FUTURE ONCOLOGY

TECHNOLOGY, PRODUCTS, MARKETS AND SERVICE OPPORTUNITIES

A NEW MEDICINE PUBLICATION | JUNE/JULY 1997 | VOLUME 3, NUMBER 2/3

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

OVARIAN CANCER — PART IV
NOVEL THERAPEUTIC APPROACHES IN DEVELOPMENT

MECHANISMS IN MALIGNANCY

THE CELL CYCLE

MEETING COVERAGE

DEMYSTIFYING THE ROLE OF CYTOKINES IN TUMOR IMMUNOBIOLOGY

REPORT FROM THE 88TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH APRIL 12-16, 1997 SAN DIEGO, CA

Next Issue: Epidemiology, etiology pathogenesis, etc., of head and neck cancer and a comprehensive update of the clinical and market status of taxanes.

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

OVARIAN CANCER — PART IV

NOVEL THERAPEUTIC APPROACHES IN DEVELOPMENT

Numerous agents are in development for the treatment of ovarian cancer (see Exhibit 1). Many of these agents, currently in preclinical or phase I clinical trials, are addressing most solid tumors, in addition to ovarian cancer. Ovarian cancer has been the beneficiary of two recent key chemotherapeutic drug approvals but both drugs, paclitaxel and topotecan, were nearly abandoned old drugs that were brought back to life thanks to better understanding of how to administer chemotherapeutics to maximize effectiveness and prevent/manage toxic side effects. Re-engineering old drugs is the hallmark of current anti-cancer drug development. Successes such as that of Taxol, whose sales are expected to surpass the $1 billion mark in 1997, are encouraging re-evaluation of many other agents considered unworthy of pursuit a few years ago. Maybe, use of these traditional chemotherapeutics in conjunction with better patient selection to match tumor type to chemotherapeutic regimen, new drug combinations and high-dose chemotherapy and tumor response enhancement achievable by immunotherapy and gene transfer techniques, will finally defeat the most resistant tumors and result in long-lasting responses.

ANALOGS, DERIVATIVES AND FORMULATIONS OF AGENTS APPROVED FOR THE TREATMENT OF OVARIAN AND OTHER CANCERS

Although three classes of anti-cancer agents with different modes of action, i.e. platinum-based drugs, taxanes and topoisomerase I (topo I) inhibitors, have been approved as first, second and third-line treatment for ovarian cancer (see FO, pp 545-562), the goal of effectively managing refractory, recurrent and/or metastatic ovarian cancer has not been achieved.

Anthracyclines

The value of adding doxorubicin to a platinum-based regimen for treatment of ovarian cancer is still in debate. Also, doxorubicin is being evaluated in platinum-refractory disease. Various analogs and formulations of doxorubicin are in development for the treatment of ovarian cancer, including liposomal doxorubicin (Doxil, Caelyx; Sequus Pharmaceuticals), described in FO, pp 554-557.

Anthra Pharmaceuticals (Princeton, NJ) is developing AD32, a lipophilic anthracycline that is an analog of doxorubicin. In July 1997, Medeva (London, UK) announced that it acquired exclusive USA marketing rights for AD32 for bladder cancer, acquired a 1% equity interest in Anthra, and also has rights to all indications for

AD32 in the USA. Medeva paid development and licensing fees of about $8 million on signing, will pay $2.125 million for production of three validation batches of AD32 and an additional $5.5 million on regulatory approval for treatment of refractory bladder carcinoma ***in-situ*** (CIS), and another $10.5 million upon approval of a second indication as adjuvant therapy in papillary bladder cancer. Medeva will also pay royalty on sales. An earlier worldwide R&D and licensing agreement, entered in October 1995 with Schering AG, was dissolved in 1996. A phase III randomized study of intraperitoneal AD32 versus altretamine in ovarian cancer patients who have failed platinum and paclitaxel chemotherapy is being conducted at the University of California, Irvine (UCI) Cancer Center; Philip DiSaia is the primary investigator.

Platinum Analogs

Among platinum analogs being evaluated in clinical trials are tetraplatin, oxaliplatin (L-OHP), DWA2114R, enloplatin, lobaplatin, JM216 and BMS-182751. Also, Sequus Pharmaceuticals has initiated phase I clinical trials of SPI-077, a liposomal formulation of cisplatin, for treatment of ovarian cancer. The key objective in developing new platinum analogs is reduction of toxic side effects and circumvention of cisplatin resistance.

Oxaliplatin (L-OHP), a diaminocyclohexane (DACH) platinum analog, is being evaluated in a randomized phase II clinical trial, conducted by the EORTC Gynecological Cancer Cooperative Group (Protocol ID: EORTC-55951), in combination with paclitaxel, in patients with platinum-pretreated refractory advanced ovarian cancer. Analysis of a 12-patient compassionate use program, using a combination of paclitaxel (135-175 mg/m^2) as a 3-hour infusion, followed by L-OHP (100-130 mg/m^2) as a 2-6-hour infusion, every three weeks, revealed low toxicity and 1 CR, 3 PR and 5 SD, among 8 evaluable patients (Faivre S, etal, ASCO97, Abs. 1315:369a). L-OHP is under development by Debiopharm (Lausanne, Switzerland) which has granted Sanofi (Paris, France) an exclusive European license. In a multi-center phase II/III clinical trial of L-OHP versus cisplatin, both in association with cyclophosphamide, L-OHP exhibited somewhat lesser activity (overall response rate was 51.5% versus 65% with cisplatin and median progression-free survival was 20.9 months versus 26.2 months) but was better tolerated than cisplatin (Misset JL, ASCO97, Abs. 1266:354a).

Taxanes

Paclitaxel (Taxol; Bristol-Myers Squibb) has emerged as the premier second-line treatment of ovarian cancer in the USA and was approved as first-line therapy in Europe in 1996. It is believed that there is extensive off-label use of paclitaxel as first-line therapy in ovarian cancer in the USA where the combination of paclitaxel and cisplatin or carboplatin, is considered standard first-line therapy based on results from the Gynecologic Oncology

Exhibit 1
Selected Agents in Development for the Treatment of Ovarian Cancer

Developer ❑ Affiliates	Generic Name ❑ Number ❑ Brand Names	Description ❑ Mechanism ❑ Target ❑ Administration Route	Clinical Status ❑ Indications
Aastrom Bioscience ❑ Cobe BCT, SeaMED, Ethox, Immunex, Anchor Advanced Products, U Michigan	Cell Production System (CPS)	Cell culture system ❑ cell therapy using stem cells; *ex vivo* expansion of peripheral blood progenitor cells ❑ lymphoid blood cells ❑ *ex vivo*	Phase I (2/97)➢USA ❑ solid tumors
Agouron Pharmaceuticals ❑ Hoffmann-La Roche	AG-337 ❑ Thymitaq	Thymidylate synthase inhibitor ❑ cell cycle regulator; apoptosis inducer ❑ IV, PO, intraperitoneal, intramuscular	Phase I➢USA ❑ solid tumors (see FO, p 55)
Allergan Ligand Retinoid Therapeutics (ALRT) ❑ NCI	9-cis retinoic acid (9cRA) ❑ ALRT 1057 (formerly LGD 1057) ❑ Panretin (Oral and Topical)	Synthetic analog of naturally-occurring hormone; retinoid ❑ intracellular receptor agonist; receptor/ligand interaction ❑ panagonist of RAR/RXR receptors ❑ PO, topical	Phase II/III (6/97)➢USA (protocol IDs: LIGAND-L1057-32, NCI-V96-1051; also see FO, pp 313, 365, 451)
AltaRex ❑ Biomira (licensor), Medac (licensee, Western Europe), MerckFrosst Canada (manufacturer)	MAb B43.13 ❑ Ovarex	Autologous vaccine; idiotypic MAb; immunomodulator ❑ binds with high affinity to CA 125; stimulates the immune system through generation of autologous antigen mimics ❑ IV (without adjuvant)	Phase II/III (b4Q/96) ➢Canada, Europe; phase IIb (planned 2Q/97) ➢USA ❑ advanced ovarian cancer
AltaRex ❑ U Alberta	Hypocrellin	Natural product, synthetic analog; photosensitizer; immunophotodynamic therapy ❑ destroys tumors when photoactivated ❑ injectable	Preclin (10/97)➢Canada
Anika Therapeutics ❑ Tufts U (licensor)	Hyaluronic acid (HA) oligosaccharides	Natural product extract; carbohydrate ❑ receptor/ligand interaction; binds and inhibits HA receptors	Preclin (12/96)➢USA
Ansan ❑ Bar-Ilan Research and Development, Cancer Therapy and Research Center	AN9 ❑ Pivanex	Natural product, butyric acid synthetic analog; formulation ❑ apoptosis enhancer; promotes cellular differentiation ❑ injectable, intraperitoneal, intra-arterial	Phase I (b11/95) (3/97)➢USA ❑ solid tumors and hematologic malignancies (see FO, p 31)
Anthra Pharmaceuticals ❑ Medeva (licensee, USA, 7/97); Prodesfarma (licensee, Portugal and Spain)	AD-32	Lipophilic anthracycline derivative; doxorubicin analog; topoisomerase II inhibitor ❑ interferes with DNA synthesis by binding nucleic acids and intercalating between base pairs ❑ intravesical, intraperitoneal	Phase III (12/96)➢USA
AntiCancer ❑ Shionogi	Recombinant methioninase (rMETase) ❑ AC9501 ❑ ONCase	Chemical modulator; cell cycle regulator ❑ arrests tumor cells before mitosis; induces apoptosis ❑ targets a metabolic defect in all types of tumors	Phase I (1/96)➢Japan ❑ solid tumors (see FO, p 18, 253, 488)
Antigenics (OncoAntigenics) ❑ Fordham U, Mount Sinai School of Medicine	Heat shock protein (hsp70 and gp96)-based vaccines	Peptide antigen and heat shock protein (hsp70 and gp96) complexes; immunomodulator; autologous vaccine ❑ complexes formed from the binding of heat shock proteins to antigen peptides, promote antigen presentation and activate CD8 T cells	Phase I (b5/95)➢Germany (see FO, p 353)
Aphton	Gonadotrophin-releasing hormone (GnRH) immunogen ❑ Gonadimmune	Synthetic peptide antigen; anti-hormone immunogen; immunomodulator ❑ induces antibodies that block GnRH secretion ❑ injectable	Phase I/II➢USA ❑ breast cancer, prostate cancer (see FO, pp 320-321); may be applicable to ovarian cancer

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Aronex Pharmaceuticals ❑ Boehringer Mannheim, NIH	AR209	Conjugated toxin ❑ delivers pseudomonas exotoxin to cancer cells ❑ targets erbB-2 oncoprotein ❑ injectable	Preclin (2/97)➢USA ❑ breast cancer; may be applicable to ovarian cancer (see FO, p 491)
Asta Medica	Lobaplatin ❑ D-19466	Platinum-based drug	Phase II (6/95)➢The Netherlands ❑ refractory or relapsed ovarian cancer
Asta Medica ❑ Tulane U, Nippon Kayaku	Cetrorelix ❑ SB-75, SB-075	LH-RH antagonist; suppresses sex hormones ❑ subcutaneous	Phase II (6/97)➢USA (see FO, p 306)
AVAX Technologies ❑ Thomas Jefferson U (licensor, 11/95)	O-Vax	Immunotherapy ❑ autologous tumor cells are removed, conjugated with a small molecule (hapten), and infused back into the patient with an adjuvant	Phase II➢USA (7/97) ❑ adjunct to ovarian tumor surgery
Banyu	NB506	Indolocarbazole derivative ❑ topoisomerase I inhibitor ❑ DNA- and RNA- polymerases inhibitor	Phase I (11/96)➢Japan
Bernardo Houssay Hospital, School of Medicine, Buenos Aires U	VRCTC-310	Natural product extract; purified snake venom fraction ❑ phospholipase A2 activity ❑ intramuscular	Phase I (5/97)➢Argentina ❑ refractory solid tumors (Costa LA, etal, ASCO97, Abs. 820:233a)
Biomira ❑ Ribi ImmunoChem Research (adjuvant licensor), Chiron (licensee, USA and Europe)	Theratope STn-KLH	Synthetic cancer-associated carbohydrate antigen vaccine; active specific immunotherapy ❑ injection	Phase II➢Canada, USA (6/97) (see FO, pp 51, 233, 348, 486)
Bristol-Myers Squibb ❑ NCI	Bryostatin 1 (BRYO) ❑ NSC-339555	Natural product extract; biological response modifier ❑ activates protein kinase C (PKC); triggers release of reactive oxygen radicals by polymorphonuclear neutrophils (PMN) and monocytes ❑ IV	Phase I (10/96)➢USA ❑ solid tumors (protocol IDs: GUMC-95332, NCI-T94-0200D)
British Biotech ❑ Tanabe Seiyaku (licensee)	Marimastat ❑ BB-2516	Matrix metalloproteinase inhibitor ❑ PO	Phase III (12/96)➢USA, UK (see FO, p 310, 194)
Calydon		Engineered adenovirus that selectively replicates in cancer cells resulting in their death	Research (8/97)➢USA (see FO, pp 392-394)
Cancer Therapeutics (Antisoma/Imperial Cancer Research Technology (ICRT) joint venture, 1996)	Theragyn	Radioimmunotherapy; yttrium-90-labeled murine IgG1 MAb (HMFG1) ❑ directed against tumor-selective, abnormally-glycosylated polymorphic epithelial mucin (PEM) ❑ intraperitoneal	Phase III (o9/96)➢UK, (b10/97)➢USA, Europe ❑ ovarian cancer in clinical remission post surgery and chemotherapy
Canji ❑ Schering-Plough	ACN-p53 TSG	p53 tumor suppressor gene-based therapy; adenovirus-mediated p53 gene transfer ❑ replacement of defective p53 with wild-type version ❑ intratumoral; bolus infusion, intraperitoneal, intrahepatic	Phase I➢USA ❑ solid tumors (see FO, pp 26, 483)
CarboMed ❑ Zeneca (licensee 7/97), NCI (licensor)	GBS toxin ❑ CM101, ZD0101	Polysaccharide exotoxin ❑ induces inflammation of tumor neovasculature and may have anti-angiogenic activity; seeks and binds pathologic neovasculature	Phase I (c12/94) (10/96)➢USA ❑ solid tumors (see FO, p 194)
CDR Therapeutics ❑ U Pennsylvania	Mim 16.1 and Mim 4D5.1	Complementarity determining region (CDR) mimics of Her2-neu MAb ❑ receptor/ligand interaction	Preclin (1/97)➢USA ❑ solid tumors

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Cell Genesys ❑ Ludwig Institute for Cancer Research, Sloan-Kettering Institute for Cancer Research	Genetically engineered T cells with MAb gene CC49	Immunomodulator; autologous vaccine; gene therapy ❑ genetically engineered T cells recognize a specific protein on the surface of tumor cells ❑ targets TAG-72 expressing tumor cells ❑ *ex vivo*	Preclin (3/97)➢USA ❑ solid tumors (see FO, pp 487-488)
Cell Therapeutics ❑ BioChem Pharma (Canadian licensee 3/95)	CT-2584, CT-2583, CT-2586, CT-3536	Small molecules; phospholipid signaling inhibitors ❑ overactivate tumor cell phosphatidylcholine phospholipase-D (PC-PLD); anti-angiogenic ❑ infusion	Phase I (b5/96)➢UK, USA ❑ refractory ovarian cancer (see FO, p 129)
Celltech Therapeutics ❑ Wyeth-Ayerst (American Home Products)	Calicheamicin ❑ CMB401 (formerly CDP671/1)	Conjugated immunotoxin; MAb linked to a toxin ❑ targets polymorphic epithelial mucin (PEM)	Phase IIa (9/96)➢UK
Cephalon ❑ Kyowa Hakko Kogyo (co-developer); Vincent T. Lombardi Cancer Research Center; Georgetown U Medical Center	CEP-2563, KT-8391	Small molecule ❑ inhibits receptor tyrosine kinase (RTK) ❑ IV	Phase I (2/97)➢USA ❑ solid tumors (protocol IDs: GUMC-96087, NCI-V96-0968; also see FO, p 325)
Corixa ❑ University of Washington (licensor)	Her-2/neu peptides	Peptide antigen; immunomodulator ❑ activates cytotoxic T cells ❑ targets Her2/neu antigen-positive cancer cells	Phase I (b9/96)➢USA
Corixa ❑ University of Washington (vaccine licensor), Vical (adjuvant licensor)	Microsphere-encapsulated Her-2/neu peptides with leishmania elongation initiating factor (LeIF) as an adjuvant	Peptide antigen; immunomodulator ❑ activates cytotoxic T cells ❑ targets Her2/neu antigen-positive cancer cells	Preclin (6/97)➢USA
Cortecs ❑ CRC Centre for Cancer Therapeutics at the Institute for Cancer Research at the Royal Mardsen Hospital	CELLCOM	Natural product extracts; novel natural molecules that display anti-cancer activity ❑ target ras oncogene ❑ PO	Preclin (4/97)➢UK ❑ solid tumors
Cytel	Theradigm-P53	Peptide antigen vaccine; active specific immunotherapy ❑ stimulates T lymphocytes (CTLs) ❑ targets p53 ❑ injectable	Research (3/97)➢USA (see FO, p 352)
Cytel	Theradigm-Her-2	Peptide antigen vaccine; active specific immunotherapy ❑ stimulates T lymphocytes (CTLs) ❑ targets Her-2/neu ❑ injectable	Research (3/97)➢USA (see FO, p 352)
Daiichi Pharmaceuticals		Laminin-derived synthetic peptide	Phase I➢Japan
Debiopharm ❑ Sanofi (licensee, Europe)	Oxaliplatin (L-OHP) ❑ 1670RB, RP 54780 ❑ Eloxatin, Transplatin (Sanofi)	Platinum-based drug; DNA, RNA and protein synthesis inhibitor ❑ binds DNA to produce interstrand and intrastrand DNA cross-links ❑ IV, bolus	Phase II (7/96)➢USA (see FO, pp 17, 174, 331)
DepoTech ❑ Chiron (USA), Pharmacia and Upjohn (outside the USA)	Cytarabine or ara-C ❑ DepoCyt	Ara-C formulation ❑ DNA polymerase inhibitor ❑ injectable	An NDA (rolling NDA procedure) was filed in 11/96 for the treatment of neoplastic meningitis arising from solid tumors (see FO, p 253)
Eli Lilly	Gemcitabine ❑ Gemzar	Nucleoside analog; cell cycle regulator; ribonucleotide reductase inhibitor ❑ inhibits DNA synthesis ❑ IV	Phase II (5/97)➢EU (see FO, pp 98, 478)
Genentech ❑ NCI	Her-2/neu MAb	Chimeric MAb; immunostimulant ❑ targets Her-2/neu-expressing tumor cells	Phase II (6/97)➢USA (see FO, p 489)

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Genetic Therapy (Novartis)	PA317 cells containing the herpes simplex thymidine kinase gene, carrying G1TkSvNa.7 vectors	Gene therapy; immunomodulator ❑ Injectable	Phase I/II (2/95)➢USA ❑
Genetix Pharmaceuticals ❑ Columbia U (licensor)	A12M1 retroviral (Genpak) supernatant	Gene therapy, chemoprotection ❑ transfer of human mdr-1 cDNA to CD34+ autologous bone marow cells using a retroviral vector ❑ *ex vivo*	Phase I/II➢(6/97)USA (RAC # 9306-051, approved 6/8/93, NIH approval 9/3/93) ❑ advanced ovarian cancer
Genzyme ❑ U Alabama, NCI	Anti-erbB-2 single chain sFv (Ad21) antibody gene	Gene therapy ❑ recombinant adenovirus vector-mediated delivery of anti-erbB-2 sFv ❑ intraperitoneal	Phase I➢USA ❑ previously-treated ovarian and extraovarian cancer (RAC 9509-124, 9/11/95; NIH/ORDA recommendation, 5/15/96); preclin (4/97)➢USA (in combination with IP cisplatin; Barnes M, etal, AACR97; 38, Abs. 1544:229)
Glaxo Wellcome		Natural product, water soluble 7-substituted quaternary ammonium salt derivatives of camptothecin; topoisomerase I inhibitors	Research (2/96)➢USA
Glaxo Wellcome	GW211; GI147211	Natural product, synthetic analogs of camptothecin; topoisomerase I inhibitors	Phase I/II (4/97)➢USA ❑ ovarian cancer; phase II (5/97)➢Europe ❑ relapsed ovarian cancer (see FO, p 539)
IDEC Pharmaceuticals ❑ Pharmacia & Upjohn (original developer), NCI (licensor)	9-aminocamptothecin (9-AC) ❑ NSC 603071	Natural product, synthetic analog of camptothecin; topoisomerase I inhibitor ❑ IV, PO	Phase II (8/96)➢USA ❑ recurrent ovarian cancer after paclitaxel and platinum-based therapy (NCI protocol IDs: NCI-95-C-0056B, NCI-T94-0164N, NCI-CPB-357); see FO, p 539
IDEC Pharmaceuticals ❑ Stehlin Foundation for Cancer Research, U Texas M. D. Anderson Cancer Center, NCI	9-nitrocamptothecin (9NC)	Natural product; camptothecin analog; topoisomerase I inhibitor ❑ PO	Phase II (5/97)➢USA ❑ platinum refractory ovarian cancer (daily oral 9NC at 1.5 mg/m^2 for 4 consecutive days, *q* week, resulted in 2/25 PR, 13 SD and 6 PD with a median survival of 6 months; Verschraegen C, etal, ASCO97, Abs. 1356:381a; also see FO, p 539)
Ilexus ❑ Austin Research Institute		Immunomodulator/vaccine	Phase I (11/96)➢ Australia ❑ solid tumors
ImClone Systems ❑ U California San Diego (UCSD), Rhône-Poulenc Rorer	Anti-EGFR chimeric MAb ❑ C225	MAb ❑ blocks epidermal growth factor receptors (EGFR)	Phase I/II (6/97)➢USA (see FO, pp 52, 326, 398-399 and 478)
Immunex/Wyeth-Ayerst International (Canadian rights)	Mitoxantrone ❑ NSC 301739 ❑ Novantrone	Topoisomerase II inhibitor ❑ intercalates with DNA and prevents religation of DNA strands ❑ IV	Phase I/II (6/97)➢USA
Immunomedics ❑ Center for Molecular Medicine and Immunology (CMMI)	Murine MN-14 anti-CEA MAb ❑ CEA-Cide (was ImmuRAIT-CEA)	Radioimmunoconjugate ❑ anti-CEA MAb linked to Iodine 131 ❑ IV	Phase I (8/97)➢USA (under a CMMI IND) ❑ ovarian cancer refractory to paclitaxel
Imperial Cancer Research Fund (ICRF)	NU/ICRF 505 and NU/ICRF 505/M (metabolite of NU/ICRF 505)	Natural product extract ❑ topoisomerase I and II inhibitor	Preclin (96)➢UK (Cummings J, etal, AACR96, Abs. 2444:358)
Imperial Cancer Research Fund (ICRF)	NU/ICRF 506	Natural product extract ❑ topoisomerase I and II inhibitor	Preclin (10/96)➢UK (Cummings J, etal, Biochemical Pharmacology, 1996 Oct 11, 52(7):979-90; Meikle I, etal, Biochemical Pharmacology, 1995 Jun 16, 49(12):1747-57)

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Indiana U School of Medicine	Merbarone ❑ NSC-336628	Synthetic analog of natural product; topoisomerase II inhibitor ❑ inhibits DNA synthesis and tumor growth	Phase II (2/96)➢USA ❑ recurrent epithelial ovarian cancer (minimal activity with 10% PR was observed)
Inex ❑ Duke U, Purdue U	Transmembrane Carrier Systems (TCS), Onco TCS-vincristine	Delivery system; drug is carried inside a capsule composed of a proprietary combination of lipids and polymers ❑ IV	Phase II (2/96)➢Canada ❑ colorectal cancer; phase II (4/96)➢Canada ❑ pancreatic cancer; may have utility in ovarian cancer
Inex ❑ Duke U, Purdue U	Transmembrane Carrier Systems (TCS), Onco-L TCS	Delivery system; ligand (vitamin)-targeted transmembrane carrier system containing an anticancer compound ❑ IV	Preclin➢Canada
Ingenex ❑ CellPro, U Chicago, M.D. Anderson Cancer Center	MDRx1	Gene therapy, chemoprotection ❑ introduction of mdr-1 cDNA into autologous CD34+ cells using a retroviral vector ❑ *ex vivo*	Phase I/II (b12/94)➢USA ❑ ovarian cancer (see FO, pp 112-113, 485; RAC # 9306-044, approved 6/7/93; NIH approval 12/2/93)
Institut für Klinische Hamatologie	bi-MAb OC/TR in combination with anti-FBP/anti-CD28 bi-MAb	Bispecific MAb; immunomodulator ❑ targets folate binding protein overexpressed in 90% of ovarian carcinomas and CD3 on T cells; CD28 co-stimulation	Preclin (4/97)➢Germany (Mazzoni A, etal, AACR97, Abs. 558:83)
Introgen Therapeutics ❑ RPR GenCell, NCI, M.D. Anderson Cancer Center, Sidney Kimmel Cancer Center	Ad-p53 ❑ INGN-004	Gene therapy ❑ targets p53 ❑ intra-arterial, intraperitoneal, intralesional, intratumoral	Phase I/II (b95)➢USA ❑ non small cell lung cancer and head and neck cancer; may be applicable to ovarian cancer (see FO, pp 26, 98, 483 and 510)
Isis Pharmaceuticals ❑ Novartis (Ciba-Geigy)	Isis 3521/CGP 64128A	Oligonucleotide (antisense) ❑ inhibits production of protein kinase C-α (PKC-α) expression ❑ IV	Phase I (5/97)➢USA ❑ solid tumors (FO, p 502)
Jenner Technologies ❑ Eli Lilly, Walter Reed Army Institute of Research (WRAIR)	OncoVax-CL	Immunotherapeutic/vaccine ❑ targets KSA antigen-presenting cells	Phase I/II➢USA ❑ colorectal cancer; may be applicable to ovarian cancer
Knoll AG (BASF)	Cemadotin ❑ LU 103793	Synthetic analog of natural product dolastatin 15 ❑ inhibits tubulin polymerization ❑ IV	Phase I (3/97)➢USA, Germany ❑ solid tumors (Wolff I, etal, ASCO97, Abs. 783:223a)
Lescarden	Bovine tracheal cartilage ❑ Catrix	Biological response modifier ❑ PO	Phase II (4/97)➢USA, Canada ❑ solid tumors (protocol IDs: MRMC-CTCA-9506, NCI-V96-1027)
Ligand Pharmaceuticals	LGD 1069 ❑ Targretin	Retinoid receptor ligand ❑ activates retinoid RXR receptors; induces apoptosis ❑ PO	Phase IIb (6/97)➢USA ❑ ovarian cancer with elevated serum CA 125, following complete remission (daily 600 mg/m^2 is under evaluation in protocol IDs: MSKCC-96061, NCI-G97-1140); also see FO, pp 251, 368 and 482
Matrix Pharmaceuticals	Cisplatin/adrenaline combination, IntraDose-CDDP	Drug delivery; therapeutic implant; formulation ❑ biodegradable protein matrix holds high concentrations of drug within the tumor and retards its release rate ❑ intratumoral	Phase III (b6/95)➢USA, Europe ❑ accessible solid tumors (see FO, pp 20-21, 56 and 326-327)
Medarex ❑ Dartmouth Medical School, Novartis (ww rights, 5/95), Chiron (licensor)	Bispecific MAb 520C9xH22 ❑ MDX-210	Bispecific MAb; immunomodulator ❑ induces immune responses through a target-trigger mechanism; directs monocytes and IFN-γ-activated neutophils, mediates cytotoxicity of Her-2 overexpressing tumor cells and triggers release of TNF-α, IL-6 and G-CSF ❑ targets HER-2 ❑ IV	Phase I/II (b7/94)➢USA, Germany ❑ cancers that overexpress Her-2; phase I/II (6/97)➢USA ❑ ovarian cancer (see FO, pp 322, 395 and 489-490); granted orphan drug status for the treatment of ovarian cancer in 10/93

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Meditech ❑ NCI	NSC-D614826 ❑ Viraplex, Virazone	PO, topical	Clin➢USA
Memorial Sloan-Kettering Cancer Center	1311-Mx35 F(ab')2 MAb	MAb linked to iodine 131 radioisotope ❑ intraperitoneal	Phase I (4/97)➢USA ❑ residual ovarian cancer (Steven Larson, PI)
MGI Pharma ❑ Dainippon Pharmaceutical, NCI, U California	Acylfulvenes (6-HMAF) ❑ MGI 114	Semi-synthetic compounds originating from the mushroom *Omphalotus illedens*	Phase I (b12/95)➢USA ❑ solid tumors
Milkhaus Laboratory	LDI-200 ❑ formulation of chorionic gonadotropin	Hormone ❑ induces apoptosis	Phase I/II (b10/95) USA ❑ refractory AML; may be applicable to ovarian cancer (see FO, p 274)
NCI	Etanidazole ❑ NSC-301467, SR-2508, EF5	Binds selectively to hypoxic cells ❑ IV	Phase II➢USA
NCI	Peripheral blood lymphocytes transduced with a gene encoding a chimeric T-cell receptor	Immunomodulator; gene therapy	Phase I (6/97)➢USA
NCI	Anti-CD3-stimulated peripheral blood lymphocytes (PBL) transduced with a gene encoding chimeric T-cell receptor reactive with folate binding protein	Gene therapy; PBLs stimulated with OKT3 (anti-CD3) MAb and retrovirally transduced with the anti-ovarian cancer MOv-γ chimeric receptor gene ❑ IV	Phase I (4/97)➢USA ❑ advanced ovarian cancer (protocol IDs; NCI-96-C-0011, NCI-T95-0040N)
NCI	Carboxyamidotriazole ❑ NSC-609974	Signal transduction inhibitor ❑ modulates non-voltage-gated calcium influx-regulated signal pathways ❑ PO, encapsulated micronized form	Phase I (NCI-95-C-0015D) (12/96)➢USA ❑ solid tumors (combination therapy with paclitaxel for advanced solid tumors, including breast and ovarian carcinomas; Berlin, J, etal, J Clin Oncol, 1997, 15:781-9)
NCI	Geldanamycin ❑ NSC-330507D	Cell cycle regulator ❑ inhibits tyrosine kinase; arrests cell cycle; induces increases in p53 protein by mechanism independent of DNA damage and p53-dependent pathways	Preclin (96)➢UK
NeoPharm ❑ NIH	hIL13-PE38QQR	Chimeric toxin; specific receptor/ligand interaction ❑ targets tumor cells expressing IL-13	Research (3/97)➢USA ❑ solid tumors
NeoRx ❑ Janssen Pharmaceutica NV (ww licensee, 8/97)	Humanized MAb (NR-LU-13) linked to yttrium-90 ❑ Avicidin	Pretargeting technology using streptavidin-biotin binding (MAb NR-LU-13, conjugated to streptavidin, and ^{90}Y, linked to biotin, are administered sequentially); radioimmunotherapy (RIT) ❑ IV	Phase I/II (b7/94)➢USA (with murine version of NR-LU-13) and (b2/96, o8/97) with humanized version) (see FO, pp 99-100)
NeXstar Pharmaceuticals	Daunorubicin ❑ NSC 82151 ❑ DaunoXome	Topoisomerase II inhibitor; liposomal formulation of daunorubicin ❑ maximizes selectivity of daunorubicin for solid tumors	Phase II (3/97)➢USA (see FO, pp 56, 132 and 367-368)
Novartis	SDZ PSC 833	MDR inhibitor; reverses resistance by modulating MDR expression through P-glycoprotein (P-gp) ❑ PO, IV	Phase II (5/97)➢USA ❑ refractory ovarian cancer (see FO, p 115)
OncoTech ❑ U California, Irvine	IL-4-transfected allogenic tumor cell vaccine	Immunotherapy; allogenic vaccine; gene therapy	Preclin (6/97)➢USA
Onyx Pharmaceuticals	ONYX-015	Genetically engineered E1B-deleted adenovirus ❑ infects and kills cells containing mutant p53 ❑ intralesional, intra-operative	Phase I (6/97)➢Scotland ❑ solid tumors (see, FO, pp 27-28 and 483)
Onyx Pharmaceuticals ❑ Eli Lilly, Myriad Genetics, U Utah	BRCA1 gene	Inhibitor of BRCA1 pathways	Research➢USA (see FO, p 337)

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OXiGENE	Metoclopramide ❑ Neu-Sensamide	Neutralized formulation of metoclopramide; chemosensitizer/ radiosensitizer ❑ inhibits DNA repair activity and induces apoptosis ❑ targets adenosine dephosphate ribosyl transferase (ADPRT)	Phase III➢(1996) Europe, USA ❑ inoperable non-small cell lung cancer; may be applicable to ovarian cancer
Parke-Davis	Mivobulin isethionate ❑ CI-980	Cell cycle regulator; mitotic inhibitor ❑ binds tubulin at cholchicine site ❑ infusion	Phase II (5/97)➢USA (16 patients with advanced platinum refractory ovarian cancer were treated with a 72-hour infusion of CI-980 at 4.5 mg/m^2/day, *q* 21 days; RR was 6% with 5 (31%) SD and an overall median survival of 6.3 months; Verschraegen CF, etal, ASCO97, Abs. 1354:380a)
Parke-Davis	CI-958, NSC-635371	Benzothiopyranoindazole ❑ DNA intercalator ❑ IV	Phase II (4/97)➢USA ❑ refractory or recurrent ovarian cancer (2-hour IV infusion, *q* 3 weeks, according to protocols UCCRC-8233, NCI-T96-0039H); recurrent platinum-sensitive ovarian cancer (GOG-146E)
Parke-Davis ❑ NCI	Pyrazoloacridine (PZA) ❑ NSC-366140, PD115934	Topoisomerase II inhibitor ❑ DNA intercalator; anti-tumor activity may be attributed to an enzymatic nitro reduction pathway; induction of nascent and parental DNA damage appears to be caused by avid binding of PZA to DNA that interferes with access of replication, repair and transcription enzyme complexes ❑ IV	Phase II (5/97) ➢USA ❑ refractory ovarian cancer (conducted at the Johns Hopkins Oncology Center; protocol IDs: J-9547; NCI-T95-0059H, JHOC-9547; PZA is administered as a 3-hour infusion, *q* 21 days)
Parke-Davis ❑ U Texas M. D. Anderson Cancer Center, NIH	Suramin (SUR) ❑ CI-1003, NSC-34936	Topoisomerase II inhibitor ❑ inhibits binding of EGFr, arrests tumor cell growth, and inhibits glycosaminoglycan metabolism ❑ parenteral	Phase II (2/97)➢USA ❑ platinum-resistant epithelial ovarian cancer (protocol MDA-GYN-94004, NCI-T94-0046D)
Pharmacia & Upjohn	Methoxy-morpholinyl DOX (MMDX)	Anthracycline analog; topoisomerase I and II inhibitor	Research➢USA ❑ solid tumors
Pharmacia & Upjohn	Carzelesin ❑ NSC-D-619020, U-80244	DNA antagonist ❑ IV	Phase II (protocol ID EORTC-16943O) (2/97)➢EU ❑ advanced ovarian cancer
PharmaMar	Ecteinascidin 743 ET-743	Novel marine-derived compound	Phase I (10/96)➢USA, Scotland, The Netherlands and France ❑ solid tumors (see FO, pp 173-174)
Repligen ❑ Cambridge NeuroScience		Small molecule compounds ❑ block the neuregulin-erbB signaling pathway	Research➢USA (Cambridge NeuroScience obtained a $100,000 SBIR grant from the NCI in July 1997)
Research Corporation Technologies, NeXstar, U Arizona, Arizona Cancer Center	Azonafide compound	Natural product ❑ topoisomerase inhibitor	Phase I➢USA ❑ leukemia
Rhône-Poulenc Rorer ❑ Chugai, NCI	Docetaxel ❑ NSC-628503, RP-56976 ❑ Taxotere	Taxane ❑ binds to free tubulin, promotes cellular microtubules assembly, and inhibits disassembly ❑ IV	A (5/96)➢USA ❑ second-line treatment of breast cancer; phase III➢France, Japan ❑ ovarian cancer (see FO, pp 172-185, 251, 261-262, 276 and 437)
Scotia Holdings	Meso-tetrahydroxy-phenylchlorine-based photodynamic therapy (m-THPC-PDT)	Photodynamic therapy	Clin (97)➢Austria (Rudolfstiftung Hospital) ❑ recurrent ovarian cancer (see FO, p 56)

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Sequus Pharmaceuticals ❑ Schering-Plough, Meiji Seika Pharma International	Doxorubicin encapsulated in long-circulating Stealth liposomes, coated with polyethylene glycol (PEG) ❑ Doxil (USA), Dox-sl, Caelyx (outside the USA)	Anthracycline; liposomal ❑ formulation helps evade recognition and attack by the immune system, therefore, prolonging circulatory half life and allowing for leakage through loose capillary junctions in tumors ❑ IV	A (11/95) & L➢USA, A (6/96)➢Europe ❑ Kaposi's sarcoma; phase II/III (6/97)➢USA ❑ refractory ovarian cancer (see FO, pp 554-557)
Sequus Pharmaceuticals	Cisplatin ❑ SPI-077	Platinum-based drug; liposomal formulation ❑ IV	Phase I (b12/96)➢USA ❑ solid tumors (see FO, pp 367 and 479)
Seragen	$DAB_{389}EGF$	Diphtheria toxin genetically fused to a ligand ❑ binds EGFr on tumor cells, penetrates cells and destroys their ability to manufacture proteins ❑ targets EGFr-expressing tumor cells	Phase I/II (6/97)➢USA (see FO, p 53)
Somatix Therapy (Cell Genesys) ❑ Johns Hopkins U, U Texas, Whitehead Institute, Bristol-Myers Squibb	Allogenic GVAX cancer vaccine	Immunomodulator; allogenic tumor vaccine; gene therapy ❑ tumor cells derived from tumor cell lines, transduced *ex vivo* with the gene for GM-CSF, irradiated, and then re-infused into patients ❑ subcutaneous	Phase I/II (3/97)➢USA (RAC #9408-082) ❑ solid tumors; may have utility in ovarian cancer (see FO, pp 53, 149, 323 and 355)
Sugen	Orally-available PDGF TK signaling pathway inhibitors (SU102 was put on hold)	Small molecule synthetic drug; platelet derived growth factor (PDGF) antagonist ❑ inhibits the PDGF tyrosine kinase (TK) signaling pathway ❑ PO	Preclin➢USA ❑ solid tumors
Sugen ❑ Asta Medica	Pan-Her antagonist (formerly Her2 antagonist)	Small molecule synthetic drug ❑ inhibits Her2 and also blocks the closely related Her1 and Her4 receptors	Preclin➢USA ❑ solid tumors
Sugen ❑ Asta Medica	Raf antagonist	Small molecule synthetic drug ❑ inhibits Raf, a serine-threonine kinase (STK) which, in turn, blocks the tumor forming potential of Ras	Research➢USA ❑ solid tumors
Sugen ❑ New York U Medical Center (licensor), Max-Planck Society (licensor)	SU101	Small molecule synthetic drug; platelet derived growth factor (PDGF) antagonist ❑ inhibits the PDGF tyrosine kinase (TK) signaling pathway ❑ IV, subcutaneous	Phase I/II (o6/97)➢USA ❑ ovarian cancer exhibiting aberrant PDGF TK signaling (see FO, p 195)
TAP Pharmaceuticals (Abbott Laboratories and Takeda joint venture)	Leuprolide acetate, leuprorelin ❑ TAP-144SR ❑ Lupron, Lupron Depot, Enantone, Leuplin	Hormone agonist ❑ inhibits gonadotropin secretion and suppresses ovarian and testicular steroidogenesis ❑ subcutaneous, intramuscular	L (85)➢USA, available in over 50 countries worldwide ❑ prostate cancer (see FO, p 303); phase II (96)➢Italy (in a open prospective study of 32 patients with relapsed ovarian cancer from platinum-based chemotherapy, leuprolide acetate depot (3.75 mg) was administered intramuscularly, every month until tumor progression; 4 (12.5%) patients experienced clinical and/or radiological PR, 5 (15.6%) SD and 23 (71.9%) PD; Marinaccio M, etal, Eur J Gynaecol Oncol, 1996, 17(4):286-8)

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Targeted Genetics/RGene Therapeutics ❑ Laboratoires Fournier, Aronex Pharmaceuticals, M. D. Anderson Cancer Center, U Tennessee Research Corporation (UTRC), U Pittsburgh	tgDCC-E1A ❑ RGG-0853	Gene therapy; immunomodulator ❑ *in vivo* non-viral delivery to cancer cells of E1A tumor suppressor gene complexed with DC-cholesterol ❑ targets HER-2/neu ❑ intraperitoneal, intratumoral	Phase I (b7/96)➢USA (protocol IDs: TGC-E1A-9601, NCI-V97-1127; administered intraperitoneally for up to six months); also see FO, p 484
Terrapin Technologies ❑ Taiho Pharmaceutical	TER286	Highly-modified small peptide ❑ tumor-activated compound that becomes toxic in the presence of P1 glutathione S-transferase (GST) isozyme	Preclin➢USA ❑ drug-resistant solid tumors
Therion Biologics ❑ NCI	TBS-RAS	Live vaccine based on a peptide antigen; gene therapy ❑ live recombinant pox virus vector expresses tumor-specific antigen to elicit cellular immune response ❑ targets ras-presenting tumor cells	Preclin (7/97)➢USA
Therion Biologics ❑ NCI	MUVAC	Live vaccine using a peptide antigen; immunomodulator; gene therapy ❑ live recombinant pox virus vector expresses tumor-specific antigen to elicit cellular immune response ❑ targets mucin on tumor cells	Preclin (7/97)➢USA
Therion Biologics ❑ NCI	TBC-NEU	Live vaccine; immunomodulator; gene therapy ❑ vaccinia virus vectors expressing the neu gene	Preclin (7/97)➢USA
Transgene	Genetically-engineered interferon γ (IFN-γ) ❑ RU-42369	Cytokine; interferon agonist ❑ IV	Phase II➢France
Transgene	Vero cells genetically engineered to express IL-2	Immunotherapeutic/vaccine; gene therapy	Phase I (1/97) France ❑ solid tumors
Trilex Pharmaceuticals (Titan Pharmaceuticals) ❑ U Kentucky Research Foundation, Goodwin Biotechnology (manufacturer), Genzyme Transgenics (manufacturer)	Anti-11D10 antibody ❑ TriAb	Anti-idiotypic MAb; immunomodulator ❑ binds milk fat globule MUC1 antigen ❑ intracutaneous	Phase II/III (6/97)➢USA
U Alabama Department of Obstetrics and Gynecology	177Lu-CC49	Radioimmunotherapy ❑ MAb CC49 linked to lutetium-177 ❑ intraperitoneal	Phase I/II➢USA ❑ refractory ovarian cancer (Alvarez RD, etal, Gynecologic Oncology, 1997 Apr, 65(1):94-101)
U Texas M. D. Anderson Cancer Center, Vincent T. Lombardi Cancer Research Center; Georgetown U Medical Center	Crotoxin	Natural product extract; phospholipase A2 toxin isolated from the venom of the South American rattlesnake, *Crotalus durissus terrificus*; a noncovalent heterodimeric toxin	Phase I (GUMC-95061) (12/96)➢USA ❑ solid tumors
Vertex Pharmaceuticals ❑ BioChem Therapeutic (licensor 5/96, Canadian rights)	Biricodar dicitrate ❑ VX-710	Blocks mdr-1 protein pump activity ❑ IV	Phase I➢USA; phase II (planned for 1997)➢Canada
Vital Pharmaceutical Development ❑ Research Corporation Technologies (licensor, 5/97), Arizona State U, Arizona Cancer Center, U Arizona College of Medicine, NCI	Azamitosenses	Antibiotic related to mitocycin-C ❑ inhibits DNA synthesis	Preclin➢USA ❑ solid tumors
Wyeth-Ayerst	CMB-401		Phase I (5/96)

analysis of an international multi-center study involving 143 platinum-refractory ovarian cancer patients administered Paxene (175 mg/m^2) as a 3-hour infusion (n=120) or, as rescue therapy, as a 96-hour infusion (n=23), at least 7 patients achieved a CR and 31 patients a PR, for an overall response rate of at least 32% in the 3-hour infusion group, and one of 23 patients achieved a PR (4%) and 3 patients remained with stable disease for 16, 20 and 36+ weeks, in the 96-hour infusion group (Gore, ME, etal, ASCO97, Abs. 1251:350a).

In anticipation of patent expiry of Taxol in late December 1997, numerous companies are preparing to file ANDAs for generic versions. A detailed update on the status of taxanes worldwide is the topic of an upcoming special article in FUTURE ONCOLOGY.

Docetaxel (Taxotere; Rhône-Poulenc Rorer) in combination with cisplatin, was safe to administer and demonstrated substantial activity as first-line treatment in chemotherapy-naive Stage Ic-IV epithelial ovarian cancer (EOC) patients. Among 33 evaluable patients, the docetaxel (75-85 mg/m^2) and cisplatin (75 mg/m^2) regimen produced 19 CRs (58%); only three patients (6%) had clinical evidence of disease progression. Incidence of Grade 4 fluid retention, the primary endpoint of the study, was prevented by dexamethasone pre-treatment (Vasey PA, etal, ASCO97, Abs. 1270:356a).

Topoisomerase I Inhibitors

Topoisomerase I (topo I) inhibitors are currently approved for various cancer indications. Topotecan (Hycamptin; SmithKline Beecham) has been launched worldwide for second- or third-line treatment of ovarian cancer. For a detailed discussion of topo I inhibitors, in general, and in ovarian cancer, in particular, see FO, pp 525-544.

GW211, a new water soluble camptothecin analog, under development by Glaxo Wellcome, is being evaluated in a phase II clinical trial involving 55 patients with relapsed ovarian cancer. The drug is being administered as 1.2 mg/m^2 daily infusion, for five days, every 3 weeks; dose reductions to 0.9 mg/m^2 were necessary in 51 cycles and to 0.6 mg/m2 in 2 cycles. As of May 1997, among 46 patients evaluable for response, 1 CR, 7 PR, 19 SD and 19 PD were observed, for an overall response rate of 22% (v. Oosterom AT, etal, ASCO97, Abs. 1250:349a).

Gemcitabine

Gemcitabine (Gemzar; Eli Lilly), a synthetic pyrimidine nucleoside approved for first-line treatment of patients with non-resectable Stage II, III or IV adenocarcinoma of the pancreas, is also being investigated as monotherapy, and in combination with other chemotherapeutics, in advanced ovarian cancer. Gemcitabine, a ribonucleotide reductase inhibitor, is a cell cycle regulator. In a phase II clinical trial of gemcitabine and cisplatin in chemotherapy-naive advanced EOC patients (11

with Stage III and 7 with Stage IV disease), carried out in Spain, a 30-minute IV infusion of gemcitabine (1250 mg/m^2) on days 1 and 8, in combination with cisplatin (100 mg/m^2) on day 1 of a 21 day cycle, was well tolerated. Among 17 evaluable patients, overall response rate was 61%, with 2 CR, 9 PR, 5 SD and 2 PD (Nogue M, etal, ASCO97, Abs. 1335:374a). In another phase II clinical trial involving similar populations and regimen, conducted in France, among 10 evaluable patients, overall response rate was 70% with 3 CR and 4 PR, and 3 SD. In the first 60 cycles, Grade 3 and 4 neutropenia was encountered in 13% and 5% of cycles, Grade 3 and 4 thrombocytopenia in 2% of cycles and Grade 3 vomiting in 17% of cycles. Grade 3 alopecia occurred in 5% of patients. There were two deaths and one patient required hemodialysis after one injection of the combination (Belpomme D, etal, ASCO97, Abs. 1300:365a).

DRUG RESISTANCE

Acquired drug resistance and cross resistance are the Achilles heel of ovarian cancer chemotherapy. Despite availability of three lines of therapy, represented by drugs whose anti-cancer action is based on different mechanisms, drug resistance emerges in a significant proportion of platinum-, taxane- and topo I inhibitor-based regimens. Therefore, development of drugs to overcome mechanisms of resistance or confer tumor sensitivity to certain cytotoxics, is particularly relevant in ovarian cancer. Developers are testing a number of approaches to combat the various resistance mechanisms (see FO, pp 74-79, 112-115, 129-139) exhibited in ovarian cancer or to chemosensitize tumor cells to various cytotoxic agents. Strategies range from development of agents addressing drug resistance to most cytotoxic agents to specific approaches targeting resistance to a particular chemotherapeutic.

Methionine

AntiCancer (San Diego, CA) is evaluating a tumor-selective chemosensitization approach that exploits elevated methionine requirement of tumor cells relative to normal cells, termed methionine dependence. Because the majority of all human tumors have a specific elevated requirement for the amino acid methionine, its depletion should be effective in all solid tumors and hematologic malignancies. Removal of the methionine source selectively starves cancer cells resulting in cell cycle arrest in late-S/G2, just before division, causing selective synchronous arrest of tumor cells. These synchronously arrested tumor cells are much more sensitive to apoptosis and chemotherapy. Methionine depletion is followed by methionine repletion, together with administration of standard anti-mitotic drugs to kill the synchronous wave of mitotic tumor cells. Anti-methionine chemotherapy has been proven effective in human clinical studies using a methionine-free total parental diet, even though this diet only lowered but did not eliminate methionine.

For instance, methionine depletion was shown to strongly modulate efficacy of cisplatin against the MX-t human breast carcinoma cell line grown in nude mice. When tumor-bearing nude mice on a methionine-free diet were treated with intraperitoneal cisplatin at 1 mg/kg, once a week, for three weeks, tumors relatively resistant to either methionine starvation or cisplatin alone, became very sensitive to the combination. Intratumoral platinum concentration was higher when cisplatin was combinated with methionine starvation than as monotherapy (Hoshiya Y, etal, Anticancer Research, 1996 Nov-Dec, 16(6B):3515-7).

AntiCancer's agent, ONCase, is a water-soluble recombinant methioninase enzyme that breaks down methionine in blood. AntiCancer has cloned the L-methionine α-deamino-γ-mercaptomethanelyase (methioninase, METase) gene from ***Pseudomonas putida*** in ***Escherichia coli*** using polymerase chain reaction (PCR) and produced therapeutic quantities of rMETase (Tan Y, etal, Protein Expression and Purification, 1997 Mar, 9(2):233-45). The half-life of rMETase was 2 hours when administered IV in mice. ***In vitro*** and ***in vivo*** studies demonstrated that most solid tumors and hematologic malignancies were sensitive to rMETase while normal cells were insensitive, resulting in no toxicity at effective therapeutic doses. ONCase and is available in high purity crystalline or lyophilized form.

Recombinant ONCase is currently undergoing a phase I clinical trial offshore, sponsored by Shionogi (Osaka, Japan), AntiCancer's collaborator in this area. No toxic side effects have been observed in more than 15 treated patients. As predicted, the level of methioninase in patients' blood decreased 200 fold to below 0.1 uM, which should be sufficient to successfully block tumor growth as demonstrated in preclinical studies with ONCase and in clinical studies using a methionine-free diet. In a pilot phase I clinical trial, a two-hour IV infusion of 5,000 units (0.4 g) and 10,000 units (0.8 g) and a ten-hour infusion of 20,000 units (1.6 g) of methioninase were administered to three patients with advanced breast cancer without any observed toxicity (Tan Y, etal, Anticancer Research, 1996 Nov-Dec, 16(6C):3937-42).

Molecular Markers

Manipulation of various tumor cell genes may also reduce resistance or enhance chemosensitivity of such cells to cytotoxic agents. Anti-TGFR antibodies appear to increase the sensitivity of cell lines to Taxol and cisplatin. Transfer of p53 gene into ovarian cancer cells increases sensitivity to cisplatin. Transfer of BAX, an apoptosis inducer, may maintain tumor cell sensitivity of tumor cells to paclitaxel.

Resistance to Platinum-based/Alkylating Agents

Resistance to platinum-based drugs such as cispaltin and carboplatin, that represent first-line treatment for ovarian cancer, is particularly devastating. Numerous

mechanisms potentially contributing to drug resistance after exposure to alkylating and/or platinum agents ***in vivo*** have been identified, including multidrug resistance (MDR) attributed to P-glycoprotein (P-gp) that acts as an energy-dependent efflux pump, decreasing intracellular drug accumulation and diminishing drug cytotoxicity; rise in intracellular glutathione or metallothionein levels; and capacity of malignant cells to efficiently repair damaged DNA. It was also reported that cisplatin-DNA adducts bind several cellular proteins, termed cisplatin-damaged-DNA recognition proteins, including some that enhance survival of cells by mediating DNA repair and others that hasten death by conferring sensitivity to the drug (Chao CC, Journal of the Formosan Medical Association, 1996 Dec, 95(12):893-900).

Studies of resistance mechanisms have identified a number of agents that reverse cisplatin resistance ***in vitro.*** Different resistance mechanisms arise ***in vitro***, depending on tumor cell exposure to cisplatin. In general, chronic, long-term exposure to rising concentrations of cisplatin seems to lead to permanent elevations in glutathione and metallothionein levels. Once-a-week pulsed administration leads to changes in folate metabolism and oncogene expression while once-a-month acute administration causes defects in drug accumulation (Chao CC, ibid). However, ***in vitro*** effects do not accurately reflect ***in vivo*** situations; in the ***in vivo*** environment, a myriad of additional players such as oncogenes and protein kinase signal transduction pathways, are introduced that interact in a variety of ways. It is this important difference between tumor cell behavior ***in vitro*** and ***in vivo*** that has marred utility of chemosensitivity testing because laboratory findings do not correlate with clinical effectiveness.

A study attempting to establish clinical significance of such markers as metallothionein (MT), heat-shock protein-27 (hsp-27) and glutathione-S-transferase π and α (GST π, GST α), that have been implicated with drug resistance ***in vitro*** in ovarian cancer, found that expression of all four markers did not help predict chemoresistance ***in vivo***. This is not unexpected because ovarian cancer is a highly heterogeneous neoplasm as reflected in the expression of chemoresistance markers. Chemoresistance is more likely multi-factorial and confirms the complexity of the ***in vivo*** model (Germain I, etal, International Journal of Gynecological Pathology, 1996 Jan, 15(1):54-62).

Agents being evaluated in attempts to combat drug resistance to platinum-based regimens include dipyridamole, that may reverse cellular accumulation defects; hydroxyurea, pentoxifylline, and novobiocin, that inhibit DNA repair; ethacrynic acid and buthionine sulfoximine (BSO) that inhibit the glutathione system; and cyclosporine, rapamycin that inactivates a serine/threonine kinase p70(s6k), tamoxifen, and calcium channel-blockers that inhibit relevant signal transduction pathways.

Intracellular glutathione levels are considered to be central to cancer cell resistance to platinum coordination complexes. Glutathione protects cells from the cytotoxic effects of alkylating agents and platinum compounds. However, results from small clinical trials of buthionine sulfoximine (BSO), a synthetic amino acid that irreversibly inhibits glutamylcysteine synthesis and lowers intracellular glutathione levels (Cancer 1995;76:2028-2033), and ethacrynic acid, an inhibitor of glutathione-S transferase (GST), proved disappointing. In a phase I clinical trial of BSO in combination with melphalan (L-PAM), patients were treated with two cycles of BSO; one with BSO alone, administered IV every 12 hours for six doses, and another, one week later, with the same BSO regimen as cycle one but with IV melphalan (15 mg/m^2) administered one hour after the fifth dose. BSO doses were escalated from 1.5 g/m^2 to 17 g/m^2 in 41 patients. The only toxicity attributable to BSO was Grade 1 or 2 nausea/vomiting in 50% of patients. Recommended BSO dose for this schedule is 13 g/m^2, which will be used in phase II trials to be conducted in ovarian cancer and melanoma (O'Dwyer PJ, etal, Journal of Clinical Oncology, 1996 Jan, 14(1):249-56). Researchers at Fox Chase Cancer Center (Philadelphia, PA) also plan a phase I clinical trial of BSO plus carboplatin, and phase I-II clinical trials of aphidicolin plus carboplatin, to determine the toxicity and biochemical effects of BSO and aphidicolin, administered in combination with carboplatin. Aphidicolin, an inhibitor of DNA polymerase α and δ, was shown to potentiate cytotoxicity of cisplatin in preclinical models (Runowicz CD, 1995 Nov 15, 76(10 Suppl):2028-33). Endpoints of the trials are glutathione levels, BSO and aphidicolin pharmacokinetics, and DNA platination.

These same researchers recently isolated a human cDNA clone coding for γ-glutamylcysteinesynthetase (γ-GCS), the rate-limiting enzyme in glutathione synthesis, and demonstrated that the γ-GCS gene is expressed at very high levels in resistant tumor cells. In preclinical studies, an expression vector, containing a full length human γ-GCS cDNA, will be introduced into cisplatin-sensitive ovarian cancer cells to observe changes in γ-GCS enzyme activity and glutathione levels, and evaluate the effect of altered glutathione synthesis on drug sensitivity, in order to establish any causal relationship between glutathione levels and platinum resistance.

TER286, a dual-action agent in development by Terrapin Technologies (South San Francisco, CA), is also exploiting elevated GST activity in tumor cells. TER286 is a highly-modified small peptide (800 MW) consisting of a targeting moiety of a three-amino acid peptide analog of glutathione S transferase (GST), and a toxic alkylating agent that is linked to the cysteine amino acid component of the peptide. TER286 targets the P1-1 isozyme of GST (GST-P1)-1 which catalytically cleaves the molecule freeing the cytotoxic component. TER286 is designed to be activated only in the presence of GST-P1-1 enzyme that is elevated (2-to 10-fold higher than in normal cells) in

many drug-resistant solid tumors. TER286 has exhibited excellent efficacy in animal tumor model systems and in primary human tumor cells in culture. In animals, TER286 also exhibits significantly reduced bone marrow toxicity compared to currently available therapies. However, because the agent is expected to also target GST isozymes in normal cells it is expected to exhibit some toxicity. According to Daniel D. Von Hoff, MD, Director of the Institute for Drug Development, Cancer Therapy & Research Center, and Clinical Professor of Medicine, Division of Medical Oncology at The University of Texas Health Science Center (San Antonio, TX) whose research team performed laboratory studies on the compound, tumor cells from six of 13 breast cancer patients, responded to taxol while tumor cells from six other patients responded only to TER286. Two USA patents (#5,545,621 and 5,556,942) have been issued covering TER286 and analogs.

In August 1997 Terrapin announced that it entered into a collaborative agreement with Taiho Pharmaceutical (Tokyo, Japan). Under terms of the agreement, Taiho will provide research and development funding, and Terrapin will be responsible for advancing TER286 through testing and preparation of an IND application. Upon successful completion of the IND-enabling studies, Taiho and Terrapin an expected to enter into a licensing agreement, providing Taiho with commercialization rights in Japan and possibly other Asian countries.

Metallothioneins, (MT), a major zinc-binding intracellular protein thiol, has been associated with cytoprotection from heavy metals (see FO, pp 505 and 509), antineoplastic drugs, mutagens, and cellular oxidants. MTs, a group of small molecular weight (6-7 kDa) cytoplasmic proteins, have been reported to be induced in various tissues by alkylating agents. Cisplatin resistance of tumor cells with high concentration of metallothionein has been observed in cultured cells and transplanted tumors. As a corollary, increases in metallothionein concentration in normal tissues of tumor-bearing animals prevent side effects of anti-cancer drugs. Also, metallothionein expression appears to be a marker of aggressive tumor behavior in breast cancer.

Resistance to Taxanes

The taxanes are inducers of the MDR gene ***in vitro***. As taxanes move to first-line therapy in ovarian cancer, the specter of drug resistance becomes more ominous. One current approach to improve the therapeutic index of taxanes involves concomitant administration of the cyclosporin analogue PSC833, that improves cellular penetration of taxanes.

SDZ PSC 833, under development by Novartis (Sandoz), is a cyclosporinD analog that reverses resistance by modulating MDR expression through P-glycoprotein (P-gp). It is administered either PO or IV, and is also being evaluated with other chemotherapeutics in solid tumors. In an ongoing phase II clinical trial, SDZ PSC 833 was combined with paclitaxel in 52 heavily pre-treated patients with known paclitaxel-refractory advanced ovarian carcinoma. Starting dose of paclitaxel (70 mg/m^2) was selected based on results from a phase I clinical trial identifying this dose as having similar tolerability to standard single-agent paclitaxel dosing (175 mg/m^2). Responses were observed in 5/49 (10%) patients (3 were too early for evaluation), representing 2 CRs (lasting 12+, 3+ months) and 2 PRs (lasting 9+, 3+ months). Five additional patients had biochemical responses with reduction of CA125 ranging from 60% to 80% of pre-treatment values. Stable disease was documented in 13; 8 patients were withdrawn after one treatment cycle because of deterioration in performance status, presumably attributable to treatment failure, and disease progressed in 23. Hematologic toxicity included Grade 4 neutropenia in 43% of patients and Grade 4 thrombocytopenia in 4%. This trial demonstrated that the SDZ PSC 833/paclitaxel regimen is safe, tolerable, and results in renewed paclitaxel responses in patients with known paclitaxel refractory disease, presumably via modulation of paclitaxel resistance by PSC 833. Investigation of PSC 833 may be warranted following first-line paclitaxel-based therapy, in view of renewed responses in the heavily pre-treated population enrolled in the current study (Fields A, etal, ASCO97, Abs. 1254:351a).

Chemoprotection

The flip side of preventing drug resistance is to exploit it in chemoprotection. This approach attempts to increase drug resistance of normal hematocrit stem cells to protect them against high doses of chemotherapy employed to destroy tumor cells. Multi-drug resistant genes may be transferred into patients' stem cells ***ex vivo***, and then re-transfused to repopulate the bone marrow with drug-resistant stem cells before initiation of chemotherapy. To date, this approach was successful but limited to 2-3 cycles of new red blood cells.

Among various approaches in development to protect hematopoietic cells from chemotherapy, two pursued by Genetix Pharmaceuticals (Cambridge, MA) and Ingenex (Menlo Park, CA), involve transfer of the mdr-1 gene encoding P-gp into hematopoietic stem cells. Both approaches have received approval for human trials and are currently in phase I.

Genetix Pharmaceuticals, a private company, is evaluating transfer of MDR retroviral supernatant (A12M1) in autologous CD34+ cells ***ex vivo***. The company has licensed the retroviral packaging cell line from Columbia University (New York, NY) where a phase I clinical trial of A12M1 is also taking place. In this trial, CD34+ cells, obtained from whole bone marrow and/or peripheral blood, are incubated for 48 hours with growth factors IL-3, IL-6 and stem growth factor provided by Amgen (Thousand Oaks, CA) and then exposed for 24 hours to two charges of A12M1. This process resulted in

MAb in vitro post transduction/pre infusion bone marrow

Exhibit 2
Estimated Potential Market for Therapeutic and Prophylactic Vaccines for Ovarian Cancer in Selected World Regions

Region	Rx Vaccine[1]		Rx Vaccine with Annual Booster[1]		Prophylactic Vaccine[2]		Prophylactic Vaccine with Annual Booster[2]	
	(#)	Market ($ mil)	(#)	Market ($ mil)	(#)	Market ($ mil)	(#)	Market ($ mil)
USA	9,176	22.9	23,976	37.7	14,800	7.4	74,000	28.1
North America	10,013	25.0	26,163	41.2	16,150	8.1	80,750	30.7
Europe[3]	16,483	41.2	43,068	67.8	26,585	13.3	132,925	50.5
Japan	2,211	5.6	5,778	9.1	3,567	1.7	17,835	6.8
Triad[3]	28,707	71.8	75,009	118.1	46,302	23.1	231,510	88.0

[1] *Therapeutic vaccine, administered once-$2,500; annual booster-$1,000*
[2] *Prophylactic vaccine, administered once-$500; annual booster-$350*
[3] *Excluding the former USSR*
Note: *Market estimates are at the 5th year post-introduction*

IMMUNOTHERAPY/VACCINES

Immunotherapy employing a variety of strategies is being actively pursued in ovarian cancer. However, because of the relative rarity of this cancer, market opportunities in terms of candidate populations, are rather limited (see Exhibit 2).

AltaRex

AltaRex (Edmonton, Alberta, Canada) is developing Ovarex therapeutic vaccine, comprising of anti-idiotypic MAb B43.13 that binds with high affinity to CA 125-expressing tumor cells, and elicits an immune response via generation of autologous antigen mimics. Ovarex is administered intravenously (2 mg/dose) without an adjuvant. Studies suggest that both induction of the anti-idiotype network as well as cellular mechanisms account for the observed therapeutic effect.

MAb B43.13, incorporated into Ovarex, was licensed from Biomira (Edmonton, Alberta, Canada), pursuant to an agreement entered in November 1995. AltaRex paid an upfront licensing fee and will make royalty payments. In August 1996, AltaRex entered into a strategic partnership with Medac (Hamburg, Germany). In return of Western European marketing rights for Ovarex, Medac is obligated to fund European clinical trials and pay product registration fees. Medac has also agreed to purchase Ovarex from AltaRex at specifically agreed upon transfer prices. MerckFrosst (Pt. Claire-Dowol, Quebec, Canada) manufactures Ovarex for AltaRex. Ovarex has been designated an orphan drug.

Ovarex has been in development since 1989. Ovarex entered phase II/III clinical trials that will enroll more than 400 women with advanced ovarian cancer, in the fourth quarter 1996. A 15-center, placebo-controlled, double-blind, randomized phase IIb clinical trial, involving over 200 women with Stage III and IV ovarian cancer who have had a complete clinical response to a primary treatment protocol, was initiated in the USA in the second quarter of 1997 after FDA approval was gained in May 1997. Previous studies conducted by AltaRex, involving more than 200 ovarian cancer patients, demonstrated an apparent survival advantage as well as a strong immunological response as a consequence of anti-idiotype induction.

AltaRex is also developing two other technologies expected to begin phase I/II clinical trials in the fourth quarter of 1997, to be used in combination with Ovarex for treatment of ovarian cancer:

- ImmunoRadioTherapy, that involves administration of radioisotope-conjugated MAbs to simultaneously kill residual tumors post-operatively and induce immune responses
- ImmunoPhotodynamic Therapy, that uses laser light to activate a photosensitizer-conjugated MAb administered via IV pre-operatively to induce tumor killing and an immune response during surgery

AVAX Technologies

AVAX Technologies (Kansas City, MO) is an early-stage biopharmaceutical company developing technologies and products for treatment of cancer and other life-threatening diseases. Incorporated in January 1990 as Nehoc, the company changed its name to Appex Technologies and merged into Walden Laboratories in 1992. Walden Laboratories changed its name to AVAX Technologies in March 1996. The company plans to initially develop immunotherapeutics and chemotherapeutics for cancer targets.

In November 1995, AVAX licensed rights from Thomas Jefferson University (Philadelphia, PA) to a patent and patent applications, relating to a modification process, AC Vaccine technology, that produces autologous cell vaccines, in return of $10,000 license fee, milestones and royalties. AVAX also issued 229,121.5 shares

of common stock each to Thomas Jefferson University and Dr. David Berd, inventor of the AC Vaccine technology. The company's immunotherapeutic approach uses hapten-conjugated tumor antigen to stimulate an immune response to the unmodified tumor antigen. Patient's tumor cells are removed, conjugated with hapten, and infused back into the patient with an adjuvant. Initially, the AVAX approach will be used as an adjunct to tumor surgery.

The AC Vaccine technology, currently evaluated in clinical trials in metastatic melanoma, may have utility in the treatment of other cancers, including ovarian, breast, prostate, lung and colorectal cancer and acute myelogenous leukemia (AML). AVAX entered into a sponsored research agreement with Thomas Jefferson University to provide annual research funding for the development of additional immunotherapies based on the AC Vaccine technology. As of July 1997, patient accrual was underway for a phase II clinical trial of O-Vax, the AC Vaccine for ovarian cancer, at Thomas Jefferson University.

Biomira

Biomira is testing its Theratope STn-KLH vaccine in ovarian cancer in phase II clinical trials in the USA and Canada. At the Sixth International Congress on Anti-Cancer Treatment meeting in Paris, France in February 1996, Dr. Tom Ehler, of the British Columbia Cancer Agency (BCCA; Vancouver, Canada) presented an analysis of a phase II clinical trial of patients with metastatic ovarian cancer treated at the BCCA and the Cross Cancer Institute (CCI; Edmonton, Canada). Among 26 evaluable patients treated with Theratope, following platinum-based chemotherapy, 15 with a strong immune response to the vaccine experienced a significantly prolonged median survival of 20.2 months as compared with a survival of 11.7 months for 11 patients with lower levels of immune response.

On May 5, 1997 Biomira and Chiron (Emeryville, CA) announced the formation of a partnership to co-develop Theratope, initially focusing on the treatment of metastatic breast cancer, although additional indications may be pursued. Pursuant to the agreement, the companies will equally fund ongoing R&D, clinical development and regulatory activities involving the vaccine, in the USA and Europe. In addition, Chiron will make an upfront licensing and milestone payment in return of exclusive USA and European marketing and distribution rights. Biomira maintains manufacturing responsibility and Canadian rights, and revenues from sales of Theratope will be shared.

Corixa

Corixa (Seattle, WA) begun phase I clinical trials in September 1996 with a vaccine consisting of naked Her-2/neu peptides, administered in conjuction with GM-CSF. In the trial, 60 patients will be treated for six months with monthly administration of the vaccine. Her-2/neu protein was shown to generate potent T cell immune responses in ***in vitro*** animal and human cells studies. Corixa acquired exclusive worldwide license to Her-2/neu technology in all fields from the University of Washington (Seattle, WA). Corixa is also conducting preclinical studies of microsphere-encapsulated Her-2/neu peptides with leishmania elongation initiating factor (LeIF) as an adjuvant. Corixa entered into an option agreement with Vical (San Diego, CA) in April 1996, to license the LeIF gene as a single gene immunomodulator for cancer. In exchange, Vical paid an up-front option fee and, upon exercising the option, will pay another fee and milestones and royalties. This amended agreement gives Vical until October 26, 1997 to exercise its option.

Trilex Pharmaceuticals

Trilex Pharmaceuticals (Scottsdale, AZ), a biopharmaceutical company incorporated as Ascalon in May 1996, is a wholly-owned subsidiary of Titan Pharmaceuticals (South San Francisco, CA). Trilex uses anti-idiotype antibody technology to develop cancer vaccines that may have application in the treatment of 50% of solid tumors. Trilex has identified four anti-id antibodies that are mirror images of tumor-associated antigens. This anti-id MAbs elicit cellular and humoral responses, at amounts as low as 2 mg per dose, against antigens associated with adenocarcinomas, breast cancer, small cell lung cancer and melanoma.

TriAb (anti-11D10), is a murine monoclonal anti-idiotype antibody that mimics a specific epitope of the human milk fat globule (HMFG). TriAb is generated against the anti-HMFG MAb MC-10 that binds a MUC1 antigen. TriAb is in development for the treatment of breast, lung and ovarian cancer. Pursuant to a license agreement entered in May 1996 with the University of Kentucky Research Foundation (Lexington, KY), Trilex acquired an exclusive worldwide license relating to USA

and foreign patents to 11D10 and two other anti-ids as well as their fragments, derivatives or analogs. In return, Trilex will fund research at the University of Kentucky at $350,000 annually for five years, pay license fees totaling up to $470,000, and make royalty payments on product sales. Trilex has engaged Goodwin Biotechnology Inc. (GBI; Plantation, FL) and Genzyme Transgenics (Framingham, MA), to produce TriAb for clinical trials.

In a completed phase I clinical trial in advanced breast cancer, TriAb induced both humoral and cellular immune responses with minimal toxicity, such as mild erythema and induration at the injection site (Chakraborty M, etal, AACR97, Abs. 4139:616). Accrual of breast cancer patients has begun for phase II trial. Pivotal clinical trials of TriAb in ovarian and non-small cell lung cancer, to be conducted by the GOG, are planned for the fourth quarter of 1997. To augment immunogenicity of 11D10 in vaccinated breast cancer patients, without using any carrier protein or adjuvant, researchers at the University of Kentucky constructed a chimeric 11D10-GM-CSF fusion protein vaccine (Tripathi PK, etal, AACR97, Abs. 563:84).

Other

CAK1, or "mesothelin", is an antigen present on the cell surface in mesothelium and on many mesotheliomas and ovarian cancers. While the role of this differentiation antigen has not yet been determined, it may be implicated in adhesion and in the dissemination of mesotheliomas and of ovarian cancers. CAK1, therefore, is a potential target for monoclonal antibodies to be used in the diagnosis and immunotherapy of these cancers. The gene for CAK1 has been cloned and sequenced.

SMALL MOLECULE DRUGS

CDR Therapeutics

CDR Therapeutics (Seattle, WA), founded in 1996, is developing small molecule drugs that mimic binding regions known as complementarity-determining regions (CDR) of immunoglobulin superfamily proteins such as MAbs. These compounds simulate only the CDR, which represents the very small portion of a MAb that binds to a target. The company is currently developing several of these small molecules, known as CDR mimics, for the treatment of cancer, autoimmune diseases, inflammatory skin diseases, transplant rejection, and eye injuries. CDR mimics, currently in development, bind to defined cellular receptors and have been demonstrated to exert direct biological effects in ***in vitro*** and ***in vivo*** testing. In preclinical studies, certain CDR mimics have been shown to inhibit the activity of cancer-related genes (oncogenes) and suppress tumor growth ***in vivo***. The company's proprietary technology is also being used to make CDR mimics of other types of immunoglobulin superfamily proteins such as adhesion molecules, cytokines and cytokine receptors.

The company has created small molecule drugs representing less than 1% of the total protein that retain its biological activity. These agents consist of as few as five amino acids, arranged in the proper sequence and shape, to simulate the CDR of immunoglobulin superfamily proteins. The compounds are then further modified to improve their stability and bioavailability. Laboratory studies have shown that these CDR mimics recapitulate the biological effects of the parent proteins. Importantly, these small molecules readily penetrate target tissues and should have enhanced bioavailibility compared to large complex proteins such as MAbs. CDR mimics can be synthesized using standard medicinal chemistry procedures.

In August, 1996, CDR Therapeutics raised $6 million in an initial venture capital financing from such institutional investors as The Sprout Group, Alta Partners (the successor fund to Burr, Egan, Deleage), Sofinnova, and Arch Partners. In January, 1997, CDR Therapeutics obtained an exclusive license from the University of Pennsylvania (Philadelphia, PA) for technology developed by Mark Greene, MD, PhD, to create CDR mimics. A broad-based patent covering this technology was recently allowed in the USA. The company has also licensed several additional patents and patent applications from the University of Pennsylvania which cover the mimics themselves, their targets, as well as clinical applications.

In the cancer area, CDR Therapeutics has developed two CDR mimics, Mim 16.1 and Mim 4D5.1, both of which bind to a unique receptor that is overexpressed in aggressive forms of most solid tumors including breast, ovarian, pancreatic, lung, prostate, and bladder cancer. These CDR mimics have been shown to inhibit growth of many types of cancer ***in vitro*** and ***in vivo***. The company plans to initially focus on the development of these compounds for the treatment of breast cancer.

Sugen

Sugen (Redwood City, CA) is developing SU101, a small synthetic molecule which inhibits the platelet-derived growth (PDGF) tyrosine kinase (TK) signalling pathway. PDGF is a growth factor ligand that stimulates growth of a variety of cell types through binding to the PDGF TK. Imbalances in the PDGF TK signaling pathway have been implicated in subsets of such malignancies as brain, ovarian, prostate and lung cancer and melanoma. SU101 is structurally similar to leflunomide, under development by Hoechst Marion Roussel in rheumatoid arthritis. In August 1995, Sugen entered into a CRADA with the NCI under which the NCI may participate in the clinical development of SU101. As of March 3, 1997 over 100 patients have been treated with SU101.

In an on-going phase I/II clinical trial, cohorts of patients with advanced ovarian, nsclc or prostate cancer, were administered escalating doses of SU101 (via IV infusion) as a loading dose on days l-4, with weekly

maintenance. Among 23 patients (6 ovarian) treated, Grade 2 toxicities observed included fatigue, diarrhea, fever, peripheral neuropathies, hypertension, anemia and thrombocytopenia, and Grade 1 toxicities, nausea, epigastric discomfort, headache and increased shortness of breath. A new formulation of SU101 resulted in similar values for SU101 and its active metabolite, SU20 (Rosen L, etal, ASCO97, Abs. 739:211a). In another phase I clinical trial in solid tumors, four patients (1 ovarian) who experienced stable disease, are continuing to a second cycle of therapy; three of these patients had positive PDGF staining by immunohistochemistry (VanUmmersen L, etal, ASCO97, Abs. 740:211a).

RADIOISOTOPES/RADIOIMMUNOCONJUGATES

Radioisotope-based agents appear particularly well suited for treatment of ovarian cancer because they can be delivered intraperitoneally (IP). One widely studied such agent, radioactive chromic phosphate (p^{32}), was effective in certain circumstances, but the limited penetration (3-4 mm) of the beta particles restricts its application to only minimal residual disease. In studies of IP administration of p^{32} versus cisplatin, the two regimens produced comparable 5-year survival rates (80% versus 78%). In view of little difference in long-term survival, cisplatin is favored over IP p^{32}, because approximately 5% of the patients in the p^{32} arm required surgical intervention for bowel obstruction. IP p^{32} is currently being evaluated in a phase III clinical trial by the GOG, to potentially treat microscopic disease. This study has been comparing IP p^{32} (15 mCi) versus intravenous cyclophosphamide (1 gm/m^2) plus cisplatin (100 mg/m^2), every 21 days, for three cycles. In a completed study of p^{32}, reported by the GOG (Young RC, etal, NEJM 1990;322:1021-7), that evaluated the effect of adjuvant melphalan versus intraperitoneal p^{32}, disease-free survival rate was 80% in each arm; there was no statistical difference between these groups after a median follow-up of over six years. Because of the added cost, and the risk of leukemia for the patients in the melphalan arm, p^{32} was selected as the control arm for the next trial by this group.

Radioimmunoconjugates, administered both IP and systemically, also show promise in treating ovarian cancer. Used in combination with surgery and immunotherapy, they may prove to be viable adjunctive procedures in managing metastatic disease.

Cancer Therapeutics

Cancer Therapeutics, a joint venture established in 1996, between Antisoma (London, UK) and the Imperial Cancer Research Fund (ICRF), is developing Theragyn, a single-dose adjuvant treatment that has clinically demonstrated improvements in 5-year survival in ovarian cancer. Theragyn is a yttrium-90-labeled murine IgG1 MAb (HMFG1) directed against tumor-selective, abnormally-glycosylated polymorphic epithelial mucin (PEM), expressed at high levels on the cell surface by over 90% ovarian tumors. The MAb serves as a tumor targeting vehicle, specifically delivering lethal amounts of radioactivity to the PEM-expressing cancer cells. Radioisotope is bound to HMFG1 by a CITC-DTPA chelate that confers improved complex stability relative to conventional chelate chemistry, in order to reduce bone marrow toxicity associated with unchelated yttrium-90. HMFG1 is produced by a mouse hybridoma cell line developed by ICRF and licensed exclusively to Cancer Therapeutics; the chelate (US patent US4, 622,420) is licensed exclusively from the University of California.

Theragyn targets and destroys tumor cells remaining in the peritoneum following conventional therapy and may, therefore, prevent relapse and increase survival. Increased survival was demonstrated in a phase II clinical study in which 80% patients were alive 5 years post-Theragyn treatment, compared to 55% of historically matched controls (Hird V, etal, British Journal of Cancer, 1993 Aug, 68(2):403-6). ICRF is conducting a randomized study, seeking to recruit 40 patients on active therapy. A randomized, placebo-controlled, double-blind, multinational and multi-center trial, to enroll approximately 300 patients (half on active therapy), is scheduled to be initiated by Antisoma in October 1997.

Immunomedics

Immunomedics (Morris Plains, NJ), in collaboration with the Center for Molecular Medicine and Immunology (CMMI), is developing a radioimmunoconjugate, murine MN-14 anti-CEA MAb (CEA-Cide) linked to iodine-131, for the treatment of ovarian cancer. Studies were performed at the Garden State Cancer Center (Newark, NJ), and St. Joseph's Hospital and Medical Center (Paterson, NJ) under an IND obtained by CMMI, involving 11 patients with chemotherapy-refractive epithelial ovarian cancer (Juweid M, etal, ASCO96, Abs. 1378:443). Of nine evaluable patients, one, with diffuse perineal implants ≤2 cm or less, exhibited a CR lasting eight months, following an initial PR of ten months. The patient's tumor had been refractory to paclitaxel. The patient was treated with two cycles of CEA-Cide, each at an intravenous dose of 74.4 mCi (5.3 mg), four months apart. The agent was effective despite presence of HAMA. No toxicity was seen with the first treatment and only Grade 2 leukopenia and Grade 1 thrombocytopenia with the second dose, both clearing within six weeks. The mechanism involved in the response achieved in this patient has not been identified, but it could be partially attributable to a cellular immune response against the murine MAb. Other studies have shown that ovarian cancer patients treated with murine MAbs develop T cells that proliferate ***in vitro*** against these MAbs as antigens. It is, therefore, possible that the response was the result of synergistic effects of radiation and the immune response to the murine MAb (Juweid M, etal, J Nuclear Medicine, February 1997, 38(2):257-260).

NeoRx

NeoRx (Seattle, WA) is developing a radioimmunotherapy agent, Avicidin, based on its Pretarget technology which takes advantage of the ultra-high binding affinity of two molecules, biotin and streptavidin. In this approach, an antibody specific to antigen markers on tumor cells, is conjugated to streptavidin and administered to patients. After 24-36 hours, an agent is used to clear unbound circulating conjugate from the bloodstream. Finally, a radioisotope, yttrium-90, linked to biotin, is administered which binds to the streptavidin found at the tumor sites and the beta emission of the isotope destroys the cells. In June 1997 NeoRx reported that interim data from phase I/II clinical trials, demonstrated safety and tumor shrinkage in patients with refractory prostate, ovarian and colon cancer. In addition, NeoRx noted that at 100 mCi/m^2 of yttrium-90, or approximately 6-7 times the maximum tolerated dose (MTD) of the conventional approach, the MTD for Avicidin had not yet been reached, and that the study is proceeding to the next dose level. NeoRx also has data indicating that a newly-tested clearing agent reduced blood exposure by another 50% which may allow higher doses prior to reaching the MTD for bone marrow. Clinical investigators are continuing to increase the single radiation dose level using the murine antibody, while NeoRx scientists are simultaneously preparing a humanized version that may be able to be administered repeatedly to the same patient.

In August 1997 NeoRx announced that it entered into an agreement with Janssen Pharmaceutica NV (Beerse, Belgium), a wholly-owned subsidiary of Johnson & Johnson, for the worldwide development, manufacture and distribution of Avicidin. NeoRx received a $10 million up front fee, of which 50% was for purchase of equity, and may receive up to an additional $50 million for achieving certain milestones. Janssen Pharmaceutica has agreed to assume responsibility for registration and commercialization of the product and to fund an estimated 95% of the remaining costs for product development and clinical trials. In exchange, Johnson & Johnson obtained a worldwide exclusive license to Avicidin and the right of first negotiation to subsequent oncology products employing the company's Pretarget technology. Additional oncology products that fall under this agreement will trigger royalty obligations and may result in additional milestone payments to NeoRx. NeoRx has retained broad development and marketing rights for its Pretarget technology.

University of Alabama

Researchers at the Department of Obstetrics and Gynecology of the University of Alabama at Birmingham, conducted a phase I/II clinical trial of intraperitoneal radioimmunotherapy with 177Lu-CC49, involving 27 ovarian cancer patients who failed chemotherapy, had disease confined to the abdominal cavity with or without retroperitoneal lymph node involvement, adequate organ function, and no previous radiation. Lutetium-177 is a member of the family of elements known as lanthanides or rare earths which also includes samarium-153, and yttrium-90. The most common treatment side effects were delayed, transient arthralgia (10/27) and marrow suppression at MDT of 45 mCi/m^2. One of thirteen patients with gross disease experienced >50% tumor reduction after therapy; most others with gross disease progressed (one exited the study with stable disease at 11 weeks). Seven of nine patients with nodules <1 cm progressed in ≤ 21 months, and two of nine remained without evidence of disease at 4 to 5 months. Among those with microscopic or occult disease, one relapsed at 10 months and four of five remained without evidence of disease at >6 to 35 months. Marrow suppression was the dose-limiting toxicity of intraperitoneal immunotherapy with 177Lu-CC49. Anti-tumor effects were noted against chemotherapy-resistant ovarian cancer, even at lower dose levels, and resulted in prolonged disease-free survival in most patients with microscopic disease (Alvarez RD, etal, Gynecologic Oncology, 1997 Apr, 65(1):94-101).

University of Chicago

Researchers at the Department of Obstetrics and Gynecology at the University of Chicago, performed ***in vitro*** and ***in vivo*** studies with alpha-emitting radionuclide bismuth 212 for intraperitoneal treatment of microscopic ovarian carcinoma. In ***in vitro*** studies, bismuth 212 was three times more effective in eradicating tumor cells grown in monolayers and in 800 micron spheroids. In ***in vivo*** studies, bismuth 212 was distributed uniformly after IP administration. In animal studies, hematologic toxicity was reversible with minimal organ damage. Bismuth 212 prolonged survival and cured up to 40% of animals inoculated with Ehrlich carcinoma cells. Doses administered were effective in eradicating tumor cells within the radiotolerance of normal human tissue (Rotmensch J, etal, American Journal of Obstetrics and Gynecology, 1997 Apr, 176(4):833-40).

PHOTODYNAMIC THERAPY

Similarly to radioimmunotherapy, photodynamic therapy (PDT) is also emerging as an effective adjuvant therapy for many accessible tumors, including ovarian cancers. Although PDT is initially very effective in shrinking tumors, its effects are short lived. To achieve more lasting results developers are combining PDT with immunotherapy. Numerous companies are engaged in PDT R&D, as reported in various issues of FUTURE ONCOLOGY.

In the ovarian cancer area, AltaRex is developing PDT under a license obtained in October 1996 from the University of Alberta in Canada, for commercialization of hypocrellin, a synthetic compound originally extracted from a Chinese mushroom by Dr. W. Lown. The company is initially planning to use this technology in ovarian cancer to assist surgeons in the removal of small tumor masses as well as in combination with its Ovarex

immunotherapeutic vaccine.

Interestingly, Ergo Science (Charlestown, MA) has reported that, in animal models, treatment using its benzophenothiazine-based PDT dyes (ER-470 and ER-480) induce a specific systemic anti-tumor immune response. When PDT-treated EMT-6 tumor-bearing mice which showed complete tumor regression after 26 days, were rechallenged with up to 5x105 EMT-6 tumor cells, 73% of them exhibited a complete tumor regression compared with 0% regression in controls. In addition, PDT-treated hosts were incapable of rejecting tumor challenge with other carcinoma cells, indicating that a tumor-specific immune response had been elicited. Also, a role for T cells in the PDT-induced anti-tumor immune response was confirmed because a similiar approach failed to eradicate EMT-6 tumors in nude mice. See FO, p 488.

INHIBITION OF METASTASIS

Location of ovarian cancer in the abdominal cavity facilitates non-hematogenous metastasis (shedding) of tumor cells into the peritoneal cavity that subsequently become attached onto the peritoneal mesothelial surfaces that line the bowel and abdominal wall. Researchers at the Dana-Farber Cancer Center at Harvard University (Boston, MA) demonstrated that CD44H, a major receptor for hyaluronic acid, may be partly responsible for mediating adhesion of ovarian cancer cells to peritoneal mesothelium ***in vivo***. Previously, binding of ovarian cancer cells to peritoneal mesothelial monolayers was shown to be partly inhibited by neutralizing anti-CD44 antibody ***in vitro***. This data suggests that the CD44 molecule plays an important role in ovarian cancer metastasis and inhibition of CD44 function may represent a novel approach to limit intra-abdominal spread of ovarian cancer (Strobel T, etal, Cancer Research, April 1, 1997, 57:1228-1232).

Anika Therapeutics

Anika Therapeutics (Woburn, MA) is developing therapies based on hyaluronic acid (HA) oligosaccharides that are discrete pieces of the HA polysaccharide chain. HA cell surface receptors are found in many different types of normal cells and in those involved in various disease processes. HA oligosaccharides bind to and block HA receptors, thus modifying the behavior of cells bearing these receptors. Anika has licensed this technology from Dr. Bryan Toole of Tufts University (Bedford, MA) and has filed for patent protection. Experimental studies performed at Dana-Farber Cancer Center and Massachusetts General Hospital (Boston, MA), have demonstrated that Anika's HA oligosaccharides inhibited metastasis of ovarian cancer and melanoma cells in mice.

MOLECULAR APPROACHES TO MANAGEMENT OF OVARIAN CANCER

Numerous markers have been associated with ovarian cancer (see FO, pp 516-520). However, exploiting novel markers to develop ovarian cancer therapeutic strategies is an arduous task.

BRCA1/BRCA2

Of the 5 to 10% of all epithelial ovarian cancer cases that are presumed to be familial cases, more than 70% may be attributable to BRCA1, while BRCA2 alone is linked to 30% of cases. Use of BRCA1 in the treatment of ovarian cancer, an intriguing option, is being tested by researchers at Vanderbilt University (Nashville, TN) and University of Washington (Seattle, WA) subsequently to obtaining approval from the FDA and the NIH Office of Recombinant DNA Activities (ORDA) in March 1996. In this trial, up to 15 women with refractory ovarian cancer are to be injected intraperitoneally, over four days, with 400 million to 40 billion genetically-engineered murine-leukemia retroviruses (LXSN) carrying wild-type BRCA1 genes. In preclinical testing, transfer of BRCA1 gene in nude mice bearing breast cancer xenografts, caused tumors to shrink or disappear altogether. An alternative to gene transfer, currently in evaluation, would be use of BRCA1 protein.

p53

The p53 gene is rarely mutated or overexpressed in benign, suspected or early-stage ovarian cancer; only about 15% of cases with localized ovarian cancer show mutation of p53, whereas 40-50% of ovarian cancer cases advanced to the peritoneal cavity or further, express p53 abnormalities. Thus, the gene is not effective as a means for screening for initial ovarian cancer. Research has demonstrated that transferring p53 can suppress the malignant phenotype by restoring gene function. Studies at M. D. Anderson Cancer Center showed that transferring p53 + adenovirus into normal or mutated ovarian cancer cells, inhibits their growth, suggesting that an overabundance of p53 can cause apoptosis or some other related mechanism of destruction. Numerous p53-related projects are ongoing in various cancers and many have been described in previous issues of FUTURE ONCOLOGY.

ras

Approximately 30% of human tumors contain mutant ras genes. Oncogenic ras genes promote cancer and tumor formation by disrupting normal controls on cell proliferation and differentiation.

Cortecs International (London, UK), a British-based public Australian company, discovered a group of novel, natural molecules (CELLCOM) that have anti-cancer activity. Cortecs' strategy for preventing tumor growth is use of specific molecules to block signaling pathways within tumor cells. These new molecules block activity mitogen-activated protein (MAP) kinase, an enzyme which is activated by ras. In work carried out with the CRC Centre for Cancer Therapeutics at the Institute for Cancer Research at The Royal Marsden Hospital (London, UK), Cortecs has shown that these novel molecules

display anti-tumor activity against several human cancers ***in vitro***, including ovarian, lung, colon and breast cancer and melanoma cell lines. ***In vivo*** results with the lead compound demonstrate inhibition of human ovarian tumor growth in a laboratory model without clinically observed toxic effects. Several international patents have been filed on the CELLCOM technology.

Her-2/neu (erbB-2)

Although Her-2 is an ovarian cancer marker, most of the approximately 30% of cases of ovarian tumors that over-express Her-2 are usually advanced stages of the disease; when treated with platinum-based regimens, patients with such cancers have about half the median survival compared to cases without over-expression of Her-2. This may be attributed to resistance to cisplatin therapies. Used as a therapy, over-expression of Her-2 can inhibit cell growth and results in long-term survival in mice with ovarian cancer. An extensive review of agents addressing Her-2 is presented in FO, V2 #9/10.

Anti-erbB-2 sFv (Ad21), an adenovirally delivered anti-erbB-2 intracellular single-chain antibody (sFv), was shown to cause specific cytotoxicity in erbB-2-over-expressing ovarian carcinoma cells ***in vitro***. Also, adenoviral-mediated delivery of this agent in humans appear to be effectively assayed, potentially free of vector-associated toxicity, and retain biologic utility based on tumor specificity. In a RAC-approved phase I clinical trial of recombinant adenovirus vector-mediated delivery of an Ad21 gene in previously treated ovarian and extraovarian cancer patients, being conducted at the University of Alabama at Birmingham (Alvarez RD and Curiel DT, Human Gene Therapy, 1997 Jan 20, 8(2):229-42), expression of the anti-erbB-2 sFv could be readily detected in target tumor cells by ***in situ*** hybridization (Deshane J, etal, Gynecologic Oncology, 1997 Mar, 64(3):378-85).

Epidermal Growth Factor Receptor (EGFr)

Research shows that ovarian tumor cells which have lost epidermal growth factor receptor (EGFr) expression, have a slightly better prognosis than those with intact potential for autocrine stimulation. ***In vitro*** investigations have shown that certain ovarian cancer cell lines secrete and respond to transforming growth factor-α (TGF-α), suggesting that endogenous activation of EGFr through autocrine or paracrine mechanisms may contribute to the proliferative response. However, researchers found that EGFr activation through autocrine pathways is not a major mechanism for growth of many ovarian cancer cell lines. Other EGFr-independent pathways of signal transduction may be important in the proliferation for ovarian cancer cells and, therefore, strategies designed to inhibit ovarian cancer cell growth through disruption of EGFr may not prove effective (Ottensmeier C; Swanson L, etal, British Journal of Cancer, 1996 Aug, 74(3):446-52).

THE CELL CYCLE

In order for an organism to grow, cells in the body proceed through a cyclical series of sub-cellular events, termed the "cell cycle", resulting in cell division and, thus, proliferation. All cells possess the necessary components in order to duplicate. Originally described by steps visible by light microscopy, during the cell cycle, a cell alternates between S phase (replicating its DNA) and M phase (mitosis, or cell division). Between these phases there are gaps of time required to prepare for either S or M phase, with G1 occurring prior to S phase and G2 occurring prior to M phase, and G0, being a resting state. Various inhibitory and stimulatory signals, received by the cell, determine whether the cell will divide, differentiate, arrest, repair DNA defects, or undergo apoptosis.

In humans, all somatic cells undergo a similar process of cell division. Cells that are stimulated to duplicate will enter from G0 into the G1 phase of the cell cycle, synthesizing proteins and performing other functions in preparation for DNA synthesis. DNA replication occurs entirely in S phase, which is followed by the G2 phase of the cell cycle. In G2, a cell prepares to divide into two daughter cells, with cell division (mitosis) taking place in M phase. As cells emerge from mitosis, they re-enter the G1 phase of the cell cycle. As long as sufficient stimuli are present, each daughter cell will continue to progress through the cell cycle, and again undergo cell division.

Early research in the mammalian cell cycle defined the stimulatory requirements of a variety of different cell types. For most cells, commitment to the process of cell division occurs in G1. When sufficient stimuli are present, a cell will proceed all the way through DNA replication and mitosis, and (the two daughter cells will) enter back into G1, even in the absence of further stimuli. In the subsequent G1, the cell will again require sufficient stimulation to continue through the cycle.

This article briefly reviews the complex regulatory control of the cell cycle, and identifies some of the more common mutations that are associated with cell cycle dysregulation encountered in human cancers. Information incorporated in this article was garnered from a variety of sources including reports from two meetings that took place in San Diego, CA, the 88th annual meeting of the American Association for Cancer Research, held on April 12-16, 1997, and Cell Cycle Therapeutics: Setting the Balance, sponsored by NMHCC Bio/Technology Conference Division (617 270-6004), held on June 2-3, 1997.

CELL CYCLE CONTROL

The process of DNA duplication and cell division is strictly regulated, so that cells only duplicate their DNA when conditions are perfectly favorable, and only divide

into two new cells when precise DNA duplication has been achieved. Before cells commit to making DNA, they must pass through a checkpoint. Checkpoints prevent mutated genes from being passed to the next generation. If conditions appear favorable, cells pass through the checkpoint and normal cells then begin DNA replication.

Recent findings have led to a more detailed understanding of the working of the cellular clock at the molecular level. Using this information, rational, focused diagnostic tools and therapeutic interventions are actively being designed, which may ultimately lead to improved strategies for fighting cancer and many other human diseases.

Growth Signals

Growth-stimulatory signals exert their effects on cells through a series of regulatory protein cascades, emanating from surface receptors on the outside of a cell, all the way to the nucleus. For example, many mitogens stimulate the mitogen-activated protein (MAP) kinase cascade, while a variety of stress signals activate a stress-activated protein kinase cascade. The majority of human oncogenes, including ras, the first human oncogene to be identified, act to provide continued stimulation even in the absence of mitogen. Ras itself functions in part as an intermediary in the MAP kinase cascade; in its oncogenic form it activates the MAP kinase cascade, comprising a series of protein enzyme kinases that activate each subsequent substrate by phosphorylation. MAP kinase kinase (MAPKK) will phosphorylate MAP kinase (MAPK), which will then be activated and phosphorylate MAP kinase itself, which will, in turn, activate other enzymes in this pathway.

Cyclins and Cyclin-dependent Kinases

All signals ultimately converge on regulatory proteins of the nucleus, resulting in activation of specialized proteins called cyclins and cyclin-dependent kinases (cdk), as well as other proteins. Normally, the cell is driven from one phase of the cell cycle to the next by specific enzymes controlled by several cyclins, each of which controls specific time segments of the normal cell cycle. Cyclins synchronize the cellular machinery resulting in appropriate and controlled duplication of the cell. Cyclins were first identified as having cyclical patterns of expression during the cell cycle. They share intra- and interspecies sequence similarity and, in general, form heterodimers with and provide regulatory control and substrate specificity to cdks which, in turn, activate or inhibit cell cycle proteins by specific phosphorylation. To date, more than 10 cyclins, including cyclins A, B1, C, D1, D2, D3, E, H, and 8 cdks have been cloned.

In G1, cyclins are synthesized and activated in response to growth stimuli. In particular, D-type cyclins and their cdk appear to be common targets of multiple signaling pathways. Signaling by a variety of different mitogens (including PDGF and estrogen) or oncogenes (such as ras), lead to D-type cyclin:cdk activity. In con-

trast, tumor suppressor genes such as APC, WT-1, and MTS-1 (p16), or TGF-β, inhibit G1 cyclin kinase activity. The G1 phase is not only controlled by the D-type cyclins (D1, D2, and D3), along with cdk4 or 6, but also by cyclin E and cdk2.

S and G2 phases are controlled by cyclin A and either cdk2 or cdc2 (cdk1) while G2 and M are controlled by cyclin B along with cdc2. Cyclin B:cdc2 was originally defined biochemically as the extract required for mitosis, termed maturation promoting factor (MPF). The role of other cyclins and cdks is not as well understood.

Targets of cyclins and cdks. Cyclin:cdk complexes promote growth by phosphorylating specific targets, including the retinoblastoma (Rb) protein, the pivotal protein in cell cycle regulation, and a related family of tumor suppressor proteins, p107 and p130. These proteins are also referred to as pocket proteins because they form a molecular pocket which interacts with the E2F complex and inhibits its ability to activate transcription. Rb appears to exert its growth inhibitory effects by binding critical factors, including a subset of the E2F family of transcription factors, preventing their downstream function and resulting in cell cycle block at the G1 to S phase transition. In turn, Rb is affected by several regulatory pathways, one of which contains the kip family of proteins, consisting of p21, p27 and p57. Because the Rb regulatory pathway is generally disrupted in cancer cells, understanding the function of Rb, the activities it regulates, and the proteins which affect its activity, is of fundamental importance in the development of anticancer strategies.

Rb not only inactivates free E2F, but the Rb:E2F complex can actively repress transcription from E2F-dependent genes. Upon phosphorylation by cyclin:cdk, Rb releases transcription factors which drive gene expression and cell cycle progression. Importance of this form of Rb control is demonstrated in mice that are genetically deficient in E2F1, which develop a wide variety of tumors of different histologic types, indicating that E2F-1 also functions as a tumor suppressor (Yamasaki L, etal, Cell, 1996 May 17, 85(4):537-48).

Cyclin kinases involved in G1, share some overlapping specificity. For example, Rb is phosphorylated in a sequential, co-operative fashion in G1, first by cyclin D:cdk and then by cyclin E:cdk2. In one model system, cyclin D:cdk only starts the process, and cyclins E/A:cdk2 only act on partially phosphorylated forms of Rb, to lead to full phosphorylation and inactivation of Rb, release of E2F and other factors, and subsequent transcription of genes required for S phase. Cyclins A and B with their corresponding cdk, maintain Rb in its hyperphosphorylated state throughout mitosis.

In late G1, cyclin E:cdk2 complexes also phosphorylate other targets such as histones, causing relaxation of chromatin structures and allowing DNA replication in S phase. They also provide negative feedback regulation, phosphorylating cyclin E itself and targeting it for ubiquitin-mediated destruction (Clurman BE, etal, Genes Dev, 1996 Aug 15, 10(16):1979-90). Other targets of cyclin:cdk include lamins, transcription factors, and the cell's transcription machinery. Relationship between cyclin kinases and their targets are complex. For example, cyclin E kinase activity appears necessary for E2F transcription factor activity (Zerfass-Thome K, etal, Mol Cell Biol, 1997 Jan, 17(1):407-15), while cyclin A:cdk2 phosphorylates the DP partner of E2Fs, inactivating the transcription factor (Dynlacht BD, etal, Genes Dev, 1994 Aug 1, 8(15):1772-86). In addition, recent evidence suggests a non catalytic role for cyclin D1 alone in the activation of the estrogen receptor (ER) in breast epithelium (Zwijsen RM, etal, Cell, 1997 Feb 7, 88(3):405-15).

Mechanism of Action of the Retinoblastoma (Rb) Tumor Suppressor

Douglas Dean of Washington University, in a presentation at the 1997 meeting of the AACR, described studies on the role of Rb in the G1 to S phase transition of the cell cycle. Rb blocks E2F-mediated transcriptional activation by becoming "tethered" to it. But Rb tethered to E2F also acts as a repressor, blocking activation of surrounding enhancers on promoters. The question is whether both the E2F inhibition and the repressor activities of Rb are necessary to regulate cell cycle genes.

To first determine whether E2F is required for the G1 to S transition, a dominant/negative form of E2F (CN-E2F) that lacks the transcription activation domain was created which was expected to displace wild-type E2F and block its activity. In a colony-forming proliferation assay, overexpression of Rb dramatically decreases the growth rate by arresting cells in G1. In this same assay, CN-E2F was able to block the growth-suppression of Rb, suggesting that Rb, tethered to E2F, is necessary to regulate the G1-S transition. Since CN-E2F did not itself inhibit cell proliferation, the transcriptional activation domain of E2F is not necessary for entry into S phase and, therefore, expression of genes regulated by E2F is not required for the G1-S transition, implying that the regulatory step is not the inhibition of E2F by Rb.

Although the repressor activity of Rb, tethered to E2F, is probably required for transition into S phase, it is not clear how Rb functions as a repressor. One possibility is that it mimics components of a transcription complex and recruits them into inactive complexes. The active repressor consists of domains A and B of Rb which are highly conserved and interact intra-molecularly. A pocket at A-B binds oncoproteins such as EIA. Rb activity can also be inactivated by G1 cdks which disrupt the A-B domain interaction. To investigate the molecular requirements for A-B domain repressor function, fusion genes between the A and B domains of Rb and p107, which have very similar repressor motifs, were created downstream of the Gal 4 gene and the expressed fusion proteins were assayed for repressor activity. When A and

B domains of Rb or p107 were expressed as either switched domain fusion proteins, or co-expressed as separate fusion proteins, they were active repressors, whereas neither functioned alone. Any combination of A and B domain fusion proteins was able to form an active repressor, suggesting that a relatively weak interaction is fully functional.

To study the mechanism of repression by Rb, three possible alternative models were considered, transactivation, inhibition of basal transcription complexes, and mimicking basal transcription complexes. The last model was deemed most likely because there are conformational similarities between the C-terminal portion of the transcription factor TBP and Rb A domain, and between transcription complex TFIIB and the Rb B domain. Formation of complexes between transcription factors and Rb domains were demonstrated by immunoprecipitation. TBA was co-immunoprecipitated with the Rb B domain and TFIIB with the Rb A domain. In fact, the TBA-Rb B complex forms a repressor with the same specificity as the Rb A-B pocket. Results support the model that Rb ties up transcription factors in inactive complexes. In this model, Rb is drawn to the transcriptional control region by the strong E2F interaction, and, once at the promoter, it can bind with relatively low affinity to nearby transcription factors. By doing this, Rb acts as a repressor, inhibiting expression of genes required for the G1-S transition.

E2F regulation by cell cycle-dependent changes in subcellular localization. Jacqueline A. Lees of Massachusetts Institute of Technology (Cambridge, MA), in a presentation at the 1997 annual meeting of the AACR, described her work regarding differences in the E2F-pocket protein interactions at various intracellular locations. Of the three pocket proteins, however, only Rb is found to be mutated in many cancers. There is, thus far, no evidence that mutations in p107 and p130 play any role in tumorigenesis.

The active E2F complex is a heterodimer composed of two different families of sub-units, one of which is called DP and the other, confusingly, is called E2F. There are 5 E2F proteins (E2F1-5) and all have DNA binding leucine zipper motifs and a pocket binding motif. There are only two members of the DP family, DP1 and DP2, and they contain a DNA-binding domain and a dimerization domain but no pocket binding motif. E2F1-3 are highly related, while E2F4 and 5 form a distinct family. E2F proteins are differentially expressed in the cell cycle. E2F1-3 which bind Rb are expressed at low levels (less than 20% of the total E2F activity) at the G1 through S phases of the cycle. These forms of E2F are associated with the activation of E2F genes. E2F5, which binds p130 is expressed at low levels at the G0 stage. E2F4 which binds p107, p130 and Rb, is unique in that it is expressed at high levels at all stages of the cell cycle.

To determine where E2Fs are located in the cell, MAbs to the different E2F forms were used to analyze cellular subtractions. E2F1-3 were found in nuclei, while E2F4 was located in the cytoplasm. This suggested a regulation of subcellular localization and, perhaps, an inactivation in the cytoplasm. To determine where the E2F functional complexes were located, gel shift assays were performed. In G1 there were active complexes in both the cytoplasm and the nucleus; while in G2, activity was found only in the cytoplasm. These changes mirrored the levels of E2F protein in each location. When the complexes were analyzed for the presence of pocket proteins it was found that the p130 and the p107 complexes were primarily cytoplasmic, and the Rb complex was nuclear. In G0 the predominant active E2F complex was bound to p130 in the cytoplasm; in G1 it was bound to Rb in the nucleus where it actively blocks progression to S phase and at the G1 to S phase transition, it was bound to p107 in the cytoplasm. The association of p130 and p107 with transcriptionally inactive cytoplasmic complexes may explain failure to find mutations in these genes associated with cancer.

The cytoplasmic location of E2F4 is probably attributed to the fact that it lacks a nuclear localization signal. When E2F4 was modified by the addition of the E2F1 nuclear localization signal, it was translocated to the nucleus and became associated with transcriptionally active complexes. Thus, activity correlated with the ability to localize to the nucleus. Because Rb associates with E2F4 as well as E2F1-3, these results supported the notion that cytoplasmic localization and association with E2F4 may be related to the temporary inactivation of Rb during stages of the cell cycle in which it is not needed.

Molecular mechanisms of E2F transcriptional regulation. In another presentation at the 1997 AACR meeting, Peggy J. Famham from the University of Wisconsin (Madison, WI) discussed studies of promoter element characteristics that determined the outcome of E2F regulation of transcription which is highly variable. In some cases E2F activates transcription, in others it represses it, and some E2F genes are growth-regulated. Examples of cellular promoters that are regulated by the E2F complexes are those associated with the E2F1 gene and the dihydrofolate reductase (DHFR) gene.

The E2F1 promoter is growth-regulated and E2F binding sites in the promoter are necessary but not sufficient for this type of control. Both growth regulatory elements and E2F elements must be present and probably cooperate to control transcription. Substituting factor binding sites such as NFY (a CCAAT box), YY1 and SP1 (a GC box) for the growth regulatory elements in the E2F1 promoter preserves growth regulation. E2F1 protein is sufficient for growth regulation when brought to the DNA by the Gal 4 DNA binding domain. In these experiments growth is measured as a fold induction in S phase.

To determine what promoter characteristics are important for activation or repression by E2F, experiments

addressed the possibility that either specific E2F elements or a core promoter region were involved. Mutations in the E2F site of the E2F1 promoter resulted in a stimulation of transcription, indicating that it is normally regulated by repression. In S phase a minimal E2F1 promoter is regulated by E2F-mediated activation. The model which emerged is that E2F probably functions as a negative regulator in a complex promoter, whereas in a minimal promoter E2F functions as a positive regulator. In the complex promoter, E2F interaction may block a key contact with an activator domain.

The DHFR promoter has 4 SP1 elements linked to a single E2F element. To determine whether it is regulated in a positive or negative manner by E2F, the E2F element of the promoter was moved relative to the SP1 elements. Promoters having an upstream E2F element were less active than the normal promoter with the E2F site in a downstream position. Also less active were promoters in which the downstream E2F element was partially deleted as well as those in which the normal downstream E2F element was separated from the SP1 elements by insertions. There was a gradual loss of growth regulation as the insertion sequences increased in length. These results suggest that E2F functions to activate DHFR transcription.

To determine whether binding of E2F is rate-limiting and to identify which E2F complex binds to DHFR, the gene was amplified by expression on minute chromosomes and E2F proteins were individually immunoprecipitated. In S phase, all E2Fs except E2F5, which is known to be predominantly expressed at G2, coimmunoprecipitated the DHFR promoter. E2F5 was associated with the promoter at G0/G1. E2F4 was nearly non-existent at that stage but its binding to the promoter progressively increased during G1. Promoter binding by E2F1-3 increased as the cycle progressed from mid-G1, to G1 to S phase transition. While binding of E2F to the DHFR promoter is not rate-limiting, it is possible that some higher-level complex is. Furthermore, in addition to being position dependent, E2F-mediated activation of DHFR correlates with the ability to recruit the TFIIB transcription factor.

Cyclin/cdk Regulation

Cyclins and cdk are themselves the targets of complex regulation. Activation requires several steps, including synthesis, formation, and activating phosphorylation of cyclin:cdk heterodimers. For example, cdks are themselves activated by another cyclin:cdk, cyclin H:CAK (cdk activating kinase) by phosphorylation on specific threonine residues. In addition, inhibitory phosphorylation of tyrosine residues are removed by cdc25; cdc25a, in particular, is activated as a result of the action of the proto-oncogene c-myc (Galaktionov K, etal, Nature 1996, 382:511).

Since active cyclin:cdk complexes promote cell growth, numerous overlapping inhibitory mechanisms are employed to limit their activity. Active complexes can be inhibited by dissociation of the complex, degradation, or inhibitory phosphorylation on cdk. As mentioned above, cyclin E:cdk2 phosphorylates cyclin E, targeting it for destruction. And another kinase, wee1, phosphorylates cdk on specific tyrosine residues, inhibiting cdk function.

In addition, there are naturally occurring kinase inhibitors. These include the INK4 family of proteins (p15, p16, p18, and p19) which inhibit cdk4 and cdk6, and the kip family of proteins (p21, p27, and p57) which inhibit cdk2, 4, and 6. The INK4 cdk inhibitors bind to and inactivate specifically cdk4 and cdk6, the cdks associating with D-type cyclin kinases. In cells functionally deficient in Rb, D-type cyclin kinase activity is not required for cell growth, and overexpression of INK4 inhibitors is tolerated, suggesting that the primary function of cyclin D:cdk is to phosphorylate Rb and drive cell cycle progression. This places INK4, cyclin D, and Rb all in the same pathway. Indeed, the overwhelming majority of solid tumors contain mutations in the genes of this pathway.

The kip family of inhibitors bind to and inhibit heterodimers of cyclin E, A, and to a lesser extent cyclin D. p21 (cip1) is upregulated specifically in response to DNA damage. p27 (kip1) may be a direct monitor of cell cycle status, because it falls dramatically when a cell is stimulated to enter the cell cycle, and rises when growth signals are removed (Firpo EJ, etal, Molecular and Cellular Biology, 1994 Jul, 14(7):4889-901). Mice genetically deficient in p27 are larger, with larger organs, and have increased numbers of cells, suggesting a direct role for p27 in cell cycle control (Yamasaki L, etal, Cell, 1996 May 17, 85(4):537-48).

Growth inhibitory factors also affect the cell cycle, and specifically cdk. The transforming growth factor β (TGF-β) family, for example, is a well characterized family of cytokines with anti-proliferative and differentiation effects on a variety of tissues and cultured cells. Recent evidence suggests that signals from the TGF-β receptor complex itself, via the SMAD/MAD family of regulatory proteins, activates multiple cell cycle inhibitory mechanisms including cdk inhibitors p15 and p27, as well as degradation of cdk4.

CELL CYCLE DYSREGULATION AND CANCER

Most major cytotoxic agents kill tumor cells by interfering with the process of cell cycle. However, such interference usually involves structurally damaging a cell's DNA or blocking its production, a process that is not selective for cancer cells but affects all normal cells, hence the severe toxicities associated with the various agents currently in use for the treatment of cancer. Granted, because cancer cells divide at an accelerated pace, they are more vulnerable to these cytotoxics. However, various attributes of cancer cells, such as the ability to remove offending agents via the P-gp pump or repair damaged DNA, often prevent cytotoxics from effectively

killing the tumor, adding insult to injury by making the patient doubly ill from untoward side effects as well as from the cancer itself. Treatment approaches that are more selective, such as withdrawal of molecules required by dividing cells such as methionine starvation, described on page 581 of this issue, may also circumvent the problems associated with cytotoxics but it is not clear how effective such strategies will prove ***in vivo***.

Dysregulation of cell cycle processes is of fundamental importance in the development of cancer, which is essentially a condition of unregulated cell growth. During initial stages of cell division, normal cells have checkpoints which enable them to detect mutations. In cancer cells, this checkpoint is defective and such cells can, therefore, duplicate their DNA in an unregulated manner. The molecular basis of this checkpoint is now believed to be a pathway that includes cdks, Rb and a number of regulatory proteins such as p16. In order for malignant cells (those with uncorrected genetic defects) to grow and spread, they must escape the normal regulatory processes of cell division.

Current research in cell cycle regulation aims to get to the heart of the problem by selectively manipulating effectors and signaling pathways dysregulated only in malignancy. It is obvious that cancer cells either receive different stimulatory or inhibitory signals than normal cells or process them differently. For instance, in order to divide, tumor cells may not require mitogenic signals or substratum adherence or presence of cell type-specific cytokines necessary for normal cell cycle progression. On the flip side, tumor cells may not respond to inhibitory signals avoiding apoptosis and going through the cell cycle at an accelerated pace. Understanding cell cycle dysregulation that results in uncontrolled proliferation, may lead to development of highly specific regulatory drugs that will address tumor growth at its origin.

The cell cycle is disrupted in virtually all cancers. The majority of human malignancies result from inappropriate progression through the cell cycle, either by gene mutation, dysregulation, or protein dysfunction. A promising finding is that mutations in cell cycle genes constitute the most common genetic change of tumor cell lines ***in vitro***; nearly 100% of tumor cells exhibit mutations in genes controlling progression throughout G1. Also, cyclins that drive cell cycling are overexpressed in tumor cells and proteins that restrain cell proliferation are inactivated. The D-type cyclin pathway appears to be the most corrupted.

Cellular Response to Oncogenic Mutation

The three signaling pathways most often mutated in sporadic cancers are those of ras, Rb, and p53. The "INK4/cyclin D/Rb" pathway alone is mutated in the majority of solid tumors. Many tumors contain mutations in more than one pathway, suggesting that each pathway independently provides some growth restraint, and that cancerous cells with mutations in multiple pathways have a growth advantage. Early studies documented the transforming nature of many oncogenes in tumor cell lines, and the requirement for collaboration among certain genes for oncogenic transformation. Only recently, however, has it become apparent that single mutations in normal, primary cells, may stimulate a cellular "anti-cancer" response. For example, a constitutively active oncogenic form of ras alone in a normal cell will evoke a cellular response to stop growing by activating either Rb-mediated or p53-mediated arrest. Only by having a second growth control pathway mutated (Rb or p53) will ras-expressing primary cells grow unabated (Serrano M, etal, Cell, 1997 Mar 7, 88(5):593-602). These experiments also explain the requirement for cooperation among oncogenes; not only must ras (or another oncogene) become activated, but the natural cellular "anti-cancer" response within the cell must also be disabled before oncogenic transformation can occur.

p53 protein is central in maintaining DNA integrity. In a growing cell, DNA damage (by genotoxic drugs, UV damage, or other mechanisms) will stimulate expression of p53, and, in turn, the cell cycle inhibitor, p21, which will inhibit cyclin kinases, and prevent phosphorylation of Rb. This (and other mechanisms) lead to cell cycle arrest in G1 (and to some extent S/G2) to allow for DNA repair. In the absence of p21 or p53, damaged DNA will attempt to replicate, and fragmented DNA will be subjected to mitosis, increasing the chance for mutations, translocations, or gross chromosomal abnormalities to be incorporated into the daughter cells. The mechanism by which p53 senses DNA damage is still not clear, but may involve the ataxia-telangectasia mutated (ATM) gene product and MDM-2, the protein product of another proto-oncogene, that binds to and inhibits p53.

The role of p57 (Kip2) in mouse development and Beckwith-Weidemann Syndrome (BWS), is being investigated at the laboratory of Stephen Elledge of Baylor College of Medicine (Houston, TX), to observe the effect of genetic alterations of p57 in developing mice to gain insight into the role of p57 in cell cycle regulation. The gene for p57 is located on human chromosome 11 at p15.5. Mutations and loss of heterozygosity in this area are known to be associated with sporadic cancers, BWS and Wilm's tumor in humans. The region is also known to contain two tumor suppressor genes, a rhabdomyosarcoma gene and the Wilm's tumor 2 gene. Genetic alterations in this area include imprinting, i.e., the allele-specific expression of a gene which is determined by the parental origin of the chromosome, the second allele having been inactivated by methylation. Imprinting may play a role in the development of Wilm's tumor, which is associated with the loss of the maternal allele. In BWS, which is a primarily a familial cancer syndrome with an extremely variable phenotype, there is evidence for imprinting as well as for incomplete penetrance of the genetic alteration.

The phenotypes presented by individuals with BWS are variable, but generally include gigantism. Individuals with this condition are in the 90th size percentile at birth. They have enlarged organs and tongue, unusual facial structures, such as creases in the ears, and thickened bones. Incidence of cancer among people with BWS is increased 1000-fold, but only 8% actually get cancer, usually hepatoblastoma, adreno-carcinoma and Wilm's tumor. The genetic alteration in BWS is either a paternal uniparental disomy, a lethal event embryonically in which two copies of the father's chromosomes are inherited, or there may be duplication or trisomy of the 15.5 region, or simple point mutations at that site. While there is evidence for imprinting, it is likely that multiple imprinted genes are involved in the process.

Knockout mice which lack the p57 gene have some of the characteristics of human BWS. They develop normally but tend to have a somewhat shorter and fatter body shape. They don't display the gigantism of BWS, but they are born with cleft palates and die within one day because they inhale milk into their lungs and air into their intestines. Their small intestines develop outside of the body and are then re-inserted. There is evidence of bone growth problems such as delay in ossification and thickened bones, loss of control of cell proliferation in the growth plate of bones, and there may be a defect in differentiation which is independent of cell proliferation. Examination of the bone growth plate indicates that there are abnormally low levels of p57 in the first two layers, the resting zone, consisting of stem cells, and the proliferation zone. The third zone, the hypertrophic layer, which is normally replaced by osteoclasts, doesn't differentiate properly.

Lens development in p57 knockout mice is also aberrant. The lens normally develops from a single layer of cells which migrate to the outer edge of the embryonic eye, exit the cell cycle and begin to differentiate by turning on the synthesis of a high level of crystalline. At the same time, the cell gets rid of membrane structures such as the nucleus. High levels of p57 are normally expressed on the edge of the differentiating lens. In the mutant mice, lens cells don't exit the cell cycle after migration, but do so later, and there are more vacuoles in the lens as a variable phenotype. In double mutants which don't express either of two of the members of the kip family of proteins, p57 or p27, crystalline genes aren't expressed at all. This suggests that both p57 and p27 are collaborating to control cell cycle regulation and differentiation.

Although BWS gigantism phenotypes aren't seen in p57 mouse knockout models, the mice do have renal dysplasia, adrenal cytomegaly and hyperplasia, chondrocyte proliferation and differentiation defects, abdominal muscle defects and cleft palates. Genetic analysis of human BWS patients showed that only two out of 40 had mutations in the p57 gene, so p57 defects aren't the only problem. Aberrant expression of p57 may be involved and it's possible that there is a p57 antagonist gene on chromosome 11. Further studies may lead to insights into therapeutic strategies for BWS and Wilm's tumor as well as defining the role of kip proteins in cell cycle regulation in more detail.

Apoptosis may present the ultimate check against cell cycle dysregulation and unrestrained cell growth. Under certain conditions, p53 expression will lead to apoptosis. p53 causes a G1 arrest via p21 and inhibition of Rb phosphorylation, but in the absence of functional Rb, cells can bypass this arrest and enter S phase. p53 responds to this inappropriate S phase by causing apoptosis. p53 may do so by directly activating the apoptosis gene bax, or down-regulating the anti-apoptotic gene bcl-2. Several other known inducers of apoptosis may also arise because of inappropriate S phase entry. Overexpression of E2F1 (which is normally restrained by Rb) will lead to early S phase and apoptosis, and expression of the proto-oncogene c-myc may also cause apoptosis in certain settings. For a comprehensive review of apoptosis, see FO, pp 22-31.

Viral Oncogenes

DNA tumor viruses have also shed light on the process of cell cycle and cellular transformation. Viruses such as simian virus 40 (SV40; via large T antigen), adenovirus (via E1A and E1B), and human papillomavirus (HPV subtypes 16 and 18; viral E6 and E7), are all able to co-opt the cell cycle machinery to induce malignant transformation. This process also requires inactivation of two cell cycle regulatory processes simultaneously. For example, HPV infects and induces malignant transformation of cervical epithelium. HPV inactivates the cell's "anti-cancer response" by inhibiting both Rb, with E7 and p53, with E6, thus enabling oncogenic events to proceed unimpeded.

DIAGNOSTIC/PROGNOSTIC APPLICATIONS OF CELL CYCLE

Presence of cell cycle proteins that are overexpressed in a wide variety of tumor types may correlate with prognosis. For example, breast cancers that overexpress cyclin D1 are associated with a better prognosis than those that overexpress cyclin E (Int J Cancer 69:92 (1996); Cancer Res 54:380 (1994). Since both diagnostic and prognostic information can be obtained by examining the levels of cell cycle proteins, tests for commercial use are actively in development. Among developers of diagnostic approaches based on cell cycle processes are Cascade Ontogenics (Portland, OR) and Mitotix (Cambridge, MA).

Cascade Oncogenics

Cascade Oncogenics is applying discoveries associated with the process of cell cycle to develop diagnostic and prognostic assays (see FO, pp 484-485). In addition to detection/measurements of cyclin E and ATM, Cascade

has acquired rights to a patented Therapy Selection Assay developed Dr. Karin Rodland at the Oregon Health Sciences University (Portland, OR). This assay serves as a common marker for several drugs that inhibit cell cycle progression. These drugs fall into several categories, with differing modes of action, including tamoxifen and bryostatin, as well as specific signaling pathway inhibitors. Cascade believes that, unlike other drug sensitivity assays, this assay is superior in terms of clinical utility because it can be performed within 16 hours using a simple cell suspension, can be used to determine the effects (growth promotion or inhibition) of several drugs acting on different biochemical targets, and results can be used to select tumor-specific therapy.

CELL CYCLE MODULATION AS AN ANTI-CANCER STRATEGY

Anti-cancer agents directly interfering with the cell cycle of malignant cells are expected to exhibit increased specificity and, potentially, decreased toxicity than the currently employed chemotherapeutics. Unlike normal cells that have redundant regulatory pathways, cancer cells often overexpress one pathway. Specifically inhibiting pathways overexpressed in a given tumor, may restrict proliferation of cancer cells. There are numerous opportunities to interfere within the cell cycle. However, for any potential therapy to be effective it must:

- selectively target the cell cycle of malignant cells without affecting that of normal cells
- target at least one essential step
- result in cell death, not merely cell cycle arrest (may need to be combined with other therapies)
- be either highly effective or target multiple steps, so that the tumor cannot easily develop resistance to the therapy
- target more than one abnormal process because malignant transformation of a cell almost certainly requires disruption of two or more growth control processes

Direct Inhibitors of Cyclin:cdk

Several highly selective inhibitors of cyclins and CDK have been identified. These include olomoucine and roscovitine, which are highly selective for cdk2 and cdc2, and do not appear to inhibit other cellular kinases (Progr. Cell Cycle Res, 1995, 1:351). Flavopiridol also selectively inhibits cdk2 (De Azevedo WF Jr, etal, PNAS USA, 1996 Apr 2, 93(7):2735-40). A fusion protein of cyclin D1:cdk4 has been constructed that co-operates with H-ras to transform cells; compounds which can revert the transformed phenotype of cells (without toxicity) are being screened as candidate inhibitors of either H-ras or cyclin:cdk. In addition, INK4 and kip proteins, that are naturally-occurring inhibitors of cdk activity, suppress human tumor cell growth and may be ideal candidates for use in gene therapy (Arap W, etal, Cancer Research, 1995 Mar 15, 55(6):1351-4).

Flavopiridol (L86-8275, NSC 649890), a N-methylpiperidinyl, chlorophenyl flavone, is a potent cdk1 inhibitor that arrests cell cycle progression in either G1 or G2. The drug was originally identified through the NCI screening program and is currently in development by Hoechst Marion Roussel under an NCI CRADA. In ***in vitro*** studies, researchers at the NCI used Rb-positive MCF-7 breast carcinoma cells that are wild type for p53 and contain cyclin D1:cdk4, and Rb-negative MDA-MB-468 breast carcinoma cells that are mutant p53, and lack cyclin D1:cdk4, to investigate G1 arrest produced by flavopiridol. Recombinant cyclin D1:cdk4 was inhibited potently by flavopiridol, competitive with respect to ATP. Cdk4 kinase activity increased 3-fold in flavopiridol-treated MCF-7 cells, compared with untreated cells. Flavopiridol inhibited asynchronous MDA-MB-468 cells in cell cycle progression at both G1 and G2, and the ***in vitro*** kinase activity of cdk2. Cdk2 kinase activity from either MCF-7 or MDA-MB-468 cells, exposed to flavopiridol for increasing time, showed initial increased activity (approximately 1.5-fold at 3 hours) compared with untreated cells, followed by a loss of kinase activity to immeasurable levels by 24 hours. This increased kinase activity was dependent on flavopiridol concentration added to intact cells and was associated with a reduction of cdk2 tyrosine phosphorylation. Cyclin E and A levels were not altered to the same extent as cyclin D, and neither cdk4 nor cdk2 levels were changed in response to flavopiridol. G1 arrest, observed after exposure to flavopiridol is, therefore, the result of inhibition of the cdk4 and/or cdk2 kinase activity (Carlson BA, etal, Cancer Research, 1996 Jul 1, 56 (13):2973-8). Flavopiridol is also a potent inhibitor of cdc2 (De Azevedo WF Jr, etal, PNAS USA, 1996 Apr 2, 93(7):2735-40).

Flavopiridol is currently in phase I/II clinical trials in the treatment of solid tumors. In 21 patients treated by a 72-hour continuous infusion of flavopiridol, administered every two weeks, MDT was 40 mg/m^2/72 hours. Toxicity was primarily gastrointestinal, with diarrhea being the predominant symptom; symptomatic orthostatic hypotension was also observed in several patients (Cleary, TJ, etal, AACR97, Abs 1496:222). Similar toxicities were encountered in a phase I clinical trial, also using a 72-hour infusion protocol to treat 63 patients with advanced refractory neoplasms. Administartion of anti-diarrhea prophylaxis using loperamide or cholestyramine, made it possible to raise MDT to 98 mg/m^2/day (Senderowicz AM, ASCO97, Abs. 793:226a). Two additional clinical trials of flavoperidol, sponsored by the NCI, have been approved but had not yet started as of August 1997 at Memorial Sloan Kettering Cancer Center (New York, NY). A phase I open label trial (MSKCC-9677A, NCI-T96-001) is to enroll up to 60 patients to evaluate a combination of flavopiridol and

paclitaxel. The potocol involves 24-hour central access infusion of paclitaxel on day one, followed by a 24-hour infusion of flavopiridol on day two, repeated every 21 days in responders, and escalated until MDT is reached. A phase II open label non-randomized clinical trial (MSKCC-96050, NCI-T96-0111) is to enroll between 14-35 patients with advanced refractory gastric cancer. The protocol involves a 72-hour infusion of flavopiridol, every 14 days, for at least four cycles.

Olomoucine and roscovitine, among other N6,2,9-trisubstituted adenines, exert a strong inhibitory effect on p34cdc2/cyclin B kinase. The structure-activity relationships of cdk inhibition showed that the 1, 3 and 7 positions of the purine ring must remain free, probably for a direct interaction, in which it behaves as a hydrogen bond acceptor. Removal or change of the side chain at position 2 or the hydrophobic group at position 9, dramatically decreased the inhibitory activity of olomoucine or roscovitine. Inhibition of cdk with olomoucine and related compounds arrests cell proliferation of many tumor cell lines at G1/S and G2/M transitions, and also triggers apoptosis in target tumor cells ***in vitro*** and ***in vivo*** (Havlicek L, etal, Journal of Medicinal Chemistry, 1997 Feb 14, 40(4):408-12).

Inhibitors of Activation or Activators of Inhibition of Cdk

Although CAK activate cdk by phosphorylation, blocking CAK function does not cause cell cycle arrest, suggesting that cells may have some redundant mechanism for cdk activation. Other activators of Cdk, such as cdc25 phosphatases, activate cdk by removing the inhibitory tyrosine phosphorylation. Cdc25 phosphatases are critical components of the cell cycle, exhibit a tight substrate specificity and a well-defined catalytic mechanism, are targets of oncogenes known to be involved in human carcinogenesis and their expression is altered in tumors. Cdc25a and cdc25b have been implicated in the transformation of mouse primary fibroblasts in cooperation with other oncogenes and may be directly regulated by myc. Both cdc25a and cdc25b can cooperate with H-ras to transform primary cells, and cdc25b is expressed at high levels in breast cancer, suggesting that its function may be essential for normal and malignant cell growth.

Activators of Cyclin:cdk Proteolysis

Variable expression of cyclins in the cell cycle is attributed in part to proteolytic destruction of cyclins, primarily by the ubiquitin-proteasome pathway. Degradation of cyclin E by the ubiquitin-proteasome system is regulated by both cdk2 binding and cdk2 catalytic activity. Free cyclin E is readily ubiquitinated and degraded by the proteasome but binding to cdk2 protects against degradation. It appears that cdk2 activity initiates cyclin E degradation by promoting disassembly of cyclin E:cdk2 complexes, followed by the ubiquitination and degradation of free cyclin E (Clurman BE, etal, Genes Dev, 1996 Aug 15, 10(16):1979-90). In certain tumors, overexpression of cyclins and other cell cycle proteins (in particular cyclin E) has been attributed to a failure of proteolysis. Restoring normal proteolysis, or activating proteolysis in tumor cells, may arrest their growth or kill them.

Transcriptional Factors

Cyclins and cdk affect cellular transcription in a variety of ways. Cyclin:cdk phosphorylation of Rb releases E2F transcription factors; cyclin D1 enhances estrogen receptor-mediated transcription; and cyclin:cdk complexes bind to and modulate the transcriptional co-activator p300 (Perkins ND, etal, Science, 1997 Jan 24, 275(5299):523-7). In tumor cells, one or several of these mechanisms will be abnormal, and this difference is being exploited in attempts to design therapy.

Chemoprotection

Tumor cells often lose regulatory control of cell growth. This phenotype can be exploited to enhance selectivity of chemotherapy for tumor cells over normal cells (therapeutic index). For example, TGF-β or low dose cycloheximide will induce a G1 arrest of cells with functional Rb protein. In contrast, cells that have lost Rb function (such as tumor cells) will not arrest. Because many standard cytotoxic chemotherapeutic agents are cell cycle phase specific, one can treat a mixed population of normal and tumor cells (or a patient with a tumor) first with TGF-β and then with a specific S-phase or mitotic poison. Tumor cells, which will not arrest in G1 will die in S or M phase, while their normal counterparts will be arrested in G1.

Chemosensitization

UCN-01, 7-hydroxystaurosporine, originally discovered by the NCI and currently under development by Kyowa Hakko Kogyo (Tokyo, Japan), is a protein kinase C (PKC) inhibitor that may block G2 arrest of the cell cycle following DNA damage. Abrogation of G2 arrest sensitizes cells to the effects of DNA-damaging agents by preventing cell viability protection afforded by DNA repair before mitosis. For instance, it was reported that UCN-01 enhances cytotoxicity of mitomycin-C. In ***in vitro*** studies UCN-01 was found to be a potent abrogator of G2 checkpoint control in cancer cells with disrupted p53 function. Therefore, UCN-01 enhanced effectiveness of DNA-damaging agents against tumor cells that lack normal p53 function (Wang Q, etal, JNCI, 1996 Jul 17, 88(14):956-65). Also, UCN-01 does not only act on protein kinases but also on cell-cycle dependent mechanisms, such as induction of expression of p21 protein, and inhibition of cyclin-dependent Rb kinases.

In ***in vitro*** and animal studies UCN-01 proved a potent inhibitor of proliferation of malignant glioma cells. In ***in vitro*** inhibition-recovery studies of clonogenic activity, UCN-01 exhibited both cytostatic and cytotoxic

effects. Although proliferation resumed after short-term (6- and 24-hour) exposures to UCN-01, recovery of proliferative activity in longer exposures was severely compromised. In addition, UCN-01 enhanced inhibition of glioma cell proliferation when used in conjunction with conventional chemotherapeutic agents, exhibiting synergistic effects with cisplatin and additive effects with 1,3-bis(2-chloroethyl)-1-nitrosourea. UCN-01 may be particularly suited for treatment of glioma because recent studies indicate that proliferation of malignant gliomas may result from activation of PKC-mediated pathways. In ***in vivo*** studies, administration of UCN-01 by continuous intraperitoneal infusion in nude rat models demonstrated significant activity against U-87 glioma xenografts at dose levels that were well tolerated. Therefore, it appears that UCN-01 is well tolerated ***in vivo*** at concentrations sufficient to achieve effective inhibition of PKC for the treatment of human gliomas (Pollack IF, etal, Journal of Neurosurgery, 1996 Jun, 84(6):1024-32).

A phase I clinical trial of UCN-01 (NCI-95-C-0171G, NCI-T95-0052N) in 29 patients with refractory solid tumors or lymphoma, including recurrent breast and prostate cancers that have not responded to hormone therapy, is ongoing at the NCI. Patients are treated with UCN-01 by intravenous infusion continuously for 3 days during the first week, then continuously for 2 days every 4 weeks, for as long as benefit is shown.

DRUG DEVELOPMENT AND RESEARCH PROJECTS

Most large companies with oncology drug development programs, such as Bristol-Myers Squibb, DuPont Merck, Glaxo Wellcome, Hoechst Marion Roussel, Knoll/BASF, Pfizer, Pharmacia & Upjohn, Schering-Plough, and Warner-Lambert, have also established internal R&D programs and/or outside affiliations with others engaged in R&D in cell cycle regulation and related areas.

Small Molecule Drugs

Mitotix (Cambridge, MA) is developing small molecule inhibitors of p27 and cyclin E and, in collaboration with DuPont Merck Pharmaceuticals, cyclin D1:cdk4 inhibitors (see FO, pp 253-254 and 485). Mitotix is also searching for small molecule inhibitors of the family of Cdc25 dual-specific protein tyrosine/ threonine phosphatases (PTPases), in a collaboration with BASF Pharma (Ludwigshafen, Germany). Mitotix scientists have constructed, expressed and purified a series of mutant cdc25 proteins.

Onyx (Richmond, CA) has developed screening assays to search for small molecule inhibitors of mutant cell cycle checkpoint genes which regulate DNA replication. Onyx also has initiated research efforts to identify pathways regulating the second checkpoint in the cell cycle that controls the decision to begin cell division. In May 1995, Onyx entered into a collaboration agreement with Warner-Lambert within its Cell Cycle Program. Under the terms of this collaboration, Onyx is responsible for performing research into cell cycle regulatory pathways, identifying and validating targets for drug screening, and developing assays for screening small molecules. Warner-Lambert uses these assays to screen its compound libraries, synthesizes and tests chemical analogs of classes of compounds which are identified in the screens, and conducts preclinical and clinical testing of compounds selected for development. In this agreement, Warner-Lambert is obligated, subject to certain conditions, to partially fund researchers working on Onyx projects. Each of the parties must commit an equivalent number of researchers to the collaboration. From the initial two assays transferred by Onyx, the collaboration has identified a lead compound that Warner-Lambert is advancing into preclinical study. Onyx has transferred to Warner-Lambert five more assays for high throughput screening. Onyx does not expect to commence clinical trials of any potential products from the Cell Cycle Program before 1999.

ProScript (Cambridge, MA) is a biopharmaceutical company engaged in the discovery and commercialization of small molecule drugs to treat inflammatory disease, cancer and cachexia, through the regulation of gene transcription. The company's discovery approach is based on its expertise in intracellular signaling and its identification of the ubiquitin-proteasome pathway. ProScript has entered into collaborations with Hoechst Marion Roussel and Hoffmann La Roche. ProScript, which is privately held, was financed by HealthCare Investment Corporation and Dillon, Read Venture Capital.

On April 14,1997, ProScript announced that it obtained exclusive licensing rights to patents and technical know-how from Harvard Medical School covering enzymes involved in cell cycle regulation with applications in the discovery of new treatments for cancer. As part of its arrangement with Harvard, ProScript will consult with Professor Joan Ruderman, an expert in the field of cell cycle regulation. Specifically, this technology covers enzymes that play a role in tagging certain cell cycle proteins for degradation by the ubiquitin-proteasome pathway which regulates cell division by maintaining appropriate levels of certain cyclins which act to control orderly replication of the cell.

Sugen focuses on the discovery and development of small molecule drugs which target specific signaling pathways regulated by serine-threonine kinases (STKs), involved in controlling the cell cycle, among other regulatory functions.

Peptide Mimetics

Cyclacel, a dedicated cancer R&D company based at the University of Dundee in Scotland, was established in November 1996 by Cancer Research Campaign Technology, the commercial arm of the UK charity Cancer Research Campaign (CRC), and Merlin Ventures, to research and develop cancer treatments. Merlin provided

initial funding of £250,000. In June 1997 Cyclacel received £2.5 million from the Merlin Fund and generous support from two regional bodies, Scottish Enterprise and Dundee City Council. Cyclacel acquired intellectual property arising from CRC-funded research by Cyclacel's scientific co-founders at Dundee University (Professor David Lane) and Glasgow University (Professor Alan Balmain, formerly at Glasgow, now VP at Onyx Pharmaceuticals) into the control of the cell cycle and genetically engineered treatments for tumors.

Gene therapy research, conducted at Glasgow University, centers on a new system for selective destruction of cancer cells that 'switches on' therapy in only the cells with faulty p53 protein. Clinical trials on the gene therapy treatments in certain head and neck tumors are expected to start in 1997. Cell cycle regulation research is based on a body of work by Dr. David Lane at Dundee University that has led to identification of two novel peptides derived from natural cell cycle regulatory proteins that may be used in treating diseases associated with uncontrolled cell multiplication.

One such peptide restores function of p16, a protein encoded by tumor suppressor gene CDKN2/INK4 that is deleted or mutated in a large number of human cancers. Overexpression of p16 has been shown to block the transition through the G1/S phase of the cell cycle in a Rb-dependent fashion, by inhibiting cyclin D-dependent kinases cdk4 and cdk6. Restoration of p16 function in transformed cells is, therefore, an attractive target for anti-cancer drug design. A 20-residue synthetic peptide, corresponding to amino acids 84-103 of p16, was identified that interacts with cdk4 and cdk6, and inhibits the ***in vitro*** phosphorylation of Rb, mediated by cyclin D1:cdk4. This p16-derived peptide can mediate three of the known functions of p16, i. e., interact with cdk4 and cdk6, inhibit Rb phosphorylation ***in vitro*** and ***in vivo***, and block entry into S phase. The fact that one small synthetic peptide can enter the cells directly from the tissue culture medium to inhibit Rb phosphorylation and block cell-cycle progression makes this an attractive approach for future peptidomimetic drug design (Fahraeus R, etal Current Biology, 1996 Jan 1, 6(1):84-91).

Oligonucleotide-based Drugs

Oligonucleotide-based approaches in interfering with the cell cycle are in the research stage (for additional information see, FO, pp 502-511).

Gilead Sciences (Foster City, CA) is conducting research on oligonucleotide analogs (code blockers) designed to act inside the cell to block or regulate production of disease-causing proteins. The company is developing two types of code blocker compounds, antisense compounds that interfere with the function of RNA, and triple helix compounds that inhibit gene expression by binding to the DNA double helix. In the cancer area Gilead's code blockers target cell cycle genes. In 1990 Gilead entered into a collaboration with Glaxo Wellcome

that covers R&D of code blocker compounds for all potential diagnostic and therapeutic applications; in March 1996, this collaboration was extended for another five years. The company is currently working on chemical modification or proprietary permeation enhancers, to enable its oligonucleotides enter the cell cytoplasm or nucleus, and on synthesizing novel oligonucleotide analogs that are able to penetrate cells efficiently.

Gilead Sciences researchers evaluated C-5 propyne pyrimidine-modified phosphorothioate antisense oligonucleotides (ONs) targeted against two cell cycle proteins, p34cdc2 kinase and cyclin B1, that are aberrantly expressed in breast cancer Nanomolar concentrations of ONs resulted in dose-dependent, sequence-specific, and gene-specific inhibition of both proteins in normal and breast cancer cells. However, precise binding of the antisense ONs to their target RNA was absolutely required for antisense activity. Antisense inhibition of p34cdc2 kinase resulted in a significant accumulation of cells in the G2/mitosis phase of the cell cycle in normal cells, but had little effect on cell cycle progression in breast cancer cells, illustrating the redundancy of cell cycle protein function in such cells (Flanagan, etal, Nature Biotechnology, 1996, 14(9):1139-1145).

Genomics

Many gene tranfer/gene therapy programs, involving modulation of activities of various oncogenes and/or tumor suppressor genes, including p53 and ras, that may also indirectly be considered cell cycle regulators, are not covered in this report but have been extensively reviewed in many other articles in FUTURE ONCOLOGY.

Ventana Genetics (Salt Lake City, UT) has also developed a genetic approach to identify suitable cell cycle regulatory targets for drug development.

Other

Icos (Bothell, WA) researchers have been investigating responses to DNA damage and regulation of cell cycle checkpoints by the ATM protein kinase family. Four protein kinases that form the PI3-kinase-related protein kinase (PIK) superfamily, FKBP12 and rapamycin-binding protein kinase (FRAP), DNA-dependent protein kinase (DNA-PK), ATM, and ataxia telangiectasia and Rad 3 related (ATR), are distinguished by their large size (>2500 amino acids), a common primary sequence relatedness through the carboxy-terminal protein kinase domain, and sequence similarity to the p110 lipid kinase subunit of PI3-kinase. FRAP participates in mitogenic and growth factor responses in G1 and may regulate specific mRNA translation signals. DNA-PK, ATM, and ATR are thought to activate DNA rearrangements or cell cycle arrest. Recent studies in this protein kinase family indicate an important role for ATM and ATR in a meiotic surveillance mechanism that may regulate proper chromosome transmission (Hoekstra MF, Current Opinion in Genetics and Development, 1997 Apr, 7(2):170-5).

DEMYSTIFYING THE ROLE OF CYTOKINES IN TUMOR IMMUNOBIOLOGY

REPORT FROM THE 88TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH APRIL 12-16, 1997 SAN DIEGO, CA

Cytokines are small molecules which are synthesized as precursor proteins and excreted from the cell by cleavage of a signal peptide at the cell membrane. Upon excretion, cytokines bind to one of a family of appropriate cell surface receptors, many of which share common signaling moieties, and activate regulatory processes. Messages encoded by cytokines vary because their interpretation by the receiving cell depends on the overall effect of many incoming signals, as well as the status of the cell itself. The role of cytokines is, thus, inherently complex, partly because of the variety of different cytokines, but also because of the complexity and redundancy of their effects. Development of cytokines as cancer therapeutics focuses on identifying those cytokines whose actions may result in tumor cell death and/or enhancement of immunotherapeutic strategies.

Among subjects covered by this symposium were strategies that:

- stimulate tumor-killing properties of tumor necrosis factor (TNF)
- enhance anti-tumor immune responses with Flt-3 ligand (Flt-3L) inhibit tumor growth promoting effects of IL-6 in B cell leukemia
- use IL-2 therapy to induce immune responses in immunodeficiency-associated malignancies

MECHANISMS OF GENE REGULATION BY TUMOR NECROSIS FACTOR (TNF)

Michael Karin of the University of California, San Diego (UCSD), discussed ways of enhancing sensitivity of tumor cells to TNF. Although it can kill tumors, each tumor exhibits a different degree of sensitivity to TNF. Resistance to TNF appears to be mediated by a protective factor that turns over rapidly in the cell. Cells can be made sensitive to TNF by inhibition of transcription and translation. To determine the mechanism of resistance to TNF, mutant cells were selected which failed to undergo apoptosis after exposure to TNF. Resistance to TNF-induced apoptosis in these mutants was not attributed to inactivation of either the APO-1 or nuclear factor (NF)-κB pathways, but was dependent on NF-κB activation. This suggested that the inhibitor of apoptosis is the product of a gene which is induced by the NF-κB regulatory pathway.

One potential approach to sensitizing tumor cells to TNF killing would be to inhibit the anti-apoptotic factor.

Interleukin-1 (IL-1) is such an anti-apoptotic factor which protects cells against killing by TNF. IL-1 and TNF use cell receptors that are in completely different structural classes, yet the biological activities of the two cytokines are identical. IL-1 binds IL-1RACP and IL-1R1 dimers and results in the recruitment of IL-1R-associated kinase (IRAK) to the receptor. This in turn leads to recruitment of TNF receptor-associated factor 6 (TRAF6), which activates c-jun amino-terminal kinase (JNK) p38 and NF-κB. Sensitivity to TNF-mediated tumor killing may be enhanced by interfere with TRAF function in order to inactivate the protective effect of IL-1.

FLT 3 LIGAND (FLT-3L)

Another cytokine which can augment anti-tumor immune responses is Flt-3L. Currently, Flt-3L is being evaluated as a stem cell mobilizer, as a cancer vaccine adjuvant and as a cancer immunotherapeutic. David Lynch of Immunex (Seattle, WA) discussed studies which show that Flt-3L induces expansion of antigen-presenting dendritic cells and natural killer (NK) cells that boost immune system response. Flt-3L is similar to stromal cell factor (SCF) and to CSF-1. It stimulates human and mouse hematopoietic stem cells. It also induces cells expressing CD11, a marker that is characteristic of dendritic cells which are responsible for capturing antigen and stimulating expansion of antigen-specific T cells and helper T cells.

In the June 1, 1997 issue of Nature Medicine, Immunex reported that in preclinical trials in mice, treatment with daily injections of Flt-3L for 19 days, caused complete regression of tumors in 38% of mice challenged with chemically-induced fibrosarcomas. Flt-3L injections also caused a significant decrease in the rate of tumor growth in the mice not experiencing complete regression. Five weeks after tumor challenge, mean tumor size in treated mice was less than one-third that of controls (about 60 mm^2 versus 185 mm^2, respectively). Effects of Flt-3L were dependent on both dose per treatment and the number of treatments. Administration of Flt-3L to mice resulted in complete regression of a high proportion of tumors established as much as seven days prior to treatment. Although treatment beginning 10 to 14 days after tumors were established resulted in a low rate of complete tumor rejection, even then tere was a significant decrease in tumor growth compared to controls. Because Flt-3L enhanced tumor rejection in SCID mice, which lack T and B cells, but not in NOD-SCID mice which also lack NK cells, the anti-tumor mechanism of Flt-3L most likely involves activation of NK cells. A phase II clinical trial was initiated in mid-1997 to study Flt-3L's anti-tumor activity in prostate cancer and

INDEX OF COMPANIES & INSTITUTIONS

— continued on back page

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FUTURE ONCOLOGY

PUBLISHED BY **NEW MEDICINE, INC.**

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SUBSCRIPTION INFORMATION:

- FUTURE ONCOLOGY (ISSN 1082-331X) is published as 10 issues (two double issues) per year, with a free annual index listing companies/institutions and subjects covered.
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