ANTICANCER DRUGS AND MARKETS

ANTIBIOTIC/ANTHRACYCLINE CYTOTOXICS
PART I — COMMERCIALLY AVAILABLE DRUGS

ANTICANCER DRUGS AND MARKETS

Antibiotic anticancer agents, in general, and anthracyclines, in particular, remain the mainstay in combination regimens for the treatment of many of the most prevalent advanced cancers. Several newer anticancer antibiotics, as well as novel formulations, have been introduced in the marketplace that promise to reduce the side effects of current antibiotic-based combination regimens. Also, antibiotics are currently used in combination with novel regulatory agents, in a new approach to the therapy and management of cancer. In addition, numerous novel agents belonging to the antibiotic class of cytotoxics are in development.

A list of selected marketed cytotoxic antibiotics is presented in Exhibit 1. The global market for antibiotic cytotoxics is estimated at $600 million in 2002. Although several novel agents and formulations such as epirubicin (Ellence; Pharmacia) and liposomal doxorubicin (Doxil; Johnson & Johnson), make the lion's share of the dollar market, the bulk of unit sales/prescriptions is attributable to generic versions of antibiotic cytotoxics, primarily doxorubicin, whose patents expired many years ago. Extensive use of doxorubicin is illustrated by the fact that it is incorporated in recommended combination regimens for the treatment of many malignancies, including breast cancer. It is, therefore, obvious that better versions of doxorubicin and other antibiotic cytotoxics would create a very lucrative market for developers.

APPLICATIONS OF ANTIBIOTICS AS CYTOTOXICS
Mechanism of Action

The exact antitumor mechanism of antibiotic cytotoxics has not been fully elucidated. Also, each subclass of antibiotics acts by unique mechanisms and, in most cases, exhibits multiple mechanisms of action. Most antibiotics exert their antitumor effects by interfering with the structure and function of DNA. In addition to DNA damage, antibiotics exert some of their cytotoxic effects by direct binding to DNA, inhibiting transcription of certain genes. Anthracyclines also induce apoptotic cell death.

Drug Resistance

Like with most other commercially available cytotoxics, drug resistance limits the effectiveness of antibiotics. Among well described mechanisms of multidrug resistance (MDR) are those conferred by the 170-kDa P-glycoprotein (P-gp, ABCB1), or the 190-kDa MDR-associated proteins MRP1 (ABCC1), or MRP2 (ABCC2). These membrane proteins are members of the ATP binding cassette transporter superfamily, and are responsible for the removal from the cell of many cytotoxic agents including doxorubicin. P-gp and MRP1 mediate efflux of anthracyclines from cells and reduce their nuclear accumulation. MDR is a major cause of failure of anthracycline-based chemotherapy in breast cancer.

Another source of MDR is breast cancer-resistance protein (BCRP), a novel half-transporter. BCRP is a cell-surface ABC transporter that confers resistance against mitoxantrone, doxorubicin, and the camptothecin derivatives topotecan and irinotecan (CPT-11).

Resistance to doxorubicin is described as a multifactorial phenomenon involving overexpression of defense factors and alterations in drug-target interactions. For instance, in addition to overexpression of P-gp, and MDR1, resistance to doxorubicin may be attributable to changes in glutathione metabolism, or downregulation, cellular redistribution, or mutations of toposomerase II. Also, solid tumors may exhibit intrinsic resistance to cytotxic drugs (Perego P, etal, Curr Med Chem, Jan 2001;8(1):31-7). Because anthracyclines can induce apoptotic cell death, an alternative source of drug resistance may center on cellular responses to drug-induced DNA damage, particularly in the relationship between cytotoxicity and antitumor efficacy, and apoptotic response.

One way of combating P-gp- and MDR1-associated resistance is use of modulators that can inhibit the activity of these transporters. However, although many compounds have been proven to be very efficient in inhibiting P-gp activity in animal models, results in the clinic have been rather disappointing. One problem is that drugs against MDR suppress the protein all over the body, resulting in serious toxicity.

Combinations of different classes of cytotoxics may also overcome MDR manifested when these agents are used as monotherapy. Anthracycline-based combinations were shown to produce higher clinical response rates and longer survival than single-agent approaches. For instance, it has been demonstrated *in vitro* that the combination of homocamptothecin and daunorubicin overcomes MDR in doxorubicin-resistant or etoposide-resistant breast cancer MCF7 cells. Simultaneous combination of subtoxic doses of homocamptothecin with daunorubicin resulted in the potentiation of daunorubicin activity, and was well correlated with an increase in its nuclear accumulation in etoposide-resistant MCF7 cells. Simultaneous administration resulted in a higher cytotoxic response than sequential one. Homocamptothecin also increased nuclear accumulation of daunorubicin in low MRP1-expressing doxorubicin-resistant MCF7 cells, but this enhancement was poorly correlated with the nuclear concentration of daunorubicin in these cells. Thus, in addition to the increase in daunorubicin accumulation, potentiation of daunorubicin activity by homocamptothecin in doxorubicin-resistant MCF7 cells implies a synergistic mechanism between both drugs, which remains to be further elucidated. These data suggest that the present topoisomerase I/II inhibitor combination may be of clinical interest to overcome the MDR phenotype in daunorubicin-treated breast cancer (Chauvier D, etal, AACR02, Abs. 1308:263).

Another approach to combating MDR is based on the design and synthesis of new non-cross-resistant drugs whose physicochemical properties favor their uptake by resistant cells. Studies have shown that whereas the P-gp- and MRP1-mediated efflux of different anthracycline-based drugs may not differ considerably, their kinetics of uptake do. Because uptake of drug by cells may lead to concentrations at the cellular target site high enough to achieve the needed cytotoxicity against MDR cells, increased drug lipophilicity might be one factor in improving drug cytotoxicity in MDR cells. *In vitro* studies have shown that idarubicin is more effective than daunorubicin and doxorubicin against MDR tumor cell lines, and that this increased effectiveness is related in part to the increased lipophilicity of idarubicin. Other studies have also confirmed the strong impact of lipophilicity on the uptake and retention of anthracyclines in MDR cells (Garnier-Suillerot A, etal, Curr Med Chem 2001;8(1):51-64).

Resistance may also be overcome by combination approaches using regulatory agents that block the activity of certain genes, thus enhancing the performance of cytotoxic agents. Numerous trials are currently ongoing combining antibiotic cytotoxics with agents that modulate numerous gene/protein targets deemed relevant to the malignant state (Exhibit 2).

Tariquidar (XR9576), under development by QLT (Vancouver, Canada), in collaboration with Xenova (Slough, Berkshire, UK), is a selective potent inhibitor of the action of the P-gp pump. The drug is being evaluated in several clinical trials in combination with various cytotoxics.

A multicenter, phase II clinical trial (protocol ID: TQD BRST 001) was ongoing as of February 2003, to assess if XR9576 can reverse primary doxorubicin or taxane resistance in advanced breast cancer. Clinical activity is measured by objective tumor response rates observed after treatment with XR9576 in combination with taxane- or anthracycline-containing chemotherapy, in patients previously resistant to the same agent(s). Secondary objectives are to assess the biological activity of XR9576 and evaluate MDR1 expression in these tumors. The MDR1 inhibitory activity of XR9576 is being evaluated by serial sestamibi

Exhibit 1
Selected Commercially Available Antibiotic Cytotoxics

Developer ❏ Affiliate(s)	Generic Name ❏ Number ❏ Brand Name	Description ❏ Administration Route	Location (Approval Date) ❏ Indications
Anthracyclines/Analogs			
Amgen ❏ Wyeth	Mitoxantrone ❏ NSC 301739 ❏ Novantrone	Synthetic anthracenedione structurally related to doxorubicin ❏ IV	USA (12/87) ❏ acute myelogenous leukemia (AML); USA (11/96) ❏ advanced hormone-refractory prostate cancer; Canada ❏ non-Hodgkin's lymphoma (NHL)
Baxter Oncology	Mitoxantrone ❏ Onkotrone	Generic mitoxantrone ❏ IV	Europe (6/00) ❏ advanced or metastatic breast cancer
Beacon Pharmaceuticals ❏ Laboratories Thissen	Daunorubicin	Generic version of daunorubicin ❏ injection	Europe (1/02) ❏ acute leukemia
Bedford Laboratories ❏ Ben Venue Laboratories	Daunorubicin HCl ❏ Cerubidine	Generic version of daunorubicin provided in 5 mg/ml, 4ml vials ❏ injection	USA (1/98) ❏ first-line therapy of myelogenous, monocytic or erythroid adult leukemia, or remission induction in childhood and adult acute lymphocytic leukemia (ALL)
Gensia Sicor Pharmaceuticals ❏ Abbott Laboratories	Daunorubicin HCl Injection	Generic version of daunorubicin ❏ injection	USA (1/00) ❏ acute leukemia
Gilead Sciences	Daunorubicin citrate liposome ❏ NSC 82151 ❏ DaunoXome	Liposomal daunorubicin ❏ injection	USA (4/96), Europe (95), ROW ❏ AIDS-related Kaposi's sarcoma (KS), first line
SuperGen	Daunorubicin	Generic version of daunorubicin ❏ injection	USA (11/01) ❏ acute leukemia
Bristol-Myers Squibb (BMS) ❏ Pharmacia	Doxorubicin HCl ❏ Rubex	Doxorubicin packaged in a polypropylene plastic vial ❏ injection	USA ❏ acute leukemia, Hodgkin's disease (HD), KS, neuroblastoma, soft-tissue sarcoma, small-cell lung cancer (sclc), and bladder, breast, gastric, ovarian, and thyroid cancer
Pharmacia ❏ Gensia Sicor Pharmaceuticals	Doxorubicin HCl ❏ Adriamycin	Doxorubicin hydrochloride injectable packaged in a polypropylene plastic vial ❏ injection	USA ❏ See above
Alza (Johnson & Johnson) ❏ Schering-Plough, Meiji Seika Pharma International	Liposomal doxorubicin HCl ❏ Doxil (USA); Caelyx (outside the USA)	Long-circulating Stealth liposomal formulation of doxorubicin; encapsulation of doxorubicin into liposomes coated with polyethylene glycol (PEG) ❏ IV	USA, Europe, ROW ❏ Kaposi's sarcoma, ovarian cancer
Elan Pharmaceuticals ❏ Enzon	Liposome-encapsulated doxorubicin citrate complex ❏ TLC D-99 ❏ Myocet (formerly Evacet)	Proprietary formulation of doxorubicin in phosphatidyl choline/cholesterol, designed to reduce toxicity and increase efficacy ❏ IV	Europe (8/00) ❏ breast cancer
Pharmacia	Epirubicin, epidoxorubicin ❏ Ellence (USA); Farmorubicin/Pharmorubicin (ROW)	Second generation anthracycline antibiotic, a diastereoisomer of doxorubicin ❏ IV	USA (10/99), Europe, ROW ❏ ovarian cancer, breast cancer; Europe, ROW ❏ melanoma, NHL
Gensia Sicor Pharmaceuticals	Idarubicin HCl	Analog of daunorubicin ❏ IV, PO	USA (5/02) ❏ AML
Pharmacia	Idarubicin HCl ❏ Idamycin, Zavedos	Analog of daunorubicin ❏ IV, PO	USA (12/97) ❏ acute nonlymphocytic leukemia
Mercian ❏ Shenzhen Main Luck Pharmaceuticals	Pirarubicin	Analog of doxorubicin ❏ IV	Japan (88) ❏ solid tumors and hematologic malignancy
Anthra Pharmaceuticals	Valrubicin ❏ AD32 ❏ Valstar, Valtaxin (Canada)	Lipophilic anthracycline; doxorubicin analog ❏ intravesical intraperitoneal	USA (9/98), Canada (7/00) ❏ bladder cancer

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Other Antibiotics			
Bristol-Myers Squibb (BMS)	Bleomycin sulfate ❏ Blenoxane	Mixture of cytotoxic glycopeptide antibiotics isolated from a strain of *Stretomyces verticillus*; water soluble ❏ IV, intrapleural, intramuscular, subcutaneous (SC)	USA (2/96) ❏ testicular cancer, squamous cell carcinoma, lymphoma, treatment and prevention of recurrent malignant pleural effusions
Mayne Pharma	Bleomycin sulfate	Generic bleomycin sulfate ❏ injection	USA(3/00) ❏ testicular cancer, squamous-cell carcinoma, lymphoma
Merck	Dactinomycin, actinomycin D ❏ Cosmegen	Principal component of the mixture of actinomycins produced by *Streptomyces parvullus* ❏ IV	USA, Europe, ROW ❏ gestational trophoblastic neoplasia and, in combination, for Ewing's sarcoma, Wilm's tumor, rhabdomyosarcoma, and metastatic nonseminomatous testicular cancer
Bristol-Myers Squibb (BMS)	Mitomycin C ❏ Mutamycin	Antibiotic isolated from the broth of *Streptomyces caespitosus* ❏ IV	USA ❏ in combination for advanced, refractory solid tumors, and disseminated gastric and pancreatic cancer
Medac	Mitomycin C ❏ Mito-medac	Antibiotic isolated from the broth of *Streptomyces caespitosus* ❏ IV	Europe ❏ advanced, refractory solid tumors (combination)
SuperGen	Mitomycin C ❏ Mitozytrex (Mito Extra)	Reformulation of mitomycin C that increases solubility and decreases hypersensitivity and other reactions seen with the current formulations of mitomycin C ❏ IV	USA (11/02) ❏ advanced gastric and pancreatic cancer
Bayer ❏ Ben Venue Laboratories	Plicamycin, mithramycin ❏ Mithracin	Antibiotic cytotoxic produced by *Streptomyces plicatus* ❏ IV	USA, Europe, ROW ❏ hypercalcemia associated with advanced cancer, refractory testicular cancer
Wyeth Pharmaceuticals ❏ Celltech, Fred Hutchinson Cancer Research Center, Protein Design Labs (PDL)	Gemtuzumab ozogamicin ❏ CMA-676 (CDP771) ❏ Mylotarg	Recombinant humanized anti-CD33 MAb (hP67.6) conjugated to the toxic enediyne antibiotic calicheamicin ❏ IV	USA (5/00) ❏ adult, relapsed, CD-33 positive AML
Sumitomo Pharmaceuticals	Amrubicin, amrubicin hydrochloride ❏ SM-5887, SMP-5887 ❏ Calsed	Synthetic anthracycline ❏ IV, intravesical	Japan (12/02) ❏ non-small-cell lung cancer (nsclc) and sclc

Source: *NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), January 2003.*

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scans of the tumor, and MDR1 expression is being detected by immunohistochemistry. The trial, to enroll approximately 30 patients, is being conducted at Florida Oncology Associates (Jacksonville, FL), Medical Center of Vincennes (Vincennes, IN) and at M. D. Anderson Cancer Center (Houston, TX) and Arlington Cancer Center in Texas. Graeme R. Boniface, MD, is the Study Director.

The effectiveness of XR9576 combined with docetaxel, doxorubicin, or vinorelbine, in 24 children with relapsed or refractory solid tumors, is being investigated in a phase I clinical trial (protocol ID: NCI-01-C-0091B), initiated in February 2001. IV XR9576 is administered over 30 minutes, on days 1 and 3 of course 1, and on day 1 of all subsequent courses. IV doxorubicin is administered over 15 minutes on day 3 of course 1 and on day 1, IV vinorelbine over 10 minutes on days 3 and 10 of course 1 and days 1 and 8, or IV docetaxel over 60 minutes on day 3 of course 1 and on day 1 of all subsequent courses. Patients treated with doxorubicin or docetaxel are treated with filgrastim

(G-CSF) subcutaneously (SC), beginning on day 5 of course 1, and day 3 of all subsequent courses, and until blood counts recover. Courses are repeated every 21 days for up to 8 courses in the absence of disease progression or unacceptable toxicity. Cohorts of 3 to 6 patients are treated with escalating doses of XR9576 until maximum tolerated dose (MTD) is determined, and then 12 additional patients (at least 3 <12 years-of-age and 3 ≥12 years-of-age) are treated at MTD. Intrapatient dose escalation may take place for patients in cohorts 1 and 2 after 2 courses of treatment. Patients are followed every 3 months. This study is sponsored by the NCI under the direction of Frank Milton Balis of the Pediatric Oncology Branch (Bethesda, MD).

Valspodar (PSC 833) is a non-immunosuppressive cyclosporin-D analog that reverses MDR by inhibiting P-gp. PSC 833 is a relatively nonspecific P-gp blocker. The drug had been in clinical trials for many years until Novartis discontinued its development several years ago.

Zosuquidar (LY335979), under development by Eli Lilly, is among the most potent modulators of P-ġp. LY335979 is selective for P-ġp, and does not modulate resistance by MRP1 or MRP2. When administered in combination with daunorubicin, doxorubicin or etoposide, LY335979 significantly enhanced survival of mice implanted with P-ġp-expressing murine leukemia (P388/ADR). LY335979 did not impact the pharmacokinetics of these anticancer agents, which may be attributable, in impart, to its poor inhibition of four major cytochrome P450 isozymes important in metabolizing doxorubicin and other oncolytics (Dantzig Ahm, etal, Curr Med Chem 2001;8(1):39-50).

In a dose-escalation, phase I clinical trial, conducted at Indiana University School of Medicine (Indianapolis, IN), to examine the pharmacokinetics of doxorubicin, doxorubicinol and LY335979, patients were treated with IV LY335979, continuously for 48 hours during cycle 1 on day 1, and with IV doxorubicin over 30 minutes, on day 15. In subsequent cycles, LY335979 was infused over 48 hours with doxorubicin administered 24 hours after commencing LY335979. A total of 41 patients with advanced solid tumors were enrolled into 9 cohorts. No clinically significant interaction in doxorubicin or doxorubicinol pharmacokinetics was observed with LY335979, in contrast to other P-ġp modulators (de Alwis DP, etal, ASCO01, Abs. 284:72a).

In another dose-escalation, phase I clinical trial, also conducted at Indiana University School of Medicine, using a similar protocol scheme, LY335979 was administered IV, alone, and in combination with doxorubicin (45 mg/m^2 and 60 mg/m^2) every 3 weeks to 23 patients with advanced malignancies. The drug was well tolerated with no Grade 3 or 4 toxicities noted and did not appear to augment doxorubicin-induced hematologic or cardiac toxicity. IV-administered LY335979, in combination with doxorubicin, modulated P-ġp as assessed in circulating CD56 cells (Sweeney C, etal, ASCO99, Abs. 703:183a).

LY335979, in combination with daunorubicin and high-dose cytarabine, was investigated in a multicenter, phase II clinical trial, in 52 patients with newly diagnosed secondary acute myeloid leukemia (AML, n=16), refractory anemia with excess blasts in transformation (RAEB-t, n=5) or relapsed/refractory AML/RAEB-t (n=31). Patients were treated with IV daunorubicin (45 mg/m^2), daily, over 10 minutes on days 2 to 4, and IV cytarabine (1.5 gm/m^2), over 2 hours, every 12 hours, for 8 doses, starting on day 5; patients >70 years-of-age were treated with cytarabine every 24 hours for 4 doses. Zosuquidar (480 mg/m^2) was administered daily to the first 3 patients as a 96-hour continuous IV infusion on days 1 to 4; subsequent patients were treated with 320 mg/m^2/day over 72 hours, and 4 patients were treated with a second cycle of induction on day 10 because of residual disease. The median time to recovery of ANC >1,000 or platelets >100,000 during induction was 26 and 31 days, respectively. Reversible

Grade 3 ataxia developed in 2/3 patients treated at the higher dose of zosuquidar; 11/49 (22%) patients treated at the lower dose experienced reversible Grade 3 ataxia (n=3), or confusion and agitation (n=8), attributed to zosuquidar. Responses are reported as CR, CRp (<5% marrow blasts but platelet count <100,000), PR (>5%, <20% marrow blasts), or refractory. Among 52 enrolled patients, 39 were evaluable for response following the first induction therapy; 8 patients died during induction therapy, and response was not assessed in 5 patients for other reasons. There were 15/39 (38%) CR, 9 (23%) CRp, 5 (12%) PR, and disease progressed in 10 (26%) (Cripe LD, etal, ASH01, Abs. 2492).

An NCI-sponsored, randomized, double-blind, placebo-controlled, multicenter, phase III clinical trial (protocol ID: E-3999) was initiated in December 2002, being conducted by the Eastern Cooperative Oncology Group (ECOG), to compare the effectiveness of daunorubicin and cytarabine with and without zosuquidar in treating older patients diagnosed with untreated AML, acute poorly differentiated (M5a) or differentiated (M5b) monocytic leukemia, acute myeloblastic leukemia without (M1) or with maturation (M2), *de novo* myelodysplastic syndromes (MDS), acute erythroleukemia (M6), acute myelomonocytic leukemia (M4), refractory anemia with excess blasts or excess blasts in transformation, acute myeloblastic leukemia, secondary AML, and acute megakaryocytic leukemia (M7). Trial objectives are to compare the CR rate and toxicity of daunorubicin and cytarabine, in patients treated with zosuquidar versus controls.

According to the protocol, patients are stratified by age (>70 versus 60 to 70), disease status (RAEB versus RAEB in transformation or AML), and *de novo* versus secondary disease. Patients are randomized to 1 of 2 treatment arms. In the induction stage, arm I patients are treated with IV daunorubicin over 10 to 15 minutes, and IV zosuquidar over 6 hours on days 1 to 3. Patients are also treated with IV cytarabine continuously on days 1 to 7. In arm II, patients are treated with daunorubicin and cytarabine, as in arm I, and IV placebo, over 6 hours, on days 1 to 3. Beginning on day 12, patients with aplasia are administered filgrastim (G-CSF), or sargramostim (GM-CSF) SC or IV, daily, until blood counts recover. Patients with evidence of persistent AML are eligible for a second identical course of induction chemotherapy. In the consolidation stage of this trial, beginning approximately 4 weeks after documentation of CR or measurable remission (MR), in arm I, responders are treated with IV cytarabine over 1 hour, once or twice daily, on days 1-6 and with SC or IV GM-CSF or G-CSF, beginning on day 7 or day 12 and continuing until blood counts recover. In the consolidation phase in arm II, patients who have maintained peripheral blood evidence of a remission are treated with daunorubicin, cytarabine, and zosuquidar or placebo as in the induction phase. Patients are also treated with SC or IV GM-CSF or G-CSF, beginning on day 8 or day 12 and con-

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tinuing until blood counts recover. Patients are followed monthly for 1 year, every 2 months for 1 year, and then every 3 months for 2 years, every 6 months for 1 year, and then annually thereafter. Approximately 450 patients (225 per treatment arm) will be accrued for this study within 4.1 years. Robert L. Comis, MD, of ECOG is the Study Chair.

A dose-escalation, phase I/II clinical trial of LY335979 plus doxorubicin, and LY335979 plus doxorubicin and cyclophosphamide, initiated in December 2000 at the Royal Marsden NHS Trust (London, UK), under PI Ian Judson, MD, in patients with locally advanced or metastatic solid tumors, was completed in June 2002. The objective of the trial was to determine a LY335979 dose that can be administered safely and with acceptable toxicity in combination with doxorubicin alone, and with doxorubicin plus cyclophosphamide in this setting.

Lovastatin, a drug commonly used in the clinic to treat hypercholesterolemia, may potentiate the cytostatic/cytotoxic activity of doxorubicin. In tumor cells, *in vitro*, the drug increased apoptosis induced by chemotherapeutic agents, and strengthened the antitumor activity of cisplatin and tumor necrosis factor (TNF)-α in murine tumor models.

Lovastatin also caused retardation of melanoma growth in mice treated with doxorubicin (Feleszko W, etal, JNCI 1998;90:247-8). In 2 murine and 2 human melanoma cell lines, lovastatin effectively potentiated the cytostatic/cytotoxic activity of doxorubicin *in vitro* via augmentation of apoptosis. In the B16F10 murine melanoma model *in vivo*, increased sensitivity was observed to the combined treatment with lovastatin and doxorubicin as compared to either agent alone. Lovastatin treatment also resulted in significant reduction of the number of experimental metastasis in doxorubicin-treated mice (Feleszko W, etal, Int J Cancer, 1 Jul 2002;100(1):111-8).

Cerulenin is a specific inhibitor of fatty acid synthase (FAS). When investigators at Hospital Universitario 12 de Octubre (Madrid, Spain) added cerulenin to breast cancer cell lines, before, during or after exposure to daunorubicin, it induced a dramatic increase in the cytotoxicity of daunorubicin in MCF-7/Adr cells (up to 136-fold), and in MCF-7 and SK-Br3 cells (up to 13- and 26-fold, respectively). Different synergistic interaction between cerulenin and daunorubicin was observed between anthracycline-resistant or anthracycline-sensitive cells. When cerulenin was used as a single agent, MCF-7/Adr cells were 2-fold more resistant to cerulenin than MCF-7 cells, and 5-fold than SK-Br3 cells. This effect was reversed in the presence of verapamil, a blocker of P-gp. To test the specificity of the synergism between daunorubicin and cerulenin the effects of palmitate, the metabolic end product of FAS was monitored. Simultaneous presence of palmitate with cerulenin and daunorubicin reversed the potentiating effect of cerulenin on daunorubicin toxicity. This suggests that the synergistic effects between cerulenin and daunorubicin result from FAS product depletion. Upregulation of p21[WAF1], a critical mediator of the cellular response to DNA damage, was seen after daunorubicin or cerulenin treatment alone, but their combination induced a significant downregulation of p21[WAF1], below the constitutive levels, which may be a critical molecular event mediating the synergism between daunorubicin and cerulenin in MCF-7/Adr cells (Martin AV, etal, AACR02, Abs. 4709:951).

Novobiocin, a coumermycin antibiotic, effectively reverses BCRP-mediated drug resistance in cancer cells *in vitro* and *in vivo*. Investigators at Nagasaki University, and Meiji Pharmaceutical University (Kiyose, Japan), examined whether novobiocin could reverse BCRP-mediated resistance in BCRP-overexpressing PC-6/SN2-5H2 cells that are 141-, 57.2-, and 173-fold resistant to topotecan, mitoxantrone and SN-38, respectively. A noncytotoxic dose of novobiocin enhanced cytotoxicity of topotecan by approximately 26-fold, SN-38 by 43-fold, mitoxantrone by 61-fold, and doxorubicin by five-fold, in PC-6/SN2-5H2 cells but not in the parental cells. In the resistant subline, novobiocin remarkably increased intracellular topotecan accumulation, and significantly inhibited topotecan transport into the membrane vesicles of the subline. Novobiocin competitively inhibited the BCRP-mediated topotecan transport in the subline. Similarly, novobiocin overcame drug resistance in other BCRP-overexpressing cancer cells. These findings indicate that novobiocin effectively overcomes BCRP-mediated drug resistance at clinically achievable concentrations, and is a promising reversal agent for topoisomerase I inhibitors (Shiozawa K, etal, AACR02, Abs. 2460:495).

Investigators at Greenebaum Cancer Center (Baltimore, MD) and at the Baltimore VA Medical Center also examined the modulation of cytotoxic drug accumulation and cytotoxicity in BCRP-overexpressing cells exposed to novobiocin. Daunorubicin accumulation assays in BCRP-transfected cells confirm novobiocin's ability to inhibit drug efflux. Novobiocin enhances the cytotoxic effects of multiple BCRP substrate drugs, probably through a direct effect on BCRP transport (Doyle LA, etal, AACR02, Abs. 3857:778).

Theanine (l-thanine), a component of green tea, is an amino acid structurally similar to glutamate, which is involved in the production of glutathione, an important detoxifier. Glutamate transporters are not only involved in glutamate uptake but also in cell-membrane export of doxorubicin. Theanine is said to interfere with the function of glutathione. Investigators at the University of Shizuoka, in Japan, have demonstrated that theanine decreases glutamate uptake via inhibition of the glutamate transporter, intracellular glutathione (GSH) synthesis, GS-doxorubicin conjugate level, and subsequent extracellular transport of GS-doxorubicin by the MRP5/GS-X pump. Theanine

Exhibit 2
Selected Clinical Trial Status of Novel Drugs Being Evaluated in Combination With Cytotoxic Antibiotics

Developer ❏ Affiliates	Generic Name ❏ Number ❏ Brand Name	Description ❏ Administration Route	Development Status ❏ Region ❏ Indication
Bleomycin			
IDEC Pharmaceuticals ❏ Genentech, Hoffmann-La Roche, Zenyaku Kogyo, Xoma, Celltech Group, Nippon Roche	Rituximab ❏ IDEC-C2B8 ❏ Rituxan (USA), MabThera (Europe)	Genetically engineered, chimeric pan-B antibody that binds CD20 antigen-expressing B cells, activates complement proteins, and recruits macrophages and natural killer cells ❏ infusion	Phase I (begin 10/01, ongoing 12/02)➢Europe (UK) ❏ poor-prognosis, HIV-related non-Hodgkin's lymphoma (NHL)
Daunorubicin			
Cell Therapeutics (CTI) ❏ Memorial Sloan-Kettering Cancer Center, Sam Waxman Cancer Research Foundation, Beijing U, National Cancer Institute (NCI), Sperling Sampson West, IDIS World Medicines	Arsenic trioxide (As2O3) ❏ Trisenox	Pharmaceutical-grade arsenic compound ❏ IV, PO	Phase III (begin 6/99, ongoing 2/03)➢USA, Canada, Australia, New Zealand, Puerto Rico, Europe (The Netherlands) ❏ acute promyelocytic leukemia (APL), first line
Eli Lilly ❏ Chugai Lilly Clinical Research, Chugai Pharmaceutical	Zosuquidar trihydrochloride ❏ LY335979	Potent, selective *in vitro* inhibitor of P-glycoprotein (P-gp) ❏ continuous IV	Phase III (begin 12/02)➢USA ❏ acute myeloid leukemia (AML), first line, refractory anemia with excess blasts (RAEB) in trans-formation, high-risk RAEB in transformation
Genta ❏ Molecular Biosystems, Avecia, U Pennsylvania, Aventis	Oblimersen sodium (formerly augmerosen) ❏ G3139 ❏ Genasense	An 18-mer fully phosphorothioat-ed antisense oligonucleotide which targets the bcl-2 gene; the lead compound of the Anticode (antisense) technology platform ❏ subcutaneous (SC), IV	Phase I (begin 4/02, ongoing 2/03)➢USA ❏ AML, first line, and refractory AML
Novartis Pharmaceuticals ❏ Oregon Health Sciences U, Variagenics	Imatinib mesylate ❏ STI-571, STI571 (formerly CGP-57148B ❏ Gleevec (USA), Glivec (ROW)	2-phenylaminopyrimidine derivative; bcr-abl tyrosine kinase inhibitor ❏ PO	Phase I/II (begin 5/01, ongoing 12/02) USA ❏ chronic myeloid leukemia (CML), first line
Telik	TLK199 (formerly TER199)	Inhibitor of the activity of the enzyme glutathione S-transferase P1-1 (GSTP1-1) ❏ injection, PO	Preclin ❏ TER199 reversed accumu-lation deficit of daunorubicin in MRP1-transfected NIH3T3 fibro-blasts, and maintained intracellular levels for >2 hours after daunorubicin removal (O'Brien ML, etal, AACR99, Abs. 4449:674)
Wyeth Pharmaceuticals ❏ Celltech Group, Fred Hutchinson Cancer Research Center, Protein Design Labs (PDL)	Gemtuzumab ozogamicin ❏ CMA-676 (CDP771) ❏ Mylotarg	Recombinant humanized anti-CD33 monoclonal antibody (MAb hP67.6) conjugated to the toxic antibiotic calicheamicin; the MAb portion of CMA-676 recognizes the CD33 antigen, which is expressed on leukemic cells ❏ IV	Phase III (begin 1/03)➢USA ❏ AML, first line
Doxorubicin			
Aegera Therapeutics ❏ Canadian Genetic Diseases Network	G4 AS ODN	Antisense oligonucleotides targeting mRNA encoding for the inhibitors of apoptosis proteins (IAP), including X-linked IAP (XIAP) ❏ injection	Preclin ❏ G4 AS ODN sensitized H460 cells to the cytotoxic effects of doxorubicin, paclitaxel, vinorelbine and etoposide (Hu Y, etal, AACR02, Abs. 2856:576)

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Alfacell ❑ Scientific Protein Laboratories	Ranpirnase (formerly P-30 protein) ❑ Onconase	Cytotoxic ribonuclease with antitumor properties; originally a molecule isolated from the ova and early embryos of the Northern leopard frog (Rana pipiens); small single-chain protein composed of 104 amino acid residues with a high degree of homology to the digestive enzyme pancreatic ribonuclease A ❑ IV	Phase III (begin 97, ongoing 11/02)≻USA ❑ unresectable malignant mesothelioma
BioResearch Ireland ❑ National Cell and Tissue Culture Centre (NCTCC) at Dublin City U		Method for enhancing activity of existing cytotoxics such as vincristine or doxorubicin by combining them with a different class of drugs used for other indications	Preclin (ongoing 11/02)≻Europe (Ireland) ❑ solid tumors
Cell Pathways ❑ Paladin Labs, Aventis Pharma, Roche Laboratories, Eli Lilly, GlaxoSmithKline	Exisulind, sulindac sulfone ❑ FGN-1 ❑ Aptosyn (formerly Prevatac)	Sulfone metabolite of the non-steroidal anti-inflammatory drug (NSAID) sulindac; member of the class of pro-apoptotic drugs termed selective apoptotic antineoplastic drugs (SAAND) ❑ PO	Preclin ❑ according to published data and company reports, Aptosyn has demonstrated synergistic or additive activity *in vitro* and in animal experiments when administered in combination with doxorubicin
Celgene ❑ EntreMed, Children's Hospital (Boston), Pharmion, Bristol-Myers Squibb, Royalty Pharma	Thalidomide ❑ Thalidomid, Thalomid	Antiangiogenic compound; may block certain growth factors such as bFGF and VEGF ❑ PO	Phase II (begin 9/00, ongoing 11/02)≻USA ❑ advanced primary liver cancer/hepatocellular carcinoma, or localized unresectable primary liver cancer
Corixa ❑ Dana-Farber Cancer Institute, GlaxoSmithKline, Boehringer Ingelheim, MDS Nordion, U Michigan, Amersham Health	Iodine 131 (^{131}I) tositumomab ❑ Bexxar	Iodine-131-labeled anti-CD20 (anti-B1) pan-B-cell murine MAb ❑ IV	Phase III (begin 3/01, ongoing 11/02) ≻USA (combination) ❑ first-line, CD20-antigen positive, bulky Stage II/III/IV, Grade I-III, follicular NHL; phase II (begin 7/00, ongoing 11/02)≻USA (combination) ❑ intermediate-grade, newly diagnosed NHL; phase II (ongoing 11/02)≻USA (combination) ❑ mantel-cell lymphoma, first line
DOR BioPharma	Disaccharide tripeptide glycerol dipalmotyl ❑ GMDP-GDP ❑ ImmTher	Lipophilic disaccharide peptide related to muramyl dipeptide (the minimum unit of the mycobacterial cell wall that is immunologically active) that acts as a systemic macrophage activator ❑ injection	Phase III (begin 3/01, ongoing 11/02)≻USA (combination) ❑ newly diagnosed follicular NHL; phase II (begin 11/97, ongoing 11/02)≻USA (combination) ❑ newly diagnosed, high-risk, Ewing's sarcoma
Eli Lilly ❑ Chugai Lilly Clinical Research, Chugai Pharmaceutical	Zosuquidar trihydrochloride ❑ LY335979	See previous record	Phase I (completed 01 and 98)≻USA (combination) ❑ advanced solid tumors
EntreMed ❑ Bristol-Myers Squibb, Children's Hospital (Boston), Aventis Pharma, Tetrionics, U Iowa	2-methoxyestradiol (2-ME2), 2ME2 ❑ Panzem	Nonestrogenic endogenous metabolite of estradiol ❑ PO	Preclin ❑ in an *in vitro* evaluation, addition of 2-ME2 moderately enhanced the growth-inhibitory effect of doxorubicin in breast cancer cell lines (Liu ZJ, etal, AACR01, Abs. 1099:205)
Genentech ❑ ImmunoGen	Bevacizumab ❑ rhuMAb-VEGF, NSC-704865 ❑ Avastin	MAb antagonist of vascular endothelial growth factor (VEGF); angiogenesis inhibitor ❑ IV	Phase II (begin 11/01, ongoing 2/03)≻USA (multimodality) ❑ locally advanced breast cancer; phase II (begin 4/01, ongoing 2/03)≻USA ❑ previously untreated Stage IIIb or IV inflammatory breast cancer

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Genentech ❑ Hoffmann-La Roche, U Pennsylvania, Protein Design Labs (PDL), ImmunoGen	Trastuzumab ❑ Herceptin	A recombinant DNA-derived humanized MAb (4D5) targeting the HER2 protein on tumor cells; an IgG1 kappa mmunoglobulin ❑ infusion	Phase III (begin 10/00, ongoing 2/03) ➢USA, Canada ❑ node-positive breast cancer overexpressing HER2; phase III (begin 5/00, ongoing 2/03) ❑ ➢USA HER-2-overexpressing breast cancer; phase I/II (begin 8/99, ongoing 2/03)➢USA ❑ locally advanced (Stage IIIb), inflammatory, or metastatic (Stage IV) breast cancer overexpressing HER2 (2+ or 3+)
Genta ❑ Molecular Biosystems, Avecia, U Pennsylvania, Aventis	Oblimersen sodium (formerly augmerosen) ❑ G3139 ❑ Genasense	See above	Phase I/II (begin 12/02)➢USA ❑ recurrent, advanced or metastatic adult primary liver cancer/ hepatocellular carcinoma, or localized unresectable primary liver cancer
GlaxoSmithKline	GF120918	Potent multidrug (MDR) P-gp and breast cancer resistance protein (BCRP) inhibitor ❑ PO	Phase I (completed 98)➢Canada, Europe (UK, The Netherlands), USA ❑ advanced solid tumors
GlaxoSmithKline	506U78	Purine nucleoside analog; prodrug of guanine arabinoside ❑ IV	Phase II (begin 4/01, ongoing 11/02)➢USA ❑ newly diagnosed, pediatric, acute lymphoblastic leukemia
Hybridon ❑ Louisiana State U, U Alabama at Birmingham, Genzyme Molecular Oncology, U Naples	GEM 240	Antisense mixed-backbone RNA/DNA oligonucleotide targeting the mdm2 oncogene ❑ injection	Preclin ❑ GEM 240 exhibited a synergistic growth inhibitory effect when combined with different classes of antitumor agents, including doxorubicin (Tortora G, etal, AACR99, Abs. 4820:730, and Int J Cancer, 1 Dec 2000;88(5):804-9)
ImClone Systems ❑ U California San Diego, Aventis Pharma, Merck KGaA, Bristol-Myers Squibb	Cetuximab ❑ IMC-C225 ❑ Erbitux	Chimerized MAb directed against the epidermal growth factor receptor (EGFr) ❑ IV	Phase Ib/IIa (begin 1/96, completed 12/98)➢USA ❑ hormone-refractory prostate cancer
Immunomedics ❑ Center for Molecular Medicine and Immunology (CMMI)	hMN-14 ❑ CEA-Cide	Yttrium-90- or iodine-131-labeled humanized MAb (hMN-14) directed against carcinoembryonic antigen (CEA) ❑ IV	Phase I/II (begin 8/98, closed 01)➢USA ❑ thyroid cancer
Introgen Therapeutics ❑ Oncormed, U Texas M.D. Anderson Cancer Center, Sidney Kimmel Cancer Center	Ad5CMV-p53, Ad-p53 ❑ RPR/INGN-201, RPR/INGN 201, INGN 201 ❑ Advexin	E1/E3-deleted, replication-defective adenoviral vector containing wild-type p53 cDNA under the control of a CMV promoter ❑ intraperitoneal, intralesional, intratumoral, IV, intra-arterial, bronchial lavage, intravesical, intracranial, intracerebral	Phase II (begin 2/02, ongoing 11/02)➢USA ❑ Stage IIIa/IIIb (excluding inflammatory breast carcinoma), or localized Stage IV breast cancer, or male breast cancer
Janssen Pharmaceutica ❑ Kyowa Hakko Kogyo, Ortho Biotech	Tipifarnib ❑ R115777 ❑ Zarnestra	Farnesyl protein transferase inhibitor; imidazole that inhibits activated p21 ras ❑ PO	Phase I/II (being planned as of 11/02)➢USA ❑ locally advanced (Stage IIIb), metastatic (Stage IV), inflammatory, or recurrent breast cancer
Millennium Pharmaceuticals ❑ Pharsight	Bortezomib ❑ MLN341 (formerly LDP-341, PS-341 & MG-341) ❑ Velcade	Boronic acid depeptide derivative that is a potent selective and reversible proteasome inhibitor ❑ IV	Phase (begin 6/01, ongoing 12/02)➢USA ❑ advanced solid tumors
Mitsui Pharmaceuticals	MS-209	Quinoline derivative that reverses P-gp-mediated MDR by inhibiting spingomyelin synthase ❑ PO	Preclin ❑ MS-209 significantly enhanced doxorubicin toxicities in SN-38-resistant PC-6/SN2-5 small-cell lung cancer (sclc) cells with increased SN-38 intracellular accumulation (Kawabata S, etal, AACR99, Abs. 4491:680)

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Molecules for Health	Didox, Trimidox	Synthetic small molecule inhibitor of deoxynucleotide synthesis, the rate-limiting enzyme of *de novo* DNA synthesis; Trimidox is a more potent analog of Didox ❏ PO	Phase II (completed 00) Europe ❏ breast cancer
ONYX Pharmaceuticals ❏ PolyMASC Pharmaceuticals	CI-1042, ONYX-015, dl1520	Genetically engineered E1B-55kD gene-deleted group C adenovirus that replicates in and lyses cells lacking p53 activity ❏ intralesional, intratumoral, intraoperative, mouthwash, intra-arterial, IV, intracranial, intracerebral	Phase I/II (begin 1/00, ongoing 11/02)➣USA ❏ recurrent or metastatic (Stage IVa or IVb) soft tissue sarcoma
Pfizer Global Research and Development	AG2037	Antifolate; potent glycinamide ribonucleotide formyl transferase (GARFT) enzyme inhibitor ❏ IV, PO	Preclin ❏ in P388 murine lymphoma monocyte cell assays, doxorubicin-resistant (P388/ADR) cells were not resistant to AG2037, and simultaneous AG2037-doxorubicin administration yielded additivity in both P388 and P388/ADR cells (Neuffer HB and Boritzki TJ, AACR01, Abs. 1579:293)
Pharmacyclics ❏ NCI, U Texas, Hoechst Celanese, Abbott Laboratories	Motexafin gadolinium ❏ Xcytrin	Gadolinium texaphyrin (Gd-Tex) that selectively accumulates in cancer cells sensitizing them to radiation ❏ IV	Phase I (begin 2/02, ongoing 11/02)➣USA ❏ advanced leukemia, lymphoma, or multiple myeloma
PharmaMar ❏ Ortho Biotech, U Illinois	Ecteinascidin 743 ❏ ET-743, NSC 648766 ❏ Yondelis	A novel marine compound derived from the tunicate *Ecteinascidia turbinata*; DNA minor groove, guanine-specific interacting agent that also targets topoisomerase I, inducing topo I-mediated protein-linked DNA breaks; cytotoxic alkaloid ❏ intermittent or continuous IV	Phase I (ongoing 9/02)➣Europe ❏ soft tissue sarcoma or breast cancer, previously treated only with adjuvant chemotherapy
Progen Industries ❏ Medigen Biotechnology, Australian National U, Griffith U	Phosphomannopentaose sulfate ❏ PI-88	Sulfated oligosaccharide is a GAG mimetic, which binds to the heparin binding site of angiogenesis-specific growth factors, and prevents heparin-dependent proliferation of endothelial cells; antimetastatic ❏ IV, subcutaneous	Preclin ❏ preliminary results using PI-88 combined with doxorubicin, 5-FU and vinorelbine, indicate that the addition of PI-88 delays tumor growth (Pavlakis N, etal, AACR02, Abs. 2605:525)
Sanofi-Synthelabo ❏ SRI International	Tirapazamine ❏ SR-259075 ❏ Tirazone	Benzotriazene cytotoxic that is bioreduced under hypoxic conditions to an active free radical species which cause single- and double-strand DNA breaks ❏ IV	Phase II (begin 11/01, ongoing 12/02)➣USA, Canada, Australia, New Zealand, Europe, Puerto Rico ❏ pediatric rhabdomyosarcoma
Telik	TLK199 (formerly TER199)	See above	Preclin ❏ TER199 significantly reversed the resistance of MRP1-transfected NIH3T3 cells against doxorubicin (O'Brien ML, etal. AACR99, Abs. 4449:674)
Therion Biologics ❏ Aventis Pasteur	CEA-TRICOM, rF-TRICOM, rF-B7.1/ICAM-1/LFA-3	TRIad of COstimulatory Molecules (TRICOM) is a live recombinant fowlpox virus expressing a triad of costimulatory molecules (B7.1, LFA-3, and ICAM) ❏ injection	Phase I/II (begin 11/02)➣USA (multimodality) ❏ metastatic breast cancer, first line
Vertex Pharmaceuticals ❏ Shire BioChem	Biricodar dicitrate ❏ VX-710 ❏ Incel	Blocks activity of the MDR1 protein pump to combat MDR; resensitizes cancer cells to chemotherapy ❏ IV	Phase II (completed 5/99)➣USA ❏ primary, inoperable, hepatocellular carcinoma

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Vertex Pharmaceuticals	Timcodar dimesylate ❑ VX-853	Inhibitor of MDR mediated by MDR1 and MRP ❑ PO	Phase I/II (begin 4/96, closed 1/01) ➢USA ❑ advanced solid tumors
Vion Pharmaceuticals ❑ Yale U	3-AP, OCX-191 ❑ Triapine	Ribonucleotide reductase inhibitor ❑ parenteral, IV	Preclin ❑ combination of Triapine with various classes of DNA-damaging agents, including doxorubicin, resulted in synergistic inhibition of the L1210 leukemia cell line, and resulted in long-term survival of tumor-bearing mice treated with several dose levels of the combinations (Finch RA, etal, Biochem Pharmacol, 15 Apr 2000;59(8):983-91)
Xenova Group ❑ QLT	Tariquidar ❑ XR9576	Selective potent inhibitor of the action of the P-gp pump; may prevent chemotherapy failure attributed to drug resistance ❑ IV, PO	Phase I (begin 2/01, ongoing 11/02) ➢USA ❑ refractory or recurrent solid tumors, including rhabdomyosarcoma and other soft tissue sarcomas, Ewing's sarcoma, osteosarcoma, neuroblastoma, Wilms' tumor, liver tumors, germ cell tumors, and primary brain tumors; phase IIa (completed 10/00)➢Europe (UK) ❑ solid tumors; phase I (begin 2/01, ongoing 12/02)➢USA ❑ pediatric solid tumors; phase II (ongoing 12/02) ➢USA ❑ advanced, refractory, breast cancer
YM Biosciences ❑ U Manitoba	Tesmilifene ❑ DPPE (formerly BMS217830)	Novel antihistamine with potent affinity for growth-regulatory intracellular receptors; enhances cytotoxic effects of chemotherapy with no additive unwanted side effects ❑ IV	Phase III (closed 11/99)➢Canada and phase II (completed 98) ❑ metastatic breast cancer
Epirubicin			
Genentech ❑ Hoffmann-La Roche, U Pennsylvania, Protein Design Labs (PDL), ImmunoGen	Trastuzumab ❑ Herceptin	See above	Phase III (begin 4/01, ongoing 12/02) ➢USA ❑ metastatic breast cancer
GlaxoSmithKline	GF120918	See above	Phase I (completed 02)➢Europe (France) ❑ advanced, refractory solid tumors
Idarubicin			
Genentech ❑ ImmunoGen	Bevacizumab ❑ rhuMAb-VEGF, NSC-704865 ❑ Avastin	See above	Phase II (begin 9/01, ongoing 2/03)➢USA ❑ chronic myelogenous leukemia (CML) in blastic phase
Novartis Pharmaceuticals ❑ Oregon Health Sciences U, Variagenics	Imatinib mesylate ❑ STI-571, STI571 (formerly known as CGP-57148B) ❑ Gleevec (USA), Glivec	See above	Phase I/II (begin 2/03)➢USA ❑ initial bone-marrow relapse in pediatric patients with acute lymphoblastic leukemia (ALL)
Liposomal Doxorubicin (Doxil)			
AnorMED ❑ Cancer Research Campaign	ZD0473 (was AMD473)	A novel sterically hindered platinum complex designed primarily to be less susceptible to inactivation by thiols; third generation compound with activity against cisplatin- or carboplatin-resistant tumors ❑ infusion, PO	Phase I (begin 11/00, ongoing 11/02)➢USA ❑ solid tumors or lymphoma
Genentech ❑ Hoffmann-La Roche, U Pennsylvania, Protein Design Labs (PDL), ImmunoGen	Trastuzumab ❑ Herceptin	See above	Phase II (begin 10/00, ongoing 12/02) ➢USA, South Africa ❑ metastatic breast cancer

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Telik	TLK199 (formerly TER199)	See above	Phase I (begin 12/02)≻USA platinum-refractory ovarian cancer; phase I/IIa ❑ myelodysplastic syndrome (MDS)
Mitomycin			
Genentech ❑ ImmunoGen	Bevacizumab ❑ rhuMAb-VEGF, NSC-704865 ❑ Avastin	See above	Phase II (begin 8/02)≻USA ❑ advanced, unresectable liver cancer, in combination with chemoembolization
ONYX Pharmaceuticals ❑ PolyMASC Pharmaceuticals	CI-1042, ONYX-015, dl1520	See above	Phase I/II (begin 1/00, ongoing 12/02) ≻USA (combination) ❑ metastatic sarcoma
Pierre Fabre Oncologie	Vinflunine ❑ F12158 ❑ Javlor	Bi-fluorinated, second-generation Vinca alkaloid, synthesized from vinorelbine using superacidic chemistry ❑ IV, PO	Preclin ❑ significantly superior results were obtained in a P388 model, by combining optimal single doses of vinflunine with mitomycin C, compared to single-agent treatment; combining minimally effective doses of vinflunine with those of mitomycin C also proved additive while in contrast, a combination of vinflunine with either doxorubicin or etoposide did not enhance antitumor activity (Kruczynski A, etal, AACR02, Abs. 1333:268)
Mitoxantrone			
Wyeth	CCI-779	See above	Phase I (begin 7/02)≻USA, Australia relapsed or refractory, pediatric, AML, or myelodysplastic syndrome (MDS)
Yamanouchi Pharmaceutical	YM598	Orally available selective endothelin A (ETA) receptor antagonist ❑ PO	Phase II (ongoing 11/02)≻USA, Australia, Europe (Belgium, France, Germany, Ireland, Poland, Spain, UK) ❑ androgen-independent, metastatic prostate cancer

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), January 2003.

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enhances the antitumor activity not only of doxorubicin but also of cisplatin or CPT-11 (Sadzuka Y, etal, Yakugaku Zasshi, Nov 2002;122(11):995-9). Theanine increases the concentration of doxorubicin in a tumor *in vivo* through inhibition of the glutamate transporter via the GSH S-conjugate export (GS-X) pump (Sadzuka Y, etal, Toxicol Lett, 15 Sep 2001;123(2-3):159-67).

Reports from a number of studies, conducted *in vitro* and in animal models, have indicated that theanine reverses lipid peroxidation in cardiac tissue, which doxorubicin causes to double, reverses loss of glutathione peroxidase, an enzyme that is depleted by the extra free radicals, suppresses liver metastases, and mitigates idarubicin cytotoxicity in drug-resistant leukemia cells. However, none of these findings have been tested in human trials.

Similar activity has been demonstrated with dihydrokainate (DHK), another glutamate transporter inhibitor. DHK also inhibits doxorubicin efflux significantly and reduces the glutamate uptake. Combination of DHK with doxorubicin enhances the latter's antitumor activity by 1.8-fold. Concentration of doxorubicin in tumors significantly increases when combined with DHK, and is correlated with reduced tumor weight. DHK also tends to reduce doxorubicin concentration in normal tissues. DHK acts differently in tumor and normal tissues because different isoforms of glutamate transporters are expressed in these tissues (Sadzuka Y, etal, Cancer Lett, 28 May 2002;179(2):157-63).

Toxicity

Treatment with anthracyclines, and particularly doxorubicin causes significant cardiotoxicity that may be manifested as acute arrhythmia or chronic cardiomyopathy. Acute arrhythmia, usually occurring during or within 24 hours of doxorubicin administration, is generally benign and does not compromise future treatment. Chronic cardiomyopathy, the most severe form of doxorubicin cardiotoxicity, is dose-related with an incidence <1% at cumulative doses <550 mg/m^2, and up to 30% at cumu-

lative doses of 560 mg/m² to 1155 mg/m². Daunomycin causes much the same type of cardiomyopathy as doxorubicin, with an incidence of 1.5% at a total dose of 600 mg/m² and 12% at 1000 mg/m². Idarubicin is also associated with similar cardiotoxicity. Mitoxantrone is also associated with cardiomyopathy, especially in patients previously exposed to doxorubicin, but the incidence is lower than that seen with doxorubicin. Cumulative dose recommendations for minimizing the occurrence of mitoxantrone-induced cardiomyopathy are 160 mg/m² in patients without prior doxorubicin exposure and 100 mg/m² in patients pretreated with doxorubicin.

Although the mechanism of this cardiotoxic effect is not fully understood, toxicity may be the result of free radical formation, resulting in the generation of reactive oxygen species such as superoxide anion, hydroxyl radicals and hydrogen peroxide. Heart tissue is particularly vulnerable to doxorubicin-induced oxygen radicals because it lacks well developed antioxidant defenses. Antioxidants such as vitamin E, vitamin A, and aspirin can interrupt this process. However, because formation of free radicals considered to be a critical factor in the pathogenesis of the toxicity of doxorubicin is iron-mediated, only iron chelators have been shown to be effective in protecting patients from doxorubicin-induced cardiotoxicity. This mechanism of action of anthracyclines also contributes to their cell toxicity arising from a drug-DNA covalent bonding. This mechanism involves the iron complex of these agents, inducing oxidative stress to yield formaldehyde, which then mediates covalent attachment to G-bases of DNA. The combination of covalent and noncovalent drug interactions serves to virtually crosslink DNA (Taatjes DJ and Koch TH, Curr Med Chem 2001;8(1):15-29).

Anthracyclines can also downregulate GATA-4 activity. The GATA-4 transcription factor is an important regulator of cardiac muscle cells. The mechanism of decreased GATA-4 activity acts at the level of the GATA-4 gene, because anthracyclines caused significantly decreased levels of GATA-4 protein and mRNA. The rate of decline in GATA-4 transcript levels in the presence of actinomycin D was unaltered by anthracyclines, indicating that these agents may directly affect GATA-4 gene transcription. To determine whether decreased GATA-4 levels are functionally related to cardiac muscle cell death induced by anthracyclines, the ability of ectopic GATA factors to rescue anthracycline-induced apoptosis was tested. Adenovirus-mediated expression of either GATA-4 or GATA-6 was sufficient to attenuate the incidence of apoptosis. Furthermore, suppression of GATA-4 DNA-binding activity by a dominant negative mutant of GATA-4 induced apoptosis (Kim Y, etal, Mol Pharmacol, Feb 2003;63(2):368-77).

Numerous approaches, have been attempted to reduce the toxic effects of anthracyclines, including liposomal and targeted formulations as well as novel analogs. For instance, a combination of reduced uptake and limited conversion to secondary alcohol metabolite formation, seems to render the novel disac-charide analog, MEN 10755, under development by Menarini Group (Florence, Italy) less cardiotoxic than doxorubicin or epirubicin (Minotti G, etal, Br J Pharmacol, Nov 2001;134(6):1271-8).

Bisoxopiperazines, especially dexrazoxane (Zinecard; Pharmacia), provide protection against anthracycline-induced cardiomyopathy. Dexrazoxane (ICRF-187), a bis-dioxopiperazine that strips iron from the doxorubicin-iron complex, thereby preventing free radical formation, has been approved to prevent and/or reduce anthracycline-induced cardiotoxicity in women with metastatic breast cancer who have been treated with a total cumulative dose of doxorubicin of 300 mg/m², and who would benefit from continued treatment. It is recommended that dexrazoxane be administered IV 30 minutes before doxorubicin, at a ratio of 10:1 of dexrazoxane:doxorubicin. For example, with a doxorubicin dose of 50 mg/m², the dose of dexrazoxane should be 500 mg/m².

Dexrazoxane chelates and removes free iron and iron associated with the doxorubicin-iron complex and, therefore, prevents formation of free radicals, lipid peroxidation, and cardiotoxicity. Sequestration of iron and its effect on cellular iron homeostasis may also contribute to its protective effect against doxorubicin cardiotoxicity. Dexrazoxane inhibits binding of doxorubicin to red blood cells in a concentration-dependent manner. Pharmacokinetic analysis showed that pretreatment with dexrazoxane (100 mg/kg) significantly reduced the area under the plasma concentration-time curve of doxorubicin, its mean residence time, and its plasma clearance. Similar reductions were also shown with the pharmacokinetic analysis of doxorubicin associated with blood cells. Therefore, the cardioprotective effect of dexrazoxane is attributable, in part, to its interaction with hemoglobin and red blood cells (Vaidyanathan S and Boroujerdi M, Cancer Chem Pharm 2000;46(2):93-100).

Although relatively safe, the combination of dexrazoxane and doxorubicin is associated with an increase in hematologic toxicity (myelosuppression), which may limit its clinical utility.

Flavonoids can protect against doxorubicin-induced cardiotoxicity without compromising its antitumor activity, as was shown with flavonoid 7-monohydroxyethylrutoside (monoHER). However, a drawback of monoHER therapy is the relatively high dose needed to obtain complete protection. When a synthesized version of monoHER with improved antioxidant properties, VUF 5434, was evaluated in mice, the compound was not toxic and demonstrated cardioprotective properties when administered IV in combination with doxorubicin (van Acker F, etal, NCI-EORTC-AACR02, Abs. 362).

Ribonucleotide reductase inhibitors Didox and Trimidox, under development by Molecules for Health (Richmond, Virginia), that potentiate the effects of doxorubicin (Fritzer-Szekeres M, Life Sci 1998;63 (7):545-52),

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also confer protection against anthracycline-induced cardiotoxicity (Elford HL, etal, AACR01, Abs. 450:83). Didox and Trimidox act as free radical scavengers. Unlike hydroxyurea, a ribonucleotide reductase inhibitor that is not a strong free radical scavenger but an effective iron chelator, Didox, as well as Trimidox, had a significant effect on doxorubicin-induced free radical generation in heart tissue.

Dosing schedule modifications of doxorubicin administration have also been attempted as a means of reducing its cardiotoxicity, while at the same time maintaining its cytotoxic action. One approach to reduce cardiotoxicity is to keep peak plasma level of doxorubicin muted, by using continuous infusion rather than by bolus dosing. Although shown to be cardioprotective in adults, this approach has not worked on children who are particularly susceptible to anthracycline-induced cardiac toxicity.

Investigators at the University of Rochester Medical Center (New York, NY) studied 121 children with acute lymphocytic leukemia (ALL) who were administered 360 mg/m^2 of doxorubicin in 30-mg/m^2 doses every 3 weeks. In total, 57 children were randomized to bolus administration, within one hour, and 64 to continuous infusion over 48 hours. Evaluation at 1.5 years after ALL diagnosis showed that continuous infusion was no help in avoiding dilated cardiomyopathy or inadequate left ventricular hypertrophy. Both groups showed significant abnormalities of left ventricular structure and function (Lipshultz SE, etal, Clin Oncol, 15 Mar 2002;20(6):1677-82).

COMMERCIALLY AVAILABLE ANTHRACYCLINES

Anthracyclines, developed in the 1960s, are a class of antitumor agents with the widest spectrum of activity against human cancers; only a few major cancers such as colon cancer, are unresponsive to anthracyclines. Despite the long history of use of anthracyclines in the clinic, their mechanism of action is not completely understood and has been the subject of considerable controversy. Among potential mechanisms are DNA synthesis inhibition, free radical formation and lipid peroxidation, DNA binding and alkylation, DNA cross-linking, interference with DNA strand separation and helicase activity, direct membrane effects, and the initiation of DNA damage via the inhibition of topoisomerase II (Gewirtz DA, Biochem Pharmacol, 1 Apr 1999;57(7):727-41). Also, anthracyclines interfere with the mechanisms that regulate gene expression in tumor cells.

A list of marketed anthracyclines is presented in Exhibit 1. The major drugs commercialized worldwide are daunorucicin, doxorubicin, epirubicin, idarubicin, and mitoxantrone. Several liposomal formulations of approved anthracyclines have also been commercialized.

Valrubicin (AD32, Valstar; Anthra Pharmaceuticals), a lipophilic anthracycline, was approved on September 25, 1998 for intravesical therapy in BCG-refractory carcinoma *in situ* of the urinary bladder in patients for whom immediate cystectomy would be associated with unacceptable morbidity or mortality. This drug's narrow indication and limited commercial opportunity appears to have caused the demise of its developer.

Another anthracycline, a lipophilic analog of doxorubicin, is pirarubicin (THP), commercialized in Japan 1988 by Mercian (Tokyo, Japan), and also marketed in China by Shenzhen Main Luck Pharmaceuticals (Shenzhen, China). The drug is said to be less toxic than doxorubicin in terms of cardiotoxicity, alopecia and GI toxicities, as confirmed in clinical trials. In a preclinical trial, short peritoneal lavage using pirarubicin prevented development of peritoneal carcinomatosis in rats (Chauffert B, etal, AACR02, Abs. 2915:588).

Daunorubicin

Daunorubicin (daunomycin), a generic monointercalating anthracycline available from multiple sources (Exhibit 1), is used widely in the treatment of cancer. Daunorubicin differs from doxorubicin only by a single hydroxyl group. Daunorubicin inhibits synthesis of nucleic acids with particularly rapid and potent effects on DNA. The drug accumulates in cell nuclei and intercalates into DNA, quantitatively inhibiting DNA replication, and gene transcription both *in vivo* and *in vitro*, and causing topoisomerase II poisoning.

In January 1998, Bedford Laboratories (Bedford, OH) gained approval of Cerubidine (daunorubicin hydrochloride). Cerubidine is provided as a sterile reddish lyophilized powder in vials for IV infusion.

Along with its approved indications, daunorubicin is being evaluated in numerous ongoing clinical trials, in combination with other cytotoxics or regulatory agents (Exhibit 2). The drug is being evaluated in refractory acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), low-and intermediate-grade refractory non-Hodgkin's lymphoma (NHL), metastatic breast cancer, mesothelioma, advanced multiple myeloma, among other indications.

DaunoXome, a liposomal formulation of daunorubicin, was originally approved in the USA in April 1996, as first-line treatment of advanced AIDS-related Kaposi's sarcoma (KS). Gilead Sciences (Foster City, CA) acquired rights to DaunoXome via a merger with Nextar in July 1999. Currently, DaunoXome is approved for sale in more than 20 countries for the treatment of AIDS-related KS. Global sales of this drug have been limited by the narrow indication for which it has been approved. Worldwide sales reached a maximum level of $5.2 million in 1997, but declined to $4.4 million in 2000 and to $4.1 million in 2001.

Recent reports indicate that cardiac toxicity may limit the usefulness of DaunoXome in treating children with relapsed or resistant tumors. In a phase I clinical trial, conducted in Europe, 2 children both having been previously treated with anthracycline-based chemotherapy, died

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of acute cardiac toxicity following 4 courses of DaunoXome. In addition, cardiac toxicity was observed in 12 other patients (2 anthracycline-naïve), diagnosed by reduction in fractional shortening and/or ejection fraction. No correlation with pharmacokinetic parameters was identified in patients with cardiotoxicity (Lowary S, etal, ASCO02, Abs. 435:109a).

Doxorubicin

Doxorubicin, the oldest of the commercialized anticancer antibiotics, is a critical component of many combination regimens against some of the most common advanced malignancies, including hematologic malignancies such as acute leukemia and lymphoma, and many solid tumors, including breast cancer.

Doxorubicin is administered by fast running IV infusion, commonly at 21-day intervals. Doxorubicin may also be administered by bladder instillation.

Common toxicities include nausea and vomiting, myelosuppression, alopecia, and mucositis, and local extravasation may cause severe tissue necrosis. Because doxorubicin is largely excreted by the biliary tract, an elevated bilirubin concentration is an indication for dose reduction. Cardiac toxicity is a serious complication of doxorubicin exposure, as described above. Cumulative doses are kept <450 mg/m^2 because symptomatic and potentially fatal heart failure is common above this dose. Cardiac monitoring, for example by sequential radionuclide ejection fraction measurement, may assist in safely limiting total dose. Some evidence suggests that weekly low-dose administration may be associated with less cardiac damage.

Numerous formulations/administration approaches are in development to lower the toxicity of doxorubicin without compromising its effectiveness, as described below and in Part II of this report.

Intrahepatic administration of doxorubicin is being evaluated in the treatment of cancer metastasized to the liver. Delcath Systems (Stamford, CT) has developed a system that isolates the liver from the general circulatory system so that chemotherapeutics such as doxorubicin can be administered directly to the liver. Delcath's delivery system uses specially configured patented catheters to isolate and perfuse single organs, or a region of the body, with high doses of toxic drugs, allowing only minimal amounts to enter general circulation. This highly controlled drug delivery approach allows cytotoxic agents to be administered at much higher doses than would normally be possible by conventional methods. Using the closed Delcath system, chemotherapy is delivered to the liver, and blood is cleansed of the drug before it is returned to the patient's circulatory system so as to protect other parts of the body from the harmful side effects of chemotherapy.

The Delcath procedure requires a team of doctors, including a cancer surgeon to oversee the procedure, an interventional radiologist to insert the catheters, the attending oncologist who determines the dosing, a perfusionist who monitors the blood's circulation outside the body, and an anesthesiologist who administers local pain control. This minimally invasive approach replaces invasive surgical perfusion that has been in use for the past eight years for the treatment of inoperable cancer of the liver. The surgical procedure requires roughly 11 days of hospitalization and can only be performed once in a patient's lifetime, whereas the Delcath procedure is performed on an outpatient basis and can be repeated many times over at different intervals, if appropriate.

Delcath is conducting a pivotal phase III clinical trial of doxorubicin for inoperable metastatic liver cancer at two sites, the Sydney Melanoma Unit, Sydney Cancer Centre, in Australia, and New York University Medical School.

Liposomal doxorubicin was developed to improve the pharmacokinetic and safety profile of free doxorubicin and increase its efficacy. Liposomal formulations may allow administration of higher doses and overcome resistance mediated by MDR1. Preclinical studies have shown a clear superiority of liposomal over free doxorubicin against various xenografts in nude mice.

Currently two products, Doxil, that has been approved by the FDA and launched in many markets, and Myocet, approved outside the USA, are addressing a variety of cancer indications.

Despite similar effectiveness and a more attractive toxicity profile, liposomal doxorubicin has not competed successfully on the market with its free counterpart, and sales have generally been disappointing. The obvious reason for this is the drugs' high price. Oncologists have not been convinced of the cost benefit advantage of liposomal doxorubicin, and reserved its use in cases where free doxorubicin would be contraindicated. In 2000, sales of Doxil were about $85 million. However, its acquisition in 2001 by Johnson & Johnson is said to have accelerated this drug's revenue both in the USA and abroad.

Doxil (USA)/Caelyx (abroad), a formulation of doxorubicin encapsulated in long-circulating Stealth liposomes (see FO, pp 554 and 557), originally developed by Sequus Pharmaceuticals that was subsequently acquired by Alza, was approved on December 29, 1998 for the treatment of AIDS-related KS in patients whose disease had progressed on prior combination chemotherapy, or in patients who are intolerant to such therapy. In June 1999, Doxil also gained supplemental approval for the treatment of metastatic ovarian cancer, refractory to both paclitaxel- and platinum-based chemotherapy regimens. This drug is currently marketed by Johnson & Johnson that acquired Alza in July 2001.

The most common side effects reported with Doxil therapy include neutropenia, anemia, nausea, hand-foot syndrome (palmar-plantar erythrodysesthesia or PPE), stomatitis, vomiting, diarrhea, constipation, appetite loss, tiredness, weakness, rash and some hair loss. Some patients

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experience infusion-related and skin reactions, and in 17% of patients, PPE is moderate to severe. Also, some patients suffer severe cardiac side effects.

Doxil has been evaluated in phase II clinical trials, alone and in various combinations, in anthracycline-refractory metastatic breast cancer, as first-line therapy in advanced soft tissue sarcoma, in mesothelioma, in the neoadjuvant setting in squamous cell carcinoma of the head and neck, as second-line monotherapy in advanced breast cancer, in combination with paclitaxel in the treatment of epithelial ovarian carcinoma and metastatic breast cancer, in newly diagnosed NHL, etc. Doxil was also compared with topotecan (Hycamtin; GlaxoSmithKline) and paclitaxel in platinum-refractory ovarian cancer.

Myocet (formerly Evacet), originally developed by The Liposome Company (TLC) that was subsequently acquired by Elan (Dublin, Ireland and New York, NY), is commercially available outside the USA. In August 2000, Elan received marketing authorization from the European Commission for Myocet in combination with cyclophosphamide for first-line treatment of metastatic breast cancer. Elan introduced Myocet in major European markets in the third quarter of 2001. In the USA, although Myocet's NDA was accepted by the FDA in February 1999, in September 1999, ODAC voted 9-2 against recommending it for approval. It is anticipated that Elan will sell Myocet as part of its restructuring and its intention to exit the oncology sector. In October 2002, Elan sold its manufacturing facility in Indianapolis, IN, valued at $12 million, and the inventory estimated at $8 million at time of closing, to Enzon (Piscataway, NJ). The two companies entered into a long-term manufacturing and supply agreement whereby Enzon will continue to manufacture Myocet.

After several false steps in the late 1990s, the drug is currently being evaluated in a number of combination trials. In a multicenter, phase I clinical trial, a 3-weekly dosing schedule of Myocet, in combination with weekly Herceptin and paclitaxel, was conducted in Spain to assess the cardiac safety and activity of Myocet in HER2+ locally advanced or metastatic breast cancer. Cohorts of 3 previously untreated HER2+ (IHC 3+/FISH+) patients were treated at several dose levels (DL). DL1 consisted of Myocet (40 mg/m^2), administered 3 times weekly for 6 weeks, in combination with weekly paclitaxel (60 mg/m^2) and Herceptin (2 mg/kg, loading dose 4 mg/kg), until progression; DL2 involved 40 mg/m^2, 70 mg/m^2, 2 mg/kg; DL3, 50 mg/m^2, 70 mg/m^2, 2 mg/kg; DL4, 50 mg/m^2, 80 mg/m^2, 2 mg/kg; and DL5, 60 mg/m^2, 80 mg/m^2, 2 mg/kg, respectively. Among 15 enrolled patients, 3 at each dose level, treatment with DL5 was still ongoing because only one DLT had been observed at this dose level. Two treatment-related serious adverse events involving febrile neutropenia occurred at DL4 (cycle 4) and DL5 (cycle 1, DLT). Two patients (DL2 and DL3) experienced asymptomatic reversible LVEF decreases to <50%. Most frequent toxicities were dermatologic (severe, leading to withdrawal in 1

patient), nail toxicity, neutropenia, fatigue and peripheral neuropathy. There were 8 PR (61.5%) and 3 CR (23.1%) in 13 patients evaluated for response, for an ORR of 84.6%; 3 additional patients were being included at DL5 to confirm the recommended dose. Myocet in combination with paclitaxel and Herceptin produced a high response rate in this setting with no incidence of symptomatic cardiotoxicity (Trigo J, etal, ASCO02, Abs. 242:61a).

In a dose-escalating, phase I/II clinical trial, conducted at Jewish General Hospital, McGill University (Montreal, Canada) and the University of Pennsylvania Cancer Center (Philadelphia, PA), Myocet was evaluated in combination with paclitaxel in patients with metastatic breast cancer. In the phase I segment, Myocet was administered every 3 weeks at several dose levels to determine MTD, with and without G-CSF. DLT without G-CSF was Grade 4 neutropenia. Regarding cardiac toxicity, 1 patient experienced asymptomatic LVEF decline >20% at a cumulative dose of doxorubicin of 300 mg/m^2, and 1 patient developed CHF at a cumulative dose of doxorubicin of 250 mg/m^2. In terms of efficacy, among 13 evaluable patients, there was 1 CR and 7 PR for an overall response rate of 62%, and disease stabilized in 4 and progressed in 1; 6 patients were not response-evaluable (3 were off-study during cycle 1 and 3 could not be evaluated). In this 3-week administration regimen, Myocet was well tolerated and active (Miller WH Jr, etal, ASCO02, Abs. 1937:31b).

Epirubicin

Epirubicin [Ellence (USA), Farmorubicin (outside the USA); Pharmacia] is a second generation anthracycline antibiotic that was approved in the USA in September 1999, and launched in October 1999, as a component of adjuvant therapy in patients with evidence of axillary nodal tumor involvement (Stage II/III), following resection of primary breast cancer. However, at that time, ODAC declined to recommend the drug for a broader indication in metastatic breast cancer, which was also rejected by the FDA. Ellence was also granted orphan drug status on September 14, 1999, for the approved breast cancer indication.

The drug has been available overseas for many years for a variety of indications including metastatic breast cancer, ovarian cancer, malignant melanoma and NHL. Numerous clinical trials are also ongoing using epirubicin in place of doxorubicin in combination regimens for the treatment of a variety of tumors.

Ellence is the first chemotherapy approved by the FDA for use as a component of adjuvant therapy, in combination with cyclophosphamide and fluorouracil (CEF), in the treatment of early-stage breast cancer that has spread to the lymph nodes. In phase III clinical trials the most common side effects were hematologic toxicities and nausea, vomiting, mouth sores, and hair loss. Few patients experienced damage to the heart muscle or a type of leukemia.

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In December 2002, at the 25th Annual San Antonio Breast Cancer Meeting, Pharmacia reported results from its multicenter phase III clinical trial (protocol ID: MA5), coordinated by the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG), involving women with node-positive breast cancer treated with an adjuvant chemotherapy regimen after surgery that included epirubicin. Mark Levine, MD, of McMaster University (Ontario, Canada) was the lead investigator of the study.

This clinical trial, initiated in 1989, and closed to recruitment in 1993, with a median follow-up of 106 months, has the longest follow-up of any trial that has compared an anthracycline-based chemotherapy regimen to the CMF standard regimen. The study randomly assigned 710 women with node-positive breast cancer, to be treated with one of two adjuvant chemotherapy regimens. In this phase III clinical trial, 710 premenopausal women with early-stage, operable, breast cancer that has spread to the lymph nodes after lumpectomy or mastectomy, were randomly assigned to either cyclophosphamide (100 mg/m²) PO on days 1 through 14, methotrexate (40 mg/m²) IV on days 1 and 8, and 5-FU (600 mg/m²) IV on days 1 and 8; or cyclophosphamide (75 mg/m²) PO on days 1 through 14, epirubicin (60 mg/m²) IV on days 1 and 8, and 5-FU (500 mg/m²) IV days 1 and 8. Each cycle was administered monthly for 6 months. Patients administered cyclophosphamide, epirubicin and 5-FU (CEF regimen) were also administered antibiotic prophylaxis with 2 tablets of cotrimoxazole, twice a day, for the duration of chemotherapy. In each treatment group, therapy was administered over 6 4-week cycles. With long-term follow-up, rates of secondary leukemia were unchanged from what was originally reported from this study (1.1% of patients in the CEF group versus 0.4% treated with CMF), while rates of congestive heart failure were slightly higher but acceptable in the CEF group (1.1% of patients, versus 0.3% in the CMF group).

Women treated with this adjuvant regimen were significantly more likely to survive cancer-free for 10 years than those treated with the standard CMF regimen. Being disease-free at the 10-year time point is a major milestone for patients in their quest for a potential cure; historically, >50% of breast cancer patients relapse within 10 years after initial diagnosis. Disease-free survival (DFS) rates in the groups treated with the regimen containing Ellence were 52% compared with 45% with the standard regimen. Overall 10-year survival rates were 62% in women treated with the Ellence-based regimen, compared with 58% in the comparison group. Risk reductions for mortality and disease recurrence in the Ellence-treated group were 15% and 24%, respectively.

After approval in the USA, the worldwide market for Ellence grew significantly, from $199 million in 2000 to an estimated $311 million in 2002 (Exhibit 3).

Idarubicin

Idarubicin (Idamycin; Pharmacia), an orally available anthracycline, was approved by the FDA in September 1990, for first-line treatment of AML in adults, in combination with other approved antileukemic drugs. An additional approval was obtained in February 1997 as first-line therapy in combination with other approved antileukemic drugs for the treatment of acute non-lymphocytic leukemia in adults.

Because of its oral bioavailability, idarubicin is being evaluated in the treatment of elderly patients of median age of 75 years (range=67-85 years), with metastatic breast cancer. A phase II clinical trial of chronic oral idarubicin administration in 26 elderly patients not previously treated with an anthracycline-based regimen, was initiated in Italy, in April 1999. According to the protocol, in the first 14 patients, idarubicin was administered at a daily dose of 5 mg and 10 mg, on alternate days, for 21 days, every 4 weeks. Grade 4 neutropenia developed in 6 patients and Grade 3 neutropenia in 3 for a 64% incidence. Also Grade 3 and Grade 4 thrombocytopenia, Grade 3 diarrhea and Grade 3 stomatitis occurred in 1 patient each. Because of this excessive toxicity, another 12 patients were treated with 5 mg, daily, for 21 days every 4 weeks. In this group only 2 (16.7%) patients experienced Grade 4 neutropenia and 1 (8.3%) Grade 2 mucositis. There were 5 (25%) PR (liver=3, lung=1, nodes=1) in 20 evaluable patients of median duration of 6+ months (range=4-11+ months). Disease stabilized in 8 (40%) patients for a median of 5 months. Results of this trial indicate that this regimen is safe and effective in older patients (Toffoli G, etal, ASCO02, Abs. 1528:383a).

Mitoxantrone

Mitoxantrone (Novantrone; Amgen), an agent structurally related to doxorubicin, is used in the treatment of breast cancer, NHL and adult non-lymphocytic leukemia. It is administered intravenously and is well tolerated but myelosuppression and dose-related cardiotoxicity occur; cardiac examinations are recommended after a cumulative dose of 160 mg/m².

In October 2000, the FDA also approved Novantrone as therapy for secondary progressive multiple sclerosis (MS), which served to boost its market. Worldwide sales of Novantrone were $71.2 million in 2001, and $58.3 million in the first 9 months of 2002.

OTHER COMMERCIALLY AVAILABLE ANTIBIOTIC CYTOTOXICS

There are a few non-anthracycline antibiotics on the market (Exhibit 1).

Bleomycin

Bleomycin is administered IV or intramuscularly to treat aggressive, AIDS-related NHL, adult and pediatric Hodgkin's disease (HD), and certain solid tumors such as glioblastoma multiforme (GBM), in combination with radiotherapy (RT), and germ-cell cancer; it is may also be administered by the intracavitary route for malignant effusions.

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Regarding bleomycin's toxicity, the drug is not particularly myelosuppressive. It is however, associated with dermatologic toxicity and mucositis. Hypersensitivity reactions, manifested by chills and fevers, commonly occur a few hours after drug administration, and may be prevented by simultaneous administration of a corticosteroid. The principal problem associated with the use of bleomycin is progressive pulmonary fibrosis. This is dose-related, occurring more commonly at cumulative doses >300,000 units and in the elderly.

Locally administered bleomycin by electroporation (EPT) is being evaluated using the MedPulser system developed by Genetronics Biomedical (San Diego, CA). In elecroporation an electric field is applied to a living cell that causes a transient permeability in the cell's outer membrane. This permeation is manifest by the appearance of pores across the membrane. After the field is turned off, the pores close within 30 minutes without significant damage to the exposed cells, and with the therapeutic molecules trapped inside the target cells. In March 1999, Genetronics received approval in the European Union, to affix the CE Mark to the MedPulser electroporation device used in EPT.

EPT facilitates the uptake of chemotherapeutic agents into the interior of cancer cells, and is intended to treat various malignant lesions by locally and temporarily permeabilizing targeted tissues, such as tumor cells. EPT has been evaluated in clinical trials in head and neck cancer, melanoma, basal cell carcinoma, and liver and pancreatic cancer.

In electroporation, a specific chemotherapeutic agent, with bleomycin being the drug of choice, is first injected in the targeted tissue. The entire tumor area is then electroporated using the appropriate MedPulse applicator. EPT allows the previously administered bleomycin to enter the cells and have the desired cytotoxic effect.

EPT, in combination with bleomycin, is being compared to chemotherapy alone, in a randomized (not blinded) phase III clinical trial to enroll 190 patients in each arm. In 2 multicenter phase II clinical trials (protocol IDs: EPT-97-01 and EPT-97-02), conducted in the USA and Canada, EPT-assisted bleomycin treatment was administered to 51 locoregional recurrences of head and neck cancer in 42 patients. There were no deaths attributable to the therapy. Significant side effects were tumor bed bleeding (9%) and cellulitis (6%). Necrosis, sloughing, ulceration and/or eschar formation were common tumor site reactions following EPT, and were routinely managed conservatively with local wound care. The overall response rate was 57%, involving 29 tumors. At a mean follow-up of 54 days in protocol EPT-97-01, and 84 days in protocol EPT-97-02, median survival (MST) was 5.4 months for all patients. Longest survival was 2 years 11 months. Survival at 1 year was 17%. The average size of the tumors treated was 29 cm^3, but tumor response did not appear to be correlated with tumor size. The technique was promising in

the management of locoregional disease in patients with head and neck cancer, and may impact survival (Goldfarb PM, ASCO02, Abs. 2550:183b).

Dactinomycin

Dactinomycin is principally administered IV to treat pediatric tumors, such as Ewing's sarcoma and Wilm's tumor. Mostly, the drug is currently used for the treatment of gestational trophoblastic neoplasia. Its side effects are similar to those of doxorubicin, except that cardiac toxicity is not a problem.

Gentuzumab Ozogamicin

Gentuzumab ozogamicin (Mylotarg; Wyeth) is a novel agent comprising a recombinant humanized anti-CD33 monoclonal antibody (MAb hP67.6), conjugated to the toxic antibiotic calicheamicin; the antibody portion of CMA-676 recognizes the CD33 antigen, which is expressed on leukemic cells.

Mylotarg was approved by the FDA in May 2000, for the treatment of patients aged ≥60, with CD33-positive, relapsed, AML. In March 2000, ODAC voted 11-2 that there was sufficient evidence of improved safety and acceptable efficacy to support accelerated approval for Mylotarg for this indication. The panel concluded that the drug was not as effective as standard salvage regimens, but may be safer in this aged population. Mylotarg had been under priority review by the FDA since December 1999. USA sales of Mylotarg were $20 million in 2000 and $30 million in 2001.

Mylotarg is being evaluated in a number of hematologic malignancies, alone or in combination with bone-marrow/stem-cell transplantation.

Mitomycin

Mitomycin is administered IV in combination with other cytotoxics and/or radiation therapy to treat various tumors, including upper gastrointestinal malignancies, breast cancer, anal cancer, uterine sarcoma, mesothelioma, non-small-cell lung cancer (nsclc), colorectal cancer metastasized to the liver with or without chemoembolization, peritoneal cancer, etc. It is also used by bladder instillation for superficial bladder tumors in combination with BCG.

Mitomycin causes delayed bone-marrow toxicity and, therefore, it is usually administered at 6-weekly intervals. Prolonged use may result in permanent bone-marrow damage. It may also cause lung fibrosis and renal damage.

Mitozytrex (MitoExtra; Supergen) was approved by the FDA, in November 2002, for treatment of disseminated adenocarcinoma of the stomach or pancreas in proven combinations with other approved chemotherapeutic agents, and as palliative treatment when other modalities have failed. Supergen's amended NDA had been accepted by the FDA in May 2002. Mitozytrex is based on SuperGen's patented Extra technology platform that incor-

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Exhibit 3
Historic (2000-2001) and Forecast (2002) Global Markets of Ellence

Region	2000 ($ millions)	2001 ($ millions)	Change (%)	2002E ($ millions)	Change (%)
USA	9.0	61.0	557.8	104.0	70.5
ROW	190.0	200.0	5.3	207.0	3.5
Total	199.0	261.0	31.1	311.0	19.1

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), January 2003.

porates mitomycin in a hydroxypropyl-β-cyclodextrin carrier to produce a stable, ready-to-inject solution with a commercially favorable shelf life at room temperature. Extra technology is also designed to 'shield' the drug from the injection site, thus providing protection from tissue ulceration that occurs when a vein is missed on injection, causing the drug to extravasate, or leak, to surrounding tissue. In preclinical studies, ulceration was not observed with an Extra-formulated drug, even when it was injected directly into tissue.

In a open-label, single-institution, crossover, phase I clinical trial, conducted at M. D. Anderson Cancer Center, under PI Richard Pazdur, MD, 35 patients were entered in 2 treatment arms. Each patient was treated with alternating courses of MitoExtra or mitomycin at a single

15 mg/m^2 dose via a short IV infusion. Patients were sequentially assigned to either MitoExtra or mitomycin as their first treatment course, in 6-week intervals; courses could be repeated up to 4 times in responders. MitoExtra was bioequivalent to mitomycin. Hematologic and nonhematologic toxicities were similar between the two drugs. However, there were 3 clinically significant infusion-related complications associated with mitomycin administration but none with MitoExtra. The similar pharmacokinetics of these two drugs suggest complete release of mitomycin from the MitoExtra formulation. Further studies are needed to define the pharmacodynamics, toxicity, and efficacy of this drug-carrier complex (Kozuch P, etal, Cancer, 15 Feb 2001;91(4):815-21).

Plicamycin

Another cytotoxic antibiotic, plicamycin (Mithracin; Bayer) is mostly used in the treatment of hypercalcemia associated with advanced cancer and refractory testicular cancer.

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FUTURE ONCOLOGY

PUBLISHED BY **NEW MEDICINE, INC.**

SUBSCRIPTION INFORMATION:

- FUTURE ONCOLOGY (ISSN 1082-331X) is published as 12 issues (several double issues) per subscription period, with a free index listing companies/institutions and subjects covered.
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