FUTURE ONCOLOGY

TECHNOLOGY, PRODUCTS, MARKETS AND SERVICE OPPORTUNITIES

A NEW MEDICINE PUBLICATION | OCTOBER 15, 2001 | VOLUME 6, NUMBER 8/9

STATE-OF-THE-ART IN THE MANAGEMENT OF CANCER

BLADDER CANCER — PART V
NOVEL THERAPEUTICS IN DEVELOPMENT

ONCOLOGY TRENDS

GLOBAL MARKETS OF ANTICANCER DRUGS AND ADJUNCTS

MEDICARE REIMBURSEMENT OF ONCOLOGY DRUGS

MEETING COVERAGE

GENE THERAPY OF CANCER — PART II
IMMUNOLOGIC STIMULATION
FROM THE 9TH INTERNATIONAL CONFERENCE ON GENE THERAPY OF CANCER, SPONSORED BY SIDNEY KIMMEL CANCER CENTER, SAN DIEGO, CA, DECEMBER 7-9, 2000

STATE-OF-THE-ART IN THE MANAGEMENT OF CANCER

BLADDER CANCER — PART V

NOVEL THERAPEUTICS IN DEVELOPMENT

Few novel anticancer agents are in development specifically for the treatment of bladder cancer (Exhibit 1). Also, most agents in early development, listed in Exhibit 1, are being evaluated in solid tumors. If early trials are successful, usually these agents proceed to phase II and III clinical trials in the more common cancers affecting large populations, rather than bladder cancer.

SYSTEMIC CYTOTOXIC CHEMOTHERAPHY

Because bladder cancer is rarely diagnosed as metastatic disease, and mostly affects the elderly, it has attracted limited interest as a specific target of powerful systemic cytotoxics. Rather, most efforts have centered on evaluating agents developed for other indications, in this setting (see FO, pp 1326-1333). Also, several cytotoxics having entered phase II clinical trials for bladder cancer, either failed to produce satisfactory results in this indication or were withdrawn from development in cancer altogether (Exhibit 1).

Alimta

Alimta (LY 231514), a multitargeted antifolate (MTA) that inhibits at least three enzymes (thymidylate synthase or TS, dihydrofolate reductase or DHFR, and glycinamide ribonucleotide formyltransferase or GARFT) involved in folate metabolism and DNA synthesis, under development by Eli Lilly, is in phase II clinical trials for a variety of solid tumors. A phase II clinical trial in advanced or metastatic recurrent transitional cell carcinoma (TCC) of the bladder was initiated in July 2001, at Stanford University and Palo Alto VA Genitourinary Oncology Clinic, with Sandy Srinivas, MD, as the Study Chair.

In May 1998, interim results of this phase II clinical trial were presented, in which 22 patients with metastatic TCC of the bladder were administered LY 231514 as a 10-minute infusion, every 3 weeks, at a dose of 600 mg/m^2 (first 6 patients) or 500 mg/m^2 (subsequent patients). In all, 60 courses were administered with a median of 3 cycles per patient; 5 courses (8%) were dose-reduced and 6 (10%) were delayed. Among 18 evaluable patients, hematologic toxicities included neutropenia, neutropenic fever, thrombocytopenia and anemia. Nonhematologic toxicity included skin rash that was preventable with dexamethasone, and nausea/vomiting, stomatitis, diarrhea and alopecia. There were 2 toxic deaths from septicemia and renal failure, respectively. Because of toxicity, doses were subsequently reduced to 500 mg/m^2. Regarding patient responses, there were 6 (33%) PR, and disease stabilized in 4 (22%) and progressed in 7 (39%); 1 patient died after the first course. Responses lasted for 1.5+ to 8 months (Paz-Ares L, etal, ASCO98 Abs. 1307:339a).

Arsenic Trioxide

Arsenic trioxide (Trisenox; Cell Therapeutics) is a pharmaceutical grade arsenic compound approved in the USA in September 2000, and recommended for approval in Europe in October 2001, as an intravenous therapeutic in refractory or relapsed acute promyelocytic leukemia (APL). A phase II clinical trial of arsenic trioxide in urothelial cancer (protocol ID: CALGB 99903) is ongoing to determine the efficacy and toxicity of this drug in patients with measurable urothelial carcinoma of the bladder, urethra, ureter or renal pelvis.

CAI

CAI (NSC-609974) is a calcium influx inhibitor that alters calcium-sensitive signal transduction pathways and suppresses the proliferative and metastatic potential of malignant cells. It is antiangiogenic and antimetastatic and, through modulation of cellular calcium balance, CAI secondarily inhibits calcium-dependent signaling pathways, such as release of second messengers, protein phosphorylation and gene transcription (Alessandro R, etal, *In Vivo*, Mar-Apr 1996;10(2):153-60). CAI has been shown to induce apoptosis in bladder cancer cells *in vitro*. In a study of the efficacy of CAI in female Fischer 344 rats with TCC of the bladder, treatment with CAI (oral, IV or intravesical) did not result in a significant change in tumor stage and grade after 6 weeks, but it induced significant apoptosis, and tumor proliferation rate was decreased. Intravesical application of CAI yielded the most efficient apoptosis induction rate. There were no toxic side effects and normal urothelium was not affected. Antiproliferative and apoptosis inducing effects of CAI in rat bladder cancer justify initiation of clinical trials to treat patients with advanced bladder cancer (Perabo FGE, etal, ASCO00, Abs. 1433:363a). CAI is available in encapsulated micronized

form or a gelcap formulation and is being evaluated in various solid tumors, and is in phase III clinical trials in nsclc.

CS-682

CS-682 is a novel, orally administered, 2'-deoxycytidine-type antimetabolite, under development by Sankyo, that has exhibited a wide spectrum of antitumor activity in human tumor xenograft models (Dees EC, etal, ASCO99, Abs 800:208a, and Hanaoka K, etal, Int J Cancer, 19 Jul 1999;82(2):226-36). An N4-palmitoyl derivative of the deoxycytidine analog, 2'-C-cyano-2'-deoxy-1-beta-D-arabinofuranosylcytosine (CNDAC), CS-682's principal metabolite in plasma is CNDAC. In CS-682-treated carcinoma cells, CNDAC 5'-triphosphate (CNDACTP) is generated and incorporated into a DNA strand as CNDACMP, inducing DNA-self-strand-breakage which results in cell-cycle arrest predominantly in the G(2) phase.

In a phase I clinical trial, being conducted at the Mayo Clinic (Rochester, MN, and Scottsdale, AZ), CS-682 is being administered PO on a 3 times-a-week schedule for 4 consecutive weeks, with each cycle repeated every 6 weeks. According to the protocol, 26 patients (colon=10, prostate=3, unknown primary=2 and other solid tumors=11), either systemic chemotherapy naive (n=4), or previously treated with one regimen (n=4), two regimens (n=5), or ≥3 regimens (n=13), were treated in groups of 3, at dose levels ranging from 1.5 mg/m^2/day to 120 mg/m^2/day. Nonhematologic toxicity ≥Grade 3, such as nausea and vomiting, and fatigue, occurred in one case each. Grade 3/4 neutropenia was seen in 3 patients but there was no ≥Grade 3 thrombocytopenia (Burch PA, etal, ASCO01, Abs. 364:92a). In another interim report from this trial, among 16 evaluable patients with refractory solid tumors (colon=5, biliary=3, and one each of melanoma, carcinoid, mesothelioma, and renal, bladder, breast, unknown primary, and prostate cancer), groups of 3 were treated at dose levels ranging from 1.5 mg/m^2/day to 50 mg/m^2/day. Toxicity was manageable, with no Grade 3/4 treatment-related toxicities (Burch PA, etal, ASC000, Abs. 921L:237a).

Eflornithine

Eflornithine (alpha difluoromethylornithine HCl or DFMO), is a specific, enzyme-activated, irreversible inhibitor of ornithine decarboxylase that blocks growth of tumor cells, and the promotion and progression phases of carcinogenesis. Eflornithine, in development by Ilex Oncology (San Antonio, TX) under an exclusive, worldwide license to all USA patents for DFMO from Aventis, is in a phase III, multicenter, randomized, double-blind, placebo-controlled clinical trial (protocol IDs: ILEX-DFMO341, NCI-G99-1509, UCLA-9812049, UF-453-1998, WCCC-CO-98803), activated in April 2001, in patients with low-grade, superficial TCC of the bladder. A total of 450 patients (225 per arm), having undergone prior transurethral resection (TUR) for visible tumor, are to be accrued for this trial. Patients are stratified according to disease status (newly diagnosed versus recurrent), clinical stage (Ta versus T1), grade (Grade I versus Grade II), and focus (multifocal versus unifocal). Study objectives are to determine the incidence and severity of toxicities associated with long-term therapy, and whether treatment with DFMO is effective in preventing tumor recurrence after TUR. Treatment continues for one year in the absence of disease progression or unacceptable toxicity. Patients are followed every 3 months during the first 2 years, every 6 months for the third year, and then annually for the fourth year. The study is scheduled to conclude in 2002.

Recently, a new coated tablet preparation of DFMO has become available to replace the DFMO in use to date, which is a liquid with a concentration of 0.2 g/ml that must be drawn up into a syringe and dispensed into a small glass. This liquid form of DFMO results in significant waste and also makes compliance and blinding in clinical trials difficult. The coated tablets come in a 0.25 gram formulation and are scored. To compare the bioavailability of both preparations, 10 normal subjects were enrolled in a crossover study in which the order that they would be treated with either the liquid or tablet preparation of DFMO was randomized. The study was designed with the objective of establishing the bioequivalence of a daily 0.5 g/m^2 tablet preparation of DFMO and an equivalent liquid preparation. No statistically significant differences were seen in time-to-peak concentration, or in serum half-life (Carbone PP, etal, Clin Cancer Res, Oct 2000; 6(10):3850-4).

A phase II clinical trial (protocol IDs: WCCC-CO-9285, NCI-G97-1213) designed to establish whether DFMO has a significant biochemical effect on malignant and nonmalignant bladder tissue, to assess the interpersonal variability of urinary polyamines (PA), PA bladder tissue baseline, and induced ornithine decarboxylase activities as well as PA levels in patients without TCC of the bladder undergoing open surgery and biopsy of bladder tissue, was closed in January 1999. This two-part, randomized trial was being conducted by Paul P. Carbone, MD, at the University of Wisconsin Comprehensive Cancer Center (Madison, WI). To date, no report on this study has been published.

An earlier NCI-sponsored, multicenter phase II prospective clinical trial (protocol IDs: NCCTG-895151, NCI-P91-0008, I89-0001) of DFMO, following TUR of superficial and superficially invasive TCC of the bladder, supported use of a DFMO dose of 1 g/day for chemoprevention trials. The trial enrolled 76 patients between March 1992 and January 1994, and randomized 19 each to be administered daily DFMO doses of 0.125 g/day, 0.25 g/day, 0.5, g/day or 1.0 g/day for a planned period of 1 year; 49 patients were treated with DFMO for more than 200 days, and 35 for ≥350 days. Patients were contacted at monthly intervals to measure compliance and toxicity. Follow-up audiograms revealed no evidence of DFMO-induced ototoxicity, nor was there any other substantial toxicity attributable to DFMO. Mild nausea was noted in 6

patients, while 1 to 2 patients each complained of mild or moderate tinnitus, diarrhea, anorexia, stomatitis, peripheral neuropathy, or lethargy. No dose-response effects were associated with these complaints (Loprinzi CL, etal, J Cell Biochem Suppl 1992;16I:153-5, Loprinzi CL, etal, ASCO95, Abs. 346, and Loprinzi CL, etal, Cancer Epidemiol Biomarkers Prev, May 1996;5(5):371-4).

Etanidazole

Etanidazole is a hypoxic cell sensitizer that enhances the anticancer effects of radiation therapy and increases the effectiveness of other anticancer drugs. The drug was originally developed by SRI International (Menlo Park, CA) under a contract with the NCI, which licensed it to Roberts Pharmaceutical. The drug reverted to Shire Pharmaceutical when it acquired Roberts, and Shire licensed it to Intraop Medical (Santa Clara, CA) in August 2000. Intraop intends to initiate a multicenter, phase III clinical trial of etanidazole in colorectal cancer patients treated with intraoperative radiation (IORT) and standard chemotherapy. Etanidazole was evaluated in combination with radiotherapy in a phase II clinical trial in bladder cancer, that commenced in October 1991 and was completed in March 1994. The study, undertaken by the EORTC Radiotherapy Group, was to recruit 30 patients.

Fenretinide

Fenretinide (4-HPR), a vitamin A analog, is a synthetic retinoid originally under development by Johnson & Johnson and since reverted back to the NCI, which is conducting a phase II clinical trial in bladder cancer. The mechanism of action of fenretinide is not fully understood, but it is hypothesized that it acts independently of the nuclear retinoid receptor pathway. Preclinical studies indicate that fenretinide induces expression of TGF-β1 in association with the induction of apoptosis (Roberson KM, etal, Cell Growth Differ, Jan 1997;8(1):101-1, and Torrisi R and Decensi A, Curr Oncol Rep, May 2000;2(3):263-70).

Fenretinide was studied extensively in a large trial conducted in the late 1980s and early 1990s, designed to evaluate the effectiveness of a 200 mg oral dose, administered daily, for 5 years, in reducing the incidence of contralateral breast cancer in patients with resected early-stage breast cancer, and was also evaluated in brain cancer. In bladder cancer, the NCI is sponsoring a randomized multicenter phase III clinical trial (protocol IDs: MDA-ID-95236, NCI-G99-1621), initiated in June 2000, to evaluate fenretinide in preventing the recurrence of bladder cancer following complete surgical resection of initial tumor. Being conducted under the direction of H. Barton Grossman, MD, of M. D. Anderson Cancer Center (Houston, TX), the start date of this trial, originally planned for 1993, was delayed because of patient recruitment problems.

This trial will attempt to determine the efficacy, mechanism of action, and toxicity of fenretinide in this indication. Treatment effects in modulating the expression of retinoid receptors, chromosomal abnormalities (numerical chromosomal abnormalities and DNA ploidy), apoptosis, and autocrine motility factor receptor (intermediate endpoint markers of recurrent disease) will also be studied. Patients are stratified according to multifocal versus solitary lesion, and are randomized to one of two treatment arms in which either oral fenretinide or placebo is administered on days 1 to 25. Treatment repeats every 28 days for up to 1 year in the absence of disease progression, unacceptable toxicity, or development of a second primary cancer requiring therapy. Patients are followed every 3 months for 15 months. The projected accrual for this study is 178 patients (89 per arm) within 3 years.

In a randomized trial conducted by Italy's National Cancer Institute, the activity of fenretinide in superficial bladder cancer was assessed using DNA flow cytometry and conventional cytology as surrogate biomarkers. A total of 99 subjects with resected superficial bladder cancer (Ta or T1) were randomized to either oral fenretinide (200 mg), daily, for 24 months, or no intervention. Cystoscopy and bladder washing for DNA flow cytometry endpoints (proportion of DNA aneuploid histograms, hyperdiploid fraction, and percentage of apoptotic cells), and proportion of abnormal cytologic examinations were repeated every 4 months for up to 36 months. Regarding toxic effects, 12 subjects in the fenretinide arm complained of diminished dark adaptability, and 9 in the fenretinide arm, versus 1 control subject, experienced mild dermatologic alterations. The primary study endpoint was the proportion of DNA aneuploid histograms after 12 months; this was 48.9% in the fenretinide arm and 41.9% in the control arm. There was no difference in any other response biomarker between the two groups for up to 36 months, nor was any biomarker able to predict recurrence risk. Recurrence-free survival was comparable between the arms. These results suggest a lack of effect for fenretinide on the DNA content distribution, and the morphology of urothelial cells obtained in serial bladder washings. However, because the data was hampered by the lack of predictivity of the selected biomarkers, the researchers conclude that additional studies were necessary to assess the activity of fenretinide in preventing bladder cancer (Bruno S, etal, J Cell Biochem, Dec 1999;76(2):311-21, and Decensi A, etal, Cancer Epidemiol Biomarkers Prev, Oct 2000;9(10):1071-8).

Scientists at the European Institute of Oncology (Milan, Italy) have investigated the effect of fenretinide on circulating IGF-I, IGF-II and IGFBP-3, measured at yearly intervals during a 2-year treatment period, and 1 year after treatment discontinuation, in a predominantly male population of patients with superficial bladder cancer. There was a significant effect of fenretinide on IGF-I levels, which were further lowered after the second year of treatment, and only partially recovered after drug discontinuation. The effect of fenretinide was not modified by age in this setting as was the case with breast cancer patients. No significant effect was evident on IGFBP-3, IGF-II and the IGF-

I+IGF-II/IGFBP-3 molar ratio, which expresses the tissue availability of these mitogenic peptides, although IGF-II and the molar ratio were lowered by treatment by an overall mean of 16% and 15%, respectively. Given the increasingly recognized importance of circulating IGF in the pathogenesis of different solid tumors, these findings strengthen the rationale for studying fenretinide as a chemopreventive agent (Torrisi R, etal, Int J Cancer, 15 Aug 2000;87(4):601-5).

In a phase II, non-randomized trial (protocol IDs: MDA-DM-92037, NCI-T92-0113C), completed in December 1992, fenretinide therapy was also evaluated in patients with chemotherapy-refractory bladder tumors. Apparently, no results from this study have been reported to date.

Although fenretinide appears to be well tolerated, even after prolonged use, it lowers plasma retinol levels and may adversely affect night vision. In female breast cancer patients treated with 200 mg of fenretinide daily, 23.5% showed mild, and 26.5% moderate, alterations of measured dark adaptability, compared with just 6.5% of controls with mild alterations. Abnormal rod function improved significantly after 7 days and normalized 1 month after use of fenretinide was stopped, or vitamin A supplementation was begun, while the conventional 3-day drug suspension, or drug half dose, did not allow sufficient recovery. Alterations of conjunctival cytology were slightly higher in patients treated with fenretinide, but no clinical disorders of the ocular surface were observed (Decensi A, etal, J Natl Cancer Inst, 19 Jan 1994;86(2):105-10). Among male subjects enrolled in a bladder cancer prevention trial, a decline in plasma vitamin A levels accounted for a 41.7% cumulative incidence of diminished dark adaptability in the retinoid arm as compared to 6.8% in the control arm (Baglietto L, etal, Cancer Detect Prev 2000;24(4):369-75).

Halofuginone

Halofuginone, a low molecular weight quinazolinone alkaloid derived from a natural plant substance, is approved in the USA and Europe as Stenorol, a veterinary drug for use in feed to prevent coccidiosis in animals raised for human consumption. Halofuginone, being developed by Collgard Biopharmaceuticals (Newton, MA) as an anticancer agent, is a potent inhibitor of collagen alpha1(I) and matrix metalloproteinase (MMP)-2 gene expression. Cellular activities impeded by halofuginone include angiogenesis, cell proliferation and cell migration. Halofuginone suppresses extracellular matrix (ECM) deposition and cell proliferation. The mechanism of inhibition by halofuginone has been termed Panstasis referring to a new combination of distinct inhibitory mechanisms which results in the inhibition of tumor stromal support, angiogenesis, invasiveness, and cell proliferation (Elkin M, etal, Clin Cancer Res, Aug 1999;5(8):1982-8, and Elkin M, etal, Cancer Res, 15 Aug 1999;59(16):4111-8).

In a study conducted by lead investigator Israel Vlodavsky, PhD, at Hadassah-Hebrew University Hospital (Jerusalem, Israel), halofuginone effectively suppressed tumor progression and metastasis in mice. Halofuginone's ability to interfere with key events in neovascularization, together with its oral bioavailability and safe use as an antiparasitic agent, make it a promising drug for further evaluation in the treatment of various diseases associated with pathologic angiogenesis (Elkin M, etal, FASEB J, Dec 2000;14(15):2477-85). Administration of oral halofuginone in transplantable and chemically induced mouse bladder carcinoma resulted in a profound anticancer effect, even when treatment was initiated at advanced stages of tumor development. Although halofuginone failed to prevent proliferative preneoplastic alterations in the bladder epithelium, it inhibited further progression of chemically induced tumors into a malignant invasive stage. The antiangiogenic effect of halofuginone was also demonstrated by inhibition of microvessel formation *in vitro* (Elkin M, etal, Cancer Res, 15 Aug 1999;59(16):4111-8).

J-107088

J-107088, under development by Banyu (Tokyo, Japan), is a derivative of NB-506, an indolocarbazole compound targeting topoisomerase I. The drug is in a phase IIa clinical trial in metastatic bladder cancer.

ZD0473

ZD0473, a platinum-based drug under development by AstraZeneca, in collaboration AnorMED (Langley, BC, Canada), that licensed the drug from Cancer Research Campaign (CRC; London, UK), is being investigated in solid tumors including bladder cancer. A multicenter, open-label, two-step, CRC-sponsored phase II clinical trial was initiated in December 2000, in systemic chemotherapy-naive patients with local recurrence in the bladder and/or metastatic TCC measurable by imaging. The PI is M. Mason, MD, at Velindre Hospital (Cardiff, UK).

INTRAVESICAL CYTOTOXIC CHEMOTHERAPY

Although the intravesical route represents a unique delivery opportunity in the treatment of superficial bladder cancer (see FO, pp 1333-1335), few novel agents are being evaluated in this setting.

Amrubicin

Amrubicin (Calsed; Sumitomo Pharmaceuticals), a synthetic anthracycline, is a DNA topoisomerase II poison that induces DNA-protein complex formation followed by double-strand DNA breaks (Hanada M, etal, Jpn J Cancer Res, Nov 1998;89(11):1229-38). Preclinical studies suggest that the potent antitumor activity of amrubicin is the result of selective tumoral distribution of its highly active 13-hydroxy metabolite, amrubicinol (Yamaoka T, etal, Jpn J Cancer Res, Oct 1998;89(10):1067-73, and Noguchi T, etal, Jpn J Cancer Res, Oct 1998;89(10):1061-6). In phase I trials the dose-limiting toxicity (DLT) was leukopenia (Ogawa M, J Cancer Res Clin Oncol 1999;125(3-4):134-40).

In animal studies, the pharmacokinetics and histopathologic effect on normal bladder mucosa of amrubicin as an intravesical chemotherapeutic in superficial bladder cancer were investigated *in vivo* by instilling it into the empty bladders of Beagle dogs with bilateral cutaneous ureterostomy. In this setting, the drug scarcely passed into the blood; in only 1/5 dogs instilled with the highest dose of 80 mg of amrubicin, a serum level of 0.0248 μg/ml was detected 2 hours after administration, with serum levels in all other dogs below the detection limit. Excretion of amrubicin into the urine was also low, with the highest urinary excretion observed 6 hours after administration of 80 mg of amrubicin. The distribution of amrubicin in the bladder mucosa and muscular layer was almost equal, but the concentration of amrubicinol was 5 to 10 times higher in the bladder mucosa than in the bladder muscular layer; distributions of amrubicin in organs other than the bladder, including the cortex and medulla of the kidney, heart, lung, liver, and spleen, were very low, with that of amrubicinol even lower (Ohmori H, etal, Gan To Kagaku Ryoho, Apr 1996;23(5):601-6).

In a phase I study of amrubicin in superficial bladder cancer, conducted at Okayama University Medical School in Japan, the drug was dissolved in 30 ml of physiologic saline and injected intravesically on 6 consecutive days; the drug solution was retained for 2 hours. The starting dose was 60 mg/day, escalated to 150 mg/day in 30 mg/day increments. Among 15 patients entered into this study, 14 were eligible and assessable for toxicity, and 13 for efficacy. Incidence and severity of cystic irritabilities such as micturition pain, pollakisuria and hematuria, were related to the amrubicin dose. DLT was Grade 3 micturition pain and pollakisuria experienced by 1/3 patients at an amrubicin dose of 150 mg/day, which was the MDT. There was 1 CR (7.7%) and 4 PR (30.8%), for an overall response rate of 38.5%; the 1 CR occurred among 2 patients treated at 150 mg/day (Ohmori H, etal, Gan To Kagaku Ryoho, Apr 2001;28(4):475-82).

In an early, dose-finding, phase II clinical trial of amrubicin in superficial bladder cancer, also conducted at Okayama University Medical School using the same dosing protocol as the phase I clinical trial, 65 patients were randomly assigned to four groups, which were administered amrubicin at four dose levels. There were 63 patients eligible and assessable for toxicities, and 55 for efficacy. Response rates were related to dose levels, being 50.0% (7/14 PR) at 30 mg/day, 53.3% (8/15 PR) at 60 mg/day, 61.5% (2/13 CR + 6/13 PR) at 90 mg/day, and 69.2% (2/13 CR + 7/13 PR) at 120 mg/day. Major toxicities were cystic irritabilities, such as micturition pain, pollakisuria and hematuria that were dose related. However, incidence and severity of toxicities were not as high as those reported with other anthracyclines such as doxorubicin and epirubicin. The optimal dose of intravesical amrubicin was estimated at 90 to 120 mg, daily for 6 consecutive days (Tsushima T, etal, Gan To Kagaku Ryoho, Apr 2001;28(4):483-91). In 1998, a randomized, parallel comparative phase II clinical trial of intravesical amrubicin versus epirubicin in superficial bladder cancer was also reported at the 17th International Cancer Congress, in Rio de Janeiro, Brazil, by the Japanese Bladder Cancer Study Group. Sumitomo filed an NDA, which is still pending, for amrubicin in Japan in September 1999.

Meglumine-GLA (MeGLA)

Meglumine (N-methylglucamine), is an organic polymer prepared from D-glucose and methylamine that forms salts with acids, enhancing the water solubility of these compounds. It is commonly encountered in the form meglumine diatrizoate (Hypaque), the N-methylglucamine salt of 3,5-diacetamido-2,4,6-triiodobenzoic acid, a water-soluble organic iodine compound used intravascularly and orally as a diagnostic radiopaque contrast medium.

Meglumine-GLA (MeGLA), an N-methylglucamine salt of gamma-linolenic acid (GLA) benefits from the water solubility of GLA. GLA, the metabolite of linolenic acid, is an essential n-6 polyunsaturated fatty acid that increases the cell membrane content of its elongase product, dihomo-GLA (DGLA), from which prostaglandin E1 (PGE1) is synthesized (Fan YY and Chapkin RS, J Nutr, Sep 1998;128(9):1411-4). PGE1 possesses antiproliferative activity, and it has been hypothesized that some of the metabolic abnormalities common to all malignant cells may be attributed to the inability of cancer cells to produce PGE1, apparently as a result of the lack of the enzyme delta-6-desaturase, which converts linolenic acid to GLA (Horrobin DF, Med Hypotheses, May 1980;6(5):469-86, and Botha JH, etal, Prostaglandins Leukot Med, Jul 1985;19(1):63-77). It has been proposed that the exposure of cancer cells to exogenous GLA would bypass this block in the metabolic pathway, causing inhibition of cancer cell growth while having no effect on normal cells (Dippenaar N, etal, S Afr Med J, 2 Oct 1982;62(15):505-9, and Das UN, Nutrition, Mar-Apr 1989;5(2):106-10). Several studies have indeed demonstrated that GLA can present a specific cytotoxicity for tumor cells (Dippenaar N, etal, *ibid*, Leary WP, etal, S Afr Med J, 30 Oct 1982;62(19):681-3, Vartak S, etal, Br J Cancer, May 1998;77(10):1612-20, and Ilc K, etal, Anticancer Drugs, Apr 1999;10(4):413-7).

However, other studies have called into question whether increased prostaglandin production is in fact responsible for the inhibitory effects produced by GLA in malignant cell cultures, and suggest that some mechanism other than the interaction between GLA and prostaglandin production may be involved in GLA's observed cytotoxic effect on tumor cells. A relatively recent study has found GLA treatment to induce elevated expression of tumor suppressor gene p53, a checkpoint regulator that mediates chromosomal segregation and apoptosis, which may contribute to GLA's antiproliferative activity on tumor cells (Joubert AM, etal, Prostaglandins Leukot Essent Fatty Acids, Sep 1999;61(3):171-82). It has also been noted that the n-6 group of polyunsaturated fatty acids can increase formation of the proinflammatory cytokine TNFα, which is

involved in the generation of highly toxic peroxyl and superoxide radicals (reactive oxygen species), partly by increasing the activity of nitric oxide synthase (Darlington LG and Stone TW, Br J Nutr, Mar 2001;85(3):251-69); this may represent an important mechanism by which GLA exerts it cytotoxic effects (Ilc K, etal, *ibid*).

MeGLA was shown to exert a relatively immediate cytotoxic effect on both parental and multidrug resistant (MDR) urothelial tumor cell lines *in vitro*, with its efficacy maintained in the presence of clinically relevant serum contamination (Solomon LZ, etal, Br J Urol, Jul 1998;82(1):122-6). In adherent cell cultures, MeGLA caused >95% reduction in residual viable biomass, compared to <50% reduction with other tumoricidal agents such as mitomycin and epirubicin. In suspended cell cultures, MeGLA prevented colony formation as effectively as either mitomycin or epirubicin in drug-sensitive (parental) cells, but only MeGLA entirely prevented colony formation in MDR cell lines. In a serum-free environment, MeGLA demonstrated a 10-fold enhancement in lytic efficacy against the parental superficial urothelial cancer cell lines MGH-U1 and RT112, compared to application in the presence of serum; a similar enhancement of efficacy was seen in the MDR clones of these cell lines. *In vivo*, intravesical administration of MeGLA in Wistar rats resulted in minimal systemic absorption (less than 2%) from the bladder; the most common destination for absorbed GLA was the liver. The drug was well tolerated, with no adverse effects observed (Solomon LZ, etal, J Urol, Dec 1998;160(6 Pt 1):2280-3).

Because topical drug application by intravesical administration, in the treatment of superficial bladder cancer is limited to a maximum duration of 2 hours, an experiment was conducted to determine if GLA is cytotoxic over such short drug exposure times. Cell lines MGH-U1 and RT112 were exposed to MeGLA for time intervals ranging from 30 minutes to 2 hours, at drug concentrations ranging up to 1000 μg/ml. Greater than 90% viable biomass inhibition was observed at a concentration of 125 μg/ml at 2 hours drug exposure; at shorter drug exposure times, higher drug concentrations were needed to induce the same effect (Solomon LZ, etal, Urol Res 1998;26(1):11-5).

A phase I clinical trial was completed in 1998, and patient recruitment in a phase IIa efficacy study of MeGLA in the treatment of superficial bladder cancer was initiated in late 1999. However, all clinical trials were suspended in December 2000, pending the outcome of an administration order granted Scotia (London, UK), the developer of MeGLA, in January 2001, in the UK. The phase I tolerability study was conducted at Southampton General Hospital in the UK, in superficial bladder cancer patients about to undergo cystectomy. A single intravesical injection of 50 ml of 1 mg/ml MeGLA was administered a few hours before surgery, and retained for a maximum of 1 hour in 7/10 patients. MeGLA was well tolerated in 9/10 patients, with pain occurring in 1; no collateral damage to normal urothelium was indicated based on histologic findings (Crook TJ, etal, UroOncology 2000;1:39-42).

SYSTEMIC IMMUNOTHERAPY

Several approaches are being considered to boost host immunity in bladder cancer by systemic administration of immunomodulating agents (see FO, pp 1337-1338) and/or therapeutic vaccines.

Bropirimine

Bropirimine is an oral immunomodulator that has demonstrated anticancer activity in *in situ* (CIS) TCC in both the bladder and upper urinary tract. Activity was also documented in patients after prior immunotherapy with Bacille Calmette-Guerin (BCG). However, despite encouraging results from phase II clinical trials, an FDA panel rejected bropirimine's NDA submitted by Pharmacia in September 1996. One of the reasons for the FDA's decision not to approve the drug for second-line treatment of CIS was the concern that disease could spread in patients in whom cystectomy is delayed while they are treated with bropirimine. Subsequently, Pharmacia discontinued development of this drug in 1999, and an intercontinental study of oral bropirimine in the treatment of BCG-naive patients with CIS of the urinary bladder was terminated at that time. Others have looked into developing this drug and Yokult Honsha (Tokyo, Japan), that was developing bropirimine in collaboration with Pharmacia for commercialization in Japan, may still proceed with the development of bropirimine in bladder cancer.

A USA phase II clinical trial was performed to estimate bropirimine's efficacy in BCG-resistant bladder CIS with a separate analysis performed in additional patients intolerant of BCG toxicity. Patients were treated with oral bropirimine (3 g), daily, for 3 consecutive days, weekly, for up to 1 year. Among 86 patients entered in the study, 21 were not evaluable. There were 21 (32%) CR among the 65 evaluable patients, including 14 (30%) of 47 BCG-resistant, and 7 (39%) of 18 BCG-intolerant patients. Overall, by intent-to-treat analysis, CR was seen in 21 (24%) of 86 patients. Most BCG-resistant patients were failures to BCG without relapse, and had been administered 12 to 36 (median=12) BCG treatments; intolerant patients were administered 4 to 11 treatments (median=6). Response duration ranged from 65 to 810 days, with median not yet reached, but >12 months. Bropirimine treatment was discontinued in 13 (15%) of 86 patients because of toxicity. Progression to invasive or metastatic disease during or immediately after therapy was documented in only 4 patients (6%), all nonresponders. Based on these findings, bropirimine may be an alternative to cystectomy for some patients with bladder CIS who have failed or have not tolerated BCG. Further evaluation to improve responses and durability is warranted (Sarosdy MF, etal, Urology, Feb 1998;51(2):226-31).

In a phase III European clinical trial, designed to compare the effects of oral bropirimine with intravesical BCG, 55 BCG-naive patients newly diagnosed with bladder CIS, were randomized to either bropirimine (n=27) or BCG (n=28). Bropirimine was orally administered at a dose of 3 g/day for 3 consecutive days with a 4-day drug-free interval on a weekly basis, for up to 1 year. BCG-Tice was instilled weekly for 2 6-week cycles. Trial dropout rates for all adverse events were 4% for bropirimine and 14% for BCG. The most frequently reported local events in the bropirimine-versus BCG-treated groups were irritative complaints (64% versus 89%) and hematuria (24% versus 61%), while the most frequently reported systemic events were fever (4% versus 21%), flu syndrome (24% versus 7%), headache (28% versus 11%), and nausea (24% versus 11%). A total of 92% of patients treated with bropirimine and 100% of those treated with BCG experienced a CR with a mean duration of 12.6 months and 12.3 months, respectively (Witjes WP, etal, Eur Urol, Dec 1999;36(6):576-81).

In a European phase II clinical study, bropirimine was orally administered to patients with recurrent, superficial bladder CIS. Seventeen CR (59%) were seen in 29 patients treated with the same dosing schedule of bropirimine. In addition, in 13 BCG failures, 6 CR were observed with bropirimine, suggesting that patients with prior BCG therapy may be salvaged by bropirimine treatment. Side effects were mild to moderate, consisting mainly of flu-like symptoms (Witjes JA, Eur Urol 1997;31 Suppl 1:27-30).

A phase II clinical trial, conducted at 38 centers in Japan, bropirimine was orally administered to patients with bladder CIS, at a dose of 750 mg every 2 hours, 3 times a day, for 3 consecutive days with a 4-day drug withdrawal period. Among 48 patients entered into the trial, 41 were evaluable for antitumor efficacy. CR was observed in 17/41 (41.5%) patients while disease stabilized in 18 and progressed in 6. Classified by disease stage, there were 7/12 (58.3%) responses in patients with primary bladder CIS, 10/29 (34.5%) in those with secondary bladder CIS, 10/22 (45.5%) in those with Grade III disease, and 5/21 (23.8%) in those previously treated with chemotherapeutic agents or BCG by intravesical or other routes. The drug was well tolerated. Adverse reactions frequently observed were Grade 2 or milder influenza-like symptoms such as fever and generalized malaise and gastrointestinal symptoms like anorexia and nausea/vomiting. Abnormalities in laboratory tests, such as an elevation in GOT/GPT, neutropenia, and leukopenia were also observed (Akaza H, etal, Gan To Kagaku Ryoho, Jan 1997,24(1):77-85).

In a follow-up investigation of 17 CR patients treated with bropirimine for CIS of the bladder in the above phase II clinical trial, disease recurred in 5 while 9 remained recurrence free for 1 year; 5 patients remained recurrence free for more than 2 years at a median follow-up of 29.1±4.2 months. The 1-year and 2-year recurrence-free rates were 70.3% and 61.5% respectively. Of the 47 bropirimine-treated patients, 11 underwent total cystectomy within a median follow-up of 31.8±5.2 months. The rates of bladder preservation after 1 year, 2 years, and 3 years were 87.2%, 80.7%, and 74.0%, respectively. Of the 39 patients who did not respond to bropirimine, or whose disease recurred after bropirimine treatment, 19 were subsequently treated with intravesical BCG. Among these patients, 13/19 (68.4%) achieved CR, indicating that bropirimine does not decrease the efficacy of BCG therapy (Akaza H, etal, Gan To Kagaku Ryoho, Nov 1999;26(13):2049-53).

MAGE-3 Protein Vaccine

A MAGE-3 antigen-based vaccine is under development by NDDO Oncology (Amsterdam, The Netherlands), in collaboration with the Ludwig Institute for Cancer Research (Brussels, Belgium), and GlaxoSmithKline (GSK) Biologicals (Brussels, Belgium) which provides the vaccine adjuvant SB AS-2 (AS02). The MAGE gene family is expressed in a variety of malignancies, including bladder cancer, but not in normal tissues except for the testis. Hence, MAGE-encoded antigens are potential tumor-specific targets for vaccine therapy. SB AS-2, a vaccine adjuvant under development by GSK Biologicals, is a proprietary oil-in-water emulsion formulation of saponin QS-21 and Detox-B SE, comprised of a derivative of monophosphoryl lipid A (MPL) bacterial endotoxin and mycobacterial cell wall skeleton (CWS). GSK Biologicals has licensed QS-21 (Stimulon) from Aquila Biopharmaceuticals (Framingham, MA), and Detox-B SE from Corixa (South San Francisco, CA).

In a phase I/II clinical trial, patients with metastatic MAGE-3-positive solid tumors who were HLA A1, A2 or B44 positive, were vaccinated with MAGE-3 protein with or without SB AS-2 adjuvant, to evaluate toxicity and response. Intramuscular immunizations were administered on days 1, 22, 43 and 64. Patients without disease progression were treated with 2 additional injections on days 106 and 148. Among 57 enrolled patients there were 49 cases of melanoma, 3 of bladder cancer, 2 of nsclc, 2 of esophageal cancer and 1 of head and neck cancer. Patients were divided into 4 groups; the first group was treated with MAGE-3 (300 mg) only, while dose levels of MAGE-3 in the other 3 groups were 30 mg, 100 mg, and 300 mg, in combination with a fixed dose of 100 mg SB AS-2. Treatment was well tolerated with mostly transient Grade 1 and 2 toxicities, with no apparent dose-related differences. The most commonly seen reactions were tenderness and redness at the injection site, fatigue, fever, flu-like symptoms, myalgia and nausea. Grade 3 myalgia and local toxicity were observed in 2 patients each. There were no hematologic toxicities. Among 36 patients treated with at least 4 vaccinations, there were 7 clinical responses, 2 PR (melanoma, bladder), 3 mixed responses (melanoma), and disease stabilized in 2 (melanoma) for 4 to 12 months. The majority of patients immunized with vaccine plus adjuvant developed a MAGE-3 antibody response. However,

no clear correlation existed between clinical and antibody responses. In conclusion, immunization with MAGE-3 protein and the adjuvant SB AS-2 was well tolerated and induced beneficial antitumor responses in a number of patients (Kruit WHJ, etal, ASCO00, Abs. 1882:479a).

Monocyte-derived Activated Killer (MAK) Cell Technology

Monocyte-derived activated killer (MAK) cell technology, under development by Immuno-Designed Molecules (IDM; Paris, France), when used in conjunction with bispecific MAbs or other protein-based tumor cell targeting approaches, turns human blood monocytes into therapeutic agents dubbed "cell drugs", including MAK cells and dendritophages. MAK cells are prepared by culturing patient mononuclear cells, obtained by apheresis, within the MAK Cell Processor, a single-use device operating under dedicated software control and containing all necessary reagents for autologous cell drug production. Circulating blood mononuclear cells are first cultured with GM-CSF or IL-13 to differentiate them into antigen-presenting cells (APC). Monocyte-derived APC are then grown in the presence of exogenous target antigens (tumor cell debris or apoptotic bodies) to become fully mature or activated APC. MAK cells express a large number of high-affinity Fc receptors and when reintroduced into the patient, act as tumor-specific killer cells. MAK cells can be combined with antibodies to enhance antitumor activity.

In February 2000, Medarex (Annandale, NJ) and IDM expanded a collaborative agreement originally entered into in December 1993, and amended in January 1995 and in December 1996, under which Medarex acquired an option to purchase exclusive USA rights to MAK when used in conjunction with its bispecific MAb (bsMAb) technologies. In particular, IDM acquired worldwide commercial rights for the use of Medarex's MDX-210, MDX-220, and MDX-22 MAbs in connection with MAK cell therapy, and certain economic interests in other Medarex products, including MAbs MDX-RA and MDX-447. Currently, MAK cell therapy is being studied in combination with MDX-22 (anti-tCD14) MAb and MDX-210 bispecific (anti-CD64 and anti-HER2/neu) MAb.

Phosphodiester Oligonucleotides as Immunomodulators

A new class of synthetic molecules with potential anticancer activity and immune modulating properties, under development by Bioniche (Belleville, Ontario, Canada), are composed of short, single-stranded, synthetic phosphodiester DNA oligonucleotides, containing a guanine-thymine motif. At the 5th International Symposium on Predictive Oncology and Therapy held in Geneva, Switzerland, on October 28-31, 2000, Bioniche presented results of a study that showed that such constructs inhibit cancer cell-cycle progression and cellular division, and are potent inductors of apoptosis. This anticancer activity is associated with the apoptosis-related activation of caspase-3 and mitochondrial membrane disruption, and is independent of cell cycle regulators or CD95/Fas/APO-1 resistance, commonly termed Fas-resistance, associated with the development of multidrug resistance.

These nonantisense constructs are capable of inducing cell-cycle arrest and apoptosis in leukemia cells, as well as cells from breast, bladder, ovarian, and prostate carcinomas. Two synthetic phosphodiester oligonucleotides derived from *M. phlei* DNA containing either GpT dinucleotides within a specific sequence context (33 base length), or ApC dinucleotides within a specific sequence context (15 base length) caused a dose-dependent inhibition of human Jurkat, K562, THP-1, HL-60 and HL-60 MX-1 cell division that was associated with cell-cycle arrest in the late S-phase/G_2M of the cell cycle. *M. phlei*-derived oligonucleotides containing a GpT dinucleotide motif also blocked the cell cycle, but at the G_0/G_1/early S-phase. The *M. phlei*-derived oligonucleotides containing an ApC dinucleotide motif did not inhibit leukemia cell division. Presence of multidrug resistance or p53 mutations did not affect the ability of *M. phlei*-derived DNA or GpT oligonucleotides in inducing apoptosis. Also, induction of apoptosis was independent of Fas (CD95) signaling. *M. phlei*-derived DNA and oligonucleotides containing a GpT dinucleotide motifs induce apoptosis in leukemia cells but each causes cell-cycle arrest at different phases of the cell cycle (Filion MC, etal, NCI/EORTC/AACR00, Abs. 525). In October 2000, at the 25th Annual Congress of the Société International d'Urologie, Bioniche also presented research data showing that these synthetic oligonucleotides inhibit cellular division and induce apoptosis in a panel of human bladder tumor cell lines with known p53-p21 mutations by a mechanism implicating the proteolytic activation of caspases.

INTRAVESICAL IMMUNOTHERAPY

Bladder cancer is managed in its early stages with a topical (intravesical) immunotherapy approach, i.e. BCG, used both as a therapeutic and preventative option. Several other immunotherapeutics are also potential candidates for this approach.

BCI-ImmuneActivator (BCI-IA)

BCI-ImmuneActivator (BCI-IA), under development by Intracel (Rockville, MD), is a proprietary formulation of keyhole limpet hemocyanin (KLH) for intravesicular immunotherapy of bladder cancer. BCI-IA is composed entirely of KLH, a copper-containing, high molecular weight glycoprotein derived from the hemolymph of *Megathura crenulata* (or keyhole limpet), a giant sea mollusk found along the coast of California and Mexico. Once harvested, KLH is purified through a proprietary process that preserves its high molecular weight structure and minimizes low molecular weight degradation products. KLH is a potent immune system stimulator that induces a nonspecific inflammatory response in humans and animals. Intravesical instillation of KLH into the bladder stim-

Exhibit 1
Novel Agents in Development for the Treatment of Bladder Cancer

Developer ❑ Affiliate(s)	Generic Name ❑ Number ❑ Brand Name	Description ❑ Administration Route	Status ❑ Indication(s)
Aronex Pharmaceuticals ❑ U Texas M. D. Anderson Cancer Center	Atragen (formerly Tretinoin LF)	Lipid based, IV formulation of all-trans retinoic acid (ATRA) ❑ intravesical, IV	Phase I/II (begin 4/99, discontinued 12/99)➢USA ❑ superficial bladder cancer
AstraZeneca	ZD1839 ❑ Iressa	A quinazoline-derivative that selectively inhibits epidermal growth factor receptor tyrosine kinase (EGFr-TK)-mediated intracellular signalling pathways ❑ PO	Phase II (begin 2/01)➢USA ❑ recurrent or metastatic cancer of the urinary tract
AstraZeneca ❑ Cancer Research Campaign (CRC)	ZD9331 ❑ Vamidex, Vamydex	Thymidylate synthase (TS) inhibitor ❑ IV, PO	Phase II (ongoing 4/01)➢USA, Europe, phase I (begin 6/99, completed 8/00)➢Europe (combination) ❑ refractory solid tumors
AstraZeneca ❑ AnorMED, Cancer Research Campaign (CRC)	ZD0473 (was AMD473)	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 II (begin 12/99, ongoing 3/01)➢USA, Europe (monotherapy); phase I (begin 12/99, ongoing 3/01)➢USA, Europe (combination) ❑ advanced solid tumors
Banyu	J-107088	J-107088 is a derivative of NB-506, an indolocarbazole compound targeting topoisomerase I ❑ IV	Phase II (ongoing 9/01)➢USA ❑ refractory metastatic bladder cancer
Bio-Technology General (BTG) ❑ Weizmann Institute of Science		Cell-free, synthetic peptide-based vaccine designed to elicit a tumor-specific, cytotoxic T lymphocyte (CTL) immune response to eliminate cancer and to confer an immunity to protect the host from disease recurrence	Research (ongoing 5/01) ❑ solid tumors, TCC of the bladder
Bioniche Therapeutics	Regressin	Immunostimulant derived from mycobacterial cell wall	Phase II/III (discontinued 6/98) ➢Canada ❑ bladder cancer
Bioniche Therapeutics ❑ UroCor	Mycobacterium cell wall complex (MCC)	A cell wall composition prepared from nonpathogenic *Mycobacterium phlei* ❑ IV, intravesical	Phase II (ongoing 5/01)➢Canada, Australia ❑ carcinoma *in situ* (CIS) of the bladder
Bioniche Therapeutics	Oligomodulators	Short, synthetic, nonantisense, single-strand, phosphodiester DNA oligonucleotides, which possess pharmacologic activity independent of any genetic function ❑ injection	Preclin (ongoing 5/01) ➢Canada ❑ bladder cancer
BioNumerik Pharmaceuticals	MDAM	Nonpolyglutamylatable antifolate structurally similar to methotrexate ❑ PO, IV	Phase I (completed 2/00)➢USA ❑ solid tumors
Calydon	CV876	Cytolytic adenovirus engineered to replicate in and kill bladder cancer cells; part of the proprietary ARCA (Attentuated Replication Competent Adenovirus) technology platform ❑ injection	Research (ongoing 5/01) ➢USA ❑ bladder cancer
Canji ❑ Transgene, Genzyme Molecular Oncology	rAd/p53 ❑ SCH58500 (formerly ACN53)	Recombinant adenovirus encoding wild-type p53 ❑ intravesical	Phase I (begin 5/98, ongoing 8/01)➢USA ❑ advanced bladder cancer
Canji	Syn3	Polyamide compound that significantly enhances adenoviral-mediated transgene expression in the urothelium, with little or no toxicity to normal tissue	Research (ongoing 4/01) ➢USA ❑ bladder cancer

— *continued on next page*

Cell Pathways ❑ Paladin Labs, Aventis Pharma, Roche Laboratories, Eli Lilly, GlaxoSmithKline	Exisulind, sulindac sulfone ❑ FGN-1 ❑ Aptosyn (formely Prevatac)	Sulfone metabolite of the nonsteroidal anti-inflammatory drug (NSAID) sulindac; member of the class of pro-apoptotic drugs termed selective apoptotic antineoplastic drugs (SAAND) ❑ PO	Phase I/II (ongoing 11/00)➢USA (combination) ❑ solid tumors
Collgard Biopharmaceuticals	Halofuginone ❑ NSC 713205	A low molecular weight quinazolinone alkaloid derived from a natural plant substance ❑ PO	Preclin (ongoing 1/01) ➢Israel ❑ bladder cancer
Columbia University	001863	Antisense oligonucleotides directed against bcl-xL mRNA ❑ injection	Preclin (ongoing 3/01)➢USA ❑ bladder cancer
CytoGenix ❑ Baylor College of Medicine, Yale U School of Medicine, Columbia U	TroVec, EnzSyn	Intracellular expression systems that enable the direct enzymatic synthesis of sequence-specific, single-stranded (antisense) DNA (ssDNA) ❑ injection	Preclin (ongoing 5/01)➢USA ❑ solid tumors
Dendreon ❑ Ludwig Institute for Cancer Research	APC80NY	Autologous dendritic cells pulsed with the NY-ESO-1 antigen; part of the company's Antigen Delivery Cassette technology platform ❑ injection	Preclin (ongoing 6/01)➢USA ❑ solid tumors
Duke University Medical Center		Antitumor vaccine based on autologous dendritic cells pulsed with tumor homogenate ❑ injection	Preclin (ongoing 4/01)➢USA ❑ solid tumors
Eli Lilly	Pemetrexed disodium ❑ LY 231514 ❑ Alimta	Multitargeted antifolate (MTA) that inhibits at least three enzymes, thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT) involved in folate metabolism and DNA synthesis ❑ IV	Phase II (begin 7/01)➢USA ❑ recurrent advanced or metastatic TCC of the bladder
Enchira Biotechnology ❑ U Texas M. D. Anderson Cancer Center		Protein therapeutics targeted to antagonize EGFr and HER-2/neu receptors ❑ injection	Preclin (ongoing 6/01)➢USA ❑ solid tumors
Galderma Research & Development	CD437	Synthetic retinoic acid receptor γ (RARγ)-selective agonist that induces apoptosis through caspase activation ❑ IV	Preclin (ongoing 1/01)➢USA, Europe ❑ solid tumors
Genetics Institute (GI) ❑ Wyeth-Ayerst, Hoffmann-La Roche		Recombinant human interleukin-12 (rhIL-12) ❑ IV, subcutaneous, intraperitoneal	Phase I/II (completed 96; suspended 10/01)➢USA ❑ solid tumors; phase I (ndr 5/99) USA ❑ superficial TCC of the bladder
Genta ❑ Molecular Biosystems (Alliance Pharmaceutical), NCI, U Pennsylvania, Avecia	Augmerosen ❑ G3139 ❑ Genasense	An 18-mer fully phosphorothioated antisense oligonucleotide which targets the bcl-2 gene; the lead compound of the Anticode (antisense) technology platform ❑ subcutaneous, IV	Phase I/II (begin 8/97, ongoing 10/00)➢USA ❑ advanced solid tumors
Genzyme Molecular Oncology (GMO) ❑ Ludwig Institute for Cancer Research	Ad2/ESO-1	Recombinant adenovirus encoding the ESO-1 transgene ❑ injection	Preclin (ongoing 6/01)➢USA ❑ solid tumors
Ilex Oncology ❑ MPI Research	Piritrexim	Dihydrofolate reductase inhibitor that enters cells by passive diffusion and inhibits DNA synthesis ❑ injection, PO, topical	Phase II (discontinued 10/98)➢USA ❑ bladder cancer, advanced
Ilex Oncology ❑ Aventis Pharmaceuticals	Eflornithine; alpha difluoromethylornithine HCl (DFMO)	Specific, enzyme-activated, irreversible inhibitor of ornithine decarboxylase; inhibits growth of tumor cells and promotion and progression phases of carcinogenesis ❑ injection, PO	Phase III (ongoing 6/01)➢USA ❑ superficial bladder cancer
ImClone Systems ❑ U California San Diego, Aventis Pharmaceuticals, Merck KGaA	C225 ❑ IMC-C225 (formerly Cetuximab)	IMC-C225 is a chimerized MAb directed against epidermal growth factor receptor (EGFr) ❑ IV	Phase Ib/IIa (completed 96)➢USA ❑ solid tumors

— continued on next page

Immunex ❑ Japan Tobacco, Abgenix	ABX-EGF	Fully human anti-EGFr monoclonal antibody (MAb) ❑ IV	Phase II (ongoing 4/01)➢USA ❑ solid tumors
Immuno-Designed Molecules (IDM) ❑ Medarex	MAK	Monocyte-derived activated killer (MAK) cell technology, used in conjunction with bispecific MAb or other protein-based tumor cell targeting ❑ injection	Phase II (ongoing 2/01) ➢Europe ❑ bladder cancer
Immunomedics ❑ NCI	RNase	Inactivation-resistant RNAse conjugated to humanized antibody, using internalizing tumor antibodies, or cell-specific cytokine ❑ IV	Preclin (ongoing 6/01)➢USA ❑ solid tumors
Ingenex ❑ Baylor College of Medicine, U Texas M. D. Anderson Cancer Center, Selective Genetics	RB-94, RB94 (formerly SG-94)	Gene therapy product based on a truncated variant (p94) of the Rb tumor suppressor gene expressed by an adenovirus vector ❑ intratumoral, IV	Preclin (ongoing 3/01)➢USA ❑ solid tumors
Intracel ❑ Mentor	BCI-ImmuneActivator (formerly KLH-ImmuneActivator)	Proprietary formulation of keyhole limpet hemocyanin (KLH) for intravesicular immunotherapy of bladder cancer ❑ intravesical	Phase III (ongoing 6/01)➢USA ❑ refractory CIS of the bladder
Introgen Therapeutics ❑ NCI, U Texas M. D. Anderson Cancer Center, Sidney Kimmel Cancer Center, Oncormed	Ad5CMV-p53, Ad-p53 ❑ RPR/INGN-201, INGN-201	E1/E3-deleted, replication-defective adenoviral vector containing wild-type p53 cDNA under the control of a CMV promoter ❑ intraperitoneal, intralesional, intratumoral, IV	Phase I (ongoing 6/01)➢USA ❑ bladder cancer
Isis Pharmaceuticals ❑ Eli Lilly	ISIS 3521, ISI641A, CGP64128A	20-mer phosphorothioate antisense oligonucleotide inhibitor of protein kinase C (PKC) alpha isoform, PKC[alpha], gene expression ❑ IV	Phase II (ongoing 12/00)➢USA, Canada, Europe ❑ relapsed or refractory solid tumors; phase I (ongoing 12/00)➢USA ❑ advanced solid tumors (combination)
Isis Pharmaceuticals ❑ Elan	ISIS 2503	20-mer antisense phosphorothioate oligonucleotide inhibitor of H-ras mRNA ❑ IV, injection, PO	Phase I (completed 5/99) ➢USA ❑ advanced solid tumors; phase I (ongoing 12/00) ➢USA ❑ advanced solid tumors (combination)
Janssen Pharmaceutica ❑ Kyowa Hakko Kogyo	R115777	Farnesyl transferase inhibitor (FTI); imidazole; inhibits activated p21 ras ❑ PO	Phase II (begin 3/00, ongoing 9/01)➢USA ❑ advanced or metastatic urothelial cancer
KS Biomedix ❑ YM BioSciences, TranXenoGen	KAb102	Super-high affinity MAb with specificity for human EGFr produced using proprietary sheep hybridoma technology ❑ IV	Preclin (ongoing 6/01)➢UK ❑ solid tumors
Ligand Pharmaceuticals	LGD 1550 (was ALRT 1550)	Novel synthetic retinoic acid receptor (RAR)-selective retinoid ❑ PO	Phase I/II (ongoing 12/00) ➢USA ❑ advanced solid tumors
Medarex ❑ Dartmouth Medical, Merck KGaA, Immuno-Designed Molecules	MDX-447, H22 x H425, EMD 82633	Fully-human, bispecific MAb directed against the CD64 receptor for IgG Fc and EGFr ❑ IV	Phase II (ongoing 6/01)➢USA ❑ solid tumors
Millennium Pharmaceuticals (LeukoSite) ❑ Pharsight	LDP-341 (formerly PS-341 or MG-341)	Boronic acid depeptide derivative; potent selective and reversible proteasome inhibitor ❑ IV	Phase I (begin 6/01)➢USA ❑ advanced, refractory solid tumors (combination)
National Cancer Institute (NCI)	9-aminocamptothecin (9-AC) ❑ NSC 603071	Water-insoluble derivative of camptothecin ❑ IV, PO	Phase I/II (ongoing 5/01)➢USA ❑ solid tumors
National Cancer Institute (NCI)	Carboxyamido-triazole (CAI) ❑ NSC-609974	Synthetic inhibitor of non-voltage-gated calcium influx-regulated signal pathways; reversibly inhibits angiogenesis, tumor cell proliferation, and metastatic potential ❑ PO	Phase I (completed 12/98)➢USA ❑ solid tumors; phase I (ongoing 7/01)➢USA ❑ advanced solid tumors (combination)
National Cancer Institute (NCI)	Fenretinide (4-HPR)	Synthetic retinoid; vitamin A analog ❑ PO	Phase III (ongoing 10/01)➢USA, Italy ❑ bladder cancer

— continued on next page

NDDO Oncology ❑ GlaxoSmithKline Biologicals, Ludwig Institute for Cancer Research		Active immunization of cancer patients against tumor-specific MAGE-3 antigens ❑ intramuscular	Phase I/II (ongoing 5/00) ➢Europe ❑ metastatic solid tumors, expressing MAGE-3 antigen
NeoOncoRx ❑ NDDO Oncology	EO9 ❑ Neoquin	Bioreductive alkylating indoloquinone ❑ intravesical	Phase I/II (begin 11/01)➢Europe (UK) ❑ superficial bladder cancer
Niigata University School of Medicine		Antisense phosphorothioate oligonucleotide directed against bcl-2 mRNA ❑ injection	Research (ongoing 7/00)➢Japan ❑ bladder cancer
Pfizer ❑ OSI Pharmaceuticals	CP-609,754	Inhibitor of farnesyl transferase enzyme; may inhibit the ras oncogene ❑ PO	Phase I (ongoing 12/99)➢USA ❑ solid tumors
Pharmacia	Bropirimine ❑ U-54461S ❑ Remisar	Pyrimidinone that increases endogenous IFN and TNF-α ❑ PO	NDA (rejected 9/96; resubmitted 6/99; discontinued 01)➢USA; phase III (ongoing 6/99, ndr 01) ➢Japan ❑ recurrent superficial bladder cancer
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 (completed 97)➢USA ❑ solid tumors
PhotoCure	Hexvix	Photodynamic therapy based on ALA-ester derivatives ❑ *in situ*	Preclin (ongoing 8/01) ➢Europe ❑ bladder cancer
Prolifix ❑ Medical Research Council (MRC), BioFocus, Chugai Pharmaceutical, Eli Lilly		Small peptide that inhibits E2F, a protein that regulates the cell cycle and induces apoptosis ❑ IV	Preclin (ongoing 8/01) ➢UK ❑ cancer
Rowland Institute for Science ❑ Ergo Science (terminated 3/99)	EtNBS ❑ (formerly ER-470)	Benzophenothiazine photochemotherapeutic ❑ injection	Preclin (ongoing 6/01)➢USA ❑ solid tumors
Rowland Institute for Science ❑ Ergo Science (terminated 3/99)	2I-EtNBS ❑ (formerly ER-480)	Benzophenothiazine photochemotherapeutic ❑ injection	Preclin (ongoing 6/01)➢USA ❑ solid tumors
Schering-Plough	SCH 66336	Orally bioavailable nonpeptide tricyclic farnesyltransferase inhibitor (FTI) in the pyridobenzocycloheptene class ❑ PO	Phase II (ongoing 6/01)➢USA ❑ solid tumors; phase I/II (ongoing 5/01)➢USA (combination) ❑ solid tumors
Scotia Pharmaceuticals	Meglumine-GLA	Cytotoxic agent for use in bladder cancer ❑ intracavitary	Phase IIa (suspended 12/00)➢UK ❑ bladder cancer
Sumitomo Pharmaceuticals	Amrubicin, amrubicin hydrochloride ❑ SM-5887, SMP-5887 ❑ Calsed	Synthetic anthracycline ❑ IV, intravesical	NDA (filed 9/99, pending 5/01)➢Japan ❑ superficial bladder cancer
Tel Aviv University ❑ National Institutes of Health (NIH)		Antisense oligonucleotide complementary to activity-dependent neurotrophic factor III/activity-dependent neuroprotective protein (ADNF III/ADNP) ❑ injection	Research (ongoing 10/01) ➢Israel, USA ❑ solid tumors
Titan Pharmaceuticals ❑ GeoMed	Gallium maltolate ❑ GaM	Oral form of gallium, a semimetallic element that is known to concentrate in malignant tumors and sites of infection ❑ PO	Phase I (completed 7/00)➢USA, phase II (planned 01)➢USA ❑ solid tumors
Zycos ❑ U Massachusetts		Cancer-related protein, cytochrome P450 1B1 (CYP1B1), an ezyme expressed in various cancers but not in normal cells ❑ IV	Preclin (ongoing 10/01)➢USA ❑ cancer

*Source: NEW MEDICINE Oncology KnowledgeBASE (nm|**OK**) residing at www.oncologyknowledgebase.com, October 2001.*

ulates the production of cytokines, in particular, IL-1, which in turn stimulates the immune cascade through adomino effect (Molto LM, etal, Eur Urol 1991;19(1):74-8, and Jurincic-Winkler CD, etal, Eur Urol 1995;28(4):334-9). A tumor-specific phenomenon may also be involved in the immunomodulatory activity of KLH, as it contains oligosaccharides bearing the carbohydrate core disaccharide Gal(β1-3)-GalNAc, also referred to as Thomsen-

Friedenreich disaccharide, that is cross-reactive with the T antigen found on bladder cancer cells (Wirguin I, etal, Cancer Immunol Immunother, May 1995;40(5):307-10).

Use of KLH as bladder immunotherapy is not a new idea. KLH versus mitomycin C was evaluated in a randomized controlled clinical trial which was initiated in 1982 at the General Hospital Celle (Mainz, Germany) as a prevention of recurrence of superficial bladder cancer (Stages Ta to T1, and Grades I to III). All tumors were resected and all 44 patients enrolled in the trial were presumed to be free of tumor at initiation of the prophylactic instillations. All patients in the KLH group were first immunized with 1 mg KLH administered intracutaneously followed by monthly bladder instillations of 10 mg, while those in the control group were treated with monthly mitomycin C (20 mg). Among 21 patients in the KLH group, after a mean followup 20.7 months, disease recurred in 3 (14.2%), compared to 9/23 (39.1%) in the mitomycin C group after a mean followup of 18.3 months. The overall preventive effect was significantly better in KLH-treated patients. Subsequently, in 1984, a new single-drug study was started with KLH monotherapy administered as above. Among 81 patients in this study, there were 17 (20.9%) recurrences within a mean follow-up period of 22.8 months. According to combined results from these studies, among patients treated with KLH, 20/21 (95.2%) in the first study and 70/81 (86.4%) in the second study experienced either CR or PR, compared to 16/23 (69.5%) among those treated with mitomycin. No adverse local or systemic side effects were noted (Jurincic CD, etal, J Urol, Apr 1988;139(4):723-6).

Currently, BCI-IA is used by a number of pharmaceutical companies conducting clinical trials to determine any immunodeficiencies in patients resulting from drug treatment, or as a measure of recovery of the immune system. When used in this manner as a neoantigen, patients are immunized with BCI-IA and then tested qualitatively by skin testing, or quantitatively by ELISA. Response to BCI-IA provides information about the immune competency of the patient at the time of testing. BCI-IA is provided in a 3-ml glass vial with a volume of 1.2 ml at a concentration of 5 mg/ml in a stabilized Dulbecco's phosphate buffered saline (DPBS) formulation (pH 7.2).

In the mouse bladder tumor model MBT2, intralesional KLH significantly reduced tumor incidence, growth rate, and mortality, exhibiting antitumor activity similar to that achievable with BCG (Lamm DL, etal, J Urol, Mar 1993;149(3):648-52). In another preclinical study, mice immunized subcutaneously with BCI-IA 2 weeks prior to intravesical implantation of MB-49 murine bladder tumor cells, and then treated with intravesical instillation of BCI-IA (10 or 100 mg) at 1, 4, 7, 14, and 21 days postimplantation, demonstrated significantly decreased tumor outgrowth relative to control animals which were either not immunized prior to tumor implantation and BCI-IA treatment, or were immunized with BCI-IA and treated with the vehicle. Prior subcutaneous immunization was required to elicit the antitumor activity of BCI-IA, indicating that the mechanism of action is immune-mediated and not caused by spurious interference with tumor implantation by intravesical instillations. Animals treated with a dissociated form of KLH also exhibited decreased tumor outgrowth, although significance was not attained. In a separate toxicity study, when BCI-IA was administered subcutaneously (4 mg/kg), intraperitoneally (40 mg/kg), or intravesically (40 mg/kg), no significant gross or histopathologic abnormalities were observed, except for mild-to-moderate papillary hyperplasia in all catheterized animals (Swerdlow RD, etal, J Urol, Jun 1994;151(6):1718-22).

In January 1997, Intracel entered into a strategic partnership with Mentor (Santa Barbara, CA), whereby Mentor handles worldwide marketing and distribution of BCI-IA on an exclusive basis. Under the terms of the agreement, Intracel will receive $1 million each year for a 3-year period to fund clinical testing and the FDA approval process. Mentor has also made a $5 million equity investment in Intracel, and may make up to $3 million in milestone payments.

In Intracel-sponsored clinical trials, the maximum dosage used was a single 1.0 mg intradermal sensitizing dose followed by intravesical BCI-IA (50 mg) administered weekly for 12 weeks. Patients were treated with 2 6-week courses with a 6-week rest period between the 2 regimens. Intradermal injection of BCI-IA was found to cause mild local skin reactions in some patients, including erythema, induration, vesiculation, and less frequently, ulceration. Possible reactions to intravesical administration of BCI-IA include acute cystitis, incontinence, urinary retention, burning on urination, hematuria, increased urgency and/or frequency, dysuria, and bladder pain. Both routes of administration commonly induce mild systemic effects including asthenia, malaise, slight fever, and chills. It is unlikely but possible that BCI-IA could cause severe systemic reactions including bronchospasm, hypotension, and serum sickness. It has been recommended that BCI-IA therapy be postponed until resolution of concurrent febrile illness, urinary tract infection, or gross hematuria.

In October 2001, based on the promising results of a phase I/II dose escalation study of BCI-IA in the treatment of superficial bladder cancer and CIS of the bladder not responsive to either BCG or chemotherapy, the FDA approved initiation of a pivotal, randomized, multicenter, phase III clinical trial in patients with superficial bladder cancer who fail BCG therapy or are refractory or intolerant to this agent. The trial, which is cosponsored by Mentor, is being conducted at 15 centers in the USA and will enroll 150 patients with CIS or unresected superficial papillary bladder cancer, to evaluate the relative efficacy and safety of KLH versus doxorubicin in this setting. Donald Lamm, MD, of West Virginia University School of Medicine (Morgantown, WV) is the PI. Originally, this phase III clinical trial (protocol ID: INTRACEL-BCI-9804-04) was to have started in June 1998.

In a completed multicenter phase I/II dose-escalation study of KLH immunotherapy for CIS or residual stage Ta,

T1 TCC, or both, unresponsive to BCG or conventional chemotherapy, 64 patients were treated with weekly intravesical instillation of escalating doses of BCI-IA for six weeks. Complete response was seen in 50% of patients with CIS, 20% with residual T(a), T(1) TCC, and 33% with both CIS and residual Ta, T1 TCC. Responses occurred at all doses tested (0.4 mg, 2 mg, 10 mg and 50 mg). There were no significant differences in response according to dose, although optimal overall CR was seen with a dose of only 2 mg. Toxicity of KLH was minimal with this dosing schedule (Lamm DL, etal, Eur Urol 2000;37 Suppl 3:41-4).

Interleukin-12 (IL-12)

Principally a phagocyte-derived multifunctional cytokine, IL-12 targets natural killer (NK) cells and T lymphocytes, stimulating their immunoregulatory activities and the secretion of interferon (IFN)-γ. Laboratory and animal studies have indicated that exogenous IL-12 may provide possible enhancement of immunologic tumor recognition, and cytotoxic activity of lymphocytes against tumors through the enhanced expression of MHC class I antigen (Suzuki S, etal, Tohoku J Exp Med, Jul 1998;185(3):223-6).

IL-12 was discovered by Wyeth's Genetics Institute (Cambridge, MA) and Hoffmann-LaRoche; the two companies have cross-licensed their IL-12 patents. Wyeth-Ayerst had formed a joint venture with Genetics Institute (GI) in 1994, to develop and commercialize recombinant human IL-12 (rhIL-12), and subsequently acquired GI. IL-12 has several biologic properties that seem useful for bladder cancer immunotherapy (Clinton SK, etal, Urol Clin North Am, Feb 2000;27(1):147-55). For instance, in the MBT-2 murine bladder tumor model, systemic postoperative administration of IL-12 significantly enhanced antitumor immune response (Tzai T-S, etal, Proc Natl Sci Counc ROC (B) 2000;24(2):81-87).

Intravesical recombinant human IL-2 (rhIL-12) was evaluated in a phase I clinical trial in patients with CIS, Ta or T1 superficial TCC who failed one prior intravesical therapy or experienced ≥2 recurrences of low-grade lesions. Cohorts of 3 patients were treated at one of 5 dose levels (5 mg, 20 mg, 50 mg, 100 mg, and 200 mg), each dose prepared in 50-ml sterile saline. Patients were administered intravesical rhIL-12 weekly, for 6 weeks, with each dose allowed to dwell in the bladder for 2 hours. Patients returned for follow-up 4 weeks after the last intravesical dose. Among 13 patients treated at doses of 5 mg to 200 mg, there were no moderate, severe, or life-threatening systemic toxicities; mild fatigue was observed in one patient at the 100 mg dose level. Superficial TCC recurred within the 4 week follow-up period in 1 patient treated at the 100 mg dose level. Urine and serum samples have shown negligible induction of IFN-γ in any patient (Weiss G, etal, ASCO99, Abs 1284:334a). Although a phase I trial of rhIL-12 in combination with BCG in patients with superficial TCC of the bladder was being planned as of mid-1999, because of other competing priorities within Genetics Institute, further development of rhIL-12 for this indication was suspended.

Mycobacterium Cell-Wall Complex (MCC)

Mycobacterium cell-wall complex (MCC), under development by Bioniche (Belleville, Ontario, Canada) is a cell-wall composition prepared from *Mycobacterium phlei*, a nonpathogenic saprophytic bacterium commonly found in soil and plants, which is relatively dissimilar to pathogenic forms of mycobacteria. Preclinical research has demonstrated that MCC possesses a dual mode of action. In addition to its ability to stimulate the synthesis of anticancer cytokines such as IL-12 by cells of the immune system, MCC directly induces apoptosis in a wide range of human cancer cells without the intervention of the immune system. The ability of MCC to induce apoptosis is not affected by the presence of multidrug resistance or p53/p21 mutations.

MCC is a second-generation product, which replaced Bioniche's Regressin when it was determined that *M. phlei* DNA was responsible for the activity observed with Regressin. In a phase II/III study, discontinued in June 1998, when 68 patients were treated with Regressin for up to 78 weeks, the positive response was 52% among patients not previously exposed to any anticancer therapy.

In June 2001, Bioniche announced positive final results from an open-label phase I/II clinical trial of MCC for the intravesical treatment of CIS of the bladder refractory to BCG and chemotherapy. Initiated in August 1999, with Dr. Alvaro Morales, of Queen's University (Kingston, Ontario, Canada) as the PI, enrollment of 23 patients was completed in February 2001. MCC (4 mg) was administered weekly for 6 weeks, followed by once-a-week instillation for 3 weeks at 3 months and 6 months, for a total of 12 treatments over a 6-month period.

According to this trial's final results, 6 patients withdrew from the study before completing the full 12 treatment regimen. Among 17 patients who completed the 6-month treatment regimen, there were 59% CR, defined as one negative biopsy (absence of CIS) and one negative urine cytology. The overall positive response (CR+PR) to MCC therapy was 65% (PR is defined as one negative biopsy and one positive urine cytology). Urinalysis also demonstrated that MCC stimulated production of the apoptosis markers NuMA and sFasL as well as production of the immunostimulatory anticancer cytokines IL-12 and IL-18. MCC therapy was well tolerated with no apparent safety concerns. Bioniche expects to initiate a pivotal phase III clinical trial in the first quarter of 2002.

In March 2001, Bioniche entered into a strategic alliance with UroCor (Oklahoma City, OK) that, among other things, also provides UroCor with an option to market Bioniche's MCC for bladder and prostate cancer in the USA, subject to the negotiation of satisfactory terms.

REGULATORY AGENTS

Exisulind

Exisulind, under development by Cell Pathways (Horsham, PA) as Aptosyn, is a sulfone metabolite of the nonsteroidal anti-inflammatory drug (NSAID) sulindac, and a member of the class of pro-apoptotic drugs termed selective apoptotic antineoplastic drugs (SAAND).

Unlike radiation and many standard cytotoxic agents that exert apoptotic activity as a secondary effect in response to extreme cellular insult, SAAND compounds act by correcting a defect within a fundamental apoptosis pathway (see FO, pp 1221-1222).

In May 2000, Cell Pathways reported that in preclinical studies carried out at the University of Alabama at Birmingham, Aptosyn inhibited carcinogen-induced bladder cancer in a rodent model of urinary bladder tumorigenesis. Aptosyn prevented, in a dose-dependent fashion, formation of bladder tumors in drug-fed rats by up to 61% compared to control animals; no adverse effects on weight gain or other signs of toxicity were observed. Based on human metabolic studies, approximately 56% to 78% of an oral dose of Aptosyn is excreted unchanged in the urine (Ravis WR, etal, J Clin Pharmacol, Jun 1993;33(6):527-34), and company scientists have suggested that because high concentrations of the drug may be achieved locally in the urinary tract, Aptosyn may have utility as a chemopreventative or therapeutic for bladder cancer.

In studying the effect of exisulind on bladder tumorigenesis, induced in rats by the carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine, at doses of 800 mg/kg, 1000 mg/kg, and 1200 mg/kg, the drug was found to inhibit tumor multiplicity by 36%, 47%, and 64% and tumor incidence by 31%, 38%, and 61%, respectively. Exisulind inhibited growth of the human bladder tumor cell line HT1376, suggesting that the antineoplastic activity of the drug *in vivo* involved a direct effect on neoplastic urothelium. Exisulind also induced apoptosis as determined by DNA fragmentation, caspase activation, and morphology. Analysis of PDE isozymes in HT1376 cells showed that PDE5 and PDE4 isozymes were inhibited by exisulind. Inhibition of PDE5 appears to be pharmacologically relevant, because treatment of HT1376 cells increased cGMP and activated protein kinase G at doses that induce apoptosis, whereas cAMP levels were not changed. Immunocytochemistry showed that PDE5 was localized in discrete perinuclear foci in HT1376 cells. Immunohistochemistry showed that PDE5 was overexpressed in human squamous tumors and TCC compared with normal urothelium (Piazza GA, etal, Cancer Res, 15 May 2001;61(10):3961-8).

In February 2000, Cell Pathways announced a cooperative agreement with Eli Lilly to support clinical trials investigating the therapeutic potential of Aptosyn in combination with gemcitabine (Gemzar; Lilly) for the treatment of pancreatic, non-small cell lung, and bladder cancer. A phase I/II clinical trial (protocol ID: EX2005) with this combination is currently ongoing in the treatment of lung cancer.

Proteasome Inhibitor LDP-341

LDP-341 (formerly PS-341), under development by Millennium Pharmaceuticals (Cambridge, MA), is a potent and reversible inhibitor of the proteasome, which is the final degradative enzyme involved in an important catabolic pathway for many intracellular regulatory proteins including IκB/NF (nuclear factor)-κB, p53, and the cyclin-dependent kinase inhibitors p21 and p27. The antineoplastic effect of LDP-341 may involve several distinct mechanisms including inhibition of cell growth signaling pathways, induction of apoptosis, and inhibition of expression of cellular adhesion molecules. LDP-341 induces apoptosis in cells that overexpress bcl-2. LDP-341 exhibits a novel pattern of cytotoxicity in NCI *in vitro* and *in vivo* assays and has cytotoxic activity in a variety of xenograft tumor models.

A phase I clinical trial in 28 heavily pretreated patients with advanced solid tumors (melanoma=4, renal cell=4, nsclc=3, prostate cancer=3, colon cancer=3, adenocarcinoma of unknown primary=2, endometrial cancer=2, head and neck cancer=2, and 1 each gastric, ovary, breast, bladder and cervical cancer) was performed at Memorial Sloan-Kettering Cancer Center (NY, NY), to determine MTD, as well as to correlate determination of 20S proteasome (the 28-subunit proteolytic complex that is involved in removing abnormal proteins and other diverse biological functions) activity in whole blood with outcome. According to the protocol, 53 courses of therapy were administered at a twice weekly schedule of intravenous bolus infusions at dose levels ranging from 0.13 mg/m^2 to 1.08 mg/m^2 per dose, for 2 consecutive weeks with a one week rest period. There were no drug-related toxicities. At 1-hour post-drug infusion (the anticipated point of maximal inhibition of 20S proteasome activity), the level of 20S proteasome inhibition, as measured in whole blood, correlated with the dose delivered ranging from no significant inhibition at 0.13 to 0.25 mg/m^2, to 30% at 0.40 mg/m^2, 40% at 0.60 mg/m^2, 50% at 0.75 mg/m^2, and 60% at 0.90 mg/m^2, with the percent of 20S proteasome inhibition being remarkably consistent from patient to patient (Aghajanian C, etal, ASCO00, Abs. 736:189a).

R115777

R115777, an imidazole under development by Janssen Pharmaceutica (Titusville, NJ), is a methylquinolone derivative that is a potent, selective non-peptidomimetic inhibitor of the farnesyl transferase enzyme required in the post-translational activation of ras.

A phase II clinical trial (protocol IDs: MCC-12162, NCI-G00-1861, JRF-R115777-INT-10, MCC-IRB-5623) of R115777 in patients with advanced urothelial tract cancer, including Stage T2-4, NO-3, M1 urothelial tract (bladder, renal pelvis, ureter, urethra) TCC not curable by surgery or radiation therapy, Stage T2-4, N+, M0 unresectable mea-

surable disease, or poorly differentiated TCC, or predominant TCC with foci of squamous differentiation, or rare foci of adenocarcinoma, was initiated in March 2000, at H. Lee Moffitt Cancer Center and Research Institute (Tampa, FL) and UCSF Cancer Center and Cancer Research Institute in San Francisco. The primary study objective is to evaluate the efficacy of R115777 in terms of objective responses. Secondary objectives are to assess the safety of continuous oral *bid* treatment with R115777, and estimate the time to disease progression. The Chair is John Seigne, MD, at H. Lee Moffitt Cancer Center and Research Institute.

Thalidomide

Thalidomide is an angiogenesis inhibitor that may block certain growth factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). The efficacy of thalidomide was investigated in 5 patients with advanced solid tumors (melanoma=1, renal cell carcinoma=2, and bladder cancer=2) treated with oral thalidomide (400 mg), daily, until disease progression. Assessment for response and toxicity were made at 0, 1, and 3 months. Disease stabilized in 2 patients who continued on treatment. Evaluation of their symptoms showed an improvement in appetite and pain. Three patients progressed and two of them died of their disease. To date, thalidomide has been well tolerated with no dose reductions required. Two patients developed Grade 2 lethargy with Grade 1/2 constipation related to the drug. No neurotoxicity was detected. There were no documented Grade 3/4 toxicities. Preliminary results suggest that thalidomide is a well tolerated antiangiogenic agent that may be biologically active in solid tumors (Fata F, etal, ASCO00, Abs. 1883A:480a).

ZD1839

ZD1839 (Iressa; AstraZeneca) is an orally active quinazoline-derivative that selectively inhibits epidermal growth factor receptor tyrosine kinase (EGFr-TK)-mediated intracellular signaling pathways. Elevated levels of the EGFr are associated with various cancers and correlate with migration, resistance to radiotherapy and chemotherapy, and selective growth advantages for metastatic cells. Bladder cancer cells frequently exhibit an increased number of functional EGFr in comparison to normal urothelium. ZD1839 has shown reversible antitumor activity in a broad range of established carcinoma cell lines and tumor xenografts (Ciardiello F, etal, Clin Cancer Res 2000;6:2053-63).

When the antiproliferative activity of ZD1839 was evaluated *in vitro* in bladder cancer, all 4 cell lines (EJ28, 5637, J82, HT-1376) derived from human TCC with different EGFr expression levels, treated with ZD1839, showed a dose-dependent and specific inhibition of EGF-stimulated tumor cell proliferation. The inhibitory effect of ZD1839 was reversible at relatively low concentrations. As expected, cells stimulated with EGF had a lower proportion of cell population in G1 and G2/M phase in comparison to untreated cells. Additional treatment with ZD1839 abolished the EGF-induced alterations of cell cycle distribution. Simultaneously, ZD1839-treated HT-1376 and EJ28 cells showed an increased rate of apoptosis in comparison to the untreated control. According to these findings, inhibition of EGFr-TK may be useful in the treatment of TCC where EGFr drive is important. The antiproliferative activity of ZD1839 is currently being tested in combination with relevant cytotoxic agents (gemcitabine, cisplatin, paclitaxel) in the treatment of advanced bladder cancer (Meye A, etal, AACR01, Abs. 4320:805).

A phase II clinical trial (protocol IDs: 199/15740; SWOG-S0031), sponsored by the NCI, and being conducted by SWOG under coordinators Daniel P. Petrylak, MD, and Peter J. Van Veldhuizen, MD, was initiated in February 2001, to evaluate Iressa in the treatment of advanced cancer of the urinary tract (recurrent urethral cancer, bladder and/or TCC of the renal pelvis and ureter, TCC of the bladder, Stage IV bladder cancer, urethral cancer associated with invasive bladder cancer, and/or metastatic TCC of the renal pelvis and ureter). Trial objectives are to determine the 6-month PFS rate, overall survival and response, qualitative and quantitative toxicity , and evaluate changes in growth factor protein kinase expression before and after treatment, and at the time of disease progression in this setting. According to the protocol patients are treated with oral ZD 1839 daily, on a continual basis, in the absence of disease progression or unacceptable toxicity. Patients are followed every 6 months until 3 years after registration. A total of 30-55 patients will be accrued for this study.

GENE TRANSFER/THERAPY

CV876

CV876, under development by Calydon (Sunnyvale, CA), recently acquired by Cell Genesys (Foster City, CA), is a cytolytic adenovirus engineered to contain genes for replication "triggers" or transcriptional response elements (TRE) generally only found in cancer cells. When the virus replicates in a cell, the cell lyses and is, therefore, destroyed. This process can repeat as long as the replicated virus continues to encounter target cancer cells. CV876 has been engineered to replicate in and kill bladder cancer cells using the proprietary Attentuated Replication Competent Adenovirus (ARCA) technology platform. ARCA technology permits the creation of cytolytic viruses that replicate in and kill the targeted cancer cells 10,000 times more frequently than normal cells.

INGN-201

INGN-201, under development by Introgen Therapeutics (Austin, TX), is an E1/E3-deleted, replication-defective adenoviral vector containing wild-type p53 cDNA under the control of a CMV promoter. A phase I clinical trial was activated in December 1998, to evaluate intravesical adenoviral vector-mediated transfer of the p53 wild type gene as a treatment of locally advanced TCC of the bladder. In reporting on the preliminary results from

9 patients ineligible for cystectomy, treated intravesically with 10^{10} to 10^{12} viral particles (vp) of Ad5CMV-p53 for 20 minutes on days 1 and 4, and again after day 28 in patients without disease progression, researchers at the University of Texas M. D. Anderson Cancer Center observed no DLT after 16 courses of therapy (9 first, 4 second, 2 third and 1 fourth course), representing 32 total instillations. Shallow mucosal ulcerations were seen in 1 patient, on day 4 at 10^{12} vp, which were asymptomatic and mostly resolved by day 28. Disease progressed in 7 patients with median time-to-progression of 8 weeks, and 2 patients were clinically disease-free at 4 and 23 months follow-up, although both required surgery for recurrent and residual disease. Vector-specific p53 mRNA was detected by reverse transcriptase (RT)-PCR 72 hours following the first instillation of 10^{12} vp, but not at lower dose levels, before therapy, at day 28, or when RT was omitted. Phase I testing is continuing using 10^{12} vp with escalation to more frequent dosing schedules (Pagliaro LC, etal, ASCO01, Abs. 799:200a).

In April 2001, Aventis and Introgen signed a letter of intent to restructure their strategic alliance established in an October 1994 agreement, originally signed with RPR Gencell, now operating as Gencell (Santa Clara, CA), an independent company. Under terms of the current agreement, Introgen will assume responsibility for the worldwide development of all p53 programs under the existing collaboration between the two companies, and will obtain exclusive worldwide commercial rights to p53-based gene therapy products, including INGN 201. Aventis funded all research and development worldwide of these products, and since 1994, Introgen has earned a total of $70 million in collaborative research and development revenues from Aventis.

SCH-58500

SCH-58500 (rAd/p53, formerly ACN53) is a recombinant adenovirus encoding wild-type p53, under development by Canji, a Schering-Plough business unit. In May 1998, a dose-escalation phase I clinical trial (protocol IDs: MDA-DM-96172, NCI-T96-0073) was initiated to study the effectiveness of rAd/p53 in treating patients with locally advanced or metastatic bladder cancer. Patients are being treated with rAd/p53 intravesically, and may continue therapy every 28 days for a maximum of 6 courses in the absence of disease progression or unacceptable toxicity. Cohorts of 3 patients in Group 1 are being treated with escalating doses of Ad-p53 to define MTD. Group 2 patients are treated with Ad/p53 at MTD on days 1-4, and Group 3 patients at MTD on days 1-4 and 8-11. Patients are followed on day 28, then every 3 months for 1 year, or until disease progression. A maximum of 24 patients will be accrued for this study being conducted at M. D. Anderson Cancer Center; Lance C. Pagliaro is the Study Chair.

Canji is also developing Syn3 (see FO, p 1341), a polyamide compound that significantly enhances adenoviral-mediated transgene expression in the urothelium, with little or no toxicity to normal tissue (Yamashita M, etal, Ninth International Conference on Gene Therapy of Cancer, San Diego, CA, December 7-9, 2000, Abs. PD-104:36).

OTHER APPROACHES

Intravesical Radioimmunotherapy

Investigators at City Hospital (Nottingham, UK) targeted superficial bladder cancer by the intravesical administration of copper-67-labeled anti-MUC1 mucin murine MAb C595 (^{67}Cu-C595). Approximately 20 MBq of MAb ^{67}Cu-C595 was administered intravesically to 16 patients with superficial bladder cancer. After 1 hour, the bladder was drained and irrigated. Tissue uptake was assessed by imaging and by assay of tumor and normal tissues obtained by endoscopic resection. Tumor was correctly identified in the images of 12 of 15 patients who were subsequently diagnosed with a tumor. Assay of biopsy samples at 2 hours showed a mean tumor uptake of 59.4% of the injected dose per kilogram, with a tumor-to-normal tissue ratio of 14.6:1. After 24 hours, this decreased to 4.3% of the injected dose per kilogram, with a tumor-to-normal tissue ratio of 1.8:1. This study indicates a promising method for the treatment of superficial bladder cancer. However, although the mean initial tumor uptake was high, effective therapy of bladder tumors will require an increased retention of the cytotoxic radionuclide in tumor tissue (Hughes OD, etal, J Clin Oncol, Jan 2000;18(2):363-70).

Photodynamic Therapy (PDT)

Photodynamic therapy has been evaluated in the treatment of superficial bladder cancer for several decades with mixed results (see FO, p 1338).

Hexvix is an ester derivative of 5-aminolevulinic acid (5-ALA) used in PDT as precursor for the formation of the photosensitizer protoporphyrin IX (PpIX). Hexvix is being developed by PhotoCure (Oslo, Norway), for both the diagnosis and treatment of bladder cancer as well as other internal cancers and precancerous conditions that can be reached using an optical fiber linked to a light source, including such conditions as cervical cancer, vulvar cancer and other gynecologic disorders. Following instillation of Hexvix in the urinary bladder, pathologic tissue can be discerned by photodetection cytoscopy using illumination with blue light (375 nm to 400 nm) to excite protoporphyrin IX (PpIX) fluorescence, and/or treated by illumination with red light (635 nm) to activate the photosensitizer, resulting in the production of singlet oxygen and subsequent destruction of cancerous cells. Alternatively, a surgeon may use Hexvix in the diagnostic mode to guide a conventional surgical procedure, such as TUR or cystectomy.

Hexvix is a long-chained 5-ALA ester that has been found to decrease the precursor concentration at which maximal intracellular PpIX accumulation is observed by up to two orders of magnitude compared to unmodified 5-

ALA (Uehlinger P, etal, J Photochem Photobiol B, Jan 2000;54(1):72-80). PpIX is used both as a photosensitizer in PDT and as a fluorescence detection marker in photodiagnosis. In nucleated cells, nontoxic 5-ALA is converted into endogenous PpIX via the heme biosynthetic pathway (Steinbach P, etal, Photochem Photobiol, Nov 1995;62(5):887-95). Local internal administration of 5-ALA, or its ester derivatives, results in a temporary increase of PpIX in many neoplastic tissues, and the selective formation of PpIX can be used for photodiagnosis as well as PDT of cancer and dysplastic conditions of the urinary bladder and other organs that can be reached with a light device (Kennedy JC, etal, J Clin Laser Med Surg, Oct 1996;14(5):289-304).

However, a major drawback of 5-ALA has been its low bioavailability, necessitating the administration of high doses to reach clinically relevant levels of PpIX. In addition, the poor tissue penetration of 5-ALA allows only superficially located lesions to be treated (Kloek J, etal, Photochem Photobiol, Jan 1998;67(1):150-4). Lipophilicity is a key parameter in defining bioavailability of topically applied drugs, and prodrug esters of 5-ALA have been synthesized that are more lipophilic than the free acid, promising better tissue penetration and enhanced PpIX production (van den Akker JT, etal, Photochem Photobiol, Sep 2000;72(3):399-406).

In March 2000, a 4-year collaborative R&D agreement entered in 1996 with the Norwegian Radium Hospital Research Foundation, which is affiliated with the Norwegian Radium Hospital (NRH) both in Oslo, Norway, was extended to December 2002. The patents covering Hexvix and other esters of 5-ALA, including Metvix and Benzvix, were filed by NRH, and PhotoCure's proprietary light system, Curelight, was developed in collaboration with NRH. In addition to gaining exclusive access to 5-ALA ester technology, PhotoCure has an option to acquire all new PDT technologies developed by NRH. In exchange, PhotoCure supports the Norwegian Radium Hospital Research Foundation with R&D funding. In March 2000, PhotoCure also signed an agreement with the Swiss Federal Institute of Technology and the Municipal University Hospital (Lausanne, Switzerland), to collaborate in the development of Hexvix. Under terms of the agreement, PhotoCure is funding research, and has the right of first refusal to intellectual property resulting from this research relating to the use of Hexvix for the diagnosis and treatment of bladder cancer.

In June 2001, PhotoCure was issued European patent #0820432 covering a number of ALA derivatives, including the 5-ALA ester used in the company's first approved product, Metvix, for the treatment of certain types of skin cancer, and the ester derivatives used in Hexvix and Benzvix, being developed for bladder and gastrointestinal cancers, respectively. A similar patent, #6,034,267 relating to 5-ALA esters, was granted by the USPTO in March 2000.

ONCOLOGY TRENDS

GLOBAL MARKETS OF ANTICANCER DRUGS AND ADJUNCTS

The oncology drug marketplace took off in the late 1990s, aided by the introduction of novel agents acting on a variety of mechanisms, and by the steady encroachment of anticancer drug therapy in the treatment of nearly all stages of cancer. Of course, another major contributor to growth has been the ever increasing costs of drug therapy, attributed to price increases for existing products, and very high initial costs for novel agents.

This article provides estimated global markets in 1997 and 2000, for anticancer drugs and agents used in the treatment of complications attributed to cancer or its treatment (Exhibits 2, 3 and 4). Future articles will cover specific subcategories of anticancer and related agents, providing a comprehensive analysis of current use and future outlook.

CYTOTOXICS

The markets for cytotoxic chemotherapeutics grew rapidly in the late 1990s, boosted by the introduction and rapid acceptance of several major drugs. Global markets grew by a cumulative average growth rate (CAGR) of 20.6% between 1997 and 2000 when they reached $6.8 billion. Leading drug categories by rank are represented by taxanes, platinum-based agents, antifolates/5-FU-related agents, topoisomerase I inhibitor camptothecins, and others.

Although demand for all cytotoxic drugs expanded in the 1990s, rapid revenue growth in this market is attributed to a large extend by the replacement of older, off-patent agents with newer ones acting with similar mechanisms but exhibiting better toxicity and/or effectiveness profiles. Such drugs are priced at significantly higher levels than their older counterparts. For instance, the average wholesale price of carboplatin (Paraplatin; Bristol-Myers Squibb) is about 57.6% higher than that of generic cisplatin.

HORMONE MODULATORS

Hormone modulators, being older drugs, have been growing at a slower pace to reach an estimated $3.8 billion in 2000, posting a 6.3% CAGR from 1997. Addressing breast and prostate cancer, hormone modulators target the largest potential oncology market sector. Therefore, considerable effort is invested in developing more effective agents with a more attractive toxicity profile, and in improving chronic delivery of commercially available drugs.

ANTICANCER BIOLOGICALS

Anticancer biologicals refer to a group of agents of a biological rather than a chemical origin. The largest group is represented by monoclonal antibody (MAb)-based agents, a market created in 1995, and by systemically administered cytokines. The anticancer biologicals market

has nearly tripled in the 1997-2000 period, growing from $597.6 million to nearly $1.8 billion.

Exhibit 2
Worldwide Markets of Selected Anticancer Agents and Adjuncts in 1997 and 2000

Drug Category	WW (USA) 1997 Sales ($ mil.)	WW (USA) 2000 Sales ($ mil.)	CAGR 97-00 (%)
Cytotoxics	3,876.1	6,800.7	20.6
Hormone modulators	3,226.4	3,872.2	6.3
Regulatory agents	NA	62.0	NA
Biologicals	3,160.3	6,295.4	25.8
Anticancer biologicals	597.6	1,796.1	44.3
Adjunct biologicals*	2,562.7	4,499.3	20.6
Adjuncts	1,473.4	2,353.9	10.5
Antiemetics	985.8	1,242.0	8.0
Hypercalcemia and bone metastasis-related	238.5	631.3	38.3
Others	249.1	480.6	24.5
Anticancer agents, total	7,700.1	12,531.0	17.6
Grand total	11,736.2	19,384.2	18.2

**Excludes domestic sales of Amgen's Epogen; includes all of Johnson and Johnson's Procrit sales*

Note: These estimates favor branded drugs in major markets; another $500 million may be adding to this total to acount for generics in smaller markets

REGULATORY AGENTS

Regulatory agents represent a new category of anticancer drugs considered to be the future treatment approaches for all stages of cancer, but probably primarily early disease. The regulation rather than cytotoxicity approach to cancer management became a reality when thalidomide was commercialized in 1998 for a different indication but was used off-label to treat various cancers. The potential role of regulatory agents in the treatment of cancer was further demonstrated with the approval of imatinib mesylate or STI-571 (Gleevec; Novartis), a small-molecule inhibitor of an oncogene defect in chronic myelogenous leukemia (CML).

ADJUNCTS

In addition to anticancer agents, the management of cancer has created a large market for adjunct therapies. This market grew at a CAGR of 19.3%, from about $4.0 billion in 1997 to over $6.8 billion in 2000. It must be kept in mind, however, that these totals include revenues derived from applications of these drugs for indications other than oncology.

Biologicals

Agents included in this area represent some of the most widely used biotechnology-derived products. This market, excluding Amgen's USA Epogen sales that are confined to the renal dialysis field, grew from $2.5 billion in 1997 to nearly $4.5 billion in 2000, posting a CAGR of 20.6%. However, even without Epogen, a significant percent of these revenues are derived from applications in indications other than oncology.

Antiemetics

Antiemetics, representing a more mature market, nevertheless grew at a respectable CAGR of 8% in the 1997-2000 period, from $985 million to over $1.2 billion.

Hypercalcemia and Bone Metastasis-related Treatments

More spectacular has been the growth of hypercalcemia and bone metastasis-related agents, primarily pamidronate disodium (Aredia; Novartis) whose sales more than doubled between 1997 and 2000. Now off-patent, Aredia is being replaced by Zometa (zoledronic acid for injection), a third generation bisphosphonate approved by the FDA in August 2001, for the treatment of hypercalcemia of malignancy (HCM).

OTHERS

In addition to pharmaceuticals, numerous other products are associated with the management of cancer, among them *in vivo* diagnostic imaging and *in vitro* diagnostic and prognostic tests, and various devices. Market estimates presented in this article do not include revenues of any products other that the ones listed in Exhibits 3 and 4.

MEDICARE REIMBURSEMENT OF ONCOLOGY DRUGS

This article was prepared by Elan Rubinstein (ebra@pacbell.net) from information presented at the Reimbursement and Government Policy Forum, by Joe Bailes, MD, ASCO EVP & Chief Policy Liaison, and Terry Coleman, esq, Bennet, Turner & Coleman, during the 37th Annual Meeting of the American Society of Clinical Oncology (ASCO), held May 12-15 in San Francisco, and from the General Accounting Office (GAO) report, entitled "Medicare Payments for Covered Outpatient Drugs Exceed Providers' Cost", released in September 2001.

In view of mounting costs for outpatient drug therapy under its part B program, Medicare has been reviewing its reimbursement policies to reduce these costs. Drugs covered by part B cannot be self-administered and are related to a

physician's services, such as cancer chemotherapy. Medicare spending for these drugs totaled almost $4 billion in 1999; 9 of the top 10 of these drugs, ranked by reimbursement volume, were oncology-related. Both the administration and Congress agree, on a bipartisan basis, that pharmaceutical payments to providers through the Medicare program need to be reduced, but consensus is lacking as to how to accomplish this. In the late 1990s, the USA government attempted to rein in rising costs of drug therapy by proposing various schemes. In 1997, the Clinton administration proposed payment of pharmaceuticals based on acquisition cost, defined as the lowest price paid by physicians in the previous 6 months. However, Congress rejected this approach, and instead established payment at average wholesale price (AWP) less 5%. After one additional effort in 1998 to revive acquisition price-based reimbursement, the Clinton administration proposed reimbursement based at 83% of AWP which was also rejected. In September 2000, the Centers for Medicare and Medicaid Services (CMS), formerly the Health Care Financing Administration (HCFA), took steps to reduce Medicare's payment for part B-covered drugs by authorizing Medicare carriers, the contractors that pay part B claims, to use prices obtained in the Justice Department investigations of providers' drug acquisition costs. Subsequently this authority was retracted in November 2000 because of concerns voiced by providers. In December 2000, as part of the Medicare, Medicaid, and SCHIP Benefits Improvement and Protection Act of 2000, Congress asked the GAO to study Medicare's payments for part B-covered drugs and make reimbursement recommendations.

In summary, the GAO found that Medicare's method for establishing drug payments is flawed. Medicare payments are based on AWP which is usually neither an average nor what wholesalers charge, but a price derived by manufacturers using their own criteria. AWP are supplied by manufacturers to organizations that publish them in drug price compendia. Medicare carriers that pay claims for part B drugs, base providers' payments on published AWP. In 2001, widely available prices at which providers could purchase drugs were substantially below AWP. Consequently, Medicare payments often far exceeded these prices. Physicians and pharmacy suppliers contend that the excess payments for covered drugs are necessary to offset what they claim to be inappropriately low, or no Medicare payments for services related to the administration or delivery of these drugs, which Medicare pays separately under the physician fee schedule. This discrepancy between actual costs versus Medicare reimbursement is particularly sanguine in the oncology sector because oncologists represent the majority of physicians billing for these drugs. ASCO's position is that drug administration fees are too low, in that they lack a physician work component and do not recognize cognitive service (such as psychosocial and nutritional support) provided by nursing staff. An example of the problem of underpayment of drug administration is illustrated in the delivery of Irinotecan (Camptosar; Pharmacia) via IV push. Dr. Bailes stated that the Medicare payment for this service is $39, compared to an estimated cost of $184, according to calculations by the Clinical Practice Expert Panel (CPEP). To underscore this problem and its possible resolution, ASCO published a position paper in May 2001, titled "Reform of the Medicare Payment Methods for Cancer Chemotherapy", available at www.ASCO.org.

Oncology is one of the most affected sectors in Medicare's attempt to reduce part B drug costs. Drugs used to treat cancer accounted for most of Medicare's expenditures for all drugs billed by physicians. In 1999, claims from three specialties, i.e., hematology oncology, medical oncology, and urology, accounted for 80% of total physician billings for part B drugs. Generally, discounts on physician-billed drugs, based on wholesaler and GPO catalog prices, were significantly lower than Medicare's payment. Widely available discounts indicate that Medicare's payments for these drugs were at least $532 million higher than providers' acquisition costs in 2000. Discounts offered to providers may also be augmented with additional benefits provided to certain purchasers, as chargebacks, rebates, etc., that may further reduce the final sale price. Despite claims to the contrary, discounts were also obtainable by small providers considered unable to negotiate such favorable prices.

Medicare also pays providers much more for part B drugs than necessary compared to market-based fees paid by VA and other federal agencies. According to GAO, it should be a principle of Medicare payment policy to pay for each service appropriately and not to rely on overpayments for some services to offset inadequate payments for others. In addition, Medicare could also determine market-based fees, where appropriate, through a competitive bidding process, and pay for administration and delivery of these drugs separately. The GAO is recommending that CMS revert to the use of the basic methodology to determine practice expense payments for all services to eliminate any reductions and add about $31 million to oncology payments. The GAO is also recommending that CMS address a data adjustment it made that reduced reported oncology supply expenses to keep from paying twice for drugs that are reimbursed separately by Medicare. Substituting a supply expense estimate based on a methodology developed by ASCO would raise practice expense payments an additional $20 million, if done in conjunction with use of the basic method to calculate payments for all services.

Another problem with Medicare reimbursement is nonuniform Medicare carrier policies regarding coverage of pharmaceuticals that are self-administered. For instance, an important reimbursement issue is Medicare coverage of orally administered chemotherapeutic agents that do not substitute for injectables otherwise covered by the Medicare program; Medicare will cover an oral version

of an injectable drug but will not cover an oral drug that does not come in an injectable form. Imatinib mesylate (Gleevec; Novartis), which was recently awarded FDA approval, is an important case in point. On May 17, 2001, the Access to Cancer Therapies Act of 2001, S.913/H.R. 1624 was introduced by Senator Olympia Snowe (R-ME) and by Representative Deborah Pryce (R–OH) which, if enacted, would update reimbursement policy to cover oral anticancer agents not currently paid for by Medicare. This legislation is actively supported by the American Society of Hematology (ASH) and by ASCO, and had 198 bipartisan cosponsors for the House version and 24 bipartisan cosponsors for the Senate version.

MEETING COVERAGE

GENE THERAPY OF CANCER — PART III

IMMUNOLOGIC STIMULATION FROM THE 9TH INTERNATIONAL CONFERENCE ON GENE THERAPY OF CANCER, SPONSORED BY THE SIDNEY KIMMEL CANCER CENTER, SAN DIEGO, CA, DECEMBER 7-9, 2000

Immunologic approaches to cancer gene therapy involves the transfer of genetic material into specific target cells to enhance antitumor immune responses. Strategies may include using genes coding for tumor-associated peptides (antigens) to engineer or transduce professional antigen presenting cells (APC), or genetically engineering APC, usually pulsed with tumor antigens, to express immunostimulatory molecules or to release cytokines. Alternatively, tumor cells may be engineered to express costimulatory or MHC molecules. Also, tumor or other endogenous cells may also be genetically modified to secrete cytokines or chemokines. The sustained release of these factors into the tumor microenvironment should facilitate recruitment and activation of APC, and enhance a cell-mediated antitumor immune response (De Giovanni C, etal, Int J Immunopharmacol, Dec 2000;22(12):1025-32). This article completes the report on laboratory investigation, preclinical studies, and clinical trials of gene manipulation in human cancer therapy presented at the Ninth International Conference on Gene Therapy of Cancer (ICGTC00; San Diego, CA; December 7-9, 2000), sponsored by the Sidney Kimmel Cancer Center (San Diego, CA). Additional information on all agents in clinical/preclinical development included in this report may by found in NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), a subscription-based resource residing at www.oncologyknowledgebase.com.

GENETICALLY ENGINEERED ANTIGEN PRESENTING CELLS

Dendritic cells (DC) are phenotypically distinct, antigen-presenting cells found in lymphoid organs, skin and mucosa, and bone marrow. Their function is to internalize, process and present antigen to naive T lymphocytes with high efficiency, playing a central role in the regulation of T- and B-lymphocyte activation *in vivo*. DC expressing tumor antigens are capable of stimulating antigen-specific cytotoxic T cells (CTL) to destroy cancer cells without harming normal tissue.

Malignant Melanoma Immunotherapy

At the University of California, Los Angeles, researchers have used a replication-defective recombinant adenovirus vector (AdVMART1/DC) encoding MART-1 (or melan-A), one of the most widely expressed melanoma antigens, to transduce immature DC. MART-1 is specifically recognized by HLA-A2-restricted CD8+ CTL; immunization of C57BL/6 mice with AdVMART1/DC results in elicitation of an antigen-specific protective immune response (Ribas A, etal, ICGTC00, Abs. PD-88:S26). Typically, this response would require the presence of an intact CD4 cell compartment, as protection is abrogated in CD4 knockout (CD4KO) and CD4-antibody-depleted mice. However, in an attempt to bypass the requirement for intact CD4, a cDNA-encoding CD40-L (CD154), a ligand for the cell surface antigen CD40, under development as Avrend by Immunex (Seattle, WA), was crosslinked with AdVMART1/DC. Whereas CD40 is expressed primarily on B cells, CD40-L is expressed mainly on activated CD4+ T cells. CD40-L has demonstrated an inhibitory effect on tumor growth by binding to CD40, inducing the expression of immune costimulatory molecules (Borges L, etal, ASH98, Abs 2056:499a, and Hylander BL, etal, ASCO99, Abs 789a:205a).

When the CD40 receptor was engaged using the CD40-L crosslinked AdVMART1/DC, tumor protection was reestablished in CD4KO mice, with 17/31 (54.8%) mice completely rejecting a B16 tumor challenge. This compares favorably with 15/32 (46.8%) wild-type mice (intact CD4) being protected against B16 challenge following immunization with noncrosslinked AdVMART1/DC. In unimmunized wild-type mice, 34/34 developed tumors after B16 challenge, and 26/28 CD40KO mice developed B16 tumors after noncrosslinked AdVMART1/DC immunization. Protective immunity in CD4KO mice administered CD40-L-crosslinked AdVMART1/DC was associated with the presence of MART-1-specific IFN-g-producing cells in the spleen, which were observed to lyse B16 cells in chromium release assays.

Prostate Cancer Immunotherapy

In prostate tumors, DC are largely eliminated, which may be a key factor in the progress of prostate cancer through loss of immune surveillance. University of Illinois (Chicago, IL) scientists have developed a biscistronic recombinant oncoretrovirus incorporating the cDNA for full-length prostate specific antigen (PSA), a self-antigen preferentially expressed in prostatic malignancies (Qin G, etal, ICGTC00, Abs. PD-90:S27). Full-length PSA cDNA

was chosen so as to encompass all potential epitopes for many HLA alleles. This construct was used to transduce immature DC. It also encoded a selectable cell surface marker, human CD25, to allow enrichment of transduced cells prior to transplantation, as well as *in vivo* cell migration and longevity tracking. Murine *in vivo* functional studies are being conducted to evaluate the production of PSA antigen and antibodies following immunization as well as protection against tumor challenge. In *in vitro* infection of murine Balb/c bone marrow cells, over 50% of cells with a DC phenotype stained for human CD25.

Scientists at the University of Illinois are also investigating the potential of immunogene therapy for prostate cancer by engineered expression of prostate specific membrane antigen (PSMA) which is a type II transmembrane carboxypeptidase, that is expressed at high levels in tumor cells of hormone-refractory and metastatic phenotypes. High-level expression of PSMA has also been found in the neovascular endothelium of a variety of other malignancies, but only limited amounts of the antigen are found in normal prostate tissues, salivary glands, brain, and small intestines. Investigators constructed an oncoretroviral vector, pG1/PSMA, in which the expression of human PSMA cDNA is driven by the viral LTR promoter (Peace D, etal, ICGTC00, Abs. P-93:S29). PSMA peptides corresponding to high affinity binding motifs for class I MHC molecule HLA-A2 have been shown to elicit antigen-specific CTL responses *in vitro*. As a result, it is exprected that pG1/PSMA may be used to transfect DC precursors, resulting in the presentation of multiple PSMA epitopes and targeted antitumor immune responses, with reduced potential for tumor escape.

Minigene Vaccine for Solid Tumors

At Epimmune (San Diego, CA), John D. Fikes, PhD, and his associates are developing immunogene vaccines based on class I- or class II-restricted epitopes of tumor associated antigens (TAAs) that are capable of eliciting CTL and helper T lymphocyte (HTL) immune responses against tumor cells (Fikes J, etal, ICGTC00, Abs. P-112:50). This strategy reflects the premise that epitope-based T-cell vaccines enjoy certain advantages over using the entire TAA, including easier targeting of multiple tumor self-antigens to avoid immune escape, and the opportunity to rationally design epitope analogs having greater potency and increased safety compared to native epitopes. Epimmune scientists first use computerized scanning of TAA sequences for HLA binding motif-bearing peptides to identify epitopes that bind to multiple HLA supertype alleles; selected native epitopes are then modified to create analogs having either improved HLA binding (fixed anchor analogs) or T-cell receptor engagement (heteroclitic analogs). Genetic sequences, or minigenes (EpiGenes) can then be constructed that encode a linear array of multiple epitopes from multiple TAAs to optimize vaccine immunogenicity. If desired, these minigenes can be combined with other costimulatory molecules or used to transfect APCs to further enhance epitope immune system recognition. Epimmune has constructed a prototype minigene vaccine for breast, colon, and lung cancer that is composed of epitopes from four well-characterized TAAs (CEA, p53, MAGE and HER2/neu) to stimulate CTL responses; this vaccine also incorporates the company's PADRE epitope, representing a family of proprietary synthetic molecules that act as a potent, universal HTL immunostimulant. This vaccine has demonstrated strong immune response activity in human cancer cell cultures as well as animal models.

Endogenous Production of Cytokines

At the University of Pittsburgh School of Medicine (Pittsburgh, PA), investigators, have used an adenoviral vector system (DC-IL-10), to genetically modify DC to release IL-10 a pleiotropic cytokine initially considered to have anti-inflammatory properties, but which has more recently been shown to sensitize and expand antigen-specific CTL (Kim S-H, etal, ICGTC00, Abs. PD-87:S26). The antitumor activity of DC-IL-10 appears to be mediated predominantly by NK cell lysis; however, DC-IL-10 may also partially inhibit tumor growth through IL-10's ability to downregulate production of proinflammatory cytokines and chemokines, or to enhance cytotoxicity of CD8+ T lymphocytes.

In a similar approach, scientists at the Chiba University School of Medicine, together with researchers at the Chiba Cancer Center Research Institute, and Sapporo Medical College in Japan, used a recombinant, replication-defective adenoviral vector to transduce murine Balb/c bone marrow-derived DC with the cDNA for IL-2 (Gunji Y, etal, ICGTC00, Abs. PD-89:S27). When injected intratumorally into mice bearing colon 26 cancers, the IL-2-expressing DC suppressed tumor growth significantly, and prolonged the survival of the treated mice. Also, multiple injections enhanced this antitumor effect and increased infiltration of macrophages to the tumor site's periphery.

Frank C. Marini, PhD, and associates at the University of Texas M. D. Anderson Cancer Center, in collaboration with Dr. Phil Zoltick of the University of Pennsylvania (Philadelphia, PA), have used an adeno-associated virus (AAV) vector with a CMV promoter to transduce patient-derived mesenchymal stem cells (MSC) *ex vivo* with the cDNA for IFNα (Marini FC, etal, ICGTC00, Abs. O-56:S17). MSC are nonhematopoietic cells that reside in the bone marrow; autologous IFNα-transfected MSC are reintroduced into the patient's bone marrow as targeted therapy for chronic myelogenous leukemia (CML). When compared to pharmacy-grade IFNα (Intron A; Schering-Plough), AAV-CMV IFNα appeared equivalent in its biological activity to inhibit growth of CML cell lines K562 and BV173. Exposure to AAV-CMV IFNa resulted in upregulation of class I-restricted MHC antigens on CML patient samples, and when human MSCs expressing CMV-driven IFNα were injected into the tail veins of nude mice, detectable levels of IFNα appeared within three days.

Localized and regulated delivery of IFNα to the bone marrow stroma as a therapeutic modality for CML is being tested *in vivo* with the injection of IFNα-expressing MSCs into nude mice bearing K562 and BV173 CML xenografts.

GENETICALLY ENGINEERED TUMOR CELLS

In order to enhance host immunosurveillance tumor cells may be genetically engineered to express certain antigens or costimulatory molecules.

University of California San Diego

Despite the expression of B-cell differentiation antigens, clonally restricted immunoglobulin, and high levels of class I- and class II-restricted MHC molecules, neoplastic B cells from patients with chronic lymphocytic leukemia (CLL) or non-Hodgkin's lymphoma (NHL) are ineffective as APC, largely because of insufficient presentation of important costimulatory molecules, such as CD80 (B7-1) or CD86 (B7-2), that activate CTL recognizing MHC and peptide antigen complexes via the T cell receptor (Cantwell M, etal, Nat Med, Sep 1997;3(9):989-9). The ligation of CD40 on neoplastic B cells induces phenotypic changes, including the expression of such costimulatory molecules (Kipps TJ, Semin Hematol, Oct 1999;36(4 Suppl 5):3-8). At the University of California, San Diego (La Jolla, CA), Thomas J. Kipps, MD, PhD, and his associates have used a replication-defective adenoviral vector to transduce neoplastic B cells with the cDNA for the CD40-ligand, CD154 (Kipps TJ, etal, ICGTC00, Abs. O-53:S16). Leukemia cells transduced with CD154 express immune accessory molecules, and also transactivate noninfected bystander B cells, rendering them proficient in antigen presentation which results in the induction of autologous CTL capable of specifically killing both transduced and nonmodified leukemia cells (Kato K, etal, J Clin Invest, 1 Mar 1998;101(5):1133-41).

This vector system, AdCD154, has been used to treat patients with CLL in a phase I dose-escalation study (Wierda WG and Kipps TJ, Semin Oncol, Oct 2000;27(5):502-11, Wierda WG, etal, Blood, 1 Nov 2000;96(9):2917-24). In this study, autologous CLL B cells were infected with AdCD154 *ex vivo* and then returned to the patient by intravenous infusion. Nine patients with progressive CLL were administered a one-time bolus infusion of autologous AdCD154-transduced leukemia cells, of which half expressed cell surface CD154.

Increased or *de novo* expression of immune costimulatory molecules on bystander, noninfected CLL cells was observed *in vivo*, with treated patients demonstrating measurable increases in the plasma levels of IL-12, IL-6, and IFNγ within 1 to 2 days postinfusion. On average, patients experienced a >240% increase in absolute blood T-cell counts within 1 to 4 weeks of treatment. An increase in the numbers of leukemia-specific T cells was also observed as well as significant reductions in absolute lymphocyte counts and lymph node mass. Treatment was well tolerated, with no signs of autoimmune thrombocytopenia or hemolytic anemia; no DLT was observed. A phase II trial involving repeat dosing with AdCD154-infected cells is planned.

Genzyme Molecular Oncology

Genzyme Molecular Oncology (Framingham, MA) has taken a plasmid-based approach to the generalized immune-mediated inhibition of tumor growth by complexing cationic lipid with plasmid DNA lacking expressed transgene pNull (Siders WM, etal, ICGTC00, Abs. O-86:S26). Treatment of mice bearing intraperitoneal M3 melanoma tumors with lipid/pNull leads to the development of a CD8+CTL response against several M3 melanoma-associated antigens, with the level of antigen-specific lysis decreasing in the order TRP-1, gp100, and MART-1 (equal to TRP-2). For these tumors, optimal antitumor effect required delivery of lipid/pNull into a tumor-bearing compartment; however, intravenous injection of the complex was found to have therapeutic activity against lung metastases arising from intravenous injection of tumor cells. The lipid/pNull complex also demonstrated therapeutic efficacy against subcutaneous M3 tumors when administered intratumorally or peritumorally. Intratumoral injection caused tumor regression in 4/5 mice, whereas peritumoral administration reduced tumor growth rate but did not completely inhibit tumor progression. In addition, the intracavity treatment of local peritoneal tumors with lipid/pNull not only caused an immune response that eradicated the intraperitoneal tumor masses, but also resulted in systemic protection against distal metastases in the lung in nearly all animals.

Supratek Pharma

Scientists at Supratek Pharma (Laval, Quebec, Canada), complexed plasmid DNA with a nonionic block copolymer-based carrier, SP1017, to improve the efficacy of DNA vaccination by increasing gene expression in target tissues at the transcription control level. SP1017-formulated DNA produces a strong immune-mediated anticancer effect compared to naked DNA, characterized by massive immature DC infiltration of the transgene expression sites (Lemieux P, etal, ICGTC00, Abs. O-85:S25). When C57B1/6 mice immunized with an SP1017/plasmid encoding for bgalactosidase were challenged with murine B16 melanoma cells transfected with bgalactosidase, strong humoral and cellular immune responses against bgalactosidase were generated, with tumor rejection occurring in a majority of the immunized mice.

Osaka City University Medical School

Intercellular adhesion molecule-1 (ICAM-1), also referred to as CD54, is a glycoprotein bound to the cell surface, that is a ligand for leukocyte function antigen-1 (LFA-1). The ICAM-1/LFA-1 system mediates the adhesion of leukocytes, monocytes and lymphocytes to endothelial cells (Kaihara A, etal, Res Commun Mol Pathol Pharmacol, Jun 1998;100(3):282-300, and Mukai S, etal, Cell Immunol,

15 Mar 1999;192(2):122-32). LFA-1 is comprised of alphaL (CD11a) and beta2 common (CD18) chains. CD11a mediates a critical function in interactions between effector T cells, tumor cells, and host accessory cells *in situ*, leading to tumor regression (Mukai S, etal, Cell Immunol 1999 Mar 15;192(2):122-32). The ability of cancer cells to escape immunosurveillance, resulting in tumor progression and metastasis has been attributed in part to a decrease in or loss of expression of ICAM-1. In homozygous ICAM-1 gene knockout mice injected with B16 melanoma cells, ICAM-1 deficiency has been associated with a 7-fold increase in the number of metastases compared to wild-type controls (Marvin MR, etal, J Surg Res 1998 Dec;80(2):143-8).

At Osaka City University Medical School (Osaka, Japan), researchers have used a lipofectin carrier to transfect scirrhous gastric cancer cell lines OCUM-2MD3 and OCUM-2MLN with cDNA coding for ICAM-1 (Tanaka H, etal, ICGTC00, Abs. PD-91:S27). Both ICAM-1-transfected cell lines demonstrated a significant increase in peripheral mononuclear lymphocyte adhesion as well as leukocyte-mediated cytotoxicity. Mice inoculated into the peritoneal cavity with 2MD3-transfected cells exhibited significantly better survival rates than animals inoculated with unmodified 2MD3 cells.

Pennsylvania State University College of Medicine

Epidemiological and molecular biological studies provide strong evidence that malignant progression is a life-threatening consequence of human papillomavirus (HPV) infection in anogenital cancers, particularly cervical carcinoma. Scientists at Pennsylvania State University College of Medicine (Hershey, PA), have investigated the efficacy of papillomavirus (PV) early-gene-based vaccines for prevention of carcinoma development of PV-induced skin papillomas on the domestic cottontail rabbit model (Han R, etal, Vaccine, 1 Jul 2000;18(26):2937-44, Han R, etal, J Virol, Oct 2000;74(20):9712-6, Han R, etal, ICGTC00, Abs. O-84:S25). Rabbit skin papillomas were initiated by infection with cottontail rabbit papillomavirus (CRPV), and allowed to grow for 3 months. Rabbits were then immunized by gene gun-mediated intracutaneous administration of DNA plasmids encoding CRPV early genes E1, E2, E6, and E7 genes. All eight control rabbits receiving vector alone developed invasive carcinoma within 8 to 13 months, while only 2/8 vaccinated rabbits developed carcinoma at 12 and 15 months, respectively. However, although papilloma growth was suppressed in the majority of vaccinated rabbits, it was not completely eradicated. Moreover, needle-mediated intramuscular injection of CRPV early genes did not prevent papilloma formation or promote systemic papilloma regression.

Vical

Vical's (San Diego, CA) Allovectin-7 is a plasmid-based DNA immunotherapeutic being evaluated as an intralesional treatment in phase III clinical trials in melanoma and in phase II clinical trials in head and neck cancer. Allovectin-7 is a DNA/lipid complex containing the gene for human histocompatibility antigen, HLA-B7, formulated with the cationic lipid N-(1-(2,3-dimyristyloxypropyl)-N,N-dimethyl-(2-hydroxyethyl) ammonium bromide/dioleoyl phosphatidylethanolamine (DMRIE/DOPE) to facilitate cellular uptake (Stopeck AT, etal, J Clin Oncol, Jan 1997;15(1):341-9, and Hersh EM and Stopeck AT, Clin Cancer Res, Dec 1997;3(12 Pt 2):2623-9).

According to results of completed Allovectin-7 clinical studies at the Arizona Cancer Center (Tucson, AZ), 31% of evaluable patients with cutaneous and/or nodal disease demonstrated a local response, and 16% have had a systemic response. However only 19% of patients with visceral disease exhibited local responses, and no patient developed a systemic response (Hersh EM, etal, ICGTC00, Abs. O-78:S23). Time to disease progression ranged from a median of 2.6 to 15.6 months in patients with local and systemic responses, respectively, compared to 1.9 months in nonresponding patients; overall survival was also better in local or systemic responding patients (median 16.6 and 33.3 months, respectively) compared to nonresponders (8.7 months). Allovectin-7 has demonstrated an excellent safety profile (Gutheil J, etal, ICGTC00, Abs. O-96:S29), with most serious toxicities (pain, hemorrhage, pneumothorax, and hypotension) related to technical aspects of the injections or biopsies; drug-related Grade 3 adverse events have included headaches (n=1), large nodes (n=1), and an increase in tumor necrosis (n=1).

A second plasmid-based DNA immunotherapeutic under development by Vical is Leuvectin, a DMRIE/DOPE/IL-2 gene complex being evaluated in phase II clinical trials in renal and prostate cancer (Hersh EM, etal, ICGTC00, *ibid*). IL-2 is an immunocytokine that has been shown to promote proliferation and activation of NK cells both *in vitro* and *in vivo* (Clark PR, etal, Cell Immunol, 15 Sep 2000;204(2):96-104). Leuvectin produces high local concentrations of IL-2 (Stopeck AT, etal, Cancer Gene Ther, Mar-Apr 1998;5(2):119-26), and like Allovectin-7, it has demonstrated an excellent safety profile and broad therapeutic index, with no DLT observed to date. Among all patients treated with Leuvectin, drug-related Grade 3 adverse events included nausea (n=1), vomiting (n=1), rigors (n=1), pancreatitis (n=1), and abdominal pain (n=1) (Gutheil J, etal, *ibid*). In Leuvectin's phase I/II studies, 2/14 patients with renal cell carcinoma (RCC) and 1/16 with melanoma achieved PR lasting from 16 to 19 months and continuing, while disease stabilized in 2/14 RCC, 3/16 melanoma, and 6/15 sarcoma patients for 3 to 18 months and continuing. IL-2 plasmid was detected by polymerase chain reaction assay in the post-treatment samples of 29 of 46 evaluated patients (Galanis E, etal, J Clin Oncol, Oct 2000;17(10):3313-23).

Valentis

Scientists at the University of Colorado Cancer Center (Denver, CO), in collaboration with Valentis (The Woodlands,

Exhibit 3
Worldwide Markets of Selected Anticancer Agents in 1997 and 2000

Developer/ Marketer ❑ Affiliate(s)	Drug Category ❑ Administration Route	Generic Name ❑ Brand Name	Approved Indication(s)	WW (USA) 1997 Sales ($ mil.)	WW (USA) 2000 Sales ($ mil.)	CAGR 97-00 (%)
Cytotoxics, Total						
Taxanes and Related Agents						
Aventis ❑ Chugai Pharmaceutical (terminated 3/01)	Taxane ❑ IV	Docetaxel ❑ Taxotere	Breast cancer, nsclc; gastric, ovarian and head & neck cancer (Japan)	248.0	688.8 (338.9)	40.6
Bristol-Myers Squibb	Taxane ❑ IV	Paclitaxel ❑ Taxol	Breast and ovarian cancer, nsclc, Kaposi's sarcoma (KS)	940.0 (614.0)	1,592.0 (1,025.0)	19.2 (18.6)
Ivax	Taxane (generic paclitaxel) ❑ IV	Paclitaxel ❑ Onxol	Breast and ovarian cancer, nsclc, KS	NA	(35.0)	NA
Pierre Fabre ❑ GlaxoSmithKline (USA)	Semi-synthetic vinca alkaloid; norvinblastine derivative ❑ IV	Navelbine ❑ Vinorelbine	Nsclc; breast cancer (UK)	(60.6)		
Platinum-based Agents						
Bristol-Myers Squibb	Platinum-based agent ❑ IV	Carboplatin ❑ Paraplatin	Ovarian cancer and in various other combinations	437.0	690.0	16.4
Multisource (Bristol-Myers Squibb and generic suppliers)	Platinum-based agent ❑ IV	Cisplatin ❑ Platinol and others	Ovarian, bladder and testicular cancer and in various other combinations	146.01	180.0	7.2
Sanofi-Synthelabo	Platinum-based agent ❑ IV	Oxaliplatin* ❑ Eloxatin	Colorectal cancer	19.0	130.1	89.9
Antifolates, thymidylate synthase inhibitors/5-FU-related Agents						
AstraZeneca/ Institute of Cancer Research, British Technology Group (BTG)	Quinazoline antifolate; potent inhibitor of thymidylate synthase (TS) ❑ infusion	Raltitrexed* ❑ Tomudex	Palliative in advanced colorectal cancer	11.7		
Hoffmann-La Roche ❑ Nippon Roche	5-FU prodrug; tumor-selective fluoropyrimidine ❑ PO	Capecitabine ❑ Xeloda	Breast cancer, colorectal cancer	NA	83.4	NA
Hoffmann-La Roche ❑ Nippon Roche	Fluoropyrimidine, 5'-deoxy-5-fluorouridine (dFUrd), a masked form of 5-FU ❑ PO	Doxifluridine Furtulon			198.2	
Multisource	Antifolate ❑ IV	5-FU	Colorectal cancer and in various combinations	125.0	130.0	1.3
Taiho Pharmaceutical ❑ Miguel Labs, Bristol-Myers Squibb	A combination of uracil and tegafur (Ftorafur), a derivative of 5-FU ❑ PO	UFT (Tegafur + uracil)* ❑ Orzel (USA)	Colorectal cancer	650.0	700.0	2.5
Topoisomerase I Inhibitors						
Aventis ❑ Yokult Honsha	Topoisomerase I IV	Irinotecan* ❑ Campto	Colorectal cancer	17.0	139.4	101.6

— continued on next page

GlaxoSmithKline	Topoisomerase I inhibitor ❑ IV	Topotecan ❑ Hycamtin	Ovarian cancer	85.0	144.0 (96.0)	19.2
Pharmacia	Topoisomerase I inhibitor ❑ IV	Irinotecan ❑ Camptosar	Colorectal cancer	153.8	441.0 (404.0)	42.1
		Anthacyclines				
Alza ❑ Schering-Plough	Liposomal anthracycline ❑ IV	Liposomal doxorubicin ❑ Doxil (USA), Caelyx (outside the USA)	Kaposi's sarcoma (KS) and ovarian cancer	(27.0)	82.4	45.0
Gilead Sciences	Liposomal anthracycline ❑ IV	Daunorubicin citrate ❑ DaunoXome	Kaposi's sarcoma (KS)	5.2	4.4	-5.4
Multisource (Pharmacia and others)	Anthracycline ❑ IV	Doxorubicin ❑ Adriamycin and others	Various indications in combination	135.0	145.0	2.4
Pharmacia	Second generation anthracycline antibiotic, a diastereoisomer of doxorubicin ❑ IV	Epirubicin, epidoxorubicin ❑ Ellence (USA); Farmorubicin/ Pharmorubicin (outside USA)	Beast and ovarian cancer, melanoma and non-Hodgkin's lymphoma (NHL)	195.5	199.0 (9.0)	0.6
		Retinoids				
Hoffmann-La Roche	All-trans retinoic acid (ATRA) ❑ PO	Tretinoin ❑ Vesanoid	Acute promyelocytic leukemia (APL)	10.0		
Ligand Pharmaceuticals ❑ Elan, Ferrer Internacional, Alfa Wassermann	Vitamin A derivative; endogenous, non-peptide retinoid ❑ topical	Alitretinoin ❑ Panretin gel 0.1%	Cutaneous lesions of Kaposi's sarcoma	NA	0.9	NA
Ligand Pharmaceuticals ❑ Elan, Ferrer Internacional, Alfa Wassermann	Member of a retinoid subclass that selectively activates retinoid X receptors (RXR); induces apoptosis ❑ PO	Bexarotene ❑ Targretin capsules and gel	Refractory cutaneous T cell lymphoma (CTCL)	NA	6.7	NA
		Other Cytotoxics				
Axcan Pharma ❑ QLT, American Home Products, Diomed, Grupo Ferrer	Semisynthetic porphyrin compound; photosensitizer used in photodynamic therapy (PDT) ❑ IV	Porfimer sodium ❑ Photofrin	Superficial bladder cancer, palliative for obstructing esophageal cancer, early-stage, microinvasive endobronchial and late-stage nsclc, early-stage cervical cancer, cervical dysplasia, superficial and early-stage gastric cancer	3.0	11.0	54.2
Berlex (Schering AG)	Synthetic purine nucleoside ❑ IV, PO	Fludarabine phosphate ❑ Fludara	Chronic lymphocytic leukemia (CLL)	78.3 (45.6)	105.0 (80.8)	10.3 (21.0)
Cell Therapeutics (CTI) ❑ Memorial Sloan-Kettering Cancer Center, Sam Waxman Cancer Research Foundation, Beijing U, NCI, Sperling Sampson West	Pharmaceutical grade arsenic compound ❑ IV	Arsenic trioxide (As2O3) ❑ Trisenox	Acute promyelocytic leukemia (APL)	NA	(0.5)	NA

— continued on next page

Eli Lilly	Synthetic pyrimidine nucleoside ❑ IV	Gemcitabine ❑ Gemzar	Pancreatic cancer, lung cancer	175.0	559.3 (316.0)	47.3 47.3
Immunex	Synthetic anthracenedione ❑ IV	Mitoxantrone ❑ Novantrone	Acute myelogenous leukemia (AML), hormone-refractory prostate cancer	51.6	59.9	5.1
Immunex	Alkylating agent; lyophilized form of thiotepa, a nitrogen mustard derivative ❑ IV, intratumoral, intracavitary, intravesical	Thiotepa ❑ Thioplex	Breast cancer (palliative), ovarian, and superficial papillary bladder cancer, lymphoma, intracavity effusions	22.4		
Multisource (Asta Medica, Bristol-Myers Squibb and others)	DNA alkylator related to nitrogen mustard ❑ IV	Ifosfamide ❑ Ifex (USA), various brand names outside USA and generics	Germ-cell testicular cancer	140.0	140.0	0.0
Multisource (Bristol-Myers Squibb and generic suppliers)	Mustard gas derivative; biotransformed in the liver to active alkylating metabolites which cross-link tumor cell DNA ❑ PO, injection	Cyclophosphamide	Multiple myeloma, breast cancer, neuroblastoma, sarcoma, sclc, NHL,CLL			
Multisource (Bristol-Myers Squibb and generic suppliers)	Semisynthetic derivative of podophyllotoxin ❑ IV, PO	Etoposide/teniposide ❑ Vepesid/Vumon and generics	Refractory testicular tumors, small cell lung cancer (sclc)	120.0	110.0	-2.9
Multisource (Eli Lilly and others)	Salt of an alkaloid obtained from the periwinkle plant ❑ IV	Vincristine/vinblastine ❑ Oncovin,Velban	Acute leukemia	20.0	20.0	0.0
Schering-Plough ❑ Cancer Research Campaign Technology (CRCT), SuperGen	Alkylating agent ❑ PO, IV	Temozolomide ❑ Temodal (Europe), Temodar (USA)	Brain cancer	NA	121.0 (65.0)	NA
Hormone Modulators						
AstraZeneca	Aromatase inhibitor ❑ PO	Anastrozole ❑ Arimidex	Advanced breast cancer in postmenopausal women whose disease progresses following antiestrogen therapy	85.0	156.0	22.4
AstraZeneca	Synthetic decapeptide analog of LHRH ❑ subcutaneous depot	Goserelin acetate ❑ Zoladex	Palliative in advanced breast and prostate cancer; also for management of endometriosis and endometrial ablation	578.0	734.0	8.3
AstraZeneca	Nonsteroidal antiestrogen ❑ PO	Tamoxifen ❑ Nolvadex	Adjuvant treatment of breast cancer	500.0	576.0	4.8
AstraZeneca	Non-steroidal antiandrogen	Bicalutamide ❑ Casodex	Palliative in combination with an LHRH analog	202.0	433.0	28.9
Barr Laboratories	Nonsteroidal antiestrogen ❑ PO	Tamoxifen	Adjuvant treatment of breast cancer	(200.0)	(312.6)	16.9
Orion Pharma ❑ Nippon Kayaku, Schering-Plough, Shire Pharmaceuticals Group	Antiestrogen that inhibits estrogen-induced stimulation of DNA synthesis and cellular	Toremifene ❑ Fareston	Hormone-dependent or unknown hormone status metastatic breast cancer in post-menopausal women replication ❑ PO		16.2 (3.2)	NA

— continued on next page

Schering-Plough	Acetanilid, nonsteroidal active antiandrogen ❑ PO	Flutamide ❑ Eulexin	Early-stage and advanced prostate cancer	214.0	128.0 (52.0)	-15.7
TAP Pharmaceuticals ❑ Abbott Laboratories	Synthetic nonapeptide analog of naturally occurring gonadotropin releasing hormone (GnRH or LHRH)	Leuprolide acetate ❑ Lupron	Prostate cancer, endometriosis	990.0 (712.0)	959.0 (799.0)	-1.1 (3.9)
Regulatory Agents						
Celgene[2]	Antiangiogenic compound; may block certain growth factors such as bFGF and VEGF ❑ PO	Thalidomide ❑ Thalidomid, Thalomid	Cutaneous manifestations of moderate to severe erythema nodosum leprosum (ENL) in leprosy	NA	62.0	NA
Anticancer Biologicals						
Chiron	Recombinant interleukin 2 (rIL-2) ❑ injection	Aldesleukin (IL-2) ❑ Proleukin	Advanced/metastatic kidney cancer and melanoma	70.4	113.0	17.1
IDEC Pharmaceuticals ❑ Genentech, Hoffmann-La Roche, Zenyaku Kogyo	Genetically engineered, engineered, chimeric pan-B antibody that binds CD20-antigen-expressing B cells, activates complement proteins, and recruits macrophages and natural killer cells ❑ infusion	Rituximab ❑ IDEC-C2B8 ❑ Rituxan (USA), MabThera (Europe)	B-cell non-Hodgkin's lymphoma (NHL)	5.5	524.0 (424.3)	356.7
Genentech ❑ Hoffmann-La Roche, Bristol-Myers Squibb, Protein Design Labs, ImmunoGen	Recombinant DNA-derived humanized MAb (4D5) targeting the HER2 protein on tumor cells; IgG1 k immunoglobulin ❑ infusion	Trastuzumab ❑ Herceptin	HER2-positive breast cancer	NA	314.5 (275.9)	NA
Hoffmann-La Roche ❑ Genentech	Recombinant IFN α-2a with increased duration of action ❑ intramuscular, subcutaneous	Interferon (IFN) α-2a ❑ Roferon-A	Hairy-cell leukemia, AIDS-related Kaposi's sarcoma (KS), minimally pretreated chronic myelogenous leukemia (CML), adjuvant therapy of malignant melanoma, RCC, NHL	222.7	151.4	-12.1
Ligand Pharmaceuticals ❑ Elan, Ferrer Internacional, Alfa Wassermann, CoPharma	Recombinant DNA-derived cytotoxic fusion protein composed of the amino acid sequences for diphtheria toxin and IL-2 ❑ IV	Dinileukin diftitox ❑ ONTAK	Cutaneous T cell lymphoma (CTCL), recurrent or persistent	NA	(13.2)	NA
Schering-Plough ❑ Biogen[3]	Recombinant Interferon α-2b	Intron A, Rebetron, PEG-Intron	Advanced malignant melanoma and NHL	598.04	1,360.0 (769.0)	39.5

**Outside the USA only*

[1] *Bristol-Myer Squibb's Platinol only*

[2] *Used extensively off-label in cancer indications*

[3] *Nearly 50% of Intron revenues are derived from oncology indications*

Source: NEW MEDICINE Oncology KnowledgeBASE (nm|OK), September 2001.

TX), have used intratumoral injection of liposomal formulations of plasmids for IL-2, and the bacterial superantigen staphylococcus enterotoxin (SEB) in gene therapy of melanoma (Walsh P, etal, ICGTC00, Abs. P-115:53). In preclinical investigations, *in vivo* transfection with IL-2 and SEB induced vigorous and tumor-specific CTL responses in mice and canines. The combination of IL-2 and SEB was more effective than either gene alone, and in Stage III canine subjects, treatment resulted in significantly increased survival compared to historical controls. Based on these preclinical data, a phase I dose-escalation study was initiated to evaluate the safety of direct injection of the DNA/lipid complex coding for IL-2 and SEB into cutaneous melanoma metastases. Valentis is supplying the plasmids, liposomes, and final product for this trial. Sixteen Stage IV melanoma patients have been treated in 4 of 5 planned cohorts with six injections of IL-2/SEB complex spaced 14 days apart, at doses escalating through 100, 250, 500, and 1000 μg levels. Preliminary data indicates the induction of antitumor CTL responses, with the treatment being well tolerated; no patients were withdrawn from the trial or required dose reduction because of toxicity. Lymphopenia was observed in two patients, with one demonstrating a Grade 4 reaction at the 500 mg dose. Of the 16 patients treated to date, disease stabilized in 2 and progressed in 14. A final cohort will be enrolled at a 2000 μg dose level.

Researchers at Valentis are also investigating combination cytokine gene therapy using plasmids coding for IL-12 and IFNα formulated with polyvinylpyrrolidone (PVP) as a polymeric interactive noncondensing (PINC) delivery vehicle (Mendiratta SK, etal, Hum Gene Ther, 1 Sep 2000;11(13):1851-62, and Mendiratta SK, etal, ICGTC00, Abs. O-79:S23). Intratumoral injection of IL-12 DNA/PVP resulted in the rejection of 58% of Renca renal tumors and 17% of CT26 colon tumors in mice, whereas intratumoral administration of IFNα DNA/PVP induced rejection of 25% of Renca and 0% of CT26 tumors. Combination therapy with PVP-formulated IL-12 and IFNα plasmids synergistically increased antitumor response against Renca and CT26 tumors to 100% and 50% tumor rejection, respectively. *In vivo* depletion of leukocyte subsets indicated that CD8+T and NK cells were the primary effectors of the antitumor response induced by the combined cytokine gene therapy. Mice that rejected primary tumors after combined PVP/IL-12/IFNα plasmid treatment also developed protective immunity against subsequent tumor challenge. In addition, levels of mRNA for the chemokines IP-10 and TCA-3 were higher in tumors treated with the cytokine plasmid combination than in tumors treated with either cytokine gene alone.

Based on the preclinical data, Valentis initiated phase I/II clinical trials of PVP/IL-12 DNA and PVP/IFNα DNA in patients with Stage III/IV unresectable or refractory/recurrent squamous cell carcinoma of the head and neck (SCCHN). In these studies, conducted at the Dana-Farber Cancer Institute (Boston, MA) and the University of Kentucky (Lexington, KY), there were no serious drug-related adverse events in the 7 patients treated with PVP/IL-12, or the 8 patients treated with PVP/IFNα (McQuonel S, etal, ICGTC00, Abs. P-113:51). Local injection-site reactions included swelling, erythema, and pain. In the IL-12 trial there were 2 PR, while disease stabilized in 2 and progressed in 4, while in the IFNα trial, disease stabilized in 4 and progressed in 4. A new clinical study, recently initiated, will determine the MTD for the PVP/IL-12/IFNa plasmid combination, and attempt to estimate the antitumor efficacy of the cytokine combination.

Loma Linda University School of Medicine

Scientists at Loma Linda University School of Medicine (Loma Linda, CA), are investigating the potential for a synergistic antitumor response with combination gene therapy using recombinant vaccinia virus (rVV)-mediated delivery of genes for p53, IL-2, and IL-12 (Fodor I, etal, ICGTC00, Abs. PD-20:S6). Intratumoral treatment of established C6 gliomas in nude mice with rVV/p53, rVV/IL-2, rVV/IL-12, or rVV/IL-2/IL-12 resulted in the prolonged expression of p53, IL-2, IL-12, or both cytokines simultaneously; the combination of rVV/p53 with rVV/IL-12 or rVV/IL-2/IL-12 caused significant tumor inhibition compared to single modality treatment. Administration of rVV/p53 and rVV/IL-2/IL-12 was associated with the significant elevation of NK, Mac-1+ and NKT cells in the bloodstream as well as IFNγ and TNFα expression in the tumors.

King's College London

In other work with application to the engineering of tumor cells to express gene combinations, researchers at King's College London (UK) developed a strategy for the coordinated expression of multiple genes from a single expression cassette (Gaeken J, etal, ICGTC00, Abs. PD-106:38). This is achieved by constructing a single fusion protein containing linked sequences encoding cleavage sites for the Golgi-expressed endoprotease, furin, between sequences for different secreted and/or membrane-bound proteins. Endoproteolytic release of the components allows the coordinated expression of two or more biologically active proteins. Several fusion plasmids have been constructed, including IL-2 with B7.1, IL-4 with B7.1, IL-2 with IL-4, IL-12 with p53 and p40, IL-12 with p40, and IL-2 with p53. Direct plasmid transfection or vector (adenovirus and retrovirus)-mediated transduction studies have confirmed the coordinated expression of each of the encoded proteins.

Canji

Researchers at Canji have constructed recombinant adenovirus vectors (rAd) encoding for IFNα or a nonsecreted counterpart under the control of an α-fetoprotein (AFP) promoter (Ahmed CMI, etal, ICGTC00, Abs. PD-21:S6). Expression of both secreted and nonsecreted inter-

ferons was observed to result in cell proliferation inhibition and induction of MHC class I expression in cellular assays. Intratumoral injection of rAd, expressing secreted IFNα as well as nonsecreted interferon, resulted in suppression of a human hepatocellular carcinoma (HCC) cell line, Hep 3B, xenografted in nude mice. Because the AFP promoter is activated in a majority of HCC cells, these constructs may be especially useful for *in vivo* targeting of IFNα to these types of tumors, thereby reducing the side effects observed in traditional systemic interferon therapies.

Transgene

At Transgene (Strasbourg, France) scientists are using E1, E3-deleted, replication-defective adenovirus vectors incorporating a CMV promoter (Ad-pCMV) to deliver IFNγ cDNA *in situ* for antitumor gene therapy (Leroy P, etal, Res Immunol, Sep-Oct 1998;149(7-8):681-4, and Slos P, etal, ICGTC00, Abs. O-81:S24). IFNγ is a strong activator of effector cells of the immune system, stimulating antibody-dependent cytotoxicity, CTL, and NK cell functions, exhibits antiproliferative properties and represses tumor growth by inhibiting angiogenesis. However, although systemic delivery of recombinant IFNγ has demonstrated activity against RCC, myeloid leukemia, cutaneous malignant lymphoma, and ovarian carcinoma, when combined with chemotherapy, the high therapeutic doses of IFNγ required by its short serum half-life are associated with often unacceptable toxicity. Because intratumoral gene therapy can avoid systemic toxicity, Ad-pCMV-IFNγ was injected directly into established, nonimmunogenic B16F0 melanoma tumors growing in syngenic mice. In this set ting, sustained expression of IFNγ was observed within the tumor, peaking one to two days after injection and still detectable up to 14 days post-injection.

Cell Genesys

As part of its GVAX anticancer vaccine strategy, Cell Genesys is using replication-defective adenoviral or retroviral vectors to modify tumor cells *ex vivo* through gene transfer to secrete high levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), a cytokine which plays a key role in stimulating the body's immune response to target antigens (Simons JW, etal, Semin Oncol, Dec 1998;25(6):661-76). In this approach, transduced tumor cells are lethally irradiated and then administered either in a non-patient-specific or allogenic format using tumor cell lines from multiple patients, or in a patient-specific or autologous format using tumor cells from individual patients. In preclinical animal studies, immune responses generated when transduced, irradiated cancer cells were administered intradermally, were capable of eradicating small but lethal cancer cell inocula with minimal toxicity (Sanda MG, etal, J Urol, Mar 1994;151(3):622-8, Borrello I, etal, Hum Gene Ther, 10 Aug 1999;10(12):1983-91, and Borrello I, etal, Blood, 15 May 2000;95(10):3011-9).

In reporting on the experience with GVAX prostate cancer vaccines, Jonathan Simons, MD, of Emory University (Atlanta, GA), noted that although autologous, GM-CSF-secreting, irradiated tumor vaccines prepared from *ex vivo* retroviral transduction of surgically harvested cells were well tolerated in phase I trials, and found to induce T cell as well as B cell immune responses in immunocompetent prostate cancer patients (Simons JW, etal, Cancer Res, 15 Oct 1999;59(20):5160-8, Nelson WG, etal, Cancer Chemother Pharmacol 2000;46 Suppl:S67-72), no objective clinical responses were observed at the low doses of vaccine used in the studies. Within a follow-up of 51-83 weeks, disease progressed in 6 of 8 patients as indicated by ultrasensitive serum PSA analysis (Simons J, ICGTC00, Abs. O-74:S22). According to Dr. Simons, yields of the primary cell cultures limited the number of courses of vaccination, and *in vitro* expansion of tumor cells for each patient and the need to perform individualized gene transfer for autologous vaccines was considered impractical for phase II clinical trials. To circumvent this difficulty, it was decided to use GM-CSF-transduced, allogenic LNCaP and PC-3 prostate cancer cell lines, that can be easily propagated as suspension cultures in defined, serum-free mediums, for use as universal, retrovirally-transduced, GM-CSF-secreting cells to be mixed with unmodified autologous tumor cells in the formulation of a vaccine (Borrello I, etal, Hum Gene Ther, *ibid*). This allogeneic GVAX vaccine was well tolerated and safe in a phase I clinical trial in prostate cancer. While there were no CR, among 21 patients treated, disease stabilized in 15 including a decrease in the rate of PSA level rise. One patient experienced a >50% decrease in PSA levels, which was continuing after 8 months. Of the 15 patients with stable disease, 14 produced specific antibodies in their blood directed against the prostate cancer cells comprising the vaccine, whereas none of the 6 patients with progressive disease produced such antibodies.

Based on the phase I study results, Cell Genesys initiated a phase I/II open-label, multicenter clinical trial of allogenic GVAX, consisting of a 50/50 formulation of transduced, irradiated LNCaP and PC-3 cells, in advanced metastatic prostate cancer patients who failed prior hormone therapy. This trial is using an initial "priming" dose of vaccine (500M cells), followed by 12 biweekly "boost" vaccine doses of either 100M cells (low-boost) or 300M cells (high-boost), with all vaccine doses administered as intradermal injections in an outpatient setting. Observed side effects have been similar to those seen in the autologous vaccine trial, including pruritus, erythema, and swelling at vaccination sites, with no DLT yet encountered. As reported by Dr. Simons, 34 of 55 patients entered into this trial had positive bone scans and were assigned to either low-boost (n=24) or high-boost (n=10) vaccine treatment. Post-treatment follow-up revealed a trend toward a dose-dependent delay in the time to disease progression (median of 140 days compared to 85 days for high-boost and low-boost regimens, respectively). The median progression-free survival for the 21 patients who did not have positive bone scans at study entry was 179

Exhibit 4
Worldwide Markets of Selected Agents Used as Oncology Adjuncts in 1997 and 2000

Developer/ Marketer ❑ Affiliate(s)	Drug Category	Generic Name ❑ Brand Name	Approved Indication(s)	WW (USA) 1997 Sales ($ mil.)	WW (USA) 2000 Sales ($ mil.)	CAGR (97-00) %
Adjunct Biologicals						
Amgen	Recombinant granulocyte colony-stimulating factor (G-CSF) that selectively stimulates the production of infection fighting neutrophils ❑ parenteral	Filgrastim ❑ Neupogen, Gran (Japan)	Neutropenia associated with myelosuppressive chemotherapy for solid tumors, bone marrow transplantation (BMT), chemotherapy-treated acute myelogenous leukemia (AML), peripheral blood progenitor cell (PBPC) recruitment and severe chronic neutropenia	1,060.0	1,224.0	4.9
Immunex ❑ Novartis, Genetics Institute, Research Corporation Technologies (RCT)	Recombinant granulocyte macrophage colony-stimulating factor (GM-CSF) ❑ IV	Sagramostim ❑ Leukine	BMT, in AML patients >55 years-of-age undergoing induction therapy, peripheral blood progenitor cell (PBPC) mobilization, and post transplantation support	52.7	88.3	18.8
Hoffmann-La Roche	Sialic acid-containing EPO; stimulates formation of proerythroblasts and release of reticulocytes from bone marrow ❑ injection	Recombinant Epoetin β* ❑ Recormon, Marogen, NeoRecormon	Cancer-related anemia		378.4	
Ortho Biotech/ Janssen Cilag (Johnson & Johnson)	Glycoprotein that stimulates red blood cell production	Erythropoietin α (EPO) ❑ Procrit	Chemotherapy-induced anemia	1,170.0	2,709.0 (1,810.0)	32.3
Antiemetics						
Aventis ❑ Abbott Laboratories		Dolasetron mesylate ❑ Anzemet	Prevention of nausea and vomiting associated with emetogenic chemotherapy	NA	163.0	NA
GlaxoSmithKline ❑ Sankyo	5HT-3 antagonist ❑ PO, IV	Ondansetron ❑ Zofran	Prevention of chemotherapy-induced nausea	619.8 (380.3)	746.0 (514.0)	6.4
Hoffmann-La Roche ❑ GlaxoSmithKline	5HT-3 antagonist ❑ PO, IV	Granisetron HCl ❑ Kytril	Prevention of nausea and vomiting associated with emetogenic chemotherapy and radiotherapy	366.0 (240.0)	333.0 (187.0)	-3.1
Hypercalcemia and Bone Metastasis-related Treatments						
Cytogen ❑ Berlex Laboratories, CIS Biointernational, Dow Chemical	A stable complex of radioactive samarium and EDTMP, a tetraphosphonate chelator ❑ injection	Samarium Sm 153 lexidronan ❑ Quadramet	Treatment of bone pain arising from cancers which have spread to the skeleton that may be visualized by bone scans	1.0	8.5	104.1

— continued on next page

Novartis	Bisphosphonate ❑ IV	Aredia	Hypercalcemia of malignancy, treatment of skeletal osteolytic lesions resulting from multiple myeloma and breast cancer	237.50	622.8	37.9
Other Adjuncts						
Medimmune ❑ Alza (USA), Schering-Plough (abroad)	Organic thiophosphate; selective cytoprotective agent ❑ IV	Amofistene ❑ Ethyol	Chemoptotective for platinum-based chemotherapy	31.7 (20.6)	73.4 (48.4)	32.3 (32.9)
Novartis Pharmaceuticals[1]	Mimics the action of somatostatin; inhibits excess release of damaging intestinal tumor cell secretions, thus eliminating the source and cause of severe diarrhea ❑ subcutaneous, intramuscular, depot	Octreotide acetate ❑ Sandostatin; Sandostatin LAR Depot	Control of symptoms, such as diarrhea, associated with metastatic carcinoid and vasoactive intestinal peptide-secreting tumors (VIPomas)	217.4	407.2	23.3

**Outside the USA only*

[1]Approved and marketed for acromegaly as well as cancer indications

Source: NEW MEDICINE Oncology KnowledgeBASE (nm|OK), September 2001.

days. One patient with prostate cancer metastatic to the bone demonstrated a CR ongoing at 12 months, with normalization of both PSA and bone scan.

In another phase I/II open-label, multicenter clinical trial, patients with either early (n=20) or advanced (n=20) non-small cell lung cancer (nsclc) are undergoing treatment with adenoviral GM-CSF-modified autologous GVAX cancer vaccines. In an initial phase I autologous GVAX trial conducted at Harvard Medical School (Boston, MA), of 22 evaluable patients with heavily pretreated, Stage III or IV nsclc, disease stabilized in 3 with 2 cases continuing after 18 months. Also, one patient who had failed prior chemotherapy experienced a >50% reduction in tumor size at two of three disease sites (Dranoff G, J Gene Med, Mar-Apr 1999;1(2):80-3). In the present trial, initiated in February 2000, 30 nsclc patients (12 early stage, 18 advanced) have undergone tumor harvest, from sources that have included primary or recurrent lung masses (n=14), and pleural effusions (n=7) as well as brain (n=2), adrenal (n=1), lymph node (n=1), and skin (n=1) metastases (Maples P, etal, ICGTC00, Abs. PD-93:S28). The overall success rate for vaccine production has been 77% (16/20 solid tumors, 4/6 pleural effusions), with the majority of vaccines yielding greater than 107 cells. Mean GM-CSF secretion rates were 334 ng/10^6 cells over 24 hours for solid tumors and 568 ng/10^6 cells for pleural effusions.

In her presentation, Cell Genesys' Annie-Chen Tran, discussed the potential effect nonviable cells may have on cellular tumor vaccines, such as GVAX. Becuase of the multiple steps involved in the generation, storage, and on-site preparation of the large numbers of cells required for tumor cell vaccines, some loss of cell viability may be introduced before injection into a patient. This may be particularly true for retrovirally-mediated gene transfer, which requires active cell division and, hence, prolonged *ex vivo* culturing. In studying a cellular tumor vaccine model based on B16 melanoma cells engineered by retroviral transduction to express GM-CSF, tumor vaccine cells, killed by incubation in 10% DMSO, were mixed with live vaccine cells and injected into syngenic murine hosts. The dead tumor vaccine cells decreased the potency of the vaccine in a dose-dependent manner, with a detrimental effect observed at admixtures containing as little as 30% dead cells. These nonviable cells apparently inhibit the antitumor effect of an equivalent number of live vaccine cells, and the coinjection of recombinant GM-CSF with a 50% viable vaccine dose did not restore efficacy (Tran A-C, etal, ICGTC00, Abs. O-77:S23).

Thomas Jefferson University

Investigators at Thomas Jefferson University (Philadelphia, PA) are conducting a phase I clinical trial of a fully-replicating rVV vector expressing GM-CSF for intralesional gene therapy of dermal and/or subcutaneous metastases in patients with surgically incurable cutaneous melanoma (Mastrangelo MJ, etal, Cancer Gene Ther, Sep-Oct 1999;6(5):409-22, Mastrangelo MJ, etal, Adv Exp Med Biol 2000;465:391-400, and Lattime EC and Mastrangelo MJ, ICGTC00, Abs. O-75:S22). As reported by Edmund C. Lattime, PhD, of the Cancer Institute of New Jersey (New Brunswick, NJ), 7 immunocompetent melanoma patients underwent treatment with twice-weekly intratumoral injections of escalating doses (10^4 to 2 x 10^7 pfu/lesion or

10^4 to 8 x 10^7 pfu/session) of rVV/GM-CSF for 6 weeks. Systemic toxicity was infrequent, dose-related, and limited to mild flu-like symptoms that resolved within 24 hours, while local inflammation, at times with pustule formation, was consistently seen with doses of 10^7 pfu/lesion or higher. Chronically treated lesions showed a dense infiltration of CD4+ and CD8+ lymphocytes, histiocytes, and eosinophils. All patients developed an antivaccinia humoral immune response within 14 to 21 days following revaccination; however, the encoded β-galactosidase reporter gene was expressed despite the presence of antivaccinia antibodies. Recombinant GM-CSF gene function was evidenced by the presence of virally encoded GM-CSF mRNA at injection sites both early (weeks 1 and 5) and late (week 31) in the course of treatment; GM-CSF was not detected in the sera. Among evaluable patients 2 with the largest tumor burden failed to respond to treatment, 3 experienced minor responses, with regression of treated and untreated dermal metastases and progression of disease elsewhere. There was one PR, with regression of injected and uninjected regional dermal metastases and 1 CR in a patient with only dermal metastases confined to the scalp.

VA Medical Center-Long Beach

Martin R. Jadus, PhD, reported on work being conducted at the VA Medical Center (Long Beach, CA) and the University of California at Irvine, that has shown certain types of tumor cells to be susceptible to macrophage-mediated phagocytosis when retrovirally transfected with the cDNA for the membrane-associated isoform of macrophage-CSF (mM-CSF). These cells, which include immunogenic rat T9 glioblastoma and nonimmunogenic murine Hepa1-6 hepatoma, are killed by mM-CSF in a dose-dependent manner (Dan Q, etal, ICGTC00, Abs. O-76:S22). When implanted subcutaneously into syngeneic hosts, mM-CSF-transfected tumor cells are rejected spontaneously via a T-cell-dependent mechanism. Animals that reject the mM-CSF-expressing tumor cells exhibit long lasting immunity against unmodified parental cells, and this immunity can be transferred into naïve hosts. Subcutaneous immunization with viable mM-CSF-transduced T9 tumor cells has been shown to be effective in eradicating established intracranial T9 gliomas in animal models, with upwards of 80% tumor rejection observed (Zeineddine NS, etal, Immunol Lett, 1 Oct 1999; 70(1):63-8).

Genphar

Fas ligand (FasL, also referred to as CD95L), a type II transmembrane cytokine expressed on activated T cells and NK cells, is released from the cell surface as a soluble molecule after metalloproteinase-mediated digestion, and induces the apoptosis of Fas-positive target cells, including certain human glioblastoma, prostate, lung, breast, and brain cancer cell lines. Although originally described as proteins involved in the regulation of peripheral immune tolerance, evidence suggests that Fas/FasL may play an important role in carcinogenesis, tumor outgrowth, and metastasis (Owen-Schaub L, etal, Int J Oncol, Jul 2000;17(1):5-12). It has been suggested that the transduction of the FasL gene into tumor cells may generate apoptotic responses and potent inflammatory reactions that can induce the regression of malignancies (Arai H, etal, Proc Natl Acad Sci USA, 9 Dec 1997;94(25):13862-7, Drozdzik M, etal, Gene Ther, Dec 1998;5(12):1622-30, and Ambar BB, etal, Hum Gene Ther, 1 Jul 1999; 10(10):1641-8).

To maximize the adenoviral vector-mediated delivery of the FasL gene to prostate cancer cells, while limiting or eliminating cytotoxic gene expression in nontumor cells, James S. Norris, PhD, and his colleagues at the Medical University of South Carolina (Charleston, SC), in collaboration with Genphar (Mt. Pleasant, SC), have developed a replication-defective adenovirus type 5 vector (Ad5) in which the transactivator gene for tetracycline (tet) is placed under the control of a prostate-specific ARR2PB promoter, and a fusion gene for transmembrane FasL (mFasL) and green fluorescent protein (GFP) is under the control of a tet-responsive element. In this construct, mFasL-GFP retains the full activity of wild-type mFasL, while allowing for easy visualization and quantification in living as well as fixed cells which allows expression of mFasL-GFP to be regulated by tetracycline or doxycycline in a dose-dependent manner (Dong J-Y, etal, ICGTC00, Abs. O-35:S10). The expression cassette for mFasL-GFP is inserted into the cloning site near the right ITR of Ad5 to minimize background transgene expression. Compared to vectors in which the mFasL-GFP fusion gene is expressed directly from the ARR2PB promoter, in this vector configuration, the level of prostate-specific mFasL-GFP expression is amplified by binding of the transactivator to the tet-responsive promoter. This vector system (AdmFasLGFP/TET) is capable of causing 70%-98% apoptosis in various prostate cancer cell lines, including PPC-1, LNCaP, PC-3, TSU-Pr1, and DU145. In addition, high levels of mFasL-GFP are observed only in prostate cancer cells and not in cells of other origin. Although the mechanisms by which FasL/Fas interaction induces apoptosis in cancer cells are not well understood, Fas and FasL have been found to be co-localized to the same intracellular compartment, and anti-Fas neutralizing antibodies are unable to block AdmFasLGFP/TET-mediated cell death. This suggests that FasL and Fas interact prior to cell surface presentation, with intracellular FasL possibly ligating Fas within the Golgi and/or endoplasmic reticulum (Hyer ML, etal, Mol Ther, Oct 2000;2(4):348-58, and Norris JS, etal, ICGTC00, Abs. O-36:S11).

Chiba Cancer Center Research Institute

In other work conducted independently at the Chiba Cancer Center Research Institute, murine lung carcinoma cells, A11, were transfected with the genes for either membrane-type FasL (A11/mFasL) or soluble FasL (A11/sFasL). The A11 cell line is a highly metastatic phe-

notype isolated from Lewis lung carcinoma, and has been shown to be resistant to Fas-mediated apoptosis despite the expression of Fas antigen on the cell surface (Takasu M, etal, Clin Exp Metastasis, Jul 1999;17(5):409-16, Tagawa M, etal, ICGTC00, Abs. O-83:S25). These transfectants did not undergo apoptosis *in vitro*, and their growth rates were essentially the same as the parental cell line. However, while inoculation of syngenic or nude mice with A11/sFasL cells resulted in the development of tumors (subcutaneous administration) or lung metastatic foci (intravenous administration), no tumor or metastases developed in mice administered A11/mFasL cells administered subcutaneously or intravenously, respectively). Moreover, the syngeneic, but not nude, mice that rejected the A11/mFasL cells were also resistant to subsequent challenge with parental A11 cells but not irrelevant syngeneic B16 melanoma cells. These results further indicate that membrane-anchored FasL can induce antitumor effects, which are specific to FasL-expressing cells, and also suggest that mFasL-transduced tumor cells can generate antigen-specific protective immune responses *in vivo*.

INDEX OF COMPANIES & INSTITUTIONS

— *continued on back page*

INDEX OF COMPANIES & INSTITUTIONS

FUTURE ONCOLOGY

PUBLISHED BY **NEW MEDICINE, INC.**

PUBLISHER AND EDITOR: **Katie Siafaca, MS**
RESEARCH ASSOCIATE: **Samuel Park**
ASSISTANT EDITOR: **Adele Simon**
DIRECTOR OF MARKETING AND SALES: **Chris Carrubba**
CIRCULATION: **Amish Kalyani**
DESIGN & PRODUCTION: **Jill Burch**
DATABASE MANAGER: **Beth Schweitzer**

NEW MEDICINE, INC. MAILING ADDRESS:

P.O. Box 909
Lake Forest, CA 92609
Tel: 949. 830. 0448 ■ Fax: 949. 830. 0887
e-mail: info@newmedinc.com
www:http://www.newmedinc.com
www:http://www.oncologyknowledgebase.com

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.
- A one-year subscription, sent first class to U.S. addresses is US $840. A one-year subscription, sent air mail to addresses outside the U.S., is US $900.
- Volumes V2, V3, V4, and V5 are $2,400 (U.S.) and $2,460 (outside the U.S.).
- Volumes V4 and V5 are $1,400 (U.S.) and $1,460 (outside the U.S.).
- Additional subscriptions sent in the same envelope are $390 each.
- Payment must accompany your order; checks must be drawn on a U.S. bank. (A purchase order number is acceptable; however, the subscription will not begin until payment is received.) Make checks payable to NEW MEDICINE. Payment may also be made by AMERICAN EXPRESS, VISA or MASTERCARD and wire transfer; please call 949. 830. 0448.