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

CERVICAL CANCER — PART II
SCREENING, DIAGNOSIS AND STAGING

Invasive cervical cancer (ICC) is rare in the developed world and nearly 50% of new cases are diagnosed when the disease is localized (Exhibit 1). However, ICC is a major cause of morbidity and mortality in the rest of the world, underscoring the effectiveness of universal screening programs adopted in the developed world. Screening has been instrumental in curbing morbidity and mortality rates associated with invasive disease by identifying early-stage, localized ICC, resulting in 5-year survival rates in excess of 90% (Exhibit 2). However, when diagnosis involves disease that has spread regionally, the 5-year survival rate declines to just under 50%, and survival is dismal in metastatic disease. Therefore, despite its limitations, screening based on the Papanicolaou (Pap) smear test has saved thousands of lives in the past 45 years.

A variety of other tests are also being used, not so much for screening asymptomatic women, but for evaluating those at high-risk for the disease, either because of abnormal Pap smear results or other factors, for triaging cases with equivocal screening results and for staging ICC. These tests represent a continuum of care culminating with the definitive diagnostic approach, i.e. tissue biopsy. As the tests become more specialized, their costs also go up (Exhibit 3).

SCREENING

The majority of deaths attributable to cervical cancer could be prevented with early-stage detection and treatment because cancer of the uterine cervix is preceded by a precancerous, curable stage that generally progresses without symptoms over a period of years until it reaches an invasive stage. Remarkably, screening and detection of precancerous conditions is possible years before they develop into full-fledged malignancies by using the Pap smear test, a rather simple cytologic evaluation. The slow progression of preinvasive disease into invasive cancer, and easy access to visual and cytologic investigation, has allowed the development of this unique screening approach that has resulted in dramatic decreases in death rates of as much as 70% in countries with universal access to the Pap smear test. Various other screening tests are also being proposed, mostly to detect human papillomavirus (HPV). The current challenge in ICC prevention is to protect the maximum number of women at risk at a minimum cost and patient discomfort. Current programs, although remarkably effective, are still short of this goal.

Papanicolaou (Pap) Test

The Pap test, developed in the 1940s by Dr. George N. Papanicolaou, is a screening procedure for the early detection of precancerous and cancerous conditions of the uterine cervix. Retrospective analysis of the incidence and

mortality of cervical cancer illustrate the effectiveness of cervical screening. After cervical cancer screening was implemented in Finland, Sweden and Iceland in the early 1960s, a 50% decrease in the incidence of cervical cancer was observed over the next twenty years. In contrast, in Norway, that implemented screening in only one county, there were no changes in the incidence of cervical cancer. Likewise, mortality from cervical cancer in the USA decreased by 70% between 1955 and 1984 (Am J Obstet Gynec 1992; 166:1254-9), a change coincident with the introduction of mass screening programs (Obstet Gynec 1984; 63:135-9; Br J Cancer 1984; 50:367-75; Obstet Gynec 1984; 63:714-8).

Because intraepithelial lesions most frequently occur in women under the age of 40, whereas cervical cancer is most commonly seen in the fifth or sixth decade of life, the American College of Obstetrics and Gynecology and the American Cancer Society recommend that annual screening commence when a woman becomes sexually active, or reaches the age of 18. Women with 3 or more normal consecutive annual Pap smears are considered low risk and may be tested at intervals no longer than every 3 years unless they engage in high-risk activities such as have sex with multiple partners or smoke cigarettes, when annual screening is recommended. Screening guidelines vary somewhat in countries with universal programs but, generally, testing is recommended every three years, unless a higher frequency is indicated. In the UK, the recommended age to initiate screening is 20, and the cut-off 64. In the USA testing continues in women in their 70s.

Industry sources estimate that approximately 50 million Pap tests are performed annually in the USA (Exhibit 4), and another 60 million outside the USA with about 12 million of these performed in Japan, making it the second largest market in the world, and 4 million in the UK.

In the USA, approximately 24,000 laboratory and hospital facilities that offer biopsy and surgical pathology services, may also evaluate Pap smears but, usually, Pap testing is performed by large multisite facilities; it is estimated that over 35% of all Pap smears in the USA are screened by the three largest laboratories, SmithKline Beecham Clinical Laboratories (Collegeville, PA), Quest Diagnostics (Teterboro, NJ), and Laboratory Corporation of America (LabCorp; Burlington, NC).

In the USA, the cost of cervical cancer screening is reimbursed under private, state and federal programs. As of January 1, 1998, under the balanced budget agreement, Medicare expanded coverage of several preventive benefits to its 39 million beneficiaries, including regular tests for cervical cancer that include pelvic and breast examinations.

Despite the preventive nature of the Pap smear, under-screening is the most important contributor to ICC. 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 situation is even more critical in the developing world where Pap smear screening is practiced in a haphazard manner or not at all. Universal

cervical disease screening programs using Pap smear analysis are logistically challenging because they require large numbers of properly trained cytotechnologists, and sophisticated laboratory facilities and procedures, commonly unavailable in developing countries.

The consequence of underscreening in the USA is illustrated by a study conducted in an HMO population by investigators at the University of California, San Francisco and TPMG (Oakland, CA). During a 7-year period (1988-1994), reflecting 8,497,000 woman-years of membership, 3,146,000 Pap smears were performed among members of a prepaid health plan meeting the criteria of 3 years of continuous membership. Medical records of all members who developed ICC during this period were reviewed and details of patient interaction with the medical care system including clinic visits, symptoms, Pap smear results, diagnostic procedures, tumor histology and FIGO stage, treatment and outcome, were abstracted. Assessment of screening history was limited to the period from 36 months to 3 months prior to diagnosis to avoid including smears or visits which were part of the evaluation that lead to the recognition of ICC. Among 455 women diagnosed with ICC who were members continuously for 3 years prior to diagnosis, 213/455 (47%) did not have a Pap smear within the health plan, 92/455 (20%) had at least one abnormal Pap test mandating follow-up, and 150/455 (33%) had one or more Pap smear tests that were interpreted as normal or abnormal not requiring follow-up [Sung Hai-Yen, et al, 1998 Meeting of the Society of Gynecologic Oncology (SGO98)].

Pap Smear Classification System

The Pap smear is designed to identify patients with premalignant or malignant lesions. Several cytological classifications of Pap smear findings exist, with the most common being the Bethesda classification, which recognizes two categories of cytological abnormalities (JAMA 1989; 262:931-4; Obstet & Gynec 1991; 77:779-82), low-grade squamous intraepithelial lesion (LSIL) and high-grade SIL (HSIL) (see FO, pp 868). Another category, referred to as atypical squamous or glandular cells of undetermined significance (ASCUS or AGUS), requires careful triage (see FO, p 566).

Problems Associated with the Pap Smear Test

Despite public conceptions about the infallibility of the Pap smear, the test is accurate only about 80% of the time. Although this relatively high rate of false negative results appears to be a serious shortcoming at first glance, the Pap smear is a very powerful screening tool capable of preven-

| Exhibit I Stage Distribution of Cervical Cancer at Time of Diagnosis in the Triad | | | | | | |
|--|---------|-------------------|-------------|-----------|-------------|-------|
| Stage | USA (#) | North America (#) | Europe* (#) | Japan (#) | Triad** (#) | % |
| Localized | 7,453 | 8,892 | 21,857 | 5,174 | 35,923 | 51.4 |
| Regional | 4,843 | 5,778 | 14,203 | 3,362 | 23,343 | 33.4 |
| Distant | 1,189 | 1,419 | 3,487 | 825 | 5,731 | 8.2 |
| Unstaged | 1,015 | 1,211 | 2,977 | 704 | 4,892 | 7.0 |
| All Stages | 14,500 | 17,299 | 42,524 | 10,065 | 69,889 | 100.0 |

*Excluding the former USSR
**North America, Europe and Japan

ting most ICC because the relatively long interval between the appearance of intraepithelial lesions and potential ICC, provides numerous opportunities to detect and treat patients with high-risk lesions. Actually, this screening approach would have been even more powerful if done annually because at a testing interval of three years, one misread Pap smear may allow a cancer to develop within the six-year span between screenings. However, the cost of annual screening far outweighs its benefits because of the rarity of positive findings.

Currently, laboratories that perform Pap smear evaluations must adhere to Federal, state and local regulations. Subsequent to a flurry of suits from misdiagnosed patients, the government fined several large volume providers of Pap smear analysis and instituted tighter regulations, primarily in the number of tests processed by each operator to combat fatigue-related errors. Partly because physical and mental stress escalates with the number of Pap smears examined, increasing the risk of false negatives, Clinical Laboratory Improvement Amendments (CLIA) regulations, instituted in 1998, limit the number of slides (gynecologic and nongynecologic) that a cytotechnologist may screen each day to no more than 100. Some states, among them California and New Jersey further limit this number to 80.

Also, according to regulations promulgated by CLIA, clinical laboratories are required to rescreen at least 10% of the Pap smears classified as normal on first pass. To also ensure that laboratories adhere to regulations and maintain quality control, the USA government requires them to keep test records for a 5-year period, allowing those misdiagnosed to access their records to check for malpractice. In the UK, again for medicolegal reasons, cervical smears are kept for a period of at least 10 years. This practice has resulted in a valuable source of data for retrospective studies.

Industry sources have estimated that, of the approximately 50 million Pap tests performed annually in the USA, approximately 2.5 million, or 5%, are diagnosed with precancerous conditions but a very small number (.03%) of Pap smears is eventually classified as invasive cancer. When properly performed, the Pap smear test would iden-

tify over 90% of cervical cancer cases at an early stage when the disease is curable. However, as many as 15%-25% of patients with intraepithelial lesions are currently diagnosed with normal Pap smear results (JAMA 1989; 262:931-4; Obstet & Gynecol 1991; 77:779-8). The high rate of false-negative results emphasizes the need to biopsy any visible lesions of the cervix, even if the Pap smear is normal.

When medical records of the 455 women who developed ICC (see page 887) were reviewed, and diagnosis, treatment and outcome was abstracted, at a median follow-up of 4 years from diagnosis, 103/455 (22.6%) had died of their malignancy. In the period from 36 months to 3 months preceding the diagnosis of fatal ICC, 65/103 (63.1%) of these patients did not have a Pap smear within the health plan, 11/103 (10.7%) had at least one abnormal Pap test mandating follow-up care, and 27/103 (26.2%) had only normal or minimally abnormal Pap smear results which would not have engendered follow-up. Among these 27 patients are those with interval cancers (occurring after the last Pap) and those with false-negative smears. The average age at death for the fatal cases was 58 years old. This relatively small contribution of false-negative smears to death from ICC suggests that expensive new technologies intended to reduce false-negative rates will have to provide other benefits, in addition to reduction in mortality, such as contribution to ASCUS triage, reduction in medicolegal risk, etc., to be cost effective (Kinney W, et al, SGO98).

There are many factors that contribute to poor test results, such specimen quality, competency of the cytologist, laboratory quality controls, etc. Specimen quality is one of the most important contributors to the outcome of Pap-based screening. In the traditional approach, to obtain a Pap smear, the physician scrapes the surface of the uterine cervix and collects a specimen which is subsequently smeared onto a microscope slide and preserved with a fixative agent such as alcohol. This Pap smear, along with patient information, is then sent to a clinical laboratory. Sampling errors may occur in 12.3% of cases. Proper sample handling techniques such as adequate sampling of the transformation zone (boundary between squamous epithelium of the exocervix and columnar epithelium of the endocervix), use of an endocervical brush (cytobrush) and plastic spatula, recently approved by the FDA, and proper fixation techniques, can help minimize the rate of false negatives and improve the sensitivity of this test.

In 1996, the FDA approved a liquid-based preparation (LBP) technique for specimen processing that has contributed to superior smear quality, and has also enhanced

Exhibit 2
Five-Year Survivors By Stage at Diagnosis of Cervical Cancer in Selected World Regions

| Stage | USA (#) | North America (#) | Europe* (#) | Japan (#) | Triad** (#) | % |
|------------|---------|-------------------|-------------|-----------|-------------|------|
| Localized | 6,775 | 8,083 | 19,868 | 4,703 | 32,654 | 90.9 |
| Regional | 2,417 | 2,883 | 7,087 | 1,678 | 11,648 | 49.9 |
| Distant | 102 | 122 | 300 | 71 | 493 | 8.6 |
| Unstaged | 620 | 740 | 1,819 | 430 | 2,989 | 61.1 |
| All Stages | 9,914 | 11,827 | 29,074 | 6,882 | 47,783 | 68.4 |

*Excluding the former USSR
**North America, Europe and Japan

the overall quality of cytology service (Dupree WB, et al, Cancer, 25 Aug 1998, 84(4):202-206). The LBP approach uses a vial containing a preservative solution instead of a glass slide for collection of the cervical or vaginal material. The vial is sent to the laboratory where its contents are filtered to remove impurities such as blood and mucus, and processed into a thin even layer of cells deposited on a glass slide, resulting in a uniform preparation of well preserved cells. A literature review found that LBP systems increase detection of epithelial cell abnormalities, as compared to the conventional smear (Austin RM and Ramzy I, Acta Cytologica, 1998 Jan-Feb, 42(1):178-84).

Initially, it was difficult to obtain reimbursement for LBP processing but assignment of CPT codes and favorable study results appear to have paved the way for widespread use of this technique that could also enhance automated primary screening of Pap smears. At a \$7 premium over traditional approaches the LBP-based Pap smear may add \$350 million to annual laboratory costs in the USA.

Cytec (Boxborough, MA) was the first to introduce an LBP technique, the ThinPrep System, after it gained approval in May 1996 (see FO, p 563). In the ThinPrep approach, cervical cells are collected into a vial containing Preserv-cyt preservative solution and sent to the laboratory where they are automatically collected on the company's proprietary TransCyt filter, which incorporates an eight-micron membrane specifically designed to collect abnormal and cancerous cells. During the collection process, the ThinPrep 2000 Processor constantly monitors the rate of flow through the TransCyt filter in order to prevent the cellular presentation from being too scant or too dense. A thin layer of cells is then transferred to a glass slide in a 20 mm-diameter circle and the slide is automatically deposited into a preservative solution. The ThinPrep 2000 Processor processes approximately 20 to 25 patient samples per hour.

Multicenter clinical trials have shown that ThinPrep yields more satisfactory samples and detects squamous intraepithelial lesions more effectively than the conventional smear with no reduction in the specificity of the test (Lee KR, et al, Obstet & Gynecol 1997; 90(2):278-84).

As of October 1998, over 8,000 physicians used ThinPrep, 477 laboratories have been trained to perform the test, and 103 insurance companies provided coverage. Based on information provided by the insurers, the company estimates that the total number of covered lives under these 103 plans is over 85 million. The value of ThinPrep, however, is still in debate. For instance, in February 1998, Blue Cross Blue Shield's (BC/BS) Technical Evaluation Center issued a rather negative review of ThinPrep's cost benefits. Nevertheless, its clinical benefits were deemed significantly superior by at least 21 BC/BS plans that provide reimbursement for ThinPrep.

For the third quarter of 1998, Cytoc reported revenues of \$12.3 million up 75.7% from the \$7.0 million reported in the same quarter of 1997 and 34% over the 1998 second quarter. For the nine months ended September 30, 1998, revenues almost doubled to \$29.6 million, from \$15.6 million for the comparable 1997 period. These results are attributable to the growing acceptance in the USA of ThinPrep.

AutoCyte (Burlington, NC) is also planning to introduce PREP, an LBP system that uses the CytoRich method, which combines liquid preservation, selective reduction of blood and inflammation, thin-layer cell dispersion and discrete staining of specimens. AutoCyte submitted a PMA for PREP in May 1997 which was amended in March 1998. In September 1998, the FDA requested additional information in support of the company's PMA, as it had already done in February 1998, but no additional trials. In Europe, the PREP process is in routine direct-to-vial use.

In a masked, split-sample, eight-site clinical trial involving 8,983 cases, there were 7,707 satisfactory AutoCyte preparations versus 6,539 satisfactory conventional Pap smears. In the same population, AutoCyte PREP detected 376 cases of precancerous precursor lesions and 21 cases of cancer, compared to 349 and 17 cases, respectively, for the conventional Pap smear. AutoCyte PREP demonstrated a statistically significant, 31% overall improvement in the detection of squamous intraepithelial lesions and invasive cancer when evaluating cases with more than one diagnostic class difference. This improvement was supported by biopsy results (Bishop JV, et al, *Acta Cytologica*, 1998 Jan-Feb, 42(1):189-97). In a small single-center study that compared AutoCyte PREP to conventional Pap smear testing at the University of California Davis Medical Center, 642 samples were judged satisfactory and 30 precancerous by AutoCyte PREP versus 614 and 28, respectively, for conventional Pap.

In a large study, involving over 48,000 patients, conducted in a Swiss laboratory where AutoCyte's LBP method has replaced the conventional Pap smear, use of the intended direct-to-vial use of the AutoCyte method rather than the FDA-mandated split-sample clinical trial protocol where the LBP must be performed on the residual cells after the con-

ventional Pap smear was prepared, detected 0.6% cases of HSIL compared to 0.3% for the conventional Pap smear. Detection of LSIL also increased from 0.8% to 3.0% and the rate of ASCUS-classified smears declined from 3.7% to 1.6% (Vassilakos P, et al, *Acta Cytologica*, 1998 Jan-Feb, 42(1):198-202).

Morphometrix Technologies (Toronto, Ontario, Canada), founded in 1993, is also developing an automated LBP specimen processing system, CYPREP.

Automated Analysis of Pap Smears

In the USA, the Pap test represents the largest nonautomated clinical laboratory procedure. To perform this, the specimen, which typically consists of 50,000 to 300,000 cervical cells, is stained to highlight important cellular features and sealed with a protective covering. The smear is then placed under a microscope and examined by a cytotechnologist, a professional with special training in cytology, who generally needs five to ten minutes to screen each Pap smear and complete related paperwork. Typically, about 95% of all Pap smears are classified as normal. In the remaining cases, suspicious slides are reviewed by a senior cytotechnologist and slides confirmed to show signs of precancerous conditions or cancer, typically about 5% of all cases, are referred to a senior cytopathologist, who carefully reviews the Pap smear and makes a final diagnosis.

Many companies have tackled the problem of Pap cytology automation but fully automated systems have yet to prove that they are as cost effective as manual techniques in primary Pap smear screening. To date, at least in the USA, most automated systems are used in quality control to rescreen the mandated 10% of slides already evaluated by the cytologist.

Although supporters of primary screening systems claim various benefits associated with their use, such as accuracy, effectiveness, cost savings, convenience, processing speed and reliability, improved laboratory productivity via an increase in the number of Pap smears processed while maintaining or improving accuracy, and better use of the cytotechnologists in reviewing suspect Pap smears, there is considerable skepticism in the field regarding the cost-effectiveness of automated screening. Nevertheless, at least three systems are nearing commercialization worldwide and others are in development. A thorough review of LBP techniques and automated Pap smear screening, prepared by Dorothy L. Rosenthal, MD, has been published in the May 10, 1998 issue of the *Journal of the NCI*, Vol. 90, #10, pp 738-749.

AutoCyte has developed SCREEN, an automated interactive cervical cancer screening system based on high resolution imaging, originally commercialized by Roche Image Analysis Systems (RIAS), a cofounder of AutoCyte (see FO, p 563).

SCREEN combines image analysis and classification software with off-the-shelf computer hardware, to screen slides prepared using the PREP LBP cell preparation methodology. SCREEN is intended to interface with AutoCyte's Pathology Workstation to allow the clinical laboratory to archive, network and transfer images, and relocate cells of interest on the glass slide. This integrated product platform allows additional analysis to be performed without having the patient return for additional testing. SCREEN uses a series of proprietary algorithms to independently classify cells and present the cytotechnologist with images of the most suspicious cells on the sample slide, and is designed to function as an interactive support tool for the cytologist in the primary screening of cervical cells.

In July 1998, AutoCyte filed a PMA seeking FDA approval for use of AutoCyte SCREEN as a primary screening system for Pap smears. This PMA was accepted for substantive review in September 1998. The company is selling this system outside the USA. It has entered into a distribution agreement with Medical & Biological Laboratories (MBL; Nagoya, Japan) to market this system in Japan.

Morphometrix Technologies is currently developing CYMET for automated Pap test screening to be used in conjunction with its LBP system. CYMET uses a computer-aided review system (CARS) that incorporates a database of reference cells and diagnostic markers combining a distillation of cellular features with clinically relevant information, to aid the cytotechnologist in reviewing Pap smears. According to the company, CYMET can screen a Pap monolayer slide every 90 seconds.

NeoPath's (Redmond, WA) initial product line consists of two automated screening systems that integrate a high-speed video microscope, comprehensive image interpretation software to accurately analyze images and classify cells and slides, and high-speed custom field-of-view (FOV) computers to run the software at high speed to capture and analyze thousands of microscopic images from Pap smear slides. In September 1995, the FDA cleared for commercial use the AutoPap 300 QC automatic Pap screener system, the company's first product, and during the first quarter of 1996, the Health Care Financing Administration (HCFA) officially allowed clinical laboratories to use AutoPap QC in quality control review of Pap smear slides that were initially screened by cytologists as normal. In March 1998, NeoPath signed a corporate-wide agreement with UniLab (Tarzana, CA) to rescreen 100% of all eligible Pap smears using the AutoPap 300 QC System, resulting in over one million Pap smears being screened annually. A study, to be published in the January-February 1999 issue of *Acta Cytologica* on computer-assisted cytology, will include the results of an independent, prospective clinical study that describes the biopsy-confirmed performance of the AutoPap 300 QC System in screening for cervical cancer. In the study, performed by

Kaiser Permanente Medical Group's, Northern California Regional Laboratory (Berkeley, CA) and involving 35,143 conventionally prepared Pap smears from women routinely screened by the lab over a four-month period, AutoPap 300 QC System was compared with manual rescreening of a randomly selected 10% of tests deemed negative by cytotechnologists. The AutoPap identified over eight times more SIL false negative cases and had higher specificity than Kaiser's current practice. Compared to manual screening, A HIGHER rate of detection of false negatives was achieved with the AutoPap that ranged from 240% for ASCUS/SIL to 744% for LSIL and HSIL. Biopsy data showed an increase from 45% positive correlation with manual practice to 85% with the AutoPap.

NeoPath's AutoPap automatic primary Pap screening system which, based on the AutoPap QC, is the only FDA-approved fully-automated primary screening system for cervical cancer screening, took several years to bring to market. On September 27, 1996 the Hematology and Pathology Devices Advisory Panel to the FDA did not recommend for approval a supplemental PMA for use of the AutoPap as a primary screener, pending completion of additional clinical trials studies. The FDA subsequently followed the panel's recommendation. In January 1997, NeoPath introduced its next-generation AutoPap System incorporating new diagnostic algorithms that operate in coordination with the original AutoPap Screener algorithms to improve diagnostic accuracy. The company used this next-generation AutoPap System in subsequent clinical trials. In January 1998, the AutoPap System was unanimously recommended for approval as a primary screener of Pap smears and the FDA approved it in May 1998. In March 1997, NeoPath also received approval from the Japanese Ministry of Health and Welfare to market the AutoPap System as a primary screener in Japan. NeoPath distributes AutoPap Systems in Japan through an agreement with Nikon. The company has also obtained regulatory approval to sell the AutoPap System as a primary screener in Canada, Australia, New Zealand, and the Netherlands.

The AutoPap System is designed to be used on-site by general laboratory personnel, and is compatible with a wide range of staining procedures and both standard and LBP specimens. NeoPath is planning to seek approval to expand AutoPap's applications to include LBP slide screening. The system holds approximately 300 Pap smear slides at a time and analyzes a Pap smear in approximately the same time as a cytotechnologist.

In support of its PMA, NeoPath provided performance data from a large-scale, prospective, intended-use clinical study based on more than 25,000 patient slides from five clinical laboratories. According to this study, the AutoPap achieves overall greater accuracy in early diagnosis of cervical disease, reduces both false-negative and false-positive test results, and identifies a statistically significant higher rate of abnormal

smears than current practice. Also, the AutoPap was equivalent to current practice in identifying satisfactory but limited slides, unsatisfactory slides, and in detecting benign cellular changes, reactive changes, and infection.

In a recent vindication of the principles of automated screening, HCFA determined that the 25% of slides designated by the AutoPap System as "no further review" are not subject to the manual random quality control (QC) rescreening of negative cases required by current rules and regulations. In August 1998, Neopath was issued USA patent number 5,787,188 "Method for Identifying Normal Biomedical Specimens."

NeoPath markets the AutoPap System in the USA under a fee-per-use method; the company retains ownership of systems placed at customer sites and assesses customers a charge for each Pap smear slide analyzed. Abroad, however, the company's product placements have primarily consisted of outright sales. NeoPath reported \$9.7 million in revenues for the nine months ended September 30, 1998, up 28% from the \$7.6 million reported for the same period in 1997. Fee-per-use revenues for the nine months increased nearly 25% in 1998 compared to the prior year. A decrease in third quarter 1998 sales, compared to those in the second quarter of 1998, was a result of lower AutoPap System sales, particularly in Asia/Pacific markets. The company expects that future revenues will be primarily from fee-per-use, except for outright instrument sales internationally, and AutoPap sales under the Kaiser Permanente national agreement.

Neuromedical Systems (NSI; Upper Saddle River, NJ) is marketing PapNet, a computerized image processing Pap smear screening service provided to laboratories either as an adjunct to manual Pap smear screening in the USA, or as a primary screening methodology in certain markets abroad. NSI licensed the PapNet technology to NetMed (Dublin, OH) a developer and marketer of medical and health-related technologies. NetMed markets PapNet in certain territories under exclusive license.

In the USA, clinical laboratories contract with NSI to perform rescreening using PapNet. Pap smears diagnosed by a laboratory as negative or normal on an initial manual inspection, and any subsequent quality-control rescreening, are sent to NSI scanning centers. The NSI system creates a color video picture of each of the 128 images on a Pap smear slide deemed to be most likely abnormal. These images are recorded on a digital tape cassette that, together with the patient's Pap smear slide, is returned to the clinical laboratory. Trained laboratory personnel then evaluate each of the 128 video pictures for each Pap smear slide and a cytotechnologist manually rescreens any abnormal smears. PapNet rescreening of Pap smears adds approximately \$35 to each test.

Originally, NSI contracted with certain laboratories to perform Pap smear screening as a service. In mid-1998 NSI

shifted its marketing strategy from operating centralized scanning facilities providing a service, to selling the actual system, PapNet-on-Cyte, to the laboratories. This approach was initially adopted in Europe with the first PapNet-on-Cyte cervical screening scanner installed at the Churchill Clinic (London, UK) that plans to contract with private physicians and laboratories, including those that were sending slides to NSI's Amsterdam facility for processing. NeoPath recognizes revenues as both an upfront sum for the acquisition of PapNet-on-Cyte, as well as a continuing service charge per slide. In preparation to convert its service business, NSI is developing a configuration of the PapNet technology that can be easily deployed in customer labs, both in the USA and abroad.

According to various clinical trials, including a multicenter USA trial, PapNet detects significantly more cervical abnormalities than unassisted manual screening, and is significantly more effective (up to seven times more effective) in the detection of abnormalities than manual rescreening. Preliminary data, released in May 1998 from a National Health Service (NHS)-sponsored clinical trial in the UK, suggest that PapNet computer-assisted testing used in a primary screening mode, may improve the accuracy and efficiency of the cervical screening process. This prospective, blinded trial is being conducted under normal laboratory conditions at five sites in the UK under a grant from the NHS. The trial compares standard manual screening to PapNet testing in a primary screening mode. The objective is to assess both the efficacy and cost-effectiveness of PapNet as a primary screener. According to interim findings, based on 12,240 cervical smears reviewed by manual microscopic screening and compared to those of PapNet computer-assisted primary screening, cytologists were able to improve screening specificity using PapNet as compared to manual screening. Furthermore, the authors suggest that based on preliminary data this increased detection of "true" negative smears compared to manual screening, was both statistically and clinically significant. Also, under normal, routine laboratory conditions, a cytologist using PapNet was able to triage an average of 82 slides in two hours, or 1.5 minutes per slide. Using manual screening, a cytologist triaged an average of 16 slides per two-hour period, or 7.5 minutes per slide. Across the five trial sites, PapNet triage required a full microscopic review in an average of 29% of the smears. The total time for the entire screening process was an average of 4.4 minutes per slide for PapNet testing, compared to 11.4 minutes per slide for manual screening.

In February 1998, investigators from the Victorian Cytology Service (VCS), a reference laboratory in Australia, reported on a study funded by a grant from the Department of Human Services in Victoria, that evaluated the effectiveness of the PapNet system in the detection of abnormalities that may have gone undetected, despite manual screening and rescreening, on approximately 20,000 Pap smears. Although

the protocol for this study used more rigorous and restrictive review criteria of cellular images selected by PapNet than would be employed in routine practice, PapNet-assisted screening significantly increased detection of cellular abnormalities. Despite double manual screening of the Pap smears, PapNet-assisted screening detected a missed LSIL or HSIL abnormality in 1 of every 122 Pap smears.

In July 1998, NSI began a prospective multicenter PapNet-assisted primary screening trial designed to include two separate tracks, one using the conventionally-prepared Pap smears and the other LBP specimens. Both tracks will include high-risk patient populations. Because of the size and complexity of the trial and the efficiencies gained by having the same sites participate in both tracks, the tracks are staggered with the conventional track being conducted first. Data collection will take approximately six months for each track. The trial is being conducted at three investigational sites. Each site will screen 6,500 conventionally prepared Pap smears using PapNet assisted testing in a primary screening mode and also screen the same smears using manual microscopic screening. The trial is designed to measure sensitivity, specificity, and positive and negative predictive values. The company expects to invest nearly \$1.0 million on this clinical trial to provide data for a PMA supplement for the use of PapNet as a primary screener.

A study of the effectiveness of computer-assisted rescreening with PapNet testing, conducted by the Armed Forces Institute of Pathology, found that PapNet increased the detection of cervical abnormalities, despite a high-quality laboratory's double screening of smears from relatively low-risk, well-screened women. The authors concluded that detection of each additional abnormality was at a relatively high cost and noted that the value of PapNet-assisted rescreening will be higher for laboratories that screen populations having a higher prevalence of cervical disease and for laboratories that have a higher false negative rate on initial screening (O'Leary TJ, et al, JAMA, 21 Jan 98, 279(3):235-237).

NSI's revenues in 1997 were \$9,374,000 compared to \$4,729,000 in 1996, representing a 98% increase attributed to higher average unit pricing, an increased unit volume of slide processing services, and revenues from the sale of a PapNet-on-Cyte system in Europe during the fourth quarter of 1997. PapNet is in use in a primary screening mode in many parts of Europe and Asia. In July 1998, NSI obtained approval by the Medical Devices Bureau of Canada to commercialize PapNet for primary screening in Canada. In 1998, NSI expects to invest over \$4.0 million to support its base of more than 100 USA laboratory customers in the use of the PapNet system for rescreening.

In July 1996, NSI filed a lawsuit against NeoPath, alleging patent infringement, unfair competition, false advertising, and related claims. In return, in March 1997 NeoPath sued NSI charging

patent infringement related to the AutoPap System. In October 1998, Cytec and NSI settled litigation between the two companies. Terms of the settlement were not disclosed.

Cost and Reimbursement Issues Pertaining to Automated Screening

Cost and reimbursement issues remain the strongest stumbling blocks to widespread acceptance of automated primary Pap smear screening. Opinions abound as to the ultimate cost effectiveness of automated screening but the upfront costs, sometimes adding as much as \$35 per test, cannot be brushed aside particularly because this sector is extremely cost sensitive and unlikely to support high-priced approaches irrespective of outcome. Although numerous models are being used to illustrate the trade-off between the few additional abnormal cases discovered by automated screening and its high cost, it is not hard to see that even a minor increase in price per test will result in millions even billions of dollars in excess costs.

However, it is too early to dismiss automated primary screening on any grounds. Certain unanticipated developments in this field may conspire to speed up adoption of automated screening:

- shortages of trained cytologists
- higher salaries for trained cytologists
- further consolidation of testing to centralized facilities, and subsequent productivity gains from automated high-volume approaches
- findings that show that automated systems may reduce overall costs of cervical cancer screening by reducing the rate of false positives and, therefore, follow-up costs
- price reductions through competition

As a primary Pap smear screening tool, automated systems could screen all of the laboratory's Pap smears and classify them according to the level of detected abnormality as either normal, needing no further review, to be archived, or potentially abnormal and selected for review by a cytotechnologist. How such a process would impact the overall market of Pap smear screening has not been elucidated. Many pro or con arguments are not based on field data and, therefore, have failed to shed light as to the outlook of automated screening.

Like most other screening programs, cervical cancer prevention comes at a high cost because of the large numbers of women who must be screened to detect a relatively small number of true abnormalities. However, even with the use of higher-priced primary automated screening, the cost per life-year saved is in line with those of other universal screening programs. Using a medical-economic model adapted from one developed for the U.S. Congress Office of Technology Assessment, the incorporation of PapNet-assisted rescreening into a routine biennial screening protocol costs \$48,474 per life-year saved compared

Exhibit 3
Pap Smear Testing in the USA

| Testing | Annual Volume (#) | % of Total | Comments |
|--|-------------------|------------|--|
| Pap smears | 50,000,000 | 100.0 | |
| Normal Pap smears rescreened routinely | 5,000,000 | 10.0 | Currently, rescreening mandated by federal regulations |
| Normal Pap smears archived without rescreening | 11,250,000 | 25.0 | May be classified by automated primary screening systems alone; not manually screened |
| Unsatisfactory | 2,355,000 | 4.7 | Usually specimens are reobtained |
| LSIL | 1,250,000 | 2.5 | Referred for retesting and evaluation |
| HSIL | 300,000 | 0.6 | Referred for colposcopy; approximately 65,00 cases may be classified as cervical carcinoma <i>in situ</i> |
| ASCUS | 2,000,000 | 4.0 | An estimated 25% to 35% of these cases are eventually diagnosed with cervical disease; among 126,000 Pap smears, risk of diagnosis of ICC within 6 months of an ASCUS smear was estimated at .0865% or 1/1156 (Kinney W, et al, SGO98) |
| Normal | 47,500,000 | 95.0 | Overall results after all tests are completed |
| Cancer | 14,500 | .03 | This incidence only holds for regularly screened populations and is much higher among unscreened women |

with \$9,880 for a standard Pap and with \$67,918 per life-year saved for annual mammography screening for breast cancer in women aged 40-50, and \$113,000 per life-year saved for just one test for prostate specific antigen (PSA) to screen for prostate cancer (Schechter CB, Acta Cytol, 40: 1272-1282, 1996). Others, however, find that it is more cost-effective to rescreen all Pap smears manually than the obligatory 10% automatically using PapNet at \$40 per slide (O'Leary, et al, *ibid*).

Another way to measure cost effectiveness is by taking into account not a test's ability to lower the incidence of false-negative results which has not been shown to impact neither incidence or mortality of ICC, but that of false-positive results, particularly ASCUS findings. Once smears are classified as CIN or ASCUS, an expensive series of interventions follow that ultimately prove to be unnecessary. Only a very small number of the approximately 750,000 patients reevaluated every year as a result of abnormal cytology findings, will ultimately be diagnosed with disease. Because all subsequent procedures are increasingly expensive, any system capable of properly classifying more normal smears will eventually save billions in unnecessary costs and spare women the distress and discomfort associated with the process.

More definitive information regarding the various screening procedures in triaging patients with abnormal smears may be forthcoming as a result of an ongoing trial, the ASCUS/LSIL Triage Study (ALTS), initiated in November 1996 by the NCI to evaluate different ways of managing Pap test abnormalities that may be as effective as colposcopy, but less invasive and costly. Study results are expected to be available 5 years hence. ALTS is being conducted at 15 independent sites and will recruit about

7,200 cases. According to the study protocol enrollees are being divided into those with ASCUS or LSIL diagnoses, and then randomly assigned to one of three study arms, immediate colposcopy, repeat Pap smears every 6 months, or a Pap smear plus an HPV test. At enrollment, and at each follow-up visit, all study participants will also undergo cervicography. At the end of the 3-year follow-up period, all participants will be examined by colposcopy (and biopsy as appropriate), to ensure that they do not leave the trial with undiagnosed and untreated lesions.

The endpoints of ALTS are to compare the three different management options in terms of their effectiveness in the early detection of serious abnormalities that can progress to cancer and their acceptance by the patients, and to establish cost-effectiveness. In addition, investigators hope to gain information about immune system factors that may help determine whether a mild abnormality goes away without treatment, or progresses to a more severe abnormality. Although LBP cytology will be the primary method of preparing slides, in 1,000 cases ALTS will also collect and read standard smears to be paired with the thin-layer slides to see if the two methods are equivalent. Automated readers will be used to review all thin-layer slides collected during follow-up as a quality control measure. HPV testing is being performed using Digene's (Beltsville, MD) Hybrid Capture assay. Colposcopy is being tracked using a digital imaging and management system (DIMS) developed by DenVu (Tucson, AZ).

Many also argue that it would be far more cost effective to launch aggressive campaigns to educate the public in order to test the 25% of women who are not currently participating in screening programs, and educate professionals to do a better job in obtaining cervical smears and in

encouraging their patients to adhere to the recommended testing frequency guidelines. In addition to the fact that the Blue Cross and Blue Shield Association rejected both the LPB slide preparation technique and the automated primary screening systems as Pap smear screening tools because, despite their high cost, they have little impact on life expectancy, in July 1998, an official committee of the American College of Obstetricians and Gynecologists (ACOG) stated that these automated systems would not be classified as standard of care until more is known about their actual impact on survival.

Nevertheless, primary automated screening is entering the mainstream. It is expected initial market penetration to be slow until reimbursement is secured but, in the long run, the availability of automation will impact the way laboratories test Pap smears. Acceptance of automated testing will probably follow a similar path to that of LBP, few placements upfront and then a wait of a couple of years while lobbying for reimbursement. Recently, the American Medical Association (AMA) designated two CPT codes for reimbursement of automated primary screening, a first step in obtaining reimbursement for the procedure.

Other Products/Techniques

Other products and adjuncts are also on the market, or in development, to augment either manual or automatic Pap smear screening, and to provide alternative methodologies to evaluate cervical smears.

AccuMed International's (Chicago, IL) AcCell Cytopathology System comprises a family of integrated, modular, technologically-advanced products that offer seamless support for the screening and diagnosis of cervical Pap smears and other cytology specimens. Major components of the AcCell Cytopathology System are the AcCell workstation incorporating the cytology microscope, the TracCell 2000 option which automatically identifies areas on a slide that are not diagnostically relevant and forwards the information to the AcCell workstation, and Specifind a data collection and specimen tracking system outside the laboratory and into the ordering physician's office. TracCell 2000 was approved by the FDA in August 1997 for use with conventional cervical Pap smears and, in October 1998, for use with ThinPrep.

diaDexus (Santa Clara, CA), a joint venture between SmithKline Beecham and Incyte Pharmaceuticals (Palo Alto, CA), and Cancer Research Campaign Technology (CRCT; London, UK) signed, in September 1997, an exclusive worldwide licensing agreement for CRCT's novel cervical cancer screening technology. diaDexus will develop and commercialize the technology, which has the potential to improve screening for cervical cancer when used in conjunction with the Pap test. The terms of the agreement were not disclosed.

Fourier transform infrared (FTIR) spectroscopy measures the frequencies at which light is absorbed or emitted from a sample and intensities are reported in terms of absorbance, i.e.

the amount of light absorbed by a sample, or transmittance, i. e. the amount of light that passes through it. In an FTIR spectroscopy system, an interferometer modulates the intensity of individual frequencies of radiation before the signals are picked up by the detector. An interferometer scan (interferogram) is a plot of intensity versus mirror position of the area being scanned. Because the interferogram is a summation of all the wavelengths emitted by the sample it cannot be interpreted in its original form; it is rather converted into a spectrum by a computer using Fourier transformation (FT). The FT spectrum shows the sample emission at all the frequencies measured and, thus, can be used to identify the sample. Samples of cervical cells can be prepared for spectroscopic diagnosis by centrifuging those obtained by the normal Pap smear technique and applying them to an infrared transparent window. However, given the number of potential confounding variables associated with cervical cytology, a multivariate statistical or neural network analysis would appear to be necessary before the implementation of FTIR technology in clinical laboratories (Wood BR, et al, Biospectroscopy, 1998, 4(2):75-91). Investigators from Nicolet Instruments (Madison, WI) used automated infrared microscopic mapping techniques to measure the infrared spectra at fixed intervals across a cytology sample covering a 5 mm spot, and applied various post-processing techniques to the spectral results to create images revealing specific features of the sample (Lowry SR, Cellular and Molecular Biology, 1998 Feb, 44(1):169-77).

NeoPath markets the Pathfinder System that provides improved quality assurance in the clinical cytology laboratory by computerizing the cytotechnologists' microscopes. The Pathfinder System's position sensors attach to the microscope. As a cytotechnologist examines a slide, the Pathfinder System records the exact areas scanned, the location and identity of any cells or cell clusters that were electronically marked, and patient identification information. This data can then be accessed electronically. The Pathfinder System is not subject to FDA regulation. In June 1997, NeoPath acquired the Pathfinder System product line from CompuCyte (Cambridge, MA). NeoPath began selling the Pathfinder System as a stand-alone product in the third quarter of 1997 and plans to integrate the technology into the AutoPap System.

Human Papillomavirus (HPV) Screening

HPV is not an approved screening methodology for cervical cancer. However, because of the strong link between HPV infection and ICC, it is expected that HPV infection will play a role as both a screening/diagnostic marker and an immunization target, in the prevention of ICC. For instance, presence of HPV infection may be as strong an indicator of premalignancy as the Pap test. In a prospective study conducted by NCI on 21,000 women with a history of normal Pap smears, approximately 80% of those who tested positive for high-risk HPV types developed clinically significant cervical lesions within four years.

Exhibit 4
Medicare Rates for Selected Gynecologic Screening and Diagnostic Procedures

| Procedure | CPT Code | Medicare Rate (\$) | Comments |
|------------------------------|---------------|--------------------|---|
| Pap smear, standard | 88150 | 7.15 | Non-Medicare rates vary with some private payors paying 125% of Medicare level; average cost to the lab is estimated at \$11.0 to \$20.0 per test resulting in losses to the laboratory |
| Pap smear, LBP | 88142 | N/A | Rates are gap-filled until Medicare allowable rates are established, probably in 2000; laboratories charge anywhere between \$20 and \$100; added cost to the standard Pap is estimated at \$9.75 |
| Pap smear, automated QC | 88152 and | 7.15 | Medicare rates are identical with non-automated charges at \$7.50; automated QC, however, adds another \$7.50 to the cost of the standard test |
| Pap smear, automated primary | 88147-48 | N/A | Rates are gap-filled and charges vary |
| HPV DNA amplification probe | 87621x2 | 97.0 | This charge is for Digene's Hybrid Capture HPV DNA test |
| Low-risk | 87621 | 48.5 | Test kit is priced at \$33.3 |
| High-risk | 87621 | 48.5 | Test kit is priced at \$33.3 |
| Cervicography | 58999 (other) | N/A | Charges are about \$25.00 per screening |
| Colposcopy | 57452 | 59.65 | Rates vary outside Medicare and may be as high as \$400.00. |
| Colposcopy with biopsy | 57454 | 88.29 | Lab charges for biopsy may be high as \$120.00, added to colposcopy charges |

During a 1997 meeting of the International Academy of Cytology Task Force (Richart RM, et al, *Acta Cytologica*, 1998 Jan-Feb, 42(1):50-8), it was concluded that although HPV appears to be essential to the transformation of epithelial cells in cervical squamous cell carcinomas, adenocarcinomas, and their precursors, it is not sufficient, and a variety of cofactors and molecular events must take place between when HPV infection occurs and cervical cancer or its precursors develop. In its consensus position the Task Force stated that:

- a subset of HPVs are unequivocally the etiologic agents for cervical cancer and its precursors, based on the available molecular, clinical and epidemiologic data
- although their neoplastic potential varies, the great majority of cervical mucosotropic HPVs have oncogenic potential; however, because oncogenic HPV-induced epithelial transformation to a high-grade lesion or cancer is rare relative to the rate of infection, the term high-risk to define HPV infection is discouraged
- all anogenital HPVs induce LSILs, which are the morphologic correlate of a productive infection and, rarely, HPVs induce a proliferative epithelial phenotype classified as HSIL, that is the proximate cytohistologic precursor of ICC
- HPV biology and issues of practical clinical management should be reflected in the classification systems used for cytologic and histologic diagnosis

Ultimately, HPV screening is expected to be performed

in tandem with Pap on the same specimen. Many improvements in specimen collection and laboratory practices being adopted in the area of Pap screening, such as LBPs, are expected will also benefit HPV testing.

Screening for HPV is hampered by several issues, including the fact that it is present in as high as 30% of normal smears, HPV latency and subclinical infection is not well understood, and no single diagnostic test can detect all HPV infection types, making detection of HPV dependent on the diagnostic method. Considerable effort is being invested to optimize HPV screening methodologies to detect all relevant HPV types, to maximize the test's sensitivity, specificity and ease of use, and minimize its cost. A cost-benefit analysis is vital in making this test a screening modality.

However, even if universal HPV screening does not appear feasible, HPV testing improves the accuracy of screening for cervical cancer, reduces the need for costly and invasive follow-up procedures, and provides cost savings in secondary triage of patients with certain lesions, especially ASCUS and LSIL (see FO, p 566). In ASCUS cases, only patients who test positive for high-risk HPV types need undergo colposcopy, biopsy and surgery, when necessary. Those who test negative or positive for low-risk HPV types, are asked to return for a repeat Pap smear in six months, sparing them the cost and discomfort of additional tests.

HPV may also be useful in the staging of ICC. When sera were examined for the presence of antibody against E7 protein, the occurrence rates of anti-E7 antibody against HPV-16 were 14.1% (10/71) in cervical cancer cases,

0% (0/48) in CIN, and 0% (0/41) in controls; 3/115 patients with tumors in organs other than the cervix also tested positive for antibody against HPV-16 E7 protein. Serum antibody persisted for a long time after surgical removal of the cancers in all cases that were followed-up. HPV-16 DNA was detected in 50% (13/26) of cervical cancer patients, 69% (9/13) of patients with HPV-16 DNA tested antibody-positive and all patients with Stage II, III, and IV cervical cancer (8/8) harboring HPV-16 DNA were antibody-positive. In contrast, only 20% (1/5) of cervical cancer patients with stage Ia or Ib harboring HPV-16 DNA were positive for the anti-E7 antibody in sera. These findings suggest that the presence of anti-E7 antibody in serum depends on the staging of cervical cancer and extent of HPV infection (Fujii T, et al, Jpn J Cancer Res, Jan 1995, 86(1):28-34).

Infection has been traditionally diagnosed by visual inspection for the presence of warts and, indirectly, by the presence of cytologic and histopathologic changes consistent with HPV. Recently, various methodologies to detect HPV infection have been developed but diagnosis is still hampered by the inability to culture HPV and the lack of serologic tests. However, the fact that HPV detection may be accomplished on the same smear used for cytologic evaluation, may be a cost-saving approach that would become routine in the future.

The most accurate methodology in detecting HPV involves identification of HPV DNA in human tissue using Southern blot, *in situ* hybridization, or polymerase chain reaction (PCR). In PCR, the most sensitive of HPV tests, the HPV L1 region of a broad spectrum of genital mucosotropic HPV types is amplified and the amplified products are analyzed by gel electrophoresis.

DNA amplification is the prevailing approach to HPV detection in the clinical setting.

Digene has introduced the Hybrid Capture (HC) HPV DNA test, a sandwich capture, molecular hybridization assay. A specific HPV RNA probe cocktail that incorporates those HPV types linked to cervical dysplasia is used to hybridize with target DNA from clinical samples. Any RNA:DNA hybrids produced are immobilized on tubes coated with antibody to the hybrid. Detection involves an antihybrid antibody conjugated to alkaline phosphatase and a chemiluminescent substrate. The intensity of the light emitted is proportional to the amount of target DNA present. Digene offers two assays, one for low-risk HPVs (e.g. 6, 11, 42, 43 and 44) and one for high-risk HPVs (16, 18, 31, 33, 35, 45, 51, 52 and 56). Tests are listed at \$33.30 each and Medicare will reimburse as much as \$48.50 for each test or \$97.0 for both. Hybrid Capture represents a significant improvement over existing technologies because of its sensitivity, speed, ease-of-use, accuracy and ability to measure viral load.

Digene's HPV test was approved by the FDA in 1995 for follow-up screening of women with equivocal Pap smears. It is

also marketed in selected European and South American countries as a primary cervical cancer screen in conjunction with the Pap smear. Digene plans to establish a global cervical cancer screening business. In Europe, Hybrid Capture is marketed primarily through a direct sales force established with an affiliate, International Murex Technologies (Toronto, Canada), recently acquired by Abbott Laboratories.

Use of HCS HPV alone detects disease in up to 92% of the cases of cervical disease or cancer. This represents a significant improvement over the Pap smear's estimated sensitivity of 80%. HCS HPV provides objective, easy to interpret results and clearly determines if high risk HPV types are present. When combined with Pap smear testing, HCS HPV detects 95% to 100% of cervical disease and virtually all instances of cervical cancer. By reducing the number of false negatives, many cases of precancerous cervical disease will be detected earlier, when treatment is more effective and survival rates are higher.

Under a set of conservative assumptions, HPV-based triage for ASCUS Pap smears was shown to decrease follow-up Pap smears by over 50% and colposcopies by 10%, with no resulting decrease in the number of HSILs missed. The findings are based on a study performed at Kaiser Permanente (Oakland, CA) using Hybrid Capture to test for high-risk HPV DNA on ThinPrep, monolayer liquid cytology medium, collected at the time of the original Pap smear, to assess the test's ability to detect early disease, reduce the need for colposcopy or additional Pap smears, and save money (Colby CJ, et al, SGO98).

In August 1998, Digene acquired Viropath BV, a Dutch company developing tests for HPV by purchasing all of the outstanding capital stock of Viropath in exchange for 181,884 shares of newly issued Digene common stock worth about \$1.7 million. Viropath was founded by Professors Chris Meijer and Jan Walboomers from the Free University (Amsterdam, the Netherlands). Viropath, in addition to collaborating with the International Agency on Cancer Research and the World Health Organization, has been instrumental in recently initiating a clinical trial involving 40,000 women, sponsored by the Dutch government, to evaluate the utility of HPV testing as a primary screening test for cervical cancer in the Netherlands. Professors Meijer and Walboomers have also developed important intellectual property in the HPV field, which is licensed exclusively to Viropath.

Second generation hybrid capture (HC II) assays are also in development that in conjunction with LBP may help to efficiently identify HSIL in women diagnosed with ASCUS. When compared with the first-generation HCT HPV to detect CIN in women with ASCUS and LSIL, the new HC II microplate HPV test achieved a greater test sensitivity for detecting carcinogenic HPV and correspondingly HSIL compared with the currently available first-generation HC HPV test. Colposcopy examinations including cervical biopsy and

endocervical curettage, when indicated, were performed in all women to determine criterion standards for comparison. In paired swabs and LBP specimens from 242 women available for testing, the sensitivity, specificity, and positive and negative predictive values for detecting HSIL were 61.9%, 57.0%, 12.0%, and 94.0%, respectively, for HC, and 90.5%, 29.4%, 10.9%, and 97.0%, respectively, for HC II. When only women with an initial ASCUS Pap smear report were considered, HC II results were 88.9%, 40.3%, 9.1%, and 98.2%, respectively. (Ferris DG, et al, J Family Practice, 1998 Feb, 46(2):136-41).

Serology

Serological assays would be ideally suited to identify HPV infection as a screening methodology, in the diagnosis of ICC, in the clinical management of patients with ICC, and in the monitoring of HPV vaccination trials. However, no serum assays are commercially available to detect HPV. Existing peptide enzyme-linked immunosorbent assays (ELISAs) for the detection of anti-E6 and anti-E7 antibodies in human sera have low levels of sensitivity and specificity and are not suitable for diagnostic or screening applications (Meschede W, et al, J Clin Microbiol, 1998 Feb, 36(2):475-80).

Based on highly purified recombinant native proteins, investigators at Angewandte Tumorstudiologie, Deutsches Krebsforschungszentrum (Heidelberg, Germany) developed four sandwich ELISAs that detect antibodies against HPV type 16 and 18 E6 and E7 proteins. These assays exhibited a high degree of specificity for cervical cancer. Among 501 serum specimens from unselected patients with ICC, 52.9% reacted positively in at least one of the four assays. In contrast, only 2 (0.8%) among 244 serum specimens from normal controls were reactive. In all (19/19) antibody-positive patients, the HPV type identified by the assay was identical to the HPV DNA type found in the tumor, which illustrates the high degree of specificity of these assays with respect to HPV type. In a direct comparison of 72 serum specimens from patients with cervical cancer, 56% of the specimens reacted with at least one of the four protein ELISAs, whereas 40% reacted in at least one of seven peptide ELISAs covering the four antigens (Meschede W, et al, *ibid*).

In another approach, immunoglobulin-A and G (IgA and IgG) responses against HPV-16-like particles (VLPs) were tested by ELISA in 104 women with cervical abnormalities, 26 ASCUS cases and 14 cytologically normal women with HPV DNA. As controls, 130 age-matched cytologically normal women with no HPV DNA were selected from the population in which the cases were generated. The existence of HPV DNA in cervical samples was tested by a PCR-based method. Normal women positive with HPV-16 DNA were followed up at 4- to 7-month intervals for 16 to 24 months. IgA and IgG antibodies against HPV-16 VLP were frequently detected in these women repeatedly positive with HPV-16 DNA, suggesting that

persistent HPV infection is crucial for effective antibody responses against the virus. IgA response appears earlier and persists longer than IgG response. Women with HPV DNA of types 16, 31/33/35, 58, and unknown types, showed significantly higher seropositivity for both IgA and IgG antibodies than the controls. No significant seropositivity for IgA or IgG was detected in the HPV 18/45 positive group. HPV 31/33/35, 58 appear to be types close to HPV 16, whereas HPV 18/45 appear to be distinct from HPV 16 in antigenicity. IgA and IgG responses against HPV-16 VLP were more frequently observed in HPV DNA-positive women with normal cervixes, and in those with ASCUS, HSIL and cervical cancer, than in the controls. Strong IgA and IgG responses depended on HPV-16 infection in HSIL and cervical cancer, but there was no correlation between the serological responses and the status of HPV DNA in ASCUS and LSIL. Antibody positivity reflects persistent viral infection that may increase the risk for malignant progression of the cervix. This serological assay using HPV-16 VLP may therefore be useful as a new diagnostic tool supplementing cervical cytological tests (Sasagawa T, et al, Int'l J Cancer, 1998 Feb 9, 75(4):529-35).

Other Approaches

Cytokine production may be used to characterize lymphoproliferative (LP) responses to identify a subset of patients with HPV infection and SIL who will undergo spontaneous regression. Spontaneous remission rates of 50% within 2 years have been documented. In this study peripheral blood mononuclear cells from 36 untreated patients with biopsy-proven SIL and HPV infection detected by polymerase chain reaction (PCR), were cultured with overlapping peptides from the HPV 16 E7 transforming protein for 3, 7, 14, and 21 days. Production of interleukin-4 (IL-4) in culture supernatants was measured by ELISA and cytokine production was correlated with follow-up Pap and HPV status. Among those with positive LP to E7 peptides, 9/10 (90%) with high IL-4 production and 7/7 (100%) with low IL-4 production had follow-up Pap negative for SIL, whereas 11/19 (58%) patients without IL-4 production had a negative follow-up Pap. Of those with high IL-4 production, 7/9 (78%) were negative for HPV, while 3/5 (38%) of those with low IL-4 production and 7/12 (37%) without IL-4 production were negative for HPV. Of the 32 patients typed for HPV, only 1/9 (11%) of high IL-4 producers and none (0/8) of low IL-4 producers were positive for HPV 16, 31, or 33, while 4/15 (27%) without IL-4 production were positive for these HPV types. These results suggest that TH2 responses to E7 peptides, characterized by IL-4 production, are associated with regression of SIL as detected by Pap smear, and these immune responses are associated with disappearance of HPV infection, including high risk types. If larger studies confirm these preliminary results, cytokine production may provide an intermediate biomarker for differentiating those patients who will develop persistent disease from those who will spontaneously regress (Timmins P, et al, SGO98).

p53 protein polymorphisms, commonly found in many individuals, may increase sevenfold the risk of developing HPV-related cervical cancer. In cervical cancer, p53 is not defective itself but can form complexes with E6 protein of HPV that promote p53 degradation. Investigators at the Imperial Cancer Research Fund's Skin Tumour Laboratory (London, UK) found that a common polymorphism confers a susceptibility to E6-mediated degradation. This polymorphism results in the presence of either a proline or an arginine in the amino-acid sequence at position 72. When the effect of this polymorphism on the susceptibility to E6-mediated degradation was investigated, the arginine form was found to be significantly more susceptible than the proline form. Moreover, allelic analysis of patients with HPV-associated tumors revealed a striking overrepresentation of homozygous arginine-72 compared with the normal population, which indicated that individuals homozygous for arginine 72 are about seven times more susceptible to HPV-associated tumorigenesis than heterozygotes. The arginine-encoding allele, therefore, signals a significant risk in the development of HPV-associated cancers and testing for this polymorphism may be of value in identifying women at high risk for cervical cancer (Storey A, et al, *Nature*, 1998 May 21, 393(6682):229-34).

HIV Screening

Cervical cancer has been designated an AIDS-defining illness. It is the most common AIDS-related malignancy and the sixth most common initial AIDS-defining illness in HIV infected women. HIV and HPV infections seem to go hand-in-hand; in HIV-infected patients, the prevalence of HPV is 5 times that of the general population. Additionally, various cohort studies have observed that up to 40% of HIV-infected women have dysplastic changes on Pap smears, compared with less than 10% of demographically similar HIV-negative women. Progressive immunosuppression in this population is a major risk factor. Immunodeficiency resulting from HIV may allow HPV to flourish caused by inhibition of the body's cell-mediated immunity. Because the disease presents at a later stage in HIV-infected patients and is less responsive to treatment, close attention to timely Pap smears and appropriate follow-up is important in this population. Also, multifocal involvement, with perianal and extensive disease, is significantly more common with HIV.

In one retrospective review of cases of cervical cancer and AIDS in women in New York City over an 8-year period, HIV infection was diagnosed by routine testing at the time of cancer presentation in 71% of women with cervical cancer included in the study. In contrast, among women with other malignancies, HIV diagnosis preceded cancer diagnosis.

The Centers for Disease Control and Prevention recommended in 1993 that Pap smears be part of the initial medical evaluation of HIV-infected individuals. If the initial smear is normal, an additional Pap smear should be done

within approximately 6 months to rule out a possible false-negative result on the initial Pap smear. Women with 2 initial normal smears should, thereafter, undergo annual Pap smears (although most experts would recommend continued screening every 6 months in women with CD4 counts less than 200/mm³ or a prior history of HPV infection). If the initial Pap smear shows inflammatory changes, it should be repeated after a period of 3 months. Patients with Pap smears demonstrating CIN or ASCUS should be referred for colposcopy with biopsies of suspicious lesions. However, routine use of colposcopy as a screening tool was not shown to be a cost-effective alternative to Pap smears in this population.

HIV infection is also a major problem in the third world, particularly in certain African and Asian countries. For instance, among many problems encountered in instituting mass screening in Africa, is the confounding effect of HIV infection (Thistle PJ and Chirenje ZM, *Central African J Medicine*, 1997 Sep, 43(9):246-51).

Novel Markers

Nuclear Matrix Proteins

Nuclear matrix proteins (NMPs), that are cell and tissue-specific, are associated with the nuclear matrix, the nonchromatin structural component of the nucleus that governs nuclear shape and plays a dynamic role in gene organization and expression. CvC-3, a unique NMP, was detected in 20 of 20 cervical cancers and 0 of 10 normal tissues by investigators at Brigham and Women's Hospital (Boston, MA) and Women and Infants Hospital (Providence, Rhode Island) in collaboration with Matritech (Newton, MA), using high resolution two-dimensional gel electrophoresis. A cDNA clone was then selected and recombinant protein was expressed (Sheets EE, et al, ASCO98, Abs. 2160:563a). In December 1997, in a blinded study involving 320 cervicovaginal specimens, conducted at the above facilities, a monoclonal antibody (MAB 179.1)-based assay, NMP179, detected 97% of precancerous high-grade or advanced cervical dysplasia and 82% of precancerous low-grade or early stage dysplasia, with an estimated specificity of 64%. NMP179 correctly identified 30 of 30 (100%) HSIL and 55 of 79 (69.6%) LSIL (Sheets EE, et al, ASCO98, Abs. 2160:563a).

In March 1998, Matritech regained all marketing and product rights regarding NMP179 which had been granted to Bayer in a joint development and distribution agreement signed in 1995. Consequently, Bayer's option to develop and launch an automated cervical cancer instrument for use with NMP179 was terminated. Matritech was initially paid \$150,000 in 1995 and recorded \$120,000 and \$140,000 in milestone revenue from Bayer in 1996 and 1997, respectively.

Telomerase

Telomerase activity also appears to be strongly present in ICC specimens. When measured by use of a telomere repeat amplification protocol (TRAP) assay in 24 cervical cancers, one carcinoma *in situ* (CIS), and 20 CIN lesions,

strong telomerase activity was detected in 22 of 24 (91.7%) cervical cancer specimens and in one CIS specimen. Relatively weak but distinctive telomerase activity was also detectable in 1/4 CIN I (25%), 2/8 CIN II (25%), and 2/8 CIN III (25%) specimens, but was absent in adjacent nontumor cervical tissue from the same 24 cervical cancer patients and in normal cervical tissues from 11 controls. It seems that there is a progressive increase of telomerase activity in association with an increased degree of cervical malignancy and that telomerase expression may play a crucial role in cervical carcinogenesis (Pao CC, et al, *J Clin Oncol*, May 1997, 15(5):1932-7).

Cervicography

In cervicography, a photograph (cervigram or cervicogram) of the cervix is taken after application of acetic acid. The cervigram is then visually examined by an expert looking for any pathologic changes consistent with dysplasia. Cervicography alone does not seem to offer an alternative to cytology for primary cervical screening because, although simple to perform, it is less sensitive and specific than cytology. When the results of cytology and cervicography were correlated with colposcopic findings among 1,709 patients (1,447 seen for routine screening, 82 for follow-up after treatment for cervical neoplasia and 180 referred because of cytologic abnormalities), cervicography entailed more defective examinations than did cytology (8.9% versus 0.2%), and it was less sensitive (51% versus 59%) and less specific (96% versus 98%). Whatever the clinical criteria (patient's age, parity, pregnancy or history of cervical treatment), the rate of false positives with cervicography was always higher than with cytology, as was the rate of false negatives, except in pregnant women (Baldauf JJ, et al, *Acta Cytologica*, 1997 Mar-Apr, 41(2):295-301).

However, the sensitivity of cytology for detection of CIN may be effectively augmented by cervicography. Combining cytology and cervicography can decrease the number of recalls, biopsies, and unnecessary treatments and, therefore, reduce cost. Also, down-staging of invasive cancer by visual inspection seems a cost-effective alternative to the introduction of cytology in countries with limited health facilities (Schneider A and Zahm DM, *Obstet & Gynec Clinics of NA*, Sep 1996, 23(3):657-73).

In a prospective multicenter clinical trial to evaluate the performance of cervicography compared with cytology for the detection of cervical intraepithelial neoplasia, three hospitals with outpatient gynecology clinics, and three cancer screening clinics, performed cervical cytology and cervicography on 5,724 women. If one or both tests showed an abnormality suggestive of at least a LSIL, a colposcopy with directed biopsy was carried out. Cervicograms were evaluated by four experienced 'senior' assessors and by ten 'junior' assessors. Results were fully analyzed for 5,192 women (91%). A cervical biopsy in 228 women confirmed a true positive lesion in 116 cases (incidence rate=2.2%). Of these, 72 cases (62.1%) were detected

by cervicography and 64 (55.2%) by cytology; this difference was not statistically significant. Only 20 cases of CIN (17%) were concordantly detected by both tests. Senior assessors performed significantly better with a detection rate of 80.6% compared to a detection rate of 56.6% for the junior assessors. These results suggest that cervicography should be considered as a complementary test to cytology. However, although overall detection of CIN is improved, this is mainly attributable to the detection of more low-grade lesions. The lower sensitivity and specificity in high-grade lesions compared with cervical cytology is the main limitation of cervicography in screening for CIN. An important finding was that the performance of cervicography was highly dependent on the assessors' experience (De Sutter Ph, et al, *British J Obstet & Gynaec*, 1998 Jun, 105(6):613-20).

National Testing Laboratories

National Testing Laboratories (Fenton, MO) introduced cervicography in 1981 but field results were published in the late 1980s. The company either sells or rents the cerviscope and, for a fee of \$25, provides the film and reads the image for about 2,000 professionals currently using this modality. This procedure is currently only reimbursed as a triage approach, in cases identified by cytology as abnormal, and not as a primary screen.

Speculoscopy

Speculoscopy, a device developed by Trylon (New York, NY), uses chemiluminescent illumination to provide a low power magnified visual examination of cervical epithelium. This technique uses the Speculite, an inexpensive (\$50) device, as the illumination source. After exposing the cervix to a vinegar wash, the doctor can directly distinguish acetowhite regions that indicate abnormalities, from the dark blue or purple background.

In a comparison multicenter prospective study, 395 high-risk women who were referred to a colposcopy clinic, underwent a repeat cervical smear and speculoscopy followed immediately by colposcopy, to establish how these tests predict cervical histology. Biopsies of abnormal colposcopic lesions and endocervical curettage were performed when indicated. Histologic diagnoses were compared with cytology, speculoscopy and colposcopy results. Colposcopy was more sensitive than speculoscopy in the detection of cervical neoplasia (97% versus 82%) and was superior in visualizing focal lesions and vascular patterns. An antecedent acetowhite abnormality, detected during speculoscopy, was highly predictive of subsequent abnormal colposcopy (97% positive predictive value). The "overall" rate of acetowhite lesions noted during speculoscopy, was nearly half the rate during colposcopy. Colposcopy is better suited than speculoscopy to the follow-up of patients with abnormal cervical cytology because it facilitates lesion grading and assists in directing biopsies. Speculoscopy is best used as a dichotomous screening test based on the presence or absence of at least one well-demarcated acetowhite lesion, and may be more suitable than colposcopy

as an adjunct to cervical cancer screening because of its lower overall acetowhite rate (Lonky NM, et al, J Reproductive Medicine, Jul 1995, 40(7):530-6).

Trylon

In early 1998, the FDA approved broadened indications for the use of Trylon's Speculite in conjunction with a Pap smear (Pap plus speculoscopy) in all women recommended for cervical screening with pelvic examination and Pap smear. Speculite is being marketed by Pharmacia & Upjohn.

Polarprobe

The polarprobe uses the optical and electrical characteristics of cervical tissue to provide, instantaneously and noninvasively, a diagnosis of CIN or cervical cancer. This pencil-sized device, being developed in Australia, has been used to check 15,000 women for the presence of cervical abnormalities.

DIAGNOSIS AND STAGING

After screening tests identify abnormalities that may indicate invasive cancer, diagnosis is carried out by histologic analysis of biopsy samples.

Colposcopy

A colposcopy is the next step in the progression of diagnostic evaluations when the Pap smear test detects HSIL or ASCUS/HPV. Colposcopy is an endoscopic procedure that uses a low power magnification device to visually identify mucosal abnormalities characteristic of CIN or invasive cancer. By applying acetic acid solution to the cervix, a colposcope may also be used to biopsy suspicious lesions that are identified by acetowhitening. Although in itself nonspecific, whitening during colposcopy reveals cervical changes that can be caused by HPV or attributed to such lesions as CIN. A meta-analysis of 9 studies performed during the 1960-1996 period, concluded that colposcopy compares favorably with other medical diagnostic tests in terms of sensitivity and specificity (Mitchell MF, et al, Obst Gynec, Apr 1998, 91(4):626-31).

Colposcopy may also be cost-effective as a primary screening methodology compared to conventional cytology. In a prospective study performed in Italy, 3,000 consecutive self-referring women were examined by cytology and colposcopy in a blind fashion. Further assessment was based on a cytologic report or on colposcopy-directed punch biopsy. Overall, 18 high-grade lesions (CIN3=9, CIN2=9) were detected. Among four different screening scenarios compared (Exhibit 5), colposcopy was more sensitive and more cost-effective than conventional cytology screening. At least in settings where access to cytopathology may be difficult, screening by colposcopy may be considered a possible alternative (Cecchini S, et al, Tumori, 1997 Sep-Oct, 83(5):810-3).

DenVu has developed, DIMS, a digital imaging and management system that allows clinicians to capture high-resolution digital images of the cervix, vagina and vulva,

during colposcopy, by using a CCD video camera attached directly to the colposcope. This product arose from an exclusive contract awarded to DenVu by the NCI in 1995 to develop imaging and patient records software to support the ALTS clinical trial. Using DIMS, images obtained for each patient may be annotated, biopsy sites marked, computerized assessment of each lesion performed, and a comprehensive report generated after each examination and assessment. DIMS is sold worldwide.

DenVu also markets a Windows-based Pap smear and biopsy management system for primary care gynecologists that allows practices to track, follow up, and communicate with their Pap smear and biopsy patients to improve patient compliance with follow-up visits for re-examination or treatment.

Invasive Procedures/Biopsy

Histologic examination of biopsy specimens is the definitive diagnostic test for ICC. Several approaches are being used to obtain a tissue sample including punch biopsy, loop electrosurgical excision procedure (LEEP) and conization which will be discussed in Part III of this report. Conization is the most reliable method currently available for identifying early cervical lesions.

Concerns regarding the effect of biopsy on the natural history of CIN or on the treatment of ICC, have prompted several studies. According to a prospective randomized clinical trial involving 161 eligible women who underwent colposcopy over an 18 month period, directed punch biopsy trauma did not have a significant effect on the immediate natural history of CIN. It appears that tissue trauma from punch biopsy and the subsequent inflammatory and wound healing processes do not modify the course of CIN, as noted in previous natural history studies (Chenoy R, et al, British J Obst and Gynaec, 1996 May, 103(5):457-62).

Also, based on retrospectively reviewed medical records of 127 patients (cone biopsy=48) with stage Ib ICC who underwent radical hysterectomy, cone biopsy as a diagnostic procedure for ICC does not appear to result in an increased incidence of complications from subsequent radical hysterectomy or influence stage Ib ICC. There were no differences between groups in age, race, history of smoking, or associated medical disease. Of those undergone cone biopsy, 32% had deep invasion on final histopathology compared with 67% who did not have cone biopsy. In addition, 19% of those in the former group and 42% in the latter group had poorly differentiated cancer. There were no differences between groups in the incidence of intraoperative or postoperative complications, and the mean overall survival was 72.2 months versus 59.2 months, respectively (Malviya VK, et al, SGO98).

Endocervical Curettage

Endocervical curettage (ECC) is performed pre- and postoperatively to detect CIN and ICC. A retrospective study of patients undergoing cervical conization was performed to evaluate the correlation of the grade of the pre-

operative ECC and the grade of dysplasia in the conization specimen as well as the incidence of invasion in the cone specimen, with respect to preoperative ECC. In addition, an evaluation of the role of routine preoperative ECC in satisfactory and unsatisfactory colposcopy and of the need for routine ECC in the detection of postoperative residual dysplasia, was carried out. When evaluating the association of grade of preoperative ECC with respect to the grade of conization specimen, among 297/391 (76%) patients who underwent ECC as part of preoperative assessment for cervical dysplasia on Pap smear, there was perfect agreement in 36.7% of cases and agreement within one grade in 60.4% of cases. Of 17 patients with invasive disease on conization specimen who had a preoperative ECC, only those with a positive ECC had ICC at conization. None of 113 patients with a negative preoperative ECC had ICC on conization specimens. The proportion of satisfactory colposcopic examinations between positive and negative ECC was not significantly different. Follow-up of patients with positive margins of resection at the time of conization was performed with a Pap smear only in 20 patients and with a Pap smear and ECC in 53 patients. In the latter group, only 2/45 (4%) had a positive ECC with a negative Pap smear. There was good agreement between the grade of dysplasia on the preoperative ECC and on subsequent conization specimen. All patients with invasive disease on conization had positive preoperative ECC and no patients with a negative preoperative ECC had invasive disease. Therefore, the preoperative ECC is a good predictor of conization findings. Colposcopic examination, however, was not a good predictor of pathology in the endocervical canal. Routine ECC should be a part of the preoperative assessment of an abnormal Pap smear; however, routine postoperative ECC may be unnecessary in the evaluation for residual dysplasia in patients with positive margins at conization (Fine BA and Feinstein G, SGO98).

Lymph Node Biopsy

Para-aortic lymph node biopsy is a controversial but proved technique to determine the extent of spread of cancers from the uterine cervix or endometrium. Among 568 patients who underwent para-aortic lymph node sampling in conjunction with another operative procedure between 1976 and 1995 [507 (89.3%) of these patients had either endometrial or cervical cancer], para-aortic lymph node biopsies led to a survival rate of 9.1% for cervical carcinoma and 46.6% for endometrial carcinoma, and were associated with acceptable morbidity. Para-aortic lymph node biopsies

Exhibit 5
Cost comparison of Cytology and Colposcopy in Cervical Cancer Screening in the Italy

| Methodology | Procedure Cost (\$) | Relative Sensitivity (%) | Cost of detected HSIL (\$) |
|--|---------------------|--------------------------|----------------------------|
| Cytology alone | 17.98 | 44.4 | 6,743 |
| Cytology + repeat smear for ASCUS cases | 19.40 | 61.1 | 5,291 |
| Colposcopy + cytology for cases of condyloma at punch biopsy | 23.86 | 100.0 | 3,977 |
| Colposcopy alone | 22.10 | 88.8 | 4,144 |

Source: Cerchini, et al, ibid

should be part of the routine evaluation of patients with gynecologic cancers. The knowledge gained by this procedure along with appropriately administered radiation therapy can save lives (Blythe JG, et al, Am J Obst & Gynec, 1997 Jun, 176(6):1157-62; discussion 1162-5).

Pelvic lymph node (LN) dissection with frozen section analysis prior to radical surgery, is the most important negative predictor of survival in early stage cervical cancer, and may exclude patients with recurrence from exenteration. Nonsurgical treatment options are chosen in case of LN metastases. Because preparation and analysis of frozen sections takes 10-15 minutes for each LN, alternative diagnostic methods to assess LN involvement such as touch imprint cytology, that requires 5 minutes or less, may be of value. In a prospective study to determine the usefulness of cytologic imprint technique versus frozen section versus paraffin histology, 318 pelvic and para-aortic LN from 32 patients with Stage I-IV cervical cancer were bisected and submitted for frozen section after touch imprints had been prepared. Metastatic squamous cell carcinoma (SCC) was detected in 29 nodes (9.1%) by frozen section histology with 26 of these diagnosed by touch imprint and confirmed histologically. Reasons for the 3 false negatives included inadequate preparation of the touch imprint and the presence of small metastases within the lymph node parenchyma deep to the bisected surface. Permanent histology always agreed with the frozen section result. Touch imprint evaluation of pelvic LN for metastatic SCC at the time of intraoperative consultation had a sensitivity and specificity of 90% and 100%. Adequate imprint preparation is very important and multiple sections should be made through thick LN. With those precautions in mind, touch imprints may provide a sensitive, specific, and time-efficient method in diagnosing LN metastases in SCC of the cervix (Hasenburg A, et al, SGO98).

Noninvasive Imaging

In ICC, noninvasive imaging is primarily done to assess disease status, provide accurate staging, and monitor treatment outcome.

Magnetic Resonance (MR) Imaging

MR imaging is useful for the detection of invasive uterine cervical cancer lesions. It also appears that MR may be used as a substitute for conization for confirmation of definite invasive disease, particularly in the endocervix. In a prospective study to determine if definite ICC can be ruled out by negative MR findings, investigators performed T2-weighted and T1-dynamic enhancement (T1D) MR scans in 42 patients who were scheduled to undergo either hysterectomy or conization because of invasive or noninvasive lesions of the cervix. The MR images were reviewed by two radiologists without any information except for the preoperative diagnosis and at least 12 longitudinal sections were made from the surgically removed cervix to be reviewed by one pathologist not given any MR information. When MR and histologic findings, analyzed in terms of the depth of invasion, were correlated, 14 cases were considered to have a possible invasive lesion; however, 9 of them were noninvasive and 3 involved minimally-invasive disease. These observations were adjusted by T1D interpretation. Only three cases considered possibly invasive by T1D were noninvasive. Most importantly, all cases of noninvasive disease as determined by MR scanning were either noninvasive or early invasive (lesion size <4 mm). Negative findings on T2 and/or T1D MR indicated noninvasive or early ICC. However, more experience in interpretation of T2 and T1D MR findings with regard to early invasive or noninvasive lesion of the uterine cervix is needed, especially in regards to interpretation by the radiologist (Oda T, et al, ASCO98, Abs. 1430:371a).

Other Imaging Modalities

Optical coherence tomography (OCT) is a new optical imaging technique which performs non invasive, *in situ*, cross sectional tomographic imaging of microstructure in biological tissues. It is analogous to ultrasound imaging, only it measures the intensity of backscattered infrared light rather than sound (Science 254:1178-1181). OCT images in scattering tissues can achieve axial resolutions of 4-16 μm . Recently, *in vivo* catheter/endoscope-based imaging of the gastrointestinal and pulmonary tracts has been demonstrated in a rabbit model (Science 276:2037-2039). OCT *in vitro* imaging of a series of human tissues from the cervix and uterus, obtained after hysterectomies and imaged fresh, was also performed by investigators at the Massachusetts Institute of Technology (MIT; Cambridge, MA) and Massachusetts General Hospital (MGH; Boston, MA). Tissue of varying degrees of neoplastic infiltration was inspected. Microstructural and epithelial changes associated with the neoplastic nature of the tissue, such as dilated and distorted glands, were readily imaged and favorably matched to histopathology. OCT can be implemented using low-cost fiber components allowing fast, near real-time image acquisition, at 4-8 frames per second. Its all-fiber optic nature makes the system uniquely suited for interfacing to fiberoptic endoscopes (less than 1 mm in diameter), as well as laparoscopes, for *in vivo* internal body imaging

of the reproductive tract. These features, coupled with the recent experimental results, suggest that OCT could provide an effective tool for the diagnosis and assessment of neoplastic changes of gynecologic tissue (Pitris C, et al, ASCO98, Abs. 1434:372a).

PET-FDG detected 19/21 (90%) of newly-diagnosed or recurrent tumors, 100% of metastases to the cervix from other primary cancers such as melanoma, and is promising for the detection of untreated cervical cancer (see FO, pp 879-880).

SPECIAL REVIEW

TRENDS IN ONCOLOGY UPDATE ON THE STATE-OF-THE-ART IN THE MANAGEMENT OF BREAST CANCER

Major fundamental changes are occurring in the management of breast cancer on all fronts, including screening, diagnosis, prevention and treatment and, in the process, a multibillion dollar drug market is being created for this indication alone in the developed world. Developments in the breast cancer sector illustrate the potential commercial value of pharmacologic interventions that will also hold for other major cancer types, such as colorectal, prostate, and lung cancer, that affect large populations in the developed world. New management approaches will undoubtedly raise the price tag over standard therapies. Paying for expensive treatments may be feasible by the developed countries but the rest of the world would have to make much tougher decisions as how to deal with the high costs of new treatments.

Interestingly, a major shift is also occurring in market segmentation. In the past, the most significant opportunity for pharmacologic intervention involved treatment of advanced disease. However, currently, treatment of early cancer and prevention of its recurrence is creating a far larger drug opportunity. Also, these earlier interventions will change the epidemiologic dynamics of this sector by extending survival and preventing recurrence. For instance, widespread prevention programs may lower the incidence of breast cancer in postmenopausal women by as much as 45% after five years of treatment. Also, chemotherapy used in early cancer may reduce recurrence by 20%-25% in all age groups, and significantly diminish patient populations presenting with relapsed disease. Finally, use of novel and more aggressive treatment approaches may not cure metastatic cancer but will definitely extend patients' lives.

In assessing the market impact of new approaches in the prevention and treatment of breast cancer (Exhibit 7), it appears that, perhaps for the first time ever, treatments will compete with each other for the same indication. It is

not clear how oncologists will select among the various options, some offering lower costs, others less severe side effects, or marginally better outcomes. One way to decide on treatment may be to rely on profiling tumors regarding various markers and then prescribing a therapy targeting these markers. There is no question that an increasingly larger proportion of women diagnosed with breast cancer will also undergo more special tests to profile their tumor as a means of tailoring their treatment.

Another means of establishing standard treatments for the various types/stages of breast cancer is by performing comparative, head-on clinical trials. Indeed, various large clinical trials, including multinational studies, are ongoing, comparing various treatment approaches, that are expected to further alter the way breast cancer is managed.

MAMMOGRAPHY

Millions of mammograms are performed annually in the developed world (see FO, page 360). In the USA, based on about 30 million mammograms performed at an average charge of \$70, the market exceeds \$2.1 billion. As of January 1998, Medicare began expanded coverage of breast cancer screening to include annual screening mammograms for all women aged ≥ 40 and a one-time baseline mammogram for women aged 35-39. Beneficiaries will pay a 20% copayment and Medicare will pay the other 80%, even when the annual deductible is not met. Before these changes, Medicare covered annual screening mammograms for women aged 50-64, and for women aged 40-49 at high risk for breast cancer. Mammograms for women aged 40-49 at normal risk and women aged ≥ 65 were covered every two years, and a one-time baseline mammogram was covered for women aged 35-39. Beneficiaries, however, were expected to pay the 20% coinsurance including any unmet portion of their deductible.

Improvements of mammography as well as novel approaches to breast cancer screening are being pursued by numerous developers (see FO, 389-391). One technique, currently under investigation, is full-field digital mammography. Although technologically more sophisticated and offering several options, such as image enhancement and teleradiology, not possible with screen-film mammography, digital radiography has not been shown, to date, to offer a clear advantage over the latter when it comes to the diagnosis of breast cancer. According to interim results reported during the 1998 meeting of the Radiology Society of North America (RSNA) from an ongoing study to enroll 15,000 women over two and one half years, sponsored by the US Army Medical Research and Material Command (MEDCOM), 8 cancers were detected in 1,600 women. Digital radiology alone detected 2 cases, film-screen alone detected 3 cases and 3 others were detected by both techniques.

A new aid in reading mammograms, the ImageChecker M100, developed by R2 Technology (Los Altos, CA), was approved by the FDA in June 1998. This technique either converts X-rays into digital images or uses digital images themselves and applies computer-aided detection (CAD)

to analyze the image to aid radiologists in their review of mammograms. Approval was based on a clinical trial of the ImageChecker M100 involving 40,000 cases reviewed prospectively and 1,000 cases reviewed retrospectively, that demonstrated the efficacy of this approach. The ImageChecker M100 is approved as a rescreening and not as a primary screening approach.

In addition to conventional mammography, investigators are evaluating dedicated PET-FDG mammography systems (see FO, p 882) and various other approaches described in FO, pp 389-391.

PHARMACOGENETICS AND DISEASE PROGNOSIS AND TREATMENT

Interestingly, obtaining the genetic profile of a patient's tumor may become an integral part in selecting appropriate drug therapy because many drugs in development are so selective that they are only effective when used with tumors expressing certain markers. A case in point is trastuzumab (Herceptin; Genentech) that is only effective in tumors expressing HER2/neu, a protein associated with poor prognosis in advanced, metastatic breast cancer. Although the exact role of HER2 in tumorigenesis and/or tumor progression has not been fully elucidated, presence of this marker is ominous in most breast cancer cases. For instance, overexpression of HER2 may render tumors resistant to doxorubicin (Paik S, et al, JNCI, 16 Sep 1998, 90(18):1361-1370), or antiestrogens (FO, p 823).

The marriage of genetics and treatment choice is creating a new paradigm as to how cancer will be managed in the future and how therapies will be reimbursed and global markets will develop. On the one hand, the fact that a treatment option is decided by the presence of a marker ensures that it will be a unique treatment choice but, on the other hand, its application potential is limited by its link to a given marker. Therefore, if pricing of Herceptin is an indication, developers of highly specialized therapies are charging high premiums over other, broader-based therapies.

The introduction of Herceptin in the mainstream of oncology care has created an overnight market for testing for amplification of HER2 in patients with breast cancer. Three tests manufactured by Dako (Copenhagen, Denmark), Ventana Genetics (Salt Lake City, UT) that acquired the Oncor (Gaithersburg, MD) test, and Vysis (Downers Grove, IL) have been approved (Exhibit 6) and are competing in a USA and Western European market for HER2 testing, estimated by Vysis at \$36 million.

The role of many other markers in breast cancer is being investigated by many groups and programs are under way to use these markers, among them p53 and BCRA1 and BCRA2, in devising diagnostic, prognostic and therapeutic approaches for all stages of breast cancer (FO, pp 337-339).

CHEMOPREVENTION AMONG HIGH-RISK GROUPS

Chemoprevention in breast cancer is becoming a reality. Although the currently available options carry too much risk, several novel entities promise to offer a much more

Exhibit 7
Approved HER2 Tests

| Developer/Affiliate | Product | Technology | Status |
|---|--------------------------------|--|---|
| Dako/Genentech | Hercep Test | Immunohistochemical assay based on a polyclonal antibody; binding of this antibody and its subsequent visualization indicate the level of presence of antigen in tissue (originally developed by Genentech for the Herceptin clinical trials) | Approved by the FDA in September 1998 |
| Ventana Genetics (acquired the test from Oncor) | INFORM HER-2/neu | FISH DNA probe assay to determine the qualitative presence of HER2/neu gene amplification on formalin-fixed, paraffin-embedded human breast tissue | Approved by the FDA in December 1997; marketed throughout Europe since 1996 |
| Vysis | PathVysion HER-2 DNA Probe Kit | FISH DNA probe assay; directly detects both the HER2 gene and chromosome 17 on which it resides; because patients with more than two copies of chromosome 17 do not represent true amplification of the HER-2 gene, the ability to simultaneously detect chromosome 17 provides a built-in control to determine true HER2 gene amplification | Approved by the FDA in December 1998; in review by the Agence du Medicament (ADM) in France |

attractive alternative to healthy women at high risk for the disease and, in the process, also prevent osteoporosis and perhaps heart disease in postmenopausal women.

Antiestrogens

The potential role of tamoxifen and related antiestrogens in the prevention of breast cancer was discussed in detail in FO, pp 823. Despite positive results emanating from the USA clinical trials, the serious side effects associated with tamoxifen have prompted many to recommend caution in prescribing this chronic treatment to healthy subjects. For instance, the National Women's Health Network, a consumer advocacy group, has expressed concern over indiscriminate use of tamoxifen by healthy women, especially prompted by direct-to-consumer (DTC) advertising, a likely event in view of current experience with other drugs with broad appeal.

However, other newer antiestrogens may offer a more favorable benefit-to-risk profile and begin to play a role as true chemoprevention agents, carrying much lower risks and, therefore, applicable to a large number of healthy women at risk for breast cancer.

Tamoxifen is the immediate beneficiary of positive results from chemoprevention trials performed in the USA and reported earlier this year (FO, pp 821-22). However, there is considered controversy as to this drug's role in the prevention of breast cancer in asymptomatic healthy women at risk for the disease. Not only the drug carries a significant risk of life-threatening side effects, but long term trials performed in Europe did not produce similar beneficial results obtained by the USA trial which many believe was terminated too soon before its long-term effects were established. Serious complications associated with long-term tamoxifen use may mitigate its benefits. Also, it has been suggested that tamoxifen may simply delay

the onset of breast cancer rather than truly affect the underlying process of tumorigenesis, so that cancer would return after the drug administration is stopped in five years, as currently recommended.

Despite the experts' reservations, anxious women are going to request treatment and physicians will prescribe it. To assist physicians in making informed decisions on tamoxifen-based chemoprevention, the NCI began distributing, in October 1998, a software program that calculates a woman's risk for breast cancer to determine if she is a candidate for tamoxifen-based chemoprevention. Zeneca is distributing the NCI software to its clients and has also developed a slide rule version. NCI plans to augment the program in 1999 to include a benefit-to-risk analysis on each patient.

Raloxifene, a selective estrogen receptor modulator (SERM) approved by the FDA and launched in January 1998 in the USA as Evista (Eli Lilly) for the prevention of osteoporosis, may also prove as affective and less toxic than tamoxifen in the prevention of breast cancer among women at high-risk for the disease (see FO, p 822). New data, reported in December 1998 at the San Antonio Breast Cancer Symposium, indicates that Evista not only prevents osteoporosis and the development of vertebral fractures in postmenopausal women, but it also reduces the risk of breast cancer. These data, an update from those presented earlier this year at the American Society of Clinical Oncology, were gathered from 10,575 postmenopausal women enrolled in 10 randomized, double-blind, placebo-controlled osteoporosis studies. Approximately 7,700 of these women are participating in the six-year Multiple Outcomes of Raloxifene Evaluation (MORE) trial, an ongoing osteoporosis treatment study (FO, p 822). The remainder are healthy postmenopausal women enrolled in osteoporosis prevention trials who were

not enrolled in these trials based on breast cancer risk. The women, who ranged in age from 31 to 80, were taking daily Evista (60 mg) for a median follow-up of 40 months and a maximum follow-up of 55 months. Overall, there was a 55% reduction in risk for all types of breast cancer among postmenopausal women taking Evista. Also, Evista did not increase endometrial cancer risks but increased the risk of blood clots at a rate similar to that encountered in estrogen replacement therapies.

Eli Lilly will continue to evaluate the cancer preventive potential of Evista in the MORE study, the Raloxifene Use for the Heart (RUTH) study, and the upcoming Study of Tamoxifen and Raloxifene (STAR). STAR, a prevention trial slated to enroll 22,000 women aged ≥ 35 years, at high risk for breast cancer, will be conducted by the National Surgical Adjuvant Breast and Bowel Project (NSABP), under the aegis of the National Cancer Institute (NCI). STAR will determine the safety and effectiveness of Evista versus tamoxifen in reducing breast cancer incidence in women at high risk. As of October 1998, the NCI had selected 193 institutions to participate in STAR to begin in early 1999.

In December 1998, Eli Lilly announced that the FDA has agreed to a new product label for Evista that incorporates the fact that the drug may reduce the incidence of breast cancer by 50% in postmenopausal women. This information may now be brought to the attention of prescribing physicians and may influence their drug choice in favor of Evista. However, it is not clear if Eli Lilly will be able to use DTC advertising to sway consumers.

Other antiestrogens in development have been discussed in FO, pp 822-23. A private British company, Kymed GB (Salisbury, Wiltshire, UK), in collaboration with University of Bradford (UK), has synthesized, by rational design, isomerically stable molecules related to tamoxifen. The company is developing a method of producing pure Z-tamoxifen isomer expected to have a more favorable side effects profile than the currently used tamoxifen preparation that contains traces of the more harmful E-tamoxifen isomer. Kymed is scaling up the manufacture of Z-tamoxifen in cooperation with Ultrafine Chemicals (Manchester, UK). In December 1998, Duramed Pharmaceuticals (Cincinnati, OH) obtained an exclusive option to marketing rights in North America in return for royalties. Kymed GB is seeking other licensees and bulk manufacturers.

TREATMENT OF EARLY DISEASE AND ADJUVANT CHEMOTHERAPY

Breast cancer recurrence is a devastating reversal for many women considered cured after early detection and tumor excision or simple mastectomy. Antiestrogen and radiation therapy is often prescribed in early-stage disease and, increasingly, polychemotherapy is becoming part of standard therapy to cure early breast cancer.

At the 6th International Conference on Adjuvant Therapy of Primary Breast Cancer, held in St. Gallen, Switzerland, in February 1998, adjuvant chemotherapy

was not recommended only in patients with a <10% chance of relapse in 10 years; the previous cut-off was <10% chance of mortality in 10 years. Various factors, such as hormone-receptor status, tumor size, histologic and nuclear grade, and age, among others, are being considered to establish risk of recurrence in node-negative breast cancer. For a comprehensive review of the conclusions at the St. Gallen meeting, see Goldhirsch A, et al, JNCI, 4 Nov 1998, 90(21):1691-1608 and Zujewski J and Liu ET, JNCI, 4 Nov 1998, 90(21):1587-1589.

Polychemotherapy

A newly published overview of results of randomized trials involving about 18,000 women, mostly under the age of 70 years, indicates that in women aged <50 years, standard polychemotherapy (CMF or CAF) extends 10-year survival for those with node-negative disease from 71% to 78% and for those with node-positive disease from 42% to 53% (Early Breast Cancer Trialists Collaborative Group, Lancet, 19 Sept 1998, 352 (9132):930-42). Among those aged 50-69 years, results were not as favorable at 69% and 49%, respectively, representing absolute gains of 2% compared to 7% and 3% compared to 11%, respectively.

In December 1998, at the San Antonio Breast Cancer conference, researchers from the Eastern Cooperative Oncology Group (ECOG) reported that the combination of docetaxel (Taxotere; Rhône-Poulenc Rorer) and doxorubicin, is highly active for the treatment of metastatic breast cancer as indicated from a clinical trial involving 54 patients with metastatic breast cancer that had spread beyond the breast to other organs of the body. Among the 57% responders, response lasted an average of at least 9 months. Side effects were manageable and only 4% of patients developed congestive heart failure. Encouraged by these results, investigators are initiating a new trial, sponsored by National Cancer Institute (E2197: Phase III Study of Adriamycin/Taxotere versus Adriamycin/Cytosan in the Adjuvant Treatment of Node Positive and High-Risk Node-Negative Breast Cancer) that will involve nearly 3,000 women with early-stage breast cancer. Earlier this year, a similar outcome was seen with the addition of paclitaxel to standard chemotherapy in node-positive early breast cancer (FO, p 825).

Despite these findings and efforts to expand the use of chemotherapy in early-stage breast cancer, many have expressed concern about exposing women with early disease who usually do well with surgery, and sometimes radiation therapy, to the rigors of polychemotherapy. Everyone agrees that what is needed is a better means of selecting those with a less favorable prognosis using some type of a reliable marker. However, currently this is not possible.

OPPORTUNITIES AND COMPETITION AMONG BREAST CANCER CHEMOTHERAPIES IN THE USA

Competition will intensify for the early-cancer treatment market that currently represents the most promising commercial opportunity. Potential markets for the various therapies are estimated in Exhibit 7.

**Exhibit 6
Schematic of State-of-the-Art in the Prevention and Treatment of Breast Cancer
and Estimates of Steady-State Markets in the USA**

| Drug/ Combination | AWP of Drug per Regimen (\$) | Populations/ Cases (#)¹ | Annual Potential USA Market (\$ mil.) | Comments |
|--|---|---|--|---|
| Prevention in Healthy Women | | | | |
| Antiestrogens | Use of antiestrogens in prevention is controversial because of a high rate of serious side effects | | | |
| Tamoxifen | 1,212 per year (101 monthly) | As many as 29,000,000 women may be at risk | 340-1,000 | Tamoxifen has a high rate of serious side effects and will be replaced by more benign alternatives; however, currently it is the only choice |
| Raloxifene (Evista; Eli Lilly) ² | 712.8 (59.40 monthly) | <i>ibid</i> | 120-500 | Evista may eventually replace Tamoxifen for this indication in postmenopausal women; also, a more attractive side effects profile and the added advantage of osteoporosis prevention may expand the number of women opting for Evista |
| Prevention of Recurrence of Treated Early Breast Cancer | | | | |
| Antiestrogens | Use of tumor antiestrogens is standard therapy | | | |
| Tamoxifen | <i>ibid</i> | 264,000 | 320 | Tamoxifen is the only drug approved by the FDA for reducing the risk of breast cancer recurrence |
| Second line/letrozole (Femara; Novartis) | 2,246 (187.2 monthly) | 141,600 | 318 | Launched in the USA in September 1998 as a once-daily oral formulation (2.5 mg) for postmenopausal women whose disease has progressed despite antiestrogen therapy |
| Treatment of Breast Cancer | | | | |
| Early Stage (I and II) Breast Cancer/Adjuvant Chemotherapy | One of the regimens below may prevail as "standard" therapy but each offer different benefits regarding cost, convenience, side effects and outcome; in all cases, however, treatment of early disease reduced recurrence rates and extended survival | | | |
| First line/anthracycline-based regimen (CAF) | 4,600 | 55,000-147,195 | 253-677 | CAF regimen produced significant improvement in 10-year survival rates and reduced recurrence rates in women <50 years-of-age |
| First line/CMF | 2,000 | 55,000-147,195 | 110.0-294.4 | CMF regimen also produced similar results at a significantly lower cost |
| First line/paclitaxel (Taxol; Bristol-Myers Squibb) | 9,600-11,500 | 55,000-147,195 | 550.0-1,472 | Use of paclitaxel as first-line monotherapy in this setting may not prove cost-effective |
| First line/paclitaxel + CAF or CMF ² | 10,000-14,000 | 55,000-147,195 | 550.0-2,060 | Adding paclitaxel to standard regimen increased survival rate by 26% and reduced recurrence rate by 22% over CAF or CMF alone |
| First line/paclitaxel + trastuzumab (Herceptin; Genentech) ² | 28,000 | 36,800 | 1,030.3 | It is expected that this regimen would be eventually approved as first line therapy of early cancer positive for HER2 overexpression; the only problem may be an 11% incidence of heart failure associated with the combination |
| Advanced (Stage III)/ Metastatic (Stage IV) Breast Cancer | The number of patients diagnosed with advanced/metastatic cancer at presentation represents only a small fraction of the total but these patients are treated with multiple courses of different chemotherapeutics. | | | |
| First line/anthracycline-based regimen (CAF) | 4,600 | 20,260 | 93.2 | This is a standard regimen; newer liposome formulations of doxorubicin may improve therapy but will also raise costs |
| First line/CMF | 2,000 | 20,260 | 40.5 | This is a standard regimen |

| | | | | |
|---|---------------|--------|-------|---|
| First line/trastuzumab + paclitaxel | 28,000 | 6,078 | 170.2 | The approved indication for advanced metastatic disease |
| First line/trastuzumab + CAF | 23,000 | 6,078 | 139.8 | An alternative to the paclitaxel combination that may prove equally effective but carries a much higher risk (28%) of heart failure |
| Second line/paclitaxel | 9,600-11,500 | 10,000 | 100.0 | Second-line indication that will compete with docetaxel |
| Second, or third line/trastuzumab | 18,400 | 6,078 | 111.8 | Regimen to be prescribed only in the case of HER2 overexpression in women who failed anthracycline-based regimens |
| Second line/docetaxel (Taxotere; Rhône-Poulenc Rorer) | 12,000-16,000 | 10,000 | 140.0 | Docetaxel trails paclitaxel in development for the breast cancer indication; new research indicates the docetaxel may be far superior when used in combination with doxorubicin |
| Third line/capecitabine (Xeloda; Hoffmann-La Roche) | 6,890 | 5,000 | 34.5 | Xeloda may advance to second-line treatment; Xeloda was deemed comparable to paclitaxel in patients who had failed anthracycline-based therapy; its oral formulation also makes it an attractive alternative. |

¹Estimated populations reflect only newly-diagnosed cases and their course; because of the introduction of aggressive treatment of early breast cancer, fewer recurrences are expected in the future, reducing the pool of candidates treated for advanced/metastatic disease (for estimates of current chemotherapy regimens see FO, p 438, Exhibit 12)

²Treatments still in development but expected to be available in the next couple of years

Antiestrogens in the Prevention of Breast Cancer in Healthy Women at High Risk

It is not clear how many healthy women are actually at high risk for breast cancer and how to identify them. However, whatever the measure, the numbers are in the millions and the opportunity is truly immense.

Despite being approved for the chemoprevention indication, Zeneca's Nolvadex will not be able to reap the benefits in the USA market because of generic competition from Barr Laboratories (Pomona, NY) that was cleared to market tamoxifen in a 1993 out-of-court settlement with Zeneca. Barr, that purchases bulk tamoxifen from Zeneca, currently claims to control 80% of the USA market. In fiscal 1998 (ending June 30), Barr reported tamoxifen revenues of \$235.7 million. It is estimated that annualized revenues were approximately \$225 million in calendar 1997 and are estimated to reach \$255 million in calendar 1998. If indeed Barr's share is 80%, then the 1998 USA market is estimated at \$318.5 million and Zeneca's final product sales in the USA at \$63.7 million. The two companies price their drugs competitively, with the average wholesale price of 30 20 mg tablets costing about \$101.

Evista with a more favorable side-effects profile has a better outlook as a chemopreventative. If its several positive effects hold over time, it may indirectly supplant tamoxifen in women anxious about being high-risk for breast cancer who also may benefit from the osteoporosis prevention attributes of Evista.

Chemotherapy in Early Disease

Because early breast cancer is the leading diagnosis, and over 70% of those diagnosed with breast cancer suffer from Stage I or II disease, the opportunity for drugs to treat early cancer is far larger than the current market addressing advanced disease. For instance, if Herceptin was to be used as first-line therapy alone or in combination with CAF, CMF or paclitaxel, in advanced breast cancer overexpressing HER2, its potential domestic market is about \$140 million. However, if this drug is used as first-line, adjuvant, or neoadjuvant therapy in combination with any of the same drugs in early cancers overexpressing HER2, then its domestic market would rise to \$677.1 million.

Adjunct Therapies

Demand for adjunct therapies, such as antiemetics and drugs to prevent/treat diarrhea and mucositis, among others, will undoubtedly rise in tandem with increased use of pharmacologic means for managing breast cancer. Opportunities will also be enhanced for chemoprotective agents, and various approaches to sensitize tumors to therapy, including agents to combat drug resistance.

INTERNATIONAL TRENDS

The management of breast cancer is also undergoing major changes in Western Europe where about 270,000 women are diagnosed with breast cancer annually. The 1st European Breast Cancer Symposium that took place in Florence, Italy in September 29-October 3, 1998, sponsored by the European Organization for Research and Treatment

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of Cancer (EORTC-BCCG), European Society of Mastology (EUSOMA), Europa Donna, and the European Breast Cancer Coalition, was designed to bring together various organizations from different countries to agree on a unified approach to combating cancer in the region. One of the important topics of the meeting was setting up well-designed, controlled multidisciplinary clinical trials on an international basis, to

better understand the impact of ethnicity in the management of breast cancer.

A newly-formed intergroup, the Breast International Group (BIG), incorporating, in addition to Europe, also South Africa, Australia and New Zealand, also plans to conduct large multicenter clinical trials to speed up development of novel therapies and agents.

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