jurkat T lymphocytes. Treating Jurkat cells with nanomolar concentrations of WP631 produced G2/M arrest, inhibited the transcription of c-myc and p53 genes, and induced limited apoptosis during the duration of treatment. Suppression of c-myc and p53 expression, and time-dependent decline in c-Myc and p53 protein levels was associated with growth arrest. Also, there was a weak interdependence between the potent antiproliferative activity and the apoptotic response. In Jurkat T lymphocytes treated with these agents, WP631 was taken up more slowly than daunorubicin, but laser confocal microscopy and spectrofluorometric quantification showed that the drug accumulated in the cells. Despite the slow uptake rate, the antiproliferative capacity of WP631, measured as IC_{50} after a 72-hour continuous treatment, was greater than that of daunorubicin. When the propensities of daunorubicin and WP631 to promote apoptosis were compared, the major effect of WP631 was a G2/M arrest followed, after about 72 hours of treatment, by polyploidy and mitotic death. In contrast, daunorubicin induced a rapid response with classic features of apoptosis (Villamarin S, etal, Biochem Pharmacol, 1 Apr 2002;63(7):1251-8, and Villamarin S, etal, Eur J Biochem, Feb 2003;270(4):764-70).

WP744 is a novel anthracycline with enhanced proapoptotic and antileukemic activity superior to that of doxorubicin in acute myeloid leukemia (AML). WP744, a 4'-O-benzylated doxorubicin selected from a series of 4'-substituted analogs, retained its cytotoxic activity against P-glycoprotein (P-gp) and MRP-positive cells. In three AML cell lines (K562, KBM-3, and OCIM2) WP744 was significantly more potent than doxorubicin, and inhibited colony formation by AML-CFU cells from fresh bone marrow of three AML patients more strongly

than doxorubicin. Cell growth inhibition by WP744 was accompanied by apoptosis induction. WP744-induced apoptosis appears to be mediated by caspase-3 because apoptotic changes were abrogated in the presence of a caspase 3 inhibitor. Accordingly, caspase 3 activity was elevated in the lysates from drug-treated cells. WP744 induced also cleavage of apoptotic marker poly(ADP-ribose)polymerase (PARP), and was a potent inducer of apoptosis in cultured human acute lymphoblastic leukemia (ALL) CEM cells, compared to doxorubicin (Faderl S, etal, Anticancer Res, Nov-Dec 2001;21(6A):3777-84).

WP760, another new DNA-binding compound with unique anticancer properties, was identified using the combinatorial approach. Waldemar Priebe, PhD, and his colleagues at the University of Texas M. D. Anderson Cancer Center (UTMDACC; Houston, TX) created a small library of at least 80 randomly assembled molecules that were tested by the NCI for their activity against 60 different cell lines. Results showed that WP760 was selectively cytotoxic against a melanoma cell line, and two nsclc cell lines.

WP900 was designed as an enantiomer (mirror image) of naturally occurring daunorubicin, in a collaboration of researchers at the University of Mississippi Medical Center (Jackson, MS), the James Graham Brown Cancer Center at the University of Louisville, and UTMDACC. WP900 was synthesized through an arduous 32-step process and then molecularly modeled (Xiaogang Qu, etal, PNAS USA, 24 Oct 2000;97(22):12032-7). WP900 binds Z-DNA, a left-handed form of DNA, with selective affinity. This compound may lead to the development of a new class of anticancer agents that target Z-DNA. Up to that point, although compounds were available that could bind B-DNA alone, or both B-DNA and Z-DNA, none selectively bound Z-DNA.

Celiptium

Celiptium (elliptinium; 9-hydroxy-2-methylellipticinium acetate) is a derivative of ellipticine isolated from species of several genera of the Apocynaceae family, including *Bleekeria vitensis*, a Fijian medicinal plant with reputed anticancer properties. The exact mode of antineoplastic action of the ellipticenes has not been elucidated but appears to be multifactorial, involving topo II inhibition, DNA intercalation, production of cytotoxic free radicals, alkylation of target macromolecules, and inhibition of tubulin.

Celiptium, developed by Sanofi-Synthelabo (Paris, France), was approved in France in 1983 for the treatment of renal cell carcinoma (RCC), soft-tissue sarcoma, and breast cancer metastatic to the bone. In an attempt to enhance the pharmacologic potency of this pyridocarbazole template, various research groups have generated analogs of ellipticine by modification of either the C or D ring, or both, of the tetracyclic ring system. SAR studies have been very useful in the orientations of synthesis in order to obtain more effective therapeutic agents.

Distamycin A

Distamycin A, an antiviral compound, acts in a similar fashion as daunorubicin, by inhibiting RNA polymerase II *in vitro*, and both DNA and RNA synthesis in HeLa cells, and by suppressing the coordinate initiation of DNA replication in *Xenopus* oocyte extracts. Distamycin A alters DNA conformation by binding reversibly to DNA minor groove with high selectivity for A/T-rich domains (Taylor A, etal, Mol Cell Biochem, Apr 1997;169(1-2):61-72).

Minor groove binders (MGB), the most widely studied class of alkylating agents, target DNA with a much higher degree of sequence specificity than that of conventional alkylators. Generally, commercially available alkylating agents have a relatively low therapeutic index, probably attributable to the fact that these compounds cause DNA damage in a relatively unspecific manner, mainly involving guanine-cytosine rich stretches of DNA present in virtually all genes. In this manner, they induce unselective growth inhibition and death, both in neoplastic and in highly proliferative normal tissues. MGB are characterized by a high level of sequence specificity. Distamycin A is the prototype of this class of drugs. Because it is not cytotoxic against tumor cells, it has been used as a carrier for targeting cytotoxic alkylating moieties in the minor groove of DNA.

Tallimustine, the benzoyl nitrogen mustard derivative of desformyldistamycin, demonstrated strong activity against a series of experimental tumors. Tallimustine, like distamycin, selectively binds to TA-rich sequences but its cytotoxicity is not associated with DNA strand breaks and interstrand crosslinking, at variance with classical phenyl nitrogen mustards (Cozzi P and Mongelli N, Curr Pharm Des 1998;4(3)998:181-202). Development of tallimustine was abandoned when it was shown in clinical trials to cause severe bone marrow toxicity.

Although MGB have not yet fulfilled expectations, it is too early to draw definitive conclusions on this class of compounds. The peculiar bone marrow toxicity observed in the clinic both with tallimustine or CC-1065 derivatives, is not necessarily a feature of all MGB, as indicated by recent evidence obtained with brostallicin and other structurally unrelated MGB such as ET-743, under development by PharmaMar (Madrid, Spain) currently in phase III clinical trials (Marchini S, etal, Expert Opin Investig Drugs, Sep 2001;10(9):1703-14).

Brostallicin (PNU-166196), under development by Pharmacia, is a distamycin derivative with antitumor activity and much lower toxicity against human bone marrow cells than tallimustine. PNU-166196 is a synthetic, second-generation DNA MGB with an α-bromoacrylic moiety linked to a distamycin-like frame ending with a guanidino moiety. PNU-166196 is effective *in vivo* against a variety of human tumors. PNU-166196 overcomes resis-

tance to alkylating agents and camptothecins, and is equally effective in DNA mismatch-repair deficient and proficient cells. PNU-166196 is highly cytotoxicity *in vitro* and differs from other MGB by exhibiting significant *in vitro* and *in vivo* induction of apoptosis. PNU-166196 is effective *in vivo* against a variety of human tumors, including ovarian (A2780 and H207), renal (CAKI2), prostate (DU145), breast (MX1) and lung (N592) cancer. Moderate activity was also observed against colon (HCT116) and gastric (GTL16) cancer.

Because of their mode of action, MGB may be targets for DNA mismatch repair (MMR)-induced resistance. Defects in MMR are associated with predisposition to tumorigenesis and with drug resistance attributable to high mutation rates and failure to engage DNA-damage-induced apoptosis. However, unlike other MGB, MMR-deficient cells retain their sensitivity to brostallicin, indicating that brostallicin-induced cytotoxicity does not depend on functional MMR. These findings suggest testing brostallicin in the treatment of MMR-defective tumors (Fedier A, etal, EORTC-NCI-AACR02, Abs. 518, and Eur J Cancer, Nov 2002;38 (Suppl 7):155).

Unlike other cytotoxics, PNU-166196 cytotoxicity is increased both in the presence of high levels of glutathione (GSH) and glutathione S-transferases (GST), and the GSH/GST system is involved in its interaction with DNA (Geroni CM, etal, Cancer Res 2002;62:2332). PNU-166196 cytotoxicity is 3-fold higher in melphalan-resistant cells, which contain higher levels of GSH compared to the parental line, and depletion of GSH significantly decreases efficacy of PNU-166196. Also, experiments on the interaction of PNU-166196 with plasmidic DNA (pUC18/19) show a change of the DNA topology, from supercoiled to the circular form (nicking), only in the presence of GSH. No change occurred in the plasmid topology in the absence of GSH, at variance with the MGB tallimustine (Cozzi P, etal, NCI-EORTC-AACR00, Abs. 363).

Among the GST isoenzymes, GST-pi is the stronger activator of brostallicin efficacy. Levels of GST-pi are negligible in a high percentage of human prostate cancers, because of hypermethylation of the promoter region of the GST-pi gene. Treatment of prostate cancer cells with DNA methyltransferase inhibitors results in demethylation and activation of the GST-pi gene and, consequently, in the increase of the intracellular level and activity of GST-pi protein. The cytotoxic activity of brostallicin was tested against the non-GST-pi-expressing human prostate cancer cell line LNCaP in which the GST-pi promoter is completely methylated. Brostallicin is five times less cytotoxic in LNCaP cells compared with the GST-pi-expressing (with methylated promoter) Du145 human prostate cancer cells. Research to verify *in vitro* whether treatment with decitabine, procainamide, or histone deacetylase (HDAC) inhibitors could activate expression of GST-pi in LNCaP cells and, consequently, increase the antitumor activity of brostallicin, indicate that pretreatment with procanaimide that induces hypomethylation of GST-pi promoter, increases the cytotoxicity of brostallicin compared to untreated LNCaP cells. Therefore, association of brostallicin with hypomethylating agents could be synergistic in prostate cancer (Sabatino MA, etal, EORTC-NCI-AACR02, Abs. 87, and Eur J Cancer, Nov 2002;38 (Suppl 7):31).

Although the precise mechanism of interaction has not yet been identified, a clear therapeutic gain is observed in preclinical models when brostallicin is combined with other anticancer agents. In nude mice bearing the human colon carcinoma HCT-116, sequential combination of cisplatin and brostallicin delayed tumor growth significantly longer than the best delays achieved with either drug alone. Also, each agent could be administered at MTD without additional toxicity. In murine leukemia L1210 model, brostallicin and doxorubicin, administered as single agents, resulted in a 33% increase in life span, compared to 100% when used in combination. However, an increase in toxicity was observed when these drugs were administered simultaneously. Supra-additive antitumor effect is shown when brostallicin is tested in simultaneous combination with gemcitabine (Gemzar; Lilly) on L1210 leukemia, and with docetaxel in a human nsclc xenograft model A549, without any additive toxicity (Geroni C, etal, EORTC-NCI-AACR02, Abs. 79, and Eur J Cancer, Nov 2002;38 (Suppl 7):29).

In a phase I clinical trial, conducted at the Rotterdam Cancer Institute and University Hospital Rotterdam, in the Netherlands, PNU-166196 was administered as a single bolus injection, at dose levels of 0.85, 1.7, 3.4, 5.1, 7.5 and 10 mg/m^2, once every 3 weeks, for a total of 33 cycles, in 14 patients with solid tumors. Nonhematologic toxicity consisted of mild nausea and vomiting, and fatigue. Hematologic toxicity, limited to Grade 2/3 neutropenia, was noted in 3/14 patients at dose levels <10 mg/m^2. Uncomplicated short-lasting Grade 4 neutropenia, not qualifying as DLT was observed in 2/3 patients at 10 mg/m^2. There was 1 confirmed PR in a pretreated patient with gastrointestinal stromal tumor (GIST) with extensive liver metastases who continues treatment. Symptoms improved with documented stable disease, in another patient with GIST, treated with 3 cycles of therapy (Planting AS, etal, ASCO01, Abs. 379:96a).

A phase I clinical trial, being conducted at Vanderbilt-Ingram Cancer Center (Nashville, TN), is assessing the MTD, DLT, and plasma PK profile of PNU-166196, administered as a 10-minute infusion on days 1, 8, and 15 of a 28-day cycle. Among 7 patients treated with weekly doses of 0.3, 0.6, 1.2, 2.4 (n=2), and 4.8 mg/m^2 (n=2), DLT involving neutropenic fever (Grade 4 neutropenia) and Grade 4 thrombocytopenia, occurred in 1 patient at 4.8 mg/m^2. Other ≥Grade 2 non-DLT included Grade 2/3 anemia (n=2), Grade 3 thrombocytopenia (n=1), Grade 1/2 nausea (n=4), Grade 1/2 vomiting (n=3) and Grade 2/3 fatigue (n=4) (Hande KR, etal, ASCO01, Abs.380:96a).

A multicenter phase II clinical trial (protocol ID: EORTC-62011), being conducted in Europe (Belgium, France, Hungary, the Netherlands, UK), is investigating the antitumor activity of brostallicin in locally advanced, or metastatic soft-tissue sarcoma that has not responded to one prior chemotherapy regimen. Starting in August 2002, a total of 58-72 patients (40 for stratum I and 18-32 for stratum II) will be accrued. Patients are stratified by tumor type, those with tumors other than GIST versus those with GIST. IV brostallicin is administered over 10 minutes on day 1. Treatment is repeated every 21 days for at least 4 courses in the absence of disease progression, or unacceptable toxicity. Patients are followed every 2 months for 1 year, and then every 4 months for 1 year. Michael Leahy of St. James's Hospital (Leeds, UK) is Study Chair.

Duocarmycin

Duocarmycins and (+)-CC-1065, produced by the Actinomycetes Streptomyces, are a well characterized class of extremely potent antibiotic cytotoxics that have been studied in humans as cancer therapeutics. The unmodified duocarmycin compound KW-DU-86 when tested *in vitro* was found to have cytotoxic activity in the subnanomolar range on a variety of tumor cell lines.

Duocarmycins act as prodrugs because they are extremely stable to nucleophilic attack until bound to their DNA target, are not substrates for any other biologic nucleophile and also avoid several MDR mechanisms. However, they suffer from low therapeutic index and poor physicochemical properties, most notably solubility. Several novel tumor-activated prodrugs (TAP) of duocarmycin, under development, may eliminate most of the problems associated with these compounds.

CC-1065 analogs, including bizelesin and carzelesin, failed in the clinic because of significant toxicity at levels necessary for the drugs to be effective.

Bizelesin, a synthetic analog of the cyclopropylpyrroloindole antitumor antibiotic C-1065, is a bisalkylator in which the two alkylating moieties are connected with a rigid linker. Bizelesin is more sequence-selective compared to the monoalkylating adozelesin, and is a potent bifunctional alkylating agent. Monoalkylating compounds such as adozelesin react at more than one site but bizelesin reacts only where there are two suitably positioned alkylation sites.

Bizelesin was evaluated in two completed phase I clinical trials. In these trials toxicities included Grade 3/4 neurtropenia but not neutropenic fever. Nonhematologic toxicity was >Grade 2. In a phase I clinical trial (protocol ID: MAYO-930103, NCI-T93-0195), conducted at the Mayo Clinic Cancer Center (Rochester, MN) under Henry Clement Pitot, MD, as Study Chair, 19 patients with advanced solid tumors were administered escalating doses of IV bizelesin every 4 weeks for 54 courses. Treatment was well tolerated with neutropenia being the DLT. MTD and the recommended phase II dose was determined to be 0.8 μg/m^2, administered once every 4 weeks (Pitot HC, etal, Clin Cancer Res, Mar 2002;8(3):712-7).

Another dose-escalation, phase I clinical trial (protocol ID: NCI-T93-0166, SACI-IDD-93-45, UTHSC-IDD-93-45) of bizelesin in patients with advanced cancer was completed in April 2002; Eric Keith Rowinsky, MD, of the University of Texas Health Science Center (San Antonio, TX) was Study Chair. A total of 103 courses of bizelesin were administered to 31 patients as a single bolus infusion at dose levels 0.1, 0.2, 0.4, 0.53, 0.71, 0.95, and 1.26 μg/m^2, every 28 days. Toxicities included Grade 4 neutropenia (n=6) and Grade 3 neutropenia (n=4). No neutropenic fever or Grade 3 or 4 nonhematologic toxicity was observed. There was 1 PR in one patient with ovarian cancer whose disease stabilized for 14 cycles, and disease stabilized in 15, 9, and 6 cycles in 3 patients with RCC (Schwartz GH, etal, ASCO00, Abs. 921D:235a).

Carzelesin, mostly investigated in Pharmacia-sponsored clinical trials in Europe, also proved to be less effective at doses associated with tolerable toxicities. A phase II study was conducted to assess the activity of carzelesin as second- or third- line chemotherapy in patients with breast, ovarian, and head and neck cancer, and non-Hodgkin's lymphoma (NHL), and as first-line chemotherapy in patients with colorectal and gastric cancer, and melanoma. According to the protocol, 140 patients were treated with 285 courses of carzelesin (150 μg/m^2) every 4 weeks by bolus infusion. Myelotoxicity was the most common toxicity with Grade 3 and 4 leukopenia observed in 18.6% of courses, neutropenia in 20.3%, thrombocytopenia in 16.2% and anemia in 8.7%. Double nadirs were seen in 41 courses for neutrophils, 40 for leukocytes, and 3 for platelets. There was 1 PR in a patient with melanoma. At this dose and schedule, no activity was observed in the types of tumors studied (Pavlidis N, etal, Cancer Chemother Pharmacol 2000;46(2):167-71).

Panorama Research (Mountain View, CA) has synthesized novel CC-1065 analogs bearing different DNA-binding subunits (Wang Y, etal, Med Chem, 13 Feb 2003;46(4):634-7).

KW-2189, a semisynthetic duocarmycin antibiotic exerts antiproliferative effects against human tumor cell lines *in vitro*, and animal tumor models *in vivo*. In phase I clinical trials, the most noteworthy adverse effect was myelosuppression (Alberts SR, etal, Clin Cancer Res, Sep 1998;4(9):2111-7). The drug was evaluated in phase II clinical trials in various malignancies, including metastatic melanoma, and advanced RCC. However, further development of KW-2189 in these or other indications does not appear likely because of this drug's toxicity, and lack of significant antitumor activity (Markovic SN, etal, Am J Clin Oncol, Jun 2002;25(3):308-12, Small EJ, etal, Invest New Drugs, May 2000;18(2):193-7, and Rubin J, etal, ASCO00, Abs. 1198:304a).

Elsamitrucin

Elsamitrucin (BMY-28090) is a heterocyclic antitumor antibiotic related to chartreusin, under development by Spectrum Pharmaceuticals (Irvine, CA). The drug acts by inducing single-strand breaks in DNA, and by inhibiting topo I and II, thus blocking DNA replication. The drug was originally evaluated clinically, in the early 1990s, by Bristol-Myers Squibb. Spectrum is currently planning a phase II clinical trial in non-Hodgkin's lymphoma (NHL).

Enediynes

Enediynes, discovered in the 1980s, are naturally occurring antibiotics found in soil. Enediynes are characterized by a 9- or 10-membered ring containing two triple bonds separated by a double bond. Presence of this highly reactive ring structure, commonly referred to as the 'warhead,' enables these molecules to cause DNA damage in living cells. Enediynes are among the most potent antitumor agents. Depending upon their structure, enediynes either partially intercalate DNA, or bind to the major or minor grooves of DNA. Enediynes must undergo a chemical transformation to be activated. Following prodrug activation, enediynes undergo cycloaromatization reactions resulting in formation of highly reactive diradical intermediates, which can strip hydrogen atoms from DNA, interfering with mitosis by causing single-strand breaks in the DNA during spindle formation, and ultimately killing the target cell. Although enediynes are potent cytotoxics, their clinical application has been hampered by their toxic side effects. Various enediynes have been identified including calicheamicin and dynemycin.

When Ben Shen, PhD, and colleagues at the University of Wisconsin (Madison, WI) cloned and characterized the stretch of the *Streptomyces globisporus* genome necessary for biosynthesis of the 9-membered enediyne C-1027, they found that the enediyne cores of both C-1027 and calicheamicin are synthesized via a common polyketide pathway, suggesting that all enediynes are biosynthesized in the same manner (Liu W, etal, Science, 16 Aug 2002;297(5584):1170-1173).

Several of the naturally occurring members of the enediyne family of antibiotics have entered clinical trials, and this has prompted the design of synthetic enediynes. Recent efforts use chemical synthesis to identify and improve the target specificity of designed enediynes, and to establish efficient methods to achieve prodrug activation (Jones GB and Fouad FS, Curr Pharm Des 2002;8(27):2415-40). A group at the Department of Chemistry of Northeastern University (Boston, MA), headed by Graham B. Jones, has developed an efficient and versatile synthetic route to linear and cyclic enediynes, and is currently applying this methodology in a total synthesis of the naturally occurring enediyne kedarcidin.

C-1027, an enediyne antibiotic, consists of an apoprotein and a labile chromophore (C-1027-Chr), which is responsible for the biological activity of this agent. Investigators at the University of Wisconsin cloned and characterized the 85-kilobase C-1027 biosynthesis gene cluster from Streptomyces globisporus. Manipulation of genes governing C-1027 biosynthesis allowed production of an enediyne compound in a predicted manner. C-1027 exhibits potent cytotoxicity against KB carcinoma cell in tissues culture. C-1027 activity was at least 106 times higher than doxorubicin, methotrexate, or cisplatin.

Calicheamicin is the best known of the enedyines. It has entered the clinic as part of various targeted cytotoxics such as Mylotarg, commercialized by Wyeth (see part I of this article), and other targeted cytotoxics described below.

Researchers at the Pharmaceutical Sciences Division, School of Pharmacy, at the University of Wisconsin, in collaboration with Ecopia BioSciences (Saint-Laurent, Québec, Canada), cloned and characterized the biosynthetic locus coding calicheamicin. This gene cluster contains an unusual polyketide synthase (PKS) that is demonstrated to be essential for enediyne biosynthesis. PKS are multifunctional enzymes involved in the production of many naturally occurring antibiotics and other therapeutics. The PKS of calicheamicin is completely novel, has a relatively simple organization and, therefore, it appears to be possible to engineer new enediyne compounds using genetic methods. Comparison of the calicheamicin locus with the locus encoding the chromoprotein enediyne C-1027 reveals that the enediyne PKS is highly conserved among these distinct enediyne families. Contrary to previous hypotheses, this suggests that the chromoprotein and nonchromoprotein enediynes are generated by similar biosynthetic pathways (Ahlert J, etal, Science, 16 Aug 2002;297(5584):1173-6).

These findings have implications for the discovery and design of new antibacterial and anticancer agents. Ecopia BioSciences filed for patent protection on the new class of PKS, its related genes, and other genes and proteins involved in enediyne production. Having the full genetic blueprint for enediynes has provided Ecopia BioSciences the tools to rapidly discover new naturally occurring compounds of this class and possibly engineer new versions with improved pharmaceutical properties. In fact, Ecopia BioSciences has already identified a number of potential new naturally occurring enediynes using its genomics technology.

Lidamycin, another enediyne, is in development by Shenzhen Sancode Biotechnology, in China, under funding by the Chinese National Fund for Natural Science and the National Fund for New Drugs. Lidamycin at low concentrations causes mitotic cell death in human hepatoma BEL-7402 cells, and breast carcinoma MCF-7 cells, resulting in retardation at G2+M phase, enlargement of cell volume, and multinucleation (He QY, etal, Int J Oncol, Feb 2002;20(2):261-6). The agent appears to be active against liver and lung cancer.

Neocarzinostatin (NCS), an antitumor antibiotic with an enediyne-containing chromophore and an apoprotein in a 1:1 complex, was discovered by Japanese researchers in 1965. In order to overcome the major problem and limitation regarding the clinical use of NCS, such as its severe toxicity and very short half-life, a styrene maleic acid neocarzinostatin (SMANCS) conjugate was developed to make NCS more lipophilic and structurally stable (see below).

FK317

FK317, under development by Fujisawa (Osaka, Japan and Deerfield, IL), is an antitumor antibiotic that is an analog of FK973, both semisynthetically derived from FR-900482, a novel compound derived from the actinomycete, *Streptomyces sandaensis*. FK317 crosslinks MGB proteins to DNA *in vivo*. In clinical trials, FR-900482 and FK973 demonstrated clinical activity in refractory tumors, but were associated with capillary or vascular leak syndrome (CLS or VLS), a harmful side effect that resulted in their withdrawal from clinical trials. In preclinical trials, unlike FK973, FK317 demonstrated potent cytotoxic activity without associated CLS. FK-317 is metabolized to deacetylated forms; deacetylated FK-317 is incorporated into MDR cells where it interacts with DT-diaphorase to form an active metabolite. This metabolite produces DNA-DNA interstrand and DNA-protein crosslinks that lead to cell death (Naoe Y, etal, Jpn J Cancer Res, Jun 1998;89(6):666-72; Naoe Y, et al, Jpn J Cancer Res, Oct 1998;89(10):1047-54).

FK317 differs from FR-900482 and FK973 in how it induces cell death; whereas FR900482 induces necrosis, FK317 induces a necrosis-to-apoptosis switch that is drug-concentration dependent. This switch is mediated, at least in part, by modulation of the expression levels of Bcl-2. Also, FR900482, in contrast to FK317, induces expression of known elicitors of both Bcl-2 gene expression and VLS. These findings provide plausible explanation for the fact that these structurally similar drugs have different biological effects, especially with respect to VLS (Beckerbauer L, etal, Chem Biol, Apr 2002;9(4):427-41).

FK317 extends survival in mice bearing B16BL6 and Lewis lung carcinoma (LLC) tumors more potently than mitomycin C. When the effects of FK317 were compared with mitomycin C on survival time of mice bearing B16BL6 melanoma and LLC, induced by intravenous (IV) inoculation of the tumor, treatment with FK317 resulted in a significant prolongation of survival time in both tumor models. Following FK317 treatment, 4/10 mice bearing B16BL6 were disease free. In contrast, mitomycin C was not effective in prolonging survival time (Inami M, etal, Cancer Lett, 8 Jul 2002;181(1):39-45).

A phase I clinical trial to determine the safety, toxicity, and MTD of FK317 administered IV once every 28 days, was conducted at UTMDACC and at the University of Wisconsin. A total of 51 doses were delivered in 23 patients with refractory solid tumors. All patients were evaluable for response and toxicity. At an FK317 dose of 14 mg/m^2, 1/3 patients experienced Grade 3 neutropenia; no DLT was observed in an additional 3 patients treated at this level. At 18 mg/m^2, none of 8 patients developed DLT, but DLT occurred in 5/9 patients treated with 24 mg/m^2 of FK317, including 3 patients with neutropenic fever or Grade 4 granulocytopenia, a patient who developed Grade 4 pulmonary fibrosis, and another who developed Grade 3 fatigue, weakness and dyspnea. There was 1 PR in a patient with an unknown primary, and disease stabilized in 4 patients (colorectal=2, sarcoma=1, and RCC=1), lasting 4 to 5 months. MTD of FK317, administered every 28 days, is 18 mg/m^2 with granulocytopenia the predominant DLT (Wolff RA, etal, ASCO01, Abs. 377:95a).

Geldanamycin/Ansamycin and Analogs

One important target in cancer is heat-shock protein 90 (HSP90), an abundant, essential chaperone in eukaryotes that plays a major role in maintaining the integrity of a number of signaling cascades. HSP90 interacts with and stabilizes several growth-related kinases frequently deregulated in cancer, such as members of the Src family, HER2, cyclin-dependent kinases Cdk4 and Cdk6, Raf-1 and RIP. HSP90 is also required for the stability and dominant negative function of mutated p53. Because HSP90 is required for the correct folding, stability and function of a range of oncoproteins that are mutated or overexpressed in cancer, HSP90 inhibitors may provide a broad-based attack on multiple oncogenic pathways (Workman P, Cancer Detect Prev 2002;26(6):405-10). Inhibition of Hsp90 and the chaperoned protein interaction destabilize and degrade the chaperoned protein by proteasomes.

Mammalian cells contain four distinct members of the HSP90 molecular chaperone family. Cytosolic HSP90 has two isoforms, HSP90a and HSP90b, which are 76% identical, and are thought to be the consequence of a gene duplication event. A third family member, the 94kDa glucose-regulated protein (GRP94), is localized primarily to the endoplasmic reticulum, and shows 50% homology with HSP90. Lastly, the tumor necrosis factor (TNF) receptor-associated protein (TRAP-1) is primarily located in the mitochondria of mammalian cells.

HSP90 family members possess a unique pocket in their N-terminal domain that is specific to and conserved from bacteria to mammals but is not present in other molecular chaperones. This pocket binds both adenosine triphosphate (ATP) and adenosine diphosphate (ADP) with low affinity, and can weakly hydrolyze ATP (ATPase activity). Interestingly, nucleotides binding into this pocket adopt a unique bent shape that is not seen in any other context.

The benzoquinone ansamycins (geldanamycin) and radicicol that specifically interact with an ATP/ADP site at the N-terminus, and novobiocin, that binds at the C-terminus of HSP90, represent the first generation of drugs that target HSP90. These three antibiotic families disrupt the chaperone's association with its client signaling proteins, leading to their destabilization and proteolysis.

Inhibition of the ATPase activity of Hsp90 by the geldanamycin and 17-AAG results in depletion of oncogenic client proteins such as c-Raf-1, ErbB2, cdk4, and a significant induction of Hsp70. Depletion of client proteins is thought to be responsible for the antitumor effects of these compounds in cell culture and animals. However, geldanamycin's role as a therapeutic agent is limited by its toxicity, prompting ongoing efforts are discover other small molecules with similar activity.

Recent structural data indicate that while the four HSP90 family members all contain an N-terminal ATP/ADP binding pocket, the individual pockets are not identical. Therefore, using computer modeling techniques and x-ray crystal structure data, compounds can be designed that will bind preferentially to individual family members. Scientists at MSKCC have designed and synthesized a series of new compounds that bind to the ATP/ADP binding pocket of HSP90 with much higher affinities than to the adenine nucleotides. Importantly, these are simple organic molecules that are amenable to optimization through the use of combinatorial chemistry. Conforma Therapeutics (San Diego, CA) has licensed this technology exclusively from MSKCC.

Conforma's medicinal chemistry efforts are focused on expanding this series of compounds with the goal of developing analogs that are selective for individual family members. In addition, the company is developing proprietary high-throughput assays to enable screening of large compound libraries for additional scaffolds that modulate individual HSP90 family members. Identification and optimization of small synthetic molecules may result in the development of targeted, orally bioavailable anticancer agents, as well as drugs to treat such chronic disorders as inflammation and autoimmune diseases.

17-(allylamino)-17-demethoxygeldanamycin (17-AAG), an analog of geldanamycin, is a benzoquinone ansamycin antibiotic that potently inhibits Hsp90 chaperone protein function. Like its parent, 17-AAG has a broad mechanism of action, interfering with a variety of oncogenic pathways. 17-AAG is a tyrosine-kinase inhibitor that binds to HSP90, causing disruption and degradation of client protein, including such oncogenic kinases as c-Raf and AKT.

Geldanamycin also destabilizes the alpha subunit of the hypoxia inducible factor 1 (HIF-1α) via a von Hippel-Lindau (VHL)- and prolyl hydroxylase-independent pathway, inhibits transcriptional activation and stabilization of HIF-1α protein under hypoxia, and promotes destabilization of proline-mutated HIF-1α. HIF-1α serves as a transcriptional factor that regulates gene expression involved in response to hypoxia and promotes angiogenesis. HIF-1α is rapidly degraded by the proteasome under normal conditions, but is stabilized by hypoxia resulting in the transactivation of several proangiogenic genes. HIF-1α is responsible for inducing production of new blood vessels as needed when tumors outgrow existing blood supplies. HIF-1α functions as a survival factor that is required for tumorigenesis in many types of malignancies, and is expressed in a majority of metastases and late-stage tumors. Tumors overexpressing HIF-1α are highly vascular and overproduce angiogenic peptides such as vascular endothelial growth factor (VEGF), which is also a transcriptional target for HIF-1α. Geldanamycin and 17-AAG antagonize HIF-1α in situations where the protein is overexpressed, either because of abrogation of VHL function, or the hypoxic tumor microenvironment. Geldanamycin promotes degradation of HIF-1α independently of VHL status and oxygen tension, thus delineating a novel pathway for drug-induced elimination of HIF-1α. Hsp90 inhibitors may also possess antiangiogenic activity, thus extending the value as anticancer agents (Isaacs JS, etal, AACR02, Abs. 1644:332).

Geldanamycin and its analogs inhibit the transcriptional activity of HIF-1α in normoxic RCC-derived cells lacking VHL prior to loss of HIF-1α protein. HIF-1α stabilization and overexpression under normal conditions occurs in 50% of sporadic RCC and clear-cell RCC, the most common malignant neoplasm of the kidney, and one of the few human tumors known to depend on the mutation (hereditary or somatic) or silencing of a specific gene, the tumor suppressor VHL. VHL is a component of the multiprotein ubiquitin ligase complex that targets HIF-1α for proteasome-dependent degradation in normoxia. VHL interaction with HIF-1α depends on hydroxylation of two consensus prolines on HIF-1α, and hypoxia inhibits the enzyme responsible for this modification. In RCC lines that lack VHL, and stably overexpress HIF-1α under normoxia, HIF-1α protein is downregulated upon addition of geldanamycin. In untreated cells, HIF-1α protein is found associated with Hsp90 and exposure to geldanamycin disrupts this complex, followed by HIF-1α ubiquitination and proteasome-mediated degradation (Isaacs JS, etal, *ibid*).

Treatment with 17-AAG targets both solid tumors such as ovarian and prostate cancer and sarcoma, as well as hematologic malignancies such as lymphoma. Also, 17-AAG induces retinoblastoma (Rb)-dependent G1 arrest in lung cancer cell lines, and may be useful in the treatment of nsclc expressing wild-type Rb (Jiang J and Shapiro GI, AACR02, Abs. 1645:332).

Geldanamycin induces apoptosis in neuroblastoma by reducing Raf-1 and Akt. When neuroblastoma cells, SH-SY5Y, SK-N-SH and LAN-1, were treated with geldanamycin, a significant reduction of cell viability was noted, as well as the induction of apoptosis in all 3 cell lines. Treatment decreased Raf-1 and Akt levels, eliminated phos-Akt expression, and lowered caspase-9, and cleaved caspase-3 and PARP in all three neuroblastoma cell lines. Neuroblastoma cells were sensitive to the effects of geldanamycin with apoptosis noted at low doses. Geldanamycin-induced apoptosis involves reduction of Raf-1 and Akt levels, dephosphorylation of Akt and caspase-9 and caspase-3 activation. In addition, Hsp90

inhibitors, 17-AAG and EC4, significantly reduced neuroblastoma tumor growth *in vivo* when compared to controls. Mice with human Lan-1 xenografts tolerated treatment with either compound without gross systemic toxicities. Therefore, Hsp90 inhibitors are effective against inhibiting neuroblastomas *in vivo*, and may prove to be valuable chemotherapeutic agents (Sunghoon K, etal, AACR02, Abs. 4418:891, and AACR03, Abs. 781).

Geldanamycin and its analogs may also act as radiosensitizers. Alteration of HSP90 function by administration of geldanamycin or its analogs, results in significant radiosensitization, similar to that observed for hyperthermia. Inhibition of HSP90 with geldanamycin increases ionizing radiation-induced cell death. In a cervical tumor model system, exposure of HeLa and SiHa cells to these agents induced DNA-binding activity similar to that observed with both hyperthermia and NSAID, well documented radiosensitizers. Additionally, treatment of HeLa and SiHa cells with 17-AAG yielded significant radiosensitization. The increase in radiation-induced cell death was attributed to a combination of mitotic catastrophe and apoptosis, implicating effects on several signaling factors/pathways known to protect against radiation. Biochemical studies determined that 17-AAG decreased intracellular levels of HIF-1α, Akt, Erk, Raf, Lyn, CK2, and HER2 at concentrations similar to those that resulted in radiosensitization. In addition, 17-AAG also inhibited DNA-binding activities of NF-κB and Egr-1 (Bradbury CM, etal, AACR03, Abs. 779).

Radiosensitization arising from treatment with geldanamycin or its analogs may be particularly helpful in the treatment of glioblastoma multiforme (GBM). HSP90, which is not mutated in brain tumors, making it a stable therapeutic target, appears to play an essential role in malignant transformation by regulating the stability and activity of multiple oncogenic growth factor receptors, including the epidermal growth factor receptor (EGFr). EGFr is highly expressed in GBM. Hyperactive EGFr signaling, which promotes unregulated cell growth and inhibits apoptosis, is believed to contribute to clinical radiation resistance. Blockage of the EGFr signal transduction pathway may increase the cytotoxic effects of radiotherapy. In the GBM cell line U251, geldanamycin and 17-AAG increased the cytotoxic effect of radiotherapy and inhibited clonogenic growth survival in a dose-dependent manner. Moreover, 17-AAG dramatically downregulated EGFr, Erk-1/2 and Akt expression in these cells. Combination of ionizing radiation and drug treatment resulted in additive effects on EGFr, ERK-1/2 and Akt expression in human U251 GBM cells (Kislin KL, etal, AACR03, Abs. 673).

Several clinical trials with 17-AAG are currently ongoing under the sponsorship of the NCI. Phase I clinical trials are investigating various dose/administration regimens of single-agent, and combinations of 17-AAG in solid tumors and hematologic malignancies.

A phase I clinical trial (protocol ID: MSKCC-03006, NCI-5878) of 17-AAG, in combination with docetaxel (Taxotere; Aventis), in patients with progressive metastatic prostate cancer or other progressive metastatic or unresectable solid tumors, was initiated in March 2003, at MSKCC and the University of Pittsburgh Cancer Institute with David Solit, MD, of MSKCC as Protocol Chair. A total of 30 patients will be accrued for this study, to be treated with IV docetaxel, over 1 hour, and IV 17-AAG, over 1 hour, on day 1. Courses repeat every 21 days in the absence of disease progression or unacceptable toxicity. Cohorts of 3-6 patients are being treated with escalating doses of 17-AAG until MTD is determined. Patients are followed every 2 to 3 months.

A dose-escalation, phase I clinical trial (protocol ID: MAYO-MC0111; NCI-5291) to determine the MTD of a combination of 17-AAG, cisplatin, and gemcitabine in treating advanced solid tumors, was initiated in October 2002 at the Mayo Clinic Cancer Center, to enroll 15 to 30 patients. IV gemcitabine is administered over 30 minutes, IV 17-AAG over 1 hour, and IV cisplatin over 2 hours, on days 1 and 8. Courses are repeated every 3 weeks in the absence of disease progression or unacceptable toxicity. Charles Erlichman is Study Chair. This combination trial follows a dose-escalation phase I clinical trial (protocol ID: MAYO-990102; NCI-T99-0058), initiated in August 1999 also at Mayo Clinic, to determine the MTD and DLT of single-agent 17-AAG in treating patients with unresectable solid tumors. A total of 48 to 120 patients (30 to 72 for arm I and 18 to 48 for arm II) were to be assigned to one of two treatment arms. Patients in arm I were treated with IV 17-AAG, over 1 hour, on days 1, 8, and 15. Courses are repeated every 28 days in the absence of disease progression or unacceptable toxicity. Patients in arm II were treated with IV 17-AAG, over 1 hour, on days 1, 4, 8, and 11, repeated every 21 days.

A phase I clinical trial (protocol ID: 99-C-0054) of 17-AAG in treating approximately 45 adult patients with solid tumors was initiated in February 1999, to determine MTD, and establish the toxicity profile of 17-AAG, and molecular effects of 17-AAG on the protein levels of Hsp72, Lck and Raf-1 in peripheral lymphocytes. 17-AAG is administered as a 1-hour IV infusion on days 1, 4, 15 and 18 of each 4-week cycle.

An NCI-sponsored phase I clinical trial (ID: MSKCC-99037; NCI-T99-0035) that commenced in July 1999 at Cancer Research Campaign and at 4 USA centers including MSKCC, under PI Howard I. Scher, MD, is to accrue a maximum of 51 patients with advanced solid tumors. This is a two-phase dose-escalation study. Patients are treated with 17-AAG IV over 1 hour, daily, for 5 days. Courses are repeated every 3 weeks in the absence of disease progression or unacceptable toxicity. In the accelerated phase, individual patients are treated sequentially with escalating doses of 17-AAG that are increased by 100% of the previous dose until 1 patient experiences ≥Grade 3 toxicity, or 2 different patients experience Grade 2 toxicity during any course. In the standard phase, cohorts of 3 to 6 patients

are treated with escalating doses of 17-AAG until MTD. Study objectives are to determine MDT of 17-AAG in this setting, and to evaluate the effects of this drug on the expression of signaling proteins present on an individual patient's cancer at the start of treatment and, if possible, post-treatment. The drug was administered daily for 5 days by IV infusion, repeated every 21 days in 16 patients enrolled in this trial. Dosing started at 5 mg/m^2 and escalated to 80 mg/m^2. At 80 mg/m^2, DLT was diarrhea, thrombocytopenia and transient transaminitis. No objective responses were seen, but disease stabilized in 4/13 patients beyond 3 months. Multiple correlative studies were performed to evaluate changes in protein levels of HER2, androgen receptor (Ar) and MAP kinase, including pre-and post-treatment tumor biopsies, skin biopsies, and PET scans (Munster PN, ASCO01, Abs. 327:83a).

A phase I/II clinical trial (protocol ID: CRC-PHASE-I/II-PH1/074; NCI-T99-0013) was initiated in August 1999, to determine the MTD of 17-AAG in treating 20 to 40 patients with advanced cancer. IV 17-AAG is administered over 15 to 30 minutes every week This study is sponsored by the Cancer Research Campaign, under PI Ian Robert Judson, MD.

17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG; NSC-707545) is a water-soluble geldanamycin analog, created to overcome difficulties in formulating 17-AAG. Although both of these compounds inhibit signal transduction through HSP90 modulation, the efficacy and pharmaceutical properties of 17-DMAG are superior to those of 17-AAG.

In preclinical testing, 17-DMAG exhibited superior *in vitro* and *in vivo* antitumor activity compared to 17-AAG. Human melanoma cells previously shown to be responsive to 17-AAG, were selected in order to compare the anticancer activity of 17-AAG and 17-DMAG. When growth inhibition was assessed in the melanoma cell lines MEXF 276L, MEXF 462NL and MEXF 514L, 17-DMAG was more potent than 17-AAG in the sensitive MEXF 276L cell line while MEXF 514L was resistant to both compounds. Additionally, in clonogenic assays performed on a panel of 13 human tumor xenografts, the mean IC_{50} for inhibition of colony formation was lower for 17-DMAG than for 17-AAG. In *in vivo* evaluations with growing melanoma xenografts, both compounds were active at their MTD, but the growth delay in mice bearing MEXF 276L tumors was 16 days for 17-DMAG and 11 days for 17-AAG. Again, MEXF 514L xenografts did not respond. The most significant difference between the sensitive and resistant melanomas was the expression of HER2 (erbB2), which is prominently expressed in the MEXF 276L model but undetectable in MEXF 514L cells. When the modulation of HSP90 and its client proteins were assessed, 17-DMAG and 17-AAG acted identically with HSP90 protein levels declining in MEXF 276L and remaining unchanged in MEXF 514L cells; c-raf-1 protein was reduced in MEXF 276L cells, but not in MEXF 514L. No change in protein expression was observed for PI3K. There was a decline in HER2 protein levels in MEXF 276L concomitant with loss of HSP90 (Smith V, etal, EORTC-NCI-AACR, Abs. 189, and Eur J Cancer, Nov 2002;38(Suppl 7):60).

In preclinical evaluation, in dose range studies conducted in rats and dogs, designed to determine MDT and relative drug toxicity of 17-DMAG, the most common side effects were GI and hepatic toxicity, present in both dogs and rats. GI hemorrhaging was most likely the cause of death in both species (Glaze ER, etal, AACR03, Abs. 828).

CNF-101 and analogs, under development by Conforma Therapeutics, are semisynthetic versions of geldanamycin with improved pharmaceutical properties. Geldanamycin, the key starting material for all of Conforma's ansamycin-based HSP90 modulators, has been produced by the company under GMP conditions at the kilogram scale. Conforma chemists have optimized a scalable, proprietary process to synthesize these compounds, from the starting material. It is anticipated that a Conforma-sponsored IND will be filed for the first ansamycin-based HSP90 modulator in mid 2003.

In *in vitro* evaluations, CNF-101 analogs demonstrated broad ability to kill cells from numerous types of cancer, as well as robust *in vivo* activity in human cancer xenograft models in mice. CNF-101 analogs have demonstrated a striking ability to degrade signaling molecules, including HER2. Within hours of treatment with Conforma's lead HSP90 modulators, HER2 protein is completely degraded and tumor cell death ensues. Contributing to the ability of these HSP90 modulators to kill tumor cells is the fact that treated cells also show a significant inhibition of Akt, a molecule that promotes survival of tumor cells. Conforma's HSP90 modulators, therefore, eliminate the survival support that is required for cancer cells to exist in the face of their many oncogenic mutations. Rapid drug-induced degradation of HER2 and Akt has also been observed *in vivo* using human tumor xenografts grown in nude mice.

Conforma has been aggressively pursuing preclinical development of a set of three ansamycin-based HSP90 modulators. EC4, one of Conforma's novel ansamycin-based compounds, has demonstrated more activity than 17-AAG *in vivo*, although it was less active *in vitro*. 17-AAG is highly potent in conventional cell killing assays *in vitro*, but commonly fails to affect the same tumors *in vivo*. By contrast EC4 was less potent *in vitro* but more active in animal models. To explore this paradox, the cell biology properties of the two compounds were studied under pharmacologically relevant conditions. Treatment of BT474 cells with 17-AAG over a 24-hour period, followed by drug washout and incubation in drug-free medium, caused temporary cell-cycle arrest but minimal cell death, whereas EC4 induced massive apoptosis under the same conditions. This was also observed in several other tumor cell lines. EC4 maintained degradation of a range of HSP90 client proteins, including EGFr, signal transducers involved in PI-3k/Akt and Raf/MEK signal transduction

Exhibit 1
Novel Antibiotics Cytotoxics in Development

Developer ❑ Affiliate(s)	Generic Name ❑ Number ❑ Brand Name	Description ❑ Administration Route	Status➢Location ❑ Indications
Advectus Life Sciences ❑ U Kentucky, U North Carolina	P80DOX-NP ❑ Nanocure	Novel method of delivering doxorubicin across the blood-brain barrier (BBB) using a nanoparticle technology ❑ injection	Preclin (begin 7/02)➢USA ❑ metastatic brain cancer
Alza ❑ Hermes Biosciences, NCI Biological Resources Branch	Anti-HER2/doxorubicin immunoliposome	Immunoliposome combining liposome-encapsulated doxorubicin and monoclonal antibody (MAb) fragment (F5) targeting HER2 on breast cancer cells ❑ IV	Preclin (ongoing 4/03)➢USA ❑ advanced breast cancer
Ariad Pharmaceuticals	AP23573/AP23675	Sirolimus analog, an inhibitor of protein mammalian target of rapamycin (mTOR) that blocks cancer cell growth and proliferation ❑ PO	Phase I (begin 1/03)➢USA ❑ solid tumors
Ariad Pharmaceuticals	AP23675	Sirolimus analog with dual action inhibition of both tumor growth (mTOR inhibition) and bone breakdown (osteoclast inhibition) ❑ PO	Preclin (ongoing 4/03)➢USA ❑ primary or metastatic bone cancer
Ariad Pharmaceuticals	AP23841	Sirolimus analog inhibiting mTOR ❑ PO	Preclin (ongoing 4/03)➢USA ❑ primary or metastatic bone cancer
Banyu Pharmaceuticals ❑ Pharmacia	J-107088	Synthetic analog of NB-506, an indolocarbazole compound targeting topoisomerase I ❑ IV	Phase I (completed 02)➢Japan ❑ advanced solid tumors; phase II (begin 9/00, ongoing 3/03)➢USA ❑ metastatic colorectal cancer; phase II (begin 9/00, ongoing 3/03)➢USA ❑ metastatic, refractory, transitional cell carcinoma of the urothelium; phase II (begin 9/00, ongoing 4/03) ➢USA ❑ advanced squamous cell carcinoma of the head and neck
Baxter Oncology ❑ Tulane U	D-26232 (AN 207)	Targeted cytotoxic consisting of doxorubicin derivative 2-pyrrolino-doxorubicin (AN-201) linked with the carrier [D-lysine6] LHRH ❑ IV	Preclin (2/02)➢USA ❑ solid tumors
BioAlliance Pharma ❑ U Paris-Sud	Transdrug Doxorubicin	Doxorubicin-loaded nanospheres made of polyisohexilcyanoacrylate polymer; agent overcomes MDR and restores sensitivity to doxorubicin in resistant cancer cells ❑ IV	Phase II (ongoing 1/03)➢Europe (France) ❑ solid tumors
Celltech Group ❑ Wyeth	CMC-544	Immunocongugate comprising a monoclonal antibody (MAb) targeting CD22, conjugated to the enediyne antibiotic calicheamicin ❑ IV	Preclin (ongoing 12/02)➢USA, Europe ❑ non-Hodgkin's lymphoma (NHL)
Celsion ❑ Duke U, Massachusetts Institute of Technology		An approach involving a combination of BPH 800 microwave urethroplasty to deliver microwaves to tumors by focused heat and doxorubicin in heat-sensitive liposomes ❑ IV	Phase I (begin 2/03)➢USA ❑ prostate cancer; preclin (begin 5/02)➢USA ❑ liver cancer
Conforma Therapeutics ❑ Memorial Sloan-Kettering Cancer Center, Duke U, Pharmacia, Burnham Institute, Harvard U	CNF-101 and analogs including EC4	Semisynthetic geldanamycin-based agents targeting heat-shock protein-90 (HSP90) ❑ IV	Preclin (ongoing 2/03)➢USA ❑ solid tumors, lymphoma

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Exelixis	DEAE rebeccamycin ❑ XL 119 (formerly BMS-181176, BMY-27557-14, BMY-27557), NSC-655649	Water-soluble analog of the antitumor antibiotic, rebeccamycin; topoisomerase II inhibitor ❑ IV	Phase II (begin 1/99, ongoing 4/02) ➢USA; phase II (begin 7/00, ongoing 4/02)➢USA, Canada, Europe (the Netherlands, Switzerland), Australia, New Zealand ❑ neuroblastoma, refractory pediatric solid tumors, relapsed or refractory pediatric NHL; phase I (ongoing 02)➢USA (combination) ❑ refractory or recurrent solid tumors; phase I (begin 10/99, ongoing 4/02)➢USA (combination) ❑ lymphoma, metastatic epithelial and mesenchymal tumors, leukemia; phase II (begin 5/00, ongoing 4/02)➢USA ❑ advanced (Stage IIIb/IV), or recurrent nsclc; phase II (begin 6/00, ongoing 4/02)➢USA ❑ advanced (Stage IIIb/IV), or recurrent breast cancer; phase II (begin 4/99, ongoing 2/03) ➢USA ❑ advanced hepatobiliary carcinoma; phase II (begin 6/00, ongoing 2/03)➢USA ❑ advanced (Stage III/IV) or recurrent, renal cell carcinoma (RCC)
FeRx ❑ Elan	MTC-DOX	Doxorubicin absorbed in microparticles, composed of elemental iron and activated carbon, based on a proprietary technology, Magnetic Targeted Carrier (MTC), that is used for site-specific targeting, retention and sustained release of oncolytic agents to solid tumors ❑ intra-arterial, infusion, intraluminal, intravesical	Phase I/II (begin 9/01, ongoing 1/03)➢USA; phase II (ongoing 6/01)➢China; phase II/III (ongoing 8/02)➢USA, Europe, China ❑ primary liver cancer; phase I/II (begin 2/01, ongoing 3/03)➢USA ❑ metastatic liver cancer
Fujisawa Pharmaceutical	FK-317, FK317	Substituted dihydrobenzoxazine; analog of FK973, a novel compound derived from the actinomycete, *Streptomyces sandaensis* ❑ IV	Phase II (completed 01)➢Japan; phase I (ongoing 99, completed 01) ➢USA ❑ refractory solid tumors
GEM Pharmaceuticals ❑ Access Oncology	GPX-100 and GPX-150	Novel non-cardiotoxic analogs of doxorubicin ❑ IV	Phase I (begin 12/98, completed 2/01) ➢USA ❑ solid tumors, hematologic malignancy
Kirin	KRN-5500, KRN5500, NSC 650426	A semisynthetic derivative of spicamycin isolated from Streptomyces that inhibits protein synthesis in tumor cells; analog of ceramide ❑ IV	Phase I (begin 6/01, ongoing 3/03) ➢USA, phase I (closed 5/01) ➢Japan ❑ metastatic or refractory solid tumors
Kosan Biosciences	Geldanamycin analogs	Generation of geldanamycin analogs with increased solubility and potency ❑ IV	Research (ongoing 2/03)➢USA ❑ solid tumors
Kyowa Hakko Kogyo ❑ National Cancer Institute (NCI)	UCN-01, KW-2401, NSC 638850	Staurosporine analog; protein kinase C (PKC) inhibitor that may block G2 arrest of the cell cycle following DNA damage; also acts on cell cycle-dependent mechanisms, such as induction of expression of p21 protein, and inhibition of cyclin-dependent Rb kinases ❑ IV	Phase I (begin 12/01, 4/02, 5/02, 7/02, 10/02; ongoing 3/03)➢USA (combination); phase I (begin 9/02)➢Canada (combination) ❑ advanced, refractory or metastatic solid tumors; phase I (begin 7/99, ongoing 3/03)➢USA (combination) ❑ low-grade, relapsed, or refractory NHL; phase II (begin 12/01, ongoing 3/03)➢USA ❑ advanced or metastatic kidney cancer; phase I (begin 7/02,11/02, ongoing 3/03) ➢USA (combination) ❑ unresectable

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			or metastatic pancreatic adenocarcinoma; phase I/II (begin 9/02) ➢Canada (combination) ❑ chronic lymphocytic leukemia (CLL)
Medarex ❑ U Catholique de Louvain, Diatos	CPI-0004, CPI-0004Na ❑ Super-Lue-Dox	Tumor-activated prodrug (TAP) of doxorubicin ❑ IV	Preclin (ongoing 1/03)➢USA, Europe (Belgium) ❑ solid tumors
Menarini Group	MEN 10755, MEN-10755	Disaccharide anthracycline analog of doxorubicin; topoisomease II inhibitor ❑ IV	Phase I (completed 00)➢Europe ❑ solid tumors; phase II (ongoing 2/03)➢Europe (Germany) ❑ sclc; phase II (ongoing 12/02)➢Europe (Belgium, France, Italy, the Netherlands, Switzerland) ❑ advanced or metastatic ovarian cancer; phase II (begin 12/01, ongoing 2/03)➢Europe (Belgium, France, Germany, Spain, Switzerland), Israel ❑ hormone-refractory, progressive prostate cancer
Merck	L-377202, L377202	Peptide conjugate of doxorubicin that is cleaved by PSA at prostate tumor cells to leucine-doxorubicin (leu-dox) and doxorubicin ❑ IV	Phase I/II (begin 1/00, closed 11/00) ➢USA; phase IIa (ongoing 5/01) ➢USA ❑ advanced, hormone-refractory, prostate cancer
National Cancer Institute (NCI)	Geldanamycin ❑ 17-(allylamino)-17-demethoxygeldanamycin (17-AAG), 17AAG; NSC-330507	Geldanamycin derivative, a benzoquinone ansamycin tyrosine-kinase inhibitor which binds to the molecular chaperone heat-shock protein Hsp90, causing disruption of client protein complexes, resulting in degradation of such proteins, including such oncogenic kinases as c-Raf and AKT ❑ IV	Phase I (begin 2/99, 7/99, and 8/99, ongoing 4/03)➢USA, phase I (begin 10/02, ongoing 4/03)➢USA (combination) ❑ advanced, refractory, unresectable solid tumors; phase I/II (begin 8/99, ongoing 4/03)➢Europe (UK) ❑ advanced malignancies; phase I (begin 12/99, ongoing 4/03)➢USA ❑ advanced epithelial cancer, lymphoma or sarcoma; phase I (begin 3/03) ➢USA (combination) ❑ metastatic prostate cancer
National Cancer Institute (NCI) ❑ U Freiburg	17-DMAG, NSC 707545	Water-soluble analog of 17-AAG ❑ IV	Preclin (ongoing 2/03)➢USA Europe (Germany) ❑ solid tumors
NeoPharm ❑ Pharmacia (terminated 1/03)	PNU-108112 ❑ LED	Liposome-encapsulated doxorubicin (LED) ❑ injection	Phase I/II (completed 5/98)➢USA, phase II (begin 6/98, ongoing 2/02) ➢USA, phase II/III (ongoing 3/02) ➢USA ❑ late-stage, hormone-resistant prostate cancer; phase II (ongoing 3/02)➢USA ❑ recurrent or refractory breast cancer; phase II (begin 9/99)➢USA ❑ osteosarcoma; phase I (ongoing 9/99)➢USA; phase II/III (ongoing 3/01)➢USA ❑ hematologic malignancy, multiple myeloma
Novartis Pharmaceuticals	Everolimus ❑ RAD001	Derivative of rapamycin; inhibitor of mTOR kinase ❑ IV, PO	Phase I (planned 03)➢USA ❑ chronic myelogenous leukemia (CML)
Pharmacia	PNU-159548	Novel cytototoxic, a derivative of idarubicin with a low cardiotoxic potential ❑ IV	Phase II (begin 7/00, closed 7/01) ➢USA ❑ metastatic colorectal cancer; phase I (completed 02) ➢Europe (The Netherlands) ❑ advanced solid tumors; phase II (begin 5/00)➢Europe (Spain) ❑ advanced, recurrent or refractory ovarian cancer
Pro-Pharmaceuticals	Davanat-2 ❑ Galactomycin	Enhances effectiveness of doxorubicin while reducing its toxicity ❑ IV	Preclin (ongoing 1/03)➢USA ❑ solid tumors

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Seattle Genetics ❑ Icos	SGN-15 (cBR96-doxorubicin immunoconjugate)	Immunoconjugate consisting of chimeric monoclonal antibody (MAb) BR96 conjugated to doxorubicin ❑ IV	Phase II (begin 10/00, completed 02) ➢USA (combination) ❑ advanced, metastatic or recurrent breast cancer; phase II (begin 10/00, ongoing 10/01)➢USA (combination) ❑ advanced colorectal cancer; phase II (begin 10/00, ongoing 2/03)➢USA (combination) ❑ advanced, hormone-refractory prostate cancer; phase II (begin 10/02, ongoing 2/03)➢USA (combination) ❑ metastatic or recurrent nsclc; phase II (begin 8/02) ➢USA (combination) ❑ advanced ovarian cancer
Spectrum Pharmaceuticals (formerly NeoOncoRx) ❑ Bristol-Myers Squibb	Elsamitrucin ❑ BMY-28090	Antitumor antibiotic acting by inducing single-strand DNA breaks ❑ IV	Phase II (planned 03)➢USA ❑ NHL
Synt:em	SYN 2002	Doxorubicin re-engineered with Pep:trans to permit transport into the brain and also bypass P-gp-mediated multidrug resistance (MDR) ❑ infusion	Preclin (begin 2/02, ongoing 4/03)➢Europe (France) ❑ metastatic brain cancer
U Utah	HPMA-HA-DOX	Targeted delivery of doxorubicin by N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-hyaluronan bioconjugates ❑ IV	Research (ongoing 9/02)➢USA ❑ solid tumors
Wyeth	CCI-779	Structural analog of rapamycin; immunophilin-binding antibiotic, that blocks the initiation of the translation of mRNA by inhibiting mTOR ❑ IV, PO	Phase I (ongoing 4/03)➢USA (oral) ❑ advanced solid tumors; phase I (begin 9/02)➢USA (combination) ❑ refractory RCC; phase II (begin 1/00, ongoing 5/01)➢Europe (UK, Germany, Switzerland) ❑ locally advanced or metastatic breast cancer; phase I/II (begin 12/01, ongoing 4/03)➢USA ❑ malignant glioma; phase II (begin 5/01, ongoing 4/03)➢USA ❑ recurrent glioblastoma multiforme (GBM); phase II (begin 6/01, ongoing 4/03) ➢USA ❑ metastatic melanoma (Stage IV); phase II (begin 4/02, ongoing 4/03)➢USA ❑ mantle-cell NHL; phase II (begin 9/00, completed 5/02) ➢USA ❑ androgen-independent prostate cancer, (Stage IV); phase II (begin 1/02, ongoing 4/03)➢USA ❑ extensive-stage small-cell lung cancer (sclc)
Zentaris/Tulane U	AN-152	Cytotoxic produced by the conjugation of doxorubicin with [D-Lys6] luteinizing hormone-releasing hormone (LH-RH), that can be targeted to tumor cells expressing LH-RH receptors ❑ infusion	Preclin (ongoing 2/03)➢USA ❑ prostate, breast, and ovarian cancer
Zentaris/Tulane U	AN-215	Targeted cytotoxic consisting of 2-pyrrolino-doxorubicin (AN-201), of a potent derivative of doxorubicin, linked to a bombesin-like peptide carrier ❑ infusion	Preclin (ongoing 2/03)➢USA ❑ brain cancer

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Zentaris/Tulane U	AN-238	Targeted cytotoxic consisting of 2-pyrrolinodoxorubicin (AN-201), linked covalently to SST octapeptide-carrier RC-121) ❑ infusion	Preclin (ongoing 2/03)➢USA ❑ prostate cancer
Zivena (formerly BattellePharma)	Resmycin	Aerosolized doxorubicin ❑ inhaled	Phase I (begin 6/99, completed 12/02)➢USA ❑ pulmonary bronchioloalveolar carcinoma (BAC), extensive-stage or recurrent sclc, Stage IIIb or recurrent nsclc; phase I (begin 2/00, ongoing 3/03) ➢USA ❑ advanced solid tumors affecting the lungs

pathways, and cell-cycle regulators, for at least 48 hours after drug removal. In contrast, 17-AAG only briefly suppressed these proteins and the pathways they control. Similarly, treatment of BT474 with EC4 for 24 hours completely shut down cell growth for more than 20 days but the growth rate of 17-AAG-treated cells was only minimally affected at the same drug concentration. This extended duration of activity in tumor cells, may be attributed to the fact that EC4 is more potent than 17-AAG in killing cells with antiapoptotic defects, such as loss of Rb function, PTEN mutations, and Bcl-2-overexpression. These data suggest that the potency of 17-AAG may be overestimated *in vitro* under conditions of continuous exposure, and that testing of these agents in ways that more closely mimic *in vivo* constraints may be more predictive of their antitumor activity (Zhang H, etal, AACR03, Abs. 784).

Indolocarbazole Antibiotics

Indolocarbazole antibiotics, including rebeccamycin and staurosporine, comprise a therapeutically important category of antitumor agents acting by a very different mechanism compared to the better-known, commercialized antibiotic cytotoxics such as the anthracyclines. Rebeccamycin and its analogs target topo I, and staurosporine and its analogs are cell-cycle modulators that also target protein kinase C (PKC).

Staurosporine is a natural product originally isolated in 1977 from the bacterium *Streptomyces staurosporeus* (Omura S, etal, J Antibiotics 1977;30:275). The chemical structure of Staurosporine was elucidated by X-ray analysis of a single crystal and its absolute stereochemical configuration by the same method in 1994. Staurosporine, by inhibiting many kinases, has broad biologic activities, including anticancer properties. However, lack of selectivity has limited its utility.

Staurosporine is also a potent inhibitor of checkpoint 1 (Chk1). Chk1, a serine/threonine protein kinase, is involved in preventing DNA replication when DNA's integrity is compromised. Its coding region comprises a sequence of nine consecutive adenines that are a potential site of mutations in tumors with microsatellite instability (MSI). The protein encoded by the Chk1 gene plays an important role in the G2 checkpoint in mammalian cells. Blocking Chk1 expression with a checkpoint kinase inhibitor prevents cell-cycle arrest during DNA damage response, causing premature mitosis and cell death. Combining Chk1 inhibitors with chemotherapeutic agents enhances the latter's apoptotic effect on cancer cells relative to normal cells. This unique mechanisms of action of staurosporine led to the chemical synthesis of itself, and of compounds with increasingly diverse chemical structure. Hoping to find a more selective Chk1 inhibitor, investigators at GlaxoSmithKline identified SB-218078, a staurosporine analog, which inhibited Chk1 phosphorylation.

AT2433-A1, AT2433-A2, AT2433-B1 and AT2433-B2 are novel antitumor antibiotic compounds related to rebeccamycin, isolated from culture broth of *Actinomadura melliaura*. Scientists at Bristol-Myers Squibb evaluated totally synthetic fluoroindolocarbazole analogs for their *in vitro* ability to induce topo I-mediated single-strand breaks in DNA, and to kill P388 murine leukemia cells expressing topo I (Long BH, etal, AACR03, Abs. 1775). The chemical structures of AT2433-A1, AT2433-A2, AT2433-B1 and AT2433-B2 were elucidated by degradation and spectroscopic studies. AT2433-A1 and AT2433-B1 are two indolocarbazole diglycosides related to rebeccamycin. In these compounds, sequence-selective DNA interaction and topo I inhibition is controlled to a large extent by the stereochemistry of the diglycoside moiety (Facompre M, etal, Mol Pharmacol, Nov 2002;62(5):1215-27).

CEP-701 is a novel, orally active, indolocarbazole derivative under development by Cephalon (West Chester, PA), in collaboration with Kyowa Hakko Kogyo (Tokyo, Japan). CEP-701 is a synthetic analog of the indolocarbazole metabolite K252a (Ruggeri BA, etal, Curr Med Chem, Sep 1999;6(9):845-57), a low molecular-weight alkaloid isolated from the actinomycete strain K-252T, originally thought to belong to the genus Nocardiopsis (Kase H, etal, J Antibiot (Tokyo), Aug 1986;39(8):1059-65,

Nakanishi S, etal, J Biol Chem, 5 May 1988;263(13):6215-9), but now proposed as a new species of the genus Nonomuraea (Chiba S, etal, Int J Syst Bacteriol, Oct 1999;49 (Pt 4):1623-30).

CEP-701 is a potent inhibitor of neurotrophin receptor-linked tyrosine kinases (trkA, B and C) exhibiting antineoplastic activity *in vivo*, and to a lesser extent, also inhibiting PKC and kinases linked to VEGFr (flk-1) and platelet-derived growth factor receptor (PDGFr) (Miknyoczki SJ, etal AACR99, Abs. 3199:484, and Marshall J, etal, ASCO00, Abs. 713:184a). Ligand-occupied trk receptors signal via several pathways, including ras/raf/mekk and PI-3 kinase/Akt. This latter pathway is important in the phosphorylation of the proapoptotic protein Bad, sequestering it in the cytoplasm, and in phosphorylation-dependent inactivation of pro-caspase-9. CEP-701 has been shown to impede the growth of cancers in various animal models in a cell-cycle independent fashion via induction of apoptosis. CEP-701 induces cancer cell apoptosis by inhibiting kinase activity of the trk receptor, preventing its autophosphorylation. This, in turn, prevents the activation of trk-associated ras and PI-3 kinase pathways. CEP-701 also binds to calmodulin, leading to a delayed elevation in intracellular free calcium ion and subsequent activation of calcineurin, which causes the dephosphorylation of Bad, and thus its translocation to the mitochondria and downstream activation of caspases and apoptosis (Weeraratna AT, etal, AACR-NCI-EORTC99, Abs. 104).

CEP-701 exhibited broad anticancer activity in preclinical trials. CEP-701 inhibits the autophosphorylation of wild-type and constitutively activated FLT3, resulting in cell death of leukemia cells harboring the mutation. CEP-701 also inhibited growth of pancreatic tumors (Miknyoczki SJ, etal, Ann NY Acad Sci, 30 Jun 1999;880:252-62, Miknyoczki SJ, etal, Clin Cancer Res, Aug 1999;5(8):2205-12, and Miknyoczki SJ, etal, AACR99, Abs. 3199:484) and, in combination with gemcitabine, was more effective at inhibiting the growth of pancreatic xenografts in murine models than either compound alone. Oral administration of CEP 701 dose-dependently reversed NGF-induced thermal hyperalgesia in rats (Aimone LD, etal, Soc Neurosci Abstracts 2000;26(Part 2):1692). CEP-701, when combined with chemically induced androgen ablation, was more efficacious than either monotherapy, suggesting that each of these approaches produces prostate cancer cell death through complementary mechanisms (George DJ, etal, Cancer Res, 15 May 1999;59(10):2395-401).

CEP-701 is being clinically investigated both in solid tumors and hematologic malignancies. A phase II clinical trial of CEP-701 is currently ongoing for the treatment of pancreatic cancer. In recently completed phase II clinical trials in resectable and hormone-resistant prostate cancer, initiated in January 2000, CEP-701 appeared to confer clinical benefit to some patients, although certain side effects and a higher than anticipated dropout rate resulted in early termination of the study.

In a dose-escalation, phase I clinical trial, initiated in March 1998 at the Lombardi Cancer Center at Georgetown University Medical Center (Washington, DC) and the University of Chicago Medical Center (Chicago, IL), CEP-701, at doses ranging from 5 mg to 160 mg, was administered PO twice-daily for 28 days, to 29 patients with advanced, incurable prostate (n=5), pancreatic (n=4), colorectal (n=3), renal (n=4), and lung (n=3) cancer and sarcoma (n=2), melanoma (n=2), and other malignancies (n=6). Median duration of treatment was 5 weeks with 2 patients treated for >6 months. According to preliminary data, drug-related toxicities included Grade 1-3 nausea, Grade 1-2 vomiting and diarrhea, and Grade 1-3 epigastric discomfort, gastro-esophageal reflux, increased fecal frequency, and anorexia. Other toxicities included moderate muscle cramps, parasthesias and mild fatigue. No myelosuppression was observed. All patients discontinued treatment at 160 mg *bid* because of gastrointestinal symptoms; 10 patients treated at 40 mg *bid* tolerated CEP-701 without Grade 3 toxicities. Disease stabilized for >6 months in 1 patient with prostate cancer, and in 1 with sarcoma while on treatment; the remaining patients continued on the study or progressed after variable periods of treatment (Marshall J, etal, ASCO00, Abs. 713:184a).

CEP-701 may be particularly useful in the treatment of acute myeloid leukemia (AML). Constitutively activating mutations of the receptor tyrosine kinase FLT3 are present in approximately 1/3 of patients with *de novo* AML, and are associated with a poor outcome to treatment with traditional chemotherapy. The cytotoxic effects of CEP-701 on both model cell lines and primary AML blasts harboring FLT3 activating mutations, were generally additive with the effects of chemotherapeutic agents commonly used to treat AML, such as cytarabine, etoposide, and daunorubicin. Vincristine, in contrast, appeared to antagonize CEP-701-mediated cytotoxicity, an effect that was likely attributable to interactions between the cell cycle-arresting properties of these different agents. These data suggest that concurrent use of FLT3 inhibitors and chemotherapy may be of clinical benefit (Levis MJ, etal, ASH02, Abs. 4603:268b).

An open-label, single-agent, phase II clinical trial (protocol ID: C0701a/202/ON/US) of CEP-701 for treatment-refractory or relapsed AML expressing FLT3-activating mutations, was initiated in January 2002 to determine response rate. Patients are administered CEP-701 (40 mg) twice daily. A total of 37 patients are expected to enroll in this trial. The PI is B. Douglas Smith, MD of the Johns Hopkins Oncology Center.

In an interim report, among 5 enrolled patients, 3/5 either completed treatment or withdrew, and 2/5 were undergoing treatment at the time of the report. CEP-701 was detected in plasma obtained 1 hour after dosing at 40 mg *bid*, but concentrations were variable. Starting dose of CEP-701 was increased to 60 mg *bid* for 1 month with a further dose escalation to 80 mg *bid* for the second month

based on the fact that isolation of peripheral blood leukemia cells from the patients undergoing treatment at a dose of 40 mg demonstrated successful but incomplete *in vivo* inhibition of FLT3 phosphorylation. In this trial, CEP-701 toxicities were minimal and often difficult to distinguish from baseline disease-related symptoms. Commonly seen toxicities included Grade 1-2 nausea, emesis and fatigue. There were 3 cases of admissions for neutropenic fever (all culture negative) and 1 catheter-related infection. The first patient to be treated at 60 mg *bid*, a 71-year-old with a history of secondary AML following previous treatment for NHL, responded to CEP-701. The patient was refractory to cytarabine-based induction with residual leukemia with approximately 25% blasts. Subsequently to treatment with CEP-701, in combination with low-dose hydroxyurea, the patient's blood counts stabilized and remained improved after discontinuation of hydroxyurea. At day 28, the patient no longer required transfusions, had normalized peripheral blood counts, and bone marrow evaluation revealed a cellular marrow with <5% blasts, and evidence of full myeloid maturation (Smith BD, etal, ASH02, Abs. 314:85a).

DEAE rebeccamycin (XL 119), under development by Exelixis (South San Francisco, CA), is a water-soluble analog of rebeccamycin. In July 2001, Exelixis, in conjunction with an agreement with Bristol-Myers Squibb, obtained a royalty-free worldwide license to DEAE rebeccamycin. A broad clinical program, mostly sponsored by the NCI, had been undertaken in the 1990s to evaluate DEAE rebeccamycin in various solid tumors and hematologic malignancies in both children and adults.

A dose-escalation, phase I clinical trial (protocol ID: POG-9670) was conducted by the Pediatric Oncology Group (POG) to determine MTD and DLT, PK, and any antitumor activity of XL 119 in children with refractory solid tumors. A 60-minute infusion of XL 119 was administered every 21 days to 17 children with malignant tumors refractory to conventional therapy. Doses ranged from 450 mg/m^2 to 760 mg/m^2. All in all, 16 patients on 3 dose levels were evaluable for toxicities. At 760 mg/m^2, 4 patients (both heavily and less heavily pretreated) experienced dose-limiting neutropenia and thrombocytopenia, with accompanying sepsis in 3. Other toxicities were minimal, including Grade 3 transient elevation of transaminase (n=2 at 760 mg/m^2), Grade 1 fever, dizziness and headache (n=3), and Grade 3 nausea/vomiting (n=1). There was a suggestion that a saturable process of elimination existed and that the compound was metabolized by cytochrome P450. The recommended phase II dose as a 60-minute infusion every 21 days to children with solid tumors is 585 mg/m^2. Neutropenia and thrombocytopenia are the DLT (Langevin A, etal, ASCO99, Abs. 764:198a). Based on this trial, two phase II clinical trials were undertaken in children with solid tumors.

A multicenter, multinational, phase II clinical trial (protocol ID: COG-P9963), undertaken by the Children's Oncology Group (COG), was initiated in July 2000, to treat children with solid tumors (bone cancer, brain tumor, or eye cancer), or NHL. Trial objectives are to determine pharmacokinetics and response, determine and maintain a plasma drug concentration of at least 5 μg/ml, and assess toxicity. According to the protocol, patients with solid tumors are stratified according to tumor histology (neuroblastoma, Ewing's sarcoma/PNET, osteosarcoma, rhabdomyosarcoma, other solid tumors, and NHL). Patients with CNS tumors are stratified according to tumor histology (medulloblastoma/PNET, ependymoma, brainstem glioma, and other CNS tumors). XL 119 is administered IV over 1 hour on day 1. Treatment continues every 21 days for a total of 16 courses in the absence of disease progression or unacceptable toxicity. Between 90-280 patients will be accrued for this study. Anne-Marie Langevin, MD, of COG is Study Chair.

A phase II clinical trial (protocol IDs: MSKCC-98095; NCI-T98-0041) with XL 119 was initiated at MSKCC, in January 1999, in children with relapsed or refractory neuroblastoma, to determine response rate, evaluate toxicity, and identify and establish *in vitro* biologic correlates of clinical responses and toxicity. According to the protocol, patients are being treated with IV XL 119 over 30 minutes, once every 3 weeks, for 6 weeks. Patients may continue therapy in the absence of disease progression or unacceptable toxicity. This study will accrue up to 30 patients. Tanya Trippett, MD, of MSKCC is Study Chair.

A multicenter, dose-escalation, phase I/II clinical trial (protocol IDs: UTHSC-IDD-98-34; NCI-T98-0069; SACI-IDD-98-34) was initiated in October 1999 at 4 Texas medical centers with Lisa Hammond, MD, of the University of Texas as Study Chair. This trial is studying the effectiveness of XL 119 and cisplatin with or without filgrastim (G-CSF), in treating patients with advanced cancer (leukemia, lymphoma or eye cancer). Study objectives are to determine MDT, toxicity and pharmacokinetics of this regimen in this setting, and assess any antitumor effects. According to the protocol patients are divided into 2 groups. Group I includes previously untreated or minimally pretreated patients, and group II heavily pretreated patients. In group I the first patient is treated with cisplatin IV over 1 hour followed 2 hours later by IV XL 119 infused over 1 hour on day 1. The second patient in the same cohort is treated with the same drugs in the reverse order. The drug sequence for each additional patient within the same cohort is alternated with reference to the preceding patient. During each subsequent course, the study drugs are administered to each patient in the reverse order as compared to the prior course. Treatment repeats every 3 weeks for 4 courses in the absence of disease progression or unacceptable toxicity. Dose escalation is initially performed without G-CSF. Cohorts of 4-6 patients are treated with escalating doses of XL 119 and cisplatin until MTD is determined for each drug. Patients are then administered G-CSF subcutaneously, daily, beginning on day 2 and

continuing until blood counts recover for 2 days or until approximately day 15. Cohorts of 4 to 6 patients are treated with escalating doses of XL 119 and cisplatin as above. Group II patients are treated with XL 119 starting at 2 dose levels preceding the MTD from group I. Patients are followed for at least 30 days. A maximum of 40 patients will be accrued for phase I.

In this trial, sequence I consisted of XL 119 prior to cisplatin (XC) and sequence II, cisplatin prior to XL 119 (CX). MTD was determined separately with and without G-CSF in minimally-preatreated (MP), and heavily-pretreated (HP) patients. Among 13 MP patients who underwent 39 courses at 2 daily dose levels (XC 440/50 mg/m^2 or 550/50 mg/m^2) without G-CSF, and 1 daily dose level (XC 550/50mg/m^2) with G-CSF, dose-limiting hematologic toxicity (cycles 1 and 2) consisted of febrile neutropenia (2/3 patients in cycle 1, sequence CX 50/550mg/m^2) and thrombocytopenia (1/3 patients in cycle 1, sequence CX 50/550mg/m^2). Nonhematologic toxicity consisted of nausea, vomiting (Grade 3=1), fatigue, mucositis, and hypomagnesemia. One patient with adenocarcinoma of unknown primary with metastases to the liver experienced severe drug-related toxicity. There was 1 PR in a patient with nsclc, and another in a patient with adenocarcinoma of unknown primary, and a minor response in 1 patient with an adenoidcystic tumor. MTD of the XC without G-CSF in MP patients is 440/50 mg/m^2 daily. Accrual continues in MP patients with G-CSF at XC 550/50mg/m^2 daily (Hammond LA, etal, ASCO02, Abs. 380:96a).

A phase I clinical trial (protocol IDs: CWRU-4Y96, NCI-T97-0002) of XL 119 in 24 adults with refractory or recurrent solid tumors was conducted at Ireland Cancer Center at University Hospitals of Cleveland with Scot C. Remick, MD, as Study Chair. In this trial, XL 119 was administered as a 2-hour infusion, daily for 5 days, repeated every 3 weeks, to 30 evaluable patients who were treated with a total of 153 cycles at daily doses of 60, 80, 106, 141, and 188 mg/m^2. Grade 2 phlebitis occurred in all patients before use of central venous access at dose levels ≥141 mg/m^2. Grade 4 neutropenia was the DLT, occurring at 188 mg/m^2 in both previously treated and chemotherapy-naive patients. There were 2 PR, 2 MR, and 6 prolonged (>6 months) cases of stable disease. Of these, 3 patients with gallbladder cancer and 1 patient with cholangiocarcinoma experienced either MR or a significant period of freedom from progression. The recommended phase II dose of XL 119 administered daily for 5 days every 3 weeks is 141 mg/m^2 for patients with prior therapy and 165 mg/m^2 for those with no prior therapy; DLT is neutropenia (Dowlati A, etal, J Clin Oncol 2001 Apr 15;19(8):2309-18, and Dowlati A, etal, ASCO99, Abs. 694:181a).

Another dose-escalation, phase I clinical trial (protocol IDs: UTHSC-9455011077, NCI-T94-0136D) of XL 119 in advanced malignancies, excluding primary or metastatic brain tumors, was also conducted at the University of Texas Health Science Center with Gail Eckhardt, MD, as Study Chair. XL 119 was administered IV over 30 to 60 minutes, once every 3 weeks to 45 patients treated with 130 courses at doses ranging from 20 mg/m^2 to 744 mg/m^2. Myelosuppression was the principal toxicity. Severe neutropenia, often associated with thrombocytopenia, was unacceptably high in heavily pretreated (HP) and minimally pretreated (MP) patients at doses of 572 mg/m^2 and 744 mg/m^2, respectively. Nausea, vomiting, and diarrhea were common but rarely severe. Despite a heterogeneous population of MP and HP patients, the magnitude of drug exposure correlated well with the severity of myelosuppression. Antitumor activity was observed in 2 HP patients with ovarian cancer, and in 1 patient with soft-tissue sarcoma refractory to etoposide and doxorubicin. Recommended phase II doses are 500 mg/m^2 and 572 mg/m^2 IV once every 3 weeks for HP and MP patients, respectively (Tolcher AW, etal, J Clin Oncol, 1 Jun 2001;19(11):2937-47).

A dose-escalation, phase I clinical trial (protocol IDs: WCCC-CO-9493, NCI-T94-0118H), completed in 2000 at the University of Wisconsin Comprehensive Cancer Center (Madison, WI) with Jim Cleary, MD, as Study Chair, assessed XL 119 in metastatic epithelial and mesenchymal tumors and malignant lymphomas. A total of 40-45 patients were to be accrued for this study to be treated with XL 119 IV over 30-60 minutes on days 1-3, repeated every 3 weeks for 4 courses at escalated doses until MTD is determined.

A multicenter, randomized phase II clinical trial (protocol IDs: CWRU-1599; NCI-91) was initiated in May 2000, to compare the effectiveness of two XL 119 regimens in treating patients with Stage IIIb, Stage IV, or recurrent nsclc. Study objectives include comparing efficacy, in terms of response rate, in patients nsclc treated with XL 119 via 1 infusion versus 5 daily infusions, every 3 weeks, response duration, and toxicity of these two regimens. According to the protocol patients are randomized to one of two treatment arms. In arm I, patients are treated IV over 1 hour on day 1, and in arm II, on days 1-5. In both arms, treatment repeats every 3 weeks for 6-8 courses in the absence of disease progression or unacceptable toxicity. A total of 36-64 patients (18-32 per arm) will be accrued for this study. Afshin Dowlati, MD, at the Ireland Cancer Center is Study Chair.

A multicenter, randomized phase II clinical trial (protocol IDs: DFCI-99283; NCI-197; CWRU-DFCI-1199) was initiated in June 2000, to compare the effectiveness of two regimens of XL 119 in treating patients with Stage IIIb/IV, or recurrent breast cancer, including male breast cancer. The objectives and protocol are exactly the same as for the nsclc trial. A total of 42 patients (21 per arm) will be accrued for this study over 14-21 months. Harold J. Burstein, MD, of the Dana-Farber Cancer Center (Boston, MA) is Study Chair.

A phase II clinical trial (protocol IDs: WSU-C-2063; NCI-T99-0113) was initiated at the Barbara Ann Karmanos

Cancer Institute (Detroit, MI), with Maha Hadi A. Hussain, MD, as Study Chair, to evaluate XL 119 as first-line chemotherapy in unresectable Stage III/IV, or recurrent RCC. Trial objectives are to determine patient response rates and assess the quantitative and qualitative toxicities associated with this drug in this setting. According to the protocol, patients are treated with IV XL119 (165 mg/m^2) over 30-60 minutes, daily, for 5 days. Treatment continues every 21 days in the absence of disease progression or unacceptable toxicity. A total of 23 to 44 patients will be accrued for this study. According to interim results involving 16 patients (11 had disease in 3 sites, 11 had prior nephrectomy, and 9 had prior immunotherapy), 62 cycles were administered with a median of 4 (range=1-8). All patients were assessable for toxicity and 15 were assessable for response. Overall, therapy was well tolerated. Major toxicities were Grade 3/4 neutropenia (n=4), Grade 3/4 anemia (n=6), and Grade 3 thrombocytopenia (n=3), hyponatremia (n-3) and fatigue (n=3). Disease stabilized in 8/15 patients, but there were no CR or PR (Ibrahim D, etal, ASCO01, Abs. 2373:156b).

J-107088, a novel indolocarbazole topo I inhibitor under development by Banyu Pharmaceuticals (Tokyo, Japan), a Merck company, in collaboration with Pharmacia, is a synthetic analog of NB-506, a natural rebeccamycin derivative. NB-506 had been investigated in phase I clinical trials in the mid-1990s.

In a phase I clinical trial, conducted in Japan at the National Cancer Center Hospital in Tokyo and National Nagoya Hospital, J-107088 was evaluated for the treatment of various advanced solid tumors, including nsclc, uterine sarcoma, and colorectal, gastric, esophageal, and bile-duct carcinoma. A total of 24 patients were administered J-107088 over 2 hours, once every 21 days, at doses of 8, 11, 13, and 15 mg/m^2. Patients were premedicated with dexamethasone and granisetron (Kytril; Roche) before and for 3 days after J-107088 administration to prevent fatigue. Nausea, vomiting, infection, phlebitis, granulocytopenia, fever, neutropenia, constipation, and fatigue were drug-related adverse events. Dose-dependent granulocytopenia and neutropenia were also observed. Among 5 patients treated at 15 mg/m^2, DLT was noted in 3, including Grade 4 granulocytopenia, and Grade 3 constipation, infection, and ileus. J-107088 plasma rapidly increased with levels being maintained from 15 minutes to end of infusion, and then decreased rapidly following infusion. Tumor activity was seen in 2 patients with gastric and esophageal cancer (Yamada Y, etal, ASCO02, Abs. 385:97a).

In another phase I clinical trial, a similar regimen was used to treat 24 patients with advanced solid tumors. Toxicities included acute nausea, vomiting, headache, fever, and fatigue, and these symptoms proved to be dose limiting in 2/3 patients treated at 15 mg/m^2. Subsequent patients were treated with dexamethasone (20 mg) and granisetron (1 mg) before, and dexamethasone (8 mg) and granisetron (2 mg) for three days after administration of J-107088 with the objective of moderating acute toxicities. This regimen reduced the frequency/severity of symptoms when used in conjunction with dose levels of 11, 13, and 15 mg/m^2. Dose-limiting neutropenia was observed in 2/6 patients treated at 15 mg/m^2 with dexamethasone and granisetron and, therefore, additional accrual continued at the 13 mg/m^2 dose level with prophylaxis. Antitumor activity was observed in 2 patients with metastatic colon cancer refractory to CPT-11 and 5-FU, and in 1 patient with heavily pretreated breast cancer (Peck R, etal, ASCO00, Abs. 767:197a).

A different regimen of J-107088 was administered to 13 patients (RCC=4, colorectal cancer=3, nsclc=2, and 1 each gastric cancer, bronchial carcinoid, mixed mesodermal uterine tumor, and hepatocellular carcinoma) in a multiple-dose schedule of 2, 4, 5.5 and 7.5 mg/m^2, biweekly, for 2 weeks, every 4 weeks, as a 1-hour IV infusion on days 1, 4, 8 and 11 in cycles of at least 28 days. Dose limiting Grade 4 neutropenia and thrombocytopenia (lasting >5 days) occurred in 2/3 patients at 7.5 mg/m^2. The recommended phase II dose on this schedule is 5.5 mg/m^2 (Lionel L, etal, ASCO00, Abs. 688:177a).

A multicenter, phase II clinical trial (protocol IDs: PRA-RC749J202-01; BANYU-PRA-RC749J202-01) was initiated in the USA in September 2000; Siu-Long Yao of Pharmaceutical Research Associates (McLean, VA) is Study Chair. According to the protocol, IV J-107088 (13 mg/m^2) was administered every 3 to 4 weeks to irinotecan-refractory patients (n=19) or CPT11-naïve but 5-FU refractory (n=13) patients with metastatic colorectal cancer. Neutropenia was the predominant adverse effect, with Grade 3/4 neutropenia occurring in 8 patients. Other infrequent adverse events included abdominal pain (Grade 3/4 in 3 patients) and Grade 3 diarrhea (n=2), nausea (n=1), vomiting (n=1) and fatigue (n=1). Although none of the irinotecan-refractory patients met criteria for PR, 5/18 patients experienced tumor shrinkage and a prolonged estimated median time-to-progression (TTP) of 6 months. Among 15 irinotecan-naïve patients, 2 experienced a sustained >75% reduction in tumor mass, and a sustained >50% decrease in CEA was observed in 2 (Perez RP, etal, ASCO02, Abs. 632:159a).

Additional multicenter, phase II clinical trials are being conducted in the USA in metastatic bladder cancer (protocol IDs: PRA-RC749J204-01; BANYU-RC749J204-01; PRA-WIRB-20010518), and head and neck cancer (protocol IDs: PRA-RC749J205-01; BANYU-RC749J205-01; PRA-WIRB-20001963).

PKC412, under development by Novartis, is a PKC inhibitor. PKC-412 inhibits angiogenesis by blocking the phosphorylation of VEGF, and also reverses the efflux and function of P-gp. Investigators at Dana-Farber Cancer Institute (Boston, MA), Brigham and Women's Hospital (Boston, MA) and Novartis, demonstrated that PKC412 directly inhibited FLT3 tyrosine kinase activity. Balb/c mice treated with marrow transduced with a FLT3-ITD-

expressing retrovirus developed myeloid leukemia fatal in 60 to 90 days. When these mice were treated with vehicle or PKC412 starting on day 32 after transplant, at the termination of the study on day 90, 13/13 control mice had progressive leukemia, manifested by marked splenomegaly and leukocytosis, while 0/13 mice treated with the drug developed overt signs of leukemia (Weisberg E, etal, ASCO02, Abs. 16:5a).

PKC412 was also shown to be effective as a single agent against experimental NCI H-460 lung tumor xenografts in immunosuppressed nude mice, inhibiting tumor growth in a dose-dependent manner. PKC412 produced an antitumor effect comparable to such conventional antitumor agents as doxorubicin, cisplatin, carboplatin, gemcitabine, cyclophosphamide, but not paclitaxel, but was much better tolerated than these agents. PKC412 improved the antitumor activity of paclitaxel by delaying the post-treatment regrowth of tumors in mice that had been treated with both agents as compared to paclitaxel alone. However, there was evidence of decreased tolerability of the combination. The combination of PKC412 with cisplatin produced an indifferent or weak combination effect against NCI H-460 tumors, whereas the combination of PKC412 with carboplatin produced a weak combination effect against NCI H-69 lung tumor xenografts (O'Reilly T, etal, AACR01, Abs. 4889:911).

In a dose-escalation, phase I clinical trial of PKC412, combined with 5-FU, 33 patients with advanced solid tumors were administered PKC412 PO, daily, combined with continuous infusion 5-FU (200 mg/m^2/day), administered on days 1-21/28. Mild DLT included emesis, fatigue, and mucositis. No DLT was observed in the first 4 patients treated with PKC412 at 225 mg/day, but 3/4 discontinued treatment because of Grade 2 emesis/stomatitis. Among 5 additional patients enrolled at a dose of 225 mg/day, 1 experienced Grade 3 fatigue and nausea, and 1 developed worsening hyperglycemia (Grade 1/3) manageable with appropriate antidiabetic medication. This patient subsequently developed Grade 2 nausea and vomiting which led to treatment discontinuation. The recommended phase II dose of PKC412 is 150 mg/day when combined with 5-FU (Garcia-Carbonero R, etal, ASCO01, Abs 329:83a).

A dose-escalation, phase I clinical trial of PKC412, in combination with paclitaxel and carboplatin, was conducted to determine MTD, pharmacokinetics, and safety in 38 patients with untreated Stage IIIb/IV nsclc. Patients were administered PKC412, PO, daily, for 21 days per cycle. Paclitaxel (175 mg/m^2) was administered over 3 hours and carboplatin (AUC=5) over 1/2 hour on day 2. Treatment was repeated every 21 days for a maximum of 6 cycles. In six cohorts, PKC412 doses were escalated from 25 mg/day to 225 mg/day. A definitive MTD of PKC412 in combination with paclitaxel and carboplatin could not be reached within this dose range. MTD was defined as 225 mg/day because of increasing toxicity, and based on results observed in a parallel study. The most common adverse events resulting from chemotherapy were muscle and bone pain, leukopenia, vomiting, diarrhea, and polyneuropathy. Adverse events resulting from PKC412 were increasing nausea, vomiting and diarrhea. In 28 evaluable patients, there was 1 CR and 10 PR (Fischer T, etal ASCO01, Abs 1322:331a).

In a dose-escalation, phase I clinical trial, 20 patients with advanced nsclc were treated with fixed doses of gemcitabine (1000 mg/m^2) on days 1, 8, and 15, cisplatin (100 mg/m^2) on day 2, every 4 weeks, and PKC412 PO daily at a starting dose of 25 mg/day (11% of the single-agent MTD); 8 patients were escalated to a daily dose of 50 mg, 4 patients to 100 mg, and 4 patients to 150 mg. DLT was represented by one case of Grade 3 headache and one case of Grade 3 neutropenia at 50 mg, and 3 cases of Grade 3 diarrhea at 150 mg. Most of the other toxicities including neutropenia, thrombocytopenia, and nausea and/or vomiting, were considered to be related to the cisplatin/gemcitabine chemotherapy. Among 13 evaluable patients, there were 2 PR, and disease stabilized in 6 and progressed in 5. There were 3 PKC412-related DLT at the maximum daily dose of 150 mg. The recommended daily dose is 100 mg. Addition of PKC412 to standard cisplatin/gemcitabine chemotherapy did not significantly increase toxicity of this combination (Monnerat C, etal, AACR01, Abs. 1941:360).

A multicenter, open-label, phase II clinical trial (protocol IDs: DFCI-01227, MSKCC-02021, NORVARTIS-CPKC412A2104, NCI-G02-2107) of PKC412 was initiated in March 2002 at the Dana-Farber Cancer Institute, MSKCC, and UTMDACC, for the treatment of relapsed AML with FLT3 mutations, and high-risk myelodysplastic syndrome (MDS). Trial objectives are to determine, preliminarily, antitumor activity of PKC412, assess the safety of this drug, determine its pharmacodynamic activity, in terms of cellular FLT3-ITD inhibition, and assess changes in markers of efficacy and toxicity. A total of 8 to 12 patients will be accrued for this study to be treated with oral PKC412, 3 times daily. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Virginia Klimek, MD, of MSKCC is Study Chair.

UCN-01 (7-hydroxystaursporine), under development by Kyowa Hakko Kogyo, is a staurosporine analog that was originally developed as a specific inhibitor of PKC. However, subsequently, it was shown that UCN-01 exhibits multiple mechanisms of action that contribute to its anticancer properties. As an example, UCN-01 induces apoptosis in human leukemia cells, a function that appears to be unrelated to PKC inhibition. In addition, UCN-01 also blocks G2 arrest of the cell cycle following DNA damage. It also acts on cell-cycle-dependent mechanisms, such as induction of expression of p21 protein, and inhibition of cyclin-dependent Rb kinases.

UCN-01 exhibits antiproliferative activity against a variety of cell types, and induces either G0/G1 or G2/M arrest, depending upon the p53 or pRb status of the cell. UCN-01 functions as a G2/M checkpoint abrogator based on its capacity as an inhibitor of Chk1 function. Inhibition

of Chk1 leads to dephosphorylation, which prevents cells from arresting in G2/M and thereby interferes with DNA repair. Loss of the G2/M checkpoint sensitizes cancer cells to DNA damaging agents, particularly in cells defective in p53 tumor suppressor function. In *in vitro* studies, UCN-01 was a potent abrogator of G2 checkpoint control in cancer cells with disrupted p53 function. Therefore, UCN-01 may enhance effectiveness of DNA-damaging agents against tumors whose cells lack normal p53 function. UCN-01 potentiates the antitumor activity of several cytotoxic agents, including camptothecin and cisplatin. It also interacts synergistically with multiple S-phase specific agents, including fludarabine, ara-C, and gemcitabine, to induce apoptosis in human leukemia cells. UCN-01 also potentiates radiosensitivity of breast cancer cells (Wang Q, etal, JNCI, 17 Jul 1996;88(14):956-65 and Cartee L, etal, Intl J Onc 2002;21:351-359).

Several phase I clinical trials, investigating various treatment regimens with UCN-01 as monotherapy in the treatment of solid tumors or lymphoma have been completed. Phase I clinical trials were conducted in Japan and the USA using different administration schedules. In the Japanese trial, UCN-01 was administered as a 3-hour infusion every 3 weeks at the starting dose of 0.65 mg/m^2, which was 1/6 of the toxic dose in dogs. A total of 16 patients were entered in the trial. Toxicities were mild including diarrhea, nausea and arrhythmia; no Grade 3/4 toxicity was observed. The pharmacokinetics of UCN-01 in humans displayed distinctive features from those in animals, i.e., extremely long half-life, and low systemic clearance and distribution volume. The dose intensity in the Japanese trial is much higher than the 72-hour infusion schedule adopted in the USA trial, which reported less severe toxicity (Tamura T, etal. ASCO99, Abs. 611:159a).

In the USA, a 72-hour continuous infusion (CIV), every two weeks, was employed in a dose-escalation, phase I clinical trial of UCN-01 to treat 44 patients with advanced solid tumors. Because of the unexpected prolonged half-life (4 weeks) of UCN-01, the protocol was amended to retreat every 4 weeks, starting at a dose of 12 mg/m^2/day, and CIV duration was reduced to a total of 36 hours for the second and later courses. DLT included pulmonary toxicity, self-limited hyperglycemia, lactic acidosis with hyperglycemia, nausea/vomiting, and transaminitis. Other reversible toxicities included Grade 2 myalgias, hypotension, and headache. MTD was 42.5 mg/m^2/day (Senderowicz AM, etal, ASCO99, Abs. 612:159a).

A dose-escalation phase I clinical trial (protocol IDs: JHOC-98012305, NCI-T97-0083) of IV UCN-01, administered over 3, 2 or 1 hour(s), every 4 weeks, in patients with advanced solid tumors or chronic lymphoproliferative disorders, was completed in September 2001 at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University (Baltimore, MD). The first dose level was administered over 3 hours, the next over 2 hours, and the next and subsequent doses over 1 hour.

A phase I clinical trial was conducted to evaluate UCN-01, administered as a short infusion (1 to 3 hours) every 28 days. Using an accelerated titration design, 6 dose levels were evaluated ranging from 3 mg/m^2 over 3 hours to 95 mg/m^2 over 1 hour, in 15 patients (colorectal=5, prostate=3, RCC=2, and 1 each cervical, esophageal, breast and lung cancer, and carcinoid). There were 37 courses evaluable for toxicity. At doses up to 68 mg/m^2 over 1 hour, toxicity was mild and reversible, including Grade 2 nausea/vomiting, and Grade 2 fatigue, fever, and hyperglycemia. However, at 95 mg/m^2 over 1 hour, dose-limiting hypotension occurred in one patient who experienced syncope, and readily reversible respiratory arrest. The 2 prior patients treated at that dose level also experienced Grade 1/2 hypotension. Under the assumption that hypotension is related to rapid infusion, the duration of infusion was lengthened to 3 hours, with accrual continuing at that dose level. There have been no responses thus far, but disease stabilized in 1 patient with cervical cancer for over one year who remains on the study (Dees E, etal, ASCO00, Abs. 797:205a).

UCN is being evaluated in several phase I clinical trials in combination with other cytotoxics. A dose-escalation phase I clinical trial (protocol ID: JHOC-J0173, NCI-5528) was initiated in October 2002 at Sidney Kimmel Comprehensive Cancer Center, to determine MTD and recommend phase II dose of UCN-01, in combination with irinotecan, in patients with advanced solid tumors, primarily lung, ovarian, and gastrointestinal cancer. Ross Donehower, MD, is Protocol Chair. IV UCN-01 is administered over 3 hours on day 1, and IV irinotecan over 90 minutes on days 1 and 8. Courses are repeated every 21 days in the absence of disease progression or unacceptable toxicity.

An NCI-sponsored, dose-escalation, multicenter, phase I clinical trial (protocol IDs: PMH-PHL-005; NCI-5518) was initiated in September 2002 to establish MTD and recommended phase II dose of the combination of UCN-01 with topotecan in treating patients with advanced solid tumors. Hal W. Hirte, MD, of Cancer Care Ontario-Hamilton Regional Cancer Centre in Canada, is Study Chair. According to early results from this trial, the combination of UCN-01 and topotecan was relatively well tolerated with some preliminary evidence of efficacy. Both agents were administered through central venous access, every 21 days, in escalating doses, to patients with refractory solid tumors (ovarian=6, colon=1, salivary gland=1, cervix=1). On day 1, following antiemetic prophylaxis with dexamethasone and a 5HT3 inhibitor, UCN-01 was infused over 3 hours, followed by topotecan over 30 minutes; on days 2 to 5, patients were administered only topotecan. Among 9 patients entered in the first 2 cohorts, 3 progressed and 1 died. Most drug-related adverse events were mild (Grade 1/2). Nonhematologic Grade 3/4 toxicity consisted of transient hyperglycemia (n=2), fatigue (n=2), hypotension (n=1), and hypokalemia (n=1). Grade 3/4 hematologic

abnormalities included neutropenia (n=6) and neutropenia with infection (n=1), leukopenia (n=5) and leukopenia as a DLT (n=1), lymphopenia (n=2), and thrombocytopenia (n=2). Among 6 patients assessable for response, there was 1 uncofirmed PR in an ovarian cancer case, and disease stabilized in 3, and progressed in 2. Accrual continues at dose level 3 involving UCN-01 at 90 mg/m^2 and topotecan at 1.0 mg/m^2 (Hotte SJ, etal, AACR03, Abs. 5355).

A dose-escalation, phase I clinical trial (protocol IDs: NCI-02-C-0241, NCI-5694) of UCN-01 and prednisone in refractory solid tumors and lymphoma, began in July 2002 to determine toxicities and MTD of this combination. Expected enrollment is 24. During the first 5 days of each cycle, patients are treated with oral prednisone. On day 3, UCN-01 is administered IV. For the first cycle, UCN-01 is administered over 72 hours, and over 36 hours for the remaining cycles.

In May 2002, an NCI-sponsored, phase I clinical trial (protocol IDs: NCI-NAVY-01-04, NCI-5535, NCI-02-C-0222) of UCN-01, in combination with 5-FU and leucovorin (LV), was initiated for the treatment of metastatic or unresectable cancer. The purpose of this study is to determine the recommended dose of 5-FU with fixed doses of leucovorin and UCN-01. On day 1, patients are administered IV LV (500 mg/m^2), over 2 hours, and a mid-infusion bolus of 5-FU. For the first cycle, IV UCN-01 (42.5 mg/m^2) is infused over 72 hours, and over 36 hours for subsequent cycles. Treatment cycles repeat every 4 weeks. A total of 24 patients will be accrued for this study. Jean L. Grem, MD, of the NCI, is Protocol Chair.

In April 2002, an NCI-sponsored, dose-escalation, phase I clinical trial (protocol IDs: MSGCC-0143; NCI-5533) of UCN-01, in combination with carboplatin, was initiated for the treatment of advanced solid tumors. On day 1, patients are being administered IV carboplatin over 1 hour, followed by IV UCN-01 over 3 hours. Treatment cycles repeat every 3 weeks for up to 6 cycles in the absence of disease progression or unacceptable toxicity. Approximately 3-30 patients are expected to enroll. Martin J. Edelman, MD, of the University of Maryland Greenebaum Cancer Center (Baltimore, MD) is Study Chair.

In December 2001, an NCI-sponsored, dose-escalation phase I clinical trial (protocol IDs: WUSM-SCC-0102; NCI-5582) of UCN-01, in combination with irinotecan, was initiated at Washington University School of Medicine (Seattle, WA) with Paula M. Fracasso, MD, as Study Chair, for the treatment of metastatic or unresectable solid tumors. Patients are administered IV irinotecan, over 90 minutes, on days 1, 8, 15, and 22, and IV UCN-01, over 3 hours, on days 2 and 23. Treatment cycles repeat every 42 days in the absence of disease progression or unacceptable toxicity. Approximately 18-21 patients will be accrued for this study.

An NCI-sponsored, multicenter, dose-escalation, phase I clinical trial (protocol IDs: CHNMC-PHI-28; NCI-T99-0065; DMS-9934; NCI-2331) of UCN-01, in combination with cisplatin, was initiated in March 2001 at the Beckman Research Institute at City of Hope (Duarte, CA) with David R. Gandara, MD, as Study Chair, for the treatment of advanced or metastatic solid tumors. This trial was closed in December 2002. On day 1, patients were administered IV cisplatin over 1 hour. Beginning on day 2, IV UCN-01 was administered over 36-72 hours. Treatment cycles repeated every 4 weeks in the absence of disease progression or unacceptable toxicity.

An NCI-sponsored, dose-escalation, phase I clinical trial (protocol IDs: MSKCC-9924; NCI-T99-0037) of UCN-01, in combination with 5-FU, for the treatment of advanced or refractory solid tumors, was initiated in July 1999 at MSKCC with Gary K. Schwartz, MD, as Study Chair, and was completed in December 2002. IV 5-FU was administered over 24 hours on days 1, 8, 15, and 22. For the first course, IV UCN-01 was administered over 72 hours on day 2, and over 36 hours. In subsequent courses. Treatment cycles repeated every 4 weeks in the absence of disease progression or unacceptable toxicity.

A phase II clinical trial (protocol IDs: MSKCC-02049; NCI-5509), initiated in November 2002 at MSKCC with Gary K. Schwartz, MD, as Study Chair, is evaluating the response rate, TTP, and overall survival of 12-37 patients with gemcitabine-refractory metastatic pancreatic cancer treated with UCN-01 and 5-FU. Treatment consists of IV 5-FU, over 24 hours, on days 1, 8, 15, and 22, and IV UCN-01, continuously, over 72 hours (course 1 only), beginning on day 2. In subsequent courses, UCN-01 is infused over 36 hours. Courses are repeated every 28 days in the absence of disease progression or unacceptable toxicity. An NCI-sponsored, dose-escalation, phase I clinical trial (protocol IDs: MDA-DM-01553; NCI-5510) of UCN-01 in combination with gemcitabine in unresectable of metastatic pancreatic cancer was initiated in July 2002 at UTM-DACC with Linus Ho, MD, as Study Chair. Patients are administered gemcitabine IV over 1 to 2 hours, on days 1 and 8, and IV UCN-01 over 3 hours, on day 1. Treatment cycles repeat every 3 weeks in the absence of disease progression or unacceptable toxicity.

An NCI-sponsored, dose-escalation, phase I clinical trial (protocol IDs: NCI-99-C-0127, NCI-T99-002) of UCN-01, in combination with fludarabine, was initiated in July 1999, for the treatment of low-grade, relapsed or refractory lymphoma and leukemia, including chronic lymphocytic leukemia (CLL), and hairy-cell leukemia (HCL). For the first course, patients are administered UCN-01 by CIV over 72 hours, together with fludarabine infused over 30 minutes, daily, on days 1 to 5 of each course. In subsequent courses, UCN-01 is administered as a 36-hour CIV together with fludarabine, over 30 minutes, on days 1 to 5. Treatment cycles repeat every 4 weeks. Approximately 20 patients will be accrued for this study. Wyndham Hopkins Wilson, MD, at the Center for Cancer Research, at the Warren Grant Magnuson Clinical Center (Bethesda, MD) is Protocol Chair.

A multicenter, dose-escalation phase I/II clinical trial (protocol IDs: PMH-PHL-006; NCI-5538) combining UCN-01 with fludarabine in treating relapsed or refractory CLL or lymphocytic lymphoma, was initiated in September 2002, at Princess Margaret Hospital (Toronto, Canada) under the direction of Michael Crump, MD. A total of 12 patients will be accrued for the phase I portion, to be treated with escalating doses of UCN-01 until MTD is determined. IV UCN-01 is administered over 3 hours, on day 1, and IV fludarabine, over 30-60 minutes, on days 1-5. Treatment is repeated every 4 weeks for up to 6 courses in the absence of disease progression or unacceptable toxicity. Subsequently, 18-46 additional patients are to be treated with UCN-01 and fludarabine as above at the recommended phase II dose.

A dose escalation, multicenter, phase I clinical trial (protocol IDs: MDA-DM-99165, NCI-T99-0100) of cytarabine, combined with UCN-01 in treating refractory or relapsed AML or myelodysplastic syndrome (MDS), was closed in September 2000 at UTMDACC; Jorge Cortes, MD, is Protocol Chair. IV cytarabine was administered over 24 hours, on days 1 to 4 of each course, and IV UCN-01, over 24 hours, on days 2 to 4 of course 1, and over 36 hours beginning on day 2 of subsequent courses. Cohorts of 3 to 6 patients were treated with escalating doses of cytarabine until MTD was determined. Treatment was repeated every 4 weeks for a maximum of 4 courses in the absence of disease progression or unacceptable toxicity.

An NCI-sponsored, phase II clinical trial (protocol IDs: UCSF-NCI-5522; NCI-5522) of UCN-01 for treatment of Stage III/IV kidney cancer, was initiated in December 2001 at UCSF Cancer Center and Cancer Research Institute (San Francisco, CA) with Brian I. Rini, MD, as Study Chair. Patients are administered IV UCN-01, over 3 hours, on day 1, every 3 weeks, in the absence of disease progression or unacceptable toxicity. Approximately 21-61 patients will be accrued for this study.

Mithramycin Analogs

Mithramycin/plicamycin (Mithracin; Bayer) is an aureolic acid anticancer antibiotic produced by *Streptomyces argillaceus*. A type II polyketide synthase (PKS) produces the aglycon of mithramycin, and various post-PKS enzymes act upon this aglycon to yield mithramycin. Mithramycin's unique spectrum of activity includes inhibition of both cancer growth and bone resorption. Although approved as an anticancer agent many years ago, toxicity has limited Mithracyn's widespread clinical use; it is currently used in managing hypercalcemia of malignancy.

Mithramycin preferentially binds to GpG (or CpC) dinucleotides in GC-rich DNA, and inhibits transcription of genes by blocking binding of Sp factors to DNA. Various oncogenes, such as c-myc and c-src, are dependent on Sp1 binding for activity. Compounds that bind to the crucial Sp binding sites in the c-myc and c-src promoters inhibit their expression and, consequently, decrease viability of human cancer cells that depend on c-myc and c-src transcription for survival. Mithramycin inhibits transcription of the c-src proto-oncogene by inhibiting Sp1/Sp3 binding to the c-src promoter region, thereby decreasing the subsequent expression of its gene product in human cancer cells. Transcription of c-src, a gene implicated in many human cancers, is also required for osteoclast-dependent bone resorption. Therefore, this gene represents not only an important anticancer target, but also a potential lead target through which mithramycin acts against osteoclastic bone resorption.

Based on its mechanism of action, mithramycin is serving as a lead compound for synthesis of DNA binding molecules that could interfere with gene expression by antagonizing activity of specific transcription factors. However, because chemical synthesis of mithramycin derivatives has proven difficult, a combinatorial biosynthetic approach was used to produce novel compounds with specific activity-increasing features. The functions of many of the enzymes encoded by the 34 genes identified so far in the mithramycin gene cluster have been assigned through data bank comparison, and by insertional gene inactivation followed by analysis of the accumulated mithramycin intermediates. Through this process, a number of mithramycin analogs were identified that exhibit various degrees of activity against human cancer cells. These analogs, were compared for their binding to the promoter regions of c-myc and c-src, and for their ability to compete with Sp factors for binding to DNA.

Small modifications in structure had a significant effect on the DNA binding of these analogs. Alteration of the mithramycin 3-pentyl side chain led to mithramycin SK, a novel aureolic acid-type antitumor compound, generated by combinatorial biosynthesis by researchers at the University of Kentucky (Lexington, KY) and Universidad de Oviedo, in Spain, with the same DNA binding specificity, but with lower binding affinity than mithramycin. Interestingly, this compound was comparable to mithramycin in promoter reporter, gene expression, and cytotoxicity assays. Thus, given its weaker interaction with DNA, this compound may be less toxic that mithramycin, while maintaining similar efficacy (Remsing LL, etal, AACR02, Abs. 2434:490 and (Remsing LL, etal, AACR03, Abs. 447). Mithramycin SK exhibits an improved therapeutic index compared to mithramycin in *in vitro* antitumor and toxicity assays. In initial *in vitro* anticancer assays, pursued by the NCI against 60 human cancer cell lines, as well as an *in vitro* toxicity assay, mithramycin SK exhibited an up to two orders of magnitude better antitumor activity, and two orders of magnitude lesser toxicity than the parent drug mithramycin (Remsing LL, etal, AACR03, Abs. 458).

Prodigiosins

Prodigiosins are natural products belonging to a family of tripyrrole red pigments that are produced by microorganisms such as Streptomyces and *Serratia marcescens*. They were first isolated in 1929, and studied as antibiotic

and cytotoxic agents in the 1960s, but were not developed clinically because of their high systemic toxicity. Subsequently, a simple and elegant synthesis of the prodigiosins was developed, which allowed the preparation of a number of analogs, triggering renewed interest in the prodigiosin group of natural products. The cytotoxic properties of these compounds are linked to their ability to facilitate oxidative DNA damage (Manderville RA, Curr Med Chem Anti-Cancer Agents 2001;1(2):195-218).

Prodigiosin induced apoptosis in several cancer cell lines including Jurkat-T cells. It acted rapidly and potently in tumor cell lines without causing significant toxicity in nonmalignant cells. Prodigiosin induced apoptosis in DLD-1 and SW-620 human colon adenocarcinoma cells by the characteristic DNA laddering pattern and condensed nuclei, or apoptotic bodies. Metastatic SW-620 cells were more sensitive to prodigiosin than DLD-1 but a significant decrease in the viability of NRK cells was not observed (Montaner B, and Perez-Tomas R, Life Sci, 16 Mar 2001;68(17):2025-36). Prodigiosin also induces apoptosis in HGT-1 human gastric cancer cells (Díaz-Ruiz C, etal, Histol Histopathol 2001;16:415-421), and in hematopoietic cancer cells with no significant toxicity in nonmalignant cells (Montaner B, etal, Br J Pharmacol, Oct 2000;131(3):585-93).

Rapamycin/Sirolimus

Rapamycin (sirolimus), a microbial macrolide initially discovered by Suren Sehgal in 1975 in a soil sample from Rapa Nui (Easter Island), is a fermentation product of *Streptomyces hygroscopicus*. Rapamycin (Rapamune; Wyeth) acts as an immunosuppressive preventing allograft rejection. It was approved as an immunosuppressant in September 1999 in the USA, and has been commercialized for this indication worldwide. Rapamycin acts as an immunosuppressive, antiproliferative, and antifungal agent, and may, therefore, play a role in transplantation immunology, cardiovascular medicine, oncology, and in treating autoimmune diseases such as rheumatoid arthritis. Recently, it was shown that rapamycin suppresses replication of arterial smooth muscle cells, and may be useful in preventing arterial lesions in chronic rejection and, even more important, following balloon angioplasty. As a result, a new generation of drug-eluting coronary stents coated with sirolimus are in development to reduce restenosis in human coronary arteries.

Rapamycin is a highly specific inhibitor of the FKBP12-rapamycin-associated protein (FRAP), also referred to as mammalian target of rapamycin (mTOR or TOR). TOR is a phosphoinositide 3-kinase (PI3K)-related protein serine/threonine kinase, and a central controller of eukaryotic growth and proliferation. It is highly conserved in all eukaryotic organisms from yeast to humans. TOR is a large molecule of Mr 300,000 with heat repeats, protein-protein interaction domains at its amino terminus, and a protein kinase domain at its carboxy terminus. TOR regulates cell division as well as the size of individual cells, organs and animals, and is directly linked to the pathogenesis of several human diseases. When activated by external growth factors through the PI3K/Akt signaling pathway, TOR is a central controller of both cell growth and cell division. Inhibition of TOR in cells elicits an antiproliferative and growth-arresting cellular response mimicking the response to nutrient starvation, thus validating TOR as a promising therapeutic target for cancer. In a hypoxic environment, increases in mass of solid tumors is dependent on the recruitment of mitogens and nutrients. As a function of nutrient levels, particularly essential amino acids, TOR acts as a checkpoint for ribosome biogenesis and cell growth. Ribosome biogenesis has long been recognized in the clinic as a predictor of cancer progression; an increase in size and number of nucleoli are associated with the most aggressive tumors and a poor prognosis (Kozma SC, etal, AACR02, Abs. 5628:1136).

Rapamycin appears to exhibit broad anticancer properties, with potential therapeutic applications in both solid tumors and hematologic malignancies. Rapamycin inhibits cell-cycle progression in a variety of hematologic cell types, including human B cells, and has shown activity against a broad range of human tumor cell lines. *In vitro* studies indicate that rapamycin is active against proliferating malignant tumor cells in B-cell chronic lymphocytic leukemia (B-CLL). Rapamycin interferes with expression of many critical molecules for cell regulation in cycling B-CLL cells. In particular, expression of cyclin D3, cyclin E, cyclin A and suvivin was reduced, inducing a G1 arrest in proliferating tumor cells. Rapamycin treatment also inhibited cyclin-dependent kinase 2 (Cdk2) activity by preventing upregulation of cyclin E and cyclin A. Rapamycin induced cell-cycle arrest in proliferating B-CLL cells, and inhibited phosphorylation of p70s6 kinase. By inducing a G1 arrest in proliferating tumor cells, rapamycin may prove useful in the treatment of B-CLL (Decker T, etal, Blood, 1 Jan 2003;101(1):278-85). A phase II clinical trial is being planned by researchers at the Technical University of Munich, Germany, using rapamycin in patients with progressive B-CLL, after treatment with fludarabine. In addition, it is hypothesized that maintenance therapy with rapamycin after remission induction with conventional chemotherapy or stem cell transplantation, may prove a promising approach in preventing disease progression.

Using *in vitro* techniques as well as a transgenic mouse model of leukemia/lymphoma that develops precursor B leukemia/lymphoma at 4 to 7 months of life, investigators have shown that rapamycin is effective against ALL. Growth of human and murine ALL cell lines exposed to rapamycin was significantly inhibited within 72 hours at a level easily achievable in patients. In addition to growth arrest in these leukemic cells, rapamycin also induced apoptosis. Rapamycin's inhibitory effect was reversed upon treatment with IL-7, with complete reversal seen in some ALL cell lines, and partial reversal seen in others. IL-7 increases phosphorylation of p70S6 kinase, while in cells

treated with rapamycin or rapamycin and IL-7, there are profound decreases in phosphorylation of this target. These results suggest that both the IL-7 and TOR pathways alter the phosphorylation state of p70S6 kinase. However, the ability of IL-7 to reverse rapamycin- induced growth inhibition may involve another downstream target protein such as p27kip1 or cdk2, or be independent of p70S6 kinase, thus resulting in a competition between parallel growth and inhibitory signals. The effect of IL-7 in modulating response to rapamycin suggests the importance of IL-7-mediated signaling in malignant as well as normal early B cells (Brown VI, etal, ASH02, Abs. 3012:761a).

Rapamycin may play a dual role in the management of solid transplants, by acting as an immunosuppressant and by preventing malignancies arising from immunosuppression. Conventional immunosuppressive drugs that are used effectively to prevent immunologic rejection of organ transplants, place individuals who take them at risk for the development and recurrence of cancer. This occurs because immunosuppressants, by impairing the organ graft recipient's immune surveillance, heighten cancer incidence and metastatic progression (Guba M, Nat Med, Feb 2002;8(2):128-35). Rapamycin, in combination with rituximab (Rituxan; Idec Pharmaceuticals) may prove an effective treatment for post-transplant lymphoproliferative disease (PTLD), an uncommon but life-threatening complication of solid-organ and blood stem-cell transplants. PTLD responds poorly to therapy, including reduction of immunosuppression, interferon, antivirals or chemotherapy. The rationale for combining rituximab and rapamycin stems from the fact that a few cases of PTLD have been successfully treated with rituximab, and experimental studies suggest that rapamycin inhibits growth of human Epstein-Barr virus-transformed B lymphocytes. Two cases of PTLD after renal transplantation were successfully treated with rituximab in association with rapamycin, indicating that this regimen may prove an effective and safe treatment for PTLD (Garcia VD, etal, Transpl Int, Mar 2003;16(3):202-6).

Rapamycin is also proving effective as an inhibitor of cancer metastasis. Experimentally, rapamycin inhibited metastatic tumor growth and angiogenesis in *in vivo* mouse models. In addition, normal immunosuppressive doses of rapamycin effectively controlled growth of established tumors. In contrast, the most widely recognized immunosuppressive drug, cyclosporine, promoted tumor growth. From a mechanistic perspective, rapamycin showed antiangiogenic activities linked to a decrease in production of VEGF, and to a significantly inhibited response of vascular endothelial cells to stimulation by VEGF. Thus, use of rapamycin, instead of cyclosporine, may reduce the chance of recurrent or *de novo* cancer in high-risk transplant patients (Guba M, Nat Med, Feb 2002;8(2):128-35).

A regimen of rapamycin and cyclosporine, that was effective in reducing acute rejection of renal allografts, may also prevent RCC progression as well, and has the potential to prevent mortality from RCC in patients with end-stage renal disease (ESRD) with renal allografts. RCC is common, and is 10 to 100 times more frequent in patients with ESRD who are candidates for renal transplantation. Treatment of metastatic RCC is largely ineffective and is further undermined by immunosuppressive therapy in transplant recipients. Rapamycin may offer a treatment option that prevents transplant rejection while constraining RCC progression (Luan FL, etal, Kidney Int, Mar 2003;63(3):917-26).

Recently, scientists have shown that rapamycin not only suppresses immune system T cells, similarly to other antirejection drugs, but also inhibits the function and activation of dendritic cells (DC). Rapamycin has a unique and profound inhibitory effect on DC function, which seems to be, at least in part, mediated by the FKBP immunophilins (Monti P, etal, Transplantation 2003 Jan 15;75(1):137-45). Rapamycin specifically induces apoptosis in DC but not in other myeloid cell types. Rapamycin induces apoptosis in human monocyte-derived DC by disarming granulocyte-macrophage colony-stimulating factor (GM-CSF), that allows DC to proliferate, and also affects proliferation of blood precursor and stem cells, which if allowed to grow unchecked, result in leukemia and other malignancies. GM-CSF preserves DC survival specifically via the PI3K/TOR signaling pathway, which is abrogated by rapamycin at the level of TOR. Disruption of this GM-CSF signaling pathway induces loss of mitochondrial membrane potential, phosphatidyl-serine exposure, and nuclear changes. Apoptosis of these nonproliferating DC is preceded by an upregulation of the cell-cycle inhibitor p27(KIP1). Both overexpression of p27(KIP1) and disruption of the GM-CSF/PI3K/TOR signaling pathway decreased expression of the antiapoptotic protein mcl-1. This TOR/p27(KIP1)/mcl-1 survival seems unique for DC and may provide novel opportunities to influence immune responses by specific interference with the life span of these cells (Woltman AM, etal, Blood, 15 Feb 2003;101(4):1439-45). These findings imply that rapamycin could arrest the growth factor signals that are involved DC-triggered autoimmune diseases and blood malignancies, such as AML.

AP23573/AP23675, AP23675 and AP23841 are sirolimus analogs under development by Ariad Pharmaceuticals (Cambridge, MA). These compounds act on TOR, inhibiting both proliferation of tumor cells and osteoclast activity. In the bone microenvironment, metastasized cancer cells produce activating factors such as parathyroid hormone (PTH)-related protein (PTHrP) that stimulate osteoclast-mediated bone resorption. Bone-derived growth factors, such as TGF-β and IGF1, are subsequently released, promoting cancer-cell proliferation and amplification of a cycle that gives rise to osteolytic consequences. Antiresorptive agents such as the biphosphonates, decrease bone lesions as well as tumor burden

in vivo, but there is no evidence that these agents, at therapeutic concentrations, have a direct effect on cancer cells. Therefore, there is a need for therapeutic agents that act directly and potently on both processes of bone breakdown and tumor growth (Metcalf C III, etal, AACR03, Abs. 717).

AP23675 has demonstrated nanomolar *in vitro* inhibition of both human osteoclast activity and proliferation of multiple human tumor cell lines. Potent and sustained *in vivo* antiresorptive activity has also been observed in a mouse model of PTH-induced hypercalcemia when AP23675 is administered either orally or intraperitoneally. Further evaluation of AP23675 in animal models of primary bone cancer and bone metastasis is underway (Metcalf C III, etal, AACR03, Abs. 717).

CCI-779 is an ester derivative of rapamycin that inhibits kinase activity of TOR resulting in inhibition of the translational regulators p70S6 kinase and 4EBP-1. In preclinical trials, CCI-779 was shown effective in a variety of solid and hematologic malignancies, triggering initiation of a clinical evaluation program. CCI-779 has completed dose-escalation phase I monotherapy clinical trials, and is currently in phase II trials, alone, or in combination with other anticancer drugs. The clinical development of CCI-779 is optimized by assessment of target inhibition in patients treated with this agent by reliably measuring the biological activity of CCI-779 in clinical patient specimens using a p70S6 kinase assay (Peralba J-M, etal, AACR02, Abs. 4961:1000).

The drug was evaluated in an IV as well as orally administered mode. In a phase I clinical trial, CCI-779 was administered as a 30-minute IV infusion, daily, for 5 days, every 2 weeks, in 51 patients with solid tumors who were treated with 262 courses at daily doses ranging from 0.75 mg/m^2 to 19.1 mg/m^2. Isolated, asymptomatic, Grade 3 hypocalcemia at the 2.16 mg/m^2/day dose level was the only DLT noted. Other generally mild-to-moderate toxicities noted were neutropenia, thrombocytopenia, rash, mucositis, hypertriglyceridemia, and allergic reactions. Minor antitumor responses and/or prolonged (>4 months) stable disease were seen in several drug-refractory malignancies including soft-tissue sarcoma (n=3), and cervical (n=1), uterine (n=1), and renal cell (n=3) cancer (Hidalgo M, etal, ASCO00, Abs.726:187a; Ann Oncol, Oct 2000;11(Suppl 4):133).

In another phase I clinical trial, CCI-779 was administered as a weekly 30-minute IV infusion in patients with advanced solid tumors. In an interim report, based on 15 patients treated at weekly doses of 7.5 (n=1), 15.0 (n=2), 22.5 (n=1), 34.0 (n=3), 45.0 (n=3), 60.0 (n=2), 80 (n=1), 110 (n=1), and 165 mg/m^2 (n=1), no DLT was observed. Grade 1/2 skin toxicity including dryness with mild itching (n=7), eczema-like lesions (n=3), subacute urticaria (n=1), and aseptic follicles (n=10), was observed at each dose level without any evidence of dose-effect relationship. Skin biopsies showed folliculitis and superficial pericapillar dermatitis; 5 patients experienced reactivation of perioral herpes lesions. Grade 1/2 mucositis was observed in 9 patients, and all patients treated with 8 doses experienced Grade 1 nail changes. Thrombocytopenia was observed in 4 patients treated at weekly doses of 34 mg/m^2 (Grade 3), 45 mg/m^2 (Grade 2), 60 mg/m^2 (Grade 3) and 80 mg/m^2 (Grade 1); treatment was delayed in 3 patients. An asymptomatic increase of triglyceridemia and cholesterolemia levels was observed in 8 and 4 patients, respectively. Decreases in testoteronemia associated with increased levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were observed in 5/6 men treated with more than 4 doses at weekly doses over 15 mg/m^2. No significant immunosuppression was observed. Among 13 patients evaluable for efficacy, there was 1 PR in a patient with an IL-2/INFα-resistant metastatic RCC, treated with a weekly dose of 15 mg/m^2, 3 minor responses, and disease stabilized in 5 (Alexandre J, etal, AACR00, Abs. 3897:613).

In March 2002, the FDA designated CCI-779 as a "fast-track" drug for the treatment of RCC after failure of initial therapy. A dose-escalation, multicenter, phase I clinical trial (protocol IDs: MSKCC-02023, NCI-G02-2104, W-AR-3066K1-124-US, WYETH-C-C0125-32) of CCI-779 and IFNα, in patients with locally advanced or metastatic RCC, was initiated in September 2002 at MSKCC, with Robert Motzer, MD, as Protocol Chair. Approximately 50 patients will be accrued for this study to be treated with subcutaneous IFNα 3 times a week. Beginning on week 2, patients are also administered IV CCI-779, over 30 minutes, once weekly. Treatment continues in the absence of disease progression or unacceptable toxicity.

In a randomized, double-blind, phase II clinical trial of IV CCI-779, administered at 25, 75, or 250 mg weekly to 110 patients with advanced RCC, the fewest dose modifications occurred in the 25 mg treatment group. Overall, the most common drug-related adverse events, based on 92 patients, included rash (72%), mucositis (65%), asthenia (39%), nausea (36%), and acne (30%). The most common laboratory adverse events included thrombocytopenia (24%), hypertriglyceridemia (24%), and anemia (23%). Adverse events were largely Grade 1 or 2. The most frequent Grade 3/4 toxicities were hyperglycemia and anemia. A nonspecific pneumonitis, the majority asymptomatic, was also observed. Median TTP and overall survival were 5.8 and 12 months, respectively. At least 15 patients remained on the study for more than 1 year. CCI-779 is well tolerated and active in RCC, with no substantial differences in antitumor activity among the 3 dose levels tested. While the response rate was low with no CR in these heavily pretreated patients, the high rate of PR/MR/SD and extended median TTP and survival are promising (Atkins MB, etal, ASCO02, Abs. 36:10a).

CCI-779 has demonstrated preliminary clinical activity for patients with advanced, refractory, breast cancer who have failed prior chemotherapy. The safety and activity of two dose levels (75 and 250 mg) of IV CCI-779, adminis-

tered weekly, were assessed in a multicenter, randomized, open-label, 2-dose-level, phase II clinical trial, that was initiated in January 2000, in patients with refractory. locally advanced or metastatic breast cancer. In the completed first stage of the trial, there were 2 objective PR in the first 16 evaluable patients at each dose group. Responses were noted in liver, lung and chest wall metastases. Patients in both dose groups continue beyond 24 weeks. Grade 3 or 4 events were reported in at least 2/32 patients evaluable for toxicity, including leukopenia, infection, mucositis, diarrhea, gamma-glutamyl-transpeptidase elevation, hyperglycemia, hypokalemia and depression. Frequent adverse events (at least 20% patients reporting) were generally Grade 1 or 2, and often resolved without requiring discontinuation of CCI-779. As of November 2001, there were 85/110 patients randomized as both cohorts are expanding to include 50 evaluable patients at each dose level to complete the study (Chan S, etal, ASCO02, abs.175:44a).

An NCI-sponsored, multicenter, phase II clinical trial (protocol IDs: CHNMC-PHII-27, NCI-29, CHNMC-IRB-99167) was initiated in June 2001 at the City of Hope with Kim Allyson Margolin, MD, as Study Chair, to determine the antitumor activity of CCI-779, in terms of progression-free survival (PFS), and its toxicity in patients with metastatic melanoma. According to the protocol, patients are administered IV CCI-779, over 30 minutes, on day 1. Treatment repeats every 7 days in the absence of disease progression or unacceptable toxicity, and patients are followed until death.

An NCI-sponsored, multicenter, phase I/II clinical trial (protocol IDs: 199/16092; NABTC-0101) was initiated in December 2001, being conducted by the North American Brain Tumor Consortium (NABTC), in adult patients with recurrent brain tumors, anaplastic oligodendroglioma, GBM, adult anaplastic astrocytoma, and mixed glioma. The trial objectives are to determine MDT of CCI-779, its safety profile and pharmacokinetics, and its efficacy, in terms of survival and objective response, in this setting. Patients in the dose-escalation, phase I part of this trial must be currently receiving enzyme-inducing antiepileptic drugs (EIAED). Patients in phase II are stratified according to use of EIAED (yes versus no) and disease type (GBM with stable neuroimaging after radiotherapy versus recurrent malignant glioma). A total of 36 patients will be accrued for phase I, and 87 for phase II. Susan Chang, MD of the NABTC is Study Chair.

Another multicenter, phase II clinical trial (protocol ID: NCCTG-N997B) was initiated in May 2001 by the North Central Cancer Treatment Group (NCCTG) with Evanthia Galanis, MD, as Protocol Chair, to determine the efficacy of CCI-779, in terms of the percentage of patients with recurrent GBM who are progression-free at 6 months, as well as TTP, time-to-death, toxic effects of this drug, and its pharmacokinetics in patients on P450-inducing anticonvulsants. Patients are stratified according to concurrent P450 anticonvulsant use (yes versus no), and are treated with IV CCI-779, over 30 minutes, once weekly, for 4 weeks. Courses repeat every 4 weeks in the absence of disease progression or unacceptable toxicity.

An NCI-sponsored, multicenter, randomized, double-blind, placebo-controlled, phase II clinical trial (protocol ID: UCLA-000606401; NCI-G01-1917; GENE-C9940-32; UCLA-CCI-779; W-AR-3066K1-201-US) was initiated in September 2000, with CCI-779, in patients with androgen-independent prostate cancer undergoing treatment with gonadal ablation such as luteinizing hormone-releasing hormone (LH-RH). Trial objectives are to determine the safety of CCI-779, and its effects on prostate-specific antigen (PSA) levels, and assess the pharmacokinetic parameters of CCI-779, and its possible pharmacodynamic relationship with clinical response and patients' QoL. This trial was completed in May 2002. According to the protocol, patients were randomized to 1 of 4 arms. In arm I patients were treated with low-dose IV CCI-779, over 30 minutes weekly, in arm II with high-dose IV CCI-779 over 30 minutes weekly, while the same dosing schedule was repeated in patients in arms III and IV who were treated with placebo. Treatment continued in the absence of disease progression or unacceptable toxicity. Patients on placebo who develop progressive disease may cross over to the equivalent dose of CCI-779. QoL is being assessed at baseline, at weeks 4, 8, 12, 24, and 36, and at a final/crossover visit. Patients are followed every 3 months. Approximately 150 patients were to be accrued for this trial. Diane Prager, MD, at Jonsson Comprehensive Cancer Center (Los Angeles, CA) is Study Chair.

A randomized multicenter phase II clinical trial (protocol ID: E-E1500), initiated in January 2002 with Kishan J. Pandya of the Eastern Cooperative Oncology Group (ECOG) as Study Chair, to compare the effectiveness of different doses of CCI-779 in treating patients with extensive-stage sclc, is to enroll 80 patients within 2.7-3.2 years. Patients are randomized to 1 of 2 treatment arms. In arm I, patients are treated with low-dose IV CCI-779, over 30 minutes, once weekly, while in arm II they are administered high-dose CCI-779 according to the same schedule.

A multicenter, phase II clinical trial (protocol ID: NCCTG-N0186), initiated in April 2002 with Thomas E. Witzig, MD, of the NCCTG as Protocol Chair, to assess the effectiveness of CCI-779 in previously treated patients with mantle-cell NHL, will accrue 35 patients within 2 years. According to the protocol, IV CCI-779 is administered over 30 minutes on days 1, 8, 15, and 22. Courses are repeated every 28 days in the absence of disease progression or unacceptable toxicity. Patients with stable disease are treated with a maximum of 6 courses, those with PR with a maximum of 12 courses, and those with CR are treated with 2 additional courses beyond CR.

A phase I clinical trial involving patients with advanced solid malignancies, is being conducted at the Institute for Drug Development, Cancer Therapy and Research Center

(San Antonio, TX) and Mayo Clinic, to determine the safety/tolerability, MTD, pharmacokinetics, and bioavailability of CCI-779, administered orally, daily, for 5 days, every 2 weeks. Prior to initiation of treatment, absolute bioavailability was assessed by administering a single dose of CCI-779 IV or orally, followed one week later by the other route. The IV dose was 20% of the planned oral dose. During treatment, CCI-779 dose-escalation to the next level was based on toxicities observed during the first 2-week course. The starting oral dose was 25 mg, daily, for 5 days. The dose was doubled for subsequent cohorts until Grade 2 or higher drug-related toxicity was observed. At least 3 patients were evaluated at each dose level. Among 24 patients treated with 149 total courses of CCI-779 (median courses=6, range=1 to 21) at 25-, 50-, 75-, and 100-mg dose levels, 2/6 patients experienced DLT, consisting of Grade 3 stomatitis, AST elevation, or solar-plantar desquamative rash, at the 100-mg dose level. The recommended MTD of oral CCI-779 is 75 mg, daily, for 5 days, every 2 weeks, although several patients required treatment delays of 1 to 2 weeks at this dose level. Preliminary evidence of antitumor activity of oral CCI-779 included disease stabilization for 8 to 9+ months in RCC, nsclc, myxoid chondrosarcoma, mesothelioma, and leiomyosarcoma. Preliminary pharmacokinetics indicate moderately rapid absorption, dose-related increases in exposure, and formation of sirolimus as a major metabolite (Forouzesh B, etal, EORTC-NCI-AACR02, Abs. 168, and Eur J Cancer, Nov 2002;38, Suppl. 7, Nov 2002:54).

The combination of CCI-779 with antiestrogens was also active in preclinical models of hormone-dependent breast tumors. The combination of non-inhibitory doses of CCI-779 with suboptimal doses of the selective estrogen receptor modulator (SERM) ERA-923, an estrogen receptor-α (ER-α) antagonist, under development by Wyeth, synergistically inhibited growth of MCF-7 cells. Synergy was found across a wide range of doses, and could be also achieved by combining CCI-779 with other antiestrogens such as raloxifene and 4-hydroxy-tamoxifen. *In vivo*, the combination of CCI-779 and ERA-923, at certain doses and schedules, completely inhibited tumor growth while these agents individually were only partially effective. Although the mechanism underlying the synergism remains to be elucidated, results pointed to CCI-779's ability to block transcriptional activity mediated by ER-α as well as an increase in G1/G0 arrest, attributable to the combination of these drugs (Zhang Y, etal, AACR03, Abs. 3715).

RAD1000 (everolimus), a derivative of rapamycin under development by Novartis, is also an inhibitor of TOR kinase. RAD1000 is being currently evaluated in a multicenter, double-blind, randomized, crossover, phase I clinical trial, comparing the pharmacokinetics, safety and tolerability of two different single oral doses of RAD001 in stable lung and heart/lung transplant recipients with pancreatic insufficient cystic fibrosis, and without cystic fibrosis. Also, a one-year, randomized, multicenter, open-label, parallel group, phase III clinical trial of the efficacy and safety of RAD001 tablets versus antilymphocyte globulin and azathioprine, is ongoing in lung or heart/lung transplant recipients with bronchiolitis obliterans syndrome. Ramona Doyle, MD, of Stanford University is the PI.

Like rapamycin, RAD1000 is expected to exhibit broad anticancer activities. RAD001 inhibits growth of mature B lymphocytes, and may be active against EBV lymphoproliferative disease. Also, like other TOR inhibitors, it may inhibit spread of metastases, and exhibits antiangiogenic properties in preclinical models of solid tumors. RAD001 may be particularly effective against ALL. In preclinical trials, everolimus suppressed growth of ALL cells *in vitro* and was active *in vivo* against leukemia/lymphoma in a transgenic mouse model. RAD001 inhibited growth >50% to 90% in ALL cell lines. Daily oral administration of RAD001 extended by approximately 3-fold survival of transgenic mice with advanced leukemia with high white blood counts, large nodal masses, and massive hepatosplenomegaly, compared to untreated controls. In addition to extending survival, RAD001 induces disappearance of nodal masses and normalization of peripheral WBC counts (Brown VI, etal, ASH02, Abs. 3012:761a). Based on these preclinical data, phase I clinical trials are planned using RAD1000 in pediatric patients with refractory or relapsed leukemia or lymphoblastic lymphoma.

Resveratrol

Resveratrol, a polyphenolic phytoalexin present in grape skin, eucalyptus, spruce and peanuts, belongs to a class of antibiotic compounds produced as a part of a plant's defense system against disease. Resveratrol may be used as a potentiator of anticancer drugs, and as a cancer chemopreventive. Resveratrol acts as a chemopreventive based on its striking inhibitory effects on cellular events associated with cancer initiation, promotion, and progression. This drug's anticancer activity, and its simple structure suggest that, in principle, resveratrol can be a lead molecule for the discovery of new chemotherapeutic agents.

Resveratrol is a weak estrogen agonist, and is also considered an estrogen antagonist. It competes in a dose-dependent manner with estrogen for the estrogen receptor. The chemopreventive activity of resveratrol in human breast epithelial cells and breast cancer cells may be attributed to its regulation of cathepsin D. Because of its dose-dependent estrogen antagonist effect, resveratrol reduces the extracellular routing of cathepsin D by MCF-7 cells, and decreases secretion of cathepsin D. Also, the concentration of mRNA encoding for cathepsin D, decreased in the presence of resveratrol, and resveratrol inhibited MCF-7 breast cancer cell proliferation in a dose-dependent manner (Vyas S, etal, AACR02, Abs. 4097:825).

Resveratrol is also a potent enhancer of apoptosis induced by anticancer drugs such as doxorubicin, etoposide or cisplatin, while treatment with cytotoxic drugs or

resveratrol alone induced only minimal apoptosis. Also, drug-induced apoptosis is strongly enhanced by resveratrol through p21-mediated cell-cycle arrest and downregulation of survivin independently of wild type p53 in a variety of tumors. Resveratrol-induced sensitization of cytotoxic drugs was mediated by rapid induction of p21 protein and cell-cycle arrest at the G1/S phase; apoptosis preferentially occurred in cells arrested at G1/S. Resveratrol-induced p21 expression was independent of wild type p53 function. Importantly, resveratrol-mediated potentiation of drug-induced apoptosis was significantly reduced in p21-deficient colon carcinoma cells, or by pretreatment with p21 antisense oligonucleotides. Likewise, ectopic expression of p21 strongly enhanced drug-induced apoptosis. Resveratrol-induced cell-cycle arrest resulted in rapid downregulation of survivin protein, which was prevented by the proteasome inhibitor lactacystine. Importantly, survivin antisense oligonucleotides enhanced drug-induced apoptosis indicating that resveratrol induced sensitization of apoptosis by proteasomal degradation of survivin, and may function as a natural survivin antagonist. Most importantly, there was a synergy between resveratrol and chemotherapeutics in different tumor cells derived from leukemia, neuroblastoma, malignant brain tumors, melanoma, colon or breast cancer, and even in primary tumor cells obtained from patients. Thus, the combination of resveratrol with cytotoxic drugs may be a novel strategy to overcome resistance of various tumors (Fulda S, etal, AACR03, Abs. 663).

Various resveratrol-like derivatives have been prepared to identify compounds with potent apoptotic activity against tumor cells. These synthesized compounds were tested *in vitro* to see if they induce cell growth inhibition and apoptosis in several sensitive, MDR and apoptosis-resistant leukemia cells. Two compounds showed activity comparable to that of daunorubicin, and greater than that of etoposide or citarabine. They were also potent apoptosis-inducing agents active in MDR cell lines, and in cells resistant to the apoptotic effects of several chemotherapeutics, including cisplatin, 5-FU and citarabine (Tolomeo M, etal, AACR03, Abs. 453).

Investigators at Institut de Recherche contre les Cancers de l'Appareil Digestif (IRCAD; Strasbourg, France) synthesized a methylated derivative, R3, of resveratrol and tested its anticancer properties on the human colonic cancer cell line Caco-2. R3 was 100-fold more active than resveratrol. The drug inhibited by 2-fold the activities of two rate-limiting enzymes of polyamine synthesis (ornithine decarboxylase and s-adenosylmethionine decarboxylase), leading to the intracellular depletion of polyamines, which are growth factors for cancer cells. At this concentration R3 caused cell-cycle arrest at G2/M but did not induce necrotic cell death or apoptosis. R3 exhibited very high affinity for the colchicines-binding site of tubulin and disrupted the microtubule spindles. The cis conformation is an absolute requisite for the optimal antiproliferative activity of R3 (Schneider Y, etal, AACR02, Abs. 1326:267, and Nutr Cancer 2001;39(1):102-7).

Salinospora

A group of researchers at Scripps Institution of Oceanography at the University of California, San Diego, led by Dr. William Fenical, director of the Center for Marine Biotechnology and Biomedicine (CMBB), have reported the discovery of 12 strains of *Salinospora*, a novel group of bacteria that produce molecules with potential in the treatment of infectious diseases and cancer (Feling RH, etal, Angew Chem Int Ed Engl, 20 Jan 2003;42(3):355-357). This new bacteria, belonging to the family of Actinomycetes, was discovered in ocean sediments. Terrestrial actinomycetes are the source of natural antibiotics, including important drugs such as streptomycin, actinomycin, and vancomycin. These findings provide the first conclusive evidence of the widespread occurrence of indigenous actinomycete populations also in marine sediments (Mincer TJ, etal, Appl Environ Microbiol, Oct 2002;68(10):5005-11).

Using new methods and tools, including a miniaturized sampling device that efficiently captures samples from the deep ocean, scientists obtained bottom mud from depths of over 1,000 meters, from the Atlantic and Pacific Oceans, the Red Sea, and the Gulf of California. They also developed new methods for sifting through these samples, which contain roughly one billion microorganisms per cubic centimeter, culturing the microorganisms, identifying them by genetic methods, and screening their metabolic products for anticancer and antibiotic properties.

The new genus Salinospora, a type of actinomycete bacteria found in tropical and subtropical oceans, but never seen before on land, were identified by genetic and culture analysis. Among 100 strains of these organisms evaluated, 80% produced molecules that inhibited cancer cell growth, and roughly 35% could kill pathogenic bacteria and fungi. A compound from this discovery, dubbed Salinosporamide A, is a potent inhibitor of cancer growth, including human colon carcinoma, nsclc, and, most effectively, breast cancer. These discoveries have been patented by the University of California, and licensed to Nereus Pharmaceuticals (San Diego, CA) for subsequent development.

Spicamycin

Spicamycin, a nucleoside antitumor antibiotic isolated from Streptomyces alanosinicus, that contains a nucleoside base, a carbohydrate, an amino acid and a lipid fragment, is a veritable combinatorial library of building blocks. The relationship between these components may be varied to arrive at unique derivatives of this natural product. The potential of spicamycin as a new class of antitumor agent stimulated SAR studies that found that the dodecanoyl derivative (R=decanoyl) exhibited antitumor activity against human gastric cancer cell line SC-9 that was superior to that of mitomycin C.

KRN 5500, under development by Kirin (Tokyo, Japan), is a novel semisynthetic antitumor compound with a unique structure, derived from the antibiotic spicamycin. The main mechanism of action of KRN5500 is inhibition of protein synthesis (Matsumura Y, etal, ASCO99, Abs 841:219a). Among several metabolites of KRN 5500, only spicamycin amino nucleoside glycine (SAN-Gly) showed a potent inhibitory activity against protein synthesis in reticulocyte lysates. SAN-Gly is the intracellular active metabolite, and conversion of activity from KRN 5500 to SAN-Gly is the major determinant of KRN 5500 cytotoxicity (Kawai H, Gan To Kagaku Ryoho, Sep 1997;24(11):1571-7, and Kenney S, etal, AACR02, Abs. 2034:409).

Based on its mechanism of action, KRN5500 is expected to be useful in the treatment of various solid tumors such as colorectal, gastric, lung, breast, and esophageal cancer, as well as leukemia and lymphoma. KRN5500 is also expected to be a suitable candidate for combination chemotherapy. In preclinical trials, KRN5500 demonstrated activity in nsclc, in combinations with cisplatin, carboplatin or etoposide (Kanzawa F, etal, Cancer Chemother Pharmacol 1999;43(5):353-63).

Phase I clinical trials with KRN5500 have been ongoing since 1997, in collaboration with the NCI, to determine MTD, DLT, and pharmacokinetics of KRN5500, and assess its safety profile, and any antitumor activity. Several phase I clinical trials have been completed that tested KRN5500 at various doses and administration schedules, as a 1-hour, 2-hour and 72-hour IV infusion. Various solid tumors were represented in these trials, including refractory stomach, colorectal and lung cancer. No responses have been reported from these trials (Prakash S, etal, ASCO00, Abs. 788:202a, Matsumura Y, etal, ASCO99, Abs 841:219a, and Clark JW, etal, ASCO98, Abs 887:231a).

An NCI-sponsored, dose-escalation, phase I clinical trial (protocol IDs: NCI-T96-0004, WSU-1294) of KRN5500 for treatment of solid tumors was completed in March 2001. Patients were administered IV KRN5500 on days 1 to 3, every three weeks. Those with stable disease, PR or CR continued treatment for up to 6 months beyond CR. Patricia LoRusso, MD, of the Barbara Ann Karmanos Cancer Institute was Study Chair.

Another NCI-sponsored, dose-escalation, multicenter, phase I clinical trial (protocol IDs: DFCI-00102; NCI-1653) of KRN5500 for the treatment of solid tumors, was initiated in June 2001 at the Dana-Farber Cancer Institute with Joseph Paul Eder, MD, as Study Chair. A total of 20 to 40 patients are to be accrued for this ongoing trial.

Taurolidine

Taurolidine was first designed and synthesized in the 1970s as a broad spectrum antibiotic and was used clinically at high doses without systemic toxicity. Subsequently, taurolidine was shown to possess cytotoxic properties with demonstrated activity in selected human tumor cell lines and in nude mice bearing human tumor xenografts.

In DU145 human prostate tumor cells, taurolidine induced cell death associated with accumulation of DNA debris in the sub-G0/G1 region, and increased membrane phosphotidylserine externalization, both suggesting the induction of apoptosis. Because of its low toxicity, taurolidine may be effective in the treatment of androgen-independent prostate cancer. Studies to assess this possibility in mice bearing xenografts of DU145 tumor cells are underway (Darnowski JW, etal, AACR02, Abs. 4737:957).

Taurolidine exhibits cytotoxicity against multiple myeloma cells. Apoptosis was induced in a dose-dependent manner in RPMI 8226 cells treated with taurolidine. A unique mechanism of this agent's activity in these cells is attributed to targeting survival signals signal transducer and activator of transcription 3 (STAT3) and FLIP, an apoptosis-modulating protein, shifting the delicate balance between life and death (Chatterjee D, etal, AACR02, Abs. 4340:876).

TARGETED CYTOTOXIC ANTIBIOTICS

Despite the incredible effort invested in the development of novel cytostatic and regulatory agents, cytotoxic chemotherapy, associated with relatively high toxicity, still remains the only option in the treatment of advanced/metastatic cancer. Therefore, the quest for better, more efficacious, and less toxic approaches to the delivery of cytotoxic agents has intensified. One approach, targeted delivery, promises to confine cell killing to malignant cells, thus reducing damage to collateral tissues.

Design of targeted therapeutics involves exploiting the difference between the structure and function of malignant cells compared with normal cells. Differences include overexpression of surface receptors on cancer cells, rendering them more sensitive to treatment regimens that target these surfaces molecules, and differences in blood supply within and around tumors compared with normal tissue, that can be exploited by incorporating cytotoxics in various carrier systems.

Two basic types of tumor targeting approaches are used, passive and active. Passive tumor targeting involves transporting anticancer agents through the bloodstream to tumor cells using a "carrier" macromolecule, or using prodrugs whose cytotoxic properties are activated at the tumor site. Active tumor targeting involves attaching a receptor molecule to the anticancer drug to create a new agent that actively seeks to bind to a complementary surface molecule. By targeting the anticancer agent to the affected cells, more of the drug should enter tumor cells, thus amplifying response to treatment and reducing toxic effects to normal tissue. Examples of active targeting approaches include monoclonal antibodies (MAb), growth factors, and other markers linked to cytotoxic moieties.

Targeting delivery of antiproliferative drugs by conjugating them to cell-surface receptors that are overexpressed

on cancer cells, is a challenging drug development approach. Every component of these constructs including the receptor, the ligand, the linker and the warhead must be optimized for the agent to be safe and effective. The ligand must interact uniquely with the target surface molecule/receptor of a given tumor type. The target receptor, in turn, should be chosen to deliver the construct intracellularly to tumor cells. The toxic moiety, or warhead, and the ligand must be joined by an appropriate linker so that the conjugated drug remains stable in circulation but is easily cleaved upon internalization into tumor cells, and must be powerful enough to destroy cells expressing the targeted cell-surface protein. Compounds are in development using various warheads, and ligands targeting a number of cell-surface receptors.

Biotinylated CBI-bearing CC-1065 Analogs

As indicated above CC-1065 and its analogs failed in the clinic because of significant toxicity. CBI analogs of CC-1065, and the duocarmycins, contain dimeric monocyclic, bicyclic, and tricyclic heteroaromatic replacements for the DNA-binding domain (Boger DL, etal, J Org Chem, 5 Oct 2001;66(20):6654-61). To avoid toxicity to healthy tissue, Panorama Research has created biotinylated CBI-bearing CC-1065 analogs that take advantage of the fact that biotin binds strongly to proteins avidin and streptavidin. A biotinalyted CBI-bearing CC-1065 analog, 6, caused apoptosis of U937 leukemia cells. This agent may serve as a model compound to explore the usefulness of nonradioactive small-molecule anticancer drugs as a pretargeting strategy in cancer imaging and therapy (Wang Y, BMC Chem Biol 2002;2(1):1).

Biotin binding to proteins avidin and streptavidin has been exploited and clinically evaluated in anticancer radioimmunoconjugates. In this approach, a nontoxic tumor-cell-specific MAb conjugated with avidin/streptavidin, is administered to patients first. After the MAb binds to tumor cells, usually 24 to 48 hours later, a clearing agent is introduced that removes any residual circulating MAb in the patient's blood. Lastly, a toxic biotin-radioisotope conjugate is administered, which rapidly binds to tumor cells with high specificity because of its small size and the tight binding between biotin and avidin/streptavidin.

Bispecific MAb

Preclinical results have demonstrated that pretargeting using bispecific MAb can deliver chemotherapeutics, such as doxorubicin, selectively and at higher concentrations to tumors than is possible with conventional systemic approaches, currently mostly used to selectively deliver a radioactive payload to tumors. However, several developers are also planning to adapt this technology to deliver cytotoxic agents. One such construct is being developed by IBC Pharmaceuticals, a subsidiary of Immunomedics (Morris Plains, NJ). In March 2002, Immunomedics was granted by the USPTO, patent # 6,361,774 covering a new method for targeting drugs to diseased tissues, including cancer. In this invention an enzyme that converts a prodrug to an active form is attached to a MAb. When a bispecific MAb is used, one arm of the MAb binds to a tumor cell, and the other to an enzyme that activates a prodrug to a more toxic form *in situ*.

CMC-544

CMC-544 is an immunoconjugate under development by Celltech Group (Slough, Berkshire, UK), in collaboration with Wyeth. CMC-544 consists of a derivative of calicheamicin γ conjugated to a humanized IgG4 anti-CD22 MAb (G544), using the same conjugation technology as in CMA-676 (Mylotarg; Wyeth). CMC-544 binds CD22 with high affinity.

In preclinical evaluations *in vitro*, in a series of human CD22+ B lymphoma lines, CMC-544 was 20-fold more potent than CMA-676. In RL and RAMOS B lymphoma xenograft models in nude mice, intraperitoneal CMC-544, administered post-tumor-staging, strongly inhibited B-lymphoma growth. In *in vivo* evaluations, CMC-544 also caused complete regression of both small and large established tumors, and produced cures based on >100 tumor-free days. In contrast, the CD33-targeted conjugate, CMA-676, used as a negative control, failed to impact growth of CD22+ CD33- B lymphomas. In addition, CMC-544, but not CMA-676, significantly prolonged survival (>80 days) of mice in a model of systemically disseminated B lymphoma (DiJoseph JF, EORTC-NCI-AACR02, Abs. 500, and Eur J Cancer, Nov 2002;38(Suppl 7):150).

Cytotoxic Peptide Conjugates Targeted to their Receptors on Tumors

A group at Tulane University School of Medicine (New Orleans, LA) and Veterans Affairs Medical Center (New Orleans, LA) have been developing targeted cytotoxic peptide conjugates comprising hybrid molecules composed of a peptide carrier which binds to receptors on tumors, and a cytotoxic moiety. These constructs were originally under development by Degussa, which spun them off in Zentaris (Frankfurt, Germany), recently acquired by Æterna Laboratories (Quebec, Canada).

AN 152 and AN-207 are targeted cytotoxics constructed by linking doxorubicin or 2-pyrrolino-DOX (AN-201), a potent derivative of doxorubicin, to [D-Lys6] luteinizing hormone-releasing hormone (LH-RH), respectively. Both these conjugates exhibit high-affinity binding and are much less toxic and more effective *in vivo* than their respective radicals in inhibiting tumor growth in LH-RH-receptor-positive models of human ovarian, breast, or prostate cancer.

Receptors for LH-RH are found in 80% of human ovarian tumors. In LH-RH receptor-positive ES-2 human ovarian cancer, treatment with AN-207 significantly decreased expression of mRNA for EGFr, and HER2 by 27% and 34%, respectively, as compared to controls and reduced the

receptor protein levels of EGFr and HER2 by 35% and 36%, respectively. These results indicate that cytotoxic LH-RH analog AN-207 could be considered for chemotherapy of ovarian cancer expressing LH-RH receptors (Arencibia JM, etal, Anticancer Drugs, Oct 2002;13(9):949-56).

AN-215 is a targeted cytotoxic consisting of AN-201 conjugated to bombesin/GRP antagonists as carrier molecules. AN-215 may find application in the treatment of small-cell lung, brain, colorectal, gastric, pancreatic, breast, and prostate cancer.

Because some brain tumors, such as GBM express high levels of receptors for bombesin/gastrin releasing peptide, AN-215 activity was evaluated on the growth of U-87MG human GBM xenografted into nude mice. Treatment with AN-215 significantly extended tumor-doubling time from 4.54+/-0.2 days to 8.18+/-1.8 days, and inhibited tumor growth as demonstrated by a 69.6% reduction in final tumor volume, and a 64.6% decrease in tumor weight compared to controls. Cytotoxic radical AN-201, used alone at the same dose, was ineffective. The antitumor effect of AN-215 could be blocked by pretreatment with an excess of a bombesin antagonist, indicating that the action of this cytotoxic analog is receptor-mediated (Szereday Z, etal, Br J Cancer, 22 Apr 2002;86(8):1322-7).

AN-238 and AN-162, are synthesized targeted cytotoxics consisting of AN-201, or doxorubicin, respectively, linked to octapeptide RC-121. Octapeptide analogs bind with high affinity to certain somatostatin receptors (SSTr) expressed in various human neoplasms. Cytotoxic somatostatin analog AN-238 efficaciously inhibits growth of human breast or prostate cancer expressing SSTr 2 and 5, and can be used for receptor-targeted chemotherapy in the treatment of pancreatic, colorectal, and gastric cancer as well as brain tumors and nsclc.

C-terminal gastrin heptapeptide-targeted cytotoxics are small molecule conjugates designed by investigators at UTMDACC that target plasma membrane receptors on tumor cells. A number of highly potent antiproliferative and toxic moieties have been tested in a model system where the ligand is the C-terminal gastrin heptapeptide that targets the gastrin receptor with high affinity. This heptapeptide can be modified at will on the N-terminus without losing binding affinity for the receptor. The gastrin receptor is overexpressed in pancreatic and gastrointestinal tumors. A variety of alkylating agents, topoisomerase inhibitors such as doxorubicin and ellipticine, folate inhibitors such as methotrexate, and microtubule binding agents such as derivatives of marine products hemiasterlin and dolastatin 15, were attached to heptagastrin using lysosomal protease-sensitive or acid-sensitive linkers. Activity of some of the conjugates tested was potent and highly selective for the tumor target (Tarasova NI, etal, AACR02, Abs. 699:140).

Davanat-2

Davanat-2 (galactomycin), under development by Pro-Pharmaceuticals (Newton, MA), enhances effectiveness of doxorubicin while reducing its toxicity. In preclinical trials, glyco-upgrade doxorubicin was significantly less toxic than the free drug.

Dynavat technology capitalizes on the natural properties of glycomolecules to attach selectively to binding sites in tumors. Dynavat technology is used to reformulate existing anticancer drugs with nontoxic carbohydrate-based compounds that recognize and adhere to specific binding sites on the surface of cancer cells. The construct combines a pharmaceutical compound, such as a chemotherapeutic agent, with a spacer and a galactose moiety. The spacer is covalently linked to the therapeutic agent at a first site, and to the galactose moiety by an ether bond at a second site to form a conjugate. This approach permits administration of an effective dose of the conjugate at a lower toxicity.

Davanat-1 (Pro-5FU), a formulation of 5-FU, entered a multicenter, open-label, phase I clinical trial in February 2003, in patients with solid tumors who have failed established surgical, radiation, and chemotherapeutic regimens.

HPMA-HA-DOX

Biochemical targeting may be used for selective delivery of anticancer agents to cancer cells. An N-(2-hydroxypropyl)methacrylamide (HPMA)-HA polymeric drug delivery system was developed at the University of Utah (Salt Lake City, UT) for targeted delivery of doxorubicin to cancer cells based on the observation that overexpression of hyaluronan (HA) receptors on cancer cells results in enhanced endocytotic uptake of drug conjugates. HA-doxorubicin bioconjugates (HA-DOX), and HPMA copolymer-doxorubicin conjugates containing HA as a side chain (HPMA-HA-DOX) were synthesized and their cytotoxicity was evaluated *in vitro* in cell culture.

Cytotoxicity of the HPMA-HA-DOX-targeted bioconjugate was higher against human breast (HBL-100), ovarian (SKOV-3), and colon (HCT-116) cancer cells when compared to the nontargeted HPMA-DOX conjugate. Using fluorescence confocal microscopy it was shown that targeted HPMA-HA-DOX conjugates were internalized more efficiently by cancer cells compared to nontargeted HPMA-DOX conjugates. There was minimal toxicity associated with either HPMA-DOX or HPMA-HA-DOX in mouse fibroblast NIH 3T3 cells. Internalization of polymer conjugates was correlated with their cytotoxicity. The HA-modified HPMA copolymer showed lower toxicity attributable to receptor-mediated uptake of the macromolecular drug (Luo Y, etal, Pharm Res, Apr 2002;19(4):396-402).

Immunoliposomes

Immunoliposomes are biologic agents that link a liposome-encapsulated cytotoxic to a MAb targeting receptors on various cancer cells. These MAb become internalized

in these cells delivering their payload intracellularly to target cells. One of the requirements of immunoliposomes is receptor-mediated endocytosis followed by endosomal escape of immunoliposome-entrapped drug molecules, usually induced by pH-dependent fusogenic peptides, or proteins, incorporated in the liposomal carrier. This 'Trojan horse' approach of delivery requires binding and internalization of immunoliposomes as a first step in the cytosolic delivery of entrapped drugs. Successful receptor-mediated endocytosis of receptor-bound immunoliposomes is determined by the type of receptor, provided that immunoliposomes are small enough (<150 nm).

Choice of the target receptor for intracellular delivery of immunoliposomal drugs is of major importance. However, not all MAb recognizing the same receptor mediate internalization of the receptor/MAb complex. Different MAb recognizing different epitopes on the same receptor may be processed by cells in different ways. In addition, internalizing capacity of cell-surface receptors is dependent on the type of cell that expresses the cell-surface receptor.

Immunoliposomes are stable, long-circulating, nonimmunogenic, and versatile drug carriers. The modular organization of immunoliposomes makes possible a combinatorial strategy for the generation of new therapeutics. MAb fragments, derived from available MAb, or newly selected from phage antibody libraries, may be coupled to an appropriate liposomal drug, chosen from a repertoire of liposomal drugs. Immunoliposomes incorporating doxorubicin or other warheads are in development targeting a variety of receptors including HER2, EGFr, VEGFr2 (Greiser U, etal, AACR02, Abs. 2060:414), and various tumor-associated antigens (TAA).

In addition to targeting cytotoxics to tumor cells, immunoliposomes may be used in a variety of applications including ferrying genes for gene therapy, and may evolve into therapeutic modalities for a variety of diseases in addition to cancer. Several features of immunoliposomes make them particularly suited as targeted therapeutics.

Hermes Biosciences (South San Francisco, CA) and its academic affiliates developed immunoliposomes for intracellular delivery of drugs by linking them with internalizing MAb fragments directed against receptors such as HER2 (ErbB2) on cancer cells, and VEGFR2 (Flk-1/KDR) on endothelial cells. These immunoliposomes are capable of delivering promising anticancer compounds, provided by the NCI Developmental Therapeutics Program, that were selected based on their potent anticancer activity, unusual mechanisms of action and drug resistance, and suitability for active loading into liposomes with high efficiency and high capacity (Drummond DC, etal, AACR02, Abs. 2077:418).

Antigen 15-3 (CA15-3) targeting with a CA15-3 antibody-doxorubicin-loaded liposome complex also increased doxorubicin cytotoxicity against the MCF-7 human breast cancer cells; approximately two-thirds of human breast cancers express high levels of CA15-3 on their cell surface. Investigators at the Mastology Research Institute (Baton Rouge, LA) bound antihuman CA15-3 antibody (CA15-3Ab) and mouse normal IgG, to the PEG terminus of Doxil. *In vitro* results suggest that a CA15-3 antibody can selectively deliver antineoplastic drugs to cancer cells, but further studies of this CA15-3Ab-Doxil complex in animal models and human breast cancer patients will be needed to prove clinical efficacy (Jiang X, etal, AACR02, Abs. 3663:415).

Anti-HER2/doxorubicin immunoliposomes were developed by investigators at the Bay Area Breast Cancer Translational Research Program, a Specialized Program of Research Excellence (SPORE), sponsored by the NCI, with the goal of improving diagnosis, prognosis, and therapy of breast cancer. This technology was subsequently licensed to Hermes Biosciences, a start-up launched by six UCSF faculty members in 1998. Hermes scientists have developed a new generation of molecularly targeted drug delivery technology, including the proprietary S.M.A.R.T. (stabilized, modularly-assembled, receptor-targeted) immunoliposome technology, using antibody-guided liposomes directed against cell-surface receptors on cancer or other target cells. S.M.A.R.T. anti-HER2 immunoliposomes efficiently bind to and become internalized by cancer cells *in vitro* and *in vivo*, resulting in targeted intracellular drug delivery. Anti-HER2/doxorubicin immunoliposomes combine the tumor-targeting ability of certain anti-HER2 MAb with the pharmacokinetic and drug delivery capabilities of sterically stabilized liposomes. This delivery approach results in a striking enhancement of therapeutic efficacy compared with the best nontargeted liposomes on the market, and is superior as well to treatment with anti-HER2 MAb alone, or in combination with chemotherapy. Anti-HER2 immunoliposomes can be used to deliver potent small molecules in addition to doxorubicin, including other FDA-approved chemotherapeutic drugs as well as novel compounds encapsulated in liposomes.

Immunoliposomes loaded with doxorubicin demonstrated antitumor efficacy in multiple HER2-overexpressing models. Each component of the immunoliposome construct, the MAb fragment, liposomal drug, and linker was optimized and developed for clinical trials. MAb scFv fragment F5 against HER2 resulted in tumor cell-specific uptake and improved efficacy in a HER2-overexpressing breast tumor xenograft model in nude mice. Investigators at the University of California San Francisco (UCSF) evaluated the pharmacokinetics and therapeutic efficacy of anti-HER2 immunoliposomes containing doxorubicin in a series of animal models. In single- and multiple-dose studies in normal rats, immunoliposomes remained in the circulation for long periods of time, identical to that of sterically stabilized liposomes. These constructs were effective in 4 different HER2-overexpressing tumor xenograft models, causing growth inhibition, regression, and cures. These results demonstrated that encapsulation of doxorubicin in

anti-HER2 immunoliposomes greatly enhanced its therapeutic index, both by increasing antitumor efficacy and by reducing systemic toxicity. These constructs were significantly superior to all other treatments tested, including free and liposomal doxorubicin, and the anti-HER2 MAb trastuzumab (Herceptin; Genentech). Anti-HER2 immunoliposomes containing doxorubicin were superior to combinations consisting of free MAb plus free or liposomal doxorubicin (Park JW, etal, Clin Cancer Res, Apr 2002;8(4):1172-81).

Local hyperthermia (HT) increased solid tumor uptake and antitumor efficacy of liposomal anticancer drugs, including HER2-targeted immunoliposomal doxorubicin (F5-ILs-DOX, 90-100 nm) that was prepared from sterically stabilized liposomal doxorubicin (Doxil) by micellar incorporation of MAb scFV fragment F5 conjugated to PEG lipid (Kirpotin DB, etal, AACR02, Abs. 2069:416).

In April 2002, Alza (Mountain View, CA), a Johnson & Johnson subsidiary, licensed the anti-HER2/doxorubicin immunoliposome technology from UCSF, and Hermes Biosciences. Anti-HER2 immunoliposomes containing Hermes' proprietary antibody fragment F5 and the chemotherapy drug doxorubicin are being readied for clinical trials under the sponsorship of the NCI. In 2001, GMP production of anti-HER2 immunoliposomes was undertaken in conjunction with the NCI Biological Resources Branch.

EGFr-targeted/epirubicin immunoliposomes are also in development by Hermes Biosciences, in collaboration with Pharmacia. Immunoliposomes are used to target epirubicin to cancer cells overexpressing the EGFr family of receptors. Unilamellar vesicles (85-95 nm) composed of distearoylphosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (DSPE), were loaded with epirubicin using a gradient-based method. Anti-HER2 scFv or anti-EGFr Fab(C225)-PEG-DSPE were conjugated to thiol-reactive maleimido-PEG-DSPE, and incorporated into epirubicin-loaded liposomes using a micellar strategy. *In vitro* studies in HER2-overexpressing BT-474 cells demonstrated increased internalization of F5-ILs-EPI compared to nontargeted Ls-EPI, and a 22-fold increase in cytotoxicity. C225 Fab-ILs-EPI showed similar target-specific activity in EGFr-overexpressing MDA-MB-468 breast cancer cells. *In vivo* efficacy studies using these two receptor-overexpressing xenograft models are currently underway (Guo Z, etal, AACR03, Abs. 762).

MCC-465 is an immunoliposome-encapsulated doxorubicin, under development by Mitsubishi Pharma (Tokyo, Japan). In this construct, the liposome is tagged with PEG and MAb GAH, the F(ab')2 fragment of a human IgG1 antibody. A multicenter, dose-escalation phase I clinical trial was conducted in Japan at National Cancer Center Hospital and Cancer Institute Hospital, in Tokyo and at Ibaraki Prefectural Central Hospital with MCC-465 in patients with metastatic or recurrent stomach cancer. At the time of the initiation of this trial, 90% of stomach cancer tissue stained positively with GAH, while normal tissue was always negative. MCC-465 was administered as a 1-hour IV infusion, once every 3 weeks, at a starting dose of 6.5 mg/m^2. A total of 62 courses at dose levels ranging from 6.5 to 45.5 mg/m^2 were delivered to 23 patients who had been exposed to 1 to 5 regimens of previous chemotherapy. An acute transient reaction to infusion was commonly observed in 16 patients. Symptoms were characterized by flushing, chest discomfort, itching, chills, fever and shivering. In one instance, chills and shivering were accompanied by Grade 3 hypertension. Other toxicities included Grade 3 nausea, Grade 4 myelosuppression, Grade 1 skin toxicities, and Grade 2 mucositis. DLT was Grade 4 neutropenia and Grade 3 appetite loss. There were no antitumor responses among 20 evaluable patients but disease stabilized in 10. Tumor markers were decreased slightly in 13 patients. The pharmacokinetic profile of this agent was similar to that of Doxil (Matsumura Y, etal, ASCO02, Abs.1884:19b).

SGN-15

SGN-15 is an immunoconjugate consisting of chimeric MAb BR96, targeting cell-surface Lewis y antigen expressed on many tumor types, chemically linked to doxorubicin with an average of eight drug molecules per each MAb molecule. SGN-15 binds to Lewis y antigen, becomes rapidly internalized, and releases its drug payload at the low pH present within the cell through acid catalyzed hydrolysis in the endosome. Toxicology studies demonstrated that side effects normally associated with doxorubicin, specifically cardiotoxicity and neutropenia, were not limiting in any of the species tested. However, as a single agent, SGN-15 was only modestly active in humans.

Cell-cycle analysis indicates that SGN-15 is most effective against S-phase cells, yet cells exposed to even subtoxic levels progress to and arrest in G2/M. Cell cycle positioning is likely attributed to doxorubicin-induced inhibition of topo II and resultant accumulation of target cells in G2 phase. Interestingly, preclinical studies have shown that combining docetaxel and SGN-15 in animal models of cancer results in tumor regression. Docetaxel, a taxane, stabilizes microtubule formation resulting in maximum cytotoxicity in G2/M phase. Because active endocytosis also requires functional microtubules, internalization of SGN-15, and resultant doxorubicin-induced apoptosis, are antagonized by coincident treatment with taxanes. However, delayed treatment facilitates selective sensitization to these cytotoxics. Sequential exposure of tumor cells with SGN-15 followed by a taxane at progressively delayed times up to 24 hours, produces progressively increasing synergy between the two agents in effecting tumor-cell killing. The synergy obtained by staged administration *in vitro* is reflected in antitumor efficacy *in vivo* against xenograft models of human lung and colon tumors that cannot be achieved by either agent alone. The staged combination elicited complete regression of established

human tumor xenografts using levels that are achievable in human (Wahl AF, etal, AACR-NCI-EORTC99, Abs. 86).

A multicenter, phase II clinical trial (protocol IDs: SGEN-UAB-9912; UAB-991), funded by Aventis, was initiated in October 2000 at the University of Alabama, Birmingham (UAB), using SGN-15 in combination with docetaxel to treat metastatic or recurrent breast cancer. Study objectives were to determine the toxicity and safety profile of SGN-15 and docetaxel as well as the clinical response rate and duration of response in patients treated with this regimen. According to the protocol, patients were treated with IV SGN-15, over 2 hours, and with IV docetaxel, over 30 minutes, on day 1 of weeks 1 to 6. Treatment was repeated every 8 weeks for up to 6 courses in the absence of disease progression or unacceptable toxicity. A maximum of 45 patients were to be accrued for this study. Lisle M. Nabell, MD, of UAB is the PI. The trial was completed in December 2002, and although multiple objective responses were observed, it was decided not to pursue SGN-15 in combination with docetaxel in this setting.

A multicenter (n=21), randomized, open-label, active-control, parallel-assignment, safety, and efficacy, phase II clinical trial (protocol ID: SG0001-015) of SGN-15, in combination with docetaxel, in advanced prostate cancer, was initiated in October 2000. The drugs are being administered weekly with SGN-15 (200 mg/m^2) administered prior to docetaxel (35 mg/m^2) in the combination arm. A single course of therapy is defined as 6 weekly doses, followed by a 2-week rest period. Patients are treated with a minimum of 2 courses of therapy. Additionally, repeat dosing with subsequent cycles is possible for patients who remain eligible, and have tolerated therapy, while patients with evidence of tumor progression or intolerable toxicity are removed from the study. Follow-up assessments include adverse event reporting, clinical laboratory studies, and QoL assessment. As of November 2002, a sufficient number of patients were enrolled for an interim analysis that will include data from patients who have already been treated, and those recently enrolled, to guide the future strategy for this program.

A randomized, open-label, phase II clinical trial (protocol ID: SGEN-SG0002-015) of SGN-15, in combination with docetaxel in Stage IV or recurrent nsclc, was initiated in October 2000, at Oregon Cancer Center (Portland, OR). According to the protocol, patients are stratified by gender and performance status, and randomized to 1 of 2 treatment arms. In arm I, patients are treated with IV SGN-15, over 2 hours, followed by IV docetaxel, over 30 minutes, once weekly, for 6 weeks. In arm II patients are treated with docetaxel alone as in arm I. Treatment in both arms repeats every 8 weeks for 6 courses in the absence of disease progression or unacceptable toxicity. QoL is assessed at baseline and on day 1 of each course. Patients are followed at 6-8 weeks. A total of 60 patients (40 in arm I and 20 in arm II) will be accrued for this study. As of November 2002, the trial has demonstrated encouraging results. The regimen containing SGN-15 is well tolerated, allowing for the implementation of an intrapatient dose escalation of SGN-15.

In August 2002, Seattle Genetics initiated a multicenter, phase II clinical trial of SGN-15, in combination with gemcitabine (Gemzar; Lilly), in patients with advanced ovarian cancer. The trial will compare treatment with the combination of SGN-15 and Gemzar to Gemzar alone. Approximately 70 patients will be enrolled in the trial.

Vitamin-mediated Targeted Delivery

It has been known for sometime that vitamin B12 and folic acid are essential for tumor growth and, as a result, receptors for these vitamins are upregulated in proliferating cancer cells. By coupling a drug to an appropriate vitamin, the vitamin serves as a carrier to increase the amount of drug at the disease site relative to its normal distribution. Vitamin B12 receptor overexpression occurs in breast, lung, bone, thyroid, colon, prostate and brain cancer, and in leukemia and lymphoma, while folate receptor overexpression occurs in breast, lung, ovarian, endometrial, renal, colon and brain tumors. Panorama Research scientists are using vitamin B12 and folate to more effectively target anticancer drugs to solid tumors.

PRODRUGS

One approach to increase the selectivity of cytotoxic agents by delivering them specifically to malignant cells, is using prodrugs that are activated only in the vicinity of or within tumor masses. The ideal prodrug must be stable in the circulation and not be able to enter cells, and activated by enzymes specifically released by tumor cells. Theoretically, extracellularly tumor-activated prodrugs (ETAP) would ensure low toxicity and high efficacy.

CPI-0004Na

CPI-0004Na (N-succinyl-ß-alanyl-L-leucyl-L-alanyl-L-leucyl-Dox), a new extracellularly tumor-activated prodrug of doxorubicin, is under development by Medarex (Princeton, NJ), in collaboration with Diatos (Paris, France) and Université Catholique de Louvain (Louvain-la-Neuve, Belgium). In lethality studies performed in mice, this compound was up to 4.6 times less toxic than IV doxorubicin, and up to 16.2 times less toxic after intraperitoneal administration. Pharmacokinetics and tissue distribution data indicate that this reduced toxicity is attributable to a lower uptake of doxorubicin in normal tissues after treatment with CPI-0004Na than after administration of an equimolar dose of doxorubicin. Because heart exposure to doxorubicin is reduced >10-fold, higher doses of CPI-0004Na than of the parent drug could be used to treat nude mice bearing human breast (MCF-7/6) and colon (LS-174-T and CXF-280/10) tumors. In all three models, the prodrug's efficacy was much improved compared with doxorubicin. Particularly, LS-174-T tumors that do not respond to doxorubicin were inhibited by 68% after treat-

ment with CPI-0004Na. Tissue distribution studies performed with MCF-7/6 tumor-bearing nude mice and comparing CPI-0004Na and doxorubicin confirmed that the improved activity of the prodrug is actually the result of selective generation and uptake of doxorubicin at the tumor site. Doxorubicin levels in tumor tissue were 2-fold higher after treatment with CPI-0004Na than after treatment with an equimolar dose of doxorubicin, whereas normal tissue levels were reduced 1.4-29-fold (Dubois V, etal, Cancer Res, 15 Apr 2002;62(8):2327-31).

CRX-103

CRX-103 is a novel tetrapeptide duocarmycin-analog tumor-activated prodrug (TAP) of CRX-395, a modified duocarmycin A, under development by Corixa (South San Francisco, CA). Investigators addressed the issues of potency, inclusion of an attachment group, identification of an optimal site for attachment, and improved solubility, to create CRX-103. CRX-103 was developed by adding an enzyme-cleavable tetrapeptide, to increase tumor specificity and protect against nonspecific systemic toxicity of CRX-395. Chemical modifications of CRX-395 were mostly targeted at the DNA binding domain, which was extended to increase potency, modified to identify a suitable attachment group, and optimized to avail of a site for attachment of the TAP peptide. To create the TAP construct, β-ALAL TAP peptide was chosen because its sequence exhibited tissue-specific activation in solid tumors. The optimized attachment group is the amino group (without need for a linker) and the optimized site of attachment is the 7-position of the distal ring. Addition of a N-methylpiperazinyl carbamate solubilizing group at the 4-hydroxyl position increased aqueous solubility to over 2 mg/ml, allowing for *in vivo* testing of CRX-103 (Ng HP, etal, AACR02, Abs. 2430:489).

In vitro, CRX-395 exhibited nanomolar potency in inhibiting proliferation of HL60 cells. CRX-103 is relatively stable and inactive in plasma and in tumor cells *in vitro*. On a molar basis, approximately 100% more of CRX-103 was well tolerated than CRX-395, indicating that the prodrug protected animals from systemic toxicity. CRX-103, unlike doxorubicin, when dosed at various single and repeat dose-levels and regimens, was well tolerated and significantly inhibited growth of the doxorubicin-resistant HT-29 colorectal carcinoma xenograft, prolonging survival of tumor-bearing mice. Moreover, CRX-103 showed superior efficacy to CRX-395 in terms of tumor-growth inhibition and extension of survival. CRX-103 was further evaluated in the aggressive doxorubicin-resistant LS174T colorectal carcinoma, and in doxorubicin-sensitive LNCaP prostate carcinoma models. A single dose of CRX-103 significantly inhibited LS174T tumor growth by 81% and extended survival of tumor bearing mice by 56%. CRX-103 induced 91% inhibition of LNCaP tumors and was well tolerated (Pan C, etal, AACR02, Abs. 2057:413).

Epidoxoform

Various formaldehyde conjugates of commercial anthracyclines, including daunoform, doxoform, and epidoxoform, were synthesized as improved chemotherapeutics. When the activity of these conjugates was contrasted with those of the corresponding free drugs, conjugates were taken up better, were retained longer, and were more toxic to a wide variety of tumor cells. The kinetics of drug release from doxoform- and epidoxoform-treated MCF-7/Adr cells was biexponential and correlated with the biexponential kinetics of drug release from extracellular DNA. The lead conjugate, epidoxoform, demonstrated increased toxicity relative to free doxorubicin and epidoxorubicin in various cell lines, which was especially evident in the more resistant cell lines.

In a mouse model of breast cancer, in all efficacy trials, a significant difference was evident in tumor volume between epidoxoform- and epidoxorubicin-treated mice and controls. In mice treated with a two-dose regimen, significantly increased efficacy was found between epidoxoform compared to epidoxorubicin (Dernell WS, etal, Cancer Invest 2002;20(5-6):713-24).

L-377202

L-377202, under development by Merck is a novel peptide conjugate of doxorubicin that, upon cleavage by membrane bound prostate specific antigen (PSA), releases the active metabolites leucine-dox (Leu-Dox) and doxorubicin.

To evaluate safety and pharmacokinetics, and determine the recommended dose for phase II clinical trials, of L-377202, 19 patients with advanced hormone-refractory prostate cancer (HRPC) were enrolled in a phase I clinical trial and treated IV with 71 cycles of L-377202 at escalating dose levels of 20 (n=1), 40 (n=3), 80 (n=4), 160 (n=3), 225 (n=6), and 315 mg/m^2 (n=2), once every 3 weeks. L-377202 was well tolerated. Dose limiting Grade 4 neutropenia was noted in 2/2 patients administered 315 mg/m^2 (both patients were able to resume therapy at 225 mg/m^2), and the recommended phase II dose is 225 mg/m^2, which induced Grade 4 neutropenia in 1/6 patients. Among 5 patients who completed at least 3 cycles of therapy at 225 mg/m^2 and 315 mg/m^2 dose levels, PSA declined >75% in 2 and stabilized in 1. There were no responses at dose levels <225 mg/m^2. L-377202 was well tolerated. L-377202 was cleaved to produce detectable levels of the active metabolites Leu-Dox and doxorubicin (DiPaola RS, etal, 2002 Annual Retreat on Cancer Research, 24 April 2002, Princeton, NJ).

Plasmin-targeted Doxorubicin

Prodrugs 1 and 2 of doxorubicin were synthesized, designed to become selectively activated in tumor tissue, to increase the drug's therapeutic index. These prodrugs contain a tripeptide specifier directly recognized by the tumor-associated enzyme plasmin, which is present in elevated levels in invading tumors. Prodrug 1, in which the

specifier was connected to doxorubicin via a conventional 1,6-self-elimination spacer, was selective against urokinase-type plasminogen activator (u-PA)-transfected MCF-7 cells. In prodrug 2, a novel elongated self-elimination spacer system was incorporated between the tripeptide and the parent drug, to increase the efficiency of plasmin activation. A series of prodrugs of doxorubicin and paclitaxel were designed and synthesized including those containing several elongated spacer systems, increasing the efficiency of drug release *in vitro* in comparison with a conventional spacer.

Plasmin-activated prodrugs 1 and 2 were evaluated both *in vitro* and *in vivo* in murine EF43.fgf-4 tumor cells, and compared to free doxorubicin. The two prodrugs were toxic *in vitro,* in EF43.fgf-4 cells, similar to free doxorubicin, confirming prodrug conversion. Furthermore, in the presence of the selective plasmin inhibitor Trasylol, the prodrugs were much less toxic against EF43.fgf-4 cells, indicating a plasmin-mediated drug release. In sharp contrast to doxorubicin, both prodrugs significantly reduced the volume of EF43.fgf-4 tumors in Balb/c mice without discernable systemic toxicity. In large EF43.fgf-4 tumors, the elongated spacer-containing prodrug 2 was significantly more effective in reducing tumor growth than prodrug 1 (De Groot FMH, etal, AACR02, Abs. 2064:415).

NOVEL FORMULATIONS

Different carrier macromolecules are being investigated as each provides advantages such as specificity and protection of the anticancer drug from degradation, arising from their structure, size and particular interactions with tumor cells.

Liposomal/PEG Formulations

Liposomal drug delivery systems offer the potential for passive accumulation in solid tumors based on their long circulation, restricted volume of distribution, and hyperpermeable tumor vasculature. However, liposomes have several serious limitations. Liposomes bond peculiarly with serum proteins (opsonization), and are taken up by reticuloendothelial system (RES) cells in the liver and spleen, which reduces their effectiveness as carriers of drugs that need to remain in the circulation long-term, or be targeted to tissues other than RES. PEG modification of the liposome surface forms a fixed aqueous layer around the liposomes by interaction between the PEG-polymer and water molecule, and prevents attraction of opsonins. PEG-modified liposomes also escape trapping by the cells of RES, and have a prolonged circulation time.

Liposome encapsulated doxorubicin (LED), under development by NeoPharm (Lake Forest, IL), is based on the company's synthetically derived cardiolipin (NeoLipid technology), a proprietary, electrostatic liposome encapsulation delivery system, that takes advantage of the naturally occurring electrical charge of a drug to combine it with proprietary oppositely charged lipids. The drug, and the liposome structure surrounding it, thus become strongly attracted to each other, resulting in a stable product during storage and after reconstitution, and IV administration. The construct is also less expensive and easier to manufacture and deliver because there is less waste as a result of the inherently greater stability of the electrostatic liposome structure. The company has been improving the liposomal formulation incorporated in this construct, and now refers to it as Easy-To-Use (ETU) NeoLipid.

NeoPharm had entered into a definitive agreement with Pharmacia to develop and market liposomal encapsulated paclitaxel (LEP) and liposomal encapsulated doxorubicin (LED), in February 1999. Both LEP and LED are currently the subject of an arbitration between NeoPharm and Pharmacia. A hearing on the arbitration is scheduled to begin on May 28, 2003. On November 21, 2002, NeoPharm terminated the License Agreement because Pharmacia had stopped all development of LEP and LED. Pharmacia disputes the propriety of the termination, and has included NeoPharm's termination of the License Agreement as part of the arbitration hearing. Because of these events clinical development of LED has been interrupted while undergoing phase II clinical trials in various indications, including prostate and breast cancer, osteosarcoma and multiple myeloma. Other NeoLipid formulations in development include Liposome Encapsulated Mitoxantrone (LEM), and SN-38 (LE-SN38).

MTC-DOX

In MTC-DOX, under development by FeRx, doxorubicin is absorbed in microparticles composed of elemental iron and activated carbon, based on a proprietary technology, Magnetic Targeted Carrier (MTC), that is used for site-specific targeting, retention and sustained release of oncolytic agents to solid tumors. MTC are microparticles, composed of elemental iron and activated carbon. Using a small, externally positioned magnet to create a localized magnetic field within the body, arterially administered drugs, which are adsorbed to MTC, can be targeted to specific sites. The physical force created by the magnetic field induces transport of the MTC through the vascular wall, leading to localization and retention of particles at the desired site. MTC are under investigation to determine if they can reduce drug toxicity created by nonspecific systemic exposure of larger doses, while still achieving an efficacious concentration at the desired site of action in the body with reduced doses.

The clinical development of MTC-DOX has concentrated on the treatment of advanced primary or metastatic hepatocellular carcinoma (HCC). However, MTC-DOX can also be targeted and retained within specific locations in the bladder allowing for greater exposure and specific deposition of the drug at the tumor site compared to intravesical administration of doxorubicin alone (Ji C, etal, AACR02, Abs. 2073:417). Delivery of MTC via intra-arter-

ial, intravesical and intraluminal routes of administration, is possibly applicable to treatment of a variety of solid tumors, such as cancer of the esophagus, stomach and colon.

A phase I/II clinical trial (protocol IDs: FERX-MTC-DOX-003, NCT00041808, UCSF-00454, UCSF-H5535-17999-01D) to determine the safety, tolerance, and preliminary activity of MTC-DOX administered intra-arterially via hepatic artery catheterization in patients with primary or metastatic hepatocellular carcinoma (HCC), was initiated in July 2001. According to a presentation in March 2003, by Mark W. Wilson, MD, Assistant Professor of Radiology, UCSF Medical Center, at the 28th Annual Scientific Meeting of the Society of Interventional Radiology, in Salt Lake City, UT, MTC-DOX was delivered to 33 patients via selective hepatic arterial catheterization. Tumor localization was confirmed by MRI post administration. Tumors treated ranged in size from 3 to 222 cm^2.

Tumor localization of MTC-DOX was achieved in 31 of 33 patients. Even with high doxorubicin doses among the MTC-DOX patients, measurements of doxorubicin in plasma were undetectable or low. Low levels seen with MTC-DOX indicate the possibility that MTC technology may be used to reduce or eliminate the toxic side effects associated with anticancer agents. As of 24 August 2002, MST for all patients was 7.5 months, and survival for patients treated above a minimally effective dose of 0.37 mg of doxorubicin/cm^2 of tumor area was 11.5 months. There were no clinically significant toxicities with intra-arterial administration of MTC-DOX, and there was possible activity against HCC. The lesion control rate in the study was 83%, and MST exceeded that reported for historical controls.

Phase II/III clinical trial (protocol IDs: FERX-MTC-DOX-004, NCT00034333) to evaluate safety, tolerance, efficacy, and survival of MTC-DOX treatment in unresectable HCC, sponsored by FeRx, was initiated in March 2002. Conducted in the USA, Hong Kong, and Thailand, the trial is expected to enroll 240 patients. MTC-DOX is administered by intrahepatic delivery every 3 weeks. Treatment is continued until 6 treatment cycles are completed, the tumor grows, disappears, or a side effect is experienced causing the patient to be removed from the study. Follow-up visits are on days 3, 10, and 21 following treatment in the first cycle and days 7 and 21 for the remaining cycles, and also 60 days after the last treatment cycle.

P80DOX-NP

P80DOX-NP is a novel method for delivering doxorubicin across the blood-brain barrier (BBB) using a nanoparticle technology. Advectus Life Sciences (West Vancouver, Canada) is collaborating with the University of North Carolina at Chapel Hill (UNC-CH), in the development of P80DOX-NP. Also, in June 12, 2002, Advectus Life Sciences entered into an agreement with the University of Kentucky Research Foundation Center for Pharmaceutical Science and Technology for manufacture of the drug under cGMP conditions and stability studies.

In preclinical evaluations at the University of North Carolina Brain Tumor Center (Chapel Hill, NC), survival was measured as a surrogate for intracranial tumor progression/regression on rodent brain metastases arising from various malignancies. In *in vitro* studies on eight metastatic malignancies, an excellent dose response curve was observed in all cancer cell lines tested with some variability to drug sensitivity. Also, free doxorubicin and P80DOX-NP were equally effective. The drug did not lose efficacy during the nanoparticle formulation or shipping, and handling of the compound was appropriate and effective. The drug is currently being evaluated in preclinical trials, *in vivo*, in colon, breast and lung and kidney cancer, glioma, and melanoma.

Resmycin

Resmycin (doxorubicin HCl inhalation solution) is under development by Zivena (Columbus, OH), formed in December 2002, to commercialize the oncology intellectual property that was previously in the BattellePharma (Columbus, OH) portfolio. The company has been issued method patents covering use of several classes of chemotherapy drugs to treat cancer by inhalation. Drugs covered by these patents represent a source of proprietary products with different mechanisms of action and proven efficacy in treating cancer. The company also has a proprietary delivery system designed to eliminate the emission of fugitive aerosols.

Resmycin is a doxorubicin aerosol generated by Oncomyst, an inhalation device developed by BattellePharma specifically for inhaled chemotherapy. In clinical trials, Resmycin treatment resulted in the delivery of higher concentrations of doxorubicin to the lung than those achieved with standard doses administered IV, but without many of the toxicities, like bone marrow suppression and hair loss.

In an animal study, when compared to a radiolabeled dose of doxorubicin administered IV, the bioavailability of inhaled drug was approximately 68%. Immediately following inhaled exposure, 72% of the dose was present in the lungs compared to only 2% of the IV dose during the same time period. By 96 hours post-inhalation, 12% of the dose remained in the lungs as compared to less than 1% after IV. Plasma levels following IV administration were 2- to 4-fold higher than those following inhalation, thereby exposing non-target tissues such as the liver, spleen, kidneys and heart to correspondingly higher levels of drug (Zutshi A, etal, AACR99, Abs. 2754:416).

In 24 pet dogs with spontaneously arising primary and metastatic lung cancer including sarcoma, carcinoma and malignant melanoma, treated with either paclitaxel or doxorubicin via the inhalation route every 2 weeks, 7 (29.2%) responses were noted including 6 PR and 1 CR; there were 4/18 (22.2%) responses to doxorubicin, and 3/15

(20%) responses to paclitaxel. Responses were noted with osteosarcoma, liposarcoma, hemangiosarcoma, undifferentiated sarcoma, and breast cancer. No systemic toxicities were observed with either drug. Local (pulmonary) toxicity was not observed with paclitaxel. However, changes consistent with pneumonitis/fibrosis were observed in 3 dogs treated with doxorubicin, only one of which showed clinical signs, which were responsive to steroid and antitussive therapy (Vail D, etal, AACR99, Abs. 2752:416).

In a multicenter phase I clinical trial (protocol ID: 00-C-0088) initiated in February 2000, and completed in December 2002, Resmycin was administered to 24 patients (sarcomas=10, nsclc=6, mesothelioma=2 and others=6) with advanced solid tumors affecting the lungs, for up to 6 courses, once every 3 weeks. Three patients, at each dose level, were treated at 0.4, 0.6, 0.8, 1.2, 1.5, 1.9, 2.4 and 3.0 mg/m^2. Inhaled doxorubicin was well tolerated at the 2.4 mg/m^2 dose level (3.0 mg/m^2) but followup has not been complete. No treatment-related toxicities above Grade 2 or mucositis were seen. Regarding toxicities, 1 patient experienced Grade 1 chest discomfort at the end of each of 3 inhalations, and 2 patients Grade 1 cough during inhalation. Grade 2 toxicities included loss of appetite (n=2), lightheadedness (n=1), and asymptomatic sinus tachycardia after inhalation (n=1). There was 1 PR in a patient with sarcoma, and disease stabilized in one patient with nsclc and another with mesothelioma (Sharma S, etal, ASCO02, Abs. 1204:302a).

In May 2001, the FDA designated Resmycin as a "fast track" product for the treatment of pulmonary bronchioloalveolar carcinoma (BAC). A protocol for a phase II combination trial was submitted at several institutions for review in February 2003, to be conducted in patients with advanced nsclc. The trial is designed to test Resmycin's effectiveness as first-line treatment with a current standard IV therapy, thus combining the benefits of local and systemic treatment.

SMANCS

Styrene maleic acid neocarzinostatin (SMANCS) is a chemical conjugate of a synthetic copolymer of styrene maleic acid (SMA) and neocarzinostatin (NCS), which dissolves in organic solvents such as pyridine and acetone, and particularly in Lipiodol. SMANCS is a simple protein capable of inhibiting DNA synthesis and inducing DNA degradation. Lipiodol is an ethyl ester of iodinated poppy seed oil in which most of the unsaturated double bonds in oleic, linoleic and linolenic acid are almost completely iodinated. When a homogeneous suspension of SMANCS with Lipiodol (SMANCS/Lipiodol) is administered intra-arterially, Lipiodol acts as a carrier of SMANCS.

SMANCS was commercialized by Yamanouchi Pharmaceutical (Tokyo, Japan) in Japan, in 1994. At about the same time the drug was evaluated in the USA in several phase I clinical trials in solid tumors, hematologic malignancies and HCC, and in phase II clinical trials in HCC, bladder and renal cancer, lymphoma, pediatric ALL, and in various combination regimens.

Many studies have demonstrated the clinical efficacy of SMANCS/Lipiodol in the treatment of HCC. Transcatheter arterial infusion of SMANCS/Lipiodol exhibited a more favorable focal therapeutic effect than epirubicin in Lipiodol in the initial treatment of HCC. However, recent clinical studies have indicated that SMANCS causes severe adverse reactions and complications. Arterial administration of SMANCS/Lipiodol, therefore, should be given as peripherally as possible via the tumor feeding arteries, to enhance the efficacy of the agent and reduce adverse effects (Abe S and Otsuki M, Curr Med Chem Anti-Canc Agents, Nov 2002;2(6):715-26).

SYN 2002

In SYN 2002, under development by Synt:em (Nimes, France) the activity/pharmacologic profile of doxorubicin has been drastically enhanced by coupling it with Pep:trans, an effective brain transport system. Pep:trans is a noninvasive drug delivery system that does not require permeabilization of the blood-brain barrier (BBB), or any surgery, or device, to deliver drugs to the brain. Pep:trans technology is based on small vectors, derived from natural peptides, which can cross the BBB with high efficiency, and without compromising the barrier's integrity. This approach maintains the integrity of the tight junctions of the BBB while transporting its cargo to all brain areas within minutes after IV administration. Pep:trans linked to a drug results in a novel, fully patentable agent. Pep:trans enables high-throughput delivery of drugs through an adsorptive-mediated endocytosis mechanism, which can only be saturated at high concentrations (micromolar range).

Pep:trans efficiently solubilizes drugs, allowing significantly lower levels of a drug to be used to obtain the same therapeutic effect, thus broadening its therapeutic window; higher brain or cell uptake results in lower systemic dosing. Pep:trans peptide linkers exhibit a good safety profile in animal models and are non immunogenic. These linkers release the active drug from the peptide directly in the brain or in the cells. These peptides are short (16-18 amino acids), making synthesis of commercial quantities economical.

The ability of doxorubicin, coupled covalently to two peptides, D-penetratin and SynB1, to cross the BBB, was studied using an *in situ* rat-brain perfusion technique, and also by IV injection in mice. In the brain perfusion studies, the very low brain uptake of free radiolabeled doxorubicin because of the efflux activity of P-gp at the BBB, was confirmed and contrasted with uptake of doxorubicin coupled to either the D-penetratin or SynB1 vectors, that was increased by a factor of 6, suggesting that the vectorized drug bypasses P-gp. Moreover, vectorization of doxorubicin led to a 20-fold increase in the amount transported

into brain parenchyma. IV administration of vectorized doxorubicin led to a significant increase in brain concentrations during the first 30 minutes post-administration, compared with free drug. Additionally, vectorization led to a significant decrease of doxorubicin concentration in the heart (Rousselle C, etal, Mol Pharmacol, Apr 2000;57(4):679-86). Additional pharmacologic studies have shown that doxorubicin in Pep:trans results in a significant decrease of doxorubicin concentrations in the heart, thus may prevent cardiotoxicity.

Transdrug Doxorubicin

Transdrug doxorubicin, under development by BioAlliance Pharma (Paris, France), consists of doxorubicin-loaded nanospheres made of a polyisohexilcyanoacrylate polymer. Transdrug doxorubicin is in phase II clinical trials. Nanoparticles represent a promising biologic vector that delivers molecules directly to targeted cells or organs, potentially allowing better penetration into the cell.

Nanospheres may also protect fragile molecules, improve absorption across mucous membranes, and enable prolonged drug activity. Preclinical studies have shown that Transdrug coupled with doxorubicin, can overcome MDR, and restore sensitivity of resistant cancer cells to doxorubicin. It has also been shown that this combination induces less resistance than doxorubicin alone.

Transdrug doxorubicin circumvented resistance of breast cancer cells overexpressing P-gp, as well as multidrug resistance-associated protein (MRP1). It overcame MDR in breast cancer cells overexpressing MRP1. Treatment of MCF7/VP cells with Transdrug doxorubicin bypassed resistance, accompanied by an increase of nuclear accumulation of doxorubicin, as well as a decrease of drug efflux (Aouali N, etal, AACR02, Abs. 1309:263).

Editor's note:
The next three issues of FUTURE ONCOLOGY will cover epidemiology, molecular markers, current treatment approaches, and novel agents in development for the management of pancreatic cancer.

INDEX OF COMPANIES & INSTITUTIONS

— continued on back page

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