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ANTICANCER DRUGS AND MARKETS

UPDATE ON SPINDLE POISONS — PART III NOVEL AGENTS IN DEVELOPMENT

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In addition to taxanes, described in Part I, and the Vinca alkaloids, described in Part II of this series on spindle poisons, numerous other compounds with antimitotic mechanisms have been identified with many currently in preclinical and clinical evaluation. Most of the compounds described in this series interact with tubulin and affect the status of microtubules. Others, like the kinesin inhibitors interfere wih the normal processes of cell diversion.

Spindle poisons were originally isolated/ extracted from natural sources such as plants, marine organisms and bacteria. Because many of these sources are rare in nature and extracting processes are cumbersome and of low productivity, synthetic and semisynthetic approaches were attempted that often succeeded, as in the case of the taxanes, to make available sufficient drug for a commercially viable operation. However, in many cases synthetic approaches proved too complex to be practical. Recently, modern drug development techniques have created new families of small molecule antimitotics discovered by high-throughput screening using tubulin as the specific target.

All compounds that interact with tubulin and microtubules affect one or more stages of polymerization. Therefore, by measuring polymerization very accurately, one can identify compounds that interact with tubulin or microtubules. Microtubules are polar polymers composed of 13 protofilaments arranged in a cylindrical fashion. These protofilaments are composed of polymerized tubulin subunits. It is established that there are three defined stages of tubulin polymerization, nucleation, elongation and steady state. However, tubulin polymerization is a complex process that has not been fully elucidated.

Cells organize microtubules into complex structures like the mitotic spindle via the centrosome, a unique organelle that nucleates microtubule polymerization from free subunits. However, how this is accomplished still remains to be described. Because cancer cells often have aberrant centrosome numbers, it is possible that there is a relationship between such abnormalities and the generation of cells with aneuploid numbers of chromosomes.

Microtubules act as directional tracks for the many motor proteins that move along them, transporting chemicals, vesicles and organelles and make up the spindle that segregates chromosomes in mitosis and meiosis.

To date, seven cytoskeletal proteins have been identified belonging to the tubulin superfamily, α–, β–, γ–, δ–, ε–, ζ–, and η-tubulin, as well as various isotypes and mutations; β-tubulin is the cellular target for a variety of microtubule interacting agents including the taxanes, epothilones, and Vinca alkaloids. In humans, the building blocks of microtubules are α- and β-tubulin heterodimers. Most eukaryotic cells can express multiple isotypes of αβ-tubulin.

Using PCR techniques, investigators identified 6 α-tubulin and 6 β-tubulin isotypes in human cells. Although the significance of this diversity is not readily apparent, recent data indicate that particular αβ-tubulin isotypes, both genome-encoded and/or derived by post-translational modification, can directly influence microtubule structure and function, validating the multitubulin hypothesis put forth over 25 years ago. Numerous αβ-tubulin mutations are also being identified. At Albert Einstein College of Medicine (Bronx, NY), investigators found point mutations in α- or β-tubulin in a series of paclitaxel- and epothilone B-resistant cell lines, and also reported the existence of a seventh human α-tubulin equivalent to the mouse α-6 tubulin isotype (Verdier-Pinard P, etal, AACR02, Abs. 3907:788).

Investigators are still seeking to understand the role of the various members of the tubulin superfamily. It was recently established that one other member, γ-tubulin, plays a role in the nucleation of microtubule assembly. The γ-tubulin complex is a very large, ring-shaped structure containing 5 proteins in addition to γ-tubulin. Cloned proteins associated with γ-tubulin are localized to the centrosome and are critical for initiation, or nucleation, of αβ-tubulin heterodimers into microtubule polymers.

Although less is known about the functions of the other tubulins, current evidence suggests that δ–, ε–, ζ– and η-tubulin all have functions associated with the centriole or basal body of eukaryotic cells and organisms (McKean PG, etal, J Cell Sci, Aug 2001;114(Pt 15):2723-33, and Dutcher SK, Curr Opin Cell Biol, Feb 2001;13(1):49-54).

Localization of δ-tubulin and ε-tubulin to the centrosome is independent of microtubules, and the patterns of localization are distinct from each other and from that of γ-tubulin; δ-tubulin is found in association with the centrioles, whereas ε-tubulin localizes to the pericentriolar material. It appears that ε-tubulin exhibits a cell-cycle-specific pattern of localization. First it associates with only the older of the centrosomes in a newly duplicated pair and, later, with both centrosomes. In this manner it distinguishes the old centrosome from the new at the level of the pericentriolar material, indicating that there may be a centrosomal maturation event that is marked by the recruitment of ε-tubulin (Chang P and Stearns T, Nat Cell Biol, Jan 2000;2(1):30-5).

Categorizing spindle poisons by structure and/or function has not been easy. These compounds are generally defined by their effect on microtubules. However, although spindle poisons interfere with tubulin assemblies, their chemical structures and/or pharmacological profiles are dramatically different. Members of each class have been subjected to structural studies seeking to define their bioactive conformation at the paclitaxel pocket in β-tubulin and microtubules. Common molecular features among

the compounds are being sought for the purpose of identifying, by prediction, a new generation of antitumor agents with greater potency, reduced side effects, and lower multidrug resistance (MDR) characteristics.

There are several classes of naturally occurring and designed compounds that conform to the definition of a spindle poison, including:

- agents that stabilize the microtubule lattice such as the taxanes, epothilones, eleutherobins, discodermolide, laulimalide, and FR182877
- Vinca alkaloid-site interacting agents that form alternate lattice contacts and polymers at microtubule ends thus destabilizing microtubule dynamics (see FO pp 1457-82)
- colchicine-site binders
- kinesins
- other antimitotics

Efforts to identify novel spindle poisons are ongoing on many fronts. Originally, most spindle poisons were derived from nature. Subsequently, new families of small molecules were also identified by high-throughput screening directed against tubulin as the specific target, or created by combinatorial, or at least automatized chemistry.

Researchers at Cytoskeleton (Denver, CO), funded by an SBIR grand, from the National Institute of Health (NIH) have devised an assay suitable for random screening upward of 100,000 compounds monthly for tubulin ligands. This assay measures tubulin polymerization by detecting an increase in optical density over time (Davis A and Middleton KM, AACR99, Abs. 2690:406-7). Cytoskeleton specializes in the manufacture of tubulin for R&D applications.

A list of novel spindle poisons in clinical or preclinical development are listed and described in Exhibit 1. Taxanes and Vinca alkaloids were described in Part I and Part II of this article, respectively.

NOVEL MICROTUBULE STABILIZING COMPOUNDS OBTAINED FROM NATURAL SOURCES-EXTRACTS, AND SEMISYNTHETIC AND SYNTHETIC ANALOGS

A diverse group of natural compounds that interfere with microtubule dynamics have been isolated from natural sources (plants, marine organisms, bacteria). One of the most fertile sources of anticancer agents are marine organisms. Several ongoing major grants are looking for antineoplastic agents in marine organisms. A 23-year project funded by the National Cancer Institute (NCI; Bethesda, MD) is ongoing at the University of Utah (Salt Lake City, UT), under Chris Ireland, PhD. The primary aim of this program is to identify new structural classes of natural products produced by marine organisms, evaluate their potential as cancer chemotherapy agents, and continue studies directed at development of new screening protocols for discovery of antitumor agents from natural sources.

Another program, funded by the NCI at the University of California Santa Cruz under the leadership of Phillip Crews, PhD, is attempting to identify novel natural cytotoxic products from marine sponges. The overall aim of this research is to obtain new marine natural products with bioactivity against solid tumors, especially colon, breast, prostate, and lung cancer. The work is being carried out through a collaboration with the Experimental Therapeutics Investigators at Henry Ford Cancer Center (Detroit, MI).

In yet another program, the Division of Biomedical Marine Research (DBMR) at Harbor Branch Oceanographic Institution (HBOI; Fort Pierce, FL) teamed up with the University of Minnesota (Minneapolis, MN), Oregon State University (Cowallis, OR), the University of California Santa Cruz, and Novartis Pharmaceutical, on project funded by the NCI, to discover and develop novel anticancer agents using genetic material from marine microorganisms. The focus of this $4.9 million, 4.5-year grant is the discovery of new compounds with anticancer activity which act through nontraditional biochemical or molecular mechanisms. Investigators are drawing upon developing technologies in the field of microbial genomics, which integrates biologic, chemical, engineering and computer sciences.

DBMR has a highly diverse collection of marine microorganisms from deep-water marine invertebrates such as sponges and cnidarians (sea fans). Dr. Amy Wright and Dr. Peter McCarthy at HBOI isolate DNA from actinomycetes (filamentous bacteria) which is then inserted into a genetically engineered actinomycete using technology developed by Dr. David Sherman at the Biological Process Technology Institute at the University of Minnesota. The resulting clones are grown by Dr. McCarthy and his group and tested for anticancer properties at Novartis' labs. Dr. Wright and her team are responsible for the isolation and characterization of new chemical compounds discovered through this screening process.

The Center for Marine Biotechnology and Biomedicine (CMBB), located at the Scripps Institution of Oceanography (La Jolla, CA), on the campus of the University of California San Diego (UCSD), is another entity dedicated to the exploration of the novel and diverse marine resources. The focus of research of the CNBB is marine biomedicine and marine drug discovery, with an emphasis on cancer and infectious and inflammatory diseases.

Another institution with a successful track record in isolating spindle poisons from natural sources is the Cancer Research Institute (CRI) at Arizona State University (Tempe, AZ), under the direction of Dr. G. Robert Pettit. Promising novel spindle poisons discovered at ASU-CRI from a variety of marine animals and terrestrial plants, include dolastatin 10, combretastatin A4, and spongistatin.

Use of natural products as a source of new drugs is, however, hampered by the restricted availability of such resources. This is being overcome by semisynthesis and, sometime, total synthesis of some of these compounds despite their complicated chemical structures. Also, effectiveness of first generation of spindle poisons has been adversely impacted by the development of drug resistance. Currently, considerable efforts are directed in the search and synthesis of new compounds, such as small molecule tubulin inhibitors with more favorable therapeutic profiles.

Colchicine-Site Binders

Colchicine itself remains a lead in the search for tubulin-interacting agents. In addition to natural products, rationally designed drugs acting as colchicine-site binders are also being evaluated, as described in a later section of this article.

Curacin A, curacin B, and curazole, isomers of a cytotoxic compound, have been isolated and purified from a marine filamentous cyanobacterium (blue green alga), *Lyngbya majuscula* (*Oscillatoriaceae*), by William H. Gerwick, Philip J. Proteau, and Dale G. Nagle of Oregon State University.

Cyanobacteria are an ancient and diverse group of photosynthetic microorganisms inhabiting various different and often extreme environments. A high degree of biological adaptation has enabled these organisms to thrive and compete effectively in nature. In addition to curacin A, *Lyngbya majuscula* produces several other promising antifungal and cytotoxic agents, including laxaphycin A and B (Burja AM, etal, J Microbiol Methods, Feb 2002;48(2-3):207-19).

Curacins exhibit substantial activity particularly against proliferating cells and are believed to function as antimitotic agents. The cytotoxic profiles of curacins A and B and curazole share certain similarities with other antimitotic agents, such as the Vinca alkaloids and taxanes. Curacin A binds to the colchicine binding site on microtubules thereby inhibiting their polymerization and exerting a powerful cytostatic action.

Consistent with the herbicidal activity of conventional antimitotic compounds, the curacins also exhibit herbicidal properties. The effectiveness of curacins A and B and curazole in reducing a population of arthropods, such as brine shrimp, indicate that these compounds have general utility as agents for reducing populations of arthropods such as insects (Wipf P, etal, J Med Chem 2002 Apr 25;45(9):1901-17).

The development of the curacins as drugs is problematic because of their instability and poor water solubility. However, Peter Wipf, PhD, and colleagues at the University of Pittsburgh, using combinatorial chemistry, have created stabilized and more water-soluble curacin-related compounds, whose efficacy is currently being evaluated *in vivo*. These compounds have been patented and are available for licensing.

Combretastatins

Combretastatins are naturally occurring tubulin-binding compounds identified and isolated from the South African tree *Combretum caffrum*. They are selectively angiotoxic to tumor vasculature.

AVE8062, under development by Aventis Pharma (Vitry-Sur-Seine, France), is a synthetic water-soluble combretastatin derivative that inhibits tubulin polymerization. In addition to being cytotoxic to tumor cells, it is also toxic to proliferating endothelial cells, thus reducing tumor blood flow *in vivo*. In July 2001, Aventis obtained a worldwide license from Ajinomoto (Tokyo, Japan), to develop, manufacture and market AVE8062 (previously known as AC-7700) in exchange for certain milestone payments and, ultimately, royalty payments to Ajinomoto.

AVE8062 produced curative effects against advanced tumor cells in mice and prolonged survival (Nihei Y, etal, AACR98 Abs. 1143:167). When the antivascular effects of the tubulin binding agents colchicine, combrestatin-A4, and vinblastine were compared with AC-7700, the latter strongly suppressed growth of colon 26 tumor cells in a dose-dependent manner and reduced tumor perfusion with a close correlation between these two effects. Although the maximum tolerated dose (MTD) of colchicine and combrestatin-A4 also induced hemorrhagic tumor tissue necrosis, sporadic intact spots were left in the tumor tissue with no or marginal suppression of tumor perfusion and growth. Also, although vinblastine at MTD strongly inhibited tumor growth, only a marginal effect was seen against tumor perfusion. Histopathologic analysis of colon26 tumors showed that vinblastine increased the population of tumor cells in the mitotic phase (Nihei Y, etal, AACR98, Abs. 324:47).

Combretastatin A-4 prodrug (CA4P), under development by OXiGENE (Watertown, MA), is a poorly soluble tubulin-binding compound (combrestatin A-4 disodium phosphate or CA4DP) that is a selective inhibitor of endothelial cell proliferation, acting by interrupting microtubule assembly. CA4P induces extensive tumor cell loss through direct cell killing, and also by the induction of intratumoral vascular shutdown. CA4P acts as a tumor-selective angiotoxic by attacking proliferating vascular endothelial cells with significantly amplified phosphatase activity as compared to quiescent vascular endothelial cells. CA4P is effective at doses that do not harm normal cells.

OXiGENE obtained an exclusive, worldwide license for the commercial rights to the combretastatin technology, including the lead compound CA4P, from Arizona State University, in August 1999. In June 2002, OXiGENE inlicensed patent rights to an enhanced formulation of CA4P as well as other pending patents from Bristol-Myers Squibb (BMS). This exclusive agreement with BMS gives OXiGENE the worldwide rights to manufacture and commercialize products based on this enhanced CA4P formulation, whose advantages include improved stability and longer shelf life. BMS developed this formulation during

Exhibit 1
Novel Spindle Poisons and Formulations

Developer ❑ Affiliate(s)	Generic Name ❑ Number ❑ Brand Name	Description ❑ Administration Route	Status ❑ Indication(s)
Abbott Laboratories	A-293620/A-318315	Small molecule antimitotics that bind to the colchicine site of tubulin; inhibitor of tubulin polymerization ❑ PO	Preclin (ongoing 7/02)➢USA ❑ solid tumors
Abbott Laboratories	A-289099	Indole-oxazoline derivative with antimitotic activity through the disruption of microtubules ❑ PO	Preclin (ongoing 7/02)➢USA ❑ solid tumors
Abgenix ❑ ImmunoGen, Japan Tobacco		Fully human antibodies generated with XenoMouse technology, combined with maytansinoid tumor-activated prodrug (TAP) technology ❑ injection	Research (ongoing 5/02)➢USA ❑ cancer
Angiogene ❑ MediciNova	ANG 600 Series (AGN615)	Benzimidazole carbamate analogs; angiotoxic agents ❑ IV	Preclin (ongoing 8/02)➢USA ❑ solid tumors
Arizona State University	Dolastatin 10 ❑ NSC 376128	A peptide isolated from the shell-less marine mollusk, *Dolabella auricularia*, an Indian Ocean sea hare; inhibits microtubule assembly and tubulin polymerization ❑ IV	Phase II (completed 02)➢USA ❑ advanced renal cell carcinoma; phase II (begin 10/98)➢USA ❑ metastatic prostate cancer; phase II (closed 1/02)➢USA ❑ advanced breast cancer; phase II (begin 4/00) ➢USA ❑ lymphoma and chronic lymphocytic leukemia (CLL); phase I (closed 11/01)➢USA ❑ acute leukemia, CLL and myelodysplastic syndrome (MDS)
AstraZeneca ❑ Angiogene Pharmaceuticals	ZD6126 (formerly ANG453)	Colchicine prodrug that disrupts the tubulin cytoskeleton of neoendothelial cells, causing selective destruction of tumor vasculature and producing massive tumor necrosis ❑ IV	Phase I (ongoing 7/02)➢USA, Europe, Phase I (begin 1/02, ongoing 7/02)➢Europe (UK) ❑ refractory solid tumors
Aventis Pharma ❑ Ajinomoto	AVE8062 (formerly AC-7700)	Combretastatin A4 (CS-A4) derivative; angiotoxic ❑ IV	Preclin (ongoing 8/02)➢Japan, Europe ❑ advanced solid tumors
Baxter Oncology (Asta Medica)	D-24851	A novel small molecule drug that acts like a tubulin interacting agent by destabilizing microtubules ❑ PO	Preclin (ongoing 7/02)➢USA, Europe (Germany) ❑ solid tumors
Baxter Oncology (Asta Medica)	D-64131	An acylindol that acts by arresting cell division in the G2/M phase of the cell cycle and inhibiting tubulin polymerisation by destablization ❑ PO	Preclin (ongoing 7/02)➢USA, Europe (Germany) ❑ solid tumors
Boehringer Ingelheim ❑ ImmunoGen	Bivatuzumab mertansine ❑ BIWI1	Anti-CD44v6 antibody combined with maytansinoid (DM1) tumor-activated prodrug (TAP) ❑ IV	Preclin (begin 11/01, ongoing 7/02)➢USA, Europe ❑ solid tumors, squamous cell carcinoma
Bristol-Myers Squibb ❑ Gesellschaft für Biotechnologische Forschung (GBF)	Epothilone B, desoxyepothilone B, aza-EpoB ❑ BMS-247550, NSC 710428, NSC 710428D	Epothilone B, is a semisynthetic analog of a spindle poison derived from the myxobacterium bacterial strain, *Sorangium cellulosum* ❑ IV	Phase I (begin 9/00, ongoing ongoing 3/02)➢USA; phase I (begin 1/02, ongoing 3/02)➢USA (combination); phase I (begin 10/01, ongoing 1/02) ➢USA (pediatric); phase I/II (begin 8/01, ongoing 5/02)➢USA ❑ advanced, refractory solid tumors or lymphoma or refractory pediatric neoplasms; phase I/II (begin 7/01, ongoing 3/02)➢USA (combination), phase II (begin 6/01, ongoing 2/02) ➢USA ❑ androgen-independent, metastatic, hormone-refractory prostate cancer; phase II (begin 5/01)➢USA ❑ advanced bladder cancer; phase II (begin 7/01, ongoing

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			3/02)➢USA ❑ advanced pancreatic adenocarcinoma; phase II (begin 6/01, closed 5/02)➢USA ❑ advanced or metastatic soft tissue sarcoma; phase II (begin 10/01, ongoing 3/02)➢USA ❑ hepatobiliary cancer; phase II (begin 9/01, ongoing 4/02) ➢USA ❑ metastatic colorectal cancer; phase II (begin 2/01, closed 4/02, ongoing 5/02)➢USA ❑ recurrent, metastatic gastric cancer; phase II (begin 2/01)➢USA ❑ metastatic breast cancer; phase II (begin 7/02)➢USA; phase II (ongoing 8/02)➢USA ❑ advanced, refractory ovarian cancer, or primary peritoneal cancer; phase II (begin 2/02)➢USA ❑ kidney cancer; phase II (approved 4/02)➢USA ❑ metastatic or recurrent head and neck cancer; phase II (begin 2/02, ongoing 8/02)➢USA ❑ Stage IV malignant melanoma; phase II (begin 5/02)➢USA ❑ refractory nsclc
Bristol-Myers Squibb ❑ Gesellschaft für Biotechnologische Forschung (GBF)	BMS-310705	Water-soluble and chemically stable semisynthetic epothilone B with potent parenteral and oral antitumor activity against models of taxane-sensitive and resistant human tumors *in vivo* ❑ PO, IV	Phase I (ongoing 5/02)➢Europe (Switzerland) ❑ refractory solid tumors; phase I (ongoing 5/02) ➢USA ❑ advanced cancer
Cell Pathways	CP461 and CP-248	Selective apoptotic antineoplastic drugs (SAAND) that inhibit a cGMP phosphodiesterase and also interfere with the normal function of the spindle apparatus during cell division ❑ PO	Phase II (begin 7/01, ongoing 9/02) ➢USA ❑ advanced or metastatic renal cell carcinoma; phase II (begin 3/02, ongoing 9/02)➢USA ❑ relapsed or refractory chronic lymphocytic leukemia (CLL); phase II (begin 8/01, ongoing 9/02)➢USA ❑ metastatic prostate cancer
Eisai ❑ Abbott Laboratories	ABT-751, E7010	Orally-active sulfonamide that inhibits tubulin polymerization ❑ PO	Phase I (begin 3/02)➢USA ❑ pediatric solid tumors; phase I (completed 98)➢Japan ❑ refractory solid tumors
Eisai Research Institute	E7389, NSC-707389 (formerly ER-086526)	Synthetic analog of the marine natural product halichondrin B ❑ IV	Preclin (ongoing 5/02)➢USA, Japan ❑ cancer
Eli Lilly ❑ U Hawaii	LY355703	Synthetic analog of naturally occurring cryptophycin 52 (C-52) isolated from cyanobacteria (blue algae); potent antiproliferative agent active against cancer cells exhibiting an MDR phenotype ❑ IV	Phase I (ongoing 3/02)➢USA ❑ advanced solid tumors; phase II (ongoing 3/02)➢USA ❑ hormone-insensitive, metastatic prostate cancer; phase II (discontinued 01)➢ Europe (Germany) ❑ first-line or salvage treatment of advanced (Stage IIIb) or metastatic (Stage IV) nsclc
EntreMed ❑ Bristol-Myers Squibb, Children's Hospital at Harvard Medical School, Aventis Pharma, Tetrionics, U Iowa	2-methoxyestradiol (2-ME2), 2ME2 ❑ Panzem	Nonestrogenic endogenous metabolite of estradiol ❑ PO	Phase I (ongoing 5/02)➢USA ❑ advanced breast cancer; phase I (ongoing 5/02)➢USA (combination) ❑ metastatic, refractory breast cancer; phase II (ongoing 5/02) ➢USA ❑ stable or relapsed multiple myeloma; phase II (ongoing 5/02) ➢USA ❑ hormone-refractory prostate cancer; phase I (begin 9/01, ongoing 5/02)➢USA ❑ advanced solid tumors
Fujisawa Pharmaceutical	FR182877 (formerly WS9885B)	Synthetic analog of a bacterial metabolite that exhibits similar activity as paclitaxel ❑ IV	Preclin (ongoing 8/02)➢USA, Japan ❑ solid tumors

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Genentech ❑ ImmunoGen, Industrial Research	Trastuzumab-DM1	Immunoconjugate of trastuzumab and DM1, an inactive prodrug until it binds to cell-surface HER2, is internalized, and releases active DM1 intracellularly ❑ injection	Preclin (ongoing 6/02)➢USA ❑ solid tumors
GlaxoSmithKline (GSK) ❑ Cytokinetics	SB-715992 (CK0238273)	Novel small molecule inhibitor of kinesin spindle protein (KSP); antimitotic expected to be more specific than other spindle poisons and, therefore, cause fewer side effects than existing therapeutics ❑ PO	Phase I (begin 8/02)➢USA ❑ solid tumors
Hoffmann-La Roche	R440 (previously Ro 31-7453)	Novel bisindolylmaleimide; cell-cycle inhibitor and apoptosis inducer ❑ PO	Phase I (closed 1/02)➢USA; phase I (ongoing 02)➢USA, Korea (combination) ❑ solid tumors, advanced nsclc; phase II (ongoing 7/02)➢USA ❑ advanced breast cancer; phase II (ongoing 7/02)➢USA ❑ advanced nsclc; phase II (begin 9/01, completed 9/02)➢USA ❑ refractory, recurrent or metastatic colorectal cancer; phase I (ongoing 02)➢USA ❑ acute myeloid leukemia (AML)
Ilex Oncology ❑ BASF Pharma, Arizona State U	Cemadotin (first-generation; formerly LU 103793, NSC D-669356) ❑ ILX-651 (second-generation; formerly LU 223651, BSF 223651)	Water-soluble, synthetic, pentapeptide analog of dolastatin 15, a natural cytotoxic depsipeptide derived from the shell-less marine mollusk, *Dolabella auricularia*, an Indian Ocean sea hare; inhibits microtubule assembly and tubulin polymerization in a fashion analogous to taxanes ❑ PO, IV	Phase I (ongoing 8/01)➢USA (PO), phase I (begin 6/01, ongoing 5/02)➢USA (IV) ❑ refractory solid tumors
ImmunoGen ❑ Takeda Chemical Industries, British Biotech, GTC Biotherapeutics	huN901-DM1/ BB-10901TAP	Anti-CD56 humanized MAb huN901 conjugated to maytansinoid compound DM1 ❑ IV	Phase I/II (begin 5/01, ongoing 5/02) ➢USA ❑ relapsed or refractory small-cell lung cancer (sclc); phase I (begin 8/02)➢Europe ❑ sclc
ImmunoGen ❑ GlaxoSmithKline, Pharmacia, Takeda Chemical Industries, Industrial Research	huC242-DM1 ❑ SB-408075	Antimucin humanized MAb huC242 conjugated to maytansinoid compound DM1; tumor-activated prodrug ❑ injection	Phase I/II (begin 12/99, ongoing 6/02)➢USA ❑ advanced, refractory, solid tumors, pancreatic cancer, nsclc, colorectal cancer
ImmunoGen	My9-6-DM1	Tumor-activated prodrug produced by linking MAb My9-6, which targets myeloid leukemia cells, with DM1 ❑ IV	Preclin (ongoing 7/02)➢USA ❑ acute myelogenous leukemia (AML)
Kosan Biosciences ❑ Memorial Sloan-Kettering Institute for Cancer Research, Stanford U, Harvard College	Epothilone D (EpoD) or desoxyepothilone B (dEpoB) ❑ KOS-862	Polyketide natural product belonging to a novel class that is structurally distinct from the other epothilones; inhibits cancer cell proliferation by a mechanism similar to that of paclitaxel but is effective against paclitaxel-resistant cells ❑ IV	Phase I (begin 10/01, ongoing 7/02)➢USA ❑ refractory solid tumors
Millennium Pharmaceuticals ❑ ImmunoGen, BZL Biologics	MLN591DM1, Anti-PSMA-DM1	Immunoconjugate consisiting of MLN591 MAb directed towards the extracellular domain of prostate-specific membrane antigen (PSMA), and Tumor-Activated Prodrug (TAP)-DM1 component ❑ IV	Preclin (begin 3/01, ongoing 7/02)➢USA ❑ prostate cancer
Nereus Pharmaceuticals ❑ U California San Diego	Halimide ❑ NPI-2352 and NPI-2358 (NPI-2350, NPI-2350a)	Novel aromatic analogs of an alkaloid natural product, isolated from *Aspergillus sp.,* a marine fungus; microtubule inhibitor ❑ IV	Preclin (5/02)➢USA ❑ cancer

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Novartis Pharmaceuticals	Discodermolide	Inhibits cancer cells by the same mechanism as paclitaxel but also is effective against paclitaxel-resistant tumors, is more potent than paclitaxel in some cancer cell lines, and may act synergistically with paclitaxel ❑ IV	Preclin (ongoing 1/02)➢USA ❑ solid tumors
Novartis Pharmaceuticals ❑ Scripps Research Institute, U Magdeburg	Epothilone B, EpoB ❑ EPO906	Synthetic analog of a spindle poison derived from myxobacterium *Sorangium cellulosum* ❑ IV	Phase IIa (ongoing 7/02)➢USA ❑ advanced breast cancer, colorectal, ovarian, and prostate cancer, and malignant melanoma; phase IIa (ongoing 7/02)➢USA, Europe (France) ❑ advanced kidney cancer
NS Pharma	HMN-214	Oral antimicrotubular agent with polo-like and cyclin-dependent kinase inhibitory activities; stilbazole derivative; prodrug of HMN-176 ❑ PO	Phase I (ongoing 5/02)➢USA ❑ advanced solid tumors
OXiGENE ❑ Arizona State U	Oxi-4503	Diphosphate prodrug form of combretastatin A1 ❑ IV	Preclin (ongoing 8/02)➢USA ❑ solid tumors
OXiGENE ❑ Arizona State U	Combretastatin A-4 disodium phosphate (CA4DP), combretastatin A-4 prodrug (CA4P)	Naturally occurring tubulin-binding compounds identified and isolated from the South African tree *Combretum caffrum*; angiotoxic that selectively attacks tumor vasculature ❑ injection	Phase I (begin 11/00, completed 01)➢USA, Europe (UK); phase IIb (ongoing 7/02); phase Ib (begin 5/02)➢USA (combination) ❑ advanced solid tumors
Salmedix ❑ U California San Diego	SDX-103	Water-soluble phosphate prodrug of indanocine that retains similar antiproliferative and proapoptotic activities of indanocine when tested in primary CLL cells and in tumor cell lines ❑ IV	Preclin (ongoing 9/02) USA ❑ B-cell leukemia
Schering AG ❑ metaGen Pharmaceuticals	ZK-EPO	Synthesized analog of natural epothilone with potent tumor growth inhibitory properties ❑ IV	Preclin (ongoing 7/02)➢Europe (Germany) ❑ solid tumors
SignalGene		2-methoxyestradiol (2ME2) analog ❑ PO	Preclin (ongoing 3/02)➢Canada ❑ solid tumors
Teikoku Hormone Manufacturing ❑ Daiichi Pharmaceutical	TZT-1027	Synthetic analog of dolastatin 10; inhibits microtubule assembly and tubulin polymerization ❑ intraperitoneal (IP), IV	Phase I (completed 02)➢Japan ❑ solid tumors; phase I (ongoing 5/02)➢Japan ❑ advanced nsclc
Tularik	T67 (previously T138067)	Pentafluorosulfonamidebenzene that binds specifically and irreversibly to beta tubulin thereby disrupting the process of cell replication and causing tumor shrinkage; active against multidrug resistant (MDR) tumors and capable of crossing the blood-brain barrier ❑ infusion	Phase I (completed 12/00)➢USA ❑ solid tumors; phase I (ongoing 5/00)➢USA ❑ MDR solid tumors; phase II (ongoing 4/01)➢USA, Europe (UK), Canada, Australia, Hong Kong, Taiwan ❑ refractory hepatocellular cancer, locally advanced or metastatic nsclc, breast and colorectal cancer and glioma; phase II (begin 10/00, ongoing 5/02)➢USA ❑ refractory, locally advanced or metastatic nsclc
Tularik	T607 (also known as T900607)	Analog of T67; targets tubulin and is active MDR tumors but does not cross the blood-brain barrier (BBB) ❑ bolus injection	Phase I (ongoing 4/01)➢USA, Canada, Europe (UK) ❑ solid tumors; phase II (begin 7/02)➢USA ❑ refractory non-Hodgkin's lymphoma (NHL), refractory ovarian cancer; phase II (begin 7/02)➢USA, China ❑ first-line treatment of liver cancer

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), August 2002.

the course of a 2-year research collaboration and licensing agreement with OXiGENE that was terminated in October 2001. This new agreement also provides for additional patent rights, including methods of administering CA4P in combination with other therapies such as chemotherapy, immunotherapy or radiation therapy, which were codeveloped by OXiGENE and BMS during the course of the collaboration.

In preclinical studies CA4P proved effective against a panel of malignant human B-lymphoid cell lines [pre-B acute lymphoblastic leukemia (Reh), chronic lymphocytic leukemia (WSU-CLL), diffuse large cell lymphoma (WSU-DLCL2) and Waldenstrom's macroglobulinemia (Nabha SM, etal, AACR00, Abs. 379:59), in experimentally allotransplanted hemangioendotheliomas (Bohle AS, etal, Int J Cancer, 15 Sep 2000;87(6):838-43), in mice with transplanted C3H mammary carcinoma and spontaneous tumors (Horsman, M.R, etal, AACR98, Abs 1142:167), and in experimental and human breast cancer models *in vivo* (Chaplin DJ, etal, AACR98, Abs 662:97-8), among others.

The most effective approach with CA4P appears to be in combination with cytotoxic drugs. CA4P was effective in an animal model of Kaposi's sarcoma (KS), either alone or in combination with vinblastine or cisplatin (Li L, etal, AACR00, Abs. 2769:435, and Li L, etal, Acta Oncol 2002;41(1):91-7), and in mice bearing KHT sarcoma, alone or in combination cisplatin (Siemann DW, AACR98, Abs 1895:277-8). CA4P also proved effective in combination with antibody targeted therapies. In colorectal xenograft models, CA4P alone caused selective shutdown of tumor vessels leading to hemorrhagic necrosis of all but an outer rim of well vascularized cells, but the tumors continued to grow. Radioimmunotherapy alone using a ^{131}I-labeled anti-CEA antibody, produced no cures, but when combined with CA4P, tumors were eradicated in 90% of mice (Pedley RB, etal, AACR00, Abs. 505:79).

When the action of CA4P and another vascular targeting agent, dimethylxanthenone acetic acid (DMXAA), was investigated in rodent sarcoma and human breast and ovarian cancer tumor models, treatment with either agent was found to damage existing neovasculature leading to a rapid vascular shutdown. Histologic evaluation showed morphologic damage to tumor cells within a few hours after drug exposure, followed by extensive central tumor necrosis and neoplastic cell death as a result of prolonged ischemia. When fixed doses of CA4P or DMXAA were combined with a range of doses of cisplatin or cyclophosphamide, tumor cell kill was increased 10- to 500-fold compared to that seen with chemotherapy alone. However, inclusion of the antivascular agent did not increase bone marrow stem-cell toxicity associated with these anticancer drugs (Siemann DW, etal, AACR00, Abs. 3344:525).

CA4P may also act as a radiosensitizer. Preclinical studies correlate the reduction of blood flow to tumors by CA4P (Landuyt W, etal, AACR99, Abs 464:70), using lower doses of radiation with the ability to induce efficacious tumor death by necrosis (Horsman MR, etal, AACR99, Abs 4225:641). Similar effects were observed in combination with hyperthermia (Eikesdal HP, Radiotherapy and Oncology 2001;60:147-154). Research studies have shown that nitric oxide (NO) partially protects tumors against CA4P. Systemic nitric oxide synthase (NOS) inhibition improves the tumor:normal tissue ratio of CA4P-induced vascular damage. The protective action of NO may result from an antineutrophil action (Tozer GM, etal, AACR00, Abs. 4127:649).

The clinical program with CA4P began in November 2000, when Bristol-Myers Squibb filed an IND with the FDA to initiate a phase I clinical trial of CA4P for treatment of advanced/refractory solid tumors. Subsequently, 3 phase I clinical trials were completed in the USA and the UK. Results from these trials indicate that treatment with CA4P results in a significant reduction of blood flow to existing solid tumors and in some instances in total tumor regression.

An open-label, dose-escalation, multicenter, phase I clinical trial (protocol IDs: CRC-PHASE-I/II-PH1/066; EU-98066), sponsored by the Cancer Research Campaign (CRC; London, UK) in the UK was completed in April 2001. The trial was undertaken to determine the toxicity profile, including dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of CA4P in patients with advanced solid tumors. Secondary objectives involve determination of the pharmacokinetics of CA4P, assessment of its effects on tumor blood flow using PET and MRI scanning techniques, and establishment of the dose at which these effects occur, recommendation of a dose for phase II evaluation based on tumor blood flow effect and MTD, and evaluation of possible antitumor effects of this regimen in these patients. Gordon John Sampson Rustin, MD, from the Mount Vernon Hospital (Northwood, England) was the Study Chair.

The drug was delivered by a 10-minute weekly infusion for 3 weeks followed by a week gap, with intra-patient dose escalation until 2 instances of Grade 2 toxicity occurred. In 32 patients treated with 163 infusions, the only toxicity related to the drug at doses up to 40 mg/m^2, was tumor pain and lymphopenia. DLT was reversible ataxia at 114 mg/m^2 and vasovagal syncope and motor neuropathy at 88 mg/m^2. Other drug related Grade 2 toxicities were pain in 10 patients (6 at the tumor site), fatigue in 7, visual disturbance in 3, hypotension in 2, hypertension in 3, dyspnea in 2, nausea in 1, vomiting in 2, and headache in 1; there was 1 fatal ischemia involving a patient with previously irradiated bowel. One patient at 68 mg/m^2, experienced a partial response (PR) in liver metastases of adrenocortical carcinoma after 12 infusions that was not maintained after a gap. CA4P was well tolerated in 11/13 patients at 52 mg/m^2 or 68 mg/m^2. Tumor blood flow reduction was reproducible at these doses (Rustin GJ, etal, ASCO01, Abs. 392: 99a).

A second open-label, dose-escalation, phase I clinical trial (Study IDs: CWRU-ILEX-1Y98; NCI-G99-1502; ILEX-1Y98; ILEX-CA4P101-A3) conducted at the Ireland Cancer Center (Cleveland, OH) under PI Scot C. Remick, MD, was completed in May 2001. This trial was designed to determine MDT of CA4P when administered IV at single doses over 10-60, minutes every 21 days, to patients with advanced solid tumors. Treatment was repeated every 3 weeks in the absence of unacceptable toxicity or disease progression. Cohorts of 3-6 patients are being treated with escalating doses of CA4P until MTD. Patients were followed at 3 weeks.

A total of 107 cycles of therapy were administered to 25 patients at 4 dose levels (18, 36, 60, 90 mg/m^2) as a 10-min infusion, and 60 mg/m^2 as a 60-min infusion at 3-week intervals. Pharmacokinetics revealed rapid dephosphorylation of CA4P to combretastatin A4, with a short plasma half-life of approximately 30 minutes. There were 4 episodes of DLT involving Grade 3 pulmonary toxicity (shortness of breath) and coronary vasospasm (during cycle 2) at 90 mg/m^2, and Grade 3 pulmonary toxicity and acute myocardial ischemia at 60 mg/m^2, both with the 10-minute administration schedule. There were no further cardiac sequelae with resolution of ischemic signs and symptoms. A variable symptom complex was identified across all dose levels several hours following infusion, including faint flush, nausea and vomiting (which appears dose-related), and 11 episodes of Grade 2 tumor pain. Tumor pain was a unique side effect, which occurred in 10% of cycles. There were 7 episodes of QTc interval prolongation, only 1 of which when recalculated using Bazzet's formula was >500 msec.

The side effect profile is suggestive of an agent with systemic vascular effects and devoid of other traditional cytotoxic effects such as alopecia, stomatitis, and myelosuppression. MR tumor perfusion studies demonstrated decreases in tumor blood flow. There was 1 complete response (CR) in a patient with anaplastic thyroid cancer who remained disease-free for at least 21 months after treatment. Prolonged intervals of disease-free survival were seen in 2 patients, one with colon cancer (19 months) and one with medullary thyroid (12 months). Based on this and other phase I trials, MTD is likely between 60-65 mg/m^2 (Dowlati A, etal, Cancer Res,15 Jun 2002;62(12):3408-16, Remick S, etal, AACR-NCI-EORTC01, Abs. 393, and Remick SC, etal, ASCO00, Abs. 697:180a).

In another phase I study, conducted in the USA, 17 patients with a range of refractory solid tumors and good performance status (PS) were treated with CA4P as a daily 10-minute IV infusion for 5 days, every 21 days, at doses escalated from 6 mg/m^2 to 56 mg/m^2. CA4P therapy was well tolerated with 2 patients experiencing Grade 1 thrombocytopenia; no significant nonhematologic toxicities were observed. Disease stabilized in 2 patients with renal cell carcinoma, lasting 4 cycles. Pharmacokinetic data (n=4) showed rapid conversion of CA4P to CA4 and to CA4 glucuronide. In addition to standard response criteria, serial MRI exams are being performed to assess changes in tumor blood flow (Stevenson JP, etal, AACR00, Abs. 3469:544).

In June 2002, OXiGENE initiated a phase Ib combination clinical trial of CA4P and carboplatin in patients with advanced solid tumors including thyroid cancer. The trial is being conducted at the University of Pennsylvania Cancer Center (Philadelphia, PA). The trial, being led by Peter O'Dwyer, MD, will enroll up to 35 patients to evaluate the safety and tolerability of this drug combination.

Oxi-4503, also under preclinical development by OXiGENE, is the diphosphate prodrug form of combretastatin A1 (CA1P), a water-soluble agent that has demonstrated antitumor and antivascular effects, as shown by researchers at the University of Bradford (West Yorkshire, UK) and Arizona State University (Holwell SE, etal, AACR00, Abs. 41:214).

Investigators at Gray Cancer Institute, Mount Vernon Hospital Northwood (Middlesex, UK) have synthesized and evaluated a number of combretastatins to identify novel analogs that possess single-agent activity. Antivascular and antitumor activities of the lead compound, Oxi-4503, were compared with those of CA4P in the murine breast adenocarcinoma CaNT. At a dose of 1 mg/kg, Oxi-4503 induced a >50% reduction in functional vascular volume, which increased to ≥80% with doses ≤50mg/kg. In contrast, CA4P induced approximately 40% vascular shutdown at 50 mg/kg, but had no measurable effect at 10 mg/kg. In addition to these vascular effects, Oxi-4503 at doses of ≤400 mg/kg induced significant retardation in the growth of established CaNT tumors while no significant growth retardation was obtained with single doses of up to 400 mg/kg CA4P. Therefore, it appears that Oxi-4503 exhibits more potent antivascular and antitumor effects than CA4P when used as a single agent (Hill SA, etal, Anticancer Res, May-Jun 2002;22(3):1453-8).

While CA4P blocks blood flow in all but the periphery of a tumor, Oxi-4503 appears to destroy blood vessels in all regions of the tumor, including the periphery. In preclinical studies *in vivo*, Oxi-4503 selectively targeted tumor endothelial cells and induced endothelial cell apoptosis within 3 hours after a single dose. As a consequence vascular permeability and blood retention ensued causing destruction of the blood vessel, and inducing massive necrosis of tumor cells (Sheng Y, etal, AACR02, Abs. 148:29).

Cryptophycins

Cryptophycins comprise a 16-membered family of macrolide antimitotic compounds exhibiting extremely potent cytotoxic activity (Eggen M and Georg GI, Med Res Rev, Mar 2002;22(2):85-101). Cryptophycins target tubulin and are associated with stabilization of microtubule dynamics, microtubule depolymerization at higher concentrations, and induction of bcl-2 phosphorylation that

deactivates bcl-2 leading to apoptosis more quickly and at considerably lower concentrations than other clinically useful compounds (Eggen M and Georg GI, Med Res Rev, Mar 2002;22(2):85-101).

Cryptophycins in development as antimitotic agents are synthetic derivatives of the desipeptide cryptophycins, originally isolated from the sponge *Dysidea arenaria* near Okinawa, but subsequently also isolated from the cyanobacterium (blue-green algae) *Nostoc sp*. Cryptophycins were originally discovered in 1991 by a team of scientists (Richard Moore, Greg Patterson, Marcus Tius, Susan Mooberry, and Thomas Hemscheidt) at the University of Hawaii (Honolulu, HI). Subsequently, completion of a synthetic route to cryptophycin 24, allowed sufficiently large quantities to be produced for comprehensive evaluation of their anticancer properties (White JD, etal, Tetrahedron Lett 1998;39, 8779-8782). Three other naturally occurring cryptophycins were subsequently synthesized, and cryptophycin analogs not found in nature were created with enhanced anticancer properties, among them cryptophycin 52 (Shih and Teicher, Curr Pharm, Dec 2001;7:1259-1276).

LY355703, under development by Eli Lilly, is a synthetic analog of naturally occurring cryptophycin 52 (C-52). Lilly signed a commercial research and licensing option agreement with the University of Hawaii for cryptophycin 52 in 1996. Wayne State University (Detroit, MI) collaborated with the University of Hawaii by performing preclinical animal trials on cryptophycins and jointly filed patents on these agents. In 2000, Eli Lilly paid a $1 million milestone upon initiation of clinical trial with cryptophycin.

In the nanomolar range, LY355703 is a potent inhibitor of microtubule polymerization and, at picomolar concentrations, inhibits cell proliferation at mitosis by perturbing microtubule dynamic instability. LY355703 blocks cell cycle at G2/M, causes accumulation of cells at metaphase, and kills the cells by apoptosis. C-52 binds strongly to tubulin. Results suggest that the antitumor activity of C-52 is attributed to a potent suppression of spindle microtubule dynamics (Panda D, etal, AACR98, Abs. 1127:164).

Cryptophycin 52 has potent antimitotic, antiproliferative and cytotoxic activity *in vitro* in human tumor cell models, including several MDR lines, murine solid tumors and human tumor xenografts. It is significantly more potent and less sensitive to multidrug resistance (MDR) mechanisms than other antimitotic antitumor agents currently used in cancer therapy (Wagner MM, etal. Cancer Chemother Pharmacol 1999;43(2):115-25). Treatment of animals bearing intraperitoneal human OVCAR-2 ovarian carcinoma with cryptophycin 52 resulted in survival times that were greater than those achieved with docetaxel or paclitaxel.

Investigators at Columbia University (New York, NY) examined the effects of C-52 on 3 prostate cancer cell lines. C-52, at picomolar concentrations, decreased cell viability in LNCaP (androgen-dependent, wild type p53) and DU-145 (androgen-independent, mutant p53) cells by inducing G2/M phase arrest and subsequent apoptosis over 48 hours. The third cell line, PC-3 (androgen-independent, null p53), was less responsive to these effects of C-52. Results demonstrated that C-52 is a potent antiproliferative and apoptosis inducer of prostate cancer cells. Furthermore, this agent modulates multiple apoptosis-associated proteins and induces death via caspase-dependent and caspase-independent pathways (Drew L, etal, AACR01, Abs. 3454:642).

In a phase I clinical trial, conducted at the University of Pennsylvania Cancer Center, LY355703 was administered as a 2-hour IV infusion, on days 1 and 8, repeated every 21 days, in 25 patients with advanced solid tumors. Doses were escalated from 0.1 to 2.22 mg/m^2. Neurologic toxicity was found to be dose limiting at 1.84 and 2.22 mg/m^2. Among 4 patients treated at these doses, 2 experienced Grade 4 constipation/ileus, one with severe myalgias, and one Grade 3 motor neuropathy, all reversible. The 1.5 mg/m^2 dose level was well tolerated. An amended twice-weekly schedule was pursued in 11 patients in an attempt to improve dose intensity and avoid dose-limiting neurotoxicity. Doses of >0.75 mg/m^2 on a day 1, 4, 8, and 11 schedule every 21 days were not tolerated as a result of nausea/constipation, suggesting that LY335703 toxicity is not schedule-dependent and is related to cumulative dose. A patient with non-small cell lung cancer (nsclc), refractory to taxane-based chemotherapy, experienced a PR lasting 4 months, and disease stabilized in 5 patients, for ≥3 months. LY355703 at a dose of 1.5 mg/m^2 is recommended for phase II evaluation on a day 1 and 8 schedule. Twice weekly dosing did not result in improvement in dose intensity or tolerability (Stevenson JP, etal, Clin Cancer Res, Aug 2002;8(8):2524-9).

In a preliminary report from this trial, among 10 treated patients, other toxic effects included Grade 2 local skin reaction (10%), severe arthralgia/myalgia (10%), Grade 1/2 transient peripheral neuropathy (30%), and Grade 1 neutropenia (10%). One patient experienced a Grade 2 hypersensitivity reaction in cycle 2 and was successfully retreated with premedication. Disease stabilized for 6 months in a patient with renal cell cancer (Stevenson JP, etal, AACR99, Abs. 609:92).

Although at first glance it appeared that LY355703 might be effective in the treatment of nsclc, further evaluation did not produce favorable results. Salvage therapy with C-52 in platinum-treated nsclc was administered in patients exposed to at least one (n=25) and no more than two prior therapies (at least one with a platinum agent) for nsclc. Among prior treatments, 14 patients were administered carboplatin/paclitaxel, 4 carboplatin/gemcitabine, and 8 other regimens. C52 was initially administered at 1.5 mg/m^2, on days 1 and 8, every 21 days. Two stages of accrual were planned with the goal of determining an objective response rate of >10%. All 26 patients accrued by the Greenebaum Cancer Center (Baltimore, MD), University

of California Davis and University of Southern California (USC; Los Angeles, CA) were evaluable for toxicity, response and survival; there was one early death. A total of 78 cycles of C-52 were administered, with 10 patients treated with ≥4 cycles. Neurotoxicity in the form of Grade 3/4 pain, neuroconstipation and paresthesias was noted in 5 of the first 12 patients, prompting a reduction of the initial dose to 1.125 mg/m^2. Myelotoxicity was minimal with only 1 Grade 3 thrombocytopenia noted and no >Grade 3 neutropenia or anemia. One patient experienced Grade 4 pneumonia probably secondary to disease progression rather than C-52 treatment. MST was 4.9 months. Median time-to-progression (TTP) was 1.4 months.

Although treatment with C-52 was associated with disease stabilization in a substantial proportion of patients, the trial was terminated because no measurable response was seen in this setting. Also, neurotoxicity of C-52 at the initially recommended dose of 1.5 mg/m^2 was unacceptable. However, lack of myelotoxicity and evidence of disease stabilization at the reduced dose indicates that it may be desirable to explore alternative schedules (Hausner PF, etal, ASCO02, Abs. 2688:2176).

A preliminary analysis of a phase II clinical trial of LY355703 as first-line therapy for Stage IIIb or IV nsclc, conducted by Gesine Groth, MD, at Krankenhaus Grosshansdorf (Heidelberg, Germany), also yielded disappointing results. None of 14 patients treated with LY355703 (1.5 mg/m^2) IV over two hours on days 1 and 8 of a 21-day cycle, and with prophylactic therapy to help avoid drug hypersensitivity, experienced a tumor response. The trial was stopped before enrollment of the initial target of 18 patients and, upon treatment response, of another 22 patients. The drug failed to arrest tumor growth and also caused a range of toxicities. Nonhematologic toxicity was severe; one patient had to be hospitalized for Grade 4 diar-rhea and eventually died of lactic acidosis, which may have been drug-related. Two patients developed drug hypersensitivity despite prophylaxis. Other problems included fatigue, constipation, alopecia, myalgia, arthralgia, hypertension, neuropathy, and increased tumor pain which, in a few patients, was very severe pain and required treatment with opiates. Hematologic toxicity was mild with Grade 1/2 anemia, seen in 50% of patients (Groth G, ECCO01, Abs. 167).

A phase II clinical trial of LY355703 is being conducted by the UCLA Medical Oncology and Southern California Prostate Cancer Study Group Program in hormone-insensitive prostate cancer with rising PSA, or new bone or organ metastases, under PI Diane Prager, MD. According to the protocol, LY355703 is administered IV over 2 hours on days 1 and 8 of a 21-day cycle.

Discodermolide

Discodermolide, isolated by researchers at HBOI from the Caribbean sponge *Discodermia dissoluta*, is a potent cytotoxic marine natural compound acting on tubulin in a similar fashion as paclitaxel (see FO p 1038). In April 1998, HBOI licensed discodermolide to Novartis. However, amounts of discodermolide, isolated from the marine sponge are limited making the material extremely scarce. Also, the total chemical synthesis of discodermolide requires approximately 30 steps, and is not practical for producing the quantities necessary for commercial drug development.

Currently, this compound is the subject of NIH grant that funds research by Drs. Ross Longley and Sarath Gunasekera of HBOI, in collaboration with Dr. Robert Boeckman Jr, at the University of Rochester (Rochester, NY) and Novartis. This research focuses on the preparation and evaluation of natural and synthetic analogs of discodermolide (Isbrucker RA, etal, Cancer Chemother Pharmacol, 2001;48:29-36). Novartis has also supported research at the laboratory of Dr. Ian Paterson at Cambridge University in the UK, working to improve the synthesis of discodermolide. Dr. Paterson's approach has produced an overall yield of 8% compared to a yield of 10^{-3}% for the natural product. Also, Kosan Biosciences (Hayward, CA) is applying its genetic engineering technology towards the economical production of synthesized discodermolide.

In a wide range of human cells, discodermolide causes mitotic arrest at metaphase-anaphase transition by stabilizing microtubules. Discodermolide is active *in vivo* in P388 murine leukemia and human ovarian tumor xenograft models. In the paclitaxel-resistant human lung carcinoma cell line, A549-T12, presence of paclitaxel significantly amplified the cytotoxicity of discodermolide. Concurrent exposure of A549 cells to paclitaxel and discodermolide, at doses that do not induce mitotic arrest by themselves, caused an increase in the hypodiploid population, indicating that a possible mechanism for the observed synergy was potentiation of apoptosis. Therefore, paclitaxel and discodermolide may constitute a promising chemotherapeutic combination (Martello LA, etal, Clinical Cancer Research, May 2000; 6:1978–1987).

Dolastatins and Analogs

The dolastatins and related compounds are antineoplastic depsipeptides isolated from the sea hare *Dollabella auricularia* by groups headed by George R. Pettit, PhD (Biochem Pharmacol, 15 Jun 1990;39(12):1941-9) at Arizona State University, and K. Yamada (Mutou T, etal, J Org Chem, 6 Sep 1996;61(18):6340-6345) at Nagoya University in Japan. Dolastatins are structurally unique peptides containing unusual amino acid residues. They exhibit potent anticancer activity *in vitro*. Among all metabolites of dolastatin, dolastatin 10 and dolastatin 15, exhibit the most promising antiproliferative properties. These antimitotic agents seem to exert their activity by interacting with tubulin and inducing apoptosis.

Dr. Pettit's group first reported the isolation and structural elucidation of dolastatin 15, a seven-subunit depsipeptide. Dolastatin 15, represented by cemadotin and ILX-651, causes cell-cycle arrest at the G2/M phase by inhibiting microtubule assembly, apparently binding to tubu-

lin in a region close to the binding site of vinca alkaloids and maytansinoids (Bai R, etal, Biochem Pharmacol, 23 Jun 1992;43(12):2637-45, de Arruda M, etal, Cancer Res, 15 Jul 1995;55(14):3085-92, Basu A, etal, Int J Oncol, Oct 1998;13(4):659-64, Jordan MA, etal, Biochemistry 15 Dec 1998;37(50):17571-8, and Poncet J, Curr Pharm Des, Mar 1999;5(3):139-62).

Auristatin PHE, a pentapeptide dolavaline-valine-dolaisoleuine-dolaproine-phenylalanine-methyl ester, is a derivative of dolastatin 10. In *in vitro* evaluations, this agent arrested C. neoformans in the budding stage, possibly attributable to inhibition of tubulin (Woyke T, etal, Antimicrob Agents Chemother, Dec 2001;45(12):3580-4). Also bryostatin 1 induces differentiation and potentiates the antitumor effect of auristatin PHE in a human pancreatic tumor (PANC-1) xenograft model (Mohammad RM, Anticancer Drugs, Oct 2001;12(9):735-40).

Dolastatin 10, a five-member (dolavaline-valine-dolaisoleuine-dolaproine-dolaphenine) peptide, is a potent antimitotic that, in addition to disrupting tubulin polymerization, noncompetitively inhibits binding of vinca alkaloids to tubulin, and stabilizes the colchicine-binding activity of tubulin. Dolastatin was isolated from the Indian Ocean sea hare *Dolabella auricularia* by investigators at the Cancer Research Institute, Arizona State University (Tempe, AZ). Dolastatin 10 has been clinically evaluated in both solid tumors and hematologic malignancies.

A phase II clinical trial of dolastatin 10 was conducted at Mayo Clinic (Rochester, MN) in patients with advanced renal cell carcinoma (RCC), to evaluate the response rate and systemic toxicities including neurologic toxicity. According to the protocol, dolastatin 10 was administered as an intravenous bolus at the recommended phase II dose of 400 μg/m^2 once every three weeks. Neurologic testing was performed at baseline, six weeks, and at progression when possible. Among 30 patients, the most common Grade 3/4 toxicities included neutropenia (Grade 3=10%/Grade 4=37%), leukopenia (77%/0%), anemia (7%/3%), dyspnea (3%/3%), pleural effusion (3%/3%), fatigue (7%/0%), and constipation (7%/0%). Neurologic toxicity was mild with 27% Grade 1 sensory and 7% motor toxicity and did not appear cumulative. There were 3 (10%) PR and disease stabilized in 3 for >24 weeks. Duration of PR was 6.0, 10.3 and 16.5+ months. Median TTP was 2.2 months (Pitot HC, etal, ASCO02, Abs. 2409:1496).

An NCI-sponsored multicenter phase II clinical trial (protocol ID: NCI-T98-0019) of dolastatin 10 was initiated in October 1998 to study its effectiveness in treating patients with metastatic prostate cancer refactory to previous hormone therapy. Objectives are to determine response rate and toxicity of this regimen in this setting. According to the protocol, patients are treated with dolastatin 10 IV bolus every 3 weeks. Treatment continues for a minimum of 2 courses in the absence of unacceptable toxicity or disease progression. A total of 15-30 patients will be accrued for this study.

A phase II clinical trial (protocol ID: NCCTG-983251) to assess the antitumor activity and toxicity of dolastatin 10 in patients with advanced breast cancer, exposed to 1 or 2 prior chemotherapy regimens, was closed in January 2000. IV bolus dolastatin 10 was administered to 60 patients every 21 days. Treatment was repeated until complete remission, disease progression, or unacceptable toxicity. Those achieving CR were treated with 2 additional courses. Patients are to be followed every 3 months for 5 years or until disease progression. Michael J. O'Connell of the North Central Cancer Treatment Group was Study Chair.

In an NCI-sponsored, open-label, multicenter, phase II clinical trial (protocol ID: VCC-9802; NCI-T98-0007), initiated in April 2000, dolastatin 10 is being evaluated in indolent lymphoma (Stage III, IV, or recurrent), Waldenstrom's macroglobulinemia, or intermediate or high-risk Stages I/IV chronic lymphocytic leukemia (CLL) that progressed on fludarabine therapy unless patient cannot tolerate fludarabine. Trial objectives are to assess the efficacy and toxicity of dolastatin 10 and investigate its mechanism of action in regards to apoptosis and effects of microtubules. According to the protocol, patients are stratified by disease and treated with dolastatin 10 IV bolus every 3 weeks. Patients continue treatment until disease progression or unacceptable toxicity. A maximum of 74 patients will be accrued for this study over 15 months. Steven M. Grunberg, MD, of the Vermont Cancer Center (Burlington, VT) is Study Chair.

A phase I clinical trial (protocol IDs: MDA-DM-98187, NCI-T98-0001) of dolastatin 10 in patients with refractory or relapsed acute leukemia, myelodysplastic syndrome (MDS), or CLL in blast phase, was closed in November 2001. Approximately 25 patients were treated with dolastatin 10 IV bolus once every 3 weeks with 2-12 courses of therapy administered in the absence of disease progression and unacceptable toxicity. Patients will be followed until death. Jorge Cortes of M. D. Anderson Cancer Center (Houston, TX) was the Study Chair.

ILX-651 is a water-soluble, synthetic, pentapeptide analog of dolastatin 15 that inhibits microtubule assembly and tubulin polymerization. In early 2000, BASF Pharma (Ludwigshafen, Germany, since acquired by Abbott Laboratories (Abbott Park, IL), discontinued development of cemadotin, a first-generation analog of dolastatin 15, and BSF 223651, a second-generation agent, making this drug technology available for license. In September 2000, Ilex Oncology inlicensed BSF 223651 (now ILX-651) through an exclusive, worldwide license agreement with BASF Pharma. Considered a second-generation dolastatin analog, ILX-651 is produced through chemical modification of cemadotin to introduce resistance to hydrolysis by prolyloligopeptidases.

In preclinical pharmacokinetic studies, ILX-651 demonstrated oral bioavailability of 50% to 80%. The compound is resistant to prolyloligopeptidases *in vitro*, with

only 7% hydrolysis obtained after 24-hour incubation with recombinant enzyme. ILX-651 was cytotoxic against human HT-29 colon cancer cell cultures. It was slightly less potent *in vitro* than cemadotin, but exhibited similar inhibitory activity *in vivo*, causing complete tumor regression when administered IV in human MX-1 breast carcinoma and human LOX melanoma xenograft models, even when treatment was started at late stage. ILX-651 also significantly inhibited tumor growth in human PC3 prostate cancer, human LX-1 nsclc, and human CX-1 colorectal cancer xenograft models when administered IV. Similar excellent antitumor activity was observed when ILX-651 was administered orally to mice by gavage (Nelson CM, etal, AACR99, Abs. 1908:287).

In January 2001, Ilex submitted an IND to begin phase I clinical trials of oral ILX-651 in the USA, and in June 2001, it initiated a phase I clinical trial at the Arizona Cancer Center with Scot W. Ebbinghaus, MD, as the PI, and at Dana-Farber Cancer Institute (Boston, MA) with J. Paul Eder, MD, as the PI. In these nonrandomized, open-label studies, ILX-651 is being administered IV, daily, for 5 consecutive days, every 3 weeks. Study duration is expected to be 12 to 18 months, and projected enrollment is approximately 32 patients with advanced solid tumors.

At the phase I clinical trial (protocol ID: ILX651-101) being conducted at the Arizona Cancer Center, IV ILX651 was administered at a starting dose of 2.3 mg/m^2/day over 30 minutes for 5 days every 21 days. As of November 2, 2001, 6 patients were enrolled and 5 patients had completed at least one cycle of therapy; 4 patients were enrolled at the starting dose level (2.3 mg/m^2) and 2 patients at the second dose level (4.6 mg/m^2).

There were no drug-related hypertensive or cardiovascular events, nor have patients experienced DLT. There was no unexpected drug accumulation following multiple daily dosing. None of the patients treated at the first 2 dose levels developed antitumor responses. Evaluation of the first 3 dose levels (3.9, 7.8, 13.0 mg/m^2/day) revealed no evidence of cardiovascular toxicity or any DLT. At dose level 3.0 mg/m^2, 1/3 patients experienced Grade 3 neutropenia. It appears that the drug decays from plasma in a biphasic fashion with a mean apparent terminal phase half-life of 0.4 hours on day 1 and 0.6 hours on day 5. Whereas drug disposition appears to be linear on day 1, drug accumulation, or an alteration in pharmacokinetic behavior was noted on day 5 despite the consistently short half-life. Accrual to the study is ongoing at higher dose levels (Ebbinghaus SW, etal, AACR02, Abs. 2743:552, and Michaelson, MD, etal, ASCO02, Abs. 414:104a).

Several trials were carried out with cemadotin. Phase I clinical trials of IV cemadotin were conducted at Dana Farber/Partners Cancer Care and Beth Israel Deaconess Medical Center, Harvard Medical School (Boston, MA) in 21 heavily pretreated patients with solid tumors, of whom 9 had colorectal cancer (Lynch TC, etal, ASCO98, Abs. 808:210a, Supko JG, etal, Cancer Chemother Pharmacol 2000;46(4):319-28), and with 24-hour continuous IV infusion of cematodin at Albert Ludwigs University (Freiburg, Germany) in patients with advanced cancer (Mross K, etal, Ann Oncol, Dec 1998;9(12):1323-30). No PR or CR was noted in these trials.

In a phase II clinical trial, conducted to evaluate cemadotin's efficacy and safety in patients with locally advanced or metastatic breast cancer, the drug was administered by short IV infusion, at a dose of 2.5 mg/m^2/day for 5 days every 3 weeks. No objective responses were observed, although disease stabilized in 10 patients (Kerbrat P, etal, AACR99, Abs 3276:496).

In another phase II clinical trial involving 80 chemotherapy-naive patients (14 were not evaluable) with metastatic melanoma, treated with a similar scheme, responses included 1 CR, 3 PR and no changes were seen in 16 patients. Disease progressed early in 10 patients and later in 36. The median duration of response was 175 days (84-227), and disease stabilized for a median duration of 113 days (49-232). The median time-to-progression (TTP) was 42.5 days (14-276), and median survival time (MST) was 198 days (Bonneterre ME, etal, AACR00, Abs. 3261:511 and Smyth J, etal, Ann Oncol, Apr 2001;12(4):509-51).

BSF 223651 was also investigated in the clinic. Because preclinical testing indicated that this drug would be more effective if administered frequently, in a phase I clinical trial, BSF 223651 was administered as a 5-minute infusion daily for 5 days, every 3 weeks, to 22 patients with advanced solid malignancies. Patients were treated with 50 courses of BSF 223651 at 0.5, 1, 2, 2.5, and 3.0 mg/m^2 daily dose levels. Neutropenia was the principal toxicity on this schedule, with DLT occurring in 3/5 patients at the 3.0 mg/m^2 dose level. Transient Grade 3/4 hyperbilirubinemia and Grade 3 edema were also observed at the 3.0 mg/m^2 dose level. Other Grade 1/2 toxicities included fever, asthenia, nausea/emesis, edema, anemia and thrombocytopenia. One brief episode of Grade 3 hypertension occurred after a single treatment. There were no major responses. Based on toxicity and pharmacokinetic results, the recommended daily dose at this schedule is 2.5 mg/m^2 (Villalona-Cajero M, etal, ASCO97, Abs. 784:223a).

Symplostatin 1 is an analog of dolastatin 10 that was isolated from cyanobacteria of the genus *Symploca*. The effects of symplostatin 1 are consistent with other drugs that target cellular microtubules. Investigators at Southwest Foundation for Biomedical Research (San Antonio, TX), the University of Hawaii, and Wayne State University (Detroit, MI) found symplostatin 1 to be a potent inhibitor of cellular proliferation with IC_{50} values in the low nanomolar range and efficacy against a variety of tumors. It was shown to cause microtubule depolymerization and loss of interphase microtubules in a manner indistinguishable from dolastatin 10. However, symplostatin 1 is less potent than dolastatin 10. Symplostatin 1 causes G2/M arrest, and induces apoptosis by initiating the phos-

phorylation of Bcl-2, the formation of micronuclei, and activation of caspase 3. Symplostatin 1, evaluated *in vivo*, was active against murine colon 38 and murine mammary 16/C, but was poorly tolerated and the mice were slow to recover from the toxicity (Mooberry, SL, AACR02, Abs. 1320:266).

TZT-1027 is a synthetic analog of dolastatin 10 under development by Teikoku Hormone Manufacturing (Kanagawa, Japan). Like dolastatin 10 and 15, TZT-1027 is a seven-subunit depsipeptide that causes cell-cycle arrest in the G2/M phase by inhibiting microtubule assembly. It apparently binds to tubulin in a region close to the binding site of Vinca alkaloids and maytansinoids (Kobayashi M, etal, Jpn J Cancer Res, Mar 1997;88(3):316-27; Kobayashi M, etal, Nippon Yakurigaku Zasshi, Oct 1999;114 Suppl 1:230P-35P; Natsume T, etal, Jpn J Cancer Res, Jul 2000;91(7):737-47). In December 2000, Teikoku Hormone and Daiichi Pharmaceutical (Toyko, Japan) entered into a license agreement regarding TZT-1027. Under the agreement, Daiichi acquired rights to exclusively develop and market TZT-1027 on a worldwide basis except in Japan, where Teikoku Hormone and Daiichi Pharmaceutical will collaborate in development and marketing of the drug.

In addition to its inhibitory effect on tubulin polymerization, TZT-1027 appears to induce tumoral vascular collapse and tumor cell death, attacking the well developed vascular system of advanced tumors by a putative protein kinase-dependent mechanism, and then blocking tumor blood flow. Histopathologic examinations following administration of TZT-1027 in murine colon 26 adenocarcinoma, revealed that tolerable doses of TZT-1027 induced tumor-selective hemorrhage within 1 hour of administration, with the hemorrhage occurring mainly in the peripheral area of the tumor mass. Biochemical measurements demonstrated that hemorrhaging occurred first, followed by cessation of tumor blood flow. The vascular damage was followed by continuous induction of apoptosis of the tumor cells, tumor tissue necrosis, and tumor regression. In cultured human umbilical vein endothelial cells (HUVEC), TZT-1027 induced significant cell contraction with membrane bleeding within 30 minutes of drug administration; these cell changes were completely inhibited by K252a, a broad-spectrum inhibitor of protein kinases. Both the effects on tumor vasculature and HUVEC were greater with TZT-1027 than with vincristine (Otani M, etal, Jpn J Cancer Res, Aug 2000;91(8):837-44).

TZT-1027 also appears to modulate gene expression at the mRNA level, including the genes encoding cell-cycle and growth regulators, intermediate filament markers, apoptosis-related factors, cell adhesion, motility and invasion-related proteins, angiogenesis and invasion regulators, rho family small GTPases and their regulators, cell-cell interaction-related proteins and growth factors, and cytokines. The alteration of such genes after exposure to TZT-1027 may be involved in this agent's antitumor activity (Natsume T, etal, AACR00, Abs. 3524:553 and Kobayashi M, etal, AACR00, Abs. 1372:215). Also, in experimental animals, TZT-1027 did not exhibit the degree of neurotoxicity compared to other antimicrotubule agents (Ogawa T, Toxicol Lett, 30 Apr 2001;121(2):97-106).

A phase I clinical trial of TZT-1027, administered weekly for 3 weeks as a 1-hour IV infusion, was conducted to determine toxicities, MTD, and to assess pharmacokinetics in solid tumors. Among 40 patients treated at weekly doses from 0.3 mg/m^2 up to 2.1 mg/m^2 (level 12), the 2.1 mg/m^2 dose level was intolerable with DLT in 2/4 patients manifested as Grade 4 leukopenia, neutropenia, and Grade 3/4 constipation. Toxicity was acceptable at the 1.8 mg/m^2 level without DLT. One patient with a thymoma experienced a PR at the 1.5 mg/m^2 dose that lasted 183 days. These results suggest that TZT-1027 is a promising new tubulin polymerization inhibitor, generally well tolerated in a weekly dosing regimen. The recommended dose for further studies is 1.8 mg/m^2 once every three weeks (Yamamoto N, etal, ASCO02, Abs. 420:106a).

Another phase I clinical trial evaluated MTD and DLT of TZT-1027 in patients with advanced, therapy-resistant nsclc. The starting dose was 0.5 mg/m^2 administered as an IV infusion once every 3 weeks. Dose was escalated up to 3.2 mg/m^2. Among 29 of 31 enrolled patients, evaluable for toxicity and response, 1 developed Grade 4 neutropenia. Pharmacokinetics were dose-dependent. TZT-027 treatment was well tolerated. MTD had not been reached at 3.2 mg/m^2 (Horti J, etal, AACR02, Abs. 2744:552).

Epothilones

Epothilones, extracted from the South African soil bacteria *Sorangium cellulosum*, belong to a class of 16-membered ring macrolides that stabilize microtubule assemblies in a paclitaxel-like action (see FO, p 1038). Epothilones were originally described in 1995 by Daniel M. Bollag and colleagues at Merck (West Point, PA), from a screening program conducted to identify substances with a paclitaxel-like mode of action.

Although these compounds do not share any obvious structural similarities with paclitaxel, they exhibit a similar biologic profile *in vitro*, by influencing the behavior of microtubili including inhibition of microtubule depolymerization, and induction of apoptosis in human cancer cell lines. In preclinical trials, epothilones demonstrated antitumor activity in breast, ovarian, and colon cancer, nsclc, and malignant melanoma. Also, these agents are more water-soluble than paclitaxel, and are apparently not as susceptible to deactivation by MDR, maintaining activity against resistant tumor cell lines.

One of the first tasks in developing epothilones as anticancer drugs was their synthesis. Currently, various analogs of epothilones have been produced semisynthetically and by full synthesis. Complete synthesis of epothilones A (EpoA) and B (EpoB) was achieved in 1996 by chemists at Memorial Sloan-Kettering Cancer Center (MSKCC; New York, NY) and the Scripps Research Institute

(La Jolla, CA). The Rapid Access to Intervention Development (RAID) Program of the NCI supported the over 20-step chemical synthesis of sufficient amounts of epothilone D (EpoD) to enable Dr. Danishefsky and his coworkers at MSKCC to confirm its activity as an antitumor agent in animals, and for pharmacology and toxicology studies conducted by NCI and MSKCC. Also, scientists at Oregon State University synthesized EpoB, EpoD, and cis- and trans-9,10-dehydroepothilone D (White JD, J Am Chem Soc, 13 Jun 2001;123(23):5407-13). Semisythesis of EpoB was achieved by researchers at Gesellschaft für Biotechnologische Forschung (Braunschweig, Germany) and the process was licensed to Bristol-Myers Squibb, forming the basis for BMS-247550.

Laboratory synthesis of epothilones created the prerequisites for further biologic investigations and clinical studies. However, because of the complexity of the 20-or-more-step synthetic process, fermentation-based methods are likely to prevail as a practical approach for large-scale production.

Epothilones are polyketide compounds, a subgroup of natural products comprising many best-selling pharmaceuticals, including erythromycin, tetracycline, lovastatin, and many others. In the early 1990s, it was discovered that polyketides were made in the cells of their microorganisms by modular chemistries, i. e., there is a code in the very large genes encoding the enzymes that make the polyketides. Each gene in a polyketide gene cluster is responsible for adding a 2-carbon unit to the growing molecule. This allows developers to go into polyketide clusters, change their genes, and permutate the module to alter its structure. However, it has been difficult to generate whole new polyketide molecules to make a large library of these agents to use in drug screens to truly perform combinatorial type chemistry. Another problem with polyketide synthesis is that often the bacteria or fungi that produce these compounds are slow growers and inefficient producers.

Kosan Biosciences has solved the above problems with proprietary techniques, which enable scientists to mix, match, and alter genes in the polyketide synthesis pathway using molecular biology. Mastering the polyketide genetic pathway enables Kosan to create a polyketide library by synthesizing new molecules that may have important biologic activity. Transplantation of the complete genetic cluster into another bacteria with better growth and production characteristics, is also possible. Kosan's chemobiosynthesis technology involves inserting chemically synthesized building blocks to genetically engineered polyketide-producing cells to form novel, hybrid polyketides that have not been made by man or nature. This technology permits significant modification of the bioactivities of polyketides, or creation of proprietary versions of known high-value polyketide pharmaceuticals. Kosan scientists first reported the production of a simple polyketide in both *Escherichia coli* (E. coli) and in yeast in 1998.

Kosan has licensed various patents covering production of polyketides from Stanford University (Stanford, CA), in March 1996, from Harvard College (Cambridge, MA), in December 1998, and from Eli Lilly, in August 2002. In March 2002, the company was awarded a $230,000 Phase I Small Business Innovative Research (SBIR) grant from the National Institute of Allergy and Infectious Diseases (NIAID), to extensively engineer *E. coli* strains for improved and versatile production of polyketides. In April 2001, Kosan Biosciences was awarded a $750,000 Phase II SBIR grant from the National Institute of General Medical Sciences (NIGMS) to construct yeast strains optimized for high-level production of polyketides.

In late 1999, Kosan, simultaneously with another group cloned and sequenced the entire 56-kilobase cluster of genes in myxobacterium *Sorangium cellulosum*, which encodes six multifunctional proteins. Concomitant expression of these genes in the actinomycete *Streptomyces coelicolor* produced epothilones A and B. *S. coelicolor* is more amenable than *S. cellulosum* to strain improvement and grows about 10-fold as rapidly as the natural producer. In addition, the availability of cloned genes and a plasmid-borne expression system allowed manipulation of the epothilone biosynthetic pathway to produce novel epothilone analogs (Tang L, etal, Science, 28 Jan 2000;287(5453):640-2). *S. coelicolor* has a fast, two-hour doubling time.

There are several members in the epothilone family with the most common being epothilone B (EpoB), which is a significantly more potent inhibitor of human cancer cell growth than paclitaxel and, unlike paclitaxel, is also effective against MDR cell lines. Analogs of epothilones A and B are also being created as potential anticancer agents by various groups, including investigators at Memorial Sloan-Kettering Cancer Center, and a collaboration between Novartis Pharma, Scripps Research Institute and the University of Magdenburg.

Development of analogs of EpoB was prompted by the fact that pharmacologic evaluations in xenograft mouse models revealed that EpoB, although the most potent drug in the epothilone series, possessed poor therapeutic efficacy at MDT. At MSKCC, Dr. Samuel J. Danishefsky's group created two synthetic epothilone analogs lacking the 12,13-epoxide functionality of EpoB, 12,13-desoxyepothilone B (dEpoB or EpoD), and 12,13-desoxyepothilone F (dEpoF), an analog of dEpoB possessing an additional hydroxyl group at C21. Another epothilone analog is 15-desoxy-15-aza-epothilone (aza-EpoB, BMS-247550), under development by Bristol-Myers Squibb, that obtained the lactam version of EpoB through an elegant partial synthesis starting from EpoB. Preliminary reports by Novartis scientists on the *in vivo* therapeutic effects of dEpoB and EpoB (Altmann KH, etal, Biochim Biophys Acta, 17 May 2000;1470(3):M79-91) and by Bristol-Myers Squibb investigators regarding aza-EpoB, reveal some promising aspects of each compound.

MSKCC researchers performed numerous experiments in mice xenografts to compare the activities of dEpoB and dEpoF with comparable anticancer agents, including aza-EpoB. When the *in vitro* cytotoxicities of dEpoB, dEpoF, aza-EpoB, and paclitaxel were compared against various tumor cells in the same experimental settings, growth of all parent cell lines tested was strongly inhibited by the four compounds with similar IC_{50} values. It has been demonstrated through these comparative studies that the therapeutic effects of dEpoB and dEpoF are superior to those of paclitaxel and aza-EpoB, as well as some currently used anticancer agents such as etoposide, vinblastine, doxorubicin, and irinotecan. Results also indicate that dEpoF and dEpoB have similar chemotherapeutic effects and are curative against human K562 tumor xenografts in nude mice.

Also, despite a similar antitumor mechanism, the epothilones and taxanes show remarkably different MDR profiles. Both *in vitro* and *in vivo* studies have shown that epothilones are much less susceptible to the onset of MDR compared to paclitaxel. In experiments conducted by MSKCC, dEpoB and dEpoF exhibited little drug resistance, whereas activities of both aza-EpoB and paclitaxel were substantially diminished against drug-resistant cell lines. Also, desoxyepothilones consistently outperformed other conventional anticancer agents in inhibiting the growth of resistant tumor cells.

Although epothilones are expected to be less vulnerable to MDR, several point mutations in class I β-tubulin have been identified in paclitaxel-resistant and epothilone-resistant cancer cell lines. One point mutation, Q292E, was isolated from a selected drug-resistant A549 human nsclc cell line that was approximately 95-fold resistant to epothilone B, and 22-fold resistant to paclitaxel. Expression of mutant β-tubulin, such as class I β-tubulin containing Q292E, is a contributing factor in drug resistance towards paclitaxel and epothilone B (Wiesen KM, AACR02, Abs. 3910:789).

BMS-247550, developed by Bristol-Myers Squibb in collaboration with Gesellschaft für Biotechnologische Forschung (GBF), is a semisynthetic analog of EpoB, currently in clinical trials for a variety of indications, alone or in combination with other anticancer agents. BMS-247550 has also demonstrated preclinical activity against taxane-sensitive and resistant tumors.

Bristol-Myers Squibb has embarked in an aggressive clinical development program with BMS-247550, currently being evaluated in various solid tumors (adult and pediatric) and in lymphoma. Indications in phase II clinical development include advanced, metastatic, refractory or recurrent prostate, breast, bladder, pancreatic, hepatobiliary, gastric, colorectal, ovarian, peritoneal, kidney, and head and neck cancer, soft-tissue sarcoma, malignant melanoma, and nsclc. Detailed protocols of ongoing studies are described in NEW MEDICINE's subscriber-based online Oncology KnowledgeBASE residing at www.nmok.net. Phase II clinical trials are based on results from several phase I dose-escalation clinical trials involving various dose and infusion schedules during which BMS-247550 demonstrated activity at tolerable toxicity. Several phase I clinical trials are also ongoing.

In early human single-agent clinical trials, BMS-247550 was shown to be clinically active at relatively safe dose levels. BMS-247550 has a toxicity profile similar to taxanes at an MTD of 40 mg/m^2, infused over 1 hour, administered every 3 weeks (Tripathi R, etal, ASCO02, Abs.407:102a). In a phase I dose-escalation clinical trial to assess the pharmacokinetics of BMS-247550, the drug was administered every 3 weeks as a 1-hour constant rate IV infusion at doses of 7.4-65 mg/m^2. Based on 30 evaluable patients, the kinetics of BMS-247550, over clinically relevant doses, appear to be linear, and produce dose-dependent increase of tubulin polymerization in peripheral blood mononuclear cells (Damle BD, etal, ASCO01, Abs. 268:68a). In another phase I clinical trial, among serious toxicities noted were Grade 3 fatigue, nausea, diarrhea, and myalgia/arthralgia. Myelosuppression was minimal (Burris HA III, etal, ASCO02, Abs. 412:104a). When BMS-247550 was administered as a 1-hour infusion, every 3 weeks, at 40 mg/m^2, 1 of 6 patients experienced dose-limiting neutropenia (Mani S, etal, ASCO02, Abs.409:103a). In another administration schedule, BMS-247550 was well tolerated as a 1-hour infusion of 6 mg/m^2, daily, for 5 days, in patients previously treated with a taxane-based regimen (Agrawal M, etal, ASCO02, Abs.410:103a, and Fojo AT, etal, AACR-NCI-EORTC01, Abs. 774).

Various phase I clinical trials were also undertaken to evaluate BMS-247550 in combination with other anticancer agents, such as carboplatin, in recurrent or refractory solid tumors (Plummer R, etal, ASCO02, Abs.2125:78b), and estramustine phosphate (EMP), in chemotherapy-naive patients with progressive metastatic prostate cancer (Smaletz O, etal, ASCO02, Abs. 732:184a). A dose-escalation phase I trial of BMS-247550 and estramustine phosphate was conducted in 9 chemotherapy-naive patients with progressive metastatic prostate cancer following castration. Escalating doses of BMS-247550 (35 to 40 mg/m^2) were administered IV with estramustine phosphate (280 mg) PO, thrice daily, for 5 days, every 21 days. At 35 mg/m^2, none of 3 patients treated showed Grade 3/4 toxicity. At 40 mg/mg^2, 3 of 6 patients treated experienced Grade 3/4 neutropenia, and 1 patient Grade 3 nausea. No hypersensitivity reactions or no other significant toxicities were observed. A >50% post-therapy decline in PSA was seen in 5/5 evaluable patients. Soft tissue regression and bone metastasis improvement were also documented (Smaletz O, etal, ASCO02, Abs. 732:184a).

In addition to combination with cytotoxic agents, it is anticipated that BMS-247550 will also be evaluated in tandem with radiotherapy and with various regulatory approaches. *In vitro* studies at the Moffitt Cancer Center and Research Institute of the University of South Florida

(Tampa, FL) have shown that a combination of Apo-2L/TRAIL and/or Smac peptide and BMS-247550 may increase the latter's cytotoxic effects by potentiating extrinsic and intrinsic apoptotic signaling and effector caspase activity triggered by BMS-247550 (Griffin D, etal, AACR-NCI-EORTC01, Abs. 339). Targeting survivin and mitotic kinase also enhances EpoB-induced mitotic arrest and apoptosis of human breast cancer cells (Wittman S, etal, AACR02, Abs. 2031:408).

The proapoptotic FT inhibitor BMS-214662 produced synergistic antitumor activity in combination chemotherapy with antiproliferative cytotoxic agents. Results from combination chemotherapy studies with various preclinical human colon carcinoma models demonstrating pronounced synergistic antitumor activity of BMS-214662 with paclitaxel, CPT-11, gemcitabine and BMS-247550 (Lee FY, etal, AACR-NCI-EORTC01, Abs. 401). BMS-247550 also enhances the effects of radiation in human lung cancer cells both *in vitro* and *in vivo*, probably via G2/M blockade (Kim J-C, etal AACR02, Abs. 2405:483).

Early results from single-agent phase II clinical trials indicate that treatment with BMS-247550 is associated with significant toxicity, leading to dose reductions. In a multicenter phase II clinical trial (protocol IDs: MAYO-MC007C, NCI-3852), initiated in June 2001 at the Mayo Clinic (Rochester, MN, Scottsdale, AZ, and Jacksonville, FL), University of Wisconsin (Madison, WI), Washington University (St Louis, MO), and Karmanos Cancer Institute at Wayne State University (Detroit, MI), the toxicity of BMS-247550 was determined in patients with advanced soft-tissue sarcoma. No prior chemotherapy was allowed for metastatic disease except adjuvant chemotherapy. After premedication with an oral H1 and H2 blocker, BMS-247550 (50 mg/m^2) was administered IV over 1 hour every 3 weeks, with treatment repeated every 21 days in the absence of disease progression or unacceptable toxicity. Patients with CR were treated with 2 additional courses. Patients are to be followed every 3 months for 1 year, every 4 months for 1 year, and then every 6 months for 3 years. Scott Okuno, MD of the Mayo Clinic Cancer Center is the PI. This study was reported closed as of May 2002.

Among 24 patients accrued in this study, the median follow-up of living patients was 42 days. Among 22 patients evaluable for toxicity, 14 (64%) experienced Grade ≥3 toxicities possibly related to treatment; 11 experienced ≥Grade 3 nonhematologic toxicities, including constipation (n=4), myalgia (n=2), dyspnea (n=2), rash (n=1), fatigue (n=2), pain (n=3), urinary retention (n=1), pleural effusion (n=1), mucositis (n=1), stomatitis (n=1), and arthralgia (n=1). Neutropenia and leukopenia ≥Grade 3 occurred in 41% and 23% of patients, respectively. One patient died from septic shock (Okuno SH, etal, ASCO02, Abs. 1645:412a).

In a multinational, multicenter, phase II clinical trial, being conducted in the USA and Europe, BMS-247550 (50 mg/m^2) was administered as a 1-hour infusion, every 21 days, to patients with metastatic gastric cancer previously treated with a taxane. Between January 2001 and August 2001, 23 patients were enrolled with data available on 21 patients; 5 of the 21 patients remain on treatment. A total of 56 courses of BMS-247550 were administered. Among 21 patients evaluable for toxicity, severe (Grade 3/4) toxicities included fatigue (n=9), anorexia (n=6), nausea/vomiting (5/21), sensory neuropathy/neuropathic pain (n=3), myalgia/arthralgia (n=2), abdominal pain/cramping (n=2); diarrhea (n=2), febrile neutropenia (n=1), and neutropenia (n=8). Among 20 patients evaluable for response, there were 2 PR (10%) and disease stabilized in 9 for an overall response of 55%. In order to reduce cumulative neurotoxicity and improve the therapeutic index, the regimen was amended to 6 mg/m^2, administered daily for 5 days over 1 hour, every 21 days, a schedule which has been associated with no Grade 3 neuropathy in phase I evaluation (Ajani JA, etal, ASCO02, Abs. 619:155a).

In a phase II clinical trial, BMS-247550 was administered at 50 mg/m^2 as a 1-hour infusion every 21 days to taxane-refractory or taxane-naïve patients with metastatic breast cancer. Among 7 evaluable patients with taxane-refractory disease, administered 16 courses of BMS-247550, Grade 3 toxicities included fatigue (n=1), sensory neuropathy (n=1), proctitis (n=1), stomatitis/pharyngitis (n=1), neutropenia (n=2), and thrombocytopenia (n=1). There were 2 PR (29%), and disease stabilized in 3 for an overall response rate of 71%. Among 19 evaluable taxane-naïve patients treated with 67 courses of BMS-247550, Grade 3 toxicities included myalgia/arthralgia (n=5), sensory neuropathy/neuropathic pain (n=4), fatigue (n=3), dyspnea (n=3), diarrhea (n=1), amenorrhea (n=1), reversible myocardial ischemia (n=1), and febrile neutropenia (n=1); Grade 3/4 toxicities included neutropenia (n=7 patients). There were 10 PR (53%), and disease stabilized in 8 for an overall response rate of 95%. In order to reduce cumulative neurotoxicity and improve the therapeutic index, this trial protocol was amended for all patients to a regimen of 40 mg/m^2 over 3 hours every 3 weeks (Roché H, etal, ASCO02, Abs.223:56a).

BMS-247550, administered at 50 mg/m^2 as a 1-hour infusion every 21 days to patients with nsclc previously treated with one platinum-based regimen for recurrent or metastatic disease, was investigated in a phase II clinical trial that enrolled 31 patients between February 2001 and August 2001. Among 25 evaluable patients (8 of whom remain on treatment) treated with 77 courses of BMS-247550, severe (Grade 3) nonhematologic toxicities were fatigue (n=5), sensory neuropathy/ neuropathic pain (n=5), nausea (n=2), constipation (n=2), and myalgia/arthralgia (n=1). Grade 3/4 hematologic toxicities included febrile neutropenia (n=2), neutropenia (n=10), and thrombocytopenia (n=2). No severe diarrhea was reported. Among 22 evaluable patients there were 4 PR (18%), and disease stabilized in 10, for an overall response rate of 64%. The study was amended to a randomized comparison

of 40 mg/m^2 over 3 hours every 21 days versus 6 mg/m^2 administered daily for 5 days over 1 hour every 21 days, a schedule which has not been associated with Grade 3 neuropathy in phase I evaluation (Delbaldo C, etal, ASCO02, Abs.1211:303a).

BMS-310705, a C-21 substituted derivative of epothilone B, is a water-soluble and chemically stable semisynthetic epothilone, developed by Bristol-Myers Squibb, again in collaboration with Gesellschaft für Biotechnologische Forschung. BMS-310705 can be formulated entirely in an aqueous solution and is stable in water, undergoing <5 % degradation in 24 hours. Clinical validation of the epothilones provided the impetus for a second generation program aiming to improve on some of the physicochemical properties of the class, specifically, water-solubility and chemical stability. BMS-310705 exhibits potent antitumor activity in a parenteral and oral form against models of taxane-sensitive and resistant human tumors *in vivo*. In studies involving a variety of taxane-sensitive and resistant human tumor xenografts in mice, BMS-310705 demonstrated robust antitumor activities that were superior than paclitaxel, epothilone B or epothilone D, but were similar to those achieved by BMS-247550. BMS-310705, like BMS-247550, is also active orally. Preclinical toxicologic studies in rats showed that BMS-310705 has a toxicity profile that resembles that of paclitaxel and BMS-247550 (Lee FY, etal, AACR02, Abs. 3928:792).

BMS-310705 is an active inducer of apoptosis. BMS-310705 was evaluated in a culture of ovarian cancer cells (OC-2) from ascites from a patient with Stage IIIc papillary serous ovarian cancer refractory to platinum- and paclitaxel-based chemotherapy. In all experiments, OC-2 cells were treated with BMS-310705, topotecan, or SN-38, an active metabolite of irinotecan, to evaluate any apoptosis and/or caspase activity. In OC-2 cells treated with BMS-310705, analysis of caspase activity demonstrated a 3-fold increase of initiator caspase 9, and 2-fold increase of executioner caspase 6 after 12 hours, followed by a 3-fold increase of executioner caspase 3 and initiator caspase 2 by 24 hours. Initiator caspase 8 activity was not observed at either of these periods. Analysis of NF-κB signaling pathways in OC-2 cells did not demonstrate transcriptional activation of NF-κB after treatment with BMS-310705, taxanes or topoisomerase I (topo I) poisons. Treatment of OC-2 cells with topotecan and SN-38 failed to demonstrate induction of apoptosis in OC-2 cells after 24 hours, despite confirmation of measurable topo I protein. These studies demonstrate a rapid and significant induction of apoptosis in this platinum- and paclitaxel-refractory ovarian cancer model suggesting potential activity in disease resistant to platinum and paclitaxel therapy. Activation of caspase 2 and 6 activity may suggest overlapping substrate specificity. Assessment of apoptotic signaling pathways and induction of apoptosis suggests that the action of BMS-310705 may not involve an NF-κB-dependent pathway (Uyar D, etal, AACR02, Abs. 4571:722).

A phase I clinical trial of BMS-310705 is being conducted by the South European New Drug Organization (SENDO), which coordinates preclinical and clinical activities conducted in the fields of new anticancer drugs development at the Oncology Institute of Southern Switzerland (Istituto Oncologico della Svizzera Italiana or IOSI), in Bellinzona, Switzerland, and in Milan, Italy, the Istituto Nazionale dei Tumori (INT), the European Oncology Institute (IEO) and the Mario Negri Institute. The trial is investigating this drug in patients with solid tumors who have failed at least two chemotherapy regimens.

In a phase I clinical trial of BMS-310705, administered once every 3 weeks as a 15-minute infusion, 22 patients with advanced cancer were treated at dose levels of 0.6 mg/m^2 to 40 mg/m^2; 11 evaluable patients in dose cohorts up to 20 mg/m^2 were treated with 28 courses. No DLT was observed. Toxicities per course include Grade 1 diarrhea (n=5), nausea (n=4), alopecia (n=2), neuropathy (n=2), and myalgia (n=1), Grade 2 vomiting (n=1) and anemia (n=1), and Grade 1/2 anorexia (n=2) and fatigue (n=7). Neutropenia was observed in 2 patients, Grade 2 in 1 patient at 20 mg/m^2, and Grade 3, in 1 patient at 30 mg/m^2. No hypersensitivity reactions have been reported. Among 18 evaluable patients, disease stabilized in 7 for as many as 7 cycles of treatment. MTD had not been reached at the doses used (Mekhail T, etal, ASCO02, Abs.408:103a).

EPO906, is an epothilone B, under development by Novartis. Investigators at Novartis Pharma (Basel, Switzerland) showed that EPO906 is more stable in human compared to rodent plasma, and its pharmacokinetic characteristics contribute to the promising overall profile of this drug (Brueggen J, etal, AACR02, Abs. 1064:212).

In phase I clinical trials the DLT of EPO906 (8 mg/m^2), administered once every three weeks, was diarrhea. In a phase I dose-escalation clinical trial, EPO906 (0.3 mg/m^2 to 8 mg/m^2) was administered to patients with advanced solid tumors as a short 15-minute or 30-minute IV infusion, once every 3 weeks. Blood and urine pharmacokinetics were evaluated in 50 patients for a single dose (n=8) or for 2 consecutive doses (n=42), once every 3 weeks. There was no apparent relationship between systemic drug exposure and development/severity of diarrhea that was the DLT. No drug accumulation was observed in blood after repeated doses. These results suggest that EPO906-related treatment toxicity may be determined primarily by local tissue drug binding and metabolism rates, rather than systemic drug exposure (Chen T, etal, ASCO02, Abs. 363:91a).

A phase I dose-escalation clinical trial was conducted at Princess Margaret Hospital (Toronto, Canada) and the Cancer Institute of New Jersey (New Brunswick, NJ), to determine toxicities, identify MTD, and assess pharmacokinetics of weekly infusions of EPO906 every 6 out of 9 weeks, in patients with advanced solid tumors. Among 5

patients (sclc=1, renal cancer=1, other=3) enrolled at an initial dose of 0.3 mg/m^2, there were no Grade 3/4 toxicities; 1 patient experienced transient Grade 1/2 paresthesias or dysgeusia. Among 7 patients (nsclc=2, colon=2, ovarian=1, bladder=1, unknown primary=1) treated at a second dose level of 0.5 mg/m^2, 1 experienced Grade 3 paresthesias during week 5 (Oza AM, etal, ASCO00, Abs. 921:234a, and Oza A, etal, Annals of Oncology, October 2000;Vol 11, Suppl 4:133).

All in all, 36 patients were eventually enrolled in this study, treated with doses ranging from 0.3 mg/m^2 to 3.6 mg/m^2. Regarding toxicities, 3/6 patients treated at the 3.6 mg/m^2 developed dose-limiting diarrhea. Subsequently, only 1/6 patients experienced dose-limiting diarrhea at a reduced dose of 2.5 mg/m^2. Other toxicities have been minimal. Among heavily pretreated patients there were 2 PR (breast, ovarian), and 3 minimal responses (colon, lung, ovarian). A subcutaneous tumor biopsy obtained from a patient one hour after a fifth dose of 0.5 mg/m^2, indicated that tumor drug levels were about 10-fold higher than blood levels, and well above intratumoral levels observed with effective doses in mice.

Another open-label, phase I clinical trial of EPO906 in 42 patients with advanced solid tumors (colorectal=13, breast=5, ovary=4, unknown primary=4, nsclc=3 and others=16), exposed to a median number of 2 prior chemotherapeutic regimens (range=0-6), was conducted at National Centre for Cancer Treatment (NCCT; Newcastle upon Tyne, UK) and Beatson Oncology Centre (Glasgow, UK). Starting dose was 0.3 mg/m^2 with the first 6 dose levels administered as a 30-minute infusion, every 21 days, and subsequent dose levels as a 5- to 10-minute infusion every 21 days. All in all, 11 dose levels were tested, with 2 median cycles delivered per patient (range=1-9). Because diarrhea was the DLT at 8 mg/m^2, a decrease to 7 mg/m^2 with prophylactic loperamide was studied. Other Grade 3 nonhematologic toxicities included fatigue (n=4) and nausea/vomiting (n=2). Grade 2 peripheral neuropathy was seen in 3 patients. No significant myelosuppression (Grade 1) was seen and there were no hypersensitivity reactions. The drug shows multiphasic clearance with a long half-life of approximately 3.5 days. Elimination is mainly nonrenal. There was dose proportionality and no evidence of drug accumulation. Among 36/42 evaluable patients, there was 1 PR in a patient with an unknown primary tumor, and disease stabilized in 11 patients, with 4 (breast=1, colorectal=3) exhibiting significant responses though not reaching criteria for PR (Calvert PM, etal, ASCO01, Abs. 429:108a).

Currently, several phase II clinical trials are ongoing in the USA and in Europe in advanced breast, kidney, ovarian, colorectal, and prostate cancer and malignant melanoma.

KOS-862, under development by Kosan Biosciences, is epothilone D (12,13-desoxyepothilone B, dEpoB, or EpoD), an epothilone analog lacking the 12,13-epoxide functionality. EpoD, discovered by Dr. Danishefsky's group at MSKCC, exhibits promising *in vitro* stability and *in vivo* efficacy and causes no observable toxicity at therapeutic levels. EpoD appears to be the most potent among the epothilones, with the best therapeutic index. It is also more potent in the human tumor xenograft model than other standard anticancer agents.

In August 2000, Kosan signed a collaboration and licensing agreement with the Memorial Sloan-Kettering Institute for Cancer Research for the development of dEpoB. Under this agreement, Kosan uses its technologies to produce a specific epothilone compound for clinical trials, and works collaboratively with MSKCC to develop new compounds and production methods, and to conduct clinical trials. Under the agreement, Kosan paid MSKCC an initial license fee and will pay annual maintenance fees, as well as payments for R&D costs, including costs of clinical trials, payments if clinical development milestones are reached, and royalty on product sales if any product is commercialized. In addition to the collaborative research program with at least a 2-year research term, the agreement grants licenses that include a research license, whereby Kosan and MSKCC grant each other a license to make and use methods and material covered under each of its patents, and know-how to carry out research during the research term of the agreement. Also, MSKCC granted Kosan exclusive worldwide rights, with a limited right to sublicense, to make, use, develop, and sell the licensed products.

In June 2001, Kosan was awarded a Phase I SBIR grant from the NCI, to generate epothilone analogs by genetic engineering. In January 2001, Kosan was awarded a $1 million Phase II SBIR grant from the NCI to improve its production process for EpoD. In August 1998, Kosan received a Phase I SBIR grant from the NCI to clone and characterize the epothilone gene cluster from its myxobacterial host.

Although epothilones have been chemically synthesized, the complexity of the process precludes large-scale commercial production. However, novel synthesis methods allowed the preparation of numerous analogs of epothilone D. These synthetic methods complement Kosan's fermentation-based approaches for the mass production of this agent. Currently EpoD is produced using heterologous expression in an engineered strain of the soil bacterium *Myxococcus xanthus*. When biologically produced (biol-EpoD) and chemically synthesized (chem-EpoD) EpoD was observed *in vitro*, using MCF-7, NCI/Adr, SF-268 and NCI/H460 cell lines, and *in vivo* in mice with MX-1 or CCRF-CEM xenografts, biol-EpoD demonstrated broad antitumor efficacy, comparable to chem-EpoD (Johnson RG Jr, etal, AACR02, Abs. 3925:792).

According to MSKCC investigators, dEpoB displayed a much more favorable therapeutic index despite its slightly decreased *in vitro* cytotoxicity relative to EpoB. When concentrations required for 50% growth inhibition (IC_{50}) for a variety of spindle poisons were measured in CCRF-

CEM/VBL1000 cells (2,048-fold resistance to vinblastine), dEpoB, dEpoF, aza-EpoB, and paclitaxel IC_{50} values were 0.029, 0.092, 2.99, and 5.17 mM, respectively. These values represent 4-, 33.5-, 1,423- and 3,133-fold resistance, respectively, when compared with the corresponding IC_{50} in the parent (nonmultiple MDR) CCRF-CEM cells. Continuous exposure of MDR human lung carcinoma A549 cells to sublethal concentrations of dEpoB (1.8 year), vinblastine (1.2 year), and paclitaxel (1.8 year), led to the development of 2.1-, 4,848-, and 2,553-fold resistance to each drug, respectively. Also, when the therapeutic effect of dEpoB and paclitaxel were compared *in vivo* in a mouse model by using various tumor xenografts, dEpoB was much more effective in reducing tumor size in all MDR tumors tested. Also, dEpoF was curative, similarly to dEpoB, against K562, CCRF-CEM, and MX-1 xenografts. These results indicate that dEpoB and dEpoF are efficacious antitumor agents with both a broad chemotherapeutic spectrum and wide safety margins (Chou T-C, etal, PNAS USA, 3 Jul 2001;98(14):8113-8).

Despite potent *in vitro* cytotoxicities, EpoB and aza-EpoB appeared to possess narrow therapeutic windows, as indicated by the occurrence of deaths of experimental animals even with only moderate decreases in body weight. An important toxicologic finding is that dEpoF and dEpoB may cause a 23%-29% drop in body weight in nude mice during treatment without causing lethality, whereas only a 14-20% drop in body weight associates with the administration of EpoB or aza-EpoB led to lethality. Furthermore, tumors disappeared completely with dEpoB and dEpoF without lethality, whereas EpoB or aza-EpoB caused lethality with only marginal therapeutic effects. The origin of the toxicity encountered in the epoxide-containing epothilones is yet to be determined.

Overall, EpoD exhibited the broadest and most efficacious effects, while dEpoF's therapeutic profile was similar to EpoD. In sharp contrast to EpoB and aza-EpoB, both of which contain the 12,13-epoxide, the less cytotoxic EpoD exhibited a much enhanced therapeutic range because of low host toxicity. In preclinical toxicology studies, EpoD toxicities included myelosuppression and bone marrow hypoplasia recoverable in 3 weeks. Based on results in dogs, the dose for first use in humans was set at 9 mg/m^2 (Hoch U, etal, AACR02, Abs. 2118:426).

In preclinical effectiveness evaluations, EpoD was as or more effective than paclitaxel in reducing the size of paclitaxel-sensitive human tumors implanted in nude mice. Also, EpoD exhibited potent or curative effects in paclitaxel-resistant human tumors implanted in nude mice while, in the same models, paclitaxel exhibited only moderate or minor effects, and aza-EpoB showed little therapeutic effect. In all tumors tested, EpoD showed superior therapeutic effects compared to other anticancer agents such as doxorubicin, vinblastine, camptothecin and etoposide. EpoD was well tolerated at therapeutically effective doses. A lower frequency of MDR occurred after cells were treated with EpoD than with paclitaxel (Chou T-C, etal, PNAS USA, 3 July 2001; 98(14): 8113-18).

In October 2001, Kosan initiated a phase I dose-escalation clinical trial at UCLA School of Medicine (Los Angeles, CA), to evaluate the safety, pharmacokinetics and pharmacodynamics of EpoD in patients with advanced, refractory solid tumors. Study objectives are to determine the toxicity and pharmacokinetics of escalating doses of KOS-862, administered every 3 weeks via IV infusion (150 cc/hour). Also, the study analyzed plasma specimens and evaluated tubulin polymerization in peripheral blood mononuclear cells (PBMC). Among 5 patients (colon cancer =2, and testicular, hepatocellular, and prostate cancer), early dose levels (9 and 18 mg/m^2) produced no DLT so dose escalation was continued. Toxicities of mild-to-moderate severity included emesis, and anemia. Following IV infusion, plasma concentrations of KOS-862 declined rapidly in a biphasic pattern with an elimination half-life of 5-10 hours. Evidence of increased tubulin polymerization in PBMC was observed 1-hour postdose at 9 mg/m^2 (Peter J Rosen PJ, etal, ASCO02, Abs. 413:104a).

In October 2001, the USPTO issued Kosan patent # 6,303,342 that claims methods for producing EpoD in recombinant host cells. In April 2001, the USPTO issued patent # 6,204,388, which is exclusively licensed to Kosan by the Memorial Sloan-Kettering Institute for Cancer Research. The patent claims methods relating to the *de novo* chemical synthesis of epothilones, including epothilone D and analogs, invented by Dr. Samuel J. Danishefsky and his colleagues at MSKCC.

ZK-EPO, a synthesized analog of natural epothilone is under development by Schering AG, in collaboration with metaGen Pharmaceuticals (Berlin, Germany), a public company, partially owned by Schering. ZK-EPO is a novel epoxide with potent tumor growth inhibitory properties, superior to EpoB. ZK-EPO was selected out of more than 350 synthesized analogs of the natural compounds based on its outstanding tumor growth inhibitory properties. It is suggested that the growth inhibitory potency of ZK-EPO may be attributed to its strong intracellular accumulation and preferential localization in the nuclear compartment (Klar U, etal, AACR02, Abs. 3924:791).

Eleutherobin

Eleutherobin, an extract from the West Australian soft coral found in the waters of India and Australia, was also shown to be a potent spindle poison (see FO p 1038). The complete synthesis of eleutherobin was achieved in 1997 by K. C. Nicolaou's laboratory at Scripps Research Institute. However, limited availability of the natural product and low yields of synthesized analogs have discouraged development of this agent as a cytotoxic.

FR182877

FR182877 (formerly WS9885B), under development by Fujisawa Pharmaceutical (Osaka, Japan), was isolated

from the fermentation broth of *Streptomyces sp. # 9885*, during the course of screening for novel cell-cycle inhibitors. FR182877 promotes the assembly of microtubules *in vitro* and displays cytotoxicity as potent as paclitaxel against several cancer cell lines.

When first reported, FR182877 was an unprecedented structural type, although it does share a close constitutional relationship with the more recently described natural product hexacyclinic acid. Interestingly, hexacyclinic acid and FR182877 can be reduced to the same alternating sequence of six acetate and four propionate units. The first synthesis of the reported stereostructure for FR182877 was accomplished at the Skaggs Institute for Chemical Biology, at Scripps Research Institute (Vosburg DA, etal, J Am Chem Soc 2002;124:4552-4553). It turns out that it was the unnatural enantiomer of FR182877 that was actually synthesized. Nevertheless, this strategy was applied to a gram-scale synthesis of the natural isomer (-)-FR182877.

Comparative microtubule-stabilization studies with both mirror image forms of FR182877 are ongoing. Investigators at the Evans Laboratory at Harvard University (Cambridge, MA) also recently published a synthesis of (-)-FR182877 via a nearly identical strategy. These approaches should generate significant quantities of this natural product and analogous compounds (Erik Sorensen, PhD, Associate Professor, Department of Chemistry and Skaggs Institute for Chemical Biology, Scripps Research Institute, private communication).

Halichondrin B

Halichondrin B, a complex macrolide polyether, is a potent cytotoxic derived from marine sponges by researchers at the NCI, using the standard agent database, the COMPARE algorithm. Halichondrin B was specifically examined for interactions with tubulin and for antimitotic activity, and such properties were confirmed. Halichondrin B noncompetitively inhibited binding of vinblastine to tubulin, and thus may bind at a unique site on the protein. As usual, scarcity of the natural product hampered efforts to develop halichondrin B as a new anticancer drug, until discovery of a complete synthetic route allowed synthesis of structurally simpler analog that retain the remarkable potency of the parent compound.

E7389, previously ER-086526, also NSC-707389, is a synthetic macrocyclic ketone analog of halichondrin B. E7389 exerts its highly potent *in vitro* and *in vivo* anticancer effects via tubulin-based antimitotic mechanisms which are indistinguishable from those of parental halichondrin B (Towle MJ, etal, Cancer Res, 1 Feb 2001;61(3):1013-21).

In a preclinical study of E7389, conducted on Beagle dogs and Fischer 344 rats, most toxicities were reversible (Tosca P, etal, AACR02, Abs. 5422:1095). It was also shown that E7389 induces prolonged mitotic block that leads to apoptosis (Kuznetsov G, etal, AACR02, Abs. 1318:265).

Halimide

Halimide, a novel aromatic alkaloid natural product obtained after saline fermentation of *Aspergillus sp.*, a novel marine fungus collected in the waters off the Philippines, was discovered at Dr. William Fenical's laboratory at Scripps Institution of Oceanography (see FO, p 1038). Originally licensed to Bristol-Myers Squibb in 1998, it was subsequently picked up by Nereus Pharmaceuticals (San Diego, CA).

Halimide is advantageous over clinically active tubulin depolymerizers such as vinca alkaloids, because it is not cross-resistant in MDR human colon carcinoma cells. *In vivo* antitumor studies found a 53% increase in lifespan in an intraperitoneally injected P388 murine leukemia model (Fairchild CR, etal, AACR98 Abs. 1131:165).

NPI-2352 and NPI-2358, under development by Nereus Pharmaceuticals, are analogs of NPI-2350, a halimide that showed potent, dose-dependent cytotoxic activity *in vitro* against the NCI 60 tumor cell screen and was selected by the NCI for advanced *in vivo* studies. NPI-2350 exhibited significant activity in multiple murine tumor models.

Nereus has an exclusive worldwide license from the University of California at San Diego for NPI-2350. Three patents have issued on the composition of matter and use of halimide and analogs as anticancer agents.

Mechanism of action studies indicate that NPI-2350 blocks growth of carcinoma cells by inhibiting tubulin polymerization at sites unrelated to paclitaxel and vincristine binding. Additional studies demonstrated that NPI-2350 hyperphosphorylates Bcl-2 and plans are set in motion to evaluate its effects on apoptosis. NPI-2350 exhibited activity in human colon carcinoma cells resistant to vincristine. It was also active in a paclitaxel-resistant cell line with altered tubulin and exhibited significant activity in multiple murine tumor models.

Through focused structure activity relationship studies, two modifications were introduced in the halimide structure that resulted in the generation of new potent analogs, NPI-2352 and NPI-2358, with greatly enhanced activity compared to the parent compound. These analogs retained their activity in human and murine MDR tumors that overexpress P-gp. Additional patent applications have been filed on these new families of molecules. NPI-2352 and NPI-2358 have been synthesized in gram quantities and are currently in preclinical testing in mice using human tumor xenografts.

Hemiasterlins

Hemiasterlins are natural tripeptides extracted from marine sponges *Auletta sp.* and *Siphonochalina spp.*, that induce microtubule depolymerization and mitotic arrest in cells. Three hemiasterlins have been identified, hemiasterlin, hemiasterlin A, and hemiasterlin C. In a comparative assay for inhibition of tubulin polymerization, the hemi-

asterlins were more potent than dolastatin 15, and equipotent with cryptophycin 1, but were somewhat less potent than dolastatin 10 (Gamble WR, etal, Bioorg Med Chem 1999:7:1611-15).

HTI-286, a synthetic analog of hemiasterlin, potently inhibits growth of cultured tumor cells, overcomes resistance to paclitaxel mediated by various mechanisms, and demonstrates intravenous and oral *in vivo* efficacy. HTI-286 is stable, soluble in saline, inhibits tubulin polymerization, overcomes resistance mediated by drug transporter overexpression, and tubulin mutations, and demonstrates *in vivo* efficacy by IV or PO administration.

According to investigators at Wyeth Ayerst Research (Pearl River, NY), HTI-286 inhibits polymerization of purified tubulin in cell-free assays and depolymerizes cellular microtubules. HTI-286 potently inhibited proliferation in 29 tumor cell lines. Also, cell lines (HCT15, DLD1, MX1), inherently resistant to paclitaxel, retained sensitivity to HTI-286. Consistent with other antimicrotubule agents, HTI-286 induces mitotic arrest and apoptosis. HTI-286 retains potency in tumor cell lines that overexpress drug transporters associated with MDR, MRP, or MXR resistance mechanisms and also overcomes resistance in four ovarian cell lines resistant to paclitaxel or epothilone associated with amino acid mutations in the taxane-binding site of tubulin. In xenograft tumor models, HTI-286 administered IV in saline, produced >95% growth inhibition of melanoma Lox and epidermoid KB tumors. Equivalent activity is also observed against large (1 gram) tumors. Moreover, HTI-286 inhibits growth of xenograft models where paclitaxel and vincristine are ineffective. Efficacy is also attainable upon oral administration of HTI-286 (Loganzo F, etal, AACR02, Abs. 1316:265).

Laulimalide

Investigators at the Cancer Research Center of Hawaii (Honolulu, HI) and Utah State University (Provo, UT) purified a crude extract from a Marshall Island marine sponge yielding laulimalide and isolaulimalide, a pair of macrocyclic compounds with microtubule stabilizing, paclitaxel-like activity (see FO, p 1039). Successful synthesis of laulimalide has allowed investigators to assess its anticancer activities in preclinical evaluations.

Laulimalide induces *in vitro* tubulin assembly, and, under certain reaction conditions, promotes nearly identical assembly reactions as paclitaxel, and epothilone A whether or not GTP or microtubule-associated proteins are present in the reaction mixture. In a quantitative assay for polymer formation in glutamate, however, laulimalide was about half as active as the other two drugs. Investigators are currently evaluating the ability of laulimalide to inhibit the binding of paclitaxel to tubulin and the effects of the drug on paclitaxel-resistant, epothilone A/B-resistant, and MDR cancer cells (Pryor DE, etal, AACR02, Abs. 5740:1158).

Maytansinoids

The maytansinoids are a group of ansa macrocyclic lactams first isolated from *Maytanus* species and from members of two other plant families. Subsequently, they were also discovered to be metabolites of a soil bacterium, the Actinomycete *Actinosynnema pretiosum*. Maytansine, a polyketide that inhibits cell division, is one of the most potent cell toxins known. Although this agent proved too toxic for human use, one synthesized maytansinoid, DM1, is currently being clinically evaluated as part of an immunoconjugate-prodrug construct, developed by ImmunoGen (Cambridge, MA). DM1 was synthesized from ansamitocin P3 (AP3) the key precursor for all the DM1-containing drugs in development. DM1, is 100-fold more cytotoxic than Vinca alkaloids such as vincristine and vinblastine, and about 50-fold more potent *in vitro* than paclitaxel against the human tumor cell lines MCF-7, A-431 and A-549.

DM1 has been chemically linked to monoclonal antibodies (MAb) that specifically bind to cancer cells for targeted delivery using the proprietary Tumor-Activated Prodrug (TAP) technology, developed by ImmunoGen. DM1 contains a disulfide substitute that renders it linkable to a MAb via disulfide bonds that are stable in circulation *in vivo*, but are cleaved inside a tumor cell to release active drug. DM1 was synthesized because commercially available taxanes are not sufficiently potent for use in TAP, and do not contain an appropriate chemical moiety for covalent attachment to a MAb. Once the MAb binds with its antigenic target on the cancer cell, the construct is ingested by the cell and, once inside, DM1 is activated, destroying the cell. In this way, potent anticancer drugs are exclusively delivered to the cancer cells, leaving normal cells intact.

ImmunoGen acquired the right to evaluate, and an option to license technology related to maytansines from Takeda Chemical Industries (Tokyo, Japan) which holds several patents for the production of ansamitocin, and its analogs, the first one being JP-53124692. Also, ImmunoGen was awarded a European patent # EP-00425235, covering conjugated forms of ansamitocin derivatives.

In December 2001, Industrial Research (Aukland, New Zealand), a contract manufacturer focusing on novel secondary metabolites, won the contract to provide AP3. Industrial Research will develop the process to produce enhanced yields of AP3 with a new production strain and scale up to produce enough clinical material through 2005. In July 2002, Kosan Biosciences was awarded a phase I SBIR grant from the NCI to use its proprietary gene engineering technology to develop economical methods to produce maytansine analogs and to enhance such analogs with features that will allow better control of their delivery.

ImmunoGen has licensed its TAP technology to several developers of anticancer agents and is collaborating with others to develop novel agents for the treatment of cancer,

based on this technology. The attractive property of TAP is that it may be linked to MAb targeting a variety of cell markers for the treatment of a broad range of malignancies.

Abgenix is developing fully human MAb generated with XenoMouse technology, to be used with maytansinoid TAP technology. The XenoMouse technology platform was originally developed by Xenotech, a limited partnership that was jointly owned by Cell Genesys (Foster City, CA) and JT America, Japan Tobacco's (Tokyo, Japan) USA subsidiary. The TAP technology was licensed from ImmunoGen in September 2000. ImmunoGen is eligible for $5 million in technology access fee payments, as well as potential milestone payments, and royalties on net sales of any commercialized products. In addition, Abgenix purchased $15 million of ImmunoGen common stock at $19.00 per share. This 10-year agreement provides Abgenix with a broad license to use ImmunoGen's maytansinoid TAP platform in its antibody product research efforts, and an option to obtain product licenses for a large number of antigen targets over the agreement's term. Abgenix will be responsible for manufacturing, product development and marketing of any products developed through the collaboration. ImmunoGen may produce preclinical and clinical material, at Abgenix's request, for a manufacturing payment.

Bivatuzumab mertansine (BIWI1) consists of an anti-CD44v6 antibody, contributed by Boehringer Ingelheim (Ingelheim, Germany), combined with ImmunoGen's maytansinoid TAP technology. Under terms of an agreement, entered in November 2001, Boehringer Ingelheim obtained exclusive worldwide rights to commercialize maytansinoid TAP using a MAb targeting CD44v6. Boehringer Ingelheim is responsible for the manufacturing, product development and marketing of any products resulting from this license. ImmunoGen is being paid to manufacture preclinical and initial clinical materials, and received an upfront payment and will receive milestone payments, in addition to royalties on net sales of any commercialized products. Financial terms were not disclosed.

CD44v6 belongs to a family of transmembrane glycoproteins responsible for cell-cell and cell-matrix interactions, and represents a basis for physiologic and pathologic processes, especially for homing behavior, homeostasis of the immune response, inflammation, and tumor metastasis. Expression of CD44v6 has been associated with malignant behavior such as invasive growth and formation of metastasis in various tumors including head and neck, colorectal, prostate, pancreatic, laryngeal, and gastric cancer, nsclc, multiple myeloma, basal cell carcinoma, and osteogenic sarcoma.

Cantuzumab mertansine (SB-408075, huC242-DM1) is humanized MAb huC242 directed against a mucin-type tumor-associated glycoprotein, conjugated to DM1, under development by ImmunoGen, in collaboration with GlaxoSmithKline.

Humanization of the murine antibody C242 was accomplished through variable domain resurfacing, in which the set of surface residues of a murine variable region was replaced with a human set of surface residues (Roguska MA, Protein Eng, Oct 1996;9(10):895-904; published erratum appears in Protein Eng, Feb 1997;10(2):181).

In August 1997, ImmunoGen received an SBIR grant to advance development of huC242-DM1. Also, in July 1998, ImmunoGen executed an agreement with Pharmacia under which it obtained rights to commercialize products that incorporate the C242 antibody for the treatment of cancer, in exchange for a royalty on product sales and other payments.

Preclinical dose-ranging studies were performed in both mice and cynomolgus monkeys, to evaluate toxicity, pharmacokinetics and *in vivo* stability. A high concentrations of active C242-DM1 was maintained in the serum of monkeys for 6 days without toxicity. These drug levels were similar to those measured in mice treated with doses that were 100% curative in colon cancer xenograft models. Clearance of C242-DM1 was the same as that of unmodified C242 antibody (Lambert JM, etal, AACR98, Abs 3550:522).

The antitumor activity of C242-DM1 was evaluated in human xenograft models of pancreatic cancer and nsclc using SCID mice. At doses ranging between 35% to 60% of MTD, C242-DM1 caused complete tumor regression lasting 28 to 50 days in BXPC-3 and SW1990 colon tumor cell lines, respectively. In the SU.8686 colon tumor cell line, 50% of the animals were cured and a CR lasting 92 days was observed in the remaining animals. In mice bearing human lung adenocarcinoma H441 xenografts, treatment with C242-DM1 at a dose of about 30% of MTD, resulted in complete tumor regression lasting 84 days (Chari RV, etal, Suppl Clin Can Res, Nov 1999;5:Abs. 462).

In vivo studies demonstrate that high circulating levels of C242-DM1 result in good tumor penetration and exceptional antitumor activity, as evidenced by the eradication of large human colon tumor xenografts in murine models. *In vitro*, C242-DM1 has shown to be 1000-fold more specific for killing target colon tumor cells as compared to nontarget cells. In a comparative study, C242-DM1 cured mice bearing human colon tumor xenografts at doses that were well below MTD, while the current chemotherapeutics of choice, 5-FU and CPT-11, even when used at their MTD, only caused a modest delay in tumor growth (Chari RV, etal, AACR98, Abs. 4382:643). Efficacy in tumors with heterogeneous antigen may be attributable to a bystander effect, conferred by MDR efflux transporters, and sporadic antigen expression by apparently antigen-negative cells.

ImmunoGen filed an IND in September 1999 to initiate its first phase I clinical trial with huC242-DM1 in the treatment of patients with refractory cancer. This trial was initiated in December 1999 at the Institute for Drug Development of the Cancer Therapy and Research Center

(San Antonio, TX) under PI Anthony Tolcher, MD. The trial was designed to establish the safety of huC242-DM1 in a single-dose format. Among 23 patients with solid tumors, 66 courses of SB-408075 were administered at 22, 44, 88, 132, 176, 235, and 295 mg/m^2. Dose-limiting, Grade 3, hepatic transaminase elevations occurred in 2 of 3 patients treated at 295 mg/m^2. Cumulative Grade 1 neurosensory changes were observed in 6 patients at doses ≥132mg/m^2. Other toxicities included Grade ≥2 nausea and vomiting (n=9), dose-related Grade 1 myalgia/arthralgia (n=6), and Grade 1 neutropenia (n=2). A single Grade 2 hypersensitivity reaction occurred at 22 mg/m^2 but premedication permitted rechallenge. There was no evidence of human antihuman antibody (HAMA) reactions. There was one minor response, disease stabilized in 2 (6, 6+ months), and CEA decreased significantly in 4 patients with high C242 expression (Tolcher AW, etal, ASCO01, Abs 273:69a).

In this first clinical trial, 37 patients (median prior regimens=3) with solid tumors including colorectal (n=32), pancreatic (n=4), lung (n=1) cancer with documented antigen expression, were treated with doses ranging from 22-295 mg/m^2 every 3 weeks. Dose-limiting transaminitis and the extent of hepatic metastases, precluded tolerance to doses >235 mg/m^2. Hepatic, hematologic, and neurosensory effects occurred, but were rarely severe with repetitive treatment at doses ≤ 235 mg/m^2. There were 2 MR; disease stabilized in >6 courses in 4 patients, and CEA declined in 9 patients.

Post-treatment tumor biopsies revealed intracellular immunoreactivity for DM1, concurrent with free antigen immunoreactivity, suggesting SB-408075 penetration and DM1 release in the face of unsaturated antigen. These findings, plus a strong relationship between hepatic toxicity and Cmax, and shed antigen levels decreasing by 90% (median) after the first dose, provided the rationale for a 3-times weekly schedule repeated 3 times every 4 weeks. DLT had not been consistently noted in 13 patients treated with a daily dose of 30-60 mg/m^2, which represents as much as a 230% increase in dose intensity compared with the single-dose schedule (Rowinsky EK, etal, ASCO02, Abs. 118:30a).

In September 2000, a second phase I/II clinical trial, using a weekly dosing regimen, was initiated at the University of Chicago Cancer Research Center (Chicago, IL) under the direction of Richard L. Schilsky, MD. The objectives of the phase I portion of this trial are to determine MTD and DLT of SB-408075, administered as a single IV infusion weekly with 3 weeks of treatment constituting one cycle. Patients are re-evaluated every two cycles. Weekly doses studied range from 40 mg/m^2 to 138 mg/m^2.

Among 17 patients (colon cancer=12, pancreatic cancer=2, unknown primary=2, and lung cancer=1) enrolled, 15 were evaluable for DLT/MTD. Mild to moderate toxicities included elevated liver enzymes, anemia, diarrhea, and nausea. Both fatigue and peripheral sensory neuropathy were observed that appeared to be dose-related and cumulative. One patient experienced an apparent hypersensitivity reaction during initial treatment. DLT occurred in 2 patients at the 138 mg/m^2 weekly dose level, one patient experienced Grade 3 elevation of liver enzymes after one dose and the other Grade 3 fatigue lasting 3 days after 3 doses. Disease progressed in 12 patients. Disease stabilized in one patient after six cycles, but the patient stopped the study medication for personal reasons. Clinical resolution of ascites occurred in 1 patient with peritoneal carcinomatosis associated with an unknown primary and chronic ascites and no measurable disease, after five weeks of treatment; this patient remained on the study for 25 weeks. Disease also stabilized as indicated by CT scan in 1 patient with pancreatic cancer after 18 weeks and a fall in CA19-9 from 1880 U/ml to 380 U/ml during the study. The recommended phase II dose is 115 mg/m^2/week and accrual is continuing in an expanded cohort at this dose level until 15 colon cancer patients and 5 pancreatic cancer patients have been treated (Helft PR, etal, AACR-NCI-EORTC01, Abs. 657).

In May 2001, a third phase I/II human clinical study with SB-408075 was initiated in colorectal and pancreatic cancer and nsclc, to evaluate this agent in a more dose-intensive regimen. The trial is being conducted at the Institute for Drug Development of the Cancer Therapy and Research Center (CTRC), under the direction of Anthony W. Tolcher, MD, and Eric K. Rowinsky, MD.

In June 2002, GlaxoSmithKline elected not to advance cantuzumab mertansine into phase II clinical trials under the present terms of the license agreement, which ImmunoGen plans to renegotiate. However, should ImmunoGen determine that it is not in the best interests of the company to enter into a revised agreement with GlaxoSmithKline, rights to cantuzumab mertansine would be returned to ImmunoGen, and the company would be free to develop and relicense the product as it sees fit. The agreement between the two companies to commercialize huC242-DM1 TAP was forged in February 1999. Under terms of the agreement, in addition to royalties, ImmunoGen's total revenues including upfront and milestone payments could have totaled more than $40 million. Under terms of the agreement, GlaxoSmithKline received exclusive worldwide rights to commercialize huC242-DM1, except in certain Far East territories. ImmunoGen was responsible for the product's initial assessment in humans. As of September 2000, the agreement with GSK generated payments totaling $14 million for five achieved milestones.

huN901-DM1/BB-10901TAP, is an anti-CD56 humanized MAb huN901 conjugated to DM1 using TAP technology. CD56 is a membrane-bound glycoprotein present on small-cell lung cancer (sclc), neural-derived tumors, myeloma, and myeloid leukemias (Rutishauser U, etal, Science, 1 Apr 1988;240(4848):53-7). In this construct, humanized MAb huN901, directed against CD56 is conju-

gated by disulfide links to about 3.5 molecules of DM1. Humanization of the murine antibody N901 was accomplished through variable domain resurfacing, in which the set of surface residues of a murine variable region is replaced with a human set of surface residues.

In May 2000, British Biotech (Oxford, UK) and ImmunoGen entered into a collaborative agreement to develop and commercialize huN901-DM1 for the treatment of sclc. British Biotech paid $1.5 million for the exclusive rights to commercialize huN901-DM1 in the European Union (EU) and Japan. ImmunoGen retained the rights to commercialize huN901-DM1 in the USA and the rest of the world. ImmunoGen is responsible for completing the preclinical development and the manufacturing and supply of materials for clinical trials and commercial sales. British Biotech is responsible for conducting clinical trials necessary to achieve regulatory approval in the USA, EU and Japan, and reimburses ImmunoGen for the cost of clinical trial supplies. In the event that ImmunoGen obtains regulatory approval for marketing the product in the USA, British Biotech will receive a one-time milestone payment, while ImmunoGen will receive royalties on sales made in the EU and Japan.

In December 2000, Genzyme Transgenics, now GTC Biotherapeutics (Framingham, MA), signed an agreement to produce MAb huN901 in specially bred goats that express this humanized antibody in their milk. The antibody will then be purified from the milk.

In vitro studies with huN901-DM1 demonstrated high tumor-antigen specific cytotoxicity against several human sclc cell lines. In murine models bearing human sclc xenografts, huN901-DM1 eradicated tumors for the duration of the study (200 days). Additional preclinical testing of huN901-DM1 for the treatment of sclc was conducted in nonhuman primates.

In preclinical studies, BB-10901 was combined with chemotherapeutic agents acting on different mechanisms. Low, noncurative doses of huN901-DM1 combined with paclitaxel, completely eradicated human sclc xenografts in mice. These results were a significant improvement over either low-dose huN901-DM1, or paclitaxel at MDT used alone, or the currently used combination of cisplatin and etoposide. In preclinical studies, BB-10901 eradicated sclc tumors while, under the same experimental conditions, other chemotherapies used to treat sclc, such as cisplatin and etoposide, produced only a temporary interruption of tumor growth (Chari RV, etal, AACR00, Abs. 4405:693, and Liu C, etal, AACR97, Abs. 190:29).

In May 2001, patient enrollment was initiated in a phase I/II clinical trial to evaluate the safety, pharmacokinetics and biologic activity of BB-10901/huN901-DM1 in sclc, and other neuroendocrine tumors expressing CD56. The phase I segment of this open-label, dose-ranging clinical trial is testing increasing doses of BB-10901 to evaluate the safety and MTD of the drug. Patients are treated once-weekly with IV BB-10901 for 4 weeks, followed by two weeks off, which defines one cycle of treatment that may be repeated in eligible patients. Phase II clinical trials will assess the drug's biologic activity specifically in patients with relapsed sclc who had been treated with only 1 prior chemotherapy regimen. Approximately 80 patients who have failed other treatment options are expected to participate in this study, being conducted by Frank V. Fossella, MD, at the M. D. Anderson Cancer Center, and by Anthony W. Tolcher, MD, at the Institute for Drug Development of the Cancer Therapy and Research Center (CTRC).

In this trial, starting dose was 5 mg/m^2, weekly, for 4 weeks every 6 weeks, with 3-4 patients treated per cohort until MTD, and then 6 patients treated at MTD. Among 13 patients treated at weekly doses of 5 mg/m^2 (n=4), 10 mg/m^2 (n=3), 20 mg/m^2 (n=4), and 40 mg/m^2 (n=2), there were 9 patients with sclc, 3 with neuroendocrine tumors of the lung, and 1 with a neuroendocrine tumor of the pancreas; 6 had refractory disease, 6 relapsed disease, and 1 with a neuroendocrine tumor of the lung was untreated. Regarding results, disease stabilized in 2 patients after cycle 1, but progressed after cycle 2; disease progressed in 8 patients after cycle 1, and it was too early to evaluate the remaining 3. No significant toxicity was seen up to a weekly dose of 40 mg/m^2. The trial continues at the 40 mg/m^2 weekly dose level (Fossella FV, etal, ASCO02, Abs. 1232:309a).

Subsequently, 23 patients were enrolled in the study. Of these, 11 had relapsed after responding to earlier chemotherapy treatment, 9 were refractory to previous chemotherapy, and 3 with neuroendocrine tumors had not been treated. Dosing was completed at the 5, 10, 20 and 40 mg/m^2 levels and patient recruitment and dosing at the fifth level of 60 mg/m^2 is continuing. Repeated cycles of BB-10901 at doses up to and including 40 mg/m^2 were well tolerated. No DLT was seen at these levels, nor has there been any evidence to date of hematologic or cardiac toxicity. At the 60 mg/m^2 dose level, two patients experienced DLT events, but it is unclear if these were drug-related. Pharmacokinetic analysis has found the agent to have a half-life of approximately one day at the 40 mg/m^2 dose level. One patient experienced a transient PR at the 40 mg/m^2 dose level, another patient an MR at the 60 mg/m^2 dose level, and disease stabilized in two other patients. A phase II clinical trial will be initiated following this phase I study, specifically in relapsed sclc previously treated with only 1 chemotherapy regimen.

In August 2002, British Biotech initiated a second open-label, dose-escalation phase I clinical trial of BB-10901 to evaluate the effects of the drug when administered on a more frequent dosing regimen, after being granted a Clinical Trials Exemption (CTX) by the Medicines Control Agency (UK). This trial is being conducted at the Christie Hospital (Manchester, UK) under the direction of Dr. Paul Lorigan and Dr. Malcolm Ranson of the Department of Medical Oncology, and at the Nottingham City Hospital, under the direction of Professor James

Carmichael and Dr. Penella Woll, to assess the safety, tolerability, and pharmacokinetics of increasing doses of huN901-DM1/BB-10901 and its biologic activity. The drug is being administered daily for three successive days, followed by an 18-day follow-up period. As in the phase I clinical trial, conducted in the USA, patients will be recruited with relapsed or refractory sclc, or other tumors that express the CD56 antigen. Dose will be increased in each new cohort of patients until DLT and establishment of MDT. The study is expected to be completed by mid-2003.

MLN591DM1 (anti-PSMA-DM1) is an immunoconjugate consisting of MLN591 MAb directed at the extracellular domain of prostate-specific membrane antigen (PSMA), and the TAP-DM1 component. In February 2002, ImmunoGen and Millennium Pharmaceuticals (Cambridge, MA) signed an exclusive product license to use ImmunoGen's proprietary maytansinoid TAP technology with Millennium's MLN591 antibody. In March 2001, ImmunoGen had entered into an agreement to provide Millennium access for a period of 5 years to its TAP technology to use in Millennium's antibody research efforts. At that time, Millennium had also obtained an option for exclusive product licenses for a restricted number of antigen targets during the collaboration. Terms of this agreement involved an undisclosed upfront technology access fee, potential milestone payments per antigen target, and royalties on net sales of any resulting products. Millennium is responsible for product development, manufacturing and marketing of any products resulting from this collaboration. ImmunoGen may be paid to produce preclinical and clinical material at Millennium's request.

Millennium obtained rights to MLN591 MAb in April 2001, through an agreement with BZL Biologics (Framingham, MA) to develop and commercialize antibody-based therapeutics targeting PSMA, encompassing both immunotoxin and radiolabeled products. Although the primary indication expected for products targeting PSMA is prostate cancer, this target may also be associated with other solid tumors. According to the terms of the agreement, Millennium and BZL will jointly develop products for the prostate cancer indication until a predetermined clinical decision point. Thereafter, Millennium will have full responsibility for development, manufacturing and commercialization of all antibody-based therapies for all indications, as well as all diagnostic products. Millennium will be responsible for all development costs. In addition, BZL will be entitled to milestone payments and royalties based on net sales of any marketed products. Additional financial terms were not disclosed.

BZL Biologics obtained rights to J591 MAb from Cornell University (Ithaca, NY). Researchers at New York Hospital-Cornell Medical Center (New York, NY) characterized 4 MAb, E99, J415, J533, and J591, that bind to the extracellular domain of PSMA (Liu H, etal, Cancer Res 1997;57:3629-3634). In August 2000, the USPTO issued Cornell University patent # 6,107,090, entitled "Treatment and Diagnosis of Prostate Cancer". The issued patent claims MAb that bind to the extracellular domain of prostate-specific membrane antigen (PSMAext) on living cells as both therapeutic and diagnostic agents for prostate cancer. These MAb may be used unmodified or as a targeting antibody for radioisotopes and/or cytotoxic drugs. BZL holds the exclusive worldwide license to this technology.

Murine J591 MAb was humanized by Biovation (Aberdeen, Scotland) using its proprietary DeImmunization method involving specific deletion of human B- and T-cell epitopes. DeImmunization avoids retention of immunogenic epitopes in the final MAb, thus limiting any undesirable immune reactions against the humanized MAb. Humanized MAb binds to PSMA on LNCap cells as efficiently as the original murine MAb, and should have little or no immunogenicity in man (Hamilton A, etal, AACR98, Abs. 2997:440).

The murine version of J591 MAb underwent a phase I clinical trial in 2000, in advanced, hormone-refractory prostate cancer, to evaluate its targeting attributes. In this trial, 33 patients were treated with a single dose, ranging from 0.5 to 250 mg, of the murine deimmunized form of the MAb, tagged with a radioactive tracer. Patients were imaged every other day for a week to track the antibody. Localization for both bone and soft tissue metastatic prostate cancer was demonstrated in approximately 80% of the patients.

MAb J591 has been conjugated to ^{111}indium, ^{90}yttrium, and ^{177}lutetium for therapeutic and imaging applications. Radioimmunoscintigraphy with this immunoconjugate has demonstrated excellent tumor targeting of prostate cancer sites not only in soft tissue but also in bone (Yao D, etal, Semin Urol Oncol, Aug 2002;20(3):211-218). This MAb is also currently being evaluated in a phase II clinical trial, in combination with interleukin 2 (IL-2), in the treatment of recurrent prostate cancer.

My9-6-DM1 is a tumor-activated prodrug produced by linking the MAb My9-6, which targets myeloid leukemia cells, with DM1. My9-6-DM1 was highly cytotoxic *in vitro* in THP-1 cells while antigen-negative Namalwa cells were at least 50-fold less sensitive to the conjugate. By contrast, the two cell lines were equally sensitive to the nonconjugated DM1 drug. My9-6-DM1 conjugate was the least toxic in mice in these studies (Goldmacher VS, etal, AACR02, Abs. 1265:254).

In preclinical studies, treatment of SCID mice bearing established subcutaneous HL-60 tumor xenografts with My9-6-DM1 resulted in complete eradication of the tumors at doses well below MDT that were not associated with serious toxicity. In contrast, treatment with My9-6 antibody alone had no effect on HL-60 tumor growth compared to vehicle control. Similar results were obtained when mice bearing subcutaneous THP-1 xenografts were treated with My9-6-DM1 (Lutz RJ, etal, AACR02, Abs. 4518:912).

Trastuzumab-DM1 (Herceptin-DM1) is an immunoconjugate consisting of trastuzumab linked to DM1 using TAP technology. The construct, which contains ~3.5 moles DM1 per mole of antibody, is an inactive prodrug until it binds to cell-surface HER2 on cancer cells, becomes internalized, and releases active DM1 intracellularly.

In May 2000, ImmunoGen and Genentech (South San Francisco, CA) entered into a 5-year agreement providing Genentech with broad access to ImmunoGen's TAP technology platform, including an option to obtain exclusive product licenses for a limited number of antigen targets. Genentech agreed to pay an upfront technology access fee of $3 million and make potential milestone payments, assuming benchmarks are met, of up to nearly $40 million per antigen target, and also pay royalties on net sales of resulting products. Genentech is responsible for manufacturing, development and marketing of any products arising from this collaboration, while ImmunoGen is to be reimbursed for any preclinical and clinical materials that it produces under the agreement. This agreement may be renewed for one subsequent 3-year period, for an additional technology access fee.

In *in vitro* efficacy studies in breast cancer, trastuzumab-DM1, after receptor-mediated endocytosis, induced a significant incidence of cell death among breast tumor cells expressing 1+ to 3+ HER2 levels. A 2-hour exposure to trastuzumab-DM1 resulted in substantial cell killing. Also, this immunoconjugate was cytotoxic on actively dividing cells but had no effect on growth-arrested cells. To determine the mechanism of induction of cell death by trastuzumab-DM1, breast tumor cells were pretreated with ZVAD-fmk, a caspase inhibitor, and then exposed to trastuzumab-DM1. Cytotoxic effects of trastuzumab-DM1 were reduced in the presence of ZVAD-fmk, suggesting that trastuzumab-DM1 is involved in apoptosis. Tumor cells expressing normal levels of HER2, as well as normal cells are not affected by trastuzumab-DM1 (Lewis Phillips GD, etal, AACR-NCI-EORTC01, Abs 664).

Trastuzumab-DM1 exhibited better activity than Herceptin alone in two Herceptin-responsive and one Herceptin-resistant breast tumor models. When trastuzumab-DM1 was injected into nude mice bearing tumors from MCF7.HER2 cells that express high levels of HER2, complete tumor regression was observed in all mice, whereas Herceptin alone only slowed tumor growth. The same results were obtained with tumors arising from MDA-MB-361 cells, which naturally express high levels of HER2. Trastuzumab-DM1 was also tested against a mammary tumor from an MMTV-HER2 transgenic mouse (designated HER2-Fo5), which expresses high levels of HER2 and grows aggressively when transplanted into the mammary fat pad of nontransgenic, nude mice. Herceptin, even at high doses, had no effect on transplanted tumors that were allowed to reach 100 mm3 in size before treatment began, while trastuzumab-DM1 caused tumor volume to regress by >90% in all mice within 10 days. Tumor regrowth was observed 4-6 weeks after the last dose of trastuzumab-DM1. The relapsing tumors were HER2-positive and regressed when retreated with trastuzumab-DM1 (Schwall RH, etal, AACR-NCI-EORTC01, Abs 652).

PC-SPES

PC-SPES, originally manufactured by BotanicLab (Brea, CA), is a combination of eight herbal therapies (chrysanthemum, Dyer's woad, liquorice, reishi, san-qi ginseg, rubescens, saw palmetto and Baikal skullcap) with purported activity against locally advanced and metastatic prostate cancer, both *in vitro* and *in vivo*, and in several small clinical trials. Unfortunately, reports of contamination with traces of diethylstilbestrol (DES), coumadin (warfarin), and indomethacin (Sovak M, etal, AACR02, Abs. LB152), forced the recall of PC-SPES from the market in February 2002, and the BotanicLab to shut its doors in June 2002, much to the chagrin of many prostate cancer patients using the preparation. When available, PC-SPECS was purchased without a prescription at a cost of $250-$400 per month by patients with advanced prostate cancer, refractory to traditional hormone therapies (Pandha HS and Kirby RS, Lancet, 29 Jun 2002;359:2213-14).

The exact mechanism of action of PC-SPES has not been fully elucidated. Studies in human prostate cancer cell lines demonstrated significant dose-dependent decreases in cellular viability after exposure to extracts of PC-SPES. Clinical studies suggest that PC-SPES reduces specific antigen levels in both androgen-dependent and androgen-independent prostate cancer. Toxicity is mild, though there is an approximately 5% risk of thromboembolic events with treatment (Oh WK and Small EJ, Urol Clin North Am, Feb 2002;29(1):59-66, viii).

PC-SPES suppresses expression of α- and β-tubulin in prostate carcinoma cells and modulates microtubule bundling by paclitaxel. Depletion of the β-tubulin target antagonizes the efficacy of chemotherapeutic agents, which stabilize microtubule arrays. Also, despite PC-SPES suppression of β-tubulin isotypes which might be considered resistant to microtubule stabilization, the predominant effect is that overall depletion of the β-tubulin target suppresses efficacy of microtubule stabilizing agents (Montgomery RB, etal, AACR02, Abs. 1324:266).

A review of the literature from 1966 to October 2001, and abstracts from the meetings of ASCO from 1995 to 2001, revealed that PC-SPES has been associated with biochemical and clinical response in some patients with prostate cancer. However, no randomized studies using this agent have been reported. Based on these published reports, the mechanism of action of PC-SPES appears to be related to its estrogenic activity. Because of the limited data available, PC-SPES should not be used in place of standard androgen suppression therapy in androgen-dependent prostate cancer, but may have a role for those who failed standard treatments for androgen-independent disease and have no history of thromboembolism or abnormal bleeding. PC-SPES has a toxicity profile similar to those

of androgen suppression and estrogen therapy (De Lemos ML, Ann Pharmacother, May 2002;36(5):921-6).

Peloruside A

Peloruside A is a novel antimitotic agent with paclitaxel-like microtubule-stabilizing activity, being investigated by scientists at Victoria University (Wellington, New Zealand). Peloruside A is a secondary metabolite isolated from a New Zealand marine sponge, *Mycale hentscheli*, which is cytotoxic at nanomolar concentrations. Its 16-membered macrolide ring is similar to that of epothilone. Like paclitaxel, peloruside A arrests cells in the G2/M phase of the cell cycle and induces apoptosis. The relatively simple structure of peloruside makes it suitable for the design and synthesis of analogs with improved tumor targeting properties and reduced cross resistance (Hood KA, etal, Cancer Res, 15 Jun 2002;62(12):3356-60).

Resveratrol

Resveratrol, a phytoalexin found in grapes and wines, is a novel antimitotic interacting with microtubules with potent chemopreventive properties on intestinal carcinogenesis *in vivo* (Milner JA, etal, Nutr Cancer 2001;41(1-2):1-16). This agent was shown to exhibit anticancer activity against many different malignancies, including prostate cancer (Morris GZ, etal, Prostate, 1 Sep 2002;52(4):319-29), breast cancer (Banerjee S, etal, Cancer Res, 1 Sep 2002;62(17):4945-54), esophageal cancer (Li ZG, etal, Carcinogenesis, Sep 2002;23(9):1531-6), and leukemia (Ferry-Dumazet H, etal, Carcinogenesis, Aug 2002;23(8):1327-33 and Tsan MF, etal, Leuk Lymphoma, May 2002;43(5):983-7), among others.

Investigators at the Institut de Recherche contre les Cancers de l'Appareil Digestif (IRCAD; Strasbourg, France) have synthesized a methylated derivative of resveratrol, which is 100-fold more active than resveratrol when tested on the human colon cancer cell line Caco-2. The drug inhibited by 2-fold the activities of ornithine decarboxylase and s-adenosylmethionine decarboxylase, two rate limiting enzymes of polyamine synthesis, leading to the intracellular depletion of polyamines, which fuel growth of cancer cells. It also caused cell-cycle arrest at G2/M, demonstrated very high affinity for the colchicine-binding site of tubulin, and disrupted microtubule spindles. The cis conformation was an absolute requisite for the optimal antiproliferative activity of this drug (Schneider Y, etal, AACR02, Abs. 1326:267).

S-allylmercaptocysteine (SAMC)

S-allylmercaptocysteine (SAMC) is a water-soluble garlic *(Allium sativum)* derivative. Epidemiologic and experimental carcinogenesis studies have shown that components of garlic have anticancer activity that may be attributed, at least in part, to disruption of microtubule assembly. It has been suggested that an active allyl and a disulfide moiety may be responsible for growth inhibition and induction of apoptosis exhibited by SAMC and related compounds. SAMC acts directly on tubulin to cause microtubule depolymerization, and also inhibits the initiation of *de novo* tubulin polymerization, thus arresting cells in metaphase and triggering downstream signaling pathways that lead to apoptosis (Xiao D, etal, AACR02, Abs. 1322:266). SAMC, but not S-allylcysteine (SAC), inhibits growth, arrests cells in G2/M, and induces apoptosis in SW480 and HT29 human colon cancer cells (Shirin H, etal, Cancer Res, 15 Jan 2001;61(2):725-31).

Spongistatins

Spongistatin 1 (altohyrtin A), isolated from marine sponges of the genus *Spongia*, *Spirastrella* and *Hyrtios*, is among the most potent compounds tested against a distinctive subset of highly chemoresistant tumor types in NCI's panel of 60 human cancer cell lines. Spongistatin 1 functions by inhibiting tubulin polymerization.

Rationally designed spiroketal pyrans (SPIKET) that target the spongistatin-binding site of β-tubulin, were synthesized by Fatih Uckun, PhD, at the Parker Hughes Institute (St. Paul, MN). These agents exhibit anticancer activity by disrupting normal mitotic spindle assembly and cell division as well as by inducing apoptosis. At nanomolar concentrations, the SPIKET compound SPIKET-P caused tubulin depolymerization in cell-free turbidity assays, and exhibited potent cytotoxic activity against cancer cells as evidenced by destruction of microtubule organization, and prevention of mitotic spindle formation in human breast cancer cells (Uckun FM, Curr Pharm Des, Nov 2001;7(16):1627-39).

Vitilevuamide

Vitilevuamide, a bicyclic 13-amino-acid peptide, was isolated from two marine ascidians, *Didemnum cuculiferum* and *Polysyncranton lithostrotum*. Vitilevuamide inhibits tubulin polymerization by binding to a tubulin site distinct from that of colchicine, the Vinca alkaloids, or dolastatin 10. Vitilevuamide was cytotoxic in several human tumor cell lines with a weak correlation with several paclitaxel analogs, and was strongly positive in a cell-based screen for inhibitors of tubulin polymerization.

Vitilevuamide was active *in vivo* against P388 lymphocytic leukemia, increasing the lifespan of leukemic mice 70% at 30 μg/kg. It inhibited polymerization of purified tubulin *in vitro*, arresting cells in the G2/M phase. Vitilevuamide exhibited noncompetitive inhibition of vinblastine binding to tubulin and colchicine binding to tubulin was stabilized in the presence of vitilevuamide in a fashion similar to vinblastine. Dolastatin 10 binding was unaffected by vitilevuamide at low concentrations, but was inhibited at higher ones. GTP binding was also weakly affected by the presence of vitilevuamide. These results suggest the possibility that vitilevuamide inhibits tubulin polymerization via an interaction at a unique site (Edler MC, etal, Biochem Pharmacol, 15 Feb 2002;63(4):707-15).

MOLECULAR MOTORS-KINESINS

Molecular motors generate motion associated with intracellular trafficking, cell division, and muscle contrac-

tion. There are three main superfamilies of linear biological motors, myosins, kinesins and dyneins. Molecular motors use the energy of adenosine triphosphate (ATP) hydrolysis to move along cytoskeletal filaments; actin filaments in the case of myosins, and microtubules, in the case of dyneins and kinesins. Although earlier studies suggested that molecular motors work by very different mechanisms, it was recently shown that kinesins and myosins share a common core structure and convert energy from ATP into protein motion using a similar conformational change strategy. Many different types of mechanical amplifiers have evolved that operate in conjunction with the conserved core. This modular design has given rise to a remarkable diversity of kinesin and myosin motors whose motile properties are optimized for performing distinct biologic functions (Vale RD and Milligan RA, Science, 7 Apr 2000;288(5463):88-95). It is not clear, however, what conformational differences account for the different behavior observed between members of the kinesin superfamily regarding the direction of their motion along the microtubule, and their interaction with their cargo, among others.

The kinesin superfamily, consisting of >100 different proteins, is involved in many important cellular processes, including mitosis and meiosis, vesicle transport, and establishment and maintenance of cell polarity. Kinesins are extremely small proteins that move slowly along microtubules by converting ATP energy into unidirectional motion. Each protein consists of two heads pivoted around a neck linker, and a tail segment. The neck linker is a 15-amino-acid segment of the kinesin protein that abruptly stiffens when ATP attaches to kinesin. This stiffening throws the neck linker forward and provides the mechanical force that sets the kinesin molecule in motion along the microtubule tracks. The neck linker only attaches to certain points on the microtubule, moving along by thrusting forward the rear head of the two-headed structure.

The linked kinesins move along the microtubule by coordinating the cycling of ATP molecules, first onto one kinesin, then onto its partner with the ATP alternately attaching, releasing their energy, and detaching as spent products. A third segment, the "tail," carries freight, such as chemicals or organelles along tracks composed of microtubules that crisscross the cell's interior. Truncated kinesin molecules with only a single motor domain do not show detectable forward movement, which is consistent with the prevailing model in which the two force-generating kinesin heads operate by a hand-over-hand mechanism (Vale RD, etal, Nature 1996 Apr 4;380(6573):451-3). According to Dr. Ronald Vale of the University of California San Francisco, kinesins are very efficient engines, hauling loads up to 1,000 times their weight, and burning fuel five times more efficiently than a car engine.

Another superfamily of molecular motors is represented by the myosins that move along rails composed of actin. Myosins move far more rapidly than kinesins. A myosin motor is equipped with a flexible arm that swings forward and grabs the actin track, pulling along the main body and its attached cargo. Scientists have identified a family of 15 myosins, each specialized to a different task or muscle type. For example, one keeps heart muscles beating while others transmit nerve signals or lug chemicals from place to place in the cell.

Specific kinesins are involved in mitotic assembly and function. Mitotic kinesins are a family of cytoskeletal enzymes that perform essential roles in mitotic spindle formation and function during cell division. Inhibition of mitotic kinesins disrupts the cell cycle, thereby inducing apoptosis. Spindle poisons block the action of kinesins by disrupting the track, i. e. the mictotubules along which they move and, therefore, interrupt delivery of vital DNA to the two cells created during cell division. As a result, the cells die. However, because cells develop resistance to the cytotoxic action of many spindle poisons, investigators are targeting protein motors as a more efficient means of stopping aberrant cell proliferation.

In addition, most established mitotic spindle poisons that interfere directly with tubulin structure and polymerization, are associated with serious side effects such as neurotoxicity. By targeting kinesins that are specific and essential for mitotic spindle function, treatments may be more efficaceous and less neurotoxic. Because mitotic kinesin inhibitors differ from existing antimitotic drugs in their molecular target and mechanism of action, they are being investigated with the expectation that they will demonstrate an improved therapeutic profile. Were this potential to be fulfilled, mitotic kinesin inhibitors would offer the first novel mechanism of action in this category of cancer therapy in over 25 years.

Among the many members of the mitotic kinesin family, several have been identified as potential targets of anticancer drugs. One such kinesin, KSP, affects mitotic spindle separation at the level of the centrosome; mitosis is inhibited in the absence of KSP. Overexpression of KSP has been detected in lung, breast, and colon cancer. Because neurons do not express KSP, it may be possible to target tumors by specific KSP inhibitors without giving rise to the neurotoxic side effects often associated with microtubule inhibitors. Another kinesin, CENP-E, affects chromosome alignment. Other members include KIF4, a microtubule-based motor protein dominantly localized in the nuclear matrix and associated with chromosomes during mitosis (Oh, etal, Biochim Biophys Acta 2000;1493:219-224).

SB-715992

SB-715992 (CK0238273), a potent and selective inhibitor of KSP (Eg5), has demonstrated a broad-spectrum activity in advanced murine tumors and human tumor xenografts. SB-715992 is active against advanced human colon tumor xenografts Colo205 (complete regressions), Colo201 (complete regressions), and HT-29 (tumor growth delay). Mammary tumor xenograft MX-1 proved to be completely refractory to SB-715992, with efficacy being

dose-related. Also, SB-715992 was negative in a mouse model of peripheral neuropathy in which paclitaxel is positive. The drug was also effective in murine solid tumors, producing regressions in Madison 109 lung carcinoma and M5076 sarcoma. In pulmonary Lewis lung carcinoma, there was significant reduction in lung tumor burden, and prolongation of survival time. Multilog cell kill was also produced in mice bearing advanced systemic L1210 and P388 leukemias. In the latter tumor model, SB-715992 demonstrated optimal efficacy when administered as a single dose or on an intermittent schedule. Daily treatment or continuous infusion was minimally effective, because of the necessity to reduce the total dose in order for the drug to be tolerated (Johnson RK, etal, AACR02, Abs. 1335:269).

In June 2001, Cytokinetics (South San Francisco, CA) and GlaxoSmithKline (GSK) entered into a broad strategic collaboration to discover, develop and commercialize novel small-molecule therapeutics targeting mitotic kinesins for applications in the treatment of cancer and other diseases. Under terms of the agreement, GSK committed funding of approximately $50 million over the minimum 5-year research term, including a $14 million upfront cash payment, and a $14 million purchase of Cytokinetics preferred stock. In addition, GSK could make milestone payments to Cytokinetics ranging from $30 million to $50 million per target for products directed to each of over 10 mitotic kinesins that are the subject of collaborative activities. GSK is responsible for worldwide development and commercialization of products arising from the collaboration and Cytokinetics is eligible for royalties from the sale of any resulting products. In addition, Cytokinetics retains a product-by-product option to cofund certain development activities, thereby increasing its royalty and affording copromotion rights in North America. During the collaboration, targets may revert to Cytokinetics for independent research and development, with GSK retaining an option to resume joint activities.

In this collaboration, the companies are employing a multipronged approach using focused genomics, high-throughput screening (including Cytokinetics' PUMA technologies), automated cell-based secondary characterization technologies (including Cytokinetics' Cytometrix cellular phenotyping technology), structural biology and medicinal chemistry, to identify, characterize and optimize small molecule inhibitors of mitotic kinesin targets.

Cytokinetics was established in April 1998 by Dr. James Sabry, Professor Larry Goldstein of the University of California San Diego, Dr. James Spudich, and Professor Ron Vale of the University of California San Francisco. The company, focuses on cytoskeletal pharmacology, and plans to combine drug discovery development work with cellular bioinformatics, using its Cytometrix technology.

An open-label, phase I clinical trial with SB-715992, was initiated in August 2002, triggering an undisclosed milestone payment from GlaxoSmithKline to Cytokinetics. This first phase I clinical trial is investigating the safety, tolerability, pharmacokinetics, and pharmacodynamic profile of this KSP inhibitor. This study is being conducted at two clinical centers, CTRC Institute for Drug Development, affiliated with the University of Texas Health Science Center (San Antonio, TX), and the University of Wisconsin Comprehensive Cancer Center (Madison, WI), in patients with advanced cancer.

DESIGNED COLCHICINE-SITE BINDERS

The complex chemical structures and limited/restricted availability of compounds derived from natural products, coupled with the development of drug resistance in clinical trials involving these agents, have limited the utility of the first generation of natural products. However, synthesis of new compounds such as small molecule tubulin inhibitors mimicking the activities of naturally occurring spindle poisons, is expanding access and enhancing activities of potentially promising novel drugs.

A-289099

A-289099, under development by Abbott Laboratories, is an orally available indole-oxazoline that inhibits tubulin polymerization by binding at the colchicine site (Li Q, etal, Bioorg Med Chem Lett, Feb 2002;12(3):465-9). In a preclinical study, A-289099 depolymerized microtubules in a time- and dose-dependent fashion. After 24 hours of exposure to A-289099, most cells were arrested at the G2/M phase with an increase in subdiploid cells. When animals xenografted with M507 tumors treated with oral A-289099, were injected with a staining compound to evaluate tumor vascular flow, control animals displayed staining throughout the tumors while little or no staining was observed in treated animals, indicating a stoppage of vascular flow (Tahir SK, etal, AACR02, Abs. 1315:265).

A-293620/A-318315

A-293620/A-318315, also under development by Abbott Laboratories, are potent, orally active, small molecule antimitotics that bind to the colchicine site of tubulin, inhibit β-tubulin polymerization and cause cells to arrest at the G2/M phase of the cell cycle leading to apoptosis. These indole sulfonamides are synthetic versions of natural products created by structural modification of combretastatin A-4 (CA-4) by interchanging the cis-double bond of CA-4 with a sulfonamide bond.

A-293620 demonstrated good antiproliferative activity against various cancer cell lines including those with the MDR (+) phenotype. Microtubules of HCT-15 human colon carcinoma cells depolymerized in a time- and dose-dependent fashion after A-293620 treatment. A-293620 was not cross resistant to MDR+ cells, suggesting that it is not expelled by the P-gp drug efflux pump (Zielinski-Mozny N, etal, AACR02, Abs. 1314:264).

A-318315, a dimethylglycine prodrug of A-293620, is an orally active anitmitotic agent that also binds to the colchicine site on β-tubulin and inhibits the *in vitro* polymerization of microtubules. A-318315 has demonstrated

improved solubility and pharmacokinetic profile compared to A-293620, and dose-dependent antitumor activity in several human xenograft models, including HCT-15 and H460. A-318315 also exhibited acute, potent, and tumor-selective antivascular effects in the SC rat 9L glioma tumor model. One hour after a single dose, A-318315 reduced tumor perfusion by 83%, while muscle perfusion was reduced only by 6% as measured by dynamic contrast-enhanced MRI (DCE-MRI). Histologic examination of the tumors treated with A-318315, revealed areas of vascular damage and increased apoptotic index at the perivascular area within 6 hours. A-318315 also showed significant efficacy against the M5076 reticular sarcoma model regardless of the route of administration (Li Q, etal, AACR02, Abs. 1313:264).

ABT-751/E7010

ABT-751/E7010 is an orally active sulfonamide that binds to the colchicine site on β-tubulin and inhibits the polymerization of microtubules. The drug was developed by Eisai (Tokyo, Japan). In July 2000, Abbott Laboratories signed a licensing agreement to develop and market E7010 worldwide except in Japan and Asia where Eisai maintains exclusive rights. Under terms of the agreement, Abbott will make milestone payments and pay royalties on sales.

In a preclinical study, rats injected subcutaneously (SC) with 9L glioma cells were administered ABT-751 to assess tumor perfusion. DCE-MRI and gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA) were used to measure tissue perfusion. MRI tissue perfusion measurements were taken before and 1 hour after IV administration of ABT-751, 2 weeks after tumor inoculation. Results showed that ABT-751 (30 mg/kg) reduced tumor perfusion to 30% ± 4% and had no significant effects on heart rate and mean arterial pressure. No altered tissue perfusion was observed in surrounding skeletal muscle. Therefore, ABT-751 at is a potent agent for selectively reducing tumor tissue perfusion (Luo Y, etal, AACR02, Abs. 784:157).

In a preclinical study, after 8 hours of exposure to ABT-751, about 60% of HCT-15 cells were arrested at the G2/M phase, at 10-fold the IC_{50}. ABT-751 displayed a dose-dependent efficacy with no observable toxicities through 21 days of an extended dosing schedule. ABT-751 was less effective on a *bid* schedule than a daily schedule. Tumor regression was observed in a M5076 tumor model. ABT-751 administered PO in an HCT-15 model, inhibited tumor growth while no activity was observed in the same model administered paclitaxel and vincristine (Zielinski-Mozny N, etal, AACR02, Abs. 1323:266).

Syngeneic C57BL/6 mice bearing intact orthotopically transplanted murine colon 38 tumors, exhibit a high incidence of hepatic metastases within 1 month after transplantation, with death occurring in about 2 months. When ABT-751 was administered orally in this model for 14 days at MDT, starting 14 days after transplantation, tumor growth inhibition of 40%, compared to controls, was observed at the orthotopic site, similar to the 32% growth inhibition observed at the SC site. When ABT-751 was administered for 8 days, starting 21 days after transplantation, it significantly decreased the number of hepatic metastases (control=17.1%, ABT-751=2.6%), with 60% inhibition of tumor growth at the orthotopic site. Administration of ABT-751 daily, until death, produced a significant increase in lifespan (control=49.8 days, ABT-751=62.5 days) and, although the tumor weight of the ABT-751-treated group on the day of death was similar to that of the untreated group, there were significantly fewer liver metastases in the ABT-751-treated group (control=41.3%, ABT-751=2.0%). These data indicate that ABT-751 is capable of suppressing tumor growth at both primary and metastatic sites. Hepatic metastases were inhibited more effectively with ABT-751 than the growth of the primary tumors. Also treatment with ABT-751 increased the lifespan of an orthotopic transplantation model of murine colon 38 tumor (Funahashi Y, etal, Cancer Chemother Pharmacol 2001;47(2):179-84).

In addition to dose-related adverse events encountered in clinical trials that have included anemia, leucopenia, nausea/vomiting, peripheral neuropathy, and, occasionally, anorexia, ABT-751 was recently found to have testicular toxicity in rodent models. Repeated daily oral administration of ABT-751 to male Slc:SD rats at a dose of 75 mg/kg for 2 weeks, induced severe testicular lesions characterized by significant decrease and/or loss of seminiferous epithelial cells, which sometimes resulted in tubules with only Sertoli cells. A drug dose of 50 mg/kg, administered by the same dosing schedule, caused alteration of germ cells at various stages of spermatogenesis, with frequently observed apoptotic figures of meiotic spermatocytes (Hayakawa K, etal, J Toxicol Sci, Oct 2000;25 Spec No:173-8).

A phase I dose-escalation clinical trial of ABT-751, in patients with solid tumors, was conducted at the Kinki University School of Medicine (Osaka, Japan). When administered orally to 16 patients as a single dose, ranging from 80 mg/m^2 to 480 mg/m^2, ABT-751-associated DLT was peripheral neuropathy at 480 mg/m^2, accompanied by mild hematologic and gastrointestinal toxicities. Using a 5-day repeated-dose schedule, 41 patients were administered ABT-751 at daily doses ranging from 30 mg/m^2 to 240 mg/m^2. DLT included peripheral neuropathy and intestinal paralysis; gastrointestinal toxicity was dose-dependent but not severe, while hematologic toxicity was not dose-dependent. Pharmacokinetic analysis in the single-dose arm showed a rapid increase in the plasma levels of the drug after administration, with a half-life of 4.4-16.6 hours. Changes in the plasma levels on day 5, in the 5-day repeated-dose study arm, were almost the same as those on day 1, indicating that the drug did not accumulate.

In this single-dose study, there was a 74% decrease in spinal cord metastasis in a patient with uterine sarcoma and a minor response was observed in a pulmonary adenocarcinoma patient. In the 5-day repeated-dose study, a decrease in carcinoembryonic antigen (CEA) was observed

in a patient with stomach cancer and in squamous cell carcinoma antigen levels in one with recurrent cervical carcinoma. Based on the results of this trial, 320 mg/m^2 for single-dose administration and 200 mg/m^2/day for 5-day repeated-dose administration were recommended for any subsequent clinical trials. Because the activity of ABT-751 is time-dependent (i.e., a certain concentration of ABT-751 for more than 12 hours has been shown to be required to suppress the growth of P388 leukemia cells in preclinical models), repeating the 5-day drug course at intervals of 3 weeks may be a more effective administration schedule (Yamamoto K, etal, Cancer Chemother Pharmacol 1998;42(2):127-34).

In March 2002, a multicenter, dose-escalation phase I clinical trial (protocol ID: ABBOTT-M01-357, NCI-02-C-0141) of ABT-751 was initiated at the NCI, for the treatment of solid tumors in pediatric patients, including rhabdomyosarcoma, soft tissue sarcoma, Ewing's sarcoma, osteosarcoma, neuroblastoma, Wilms' tumors, hepatic tumors, germ cell tumors, or primary brain tumors. Trial objectives include determining toxicities, MTD, and pharmacokinetics. Patients are being administered one capsule of ABT-751, every day, for 7 days, for each treatment cycle ranging from 21 to 28 days. Treatments continue until absence of disease progression or unacceptable toxicity. Approximately 21 patients will be accrued for this trial chaired by Steve Y. Cho, MD, of the NCI Center for Cancer Research.

D-24851/D-64131

Two novel, easily accessible indole agents, D-24851 and D-64131, were identified as tubulin-destabilizing agents. Owing to their synthetic nature, potent *in vitro* and *in vivo* antitumor activity, and efficacy against MDR tumors, D-24851 and D-64131 have significant potential in cancer treatment (Bacher G, etal, Hungarian-German-Italian-Polish Joint Meeting on Medicinal Chemistry, Budapest, Hungary, 2 –6 September 2001). Both these agents were originally synthesized by ASTA Medica (Frankfurt/Main, Germany), now Baxter Oncology (Deerfield, IL), a division of Baxter International.

D-24851 acts like a tubulin-interacting agent by destabilizing microtubules (Bacher G, etal, AACR99, Abs. 1893:285), but its antitumor activity *in vivo* is superior to those of vincristine or paclitaxel. This drug, belonging to a class of colchicine-site binders, was identified by combinatorial chemistry, making its synthesis relatively simple compared to other spindle poisons. D-24851 blocks cell-cycle transition specifically at G2/M phase and leads to subsequent cell death (FO, pp 1037-8).

In preclinical trials, D-24851 demonstrated oral bioavailability, potent *in vitro* and *in vivo* antitumor activity, no neurotoxicity, and efficacy against MDR tumors. *In vitro*, D-24851 exhibited potent cytotoxic activity against a panel of established tumor cells. In several *in vivo* solid tumor models, such as AH13 sarcoma, D-24851 exhibited superior antitumor activity compared to paclitaxel or vincristine. In contrast to paclitaxel, D-24851, at curative doses, was not associated with any decrease in nerve conductance velocity. Also, MDR cell lines generated by vincristine adaptation or transfection with P-gp encoding human cDNA, that were resistant to paclitaxel, vincristine, or doxorubicin, were sensitive to D-24851 when compared to parental cells (Bacher G, etal, AACR00, Abs. 1930:303).

D-24851 exhibited potent cytotoxicity against various malignancies, including leukemia, sarcoma, and laryngeal, breast, brain and ovarian cancer both *in vitro* and *in vivo*. D-24851 also showed excellent antitumor activity when administered orally by gavage. An oral dose in the AH13 sarcoma model cured 90% of the animals, observed for up to 42 days, with no side effects (Nickel B, etal, AACR99, Abs. 4110:623). Clinical trials with this drug are anticipated to begin in the last quarter of 2002.

D-64131, an acylindol, is a small molecule that acts by arresting cell division in the G2/M phase of the cell cycle and inhibiting tubulin polymerization by destabilization.

ZD6126

ZD6126 (formerly ANG453), a colchicine prodrug, disrupts the tubulin cytoskeleton of neoendothelial cells, causing selective destruction of tumor vasculature and producing massive tumor necrosis. ZD6126 exploits differences between tumor neovasculature and established blood vessels to selectively target tumors by interfering with their blood supply. ZD6126 is currently under development as a vascular targeting agent by AstraZeneca that obtained an exclusive worldwide license for this drug from Angiogene Pharmaceuticals (Oxford, UK) in January 1999. Using novel *in vitro* and *in vivo* screening procedures, Angiogene pinpointed structural features that appear to be important for antivascular rather than antiproliferative effects on tumor cells, to identify compounds with highly selective activity towards tumor-associated endothelium both *in vitro* and *in vivo*. These compounds are related to a known class of tubulin binding agents that are structurally distinct from the combretastatins. The antivascular effects of the compounds with activity against a wide range of different solid tumor types in animal models, were attained within a large therapeutic window. One of these compounds, ZD6126, binds tubulin and is thought to induce morphologic changes in immature tumor endothelial cells which are critically dependent on their tubulin cytoskeleton to maintain shape, leading to vessel occlusion and extensive central tumor necrosis.

In animal models, ZD6126 induces tumor vascular damage shortly after injection, leading to massive tumor necrosis. *In vivo*, ZD6126 is rapidly converted into ZM445526, an inhibitor of tubulin polymerization. The effects of ZM445526 on endothelial cell adhesion to extracellular matrix (ECM) components, was investigated by an adhesion assay to test its ability to prevent attachment of human umbilical vein endothelial cells (HUVEC) or by a detachment assay to impair adhesion of already attached

cells. ZM445526 had no effect on the adhesion of HUVEC and only small effects on their detachment but caused a dramatic change in endothelial cell shape. After a 40-minute exposure to the drug, endothelial cells were seen to round up compared with their normal flattened morphology. This effect was concentration-dependent and reversible.

When confluent and nonconfluent HUVEC were compared, the change in cell shape was more significant (10-fold difference in IC_{50}) on nonconfluent proliferating cells than on confluent cells. HUVEC were very sensitive to the induction of morphologic changes, whereas NIH-3T3 mouse fibroblasts were less sensitive, and A10 rat smooth muscle cells were not affected at all. When the activity of ZM445526 was evaluated in a 3D Matrigel cord-formation assay *in vitro*, addition of ZM445526 caused a concentration-dependent loss of cord integrity, with complete disruption evident after a 30-minute exposure. This effect was completely reversible with normal cord structures regained at 24 hours. This further suggests that the antivascular activity of ZM445526 is mainly attributable to rapid morphologic changes in endothelial cells, rather than to direct cytotoxic effects. These studies provide supportive evidence that rapid and selective effects on proliferating endothelial cells may be an early event in the series of changes that lead to massive tumor necrosis observed 24 hours after ZD6126 administration in a range of tumors grown as xenografts in nude mice (Nicoletti MI, etal, AACR-NCI-EORTC01, Abs. 46).

ZD6126 acts by disrupting tubulin. Because the structure of neoendothelial cells is critically dependent on the tubulin cytoskeleton, disruption by ZD6126 causes selective damage to tumor vasculature (Honess DJ, etal, AACR-NCI-EORTC01, Abs. 49). When DCE MRI was used to investigate the dose-response effects of ZD6126 in rat GH3 prolactinomas, 14 of 15 treated tumors showed a reduction in the highly enhancing fraction, while 1 rat treated with the lowest dose showed no reduction. After imaging, the tumors were excised and assessed for necrosis. Histologic assessment indicated massive central necrosis surrounded by a viable rim of tumor cells. Data are consistent with the tumor vasculature being compromised by ZD6126 and the induction of necrosis (McIntyre DJ, etal, AACR-NCI-EORTC01, Abs. 2).

In preclinical trials ZD6126 was found to be active in lung adenocarcinoma (Blakey DC, etal, AACR02, Abs. 778:156) by inducing apoptosis leading to necrosis in tumor vascular endothelial cells (Goto H, etal, AACR02, Abs. 777:156) and in renal cell carcinoma, particularly when administered sequentially with cisplatin (Siemann DW, etal, AACR02, Abs. 2594:523). In mice bearing murine C38 colon adenocarcinoma, ZD6126 caused > 80% central tumor necrosis, although a small rim of viable tumor remained at the periphery.

Following chronic IV dosing in the rat, ZD6126 showed no evidence of peripheral neuropathy, a major DLT associated with antimicrotubule agents such as taxanes and Vinca alkaloids, or neurotoxicity. In one study, male and female rats were dosed IV with either vehicle (control) or ZD6126, administered over a 6-month period in monthly cycles. Comprehensive neuropathologic assessment revealed no changes attributable to ZD6126. Similar results were obtained when the potential for ZD6126 to induce neuropathy was assessed in 20 male and female Wistar rats administered either vehicle or low-dose or high-dose ZD6126. Both studies showed that chronic intermittent dosing of ZD6126 was well tolerated and produced no evidence of peripheral neuropathy or neurotoxicity (Horner SA, AACR02, Abs. 1338:269).

ZD6126 was also shown to enhance the effects of radiation therapy. Combination therapy with radiation and ZD6126 reduced tumor survival by 10-to 500-fold compared to radiation alone. Radiation therapy enhancement was observed when ZD6126 was administered either before or after therapy. ZD6126, when administered once weekly in combination with daily intratumoral fractionated radiation doses of 2.5 Gy, increased tumor response (Siemann DW, AM Rojiani, Int J Radiat Oncol Biol Phys, May 2002;53(1):164-71). In preclinical trials, ZD6126 coadministered with ZD6474, a potent vascular epidermal growth factor (VEGF) KDR receptor tyrosine kinase inhibitor, also blocked tumor growth and even caused tumors to regress (Wedge SR, etal, AACR02, Abs. 5351:1081).

In a preclinical study, in mice bearing murine C38 colon adenocarcinoma, DCE-MRI was found effective in serially monitoring the effect of ZD6126 on tumor vasculature. In mice bearing murine C38 colon adenocarcinoma, ZD6126 caused greater than 80% central tumor necrosis histologically, although a small rim of viable tumor remained at the periphery. Because a similar DCE-MRI approach can be used clinically, it should provide a nonambiguous measure of activity for clinical trials (Evelhoch JL, etal, AACR01, Abs. 580:107). In a phase I clinical trial, DCE-MRI was used to evaluate the vascular effects of ZD6126 on solid tumors in 6 patients who were administered ZD6126 as a single IV dose of 56 mg/m^2 (n=3), 80 mg/m^2 (n=2), or 112 mg/m^2 (n=1), every 3 weeks. The central area of tumors displayed the most effect. Further studies are being conducted to correlate DCI-MRI data with clinical outcome (DelProposto Z, etal, ASCO02, Abs. 440:111a).

Also, two new approaches, high frequency Doppler and microbubble ultrasound imaging, both were able to detect and monitor modulations in tumor blood flow caused by ZD6126. The former provides high-resolution imaging and velocimetry of the tumor vasculature in small animals, while the latter provides a means of quantitative, clinical measurement of flow in soft tissue tumors (Burns P, etal, AACR-NCI-EORTC01, Abs. 235).

AstraZeneca has embarked on a dose-escalation phase I clinical development program with ZD6126. In May 2002, interim results were presented from a dose-escalation phase

I clinical trial of ZD6126 for the treatment of refractory solid tumors. Trial objectives include determining DLT and MTD. Among 27 patients administered ZD6126 as IV doses of 5, 10, 20, 40, 56, 80 and 112 mg/m^2 over 10 minutes, every 21 days until disease progression or withdrawal, adverse events included anorexia (n=3), constipation (n=6), dyspnea (n=5), fatigue (n=5), headache (n=3), nausea (n=4), pain (n=14), and vomiting (n=3). No relationship between ZD6126 doses and incidence of adverse events became apparent. Grade 2 ischemia and Grade 3 elevation in troponin I was observed in 1 patient. Pharmacokinetic studies show that ZD6126 rapidly hydrolyzes into ZD6126 phenol which has a half-life of 2-3 hours (Gadgeel SM, etal, ASCO02, Abs. 438:110a).

In an earlier report, among 19 patients treated at 4 dose levels (5, 10, 20 and 40 mg/m^2), 12/19 were exposed to 3 or more previous treatments, 6 to 2 treatments, and 1 to only one treatment. Up to three 21-day treatment cycles were completed at the 10 mg/m^2 and 20 mg/m^2 doses, and two 21-day cycles at 5 mg/m^2 and 40 mg/m^2. Adverse events included Grade 1 leg cramps (n=1) and Grade 2 anemia (n=1). One patient had asymptomatic, reversible, Grade 2 ischemic changes on EKG and Grade 3 elevation in troponin I with subsequent demonstration of coronary artery disease (Lorusso PM, etal, AACR-NCI-EORTC01, Abs. 36).

In another dose-escalation phase I clinical trial of ZD6126 for the treatment of refractory solid tumors, patients are being administered ZD6126 doses of 5 mg/m^2 or 7 mg/m^2 over 10 minutes, every 7 days, for 4 weeks. Among 12 patients with colon, bronchial, breast, adenoid cystic, renal cell or ovarian carcinoma, treated with 11 doses, toxicities were hypokalemia and transient, reversible, decline in ejection fraction. Also, 2 patients with brain metastases displayed symptoms of intracranial pressure after treatment. MTD had not been reached at the time of this report. Elimination half-life was 2-3 hours. Of 5 patients, 4 displayed a 2-fold increase in circulating endothelial cells after 4-6 hours, indicating biological activity (Radema SA, etal, ASCO02, Abs. 439:110a).

A phase I clinical trial of ZD6126 was initiated in January 2002, for the treatment of solid tumors. A total of 6 patients are being administered a single IV dose of ZD6126 to assess metabolism, excretion, and pharmacokinetics. This trial is being conducted by PI Ian Judson, MD, at the Royal Marsden Hospital (London, UK).

OTHER NOVEL SPINDLE POISONS

2-ME2

2-ME2 (Panzem), under development by EntreMed (Rockville, MD), has a dual mechanism of action involving direct, selective inhibition of tumor-cell proliferation through G2/M arrest, and inhibition of various stages of the angiogenic cascade in tumor vasculature. In *in vitro* and *in vivo* experiments, 2-ME2 upregulated DR5 expression and synergistically enhanced TRAIL in tumor and endothelial cells (Lavallee TM, etal, AACR02, Abs. 3484:703), and also regulated VEGF-mediated angiogenesis, and the rate of tumor-cell proliferation (Banerjee SN, etal, AACR02, Abs. 1143:228). In PC-3 prostate cancer cells, 2-ME2-induced tubulin depolymerization inhibited hypoxic-inducible factor 1 (HIF-1)-α transcription, and played a role in inhibiting angiogenesis (Masbjeesh NJ, etal, AACR02, Abs. 902:180).

It has also been shown that 2-ME2 inhibits superoxide dismutase (SOD). As an example of this activity, 2-ME2 treatment of chronic lymphocytic leukemia (CLL) cells that have significantly higher basal levels of SOD compared with normal lymphocytes, resulted in inhibition of SOD, and substantial O2- accumulation and apoptosis, while no substantial changes in O2- status were seen in normal lymphocytes. Similar results are observed with 2-ME2-treated ovarian cancer cells, which exhibit higher SOD levels than normal ovarian epithelial cells (Oldham EA, etal, AACR01, Abs. 1021:190). Accumulation of SOD in endothelial cells, induced by 2-ME2, may also play a role in suppression of angiogenesis (Pelicano H, etal, AACR01, Abs. 1101:206).

In preclinical studies, 2-ME2 demonstrated effectiveness in a canine model of oral melanoma (MacEwen EG, etal, AACR01, Abs. 20:4), in a human glioblastoma xenograft model, and in an orthotopic breast cancer model in nude mice. At low doses, 2-ME2 inhibited the spread of metastatic disease in lung metastases in the B16BL6 experimental metastatic tumor model and, at higher doses, reduced surface metastases by 90% (Lavallee TM, etal, AACR01, Abs. 283:54). In an *in vitro* evaluation, 2-ME2 demonstrated a dose-dependent inhibition of growth of breast cancer cell lines MCF-7, T-47D, MDA-MB-435s, and MDA-MB-231. Adding 2-ME2 enhanced the microtubule inhibitory effect of vinorelbine or paclitaxel on human breast cancer cells, and also moderately improved the growth-inhibitory effect of methotrexate, 5-FU, doxorubicin, and mitomycin C, in the four cell lines tested. These results suggest that 2-ME2 could be used to enhance the chemotherapeutic effects of anticancer agents commonly used to treat breast cancer (Liu ZJ, etal, AACR01, Abs. 1099:205).

In January 2002, an NCI-sponsored dose-escalation, phase I clinical trial (protocol IDs: MAYO-MC0017; NCI-3356) was at the Mayo Clinic Cancer Center, initiated for the treatment of advanced solid tumors. Trial objectives include determining optimal dose and/or MTD, toxicities, pharmacokinetics, and evaluating biologic evidence for angiogenesis inhibition. Patients are being administered 2-ME2 PO daily. Treatment cycles repeat every 28 days until absence of disease progression or unacceptable toxicity. Approximately 42 to 60 patients will be accrued for this trial. Charles Erlichman, MD, of the Mayo Clinic Cancer Center is Study Chair.

In September 2001, an NCI-sponsored, dose-escalation phase I clinical trial (protocol IDs: NCI-01-C-0256; NCI-

3371; NCI-CC-01-C-0256) was initiated with 2-ME2 in advanced solid tumors. Trial objectives include determination of MTD, toxicities, pharmacokinetics, changes in PET scans, and changes in apoptosis. Patients are being administered 2-ME2 PO on day 1 followed by 2 days of evaluation. After evaluation, patients are being administered 2-ME2 every 12 hours in the absences of disease progression or unacceptable toxicity. This trial will accrue a total of 30 patients. William D. Figg, MD, of the NCI, is Study Chair.

Both single-agent and combination clinical trials are being conducted with 2-ME2 in treating breast cancer. In September 2000, following FDA approval of an IND application that had been filed in February 2000, the first patient was treated in a phase I clinical trial, to investigate the safety profile of the simultaneous administration of 5 different dose levels of oral 2-ME2, in combination with docetaxel, in advanced breast cancer. This trial was conducted at the Indiana University Cancer Center (Indianapolis, IN) with Kathy Miller, MD, as the PI.

In this trial, docetaxel (35 mg/m^2) was administered weekly for 4 of 6 weeks and 2-ME2 was administered orally once daily for 28 days, followed by a 13-day observation period in cycle one, and continuously thereafter. After a maximum of 6 cycles of combined therapy, responders and those with stable disease continued with 2-ME2 alone until progression. Among 15 patients enrolled at daily 2-ME2 doses of 200 mg, 400 mg, 600 mg, 800 mg and 1000 mg, there were no Grade 4 toxicities and MTD had not been reached. Grade 3 fatigue, diarrhea, and hand-foot syndrome occurred in 5, 4 and 1 patients, respectively. Transaminase elevations that occurred in 3 patients returned to normal with continued treatment in 2. There were no changes in estrogen, luteinizing hormone, or follicle stimulating hormone levels. There was significant interpatient variability in 2-ME2 clearance, and extensive metabolism to 2-methoxyestrone (2-ME1) with 2-ME1 levels being significantly higher than 2-ME2 levels. Overall response rate was 20% including 1 CR; disease stabilized in an additional 40% of patients. Median time-to-failure was 167 days; 2 patients remained on 2-ME2 for >1 year (Miller KD, etal, ASCO02, Abs. 442:111a). In this trial, concurrent therapy with docetaxel and 2-ME2 was well tolerated and did not alter either docetaxel or 2-ME2 pharmacokinetics.

Enrollment in two phase I clinical trials of single-agent 2-ME2 at the Indiana University Cancer Center has been closed. In a completed phase I clinical trial in previously treated patients with metastatic breast cancer, 2-ME2 was administered orally at 200-1000 mg, once daily to cohorts 1-5, or at 200-800 mg, twice daily, every 12 hours to cohorts 6-9, for 28 days, followed by a 14-day observation period. Among 31 enrolled patients, there were no Grade 4 toxicities and MTD had not been reached at the time of this report. No changes in estrogen, luteinizing hormone or follicle stimulating hormone were detected. Peak serum levels of 2-ME2 were achieved 2-4 hours after administration; T1/2 was approximately 10 hours. Plasma VEGF was undetectable in all patients continuing 2-ME2 therapy for >3 months; urine VEGF and serum vascular cell adhesion molecule 1 (VCAM-1) concentrations did not change consistently. Serum and urine basic fibroblast growth factor (bFGF) were rarely elevated at baseline. After the first treatment period, disease stabilized in 17 patients who continued therapy. Median time on 2-ME2 for all patients was 41 days (range 13-258 days). Among 5 patients it was >100 days with 3 remaining on therapy. The drug was well tolerated and did not alter hormonal status of patients with refractory metastatic breast cancer (Sledge GW, etal, ASCO02, Abs. 441:111a).

In a report from a phase I safety, pharmacokinetic, and pharmacodynamic clinical trial of oral 2-ME2 as single-agent therapy in patients with previously treated metastatic breast cancer, initiated in March 2000 at the Indiana University Cancer Center under the direction of Dr. Kathy D. Miller and Dr. George W. Sledge, administration of the agent was noted to be without serious drug-related adverse events and to cause no change in estrogen levels. The drug was administered orally, once-daily, for 28 days followed by a 14-day observation period; thereafter patients continued 2-ME2 treatment until progression (50% increase in tumor size). As of May 2001, 15 patients had been enrolled at daily doses of 200, 400, 600, 800 and 1000 mg. Enrolled patients had been heavily pretreated with a median of 4 prior therapies; 9 patients had prior high-dose therapy with stem cell support. No Grade 4 toxicities were observed, and Grade 3 toxicity occurred only in relation to disease progression. Dose escalation and determination of MTD was based on toxicity observed during the first 28-day treatment period. Disease stabilized in 10 patients after the first treatment period; 2 patients experienced a clinically significant reduction in bone pain and analgesic use. Oxygen requirements declined significantly in one patient with lymphangitic pulmonary metastases. The median duration of therapy was 72 days (range=28-176 days). An assessment of potential surrogates of antiangiogenic activity is part of this clinical trial (Miller KD, etal, ASCO01, Abs. 170:43a).

Enrollment in two phase II clinical trials of single-agent 2-ME2 is still ongoing for the treatment of multiple myeloma. In December 2000, EntreMed initiated a phase II safety and efficacy clinical trial of oral 2-ME2 in the treatment of stable or relapsed multiple myeloma, at the Mayo Clinic (Rochester, MN), under the direction of S. Vincent Rajkumar, MD, and at the Dana-Farber Partner's Cancer Consortium, under the direction of Paul Richardson, MD. In August 2001, EntreMed announced that the FDA had granted 2-ME2 orphan drug status in the treatment of multiple myeloma.

Enrollment had been completed in two phase II clinical trials in hormone-refractory prostate cancer. In January 2001, a multicenter phase II clinical trial of oral 2-ME2 as single-agent therapy was initiated at the Indiana University

Cancer Center, under the direction of Christopher J. Sweeney, MD, and George Sledge, MD, and at the University of Wisconsin Comprehensive Cancer Center under the direction of George Wilding, MD. EntreMed is also collaborating with Aventis Pharmaceuticals to evaluate 2-ME2 in a phase II clinical trial, in combination with docetaxel, in patients with hormone-refractory prostate cancer.

Benzimidazole Carbamates

Benzimidazole carbamates are anthelmintic agents with an excellent safety profile as demonstrated by years of clinical use. Among benzimidazole carbamates in use as human or veterinary antihelmintics are mebendazole, fenbendazole and albendazole. Because their anthelmintic action is mediated by binding to parasite β-tubulin these compounds were investigated regarding their action as selective vascular damaging agents, similar to other classes of spindle poisons. When vascular damage was assessed in the syngeneic breast adenocarcinoma cell line, CaNT, all 3 agents reduced functional vascular volume by 56%, 44%, and 51%, respectively. Therefore, benzimidazole carbamates represent a new structural class of vascular targeting agents (Davis PD, etal, ACR01, Abs. 2000:371).

ANG 600 series, under development by Angiogene Pharmaceuticals, consists of various bezimidazole carbamate analogs. A novel analog, ANG615, at an intraperitoneal dose of 50 mg/kg, reduced functional vascular volume by 99% at 24 hours, and induced widespread necrosis in the tumors, leaving only a rim of viable cells.

In July 2002, MediciNova (San Diego, CA) acquired exclusive worldwide rights to the ANG 600 series, in exchange for an upfront licensing payment, as well as milestone and royalty payments. MediciNova will be responsible for the future preclinical and clinical development, regulatory activities, and commercialization of the ANG 600 series. The company plans to initiate a phase I clinical program with a lead compound in 2003.

Mebendazole (MZ) binds to microtubules at a different site than the Vinca alkaloids or taxanes. MZ inhibits growth of a number of cancer cell lines derived from melanoma and from lung, pancreatic, liver, breast, prostate and ovarian cancer, in a dose- and time-dependent manner. Unlike colchicine, MZ blocks cells at the G2/M phase and then induces apoptosis without inducing tetraploidy. Interestingly, MZ has no apparent effect on normal fibroblast, HUVEC or bronchial epithelial cells. Release of cytochrome c, a mitochondria-dependent apoptotic inducer, from mitochondria to cytosol, and activation of caspases were observed in a time- and dose-dependent manner after treatment with MZ. Levels of p53 protein and p21 protein were increased after MZ treatment, but this increase had no effect on the Bcl-2 gene family. In MZ-treated A549 cells, the ratio of cytosolic/cytoskeletal tubulin was significantly lower than that in nocodazole-treated cells. Moreover, MZ effectively inhibits growth and induces growth inhibition and apoptosis in cancer cell lines that were resistant to paclitaxel or vinorelbine. *In vivo* studies show that MZ inhibits growth of subcutaneous tumors and experimental lung metastasis in a nu/nu mouse model. These findings indicate that MZ has potent antitumor activity and could have significant clinical implications (Sasaki J, etal, AACR02, Abs. 1317:265).

Introgen Therapeutics (Austin, TX) has investigated the development of MZ formulations and evaluated the drug's bioavailability under an 8-month $52,000 grant, administered by the University of Houston.

CP248/CP461

CP248 and CP461 are novel high-affinity analogs of FGN-1, a sulfone metabolite of the nonsteroidal anti-inflammatory (NSAI) drug sulindac, discovered by Cell Pathways (Horsham, PA) by targeting a new cyclic-GMP phosphodiesterase (cGMP-PDE) enzyme. These agents are collectively referred as selective apoptotic antineoplastic drugs (SAAND) that trigger apoptosis in abnormal cells by inhibiting certain cGMP-PDE overexpressed in a variety of tumor types. Inhibition of cGMP-PDE leads to activation of another intracellular signaling molecule, protein kinase G (PKG), triggering a cascade of downstream events leading to apoptosis.

CP461 and CP248 appear to act against cancer cells by inducing apoptosis at levels that are selective for cancer cells over normal ones. In addition, CP248 contains a trimethoxybenzyl group, similar to that found in the tubulin-binding compounds colchicine and colcemid that inhibit tubulin polymerization and cause G2/M arrest. Therefore, CP248 also acts as a spindle poison arresting cells in the M phase of the cell cycle. Because the anticancer properties of CP461 and CP248 result from both proapoptotic and antiproliferative activities, these and similar compounds may prove more selective, and safer cancer treatments, effective in many types of solid tumors and hematologic malignancies.

CP248 selectively inhibits cell growth and triggers apoptosis in a number of human cancer types, through inhibition of cyclic GMP-PDE 2 and 5, and also causes cell-cycle arrest by binding to tubulin and disrupting the polymerization and function of microtubules. CP248 may offer advantages for growth inhibition and apoptosis induction in neoplasia because its direct inhibition of tubulin polymerization is combined with cGMP-mediated pathways of apoptosis. CP248 possesses up to 20,000 times the apoptotic potency of exisulind.

CP248 causes G2/M cell cycle arrest in SW480 colon cancer cells by inhibition of tubulin polymerization via the colchicine-binding site. SW480 colon cancer cells treated with CP248, showed a 70% accumulation in G2/M relative to 18% for controls. Analysis with the MPM2 antibody indicated that 22% of the cells were in the M phase (mitosis) compared to 3% for controls. Immunofluorescent staining for microtubules using a monoclonal antibody to

β-tubulin revealed complete dissolution of the microtubule cytoskeleton (Fetter JR, etal, AACR02, Abs. 1319:265).

CP248 also caused treated glioma cells to arrest in M phase. When scientists at Columbia University and Cell Pathways examined the effects of exisulind and CP461 and CP248 on the growth of 4 rat glioma and 8 human glioma cells lines, they found that exisulind, CP461 and CP248 inhibited growth of the cells at IC_{50} of 150, 1 and 0.07 μM, respectively, similar to or less than the concentrations required to inhibit human colon or prostate cancer cell lines.

In preclinical studies, CP461 was shown it to be up to 100 times more potent than exisulind in triggering selective apoptosis in neoplastic (cancerous and precancerous) cells. CP461 also exhibited a broad therapeutic window in animal studies, suggesting it may offer antineoplastic effects with minimal toxicity.

CP461 is currently being evaluated in several clinical trials testing as a single agent for treatment of chronic lymphocytic leukemia (CLL), hormone-refractory prostate cancer, and advanced kidney cancer and in Crohn's disease. The clinical program was initiated in 1998, and in August 1999, in a completed phase Ia clinical trial, CP-461 demonstrated excellent tolerability with no clinically significant drug-related side effects. This single dose, crossover-design trial was conducted in 18 healthy volunteers. CP-461 was administered orally in doses ranging between 50 and 700 mg. The phase Ib segment of this clinical trial of CP-461 was completed in August 2000. Results demonstrated that CP-461 could be chronically administered orally at well tolerated doses that achieve or exceed targeted blood levels.

Subsequently, a dose-ranging phase I/II clinical trial of CP-461 was conducted at the University of Pennsylvania (Philadelphia, PA) in 18 patients with solid tumors. According to the protocol, patients were treated with CP-461 orally, twice daily, for 28 consecutive days, at daily doses ranging from 100 mg to 800 mg. Drug-related toxicities included 1 Grade 3 case of asymptomatic AST/ALT elevation, transient Grade 1/2 sensory neuropathy in 2 patients previously exposed to platinum-based therapy, and 1 case of Grade 1 alopecia. There were no cases of hematologic toxicity. Disease stabilized in 3 patients after 2 cycles of treatment. CP-461 was rapidly absorbed, and high plasma concentrations that exceed the concentrations required to induce apoptosis in cancer cells, were achieved at daily doses >200 mg (Sun W, etal, ASCO01, Abs. 459).

In July 2001, an open-label, phase II clinical trial (protocol ID: CP 461-003) was initiated at UCLA Medical Center, to evaluate CP-416 in advanced, measurable, renal cell carcinoma. Trial objectives are to evaluate the efficacy of CP-461 in locally advanced or metastatic renal cell cancer as measured by changes in disease, i.e. tumor size or tumor burden, and to evaluate the safety profile of CP461 in this setting. According to the protocol, CP-461 (200-400 mg) is being administered PO, twice daily. The trial is to enroll 14 patients to be followed-up for a minimum of three months.

An open-label, phase II clinical trial (protocol ID: CP 461-006) of CP-461 in metastatic prostate cancer, was initiated in August 2001, at Columbia Presbyterian Medical Center (New York, NY). The treatment protocol is identical to that of the phase II clinical trial in renal cancer. Trial objectives are to evaluate the efficacy of CP-461 in metastatic adenocarcinoma of the prostate, determine the duration of response and survival, and evaluate the safety profile of CP461 in this setting. The trial is expected to enroll 25 patients.

A multicenter, open-label, pilot, phase I clinical trial in patients with previously untreated chronic lymphocytic leukemia (CLL) with increasing CLL disease activity but without urgent need for standard therapy, was ongoing as of September 2002, to evaluate the biologic activity of CP-461 in previously untreated CLL patients, and assess its safety profile in this setting. According to the protocol, patients are treated with CP-461 at 200 mg or 400 mg, twice daily.

A multicenter, phase I clinical trial (protocol ID: CP 461-010) to determine the MTD of CP-461 in treating patients with relapsed or refractory chronic lymphocytic leukemia (CLL), was initiated in March 2002. Primary objectives of this trial are determination of MDT of oral CP-461 and evaluation of its safety in this setting. Secondary objectives are to evaluate the activity of CP-461 in terms of objective response rates, and to determine its pharmacokinetics. Approximately 15 patients will be enrolled in this trial to be treated with CP-461 at a daily dose ranging from 800 mg to 1600 mg. Patients are being assessed for objective response after every 2 cycles of therapy.

HMN-214

HMN-214, under development by NS Pharma (Parsippany, NJ), a subsidiary of Nippon Shinyaku (Kyoto, Japan), is an orally administered stilbene prodrug of HMN-176 that interferes with microtubule function. Among the unique *in vitro* effects of HMN-176 is potent apoptosis associated with an abnormal distribution of polo-like and cyclin-dependent kinases. HMN-176 increases the amount of cyclin proteins and collapses the prometaphase spindle body, which causes G2/M phase arrest and caspase-dependent DNA fragmentation. HMN-124 has demonstrated potent antitumor activity in a broad spectrum of human tumor xenografts.

In a phase I clinical trial, conducted at the Arizona Cancer Center, HMN-214 was administered PO to 15 patients with advanced solid tumors including colon, breast, and esophageal cancer, nsclc, and Hodgkin's disease, daily for 21 days, in 28 day treatment cycles. No DLT was observed at daily dose levels of 3 mg/m^2 and 6 mg/m^2. At a daily dose of 9.9 mg/m^2, Grade 3 hyperglycemia and myalgias was experienced by 1 patient, and another expe-

rienced Grade 3 long bone pain. At the daily dose of 6 mg/m^2, 1 patient with colon cancer had a transient decrease in CEA after 1 cycle, but progressed after 2 cycles (Taylor C, etal, ASCO02, Abs. 419:105a).

In a phase I clinical trial, conducted at the Institute for Drug Development, Cancer Therapy and Research Center, at the University of Texas, HMN-214 was investigated for the treatment of solid tumors, in 18 patients administered HMN-214 PO daily for 5 days, every 4 weeks at a dose of 6, 12, 24, 36, and 48 mg/m^2. Patients were stratified according to prior therapy into groups of heavily pretreated (HP) and lightly pretreated (LP) patients. Febrile neutropenia occurred in 1 HP patient treated at the 24 mg/m^2 dose level, and 1 HP patient at the 48 mg/m^2 dose level. In LP patients at the 36 mg/m^2 and 48 mg/m^{2}dose levels, DLT included febrile neutropenia, electrolyte disturbances, myalgias, and neuropathy. HP and LP patients appear to tolerate 24 mg/m^2 and 48 mg/m^2 doses. A minor response was observed in 1 LP patient with colorectal cancer, and disease stabilized for >7 months in 3 patients with carcinoid, and colorectal and breast cancer. MTD had not been determined at the time of this report (Patnaik A, etal, ASCO02, Abs. 418:105a).

R440

R440 (previously Ro 31-7453), under development by Hoffmann-La Roche, is a novel orally available bisindolylmaleimide that acts as a spindle poison, cell-cycle inhibitor, and apoptosis inducer. Low concentrations of R440 inhibit mitotic spindle formation, prevent chromosome aggregation, and induce hyperploidy and apoptosis. At high concentrations, R440 inhibits progression of some cell lines into S phase. Although R440 halts cells in the G2/M phase and disrupts the formation of mitotic spindles, its mode of action is mechanistically distinct from the taxanes and Vinca alkaloids. Cell-cycle inhibition by R440 is reversible for some time simply by washing out the drug. Two active metabolites are formed, each of which form inactive secondary metabolites. One of these metabolites is formed by hydroxylation of one of the methyl groups.

R440 works in a wide array of *in vivo* cancer models, causing regression of the tumor in some. It exhibited antitumor activity when administered by oral, IV infusion, and intraperitoneal routes. In 13 of 14 models, antitumor effects included growth suppression, shrinkage, and cures depending on the model and the therapeutic regimen. Tumors growing at subcutaneous, orthotopic and pulmonary sites were inhibited (Dhinga U, etal, AACR00, Abs. 218:34). R440 is expected to demonstrate activity in various solid tumors including lung, breast and colorectal cancer. Currently, the drug is being evaluated in the clinic in both solid tumors and hematologic malignancies. Phase II clinical trials are also ongoing in the USA in breast cancer and nsclc. Clinical development involves both single-agent and combination regimens.

A randomized phase I clinical trial (protocol ID: MSKCC-00118, NCI-G01-1928, ROCHE-NP15980C) to assess the impact of food on the bioavailability of R440 in patients with locally advanced or metastatic solid tumors, was closed in January 2002. A total of 10 patients were randomized to one of two treatment arms. Patients in arm I were treated with R440 under fasting conditions on day 1. After a 1-week washout period, oral R440 was administered under fed conditions. Patients in arm II underwent treatment under fed conditions as in arm I on day 1. After a 1-week washout period, fasted treatment as in arm I was administered. At the start of week 3, oral R440 could be administered every 12 hours on days 1-4 to patients in both arms. Treatment was repeated every 3 weeks for at least 24 weeks in the absence of disease progression or unacceptable toxicity. Patients were followed at 7 days. Steven Soignet, MD, of the Memorial Sloan-Kettering Cancer Center is Study Chair.

A dose-escalation phase I clinical trial, conducted at University of Texas M. D. Anderson Cancer Center, and the National Cancer Hospital (Goyang, Korea), investigated MTD, toxicity, and pharmacokinetics of oral R440, administered in combination with paclitaxel, to 23 patients with solid tumors. R440 (220 mg/m^2) was administered every 12 hours for 4 days with paclitaxel (175 mg/m^2) as a 3-hour infusion on day 1 in 3 weekly cycles. Median number of treatment cycles was 4 (range=1-8), with a total of 97 cycles delivered. Among 4 patients who completed 24 weeks of treatment, two continue to undergo chemotherapy in an extension phase. Grade 3 or 4 adverse events such as neutropenia, leukopenia, fatigue, arthralgia, constipation and vomiting, were observed in 10 patients; these are consistent with the known toxicities of R440 and paclitaxel. There were no treatment-related deaths and no evidence that the pharmacokinetics of paclitaxel changed as the dose of R440 was increased. Cmax exposure to R440 and its metabolites appeared to be slightly lower on day 4 than would have been expected from single-agent studies, suggesting a possible interaction either with paclitaxel or its premedication. The small sample size and the extent of variability preclude any definitive conclusions on the impact of paclitaxel on the pharmacokinetics of R440. Dose escalation continues to define MTD (Papadimitrakopoulou V, etal, ASCO02, Abs.1830:6b).

In a phase I clinical trial (protocol IDs: MSKCC-98099, NCI-G99-1499, ROCHE-NO15857A), conducted at Memorial Sloan-Kettering Cancer Center, 27 patients with solid tumors were treated with daily doses of oral R440 (25-800 mg/m^2) for 4 consecutive days on a 21-day cycle; median number of cycles administered were 2 (range=1–6). DLT was myelosuppression and stomatitis, occurring in 5/14 patients at the 800 mg/m^2 daily dose level; there was one death. Other adverse events included diarrhea, nausea, vomiting, fatigue and alopecia. Early indication of antitumor activity was observed in two patients with nsclc. Accrual continued at 400 mg/m^2 dose level administered in 2 divided doses, and 560 mg/m^2 as a single daily dose (Soignet SL, etal, AACR00, Abs. 3884:610).

A phase I clinical trial is being conducted at Roswell Park Cancer Institute (Buffalo, NY) to evaluate the safety of R440 treatment in acute myeloid leukemia (AML). The trial's duration is approximately two years with Maria Baer, MD, as the PI. Patients are randomly assigned to one of 2 treatment schedules. R440 is administered PO for either 7 or 14 consecutive days, every 12 hours. The initial R440 dose is 150 mg/m^2 every 12 hours for the 7-day schedule and 95 mg/m^2 for the 14-day schedule. Treatment cycles are administered every 21 days in the absence of progressive disease or substantial toxicities. For patients in the 14-day schedule, if toxicities have not resolved to ≤Grade 1 by cycle day 21, the start of the next cycle may be delayed an additional 7 days. For the 7-day schedule, subsequent cycles may begin before 3 weeks if there are no toxicities or all therapy-related toxicities resolved to ≤Grade 1. Treatment is continued for a maximum of 2 courses beyond best response or 24 weeks, whichever is shorter.

A randomized open label, multicenter, phase II clinical trial (protocol ID: MSKCC-00139; NCI-G01-1945; ROCHE-16113; ROCHE-RO31-7453), initiated in September 2001 to compare the effectiveness of two different regimens of R440 in treating patients with recurrent or refractory metastatic colorectal cancer, was completed in September 2002. Sunil Sharma of Memorial Sloan-Kettering Cancer Center is Study Chair. Approximately 160 patients (49 per treatment arm plus 61 additional patients in the arm determined to be most effective) were randomized to one of two treatment arms. In arm I patients were treated with R440 PO twice daily on days 1-4, repeated every 21 days in the absence of disease progression or unacceptable toxicity. In arm II patients were treated with R440 PO twice daily on days 1-14, repeated every 28 days in the absence of disease progression or unacceptable toxicity. Patients are being followed at 28 days and then every 3 months.

SDX-103

SDX-103, under development by Salmedix (San Diego, CA), is a water-soluble phosphate prodrug of indanocine. This patented agent was synthesized at the University of California San Diego, and licensed to exclusively to Salmedix.

Indanocine is a cytostatic and cytotoxic indanone that blocks tubulin polymerization but, unlike other antimitotic agents, induces apoptotic cell death in stationary-phase MDR cancer cells at concentrations that do not impair the viability of normal nonproliferating cells. Indanocine is selectively toxic to leukemic cells. B cells associated with chronic lymphocytic leukemia (B-CLL), but not normal B cells, are hypersensitive to indanocine. However, the biochemical basis for the hypersensitivity is unknown. Incubation of CLL cells with indanocine induces rapid activation of the stress-activated protein kinase pathway (JNK and p38 kinase), subcellular relocalization of the proapoptotic Bcl-2 family members, Bim and Bax, and increased expression of p73, a p53 functional homolog. Subsequent to these early events, indanocine activates an intrinsic apoptotic program that is characterized by the release of cytochrome c from the mitochondrial compartment, activation of caspase-9 and caspase-3, cleavage of intracellular caspase substrates, and morphologic changes associated with apoptosis. CLL apoptosis induced by indanocine was prevented by pretreatment with cell-permeable pancaspase inhibitors (Leoni LM, etal, JNCI, 2 Feb 2000;92(3):182-3).

SDX-103 retains a similar level of antiproliferative and proapoptotic activities as indanocine when tested in primary CLL cells and in tumor cell lines. The *in vivo* antitumor activity and pharmacodynamic properties of SDX-103 and other analogs are currently being evaluated in animal models. No toxicity was observed when SDX-103 was tested IV and IP in athymic nude mice at doses as high as 300 mg/kg. SDX-103 was active in mice bearing P-388 leukemia that were administered doses of 300 mg/kg IP (Leoni L, etal, AACR02, Abs. 1321:266).

T67/T607

T67 (previously T138067), a 2-fluoro-1-methoxy-4-pentfluorophenylsulfonamidobenzene, under development by Tularik (South San Francisco, CA), is a novel irreversible tubulin polymerization inhibitor. T67 belongs to a unique series of compounds that show activity *in vivo* against a variety of tumors, including those resistant to known cancer drugs. These compounds act by binding specifically and irreversibly to β-tubulin, thus disrupting the process of cell replication resulting in tumor shrinkage. T67 disrupts microtubule polymerization by covalently and selectively modifying the 1, 2, and 4 isotypes of β-tubulin at a conserved cysteine residue. When exposed to T67, cells become altered in shape, indicating a collapse of the cytoskeleton, show an increase in chromosomal ploidy and, subsequently, undergo apoptosis. T67 is also cytotoxic to tumor cell lines that exhibit substantial resistance to vinblastine, paclitaxel, doxorubicin, and actinomycin-D, and is equally efficacious in inhibiting the growth of sensitive and MDR human tumor xenografts in athymic nude mice. Therefore, T67 may be clinically useful in the treatment of MDR tumors (Shan B, etal, PNAS USA, 11 May 1999:96(10):5686-91).

T67 has demonstrated efficacy against breast, colon, ovarian, renal, lung, CNS, and prostate cancer and melanoma cell lines, and activity against cell lines expressing the MDR phenotype (Medina JC, AACR99, Abs. 4382:664).

In humans and preclinical species, T67 demonstrates clearance approximating hepatic blood flow. In addition, plasma protein binding of this compound is extensive and, therefore, drug-drug interactions arising from the displacement of T67 from albumin should not occur in the clinical setting (Wright MR, etal, AACR00, Abs. 1379:216).

Also, the efficacy of T67 is not significantly affected by expression of MDR1. T67 not only evades resistance in

cells that express the P-gp pump, but also in cell lines in which resistance to other agents is derived from mutations in β-tubulin and changes in β-tubulin isotype expression patterns. There was no increased resistance to T67 in either ovarian cancer cells 1A9PTX10 or 1A9PTX22 that carry β-tubulin mutations making them 54- and 68-fold more resistant to paclitaxel, respectively. Similarly, no resistance to T67 and its analogs was noted in the EM15 variant of the DU145 human prostate cell line with altered β-tubulin isotype expression, that is 8-fold resistant to estramustine, while PAC10 cells that are 4-fold resistant to paclitaxel were only 0.47-fold resistant to T67 (Medina JC, etal, AACR00, Abs. 1378:216).

T67 demonstrated similar inhibition of tumor growth as either vinblastine or paclitaxel in CCRF-CEM lymphoblastic leukemia xenografts in SCID mice, with reduction of the final tumor volumes of 65%, 80% and 75%, respectively, compared with control. T67 was equally effective (75%-80%), in reducing the growth rate of a vinblastine-resistant subclone whereas the efficacy of paclitaxel (40%) and vinblastine (50%) were both significantly attenuated. Treatment with T67 resulted in a 75% growth inhibition of MX-1 human mammary tumor xenografts in athymic nude mice (Schwendner SW, etal, AACR99, Abs, 1911:288).

Phase I clinical trials were initiated in March 1998, using a 3-hour infusion of T67, administered every 4 weeks, in patients with solid tumors. Initial dose escalation levels ranged from 11 mg/m^2 to 55 mg/m^2. Subsequently, 26 patients (renal cancer=5, 3 patients each with bladder, head and neck, and ovarian cancer, and 12 with other tumors) were treated with doses escalated to levels ranging from 110 mg/m^2 to 585 mg/m^2. No toxicity and linear pharmacokinetics were observed up to the 440 mg/m^2 dose level. Among 3 patients treated at 585 mg/m^2 every 3 weeks, 1 developed transient Grade I neutropenia as well as Grade 2 nausea, vomiting and diarrhea, another developed Grade 2 neutropenia and the third developed Grade 4 neurotoxicity characterized by lack of responsiveness and hearing loss. These acute symptoms resolved within 72 hours although some residual hearing loss persisted. This patient and one other treated at 585 mg/m^2, exhibited higher than predicted plasma levels of T67, suggesting the possibility of nonlinear pharmacokinetics above 440 mg/m^2. Accordingly, 440 mg/m^2 was established as the MTD for further studies. A PR was observed in one patient with liver cancer, that was maintained for more than one year (Spriggs DR, etal, ASCO00, Abs. 921I:237a).

Subsequent phase I clinical trials tested T67 at more frequently administered lower doses. In a phase I dose escalation clinical trial, designed to assess MTD, pharmacokinetics, and safety of T67, 20 patients with refractory solid tumors (colorectal cancer=9, hepatocellular carcinoma=4, nsclc=1, renal cell carcinoma=1, gastric cancer=1, prostate cancer=1, lymphoma=1, breast cancer=1, and unknown primary=1) were treated with T67 at a dose of 44, 88, 175, 200, and 250 mg/m^2, administered daily for 5 days every 3 weeks. DLT, manifested as Grade 4 neutropenia (n=1) and reversible Grade 4 encephalopathy/hearing loss (n=1), occurred in 3 patients treated at a dose of 250 mg/m^2. In addition, DLT occurred in 2/5 patients treated at 200 mg/m^2, manifested as Grade 3 neutropenia and hearing loss. MTD was 175 mg/m^2. Pharmacokinetics were linear. The most common adverse events, considered to be possibly related to the drug, were limb pain (30%), fatigue (20%), perception of feeling cold (20%), arthralgia (20%), hypokalemia (20%), burning sensation (20%), and reticulocytosis (20%). Among 15/20 patients evaluable for response, there were no CR or PR; disease stabilized in 1 (7%), and progressed in 14 (93%). Based on these data MTD and recommended phase II dose of T67, administered daily for 5 days every 3 weeks, is 175 mg/m^2 (Molpus K, etal, ASCO02, Abs. 415:104a).

In another phase I clinical trial, T167, administered as a 3-hour infusion daily for 5 days every 3 weeks, was assessed in 12 patients (colorectal cancer=6, hepatocellular cancer=2, and 1 each renal, gastric and breast cancer and nsclc) at 4 dose levels (44 mg/m^2, 88 mg/m^2, 175 mg/m^2, and 250 mg/m^2). Treatment was well tolerated at doses below 250 mg/m^2 with 1 case of Grade 1 leucopenia, 1 of Grade 2 anemia and 1 of Grade 1 thrombocytopenia. There were 2 DLT at the 250 mg/m^2 dose level; 1 patient experienced a Grade 4 transient and uncomplicated neutropenia and another reversible Grade 4 encephalopathy with reversible hearing loss. T67 was well tolerated at dose levels ≤175 mg/m^2, daily, for 5 days, but severe neurotoxicity was seen at 250 mg/m^2 (Preston G, etal, ASCO01, Abs. 443:112a).

A current pivotal indication pursued by Tularik for T67 is advanced hepatocellular carcinoma (HCC). A phase I/II clinical trial in unresectable HCC was initiated in July 2000. In the phase I portion of this clinical trial, 28 patients with HCC were treated at 110, 220, 330, 385 and 440 mg/m^2 as a weekly 3-hour infusion. One HCC patient at 110 mg/m^2 who had progressed through 3 prior therapies had a durable (13+ months) PR to T67 with symptomatic improvement. There were 2 DLT at the 440 mg/m^2 dose level involving Grade 3 reversible ataxia and neutrophil count that failed to return to 1000/mm^3 on the planned dose day, resulting in a dose omission. No acute CNS toxicity was observed below 440 mg/m^2. Treatment in HCC patients was limited by mild reversible thrombocytopenia. The current dose under evaluation is 220 mg/m^2. In the non-HCC group at 330 mg/m^2, 1 patient experienced Grade 3 leucopenia and Grade 2 neutropenia, and another Grade 3 febrile neutropenia. Other toxicities included Grade 2 infusion pain (n=1), diarrhea (n=1), nausea/vomiting (n=1) and Grade 3 neurotoxicity (n=1). The recommended phase II dose for non-HCC patients is 330 mg/m^2. This schedule was well tolerated, has shown evidence of activity; and is recommended for evaluation in phase II (Donehower RC, etal, ASCO01, Abs. 438:110a).

Based on the fact that there was 1 PR in a patient with HCC in the phase I portion of the phase I/II clinical trial, the T67-Sodium HCC Study Group conducted a phase II clinical trial designed to assess the safety and efficacy of 165 mg/m^2 of T138067-sodium infused over 3 hours once a week in two groups of patients (chemotherapy naïve=35 and one prior chemotherapeutic regimen=35) with unresectable HCC. Among 53 patients (first-line=33, second-line=20), enrolled from March to October 2001, 19/20 previously treated patients had been exposed to anthracycline-based chemotherapy. Among 21 of 33 first-line patients evaluable for response, there were 2 (10%) PR, and disease stabilized in 9 (43%), and progressed in 10 (47%). A reduction of >50% in α-fetoprotein (AFP) was observed in 3/20 (15%) first-line patients; 1 of these 3 patients had a PR. Among 11 of 20 second-line patients evaluable for response, there was 1 (9%) PR, and disease stabilized in 3 (27%), and progressed in 7 (64%). The most common adverse events considered to be possibly related to the drug, were fatigue (30%), nausea (13%), vomiting (13%), and diarrhea (10%). There were no Grade 4 adverse events (Leung TW, etal, ASCO02, Abs. 572:144a).

In August 2002, Tularik received a written response from the FDA that the design for its pivotal phase II/III clinical trial program for T67 in HCC, was satisfactory. This trial of first-line treatment with T67 in hepatocellular carcinoma is expected to enroll approximately 750 patients at numerous centers across the USA, Europe and Asia. The trial will compare survival of patients treated with IV T67 (250 mg/m^2), administered every week, to IV doxorubicin(60 mg/m^2), administered every 3 weeks and evaluate the safety and efficacy of T67 in this setting. Tularik expects to open this trial in early 2003.

In October 2000, a multicenter phase II multicenter clinical trial (protocol IDs: TULA-T2002; VU-000511) to study the effectiveness of T67 in treating patients with refractory locally advanced or metastatic nsclc, was initiated by Tularik with Sean McCarthy as Study Chair. IV T67 is administered over 3 hours on days 1, 8, and 15. Treatment is repeated every 21 days in the absence of disease progression or unacceptable toxicity. Patients are followed every 3 months after completion of the trial. Between October 2000 and October 2001, 20 patients with refractory, locally advanced or metastatic nsclc were enrolled in this trial. Prior regimens included paclitaxel/carboplatin (n=16), other paclitaxel combinations (n=3), and docetaxel/irinotecan (n=1). Among 14/20 patients evaluable for efficacy, disease stabilized in 3 (21%), and progressed in 11 (79%). The most common adverse events that may have been related to the drug, include neutropenia (23%), nausea (15%), vomiting (15%), and vein irritation (15%). There were no Grade 4 adverse events (Jahan TM, etal, ASCO02, Abs. 1282:321a).

T607 (also known as T900607) is a structural analog of T67. T607 binds to β-tubulin preventing assembly of polymeric structures thus disrupting cytoskeletal structures. T607 causes cell cycle arrest in the G2/M phase and results in apoptotic events. T607 causes cell-cycle arrest in the G2/M phase and results in apoptotic events. T607 is more water-soluble, amenable to bolus administration, and has a different DLT profile compared to T67. Also, T607 is active against MDR tumors. Animal studies indicate that T607 is distinguished from T67 because it of its reduced ability to cross the BBB.

T607 demonstrated tumor growth regression when administered to athymic nude mice bearing MX-1 mammary tumor xenografts. Several mice demonstrated a complete tumor remission for more than 60 days. However, optimal doses of T607 that caused regression of tumor growth, also resulted in dose-dependent weight loss and toxicity. Administration of a suboptimal dose of T607, simultaneously with a suboptimal dose of cisplatin, resulted in significant tumor regression in all mice. Body weight loss and toxicity were greatly reduced compared with administration of either agent at MDT. Similar synergy in tumor growth regression was also apparent with carboplatin. Additive effects of tumor growth inhibition were observed with the administration of T607 and gemcitabine, but T607 in combination with either 5-FU or doxorubicin was not effective against MX-1 mammary tumor xenografts (Schwendner SW, etal, AACR00, Abs. 1914:301). T607 was also effective at inhibiting tumor growth against DLD-1 colon, C13 cisplatin-resistant ovarian and MCF7-adriamycin-resistant mammary tumor xenografts in mice (Schwendner SW, etal, AACR00, Abs. 1919:302).

Tularik initiated two phase I dose-escalation clinical trials in USA and the UK in April 2000, and a third phase I clinical trial began in Canada in late 2000.

In a phase I clinical trial of T607, administered daily as a 60-minute infusion for 5 days every 3 weeks, in 25 patients with refractory malignancies, conducted at Vanderbilt-Ingram Cancer Center (Nashville, TN) and Montefiore Medical Center (Bronx, NY), the most common adverse effects were fatigue (33%), nausea (24%), and diarrhea (19%). There were no Grade 4 toxicities. One patient experienced a transient asymptomatic troponin elevation and 1 patient had an asymptomatic, Grade 1 decrease in cardiac ejection fraction and troponin elevation after 5 cycles of 60 mg/m^2; the ejection fraction recovered to baseline post-treatment. One patient with previously treated hepatoma experienced a durable PR (95% reduction in tumor and 99% reduction in AFP), lasting 8+ months (Lockhart AC, etal, ASCO02, Abs. 417:105a).

This same group conducted a phase I dose-escalation clinical trial of T607, administered daily as a 30-minute infusion at dose levels of 7.5 mg/m^2, 15 mg/m^2, 30 mg/m^2, 45 mg/m^2, and 60 mg/m^2, for 5 days, every 3 weeks, in 14 patients with advanced solid tumors. Toxicities included Grade 3 fatigue (n=1), anemia (n=1) and low back pain (n=1). Other ≤Grade 2 toxicities included anemia, fatigue, pain at the infusion site, vomiting, dizziness, headache,

and paresthesia. One patient with K1-anaplastic lymphoma who had failed CHOP chemotherapy, experienced a PR that lasted 5+ months (Schumaker RD, etal, ASCO01, Abs. 442:111a).

A phase I clinical trial was conducted at University of Aberdeen (Scotland) and Nottingham City Hospital, in the UK, to determine MTD, pharmacokinetics, and safety of weekly infusions of T607, in 24 patients with refractory cancer. Cohorts of patients were treated at 20, 40, 60,100 and 130 mg/m^2. At 130 mg/m^2, 2 patients experienced DLT manifested as Grade 3 abdominal pain and a platelet nadir of 80,000 cells/mm^3 resulting in an omission of a T607 dose. MTD was 100 mg/m^2, administered weekly. The pharmacokinetics of T607 were linear. The most common adverse events were nausea (52%), injection site pain/irritation (48%), vomiting (33%), fatigue (29%), and chills (24%). There were no Grade 4 adverse events; 1 patient developed an asymptomatic decline in ejection fraction after 6 cycles of 60 mg/m^2, and 1 patient experienced an inferior MI after 5 cycles of 100 mg/m^2 (Stagg RJ and Cassidy J, etal, ASCO02, Abs. 416:105a).

Tularik initiated three phase II clinical trials with T607 in July 2002. A multicenter, open-label, single-agent, phase II clinical trial is being conducted in refractory non-Hodgkin's lymphoma (NHL), and in refractory ovarian cancer; each trial is being conducted in the USA and will enroll about 35 patients. A third open-label, multicenter, phase II clinical trial is being conducted in several centers in the USA, and at Queen Mary Hospital (Hong Kong, China), under PI Raymond Chan, MD, in chemotherapy- or radiotherapy-naïve patients with hepatocellular carcinoma (HCC). Approximately 35 patients will be enrolled in this trial as well. Primary endpoints of all these trials are safety and effectiveness.

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