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

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

BREAST CANCER–PART III

DIAGNOSIS, STAGING, MONITORING AND PROGNOSIS

A multitude of methodologies are currently employed or are under investigation for the diagnosis, staging, monitoring and prognosis of breast cancer. Breast cancer screening methodologies, primarily mammography and inherited susceptibility screening, were described in Part II of this article (see FO, V2 #5, pp 358-362).

CURRENT METHODOLOGIES

Various parameters are currently used to establish prognosis and select treatment regimens in breast cancer. After a suspicious mammographic finding, diagnosis of breast cancer is confirmed by core needle biopsy using a stereotactic technique for nonpalpable lesions, fine needle aspiration (FNA), which is less invasive, painful and costly, or incisional or excisional biopsy. Because of the high numbers of false positive findings with mammography, thousands of unnecessary biopsies are performed annually (see Exhibit 1). When a diagnosis of cancer is established by biopsy, a series of tests are performed to establish the stage, prognosis and special characteristics of the tumor.

Exhibit 1
Breast Biopsies Performed in Selected World Regions in 1995

Region	Biopsy (#)
Europe*	739,167
Former USSR	162,889
Japan	264,286
United States	670,523
Canada	72,717
North America	743,240
Triad (Europe*, Japan, NA)	1,746,693

** Excludes the former USSR*

Generally, important prognostic factors include age, menopausal status, weight (overweight patients may have

a poor prognosis) and general health. Although other factors such as race are also used, a poorer outcome in nonwhites is probably attributable to tumor stage at diagnosis rather than the patient's race.

Pathologically, breast cancer is frequently a multicentric disease. However, clinical diagnosis of two or more primary cancers in a single breast is uncommon. Similarly, simultaneous bilateral breast cancer is unusual. It is more common in patients with infiltrating lobular carcinoma. Rarely is the breast involved by other tumors such as melanoma, lymphoma, or sarcoma.

Tumor Differentiation

Tumor differentiation work-ups establish such histologic variables as tumor histologic type and grade, nuclear grade and degree of tumor necrosis, among others. Tumor grading is based on the pathologist's judgment as to a tumor's state of differentiation and growth rate, depending upon the degree of tubular formation, size of cells, size of nuclei, degree of hyperchromatism and number of mitoses. Often graded on a scale of I to III or IV, with the higher numbers representing least differentiated, fastest dividing tumors presumed to have the worst prognosis, this test has serious shortcomings because a different scale is required for each type of tumor, and scoring is subjective and not always reproducible.

Although a study by the International Breast Cancer Study Group (IBCSG) has shown that tumor histologic grade is a significant prognostic factor, it is not always possible to predict tumor behavior from histologic appearance. Currently, most tumors are not graded based on a scale; rather the pathologist uses terminology such as well- or poorly-differentiated tumor. Parenthetically, a study by the National Surgical Adjuvant Breast and Bowel Project (NSABP) demonstrated that tumor nuclear grade is a significant indicator of outcome following adjuvant therapy.

Estrogen-receptor (ER) and progesterone-receptor (PR) levels in the tumor tissue are also determined at the time of tumor removal. There is substantial evidence that ER/PR status may have significant independent predictive value. Also, in stage II disease, PR status may have greater prognostic value than ER status. Establishing hormonal status, which is mandated as part of the diagnostic work-up, is commonly performed either by standard biochemical assays or by immunohistochemistry (IHC). Another tumor differentiation determination is DNA ploidy status, detected by analytic cytometry.

Proliferative Rate

Proliferative capacity, or rate, establishes the percentage of cells that are actively dividing using such measures as mitotic index, thymidine labeling index and S-phase fraction (SPF). High proliferative rates and abnormal DNA content of cancer cells strongly correlate with faster progression and earlier recurrences. Proliferative capacity is assessed by image analysis and/or flow cytometry of cytological specimens. IHC is also used visually to evaluate cell proliferation.

Neopharm (Lake Forest, IL) is developing radiosentizers broxuridine (BUdR) and idoxuridine (IUdR) as tumor cell proliferation markers to assess cancer severity for diagnostic purposes. Following patient administration of these drugs in small doses, biopsies of the tumor are treated with specific MAbs to highlight presence of BUdR or IUdR; the actively dividing cells can then be identified and counted. The company has access to data relating to the diagnostic use of BUdR and IUdR in approximately 1,300 cases of brain cancer, 250 cases of breast cancer, 700 cases of hematological malignancies and 40 cases of head and neck cancer. In October 1995, when the company met with the FDA to review use of BUdR in a diagnostic application prior to an NDA filing, the FDA requested additional information.

Tumor Invasiveness or Metastatic Potential

Axillary lymph node status (presence of tumor in the axillary nodes) by a complete dissection of levels I and II axillary nodes is the primary breast cancer staging modality and a specific and reliable prognostic indicator. Because risk of tumor recurrence in primary breast cancer is related to the number of positive axillary lymph nodes, at least 6-10 nodes must be examined histologically to determine involvement. The same number of nodes is retrieved for examination irrespective of surgical procedure employed, i.e. Halsted's operation (radical mastectomy), modified radical or simple mastectomy, or lumpectomy.

Nodal status also determines adjuvant chemotherapy regimen. However, although lymph node status is a powerful prognostic indicator, approximately 30% of node-positive patients survive for long periods whereas 25% of node-negative patients relapse within a very few years. The relationship between nodal status and breast cancer relapse and survival rates is presented in Exhibit 2.

A 1995 study by the Instituto Nazionale per lo Studio e la Cura dei Tumori (Milan, Italy) identified four independent prognostic parameters of the primary tumor, tumor size, grading, and overexpression of the HER-2/neu oncogene and the laminin receptor, that better predict outcome than nodal involvement. In a retrospective series of 500 primary breast carcinomas without palpable nodes and with a 20-year follow-up, presence of these parameters correlated only weakly with nodal status but predicted disease outcome more accurately than did nodal status (Ménard S, etal, JNCI, 8 April 1995, 87(8):607). Noninvasive methodology and the advent of biologic indicators of tumor aggressiveness may well supplant surgical lymph node dissection in the future, but until then, this procedure remains the most reliable prognostic indicator available.

Tumor size usually correlates with axillary lymph node involvement. However, it is not an important discriminant within axillary node groups except for patients with >4 involved nodes. Tumor size is a less useful prognostic indicator than axillary node involvement. Large tumor size per se does not indicate poor prognosis. For instance, in localized disease, patients with tumors >10 cm have similar survival rates as those with tumor sizes of <1 cm. Conversely, small tumor size does not necessarily indicate a favorable prognosis; some small tumors detected by mammography are aggressive and associated with distant metastases.

Exhibit 2
Recurrence and Survival Rates Based on Lymph Node Involvement

Axillary Node	Relapse Rate (%)			Survival (%)	
Status	18 months	5 year	10 year	5 year	10 year
N-	5	18-19	24	78	65
N+	33	65	76	47	25
N+ (1-3)	13	40-50	65	62	38
N+ (> 4)	52	79	86	32	13
All patients	17	40	50	64	46

Source: Adapted from Fisher B and Slack N, Surg Gynecol Obstet, 1970; 131:79

Other Breast Cancer Markers

An incredible amount of information is being amassed on cellular and molecular markers associated with malignancy. These markers are substances produced by a tumor cell or by the host during tumorigenesis or as a reaction to the presence of malignancy. Although it is anticipated that molecular biology will change the way breast and other cancers are detected, staged and monitored in the long run, to date, molecular markers have had a limited impact in the management of cancer. In breast cancer, mammography has been instrumental in the detection of breast abnormalities that in some cases were proven to be early (subclinical) cancer. However, mammography has its limitations.

Investigators are currently searching for biologic markers to be used in the early detection of breast cancer and for disease staging, monitoring and prognosis. Currently useful biologic markers such as nonspecific antigens including carcinoembryonic antigen (CEA) and tumor-associated antigens such as CA 15-3 (CA 27.29) are being used to detect advanced disease (stage III or IV) and/or monitor disease progression during and after treatment.

Numerous new markers are under investigation, including various genes and their products, but, to date, none has been shown to be more useful than established clinical and pathological criteria for diagnosing or staging cancer. Because breast or any other cancer does not conform to any standardized description, attempting to use the presence or absence of any biologic marker(s) in the diagnosis or prognosis of malignancy is at best a very precarious undertaking, as illustrated by conflicting results from numerous clinical trials. For instance, the presence of a biologic marker in a significant number of women experiencing a certain outcome does not preclude that it has no role in determining this outcome. It could very well be that other unknown or unmeasured factors are far more instrumental in determining this outcome. It is also possible that any given marker is not an independent determinant of the outcome but only plays a role in combination with other known or unknown contributing factors.

Inherited susceptibility, determined by mutations of newly identified genes such as the ataxia telangiectasia (AT) mutated (ATM) gene and BRCA1 and BRCA2 genes (see FO, V2 #4, pp 337-339), may also prove valuable as a prognostic tool. However, developing test kits to identify mutations on these genes represents a technological challenge. For instance, allelic heterogeneity of BRCA1 mutations in the population at large, coupled with the very large size of this gene, is proving very challenging in the development of accurate, quick and inexpensive assays. Various companies are developing BRCA1 and BRCA2 tests. In September 1996, Myriad Genetics announced that it plans to provide a comprehensive full-sequence testing service to identify mutations in both the BRCA1 and BRCA2 genes under the brand name BRAC-Analysis (see FO, V2 #5, p 362 and FO, V2 #4, p 337).

Investigators from the National Center for Human Genome Research (NCHGR; Bethesda, MD) have been exploring the use of DNA chips to develop a methodology to identify mutations in such genes as BRCA1. In this approach, oligonucleotides of length 15 to 20 nt and of known sequence are synthesized onto the surface of a solid support in a gridded array. As many as 60,000 oligonucleotides can be fixed on the surface of a 1 cm x 1 cm chip, so that the entire coding sequence of both strands of BRCA1, as well as all possible missense mutations and single nucleotide deletions, is represented. Determination of the presence of a given mutation is made using PCR amplification and fluorescence labeling of the BRCA1 gene from a patient sample, followed by hybridization to the chip and recording of the results using a charged-coupled device (CCD) detector. A two-color analysis, where a normal control is labeled in one color and the patient in another, allows quick identification of discrepancies between the two signals, making the process even more powerful. If the sensitivity and specificity of this approach proves adequate, it shows great promise for the rapid detection of mutations and

could be generalized to detect mutations in any gene (Collins FS, AACR96, Extended Abstracts, p 670).

Similar approaches are used by several companies to develop tests that sequence the genome of genes putatively associated with cancer and many other diseases to identify mutations/deletions deemed relevant in the diagnosis, prognosis and monitoring of disease. Prominent among such developers are Affymetrix (Palo Alto, CA) and Molecular Tool (Baltimore, MD) that are working with broad based technologies. Zeneca Diagnostics (was Cellmark Diagnostics; Germantown, MD) in collaboration with Johnson & Johnson Clinical Diagnostics (Rochester, NY) is developing a technique, Amplification Refractory Mutation System (ARMS), that also detects point mutations or small deletions in genomic DNA.

Estrogen- and progesterone-receptor proteins are valuable prognostic factors that may be used to guide therapy. In breast cancer, about one third of tumors are estrogen-receptor negative (ER-), and nearly all of these are resistant to endocrine therapy. Interestingly, a study designed to investigate the molecular mechanisms involved in the ER- phenotype found that only rarely is it the result of mutations in the coding region of the ER gene. Rather, it appears that it may be caused by deficiencies in ER expression at the transcriptional or post-transcriptional level. A search for mutations in exons 1 through 8 of the ER gene in 118 ER+ and 70 ER- primary breast tumors revealed no deletions/insertions and only two missense mutations, both in the same ER- tumor, indicating that only 1% of the tumors had point mutations in the ER gene. Neutral polymorphisms found in codons 10, 87, 243, 325, and 594 did not correlate with any clinicopatho1ogic parameters except for codon 325, which showed a strong, statistically significant correlation with family history of breast cancer (Roodi N, etal, JNCI, 15 March 1995, 87:446-51).

p53 is a tumor-suppressor gene located on the short arm of chromosome 17. Dysregulations and mutations of p53 have been detected in most tumors. However, the pathogenetic role of p53 has not been fully described. Also, conflicting results have compromised the role of assessing p53 status as a means of prognosis and effectiveness of treatment. Such conflicting results may stem from methodology lapses and can be avoided if testing approaches become more rigorous and standardized. One approach to definitively establish p53's role in breast cancer prognosis uses DNA sequence analysis of the complete p53 gene using Sequence-Based Diagnosis (SBD), a new concept for complete DNA sequencing, and then links tumor mutations with patient outcome, especially in relation to adjuvant therapy and radiotherapy. SBD, using the automated laser fluorescence (ALF) DNA sequencer developed by Pharmacia Biotech (Stockholm, Sweden), finds mutations over the entire coding sequence that may be missed using traditional molecular biology methods such as immunohistochemistry or DNA analysis. When the complete coding region of p53 was sequenced in 316 (97 node-positive, 206 node-nega-tive and 13 unknown node status) consecutively presenting breast cancer cases (from January 1987 to December 1989), followed up for a median time of 57 months, p53 status correlated with prognosis and response to adjuvant therapy. A total of 69 individual mutations in partly different sites were detected in 29 node-positive and 40 node-negative patients. Adjuvant therapy, particularly tamoxifen (Nolvadex; Zeneca), in conjunction with radiotherapy, was less effective in node-positive tumors with p53 mutations; such mutations in the conserved regions II and V of p53 were associated with worse prognosis (Bergh J, Nature Medicine, Oct 1996, 1 (10):1029-34).

HER-2/neu (c-erbB-2) oncogene is also considered to be a relevant prognostic marker in breast cancer. Activation of neu is pivotal in mammary tumorigenesis and overexpression and amplification of neu is associated with tumor progression. Mutations, however, are not predictive in node-negative breast cancer but appear to predict response to adjuvant chemotherapy in node-positive patients.

Prostate-specific antigen (PSA) may also serve as a prognostic marker in breast cancer. In a collaborative study at the Jefferson Cancer Center of Jefferson Medical College (Philadelphia, PA), the University of Toronto, Toronto Hospital, Uniformed Services University of the Health Sciences (Bethesda, MD), and Memorial Sloan-Kettering Cancer Center (New York, NY), a protein identical to prostate-specific antigen (PSA) was recently found to be produced in approximately 30% of breast tumors. Presence of PSA, based on a cut-off level of 0.03 ng/mg, was associated with early stage disease, small tumors, and ER+ tumors. Patients with PSA+ tumors, irrespective of nodal or hormonal status, had a lower risk of developing relapses and dying from the disease. It appears that presence of PSA is an independent, favorable prognostic factor and may be useful in identifying ER- node-positive patients with a good prognosis (Yu H, etal, Cancer Res, 15 May 1995, 55:2104-10).

Other markers/indicators with diagnostic, prognostic and monitoring properties include:

- Cathepsin-D, a proteinase, has been associated with the promotion of metastasis
- Urokinase plasminogen activator (uPA), also a proteinase that promotes metastasis, correlates strongly with poor clinical outcome
- Growth factor receptors on epithelial cells are also under investigation as markers; characterization of epidermal (EGF) and insulin-like (IGF-I) growth factor receptor molecules in neoplastic tissue or their measurement in patient sera, may provide clues as to the status and progression of breast cancer

- MTS1 (CDKN2/p16), the multiple tumor-suppressor gene that is a cell-cycle regulator potentially involved in genesis of many tumor types, was originally described by investigators at the University of Utah Medical Center (Salt Lake City, UT) and Myriad Genetics (Science, 15 April 1994, 264:436-40). Located on the short arm of human chromosome 9 (9p21), it is localized to a region of less than 40 kilobases and encodes the cell-cycle-regulator protein p16 of cyclin-dependent kinase 4; MTS1 is frequently inactivated through homozygous deletion in many human cancers (Brenner AJ and Aldaz CM, Cancer Res 55:2892-5, 1995) but may not be a critical genetic change in the formation of primary breast carcinoma (Xu L, etal, Cancer Res 54:5262-4, 1994; Quesnel B, etal, Br J Cancer 72:351-3, 1995; and Berns EM, etal, Br J Cancer 72:964-7, 1995)
- Cell-surface sialoglycoprotein (luminal epithelial antigen or LEA.135), expressed in normal and in a subset of neoplastic epithelial cells, is associated with a favorable prognosis in patients with lymph node-negative breast cancer (Imam S, etal, Anticancer Research, 16 (6), 1996); this marker is being evaluated as a prognostic test by Dako (Carpinteria, CA)
- Early growth response-1 (EGR-1) protein stimulates cancer cells to produce transforming growth factor-β1 (TGF-β1) which maintains normal cell growth. Lack of TGF-β1 leads to uncontrolled cell proliferation and tumor formation. Growth-arresting properties of TGF-β1 are disrupted early in the development of many kinds of human tumors including colon, breast, and pancreatic cancer. According to Dan Mercola, MD, PhD, and colleagues at the Sidney Kimmel Cancer Center (San Diego, CA) and the Burnham Institute (was La Jolla Cancer Research Foundation; La Jolla, CA), as reported in the October 15, 1996 issue of the *Proceedings of the National Academy of Sciences*, EGR-1 was below normal or undetectable in the majority of human glioblastoma, breast, and lung cancer cell lines examined
- Angiogenesis activity, measured as tumor microvessel density, is also under investigation as a prognostic factor (see FO, V1 #11, p 254 and #12, pp274-275)
- Telomerase is not present in most normal cells but is abnormally active in cancer cells causing telomere length to be maintained and, as a result, confers immortality to these cells; telomerase was detected in many different cancer types, including prostate, breast, ovarian, lung, and colon cancer, among others

IN VITRO TESTING METHODOLOGIES

Numerous R&D programs are ongoing to develop markers that can be used in the management of cancer in general, and breast cancer, in particular. This segment describes markers associated with breast cancer and briefly reviews technologies and products in development directly targeting breast cancer.

In vitro Diagnostics

In vitro diagnostics (IVDs) usually represent the most simple and cost-effective means for the detection of any disease, including cancer. Although currently there is no such test capable of diagnosing breast cancer, several *in vitro* tests have been approved for certain specialized applications and numerous are in development. In December 1995, the Immunology Devices Panel of the FDA unanimously recommended reclassification of Centocor's (Malvern, PA) IVD products from class III, which requires premarket approval, to class II which requires a 510(k) premarket notification. It is expected that this decision will encourage the introduction of new tumor marker tests.

Biomira Diagnostics (Rexdale, Ontario, Canada), a wholly-owned subsidiary of Biomira (Edmonton, Alberta, Canada), has developed Truquant BR, a radioimmunoassay (RIA)-based blood test that was approved by the FDA, in March 1996, for the detection of recurrence of cancer in women previously treated for Stage II and III breast cancer. The test uses the B27.29 MAb to detect breast cancer-associated antigen CA 27.29 (also known as CA 15-3) which is shed into the circulation by tumor cells at high levels during metastasis. This is the first breast tumor marker-based test to be commercialized in the USA. In April 1996, Biomira Diagnostics entered into an agreement with Immunex (Seattle, WA) to co-promote the test to medical oncologists in the USA and with Polymedco (Cortlandt Manor, NY) to promote and distribute the kits to the USA laboratory market. Truquant BR is also marketed in Canada, Europe, and Japan.

According to investigators, Truquant BR is highly predictive of relapse in Stage II and III breast cancer. A minimum of three assays were performed over a period of 18 to 24 months in 166 evaluable patients treated for Stage II (133 patients) and Stage III (33 patients) disease who had no clinical evidence of metastases. Truquant BR results were considered abnormal if two consecutive test results were above the normal upper limit (37.7 U/ml). During a median follow-up time of 13.5 months, cancer recurrence was detected by clinical signs or imaging in 26 patients (16%). The median lead time for Truquant BR to predict recurrence prior to clinical detection was 5 months. At 95% CI, the assay's sensitivity was 58%, specificity was 98%, positive predictive value was 83% and negative predictive value was 93%. Truquant BR was also useful in predicting bone metastases in women com-

plaining of bone pain. Of 32 women with bone pain, none of the 29 with negative CA 27.29 values were subsequently diagnosed with breast cancer recurrence but all three with positive CA 27.29 results develop recurrence. Truquant BR may prove a more cost-effective means of determining the likelihood of recurrence than *in vivo* imaging (Muss H, etal, ASCO96, Abs. 117). It is currently recommended that Truquant BR is used in conjunction with other clinical methods of early detection of recurrent breast cancer.

In May 1995, Ciba Corning Diagnostics (CCD; Norwood, MA), a wholly-ownend subsidiary of Chiron (Emeryville, CA), was granted the right to use Biomira's MAbs against breast, gastrointestinal and ovarian tumor antigens to develop *in vitro* diagnostic assays for its fully automated random access analyzers. The deal involved an upfront licensing fee and royalties on sales. At the same time, Biomira also granted Behringwerke (Liederbach, Germany) a nonexclusive right to use its antibodies against the CA 125 and CA 27.29 antigens in its fully automated immunoanalyzers in return for an upfront licensing fee and future royalties.

Centocor is marketing several oncology IVDs based on the detection of tumor-associated antigens, such as CA 15-3 used in the monitoring of breast cancer. The product is currently sold outside the USA, in certain European countries and in Japan. Centocor, in collaboration with a computer software company, is also developing a cellular diagnosis system to rapidly detect rare cancer cells in blood, bone marrow and other tissues. The Rapid Rate Event Detection (RRED) system is a computer-driven high-powered microscope that uses reagents developed by Centocor, based on its experience with Panorex, a MAb-based immunotherapy currently approved for the treatment of colorectal cancer.

Geron (Menlo Park, CA) is developing techniques to detect and quantify telomerase, a universal and highly specific marker of cancer, that may have clinical utility in cancer diagnosis, prognosis, monitoring and screening. Telomerase-based diagnostics are expected to address a broad range of cancer types. Geron has developed several proprietary assays for the detection of telomerase. A first generation product, Telomeric Repeat Amplification Protocol (TRAP) assay, detects telomerase activity in malignant tumor tissue. A second generation assay detects the RNA component of human telomerase, first cloned by Geron scientists. This assay uses proprietary *in situ* hybridization and other telomerase detection methods. Geron is the exclusive licensee of an issued USA patent on TRAP technology and a patent application is pending on the RNA approach. Preliminary data from a number of clinical studies indicate telomerase levels correlate with clinical outcome in cancer patients.

Oncor (Gaithersburg, MD) and Boehringer Mannheim (Mannheim, Germany) have licensed the TRAP assay and Dako has licensed the RNA detection technology on a non-exclusive basis for sale for research use. Oncor began selling the TRAP-eze kit in May 1996. Geron has also entered collaborative agreements with DIANON Systems (Stratford, CT) and Ventana Medical Systems (Tucson, AZ) and also established research collaborations for the study of telomerase detection with the Cleveland Clinic (Cleveland, OH), the University of Texas, San Antonio, and the University of Texas Southwestern Medical Center (Dallas, TX).

Horus Therapeutics (Rochester, NY) is developing MammAsure, a serum-based neural network-derived test combining several biomarkers, for the diagnosis of breast cancer in premenopausal women. Sample analysis is based on the company's computer-assisted deductive reasoning system (CADRS) that teaches a neural network to identify discrete changes in complex patterns of biochemical activity (see FO, V2 #1, p 290). In a pilot study involving 150 women, evenly divided by disease status (normal, breast cancer and benign disease), a version of MammAsure incorporating 5 biomarkers (CA 15-3, CEA, MAbs 115D8 and DF3 and HER-2/neu) exhibited sensitivity of 81% and specificity of 89%.

Matritech (Newton, MA) is developing breast cancer assays based on the detection of nuclear matrix proteins NMPs (see FO, V1 #1, p 28) that are present at elevated levels in the serum of breast cancer patients. The development and marketing of breast cancer products are covered by agreements with Sangtec Medical, a Swedish company which holds exclusive marketing rights in Europe, and Yamanouchi (Toyko, Japan) with similar rights in Japan and Taiwan.

Novopharm Biotech (Toronto, Ontario, Canada) signed, in April 1996, an agreement with BreasTek (Charleston, SC) to co-develop a monitoring test for breast cancer. The company announced that it would provide funding of approximately $1.5 million in the first year and $1.4 million in the second year to BreasTek and may provide additional funding for a third year at approximately the same level as the proceeding two years. As of June 30, 1996, the company had provided $393,000 in research funding to BreasTek. If the project is successful (as defined by the terms of the agreement), the two companies will establish a formal collaborative agreement. BreasTek has invested more than a decade of research to arrive at the current prototype version of the monitoring test. Unlike other tests of its kind, preclinical data from this test provide evidence of its ability to monitor Stage I recurrent breast cancer. Novopharm Biotech is a publicly traded subsidiary of Novopharm (Burnaby, BC), one of Canada's largest generic pharmaceutical companies.

Oncogene Science (Uniondale, NY) is developing cancer diagnostic tests based on oncogenes, tumor suppressor genes and other genes whose proteins are directly involved in tumor growth or metastasis. Both

serum and histologic assays are in development to aid oncologists in the confirmation, monitoring, staging, screening and prognosis of cancer. Currently, the company is focusing on breast and colon cancer, but many of the cancer markers under development are expected to have clinical utility for other malignancies, including lung, prostate, ovarian and stomach cancer. Oncogene Science has been pursuing its cancer diagnostics program in collaboration with Becton Dickinson (B-D; Franklin Lakes, NJ) under an agreement entered in October 1991 (after an earlier collaboration from 1984 to 1989). In 1995, this program's focus was changed to involve exclusively tissue-based diagnostic tests, including immunohistochemistry. As a result, B-D reduced funding for this program in fiscal 1996. It is anticipated that B-D will seek FDA approval for one or more manual immunohistochemistry tests for the pathology market in the near future. Oncogene Science is currently seeking collaborators for its serum-based cancer diagnostics.

Oncor filed a pre-market approval application (PMA) for its INFORM HER-2/neu breast cancer test for use in the management of breast cancer. As of October 1996, the FDA has accepted the clinical utility and the statistical model for determining the predictive value of this test but has asked Oncor to re-run some previously examined specimens at an independent laboratory and to co-develop a custom physician education/training program to help ensure the reproducibility of the test in the field. No new clinical trials were requested by the FDA. Concurrently with the re-running of samples, Oncor plans to proceed with the ongoing manufacturing validation and FDA inspection, required prior to final approval. Oncor has received a number of regulatory approvals for INFORM HER-2/neu breast cancer test abroad and has initiated marketing of the test in the UK, Germany, Switzerland, Denmark, Austria, the Netherlands, Ireland, Australia, and Canada.

In March 1994, Oncor acquired an exclusive worldwide license from Johns Hopkins University (Baltimore MD) for a molecular staging assay to detect small numbers of metastatic cancer cells in pathology samples. The test is more sensitive than current methods and is intended as a staging methodology for solid tumors. As of early 1996, the test was being developed by OncorMed (Gaithersburg, MD), Oncor's medical services affiliate, for application in the detection and management of breast, colon and prostate cancer.

In July 1996, Oncor announced that it had obtained an exclusive, worldwide license for a blood test, the p43 test, capable of detecting early-stage breast cancer. After FDA approval, Oncor initially intends to market this test as a companion to conventional mammograms of women at high risk for the disease. Developed by Chaya Moroz at Tel Aviv University's Sackler School of Medicine in Israel, the p43 blood test detects an early immunological event associated with the host's response to the development of primary breast cancer, particularly in women at high risk for breast cancer. According to a study of 3,400 women, including 900 with known breast cancer risk factors, such as family history, elevated levels of p43 protein strongly correlated with the presence of *in situ* and early (Stage I/II) breast cancer. In follow-up of patients after surgery, the test showed that p43 protein levels declined after removal of pre-cancerous or malignant tumors. Studies suggest that the measure of p43 may also be a highly accurate predictor of tumor development and recurrence. The company intends to market the p43 test to complement its INFORM HER-2/neu gene amplification test. In addition to its license agreement with the developers of the test, which include the National Health Institute of Israel, Oncor announced that patents covering the technology have now been issued in the USA (#4,882,270; 4,954,434; 5,120,640; and 5,283,177) and additional patents are pending.

In July 1996, OncorMed entered into a collaborative agreement with Zeneca Diagnostics under which the latter will supply OncorMed with proprietary reagents to be used in OncorMed's cancer genetic testing in the USA.

Paracelsian (Ithaca, NY) is developing two diagnostic or prognostic markers for cancer, one based on the detection of Cdk2 biomarker, associated with abnormal cell division, and the other, a test for p34 protein, a patentable marker associated with cell cycle coordination and control.

Reference Laboratory Services

Impath (New York, NY), a company that specializes in providing diagnostic, prognostic, and monitoring services in oncology, is also providing comprehensive prognostic information essential to the management of breast cancer. Impath specializes in intensive, highly technical, patient-specific service on a 48-hour turn-around basis. Impath maintains a comprehensive cancer database and tissue library that emphasizes patient outcomes and optimal treatment protocols. The company's clientele includes 4,000 physicians in over 1,000 hospitals. Currently, approximately 65% of the company's revenues are derived from the provision of prognostic and 35% from diagnostic analyses.

MRDx Diagnostics (Bothell, WA) was established by CellPro in 1995, to explore methods to identify tumor cells in the bloodstream or the bone marrow to determine presence of micrometastases before they are manifested clinically and to detect residual tumor cells that may be present in transplantation products following surgery, chemotherapy or radiation which can contribute to cancer relapse. MRDx Diagnostics provides ultra-sensitive tumor detection through cell-specific MAbs and PCR techniques that enable detection of one tumor cell in one million peripheral blood or bone marrow cells. MRDx Diagnostics currently offers its services worldwide to detect tumor cells in patients being treated for lymphomas, or breast, prostate or lung cancer.

IN VIVO IMAGING APPROACHES

Various novel approaches are improving the diagnostic and monitoring capabilities of noninvasive imaging approaches used in the management of cancer, in general, and breast cancer, in particular. Techniques are also in development in teleradiology to transmit high quality image data from remote locations to a site where the image can be read and analyzed by experts. Imaging techniques are also used to guide the needle precisely to the tumor during breast biopsies. Accurate needle placement considerably reduces patient trauma and improves the efficiency of cell collection.

Mammography

Currently, the most effective screening modality for early detection of breast cancer is mammography, an x-ray technique employed to visualize the internal structure of the breast. Studies have shown that in women ages 50 and older (postmenopausal) mammography, coupled with clinical breast examination, is effective in detecting breast cancer and, with appropriate treatment, can result in as much as a 30% to 35% reduction in deaths in this age group. Mammography is also commonly used to re-evaluate patients whose screening mammograms revealed an abnormality. It is reported that nearly one-third of screening mammograms are impossible to interpret and mammography x-ray film often contains subtle information not easily discernible to the radiologist. Also, mammography is less reliable in younger patients with denser breast tissue. A recent study reported that in case of invasive cancer (excluding DCIS), sensitivity of mammography was 58% and 75% in women in their thirties and forties, respectively, compared to 92% in women in their fifties. Among women under the age of fifty with a family history of breast cancer, sensitivity was only 69% (Kerlikowske K, etal, JAMA, 3 July 1996, 276:33-8). Also, mammography, unlike early detection methods for some other cancers (PSA in prostate cancer and Pap smears in cervical cancer) detects cancers when they are already formed and often have invaded beyond the milk ducts. Therefore, currently, investigators are actively pursuing a variety of approaches to improve the screening and diagnostic performance of x-ray mammography and to adapt other *in vivo* imaging modalities for the detection of breast cancer.

Digital mammography, is one approach that promises enhanced diagnostic capabilities by improving the sensitivity of conventional mammography, especially in radiographically dense breast tissue. Digital mammography is also expected to decrease the radiation dose per mammogram and because it is a computerized technique, allow computer-aided diagnosis and teleradiology. On the negative side, full breast digital mammography is more complex and time consuming to perform and its cost-effectiveness must be conclusively demonstrated before expensive hardware is put in place for routine use of this modality. Numerous projects are pursuing developments in digital mammography. The National Digital Mammography Development Group, with funding from the National Cancer Institute (NCI), is developing and evaluating methods to increase image quality. The National Science Foundation (Arlington, VA) is currently funding a pilot study to see if software developed for image processing in astronomy can improve the ability of digital mammography to detect early breast cancer. In progress at Johns Hopkins University (Baltimore, MD), Georgetown University's Lombardi Cancer Research Center (Washington, DC) and the Space Telescope Science Institute (STScI; Baltimore, MD), this program aims to identify microcalcifications as small as 100 microns in breast tissue to detect breast cancer at its earliest stage. Several companies, among them Fuji Medical Systems (Stamford, CT), GE Medical Systems (Milwaukee, WI), Fischer Imaging (Denver, CO) and Trex Medical (Danbury, CT) and its subsidiaries, are also developing special detectors for digital mammography. Although digital mammography has not yet received FDA approval for clinical use in the USA, it is currently being performed routinely in certain European countries and in Japan.

Ultrasound Imaging

ATL (Bothell, WA) obtained premarket approval from the FDA to use its High Definition Imaging (HDI) digital ultrasound system in conjunction with mammography and physical examination, to determine whether a biopsy is needed for suspicious breast lesions. The FDA acted upon the recommendation of its Radiology Advisory Panel which unanimously recommended approval on December 11, 1995. The company's PMA application was based on the findings of an international multicenter study involving over 1,000 women with breast lesions. Clinical investigators found that use of HDI could reduce the number of biopsies by approximately 40%.

Mammoscintigraphy/Immunoscintigraphy

Antisoma (London, UK), in collaboration with the Royal Postgraduate Medical School at Hammersmith Hospital (London, UK), completed phase I clinical trials of a technitium-99m-labeled peptide against a cancer mucin and has initiated phase II clinical trials in breast and other malignancies (see FO, V1 #12, p 277).

Biomira is developing Tru-Scint AD, an immunoscintigraphy diagnostic based on technitium-99m-labeled MAb-170 which reacts with most adenocarcinomas and is an effective vehicle to target cancer cells. Tru-Scint AD is in phase II/III clinical trials in the USA to support a PLA for its use in the management of recurrent breast cancer. A phase III clinical trial for this agent in the detection of ovarian cancer is in process. In May 1996 Biomira announced that it filed a Canadian New Drug Submission (NDS) for Tru-Scint AD for the detection of recurrent breast cancer and of primary, residual or recurrent ovarian cancer, based on data from phase II

clinical trials. The Canadian Health Protection Branch (HPB) has confirmed that the product qualifies for priority evaluation status or "fast-tracking".

Immunomedics (Morris Plains, NJ) is investigating application of its CEA-Scan (arcitumomab), approved by the FDA in July 1996, for the detection of colorectal cancer, also in breast cancer (FO, V1 #12, pp 278-279).

Targon (New York, NY), a joint venture established in October 1996 by Elan (Athlone, Ireland) and Cytogen (Princeton, NJ), is developing Oncotec, a breast cancer imaging agent based on Cytogen's OncoScint CR/OV which has been approved by the FDA for colorectal and ovarian applications (see FO, V1 #2/3, pp 39, 71-72; #12, pp 277-278; and V2 #4, pp 342-343).

Magnetic Resonance Imaging (MRI)

Although significant progress has been made in the use of MRI in breast imaging, in 1995, of a total MRI volume of approximately 8.2 million scans performed in the USA, fewer that 2% involved the breast, mostly to evaluate breast implant status. Enhanced MRI using gadolinium-DTPA, currently estimated to represent about 30% of all MRIs performed in the USA, is even less common in breast evaluations with fewer than 1% of all currently performed enhanced scans involving the breast. In contrast, breast MRI was performed approximately three times as often in Europe in 1995, with enhanced procedures accounting for 50% of all breast MRIs. Investigators are, however, optimistic as to the future of MRI in breast cancer diagnosis because of its unique ability in evaluating soft tissue. MRI is particularly useful in cases where mammography has serious limitations. However, MRI is still hampered by a high rate of false-positives and high costs, estimated at about $750 to $1,000 for unilateral or bilateral scans, respectively. (Also see FO, V1 #9 p 216 and V1 #10 pp 234-235.)

Using a recently developed dedicated breast MRI method, three-dimensional rotating delivery of excitation off-resonance (RODEO), MRI was evaluated as a tool for determining tumor response and extent of residual disease after neoadjuvant chemotherapy in 39 patients with Stage II, III, or IV breast carcinoma who were prospectively evaluated prior to and following neoadjuvant chemotherapy by MRI, physical examination, and mammography. MRI accurately predicted the pathologic determination of residual disease in 30 of 31 cases (97%) and may be used to better select patients for breast conservation after neoadjuvant chemotherapy for locally advanced disease (Abraham DC, etal, Cancer 78:91-100, 1996). However, among 172 surgical biopsies for isolated clustered breast calcifications (mammograms showed 88 round and 84 linear/irregular microcalcifications), histological analysis found 58 *in situ* carcinomas, 22 invasive carcinomas, and 92 benign lesions, while preoperative Gd-DOTA-enhanced subtraction dynamic MRI showed early contrast enhancement in 76 of 80 malignant lesions (sensitivity 95%) and in 45 of 92 benign lesions (specificity 51%); two invasive and two intraductal carcinomas did not show early contrast enhancement. Poor specificity limits the diagnostic accuracy of dynamic contrast-enhanced subtraction MRI in distinguishing benign from malignant microcalcifications detected by mammography (Gilles R, etal, Journal of Computer Assisted Tomography, 1996 Jan-Feb, 20(1):9-14).

Fonar (Melville, NY) introduced a high throughput MRI system, the QUAD 7000 or 12000 scanner, designed specifically for breast cancer scanning. Using a four-bed carousel per scanner, the system can accommodate ten patients an hour and is expected to significantly reduce costs per scan to make them compatible with mammography costs.

Positron Emission Tomography (PET)

Positron emission tomography (PET) scanning is being studied to determine whether it can supplement or replace lymph node dissection in breast cancer staging and to detect metastases. PET scans use a trace amount of radioactivity to look at tumor metabolism. Breast cancer, similar to many other cancers, exhibits an increased metabolic rate for glucose. High energy PET imaging employing such agents as fluorine-18 deoxyglucose (FDG), a sugar analog that helps visualize patterns of metabolic function, identifies spots, or markers, with increased glucose metabolism that often represent cancer cells (also see FO, V1 #6, p 165 and #9 p 216).

A 5-year study to be conducted at the University of Michigan Medical Center (Ann Arbor, MI) has been funded by the NCI to evaluate the accuracy of PET scanning in the diagnosis of breast cancer. The study will enroll more than 400 newly diagnosed breast cancer patients. PET scanning will be used to determine whether tumor cells from a primary cancer have spread to the adjacent lymph nodes. Patients will also undergo standard axillary lymph node dissection to confirm the results obtained by PET and will be followed for an extended period after their initial PET scan to see how accurate it is in determining prognosis.

Optical/Laser Imaging

Optical/laser imaging of breast cancer is also under investigation. Using a time-gated optical imaging technique, pulsed laser light at wavelengths of 780 and 830 nm is transmitted through human breast tissues and time spectra of the diffused light through the tissue are recorded in nanoseconds. This approach images oxygen concentration in the cancerous and fibrotic breasts. A two dimensional image using data from different locations is then reconstructed as a set of spectra in pixel form. Imaging consists of absorption and scattering coefficients of early arrival photons; absorption coefficients at the two wavelengths are related to oxygen concentration and blood volume. One limitation of this technique has been inadequate imaging resolution of smaller tumors

(Nioka S, etal, Advances in Experimental Medicine and Biology, 1994, 361:171-9).

Laser Doppler perfusion imaging is another technique under development to assess breast skin blood perfusion and its patterns as a prognostic indicator and treatment monitoring method in breast cancer. The laser Doppler imager (LDI) creates an image of tissue perfusion by recording the Doppler shift caused by movements of red blood cells in the backscattered light of a laser beam. This method reflects the vascularity of overlaying skin. LDI detected higher skin blood flow in benign breast changes than in symptomatic normal patients but the highest levels were recorded in patients with breast cancer (Seifalian AM, etal, International Journal of Microcirculation: Clinical and Experimental, 1995 May-Jun, 15(3):125-30).

Imaging Diagnostic Systems (Plantation, FL) is developing a mammography system, Computed Tomography Laser Mammography (CTLM) which, according to the manufacturer, is capable of distinguishing between cystic and solid lesions irrespective the size or density of the breast. CTLM is currently undergoing clinical evaluation with FDA's permission granted in February 1996.

INVASIVE PROCEDURES

Biopsy

Biopsy, an invasive procedure, is widely used to confirm suspicious or inconclusive findings from mammography or other imaging techniques. Most suspicious mammograms prove normal upon re-examination and many positive ones involve benign disease or *in situ* tumors that may never progress to invasive cancer. As more women participate in mass mammography screening programs, more are also undergoing diagnostic biopsy procedures (Exhibit 1). This in turn has resulted in a large number of unnecessary biopsies. The cost of biopsy ranges between $1,500 and $3,500 when performed in a hospital setting and from $500 to $1,200 in a clinic. Based on an average cost of $2,500 per procedure, revenues from biopsies are estimated at $1.7 billion in 1995 in the USA.

There are many types of biopsies ranging from FNAs involving aspiration of cells obtained with a small needle to core biopsies using a larger needle to withdraw multiple tissue samples to surgical removal of part or the whole lump. Open breast biopsies involve a 2-step process that begins in the radiology suite with a radiologist manually inserting a needle into the lesion and placing a wire marker that identifies its location. With the wire is left in place, the patient is moved to a surgical suite where a tissue sample is removed through an open incision. Because the wire marker is inserted manually, it is often difficult to place it with a high degree of accuracy and, as a result, a larger specimen is removed, often the size of a golf ball or larger, causing some degree of disfigurement.

Biopsys Medical (Irvine, CA) is currently marketing the Mammotome Biopsy System, a single-insertion breast biopsy system that was granted FDA 510(k) clearance in April 1995. Biopsys signed a marketing agreement with Lorad (Danbury, CT) to distribute the Mammotome in conjunction with Lorad's prone stereotactic breast biopsy table. Until recently, Lorad, a unit of Trex Medical, was selling its own stereotactic breast biopsy system. Mammotome is also compatible with the prone stereotactic breast biopsy table supplied by Fischer Imaging. Using the Mammotome, core needle biopsies can be performed in a 20-minute procedure without removing and reinserting the probe. Mammotome's list price is $7,500, with disposable probes priced at $135. As of June 30, 1996, it is estimated that the Mammotome installation base was 220 systems out of approximately 900 stereotactic breast biopsy tables in the USA. The company is planning to introduce a larger bore (11-gauge) disposable probe priced at $215. Biopsys also launched its MicroMark Clip, a 1 mm titanium surgical clip that can be delivered to the biopsy site at the end of the procedure to mark the spot for future reference. Company sales were $3.5 million in fiscal 1996 (ending July 30).

United States Surgical (USS; Norwalk, CT) introduced, during the first quarter of 1996, Advanced Breast Biopsy Instrumentation (ABBI), a minimally invasive system for breast biopsy/surgery. ABBI uses a stereotactic x-ray approach to localize the biopsy site to within one millimeter. Placement of the wire marker and removal of the tissue sample are performed in one step, using a minimally invasive technique, with the patient under local anesthesia. The ABBI instrument is inserted only once to remove the entire specimen through an incision that can be less than one inch in diameter. If the specimen proves malignant, but the entire tissue margin is free of cancer cells, the surgeon decides if additional tissue must be removed. The system consists of StereoGuide, a specially designed stereotactic table manufactured for USS by Lorad, and USS' new minimally invasive breast biopsy instrument (in 5 mm, 10 mm, 15 mm and 20 mm sizes) which is similar in appearance and function to a trocar but it incorporates a device for removing tissue.

Ductoscopy

Ductoscopy, a potential method for detecting breast cancer by inserting a tiny, flexible scope through a nipple into a milk duct, is being investigated by researchers at the Jonsson Comprehensive Cancer Center at UCLA. As described in the October 12, 1996 issue of *Lancet* by Susan Love, an adjunct associate professor of surgery at the UCLA School of Medicine and Sanford Barsky, professor of pathology at the UCLA School of Medicine and head of pathology at the Revlon/UCLA Breast Center, this new approach may provide a means for much earlier detection of breast cancer. Most breast cancer starts in the lining of the milk ducts. Breast ductoscopy allows visual examination of the inside of the milk ducts, and can

be coupled with extraction of cells to test for precancerous conditions and insertion of dye to track the relationship between tumors in the breast and ducts. Ductoscopy has been employed in the past but, unless there is fluid leakage and the ducts are distended, it did not produce useful information. In this report, for the first time, ductoscopy was performed in women who did not have fluid leakage from their nipples. Nine women who were about to undergo mastectomies under general anesthesia were examined by ductoscopy. After observing the duct lining, salt water was instilled into the duct and then withdrawn to obtain ductal cells and then dye was delivered into the duct to allow researchers to further examine the breast once it had been removed. If early findings that breast cancer may originate from the cells of a single duct are confirmed, treatment may then be directed to just one area of the breast and early tumors may be treated by instilling medication directly into an abnormal duct. Work in this area is funded with a $579,587 grant from the Department of Defense.

Intra-operative Cancer Detection

Neoprobe (Dublin, OH) completed, as of April 1996, three early phase I/II trials involving 54 breast cancer patients, of its radioimmunoguided surgery (RIGS) system using three targeting agents, TAG antibodies B72.3 and CC49, and the peptide targeting agent lanreotide. Although it appears that RIGS could assist the surgeon in finding occult tumor, determining if margins of resection were clear and identifying spread of disease within the breast, the waiting time between administration and surgery required for the two TAG antibodies would limit their potential in this application. However, lanreotide used with RIGS identified cancer positive margins within a short interval, less than three hours, between injection and surgery. Neoprobe licensed lanreotide from Biomeasure (Milford, MA).

Neoprobe also obtained an exclusive worldwide license in August 1996 to use Biomira's murine MAb-170, in its RIGS system for intraoperative detection of breast cancer. Neoprobe plans to begin a phase I clinical trial involving up to 45 women treated with breast-conserving surgery. Biomira received an upfront fee and will receive milestone payments and royalties upon commercialization. The agreement also allows Neoprobe the opportunity to use MAb-170 in the RIGS process in other indications, such as ovarian cancer. MAb-170 is the antibody component of Biomira's Tru-Scint AD. In addition, Neoprobe has access to improvements to MAb-170 and an option to license another Biomira antibody, MAb-174. In September 1996, Neoprobe entered into a marketing agreement with USS giving the latter exclusive worldwide sales and marketing rights of Neoprobe's RIGS-can CR49, for intraoperative detection of metastatic colorectal cancer. USS paid Neoprobe $2 million in 1996 and will pay an additional $3.5 million when regulatory approvals are obtained for the procedure in Europe and the USA.

MORE ON PROSTATE CANCER

This article provides additional information that came to the attention of NEW MEDICINE subsequent to the publication of FUTURE ONCOLOGY, V2 #1 and #2/3 that covered prostate cancer. These past issues, together with this addition and coverage of selected presentations at the CaP CURE retreat appearing on page 396 of this issue, represent one of the most thorough reviews of the current status of prostate cancer management and of research efforts to develop novel diagnostic and therapeutic agents in this area. A listing of agents in development for the treatment of prostate cancer, presented in Exhibit 3 of this issue, should be consolidated with Exhibit 4 found in FO, V2 #2/3, pp 313-319.

NOVEL THERAPEUTICS

Gene Therapy

Axis Genetics (Cambridge, UK), in collaboration with the John Innes Centre (Norwich, UK) and Purdue University (West Lafayette, MI), has developed Epicoat, a chimeric virus particle (CVP) technology, that creates genetically-modified plant (cowpea) mosaic viruses that express 60 identical foreign peptides on their surface; when inoculated into cowpea plants large quantities of the virus are produced which may be purified to extract the desired proteins. Axis, originally organized as a wholly-owned subsidiary of Agricultural Genetics, was acquired by its management in May 1995. The company raised £1.1 million in August 1995 and £4.4 million in October 1996. Axis is collaborating with Oxford University in the UK to develop prostate cancer therapeutics based on its Epicoat technology.

Calydon (Menlo Park, CA), a private company is focusing its development efforts on prostate cancer and other benign diseases of the prostate. The company is developing therapeutic products based on a gene that enhances and promotes prostate-specific gene expression. Calydon plans to form strategic alliances to develop and market its products in foreign and domestic markets. Calydon's lead product for prostate cancer is scheduled to enter human clinical trials in the USA in 1997 (also see this issue, p 400). Calydon's prostate cancer therapeutic is based on a new proprietary prostate-specific regulatory gene which acts as a master controlling gene to regulate expression of other genes which code for proteins or other structural genes uniquely found in prostate epithelial cells. This gene controls and limits the transcription of structural genes to prostate cells and is the first such gene described for the human prostate.

Calydon is pursuing three strategies in its drug development efforts. The company has:

- developed a virus that only infects and grows on the subtype of prostate cells that cause prostate cancer; a single injection of this virus has cured prostate cancer in animal models

Exhibit 3
Agents in Development for the Treatment of Prostate Cancer

Primary Developer/ Affiliate(s)	Generic Name/ Number/ Brand Name	Drug Type/ Target/Mechanism/ Delivery	Status/ Location/ Indication	Comments
Axis Genetics/John Innes Centre, Purdue U and Oxford U	Epicoat	Chimeric virus particle (CVP) technology/creates transgenic plants that produce large quantities of desired proteins	Preclin/UK	
Bristol-Myers Squibb/ Manitoba Cancer Treatment and Research Foundation and U Manitoba (licensor)	DPPE	Chemosensitizer/used in combination with chemo-therapeutics to enhance their activity	Phase II/Canada/ hormonally-resistant prostate cancer	
Calydon		Attenuated virus that kills prostate cancer cells or their precursors	Preclin/USA/pro-phylaxis of prostate cancer, treatment of locally advanced prostate cancer	
Calydon		Gene therapy	Research/USA	Available for licensing
Calydon		Small molecule drugs	Research/USA/ metastatic prostate cancer	
Canji/ Schering-Plough		Prostate tumor suppressor gene-based therapeutics	Research/USA	Applied for a European patent (10/95)
Cell Genesys/Ludwig Institute and Sloan-Kettering Institute for Cancer Research		Gene therapy	Research/USA	Also breast, colon and lung
Centocor/Apollon	GeneVax	Naked DNA vaccine that mimics a live attenuated virus/IM	Preclin (4/96)/USA	
Genome Therapeutics Corporation		Mapping and positional cloning to identify genes associated with prostate cancer	Research/USA	
Genta/Institute of Cancer Research; Chugai (has option for exclusive license)	Anticode G3139	Antisense oligonucleotide/ inhibitor of bcl-2/induces apoptosis	Preclin/USA	Phase I/II(11/95)/ UK/drug resistant follicular NHL
Hoechst Marion Roussel	Ramorelix/HOE013		Phase I (10/96)/USA	
Hoechst Marion Roussel	MDL 101731	Irreversible inhibitor of ribonu-cleotide diphosphate reductase	Phase II/III/USA, Europe	Also lung, breast and colon cancer
Immune Response/Sidney Kimmel CancerCenter		Gene therapy, immunotherapy	Preclin/USA/prostate and brain cancer	
Ingenex/Baylor College of Medicine (licensor)	SG-94	Gene therapy/combines a truncated variant (p94) of the rb tumor suppressor gene with a liposome or viral vector	Preclin/USA	
Introgen Therapeutics/ RPR Gencell and UTMDACC (licensor)	Ad-C-CAM	Tumor suppressor gene C-CAM, delivered by an adenoviral vector (Ad-C-CAM)	Preclin/USA	
Jenner Technologies/Ribi ImmunoChem Research	Vaccine adjuvant		Phase I/II/USA	
Maxim Pharmaceuticals	Maxamine (formerly referred to as EpiLeukin)	H2 receptor agonist (H2RA) based on histamine dihydrochloride/ specifically "blocks" the phagocyte signal that leads to NK-cell death, thereby allowing NK-cells to retain cytotoxic function/IM	Phase II/Sweden	

- identified several gene therapy applications of the prostate gene, ranging from a prostate cancer prophylactic to a treatment of organ confined cancer, which it plans to license to others
- developed proprietary screening assays based on the master gene to discover novel small molecule pharmaceutical agents for the treatment of metastatic prostate cancer

Calydon's technology is protected by USA and foreign patent applications in all relevant markets.

Genome Therapeutics Corporation (GTC; Waltham, MA), a company established 35 years ago as Collaborative Research, has reinvented itself to emerge as another participant in the burgeoning genomics field. Among its R&D efforts that currently center mostly on anti-infective agents, GTC is also developing prostate cancer therapeutics. GTC is identifying gene sequences associated with prostate cancer by positional cloning, a technique that allows researchers to distinguish between genetic material of affected individuals from those who are disease free, thereby identifying the gene or genes responsible for the disease. Genes responsible for prostate cancer may be identified using data from mapping studies that compare DNA from a patient with prostate cancer to DNA from a close disease-free relative. GTC then associates these genes with their products in biological assays. Association of the gene, its product, and the production of a biological effect permits unambiguous identification of a potential drug target. GTC is using mapping and positional cloning to identify human disease genes, bioinformatics to manage and analyze the data accumulated during its genomic studies, and functional genomics such as gene knockouts, gene transcription identification and bioassay development, to define the biological function of genes it discovers.

Genta (San Diego, CA) announced in 1996 that the NCI will fund and conduct preclinical studies of its Anticode drug G3139 and, depending on the outcome of these studies, sponsor phase I clinical trials to evaluating G3139 against various solid tumors. Genta will collaborate with the NCI on the design of such clinical studies and in the selection of tumor targets among such malignancies as melanoma, and breast, prostate and colorectal cancers. The NCI will provide funding while Genta would supply the NCI with G3139. Bcl-2 gene, the target of G3139, is a proto-oncogene involved in the inhibition of apoptosis of cancerous cells. The protein produced by the gene makes cancer cells immortal, creating a survival advantage of malignant over normal cells. G3139 is designed to inactivate the RNA that produces Bcl-2 protein, thereby preventing cellular production of the protein. High levels of Bcl-2 associated with a poor clinical prognosis have been detected in a number of solid tumors and hematologic malignancies including non-Hodgkin's lymphoma (NHL), malignant melanoma, and breast, colorectal and prostate cancer.

Ingenex (Menlo Park, CA) is developing SG-94, based on a tumor suppressor gene, for the treatment of solid tumors. SG-94 is a gene therapy product in preclinical development that combines a truncated variant (p94) of the rb tumor suppressor gene with a liposome or viral vector. Although reintroducing rb itself into rb-deficient tumor cells inhibits the growth of these cells, it sometimes does so incompletely and tumor regrowth occurs in reconstituted cells after a period of latency. Rb protein encoded by the SG-94 gene therapy product is more effective at causing suppression of tumor cells than the full-length rb gene, based on data demonstrating *in vitro* suppression of numerous tumor types tested, including tumors of the bladder, prostate, cervix, bone, breast, lung and fibrous tissue. In addition, preliminary experiments indicate the modified gene is effective in suppressing some cancer cell lines *in vitro* that continue to contain a functional native rb gene. SG-94 is intended to be delivered directly to tumor cells through local application. In collaboration with Baylor College of Medicine (Houston, TX), Ingenex is currently testing SG-94 in preclinical studies of solid tumors in murine models.

Ingenex has obtained an allowance for a USA patent application directed to DNA molecules that encode protein p94Rb used in SG-94 and, in October 1992, licensed related assigned and pending patent applications from Baylor College of Medicine pertaining to the modified rb gene, including its use in conferring senescence to tumors that forms the basis of SG-94. According to this licensing agreement, Ingenex will pay royalties based on net sales of products and processes incorporating the licensed technology, subject to certain minimum annual amounts, and a percentage of sublicensing income arising from the license of such products and processes.

Introgen Therapeutics (Austin, TX), in collaboration with RPR GenCell (Collegeville, PA) and pursuant to a licensing agreement with the University of Texas M.D. Anderson Cancer Center (UTMDACC; Houston, TX), is engaged in the preclinical testing of a tumor suppressor gene, C-CAM, delivered by an adenoviral vector (Ad-C-CAM) for the treatment of prostate cancer. Preclinical studies conducted at UTMDACC identified C-CAM as a tumor suppressor based on its ability to alter the growth rate and characteristics of prostate cancer cells in both *in vitro* and *in vivo* models of human prostate cancer. Mutations or inappropriate regulation of C-CAM may be involved in cancer of the prostate. Prostate cancer cells treated with Ad-C-CAM vectors showed significantly slower growth rates and reduced tumorigenicity. Furthermore, non-tumorigenic prostate cells became tumorigenic when C-CAM levels were experimentally reduced using an antisense RNA approach.

Immunotherapy

Centocor is developing GeneVax, a naked DNA vaccine that mimics a live attenuated virus, for various cancer indications. Developed by Apollon (Malvern, PA), a private company 33%-owned by Centocor, GeneVax uses specific sequences of DNA to elicit responses from the immune system. The MHC-l-directed peptide, coded by the DNA vaccine, is then synthesized and processed intracellularly, as though it originated from a tumor cell. Under a licensing agreement with Apollon, Centocor holds rights to develop GeneVax in the cancer field. Initially, Centocor plans to target prostate, breast and colorectal cancer.

Maxim Pharmaceuticals (San Diego, CA), a public company with a recently issued IPO, is developing Maxamine (formerly referred to as EpiLeukin), which is based on the discovery of an immune system mechanism that may allow certain biological response modifiers, such as cytokines, to achieve their full antitumor potential. Maxamine, an H2 receptor agonist (H2RA), is based on histamine dihydrochloride which specifically "blocks" the phagocyte signal that leads to NK-cell death, thereby allowing the NK-cells to retain cytotoxic function. Although IL-2 and IFN-α activate the antitumor cytotoxicity of NK-cells *in vitro* and in mice, they have not been equally effective in humans. Dr. Kristoffer Hellstrand, a Maxim founding scientist, and his co-workers discovered that specific signals transmitted by phagocytes cause NK-cells to initiate apoptosis. Maxamine maintains the activity of NK-cells and may thus improve the therapeutic efficacy of NK-cell-activating cytokines such as IL-2 and IFN-α.

Phase II human clinical trials of Maxamine for the treatment of acute myelogenous leukemia (AML) and malignant melanoma are in progress in Sweden under the company's sponsorship. Phase II clinical trials have been initiated in multiple myeloma and renal cell carcinoma and are being planned for prostate adenocarcinoma. Maxamine is being developed as an outpatient treatment administered subcutaneously in combination with cytokine therapeutics.

In 1993, Maxim entered into a technology transfer agreement with Estero Anstalt, pursuant to which Maxim exercised an option to purchase the core intellectual property and patent rights related to Maxamine technology for a total price of $700,000 plus certain royalty obligations to Drs. Hellstrand and Svante Hermodsson, the two inventors of the technology.

Medarex (Annandale, NJ) initiated, in July 1996, a phase II trial of MDX-210, a bispecific antibody, for the treatment of prostate cancer (see FO, V2 #2/3, pp 322-323). The trial will take place in England at the University Hospital (Birmingham, UK) and at the Royal Postgraduate Medical School and Hammersmith Hospital.

OTHER DEVELOPMENTS

Genomics

At the NCHGR, Francis S. Collins and colleagues, in collaboration with researchers at Johns Hopkins University, are searching for genes that confer susceptibility to prostate cancer. Based on reviews of records of a hereditary prostate cancer database established at Johns Hopkins University, now incorporating data from 2,000 families, it was found that first-degree relatives of prostate cancer victims are twice as likely to contract prostate cancer than the population at large; significatly higher risk is also encountered in those with multiple affected relatives and in those whose relatives contracted prostate cancer at an early age. Inheritance pattern analysis indicates that about 5% to 10% of all cases of prostate cancer may be caused by an inherited mutation in a dominant susceptibility gene, paralleling findings in inherited breast cancer. Based on this model, about 43% of all prostate cancers that develop by age 55 would be attributed to inherited mutations and 80% of those carrying an altered gene would develop prostate cancer by age 85. Inherited prostate cancer does not appear to be different than sporadic cancer in terms of clinical and pathological attributes.

In another development, Paul B. Fisher, PhD, and colleagues at Columbia-Presbyterian Medical Center (New York, NY) reported in July 1996 the discovery of a gene, prostate carcinoma tumor antigen-1 (PCTA-1), that appears to be involved in prostate cancer metastasis by promoting cell adhesion. PCTA-1 is expressed on malignant but not on normal cells or benign prostatic hyperplastic tissue.

Recommendations/Approvals

Hoechst Marion Roussel (Kansas City, MO) received FDA approval, in September 1996, to market Nilandron (nilutamide, RU-23908) in the USA in combination with surgical or chemical castration in advanced prostate cancer. Launched in October 1996 at a recommended oral dose of 300 mg daily for 30 days and 150 mg daily thereafter, the drug will be imported from France where it is sold, as well as elsewhere, as Anandron. In a multicenter randomized double-blind placebo-controlled trial, patients on Nilandron experienced significant improvement in bone pain (54% versus 37%) and longer progression-free survival (21.2 months versus 14.7 months) and median overall survival (27.3 months versus 23.6 months). Among various side effects associated with Nilandron, there is a risk of interstitial pneumonitis that occurs in about 2% of patients during the first three months of treatment and of hepatic impairment which occurs in 1% of patients.

Immunex' (Seattle, WA) Novantrone (mitoxantrone) was recommended for approval by the Oncologic Drugs Advisory Committee of the FDA in September 1996 for the treatment of hormone-refractory late stage prostate cancer (see FO, V2 #2/3, p 309).

Schering-Plough was granted FDA approval, in June 1996, to market flutamide (Eulexin) capsules for the treatment of locally confined Stage B2-C carcinoma of the prostate, in combination with LHRH agonists. The drug is already approved for use in combination with LHRH agonists and radiation therapy (see FO, V2 #2/3, pp 307-309) in the treatment of advanced (Stage D2) prostate cancer. Shire Pharmaceuticals (Andover, Harts, UK) plans to launch a generic version of flutamide to be sold by various partners in Europe in 1997.

MEETING COVERAGE

CaP CURE'S THIRD ANNUAL SCIENTIFIC RETREAT
INCLINE VILLAGE AT LAKE TAHOE, CA
SEPTEMBER 5-8, 1996

This article is a review of CaP CURE's third annual retreat during which scientists gathered to share the results of their investigations and to help formulate new directions for future research in the field of prostate cancer.

CaP CURE AND THE MEETING

CaP CURE (Santa Monica, CA) was founded in 1993 by Michael Milken in response to the large research funding gap that exists between prostate cancer and other diseases that claim similar numbers of lives each year (see FO, V2 #2/3). The goal of CaP CURE, and of the scientists who have been recruited to the effort, is to search for controls and cures for advanced prostate cancer. During the 1996 retreat, the group agreed that progress was made in the past year toward finding a cure, that research tools needed to advance prostate cancer research are now virtually in place, and that a number of potential therapeutics are already in clinical trials.

PROSTATE CANCER RESEARCH

Prostate cancer research has been traditionally underfunded. For example, in 1996, federal funds allocated to prostate cancer research represent approximately 12% of similar funding committed to breast cancer research and 4% of the commitment to AIDS research. To date, CaP CURE has raised approximately $25 million to augment Michael Milken's contributions. CaP CURE is serving as a catalyst for bringing together scientists from many disciplines to work on the basic problem of prostate cancer. Its goal is to explore all avenues leading to a possible cure for the advanced stage of this disease.

Contributions to the goal of curing prostate cancer are emerging from many levels. Targets for the development of therapeutic strategies are being identified by scientists pursuing basic research in the areas of apoptosis and angiogenesis as well as other areas of cell biology and biochemistry. Gene therapy and immunotherapy approaches to treatment are being developed, new cancer-specific genes are being identified and novel drugs are being discovered or synthesized in the laboratory. Other important developments involve strategies to evaluate the progression of prostate cancer, including the latest techniques of *in vivo* imaging as well as identification of markers used for prostate cancer staging. (A comprehensive review of agents in research, preclinical development and clinical trials for the management of prostate cancer was presented in FO, V2 #2/3, pp 302-328.)

THE GENETICS CONSORTIUM

An important contribution to the entire biomedical community that evolved from CaP CURE's mission was the creation, in January 1996, of the genetics consortium. Although its purpose, the study of prostate cancer, is somewhat narrow, it is expected to benefit all areas of cancer research. According to Leroy Hood, the William Gates III Professor of Biomedical Sciences and chair of the Department of Molecular Biology at the University of Washington School of Medicine (Seattle, WA), the consortium represents a systems approach to medicine. Prostate cancer may be the first medical problem to truly benefit from the wealth of information promised by the accumulation of genetic data. The basic goals of the consortium are twofold:

- to complete analysis of genetic information on high-risk prostate cancer families and integrate the information with approaches to therapy
- to promote further development of emerging technologies in genetics

Database of Patient Histories and DNA Samples (PROGRESS)

Janet Stanford of the Fred Hutchinson Cancer Research Center (Seattle, WA) reported that, since its inception, the consortium has collected medical histories and DNA samples of members of 405 high-risk families in a database dubbed PROGRESS. Families included must have had prostate cancer present in three successive generations, and there must be at least three affected individuals living, two of whom were diagnosed prior to the age of 50 and are available for sampling. Most of these families came forward after Michael Milken, General Schwarzkopf, Stuart Holden, Medical Director of CaP CURE, and Leroy Hood appeared on the Larry King Live show. To date, DNA from lymphocytes of affected and unaffected individuals from 54 families and sibling pairs have been sent for genotyping. The three-year goal is 500 families.

Work being done by the genotyping arm of the consortium at the Fred Hutchinson Cancer Research Center was reviewed by Elaine Ostrander. Her group has initiated the genetic mapping of individual members of PROGRESS families and has begun blind genotyping of the sibling pairs and trios collected by Washington University (St. Louis, MO). She and Rick McIndoe from the

University of Washington described robotics and software capable of extraordinarily rapid and efficient genetic analysis. As Helen Donis-Keller from Washington University calculated, it should be possible to accomplish the immediate genotyping goal within a year.

A number of scientists presented preliminary results on genetic analysis. Genes that are over- or under- or newly-expressed in prostate tumors, as compared with those from the normal prostate, have been identified and will be the subject of future work. According to Leroy Hood's summary of the results of the genetics consortium, so far out of 10,000 clones investigated, 150 are overexpressed in tumor tissue. Fully half of these are new genes rather than known growth regulators, cell structure genes or prostate-specific genes.

TOOLS EMPLOYED IN PROSTATE CANCER RESEARCH

Other Prostate Cancer Databases

Among tools that make prostate cancer studies possible are collections of cells lines, tissues, blood samples, DNA samples and animal models. At earlier CaP CURE scientific retreats scientists argued that a major deficiency in the study of prostate cancer was a lack of these resources. Now, there is an extensive prostate cancer database and tissue bank in place at Washington University, under the direction of William Catalona, Chair of the Division of Urologic Surgery. This Family History Database contains nearly 2,300 cancer probands and, in some cases, their relatives and spouses as controls. There is also a tissue repository of frozen samples from over 1,600 radical prostatectomies. A collection of "super families" which meet the criteria for inclusion in the PROGRESS study, and 194 sibling pairs and trios have also been assembled. An African-American study described by Helen Donis-Keller, which includes DNA from 100 cancer patients and 100 unaffected men, is also part of this collection. A database of Dr. Catalona's own series of nerve-sparing radical prostatectomies from nearly 1,500 patients is available and continuously updated. Another large database is the PSA prostate cancer screening study which includes 31,000 men enrolled for six-month screening repeats. Two thousand of them were diagnosed with prostate cancer. Dr. Catalona said that a major project will be to link these databases for everyone's use.

Animal Models

In order to study human prostate tumor tissue and the changes it goes through as it progresses from androgen-sensitive primary tumor to metastatic androgen-insensitive tumor, the tissue must be grown in an environment outside of the original host. When implanted into mice, human prostate tumor tissue xenografts continue to grow and develop enabling the establishment of cell lines. New therapies are initially tested on these mouse xenograft models. Gert van Steenbrugge of Erasmus University (Rotterdam, the Netherlands) has established xenografts in NIMR I mice from primary as well as metastatic tumors which represent both androgen-sensitive and insensitive stages of disease. Charles Sawyers of the University of California at Los Angeles (UCLA) has produced six successful xenografts of advanced stage metastatic cancer implanted into athymic SCID mice. One of these xenografts progressed from hormone-sensitivity to insensitivity and cell lines from various stages of this progression have been established.

Transgenic Animals

Transgenic animals that carry recombinant genes that induce the development of prostate cancer are another important research tool. Transgenics mimic the progression and cellular changes of human prostate cancer and enable the establishment of genetically matched cell lines to study each phase of the disease. Timothy Thompson of Baylor College of Medicine has developed a different kind of transgenic model, a transgenic prostate gland animal, in which fetal prostate tissue from one mouse was removed, genetically manipulated by infection with a retrovirus containing a cancer-inducing gene, and then grafted into another animal. Because only a few cells are infected, these cells are able to progress clonally like in human cancer. An advantage of this system is that it mimics focal events that initiate cancer and may provide clues about early events in the generation of the primary tumor as well as the selection of metastatic clones. Clonal progression may be tracked by taking advantage of unique retroviral integration sites both *in vivo*, and then in cell lines.

THERAPEUTICS

According to Eli Gilboa of Duke University Medical Center (Durham, NC), basic approaches to therapy for prostate cancer can be divided into direct and indirect strategies. Direct strategies involve interfering with the tumor-specific biochemistry of tumor cells or directly killing such cells. Indirect strategies involve immunotherapies which activate the body's own defenses for eliminating abnormal cells. Biochemical targets for direct strategies focus primarily on:

- interfering with tumor blood supply (angiogenesis)
- stimulating the biochemical pathway leading to cell death (apoptosis)
- targeting killing agents to tumor-specific markers
- altering the activity of a process that is underactive or overactive in malignant cells

Prevention of Tumor Growth by Inhibition of Angiogenesis

Judah Folkman of Harvard Medical School Children's Hospital (Boston, MA) reported on the use of angiogenesis inhibitors in treating prostate cancer. Since tumor growth is highly dependent on the development of a blood supply, inhibition of the proliferation of cells lining the blood vessels (endothelial cells) can prevent tumor

growth, and in some cases causes tumor regression (for more on angiogenesis and its role in cancer management see FO, V1 #7/8, pp 185-199 and V1 #12, pp 274-275). Although this approach results in tumor dormancy rather than obliteration, the results obtained with two angiogenesis inhibitors, Angiostatin and endostatin, have been impressive. Treatment with Angiostatin prevented metastases and reduced tumor size in mice bearing grafts of human tumors. EntreMed (Rockville, MD) has cloned, scaled-up production and purified Angiostatin, in anticipation of upcoming clinical trials. EntreMed and Bristol-Myers Squibb (BMS) signed an agreement in December 1995 under which EntreMed gave BMS exclusive worldwide rights to Angiostatin and thalidomide analogs (see FO, V1 #7/8, p. 195) as well as first refusal rights to other potential antiangiogenic compounds under development by Dr. Folkman's team at Children's Hospital. In exchange, BMS made an equity investment in EntreMed, and agreed to fund a 5-year collaborative research effort to develop these anti-angiogenesis compounds for the treatment of cancer.

In order to prevent the tumor from redeveloping Angiostatin had to be administered continuously, but long-term treatment was not associated with any apparent side effects. Dr. Folkman and others suggest that anti-angiogenesis drugs could be used as a first line of defense against progression of prostate cancer. A combinational therapeutic strategy, first using angiogenesis inhibitors to stem tumor growth and then employing another approach to eliminate tumor cells, may prove effective in the management of solid tumors.

Activation of Tumor Cell Apoptosis

John Reed of the Burnham Institute is focusing on research to reduce activity of bcl-2 as a way of inducing apoptotic tumor cell death to cure prostate cancer (for more on apoptosis and the role of bcl-2, see FO, V1 #1, pp 22-31 and V1 #12, p 274). Bcl-2, one of the factors involved in apoptosis, becomes more abundant as tumor cells assume more malignant forms. When overproduced, Bcl-2 prevents apoptosis, allowing cells which might ordinarily self-destruct to continue to grow. While the actual function of bcl-2 has not been fully elucidated, information derived from its sequence suggests that it may play a role in creating pores in membranes by forming complex structures with other proteins and then inserting itself into these membranes. The pores themselves are presumed to initiate cellular changes, such as Ca^{2+} ion influx, that lead to apoptosis. The protein-protein interactions involving Bcl-2 will be the focus of research as likely targets for therapeutic strategies.

The mitogen-activated protein (MAP) kinase pathway, another intermediate step in the apoptotic pathway, can activate an alternative pathway leading to apoptosis. Massimo Loda of Harvard Medical School Deaconess Hospital (Boston, MA) finds that prostate cancer cells overproduce a regulator of this pathway, MAP kinase phosphatase I. Like with Bcl-2, overproduction of MAP kinase phosphatase I inhibits apoptosis. Therefore, agents that block MAP kinase phosphatase I may serve as adjuncts to therapies designed to trigger apoptosis.

Suramin, a drug known to activate apoptosis under development by Parke-Davis, is now in the final stages of FDA approval and could be on the market in 1997 as noted by John Isaacs of Johns Hopkins Oncology Center (Baltimore, MD). In spite of the fact that suramin is a non-specific killing agent, according to Wilbur Leopold of Parke-Davis, it is effective in treating hormone-refractive prostate cancer with minimal side effects when delivered in an optimized treatment schedule. (For additional information on suramin, see FO, V2 #2/3, p 310.)

Retinoids and vitamin D analogs that also activate apoptosis in cancer cells are being used in clinical trials for the treatment of prostate cancer by H. Phillip Koeffler of Cedars-Sinai Medical Center (Los Angeles, CA).

Telomerase activity may be a more indirect target for activating apoptosis. David Corey of the University of Texas Southwestern Medical Center is developing peptide nucleic acids designed to inhibit this enzyme. One of the reasons cancer cells proliferate abnormally is that they don't lose telomerase activity the way that normal cells do as they age. Since telomerase repairs DNA, its loss allows DNA damage to accumulate which then triggers apoptosis. Inhibiting telomerase activity in prostate tumor tissue allows activation of normal apoptotic pathway. Because telomerase contain an active RNA component, one inhibition strategy is to block this RNA using antisense RNA-binding reagents. Peptide nucleic acids are modified forms of antisense nucleic acids which hybridize to DNA and RNA so strongly that functional molecules can't displace them. Several such reagents are now being tested.

5-HETE, an intermediate in the sphingomyelin signal transduction pathway, represents another chemotherapeutic agent that triggers apoptosis. Charles Myers of the University of Virginia Health Sciences Center (Charlottesville, VA) has identified a late intermediate in the pathway, 5-HETE, which triggers apoptosis in prostate cancer P3 cells. A modified and highly active form of this intermediate, 5-HETE lactono, has been synthesized and will be tested for use as a therapeutic agent.

Inhibition of Tumor Cell Growth by Blocking Growth Factor Receptors

One of the reasons tumor cells proliferate rapidly is that they overexpress growth factor receptors and respond more readily to the corresponding growth signals.

Epidermal growth factor receptor (EGFr) is one growth factor receptor that is overexpressed in many malignancies. Harlan Waksal of ImClone Systems (New York, NY) has been developing antagonists of tyrosine kinase receptors like EGFr such as monoclonal antibodies

(MAbs) that react with the external domain of EGFr and inhibit its function. When he combines antibody treatment with doxorubicin, he finds that complete tumor regression can be achieved in 70% of cases in a mouse xenograft model. This agent is now in phase I trials for prostate and other cancers (see FO, V2 #2/3, pp 315 and 326). Howard Scher of Memorial Sloan-Kettering Cancer Center (MSKCC) is using the humanized version of the ImClone MAb to EGFr in combination with doxorubicin to treat androgen-independent prostate cancer. This strategy successfully reduced patient PSA levels and is expected to enter phase II trials in the near future.

Immunotherapy

A simple strategy for eliminating prostate cancer would be to turn on the immune system to recognize and attack the prostate gland. But because prostate tumor tissue does not, as a rule, stimulate the immune system it is necessary to use antigens that are specific to this tumor to use as vaccines. (For more on immunotherapy in general see FO, V2 #4, pp 345-355 and for more on immunotherapy of prostate cancer see FO V2 #2/3, pp 320-323.)

Muc-1, a polyvalent carbohydrate surface antigen present in many types of cancer cells including prostate cancer cells, is being employed by Philip Livingston of MSKCC in a vaccine formulation. As of May 1996, 10 patients were enrolled in a phase I clinical trial of Muc-1 cancer vaccine at MSKCC. Currently, several vaccines employing muc-1 are in development for such indications as breast, pancreatic and colorectal cancer.

Gene-modified tumor cell vaccines represent another possible type of immunotherapy. Jonathan Simons of Johns Hopkins Oncology Center has developed gene therapy strategies designed to turn on the immune response to prostate cancer cells by genetically altering them to express granulocyte-macrophage colony stimulating factor (GM-CSF). This factor stimulates growth and proliferation of many cells of the immune system, and focuses the immune response on targeting tumor cells for elimination. In this procedure, presently in phase I trials, prostate cancer cells are removed, transfected with DNA containing the GM-CSF gene, and then reinjected. This construct, GVAX, is under development by Somatix (Alameda, CA) for treatment of various cancers.

In a similar approach, Timothy Ratliff of Washington University, in collaboration with Virogenetics (Troy, NY), is using immunostimulatory factors and cytokine genes in a non replicating canary poxvirus vector injected directly into the prostate tumor. This combination therapy worked best in mouse models when the constructs contained genes for tumor necrosis factor combined with IL-2, GM-CSF, or IL-12.

T cell response stimulation is another immunotherapy approach. Eli Gilboa has been developing strategies to elicit cellular immunity using dendritic cells loaded with unfractionated antigens from prostate tumor tissue, either in the form of peptides or proteins derived from tumor tissue, or as products of *in vitro* translation of mRNAs extracted from the tumor. These cells present all sorts of antigens to the immune system, including known as well as unknown tumor antigens, resulting in a both cellular and humural immune response. Antigens recognized as self are ignored, while unique tumor antigens stimulate a tumor-specific response. Three of these strategies are in planned or active clinical trials.

To further enhance the tumor-directed immune-stimulation activated by this approach, Alton Boynton of Pacific Northwest Cancer Foundation Northwest Hospital (Seattle, WA) used a two component system consisting of dendritic cells and zinc antigen vaccination. Dendritic cells were isolated from prostate cancer patients and the specific antigen was an HLA-A2 binding immunostimulatory peptide derived from the sequence of prostate-specific membrane antigen (PSMA). This strategy was successful in phase I trials, and phase II trials will begin later this year.

Zelig Eshhar of the Weizmann Institute of Science (Rehovot, Israel) has constructed chimeric T cell receptors he calls T-bodies designed to redirect effector lymphocytes to respond more readily to tumor antigens. T-bodies are recombinant T cell surface receptors composed of the variable region of a prostate tumor-specific antibody heavy chain and the constant region of the T cell receptor. When expressed on the surface of T cells which were removed from the patient, genetically altered and then reinjected, T-bodies activated a vigorous T cell response to the tumor.

Martin Cheever of the University of Washington has been able to activate T cell-mediated autoimmune prostatitis in rats by immunization with a prostate-specific binding protein, a major secretory protein in rat seminal fluid which binds steroids, cholesterol and proline-rich peptides. It is normally sequestered and unavailable to the immune system, but is capable of activating an immune response when delivered as isolated protein.

To avoid the complications of genetically manipulating cells *ex-vivo*, Helen Tighe of the University of California at San Diego (UCSD) injected naked DNA intramuscularly to mount a T cell response to tumor antigens which are expressed by the cells which take up the DNA plasmids. She has shown that this approach works in that it activates an immune response, and the cells involved in the response are bone marrow antigen presenting cells.

Combination of immunotherapy and chemotherapy is being evaluated by William Nelson of Johns Hopkins Oncology Center. He pointed out that most of the existing vaccine strategies can eradicate small, but not large, tumors; he believes that integration of vaccine therapy with other forms of treatment, such as surgery and chemotherapy, will be the most effective course of action for patients with aggressive hormone-refractory systemic cancers. In his scheme, cancer cell-based vac-

cines in the form of readily available LNCaP cells were combined with doxorubicin drug therapy. Doxorubicin was selected because it is not immunosuppressive, and in fact appears to be immunostimulatory when given prior to the vaccine.

Gene Therapy Approaches

Although gene therapy is one of the more difficult strategies because of the inefficiency of incorporation of genes into cells, it is also the source of some of the most innovative approaches to specific anti-cancer therapies.

Jonathan Simons described the use of a PSA-adenovirus vector construct which is only activated to produce a lethal virus infection if the PSA gene is expressed by the host cell. Release of virus from the *ex vivo*-infected cells that are reimplanted into the tumor result in the infection of neighboring cells in the tumor, which in turn are killed only if their PSA genes are active. This innovative therapy results in both spread of the vector and specific prostate cancer cell killing. This approach is being pursued by Calydon as described on page 392.

One of the major problems with gene therapy is that transfection of cells with naked DNA *in vivo* is very inefficient. One way to protect DNA from degradation en route to the target site is to deliver it encased in a lipid. Cationic liposome-mediated gene therapy for metastatic prostate cancer is being developed by Robert Debs of California Pacific Medical Center Research Institute (San Francisco, CA). Recent improvements in the procedure have made this approach more efficient for delivering new genes by intravenous injection.

Timothy Thompson of Baylor College of Medicine has pointed out that direct injection is a very simple approach to delivering DNA to the prostate for gene therapy as well as cytotoxic prodrugs. In this strategy a plasmid containing the herpes simplex virus thymidine kinase (HSVtk) gene was introduced together with the prodrug ganciclovir directly into prostate tumors. Ganciclovir is only cytotoxic when it is activated by tk, so the presence of both agents in the same cell is necessary for cell killing. This approach is in phase I clinical trials.

Other approaches to gene therapy include growth factor ablation and inhibition of the angiogenic activity of tumor cells. Norman Greenberg of Baylor College of Medicine has developed strategies for preventing the inhibition of tumor suppressor p53 and for inhibiting keratinocyte growth factor (KGF) receptor activity by using mutant genes of these factors. He is presently planning to collaborate with Avigen (Alameda, CA) to use these approaches in human gene therapy.

Josh Fidler of the University of Texas M.D. Anderson Cancer Center (Houston, TX) inhibited angiogenesis by transfecting tumor cells with the interferon β (IFN β) gene in order to down-regulate expression of basic fibroblast growth factor (bFGF), one of the growth factors needed for activation of this pathway. All of these strategies were shown to be effective in mouse model systems.

Other Therapeutic Approaches

Immunoconjugates consisting of MAbs linked to a radioactive isotope, have been an effective therapy for treating other cancers. Neil Bander of Cornell University Medical Center (Ithaca, NY) has created a panel of prostate-specific MAbs and several are in phase I/II clinical trials. One of the antibodies reacts with a novel lipid antigen that fixes complement, and was shown to be very active against the LNCaP tumor cell line *in vitro*.

Multiple-drug strategies are being tested using anticancer drugs that are already available. Christopher Logothetis of M.D. Anderson Cancer Center described the use of a four-drug protocol which alternates two drug combinations, those of vinblastine and estramustine with doxorubicin and ketoconazole, to improve tumor killing and reduce side effects. This protocol is now in phase I trials for late stage prostate cancer. Other laboratories are testing different combinations of drugs.

DELIVERY OF THERAPEUTICS TO TUMOR TARGETS

One of the major problems in treating cancer is targeting a toxic drug or therapy exclusively to tumor cells.

Targeting Drugs to the Vascular Endothelium

Two laboratories are trying to target drugs to the vascular endothelium underlying tumors. According to Stephen Reeders, Peregrine Pharmaceuticals (New York, NY) has developed a targeting agent consisting of an antibody to the vascular endothelial growth factor (VEGF) receptor as the targeting device linked to tissue factor CM, a protein which causes coagulation. He pointed out that it is only necessary to do focal damage to a blood vessel to cause its degeneration.

Jan Schnitzer of Harvard Medical School Beth Israel Hospital (Boston, MA) intends to takes this approach one step further by aiming at blood vessel targets and then killing the underlying tumor. He has created a library of blood vessel targeting sites he calls "organ-specific molecular zip code molecules." He has also developed techniques that enable him to move the endocytotic vesicles, called caveolae, in bolus fashion from one side of the endothelium to the other side as a mechanism for delivering toxic agents to the underlying tumor tissue.

Targeted Delivery of Cisplatin

One of the cytotoxic drugs that needs to be "tamed" by linking it to targeting reagents is cisplatin. This drug, which is extraordinarily effective in treating many cancers, is also highly toxic. Randolph Christen of UCSD has been studying ways to link this "warhead" to a tumor-specific "shield" that would cause the drug to be selectively retained in tumor cells. John Essigman of the Massachusetts Institute of Technology (MIT; Cambridge, MA) also has a strategy to increase the sensitivity of tumor cells to cisplatin by activating the EGF receptor using genetic manipulation techniques. Other "molecular warheads" that need taming are the DNA-cleaving

enidynes discussed by K.C. Nicholaou from Scripps Research Institute (La Jolla, CA).

PROSTATE CANCER STAGING

As prostate tumors grow and evolve, new therapeutic strategies are needed. One problem to be solved is how best to identify those turning points which signal the time to begin changing the approach to treatment. Staging strategies include monitoring for biochemical markers of advanced prostate cancer, and imaging to identify the size of the primary tumor and the existence and location of metastases.

Biochemical Markers

Monitoring biochemical markers, such as PSA levels, has been the most widely used approach to determining cancer stage. One of the new staging markers, matrix metalloproteinase (MMP), is an indication of prostate cancer metastases. A urine test for its activity has been developed by Marsha Moses of Harvard Medical School Children's Hospital (Boston, MA). Other staging markers can be detected as overexpressed tumor-specific genes by quantitative reverse transcription polymerase chain reaction (RTPCR). UroCor (Oklahoma City, OK) is developing such a test for a tumor transmembrane protein.

Other *in vitro* Staging Approaches

Other staging techniques measure changes in cell morphology or tumor vascularization. Robert Veltri of UroCor described a new marker for disease progression called quantitative nuclear grading, which is based on variations in size, shape and chromatin texture of nuclei. Michael Brawer of the University of Washington measures microvascular density (MVD) of tumors by a quantitative image analysis system. He was able to show that mutations in p53, that often accompany disease progression, correlate with increased MVD.

In vivo Imaging

High-resolution magnetic resonance imaging (MRI) described by John Kurhanewicz of the University of California at San Francisco (UCSF) is used to assess the early response to hormone ablation therapy and the extent of residual disease. A standard method of imaging tumors is MRI. A more informative modification of this method is *in vivo* magnetic resonance spectroscopy (MRSI) that allows noninvasive imaging of tissue biochemistry and cellular metabolism. MRSI takes advantage of the unique metabolism of the prostate glandular epithelial cells, which take up glucose and aspartate and uniquely produce very high levels of citrate which are pumped out and collect in the prostatic fluids. MRSI measures increases in choline and decreases in citrate concentrations which correlate with progression of cancer. This approach gives good estimates of the volume and position of the cancer and has led to significant improvements in staging accuracy.

Positron emission tomography (PET), like MRSI, identifies regions of increased metabolic activity. Michael Phelps of UCLA School of Medicine is the developer of this technology that uses a positron-emitting labeled probe that concentrates in metabolically active areas such as malignant tissue. He is developing technology for gene expression imaging in which prostate cancer metastases can be imaged using a PET probe linked to a genetic expression vector. As he pointed out, the advantage of this approach is that in a single setting all organs of the body are scanned.

Immunoscintigraphy using a MAb to PSMA for diagnosis of prostate cancer, developed by Cytogen (Princeton, NJ) as ProstaScint, was described by Robert Carretta of Sutter Roseville Medical Center (Roseville, CA). Approved by the FDA in October 1996, ProstaScint localizes to prostate tumor cells and, in patients with low PSA serum levels, it can be used to detect the presence of extensive metastases in nodes, liver and bone (for more information see FO, V2 #1, pp 287-288). It is particularly useful for recurrent disease. It picks up soft tissue lesions and lymph node disease even in the absence of evidence from MRI.

THE ANDROGEN RECEPTOR

An important turning point in the progression of prostate cancer is marked by an accumulation of alterations in the androgen receptor (AR). According to Wayne Tilley of Flinders Medical Centre at the University of South Australia (Bedford Park, Australia), half of the hormone refractory tumors he examined had mutations in the AR gene itself. Dolores Lamb of Baylor College of Medicine has characterized specific androgen receptor mutations, and described one which abolished the function of the receptor protein by blocking its ability to bind DNA. Tapio Visakorpi of Tampere University Hospital and Institute of Medical Technology in Finland, showed that amplification of the AR gene is found in 28% of recurrent tumors and suggested that amplification might be occurring as a response to hormone ablation. Adaptation to hormone therapy may also occur as a result of changes in the activity of cofactors of the receptor.

The ARA 70 cofactor, discovered by Chawn-shang Chang of the University of Wisconsin Medical School (Madison, WI) appears to allow the receptor to recognize a broader range of hormones. Clearly, this area of research is of great importance to the study of prostate cancer progression and further work may lead to strategies for preventing the loss of hormone sensitivity as well as new approaches to staging diagnostics.

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OPPORTUNISTIC INFECTIONS IN CANCER PATIENTS

FROM THE 36TH INTERSCIENCE CONFERENCE ON ANTIMICROBIAL AGENTS AND CHEMOTHERAPY (ICAAC), NEW ORLEANS, LA, SEPTEMBER 15-18, 1996

Infections, in general, and drug-resistant infections, in particular, continue to be a major cause of mortality and morbidity in immunocompromised patients. This article summarizes developments relating to treatment strategies in neutropenic or otherwise immunocompromised cancer patients with infections. (For additional information regarding infectious diseases in cancer patients and their treatment, please see FO, V1 #6, pp 159-163.)

BACTERIAL VAGINOSIS, CERVICITIS, HUMAN PAPILLOMAVIRUS (HPV) AND CIN

Currently, a growing body of scientific evidence is strongly suggesting that bacterial vaginosis acts as a cofactor with cervicitis and human papillomavirus (HPV) in the development of cervical intraepithelial neoplasia (CIN) and eventually, in some cases, cervical cancer. This piece summarizes information presented at a roundtable discussion and in scientific sessions at ICAAC96. Exhibit 4 provides definitions of the various infections that may lead to CIN and cervical cancer and Exhibit 5 estimates incidence and mortality associated with cervical cancer in the North America, Europe and Japan. Cervical cancer incidence is much higher in other world regions and has assumed epidemic proportions in some.

According to Dr. Sharon Hillier, Associate Professor of Obstetrics and Gynecology at the University of Pittsburgh and Director, Infectious Diseases, at Magee-Women's Hospital (Pittsburgh, PA), while bacterial vaginosis does not cause inflammation of the vaginal epithelium, new studies have shown an association to cervicitis; bacterial vaginosis has been found to coexist in up to 50% of women with cervicitis. Furthermore, recent investigations strongly suggest that women with bacterial vaginosis present with a higher incidence of cervical intraepithelial neoplasia (CIN). High levels of abnormal vaginal flora in bacterial vaginosis can produce nitrosoamines known to be carcinogenic. These can make it hard to keep the infection in check and, at the same time, may act synergistically with HPV to damage epithelial cells, thus possibly aiding in the development of CIN.

Dr. Laura Koutsky, Associate Professor of Epidemiology at the University of Washington (Seattle, WA) also concurred that bacterial vaginosis may be associated with the development of CIN, acting as a cofactor with HPV, because the two seem to go hand in hand. It is now necessary to study the relationship between all these infections to establish if they are separate conditions or if they are working together to predispose epithelial cells to CIN. In patients with both bacterial vaginosis and HPV, there does seem to be a higher incidence of CIN lesions. Because there is usually a long interval between formation of CIN lesions and development of invasive cancer, it is nearly impossible to study the relationship of bacterial vaginosis, CIN, HPV and cervical cancer in untreated patients. It is possible, however, to carry out randomized clinical trials to evaluate the possibility of preventing abnormalities in the cervix by employing various treatment regimens. It has been shown, for example, that women with clinically-defined cervicitis or vaginosis fared better when treated with oral doxycycline and intravaginal metronidazole gel than those treated with doxycycline alone.

Such combination therapies are more effective when vaginosis is present without cervicitis. In an earlier pilot study conducted by Dr. Jane Schwebke, Assistant Professor of Medicine at the University of Alabama (Birmingham, AL), it was found that in women with coexistent clinically-defined cervicitis and bacterial vaginosis, the treatment of cervicitis, either with doxycycline alone or in combination with metronidazole gel did not result in a normalization of the vaginal flora (Schwebke JR, etal, Infectious Diseases in Obstetrics and Gynecology, 1995, 3:119-122). In actuality, simultaneous treatment of bacterial vaginosis and cervicitis effectively eradicated bacterial vaginosis in women who present with both syndromes, particularly if the treatment included concomitant doxycycline and intravaginal metronidazole; bacterial vaginosis persisted in 95% of women on doxycycline compared to 14% on combination therapy.

There is little doubt that randomized trials with intravaginal metronidazole are warranted to see if this approach clears Papanicolaou (Pap) smears. In male urethritis, for example, it is suggested that the effect of doxycycline may be attributed to its anti-inflammatory properties. Because both doxycycline and metronidazole decrease inflammation, maybe metronidazole's effect also is anti-inflammatory rather than antibacterial in nature.

If a screening Pap smear is atypical, patients should be evaluated for bacteria infection (gonorrhea, chlamydia, other abnormal vaginal flora, anaerobes, etc). Generally, a healthy vagina is the clearest area in the body; usually only hydrogen peroxide-producing lactobacilli are present and, possibly, low levels of other microorganisms, depending on the estrogen levels which affect pH. When a diagnosis of bacterial vaginosis is made and the patient chooses to be treated, therapeutic options include oral metronidazole, oral clindamycin, intravaginal metronidazole gel or intravaginal clindamycin. Intravaginal preparations have fewer systemic effects and are better tolerated.

Exhibit 4
Infections that may Lead to Cervical Intraepithelial Neoplasia and Cervical Cancer

Bacterial Vaginosis or Nonspecific Vaginitis

Bacterial vaginosis is an infection involving both aerobes and anaerobes. Bacteria isolated from vaginal secretions of infected patients include *Gardnerella vaginalis* as well as various *Bacteroides, Peptostreptococcus,*and *Mobiluncus* species. The most prevalent type, *G. vaginalis coccobacilli,* acts synergistically with anaerobes to produce the amines responsible for the characteristic "fishy" odor associated with this infection but do not produce clinical disease if present alone. The role of curved anaerobic rods such as *Mobiluncus curtisii* is currently controversial and under investigation.

Bacterial vaginosis affects sexually active women. The common presenting symptom is a loose, often scanty, and usually nonirritating vaginal discharge which is frequently accompanied by a "fishy" odor. Differential diagnosis includes microscopic evidence of clue cells (vaginal epithelial cells studded with tiny coccobacilli) in the discharge, a vaginal pH of 5.0 or higher, and a positive whiff test.

Treatment typically targets vaginal anaerobes and consists of metronidazole (500 mg PO *bid* for seven days). Findings from recent studies suggest that a single dose of 2g may be equally effective and is preferred in cases where compliance may be a problem. For pregnant women, clindamycin (300 mg PO *bid* for seven days) may be used. Topical clindamycin (Cleocin; Pharmacia & Upjohn) and topical metronidazole (MetroGel-Vaginal; Curatek) are relatively effective alternatives. Except in women with recurrent disease, management need not involve treatment of male sexual partners.

Cervicitis

The usual manifestation of acute cervicitis is an increased mucoid or purulent cervical discharge and cervical erythema and inflammation around the cervical os. Increased cervical discharge is also seen in pregnancy and in some patients who use oral contraceptives or contraceptive intrauterine devices (IUDs). However, this discharge is generally mucoid rather than purulent and does not contain abnormally large numbers of polymorphonuclear leukocytes (PMNs).

Etiologic agents of acute cervicitis include herpes simplex virus (HSV), which is recovered from the cervix of 80% of women with primary herpes genitalis. This type of cervicitis is often accompanied by a mucoid discharge, it may not be associated with lesions of the external genitalia, but cervical ulcerations and necrosis may be present.

Cervicitis of gonococcal or chlamydial origin is usually accompanied by a purulent or mucopurulent discharge. Analysis reveals large numbers of PMNs and gram-negative diplococci in about 50% of women with gonorrhea. Other gram-negative diplococci, present in normal vaginal secretions, may result in misleading smears. Chlamydia, which is present in about 50% of women with gonorrhea, is associated with a form of cervicitis characterized by erosion, edema, and hypertrophy about the cervical os, accompanied by a purulent discharge. The lesion is generally red, and bleeding when abraded during clinical examination is common. After treatment, this hypertrophic cervicitis usually resolves to a simple cervicitis.

Women with hypertrophic cervicitis (and their sexual partners) may be treated with doxycycline (100 mg PO *bid* for seven days or single dose of 1g PO), or tetracycline (500 mg PO *qid*), or minocycline (100 mg *qhs*). Chlamydial cervicitis is treated with doxycycline (100 mg PO *bid* for seven days), or azithromycin (1g PO once), or erythromycin (500 mg PO *qid* for seven days), or ofloxacin (300 mg PO *bid* for seven days). Gonococcal cervicitis is treated with ceftriaxone (125 mg IM once), or cefixime (400 mg PO once), or ciprofloxacin (500 mg PO once), or ofloxacin (400 mg PO once), or spectinomycin (2 g IM once).

Human Papillomavirus (HPV) Infection

Prevalence of HPV infection in women in the USA is estimated to exceed 6 million. Epidemiologic studies have consistently shown that HPV infection is the most important risk factor for development of preinvasive or invasive cervical cancer.

Cervical Intraepithelial Neoplasia (CIN)

Cervical carcinoma *in situ* (CIS), the precursor to cervical cancer, is also referred to as cervical intraepithelial neoplasia (CIN) or cervical dysplasia. In 1970, Barron and Richart proposed a change in the descriptive cytology terminology (JNCI, 1970; 45(5): 1025-30). They demonstrated that, although clinically similar, the natural history of mild and moderate dysplasia is significantly different from that of severe dysplasia and CIS. The authors suggested that the terms dysplasia and CIS be replaced with CIN, grades I-III. CIN I is mild dysplasia, CIN II is moderate dysplasia, and CIN III is severe dysplasia and CIS.

— continued on next page

OTHER BACTERIAL INFECTIONS

Vancomycin-resistant Enterococci (VRE)

An increasingly frequent cause of hospital-acquired infections in the USA are vancomycin-resistant enterococci. These bacteria are resistant to virtually all currently available antibiotics including vancomycin, the agent of last resort for gram-positive infections. Vancomycin use is prevalent particularly in hematology-oncology, neurosurgery, and cardiovascular surgery but dosing in many cases may be inappropriate. In a study carried out at the University of Iowa's (Iowa City, IA) 900-bed University Hospital, a retrospective review of pharmacy data of all patients treated with intravenous (IV) vancomycin in a ten-year (1981-1991) period, revealed that use of the drug increased twentyfold, from 1,993 grams in 1981 to 19,957 grams in 1991. Use was significantly higher in the hematology-oncology area than in any other, with the reasons for use (prophylaxis, empiric therapy, or specific treatment) being almost evenly divided. Using specially devised criteria, 63% of the use was judged inappropriate. Furthermore, univariate analysis revealed that oncology patients were ten times more likely to be treated with vancomycin.

A study sponsored by the Centers for Disease Control (CDC) found that among non-surgical patients, those admitted to the hematology-oncology department accounted for almost 50% of the vancomycin charges. Because antimicrobial use is rising and these agents are a

In 1988, the National Cancer Institute (NCI) recommended another change to this terminology (J. Reproductive Med., 1992; 37(5): 383-6). NCI's system (the Bethesda System) incorporated the role of HPV in the development of precursor lesions and invasive cancers by introducing the terms low-grade squamous epithelial lesion (LSIL) and high-grade SIL (HSIL) which correlate with CIN I, and CIN II-III, respectively. Any HPV-related cytology is added to the diagnosis of LSIL or HSIL. Abnormalities that do not meet the criteria for SIL are denoted as atypical squamous cells of undetermined significance (ASCUS). Colposcopical evaluation of persistent atypical smears will subsequently show that about 20% of patients actually have HSIL or invasive cancer. Almost 600,000 women are diagnosed yearly with premalignant changes (SIL or CIN).

Cervical Cancer

An estimated 15,700 new cervical cancer cases and 4,900 deaths will occur in the USA in 1996 (Ca- Cancer J. for Clinicians, 1996; 46(1):5-27). Carcinoma of the cervix uteri, which initially manifests as a premalignant lesion, originates at the squamocolumnar junction in the endocervical canal or on the cervix. Changes that progress to cervical cancer ranges from low to high grade SIL or CIN I, II, and III, reflecting increasingly abnormal maturation of the epithelium. These premalignant lesions may persist, regress, or progress to an invasive malignancy. In untreated patients with CIN, 30-70% will develop invasive carcinoma over a period of 10-12 years. However, in about 10% of patients, lesions progress from *in situ* to invasive cancer in less than one year. As it becomes invasive, the tumor breaks through the basement membrane, invading the cervical stroma. Eventually, it may manifest as ulceration, exophytic tumor, or extensive infiltration of underlying tissue of the bladder or rectum. HSIL (CIN II-III) is more likely to persist or progress and spontaneous regression is rare, while LSIL (CIN I) often regresses without treatment. The average time for progression of CIN III to invasive cancer is 10-15 years, based on mean age at diagnosis.

In addition to HPV infection, other known etiologic factors implicated in cervical cancer include high parity, many sexual partners, young age at first intercourse, low socioeconomic status, and smoking.

The prognosis of cervical cancer is greatly affected by the stage of disease at the time of diagnosis. The Pap smear is a screening tool that, when properly done, would identify over 90% of cervical cancer cases at an early stage when the disease is curable. However, reliability of Pap tests varies with adequacy of the cytologic specimens and competency of the cytologist. Failure to diagnose invasive cancer by Pap smears may be as high as 50%, emphasizing the need to biopsy any visible lesions of the cervix, even if the Pap smear is normal. Current death rates in the USA reflect the fact that, even today, Pap smears are not done on approximately one-third of American women.

The slow progression of preinvasive disease into invasive cancer and easy access to visual and cytologic investigation provides a unique opportunity to evaluate disease status and interventions. Common treatment for preinvasive lesions include laser vaporization or excision, cryosurgery, cold knife conization, electrosurgical excision, or simple hysterectomy. Treatment strategies at the time of diagnosis such as loop electrosurgical excision procedure (LEEP) may be appropriate for women who cannot be followed up. Non-invasive modalities are currently being explored in an effort to reduce the cost and morbidity of managing preinvasive lesions, and chemoprevention clinical trials are also ongoing.

major contributor to healthcare costs, it is anticipated that they will become the target of cost-containment in hospitals, health maintenance organizations, and outpatient and home healthcare settings (Jarvis WR, "Emerging Resistance with Gram-Positive Aerobic Infections: Where do we go from here?"– A satellite symposium held as part of ICAAC96).

Results from a vancomycin-resistant *Enterococcus faecium* (VREF) outbreak in severely neutropenic cancer patients illustrated the fact that VREF bacteremia is poorly addressed by currently available antibiotics and is associated with a significantly higher risk of mortality than is vancomycin-sensitive *E. faecium* (VSEF) bacteremia. At Emory University Hospital (Atlanta, GA), over an 11-month period, VREF bacteremia occurred in 13 patients with severe neutropenia. Antibiograms of all isolates indicated identical minimum inhibitory concentrations (MICs), and all isolates were identical as demonstrated by pulsed-field gel electrophoresis. The *in vitro* antibiogram MICs were ≥ 128 µ/ml for vancomycin, 128 g for teicoplanin, ≤ 0.5 µ/ml for quinupristin/dalfopristin, ≤ 500 µ/ml for gentamicin, and ≤ 4 µ/ml for chloramphenicol. Mean duration of bacteremia was 9.8 days. Among nine patients, seven remained bacteremic until death and two until resolution of neutropenia. Mortality in neutropenic patients with VREF bacteremia was significantly higher than in neutropenic patients with VSEF identified over the same period (six of 13 versus none of seven, respectively) despite similar severity of illness at the onset of bacteremia (Jernigan JA, Abstracts of ICAAC96, p 219:J8).

Emerging Resistance to *Staphylococcus Epidermidis*

Emergence of *Staphylococcus epidermidis* as a pathogen has been aided by widespread use of catheters, prosthetic joints, valves, and other invasive medical devices, and is a growing concern, particularly for immunocompromised cancer patients. For years, *S. epidermidis* and other coagulase-negative staphylococci, isolated from the bloodstream of hospitalized patients, were considered non-pathogenic contaminants. Now, these organisms are the leading cause of nosocomial bloodstream infections. An understanding of patterns of resistance and tolerance of *S. epidermidis* to antimicrobial agents is essential for the appropriate management of such infections. More than 80% of the nosocomial *S. epidermidis* isolates are methicillin-resistant, a fact which could be predictive of true infection versus a blood-culture contaminant. In a recent study, for example, coagulase-negative staphylococci that were resistant to at least six antimicrobial agents were found to be an independent predictive factor of bloodstream infections. Resistance to quinolones is also emerging, particularly to ciprofloxacin, because of its widespread use as a prophylactic agent in neutropenic cancer patients. In addition, it appears that vancomycin and other glycopeptide antibiotics are losing their efficacy against *S. epidermidis*

organisms embedded in the biofilon environment. This multidrug resistant pattern has motivated investigators to search for alternative agents that can be used in the prophylaxis and treatment of nosocomial *S. epidermidis* infections. Agents with promising activity include minocycline and rifampin in combination and, more recently, the new injectable streptogramin, quinupristin/dalfopristin (Raad I, "Emerging Resistance with Gram-positive Aerobic Infections: Where do we go from here?" A satellite symposium held as part of ICAAC96).

Exhibit 5
Incidence and Mortality of Cervical Cancer in Selected World Regions in 1995

COUNTRIES	INCIDENCE		MORTALITY	
	(#)	Rate	(#)	Rate
Western Europe – EEC	23,961	13.4	7,402	4.1
Western Europe – non-EEC	2,062	11.9	819	4.7
Eastern Europe	12,321	22.2	5,542	10.0
Total Europe[1]	38,344	15.2	13,763	5.5
Former USSR	28,275	18.8	11,913	7.9
Japan	8,216	12.9	2,060	3.2
United States[2]	15,700	11.7	4,900	3.6
Canada[2]	1,350	8.9	390	2.6
Total North America[2]	17,050	11.4	5,290	3.5
Triad (Europe[1], Japan, North America[2])	63,610	13.7	21,113	4.5

[1]Excluding the former USSR [2]Figures are for 1996

Stentotrophomonas maltophilia

Stentotrophomonas maltophilia, an opportunistic bacterium with inherent resistance to most β-lactam antibiotics, especially carbopenems, is being increasingly recognized as a pathogen in cancer patients. At Institut Gustave Roussy (Villejuif, France), a 420-bed reference cancer center that treats approximately 11,000 new patients annually, 20,000 blood cultures and 2,500 stool cultures are performed on the 4,000 patients undergoing microbiological investigations.

Between 1982 and 1995, 436 positive *S. maltophilia* samples were reported from 387 patients, representing a steady annual increase. Of this group, 227 were diagnosed with such infections as urinary tract infection (27%), bacteremia (24%), respiratory tract infection (17%), skin infection (11%), catheter-related infection (10%), and other (11%); 140 presented with digestive tract colonization; and 20 were both infected and colonized. Among the 71 patients with bacteremia, 24 had paired stool cultures (5+ and 19- for *S. maltophilia*), indicating fecal carriage sensitivity and specificity of 20% and 98%, respectively. Empirical therapy with a given drug is avoided when the patient is known to be colonized with a resistant organism such as S. maltophilia. Among selective antibiotics, administration of imipenem (Primaxin; Merck) at a dose of 4 mg/ml is checked routinely. Until now, in this institution, imipenem was only prescribed when first-line empirical treatment failed. Because imipenem use in infected patients is indicated only when *S. maltophilia* colonization is ruled out, the strong predictive value (98%) of negative detection of this microorganism in stool samples may be an adequate tool for improving empirical antibiotic treatment in febrile cancer patients (Gautier E and Tancrade C, Abstracts of ICAAC96, p 223:J31).

New Therapeutic Modalities for Opportunistic Bacterial Infections

Quinupristin/dalfopristin (RPR 59500, Synercid; Rhône-Poulenc Rorer), an innovative injectable antimicrobial agent belonging to the streptogramin class of antibiotics, has been shown to be highly effective for the treatment of potentially deadly VREF infections. Over a 14-month period, 21 patients were enrolled into two multicenter, open-label clinical studies to evaluate the safety and efficacy of quinupristin/dalfopristin in VREF infections. Sites of infection included bacteremia (10 patients), intra-abdominal infection (5 patients), wound infection (3 patients), and urinary tract infection (1 patient). All patients had serious underlying disease and, of the 19 evaluable for efficacy, eight were neutropenic leukemics being treated by multiple antibiotics, six were on hemodialysis, and four had undergone multiple abdominal surgeries. Overall, eleven patients were cured and five registered significant improvement, representing 84% of those treated. Also, bacteria were eradicated in 90% of cases (17/19). The majority of patients tolerated the therapy fairly well, although close to half experienced some adverse effects that resolved in all cases when the drug was discontinued (Blumberg CA, etal, Abstracts of ICAAC96, p 286:LM32). Resistance to Synercid appears to be emerging in VRE. Among 24 patients treated in two open trials, three developed resistance. Another problem encountered with Synercid treatment is superinfection with other pathogens, such as *Enterococcus faecalis,* against which the drug has poor activity. In one trial, among 68 evaluable patients with VRE treated with Synercid, 15 became superinfected with *E. faecalis* (Linden P, etal, ICAAC96, p286:LM31).

Cefepime (Maxipime; Bristol-Myers Squibb), a fourth generation injectable cephalosporin that is administered as first-line monotherapy for the treatment of acute febrile

neutropenia, achieved similar clinical success rates when compared to ceftazidime (Fortaz; Glaxo Wellcome, and Tazicef; SmithKline Beecham), although bacterial pathogens were more likely to be susceptible to cefepime than to ceftazidime. In a double-blind, randomized comparison study of 317 patients with acute febrile neutropenia enrolled in 12 USA centers, 163 patients were randomly assigned to cefepime (2 g every eight hours) and 154 to ceftazidime (2 g every eight hours). Monotherapy was initiated with standardized criteria for the addition of vancomycin after 72 hours if there was no response or if indicated based on culture results. Confirmation of a bacterial focus of infection was uncommon but, when present, it was mostly attributed to *Enterobacteriaceae*. Both gram-negative and gram-positive pathogens were more likely to be ceftazidime-resistant. For cefepime, clinical responses within 24 hours post-therapy were 40% as monotherapy, 6% in combination with vancomycin and 16% when no pathogenic bacteria was isolated; failure rate was 38%. For ceftazidime, clinical response rates were 38% as monotherapy, 6% in combination with vancomycin, and 19% when no pathogenic bacteria isolated; failure rate was 37% (Hathorn J, etal, Abstracts of ICAAC96, p 284:LM22). Cefepime binds to penicillin-binding proteins resulting in defective wall synthesis and, as a result, death of susceptible bacteria. The drug is marketed in the UK, Canada, Mexico, South America and South Africa, among others, and was approved in the USA in early 1996.

Analysis of Cost-Effective Bacterial Prophylaxis

Despite the incremental cost of drug acquisition, use of fluoroquinolones for antibacterial chemoprophylaxis in neutropenic patients may be more cost-effective than trimethoprim-sulfamethoxazole (TMP/SMX, Bactrim; Hoffmann-La Roche and Septra; Glaxo Wellcome) therapy, because of reduced treatment requirement, particularly with regard to gram-negative bacteria. According to a meta-analysis of seven randomized studies involving 682 neutropenic patients comparing fluoroquinolones ciprofloxacin (Cipro; Bayer) or norfloxacin (Noroxin; Merck) with TMP/SMX, fluoroquinolones significantly reduced incidence of gram-negative bacteremia, without affecting incidence of gram-positive bacteremia, fever, or deaths attributable to infection. Mean cost of bacteremia prophylaxis for every 100 episodes of neutropenia was $2,061 for TMP/SMX, $7,385 for norfloxacin and $22,316 for ciprofloxacin. Thus, bacterial prophylaxis with fluoroquinolones resulted in an additional cost of $5,234 to $20,255 per 100 episodes of neutropenia, compared to TMP/SMX. However, because the observed reduction of risk among those treated with fluoroquinolones was 11.3% for gram-negative bacteremias, the incremental cost of using fluoroquinolones instead of TMP/SMX in neutropenic patients was reduced to between $471 and $1,792 for each episode of gram-negative bacteremia that was prevented (Cruciani M, etal, Abstracts of ICAAC96, p 296:N22).

VIRAL INFECTIONS

Opportunistic invasive fungal infections are becoming increasingly common in immunocompromized patients (see FO, V1 #6, pp 161-163). In October 1996, The Liposome Company (Princeton, NJ) established the Collaborative Exchange of Antifungal Research (CLEAR) which provides a forum for information exchange and database development as it relates to the treatment of fungal infections.

Penciclovir for Cold Sores

Penciclovir 1% cream (Vectavir; SmithKline Beecham) is the first antiviral treatment to convincingly alter the clinical course of recurrent herpes simplex labialis, commonly known as cold sores, reducing the duration of signs and symptoms whether treated in the early or late stage of the disease. In order to evaluate the efficacy and safety of penciclovir 1% cream, a multinational, randomized, placebo-controlled trial, carried out in 43 centers in Europe, Canada, and Singapore, randomly assigned 1,484 persons with recurrent cold sores (average of six episodes annually) to either penciclovir 1% cream applied every two hours, six to nine times daily (734 patients) or placebo (750 patients), within one hour of symptom onset. Patients were seen daily until crusting was gone and then every other day until healed. Clinically, classical lesions healed 29% faster (4.4 days versus 5.3 days) in patients treated with penciclovir compared to those on placebo, and pain resolved 32% faster (3.1 days versus 4.3 days) in the penciclovir group compared to those on placebo. Similarly, cessation of viral shedding occurred significantly faster (3 days versus 4 days) in patients treated with penciclovir compared to those receiving placebo (Raborn GW, etal, Abstracts of ICAAC96, p 178:H81).

Ganciclovir for Prevention of Cytomegalovirus (CMV) Disease

In a comparison of two ganciclovir (Cytovene; Hoffmann-La Roche) regimens for the prevention of cytomegatovirus (CMV) disease in adults treated by allogenic bone marrow transplants (alloBMT), a five-times weekly regimen significantly reduced overall CMV incidence and mortality compared with a three-times-a-week regimen. However, whatever the regimen, CMV disease continued to be a significant problem among patients who had received a non-T cell depleted (TCD) marrow or FK506 prophylaxis for graft-versus-host disease (GvHD). In this study, two different regimens of ganciclovir prophylaxis were administered, 5 mg/kg thrice weekly in 78 patients and 5 mg/kg five times weekly in 137 patients. Active CMV infection occurred in 28 patients (41%) and 26 (21%) in the thrice and five-times weekly regimens, respectively. Mortality attributable to CMV was 12% and 1.5%, respectively. Statistically significant risk factors for CMV disease were the

three-times-per-week regimen, a TCD marrow, and use of FK506 prophylaxis for GvHD (Maltezou HC, etal, Abstracts of ICAAC96, p 168:H26).

FUNGAL INFECTIONS

Empirical Fluconazole in Febrile Neutropenic Patients

Early empirical antifungal therapy with fluconazole (Diflucan; Roerig) reduced candidemia, persistent fever and use of empirical amphotericin B (Fungizone; Apothecon) in neutropenic patients. To determine if prophylactic fluconazole was beneficial for patient populations other than neutropenic adult alloBMT recipients (where its efficacy is already proven) and whether these benefits could be obtained in a shorter, less costly course, 843 neutropenic patients treated with chemotherapy were randomized to early empirical antifungal therapy (defined as simultaneous initiation with empirical antibacterial therapy at onset of fever) using either IV or oral fluconazole (8 mg/kg/day), or placebo. Among these patients (565 adults and 278 children), a significant reduction in fungemia and persistent fever was observed in the fluconazole-group. The most significant responses to early empirical fluconazole therapy were observed in children and in patients with hematologic malignancies. Children receiving fluconazole for less than seven days experienced the greatest benefit as demonstrated by a reduction of persistent fever and lowering of empirical amphotericin B dosage (Walsh T, etal, Abstracts of ICAAC96, Late Breakers, p 11:LB22).

Itraconazole for Antifungal Prophylaxis

In a comparison of itraconazole (Sporanox; Janssen) oral solution with fluconazole suspension for antifungal prophylaxis in neutropenic patients, both drugs provided effective prophylaxis against candida infection, while itraconazole appeared more effective than fluconazole in preventing fatal aspergillosis. In a randomized, multicenter trial, 445 patients of whom 351 were treated by chemotherapy and 230 with BMT (179 with autoBMT and 51 with alloBMT), were randomly assigned to itraconazole (25 mg/kg twice daily) or fluconazole (100 mg daily) during 581 neutropenic episodes. There were more proven systemic fungal infections (six versus one) and more fatalities (four versus none) among those treated by fluconazole rather than itraconazole. There were four cases of aspergillosis (three were fatal), one fatal case of *Candida tropicalis*, and one case of *C. kusei* in the fluconazole group and one case of *C. albicans* in the itraconazole group. Adverse events resulted in 51 instances of drug withdrawal in the itraconazole group and 13 in the fluconazole group (Morgenstern GR, etal, Abstracts of ICAAC96, p 286:LM34). In September 1996, Sporanox was deemed approvable by the FDA for the treatment of candidiasis.

Amphotericin B in Intralipid for Candidemia

Amphotericin B (Fungizone; Apothecon) directly mixed in Intralipid 20%, a lipid solution for parenteral nutrition supplied by Pharmacia & Upjohn (Saint-Quentin Yvelines, France), appears to be effective for the treatment of candidemia in neutropenic cancer patients, with the added advantage of a low nephrotoxicity. Since June 1989, 36 patients with hematologic malignancies such as acute leukemia (16 patients) and others (20 patients), were treated with amphotericin B mixed in Intralipid 20% at a final concentration of 2 mg/ml. All but two of these patients were neutropenic. Interestingly, 20 of these patients were treated prophylactically by triazoles, six with fluconazole and 4 with itraconazole for a median duration of 11 days at the time of candidemia diagnosis. All isolated Candida strains were sensitive to amphotericin B.

Amphotericin B plus Intralipid 20% was administered at a median daily dose of 1.25 mg/kg for 18 $\pm$ 12 days, within two days after the first positive blood culture. Fluorocytosine was added in 15 cases for a median duration of seven days. Overall, 20 patients (56%) were cured (median survival 805 days), seven (19%) showed improvement (median survival 90 days) and nine (25%) died from fungemia (median survival 21 days). Considering that the dose-limiting toxicity with amphotericin B generally is renal failure, there was a major significance to the fact that renal toxicity was only mild to moderate with amphotericin B-Intralipid 20% combination; mean serum creatinine levels rose from 9.7 $\pm$ 3 mg/L at baseline to only 11.6 $\pm$ 4 mg/L at completion of therapy (Caillot D, etal, ICAAC96, p 227:J48).

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