FUTURE ONCOLOGY

TECHNOLOGY, PRODUCTS, MARKETS AND SERVICE OPPORTUNITIES

A NEW MEDICINE PUBLICATION | AUGUST 1996 | VOLUME 2, NUMBER 4

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FROM THE 43RD ANNUAL MEETING OF THE SOCIETY OF NUCLEAR MEDICINE DENVER, CO, JUNE 3-6, 1996

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STATE-OF-THE-ART MANAGEMENT OF CANCER

BREAST CANCER-PART I

EPIDEMIOLOGY

Breast cancer remains the most common malignancy and the second leading cause of cancer mortality in females in most developed countries. In the USA breast cancer is expected to account for 31% of new cancer cases and 17% of cancer deaths in females in 1996. Mortality and incidence trends in Western Europe parallel those in North America. Incidence rates in the UK, Denmark, Belgium, and Switzerland are among the highest in the world. In general, there is considerable variation in incidence in Europe, with rates being higher in northern and western regions. However, survival rates are higher in Western Europe and North America. The lowest incidence rates are reported from Eastern Europe, the former USSR, Japan, and Costa Rica. However, Eastern Europe is currently experiencing the most rapid increase in incidence rates. Some of the variations in incidence rates may be the result of lack of detecting and recording cancer.

Although incidence of breast cancer is lower among African Americans, age-adjusted mortality rates are higher (27/100,000 in other races versus 35/100,000 in African-Americans) because of the more advanced stage at diagnosis and lower relative survival rates. Based on results from the Black/White Cancer Survival Study (BWCSS), researchers at the National Cancer Institute (Bethesda, MD) have estimated that the risk of dying from breast cancer was 2.1 times greater in African Americans. They also report that 40% of racial differences in survival rates could be explained by more advanced stage at diagnosis among African-American women and 15% depended on histologic differences (tumor grade). Other factors included differences in body mass index. However, one of the most probable explanations for the lower incidence and higher mortality associated with breast cancer in African-American women is lower socioeconomic status, limited access to healthcare and late diagnosis of cancer. For instance, risk of death was 2.3 times higher among women without medical insurance than those with private medical insurance. Other studies have shown that factors such as limited access to health care and screening are strong determinants of survival.

Over 632,000 women are diagnosed with breast cancer annually in North America, Europe and Japan (see Exhibit 1). In the USA 184,300 women will be diagnosed with breast cancer in 1996. Breast cancer incidence increased in the USA in the early 1980s, mostly because implementation of large scale screening identified many women with early asymptomatic breast cancer who may have never developed full blown disease in their lifetime. Similarly to experience with prostate cancer, these higher incidence rates abated in recent years in the USA but continued to rise worldwide, particularly among post-menopausal women.

Although increases in breast cancer incidence in the USA and abroad may also be artifactual, reflecting aggressive mass screening programs in many Western nations, it is unlikely that screening alone explains the upsurge in incidence rates. Other factors such as the aging populations worldwide may also be relevant.

Unlike incidence, breast cancer mortality rates have remained stable and even declined in North America and Western Europe in recent years. In the USA, overall estimated crude mortality rate of breast cancer, estimated to claim 44,300 lives in 1996, represents a 4.5% decrease from 1995 levels. Similarly, in Canada the mortality rate has decreased by 3%. The most important contributing factor to lower mortality rates are mammography screening programs that have resulted in detection of breast cancer in early and curable stages. Additionally, improvements in treatment, including the use of adjuvant chemotherapy in advanced disease, has resulted in higher survival rates. In the USA today, the overall five-year survival rate for all stages is 83%. Unfortunately, mortality rates in many nations, particularly in Eastern Europe, are still on the rise. Even in the USA, age-adjusted breast cancer mortality in African-American women is significantly higher than overall rates.

PATHOGENESIS OF BREAST CANCER

Most genetic, epidemiologic, and laboratory studies support a stochastic model of breast cancer carcinogenesis, in which a series of genetic changes contribute to the process. The effects of these genetic changes are cumulative and result in phenotypic changes associated with the evolution of malignancy. Both mutagenic (initiation and/or development of genetic changes) and mitogenic (proliferation of cells) processes contribute to the development of breast cancer.

Each breast consists of about 15 to 20 lobes, which are further divided into sections called lobules. Lobes and lobules are connected by ducts. Cancer in ductal cells is the most common type of breast cancer. Lobular carcinoma that begins in the lobes or lobules is more often found in both breasts. Inflammatory breast cancer, in which the breast is warm, red, and swollen, is rare. Incidence and mortality statistics presented in Exhibit 1 are for invasive carcinoma. Another common breast malignancy, ductal carcinoma *in situ* (DCIS), which accounts for about 13% of all cases, is difficult to distinguish from the more severe forms of invasive breast cancer. Incidence of DCIS has been rising in the USA because of aggressive screening programs (see Exhibit 2).

Little is known of the pathogenesis of breast cancer. One model assumes a progression from aberrant ductal or lobular hyperplasia to dysplasia to frank invasive cancer. However, histologically-confirmed hyperplasia is

associated with only 1.6-fold increase in lifetime risk of developing breast cancer and presence of DCIS is associated with a cumulative risk of 0.8% to 1% per year.

BREAST CANCER ETIOLOGY AND RISK FACTORS

The National Cancer Institute estimates that a woman's lifetime risk (probability) of developing breast cancer is 1 in 8 (see Exhibit 3). This probability is higher when certain risk factors are present. However, except for age, family history and high-dose ionizing radiation, studies have rarely shown a consistent association between other risk factors and breast cancer. Although many breast cancer risk factors have been proposed, their mode of action has not been scientifically explained. In addition to hormonal influences, risk factors studied range from the commonsensical (diet, smoking, age, various mutagenic factors such as radiation and occupational exposures, etc.) to the far fetched (abortion, breast feeding, etc.) to the absurd (use of condoms; it appears that some chemical in semen confers protection against breast cancer). However, in spite of extensive retrospective studies of relatively large populations, no risk factor has emerged that can be directly linked to increased risk for developing breast cancer. Several factors including diet, weight, and differences in hormonal activity may be linked to international variations in breast cancer risk but none can be categorically singled out as a definite risk factor. Like with many other neoplasms, breast cancer appears to emerge when sev-

Exhibit 1
Incidence and Mortality of Female Breast Cancer in Selected World Regions in 1995

Country	Incidence (#)	Rate[1]	Mortality (#)	Rate[1]
Belgium	8,216	159.3	2,347	45.5
Denmark	4,559	174.0	1,303	49.7
France	36,918	124.3	10,548	35.5
Germany	65,072	155.8	18,592	44.5
Greece	3,647	68.8	1,459	27.5
Ireland	2,285	128.5	653	36.7
Italy	39,591	134.8	11,312	38.5
Luxembourg	313	151.9	89	43.4
The Netherlands	12,300	157.2	3,514	44.9
Portugal	3,682	72.5	1,473	29.0
Spain	15,476	76.8	5,542	27.5
United Kingdom	54,399	182.7	15,543	52.2
Total Western Europe - EEC	246,459	137.9	72,374	40.5
Austria	5,867	143.9	1,745	42.8
Finland	2,910	111.1	831	31.7
Iceland	151	112.6	47	35.2
Malta	132	71.2	91	49.1
Norway	2,545	116.2	782	35.7
Sweden	5,572	125.7	1,534	34.6
Switzerland	5,769	158.8	1,682	46.3
Total Western Europe - non EEC	22,946	132.8	6,712	38.9
Bulgaria	3,019	67.5	1,208	27.0
Czechoslovakia	6,674	83.2	3,240	40.4
Hungary	4,083	77.4	2,552	48.4
Poland	11,668	59.3	4,841	24.6
Romania	6,318	54.5	2,605	22.5
Yugoslavia	4,713	73.0	2,892	44.8
Total Eastern Europe[2]	36,475	65.7	17,337	31.2
Europe Total[2]	305,880	121.6	96,424	38.3
Total Former USSR	99,163	65.9	35,466	23.6
Argentina	13,137	74.6	4,948	28.1
Australia	8,488	93.7	2,509	27.7
Chile	2,946	40.8	816	11.3
Costa Rica	549	32.6	133	7.9
Cuba	2,710	50.1	865	16.0
Hong Kong	1,253	43.7	333	11.6
Israel	2,305	81.2	960	33.8
Japan	24,902	39.1	6,815	10.7
New Zealand	1,764	97.4	610	33.7
Singapore	606	43.1	200	14.2
Uruguay	1,641	100.5	599	36.7
Others Total	60,302	52.3	18,788	16.3
United States[3]	184,300	136.8	44,300	32.9

— continued on next page

Other Race	167,700	143.0	39,100	33.3
Blacks	16,600	95.4	5,200	29.9
Canada[3]	18,600	123.1	5,300	35.1
Total North America[3]	202,900	135.4	49,600	33.1
Triad (Europe[2], Japan, North America)	533,682	114.8	152,839	32.9
TOTAL	668,245	100.2	200,278	30.0

[1]Per 100,000 population [2]Excluding the former USSR [3]Estimates are for 1996

eral endogenous and exogenous factors interact in a highly specific manner. This multifactorial origin of breast cancer produces conflicting results depending on the profile of the population being studied. The potential permutations and combinations boggle the mind and invariably always confuse interpretation of the results. Identifying the conditions that lead to breast cancer is probably a task that should be assigned to molecular epidemiology. Using accurate molecular profiles of statistically significant numbers of women, combined with closely monitored environmental conditions, it may be possible to conduct prospective studies over long periods of time that will pinpoint the origins of breast cancer.

Exhibit 2
Incidence of Breast Cancer *in Situ* in Selected World Regions in 1995

Country	Incidence (#)
USA[1]	28,290
North America[1]	31,152
Europe[2]	46,831
Japan	3,824
Triad (Europe[2], Japan, North America[1])	81,807
Total	102,467

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

Age

Age is the most important etiologic factor for breast cancer. The risk of developing breast cancer increases with increasing age, especially after age 45 (see Exhibit 4). This is obvious by the fact that less than 5% of all breast cancer cases occur in women under 35 years of age. However, age is a risk factor in many solid malignancies. After all, cancer is a disease of the aged.

Hormonal Risk Factors

Among the leading putative mitogenic factors are reproductive history which includes early menarche, late first pregnancy, low parity or nulliparity, and late menopause. Increased risk has also been associated with hormonal use (oral contraceptive, estrogen therapy). These risk factors are associated with hormonal changes which appears reasonable in a cancer that is hormonally modulated. However, instead of listing the above events as contributors to carcinogenesis in breast cancer it would make far more sense to study the role of hormones in breast cancer by linking hormone levels, changes in hormone release and variations between the balance of the various sex hormones to the risk of developing breast cancer.

The role of hormonal regulation in the development of breast cancer in not well defined. It is believed that total exposure to hormones increases the risk of breast cancer. The observation that mitosis increases during the luteal phase of the menstrual cycle suggests that estrogen may act as a primary stimulant for the growth of breast tumor cells and progesterone may increase this proliferation. Thus, early menarche (before age 12), late menopause (after age 55), estrogen replacement therapy, and use of oral contraceptives are all considered contributors to increased risks because they prolong exposure to estrogen. For instance, women who undergo bilateral oophorectomy have reduced risks of developing breast cancer. Although pregnancy itself increases the short-term risk of breast cancer (due to a rapid increase in estradiol levels in the first trimester), early full-term pregnancy (before age 20) is associated with a long-term reduction in estradiol levels which decreases the risk. Additional pregnancies further reduce this risk. For the same reason, nulliparity and late age at first full-term pregnancy may increase the risk of breast cancer.

Many studies of the effects of hormones on breast cancer development have been contradictory, particularly for risks related to oral contraceptive (OC) use, estrogen replacement therapy (ERT), and pregnancy. The link between OC use and breast cancer remains obscure. At various times different studies produced conflicting findings. It has been proposed that earlier formulations of OC which incorporated higher doses of estrogen may be responsible for findings that breast cancer risk rises with increasing duration of OC use in premenopausal women. However, based on a substantial number of studies, OCs do not appear to be associated with an increased risk for breast cancer. Monophasic OCs contain a constant dose of estrogen (mestranol or ethinyl estradiol) and progestin. The numerous different products on the market incorporate various combinations of these hormones. Currently about 60 million women use OCs worldwide (10 million in the USA).

Hormonal replacement therapy (HRT) has also been investigated as a risk factor in breast cancer. Some epi-

Exhibit 3
Probability of Developing Invasive Breast Cancer Over 10-year Time Intervals

Current age (years)	Risk over intervals in years (%)				
	10	20	30	40	Lifetime
30	0.43	1.99	4.29	7.47	12.80 (1 in 8)
40	1.58	3.91	7.13	10.28	12.53 (1 in 8)
50	2.41	5.74	9.01	0.83	11.33 (1 in 9)
60	3.59	7.10	9.07		9.62 (1 in 10)
70	4.13	6.45			7.08 (1 in 14)

demiologic studies have shown that long-term use of estrogen replacement therapy is associated with an increased risk of breast cancer of about 2-3% per year of use. Results from a population-based prospective study in Sweden showed a 70% increase in risk after 10 years of estrogen HRT use (NEJM 1989; 321:293). However, other large epidemiologic studies found no such association. Several large studies, designed to account for many of the methodologic issues of prior studies of hormonal use and breast cancer risk, are ongoing.

Inherited Breast Cancer

Genetic risk factors are believed to be more important in pre-menopausal disease. A family history of breast cancer increases the risk of developing breast cancer. This risk is highest for first-degree relatives of women with bilateral breast cancer that occurred in pre-menopausal years. Known genetic syndromes are believed to be responsible for approximately 5-10 % of all breast cancers. Among genes associated with hereditary breast cancer are BRCA1 (breast cancer 1) and BRCA2 (breast cancer 2) and ATM which when present in a homozygous mutated stage causes ataxia telangiectasia. (Additional information on applications of markers in the diagnosis and therapy of breast cancer will be discussed in subsequent articles in this multi-part series on breast cancer).

ATM gene may be implicated in inherited breast cancer as indicated by preliminary studies that found ATM heterozygotes to be at increased risk of developing solid tumors, particularly breast cancer. ATM appears to be involved in cell cycle regulation and determination of telomere length.

BRCA1 gene was mapped on chromosome 17q21.1 in December 1990 and isolated in October 1994. Mutations in the BRCA1 gene were first discovered by Myriad Genetics (Salt Lake City, UT) in collaboration with many other groups and the National Institute of Environmental Health Sciences (NIEHS; Research Triangle Park, NC). In April 1992 Myriad Genetics entered into a research collaboration and licensing agreement with Eli Lilly and its then subsidiary Hybritech (San Diego, CA), since aquired by Beckman Instruments (Fullerton, CA), pursuant to which the latter made an equity investment in Myriad Genetics and funded R&D for three years to discover and sequence the BRCA1 gene. Eli Lilly (Indianapolis, IN) retains exclusive rights to commercialize therapeutic products based on BRCA1. Hybritech decided not to continue internal development of diagnostics based on BRCA1 gene mutations but intends to continue making milestone payments based on an amended collaborative agreement signed in March 1992 and may sublicense the rights to the technology to a third party. Myriad Genetics has licensed exclusive worldwide rights to BRCA1 gene-related technologies from the University of Utah in October 1991. In May 1996, Eli Lilly extended its collaboration with Onyx Pharmaceuticals (Richmond, CA) to identify proteins that interact with BRCA1, define specific pathways involved in the role of this gene in tumor progression and select targets for therapeutic intervention. Onyx will conduct a one-year feasibility program funded by Eli Lilly which will also make a small investment in Onyx and pay royalties on any products that may emerge from this collaboration.

BRCA1 gene comprises 24 exons and encodes a 1,863-amino-acid protein. Over 100 mutations in BRCA1 have been identified in families with many members suffering from breast and ovarian cancer. It has been estimated that alterations of BRCA1 account for about half the inherited breast cancers or 5% of all cases of breast cancer (see Exhibit 5). The most common BRCA1 alteration is a frameshift mutation at position 185 caused by deletion of adenine and guanine (185delAG). Current estimates of the frequency of the BRCA1 mutation in the USA ranges from 1/300 to 1/800 women. The mutation is believed to occur in approximately 1% of Ashkenazi Jewish women (over 90% of the total Jewish population in the USA are Ashkenazi). A survey of 5,000 Ashkenazi Jewish women in the Washington, DC area is underway to establish the validity of this finding.

Among women with the BRCA1 mutation, the probability of developing breast cancer has been estimated to be more than 50% by age 50 and 85% by age 70. Women who are heterozygous for mutations in the BRCA1 gene have an 85% life time risk of developing breast cancer and a 63% risk of developing ovarian cancer. By age 60, approximately 77% of women who carry certain mutations of BRCA1 gene would develop breast cancer as compared to 4% of women in the general population.

BRCA1 is a tumor suppressor gene, as indicated by the fact that it is most often absent in breast tumors.

Exhibit 4
Age-Specific Incidence of Female Breast Cancer in Selected World Regions

	20-29		30-39		40-49		50-59	
Country	**Incidence (#)**	**Rate[1]**	**Incidence (#)**	**Rate[1]**	**Incidence (#)**	**Rate[1]**	**Incidence (#)**	**Rate[1]**
Western Europe - EEC	1,146	4.8	12,547	46.9	43,241	184.4	47,383	232.7
Western Europe - non-EEC	93	3.9	1,072	41.9	3,767	157.5	4,187	214.7
Eastern Europe[2]	157	2.0	1,751	23.1	6,091	76.6	6,383	108.5
Total Europe	1,396	4.1	15,370	41.7	53,099	157.1	57,953	205.6
Former USSR	426	2.1	4,760	20.4	16,560	86.2	17,354	110.0
Japan	107	1.2	1,195	15.2	4,159	42.6	4,358	79.1
United States[3]	510	2.7	8,700	39.5	33,400	174.0	30,900	241.6
Canada[3]	80	3.8	900	34.4	3,100	136.9	3,500	233.0
Total North America[3]	590	2.8	9,600	38.9	36,500	170.1	34,400	240.7
Triad (Europe[2], Japan, North America)	2,093	3.3	26,165	37.7	93,758	144.2	96,711	201.5

	60-69		70-79		80+		≥ 50	
Country	**Incidence (#)**	**Rate[1]**	**Incidence (#)**	**Rate[1]**	**Incidence (#)**	**Rate[1]**	**Incidence (#)**	**Rate[1]**
Western Europe - EEC	56,883	303.6	54,444	388.1	28,801	327.9	187,511	302.9
Western Europe - non-EEC	5,620	345.2	4,945	341.0	3,186	361.2	17,938	303.5
Eastern Europe[2]	8,608	148.0	8,353	243.3	4,669	298.3	28,013	167.8
Total Europe	71,111	271.6	67,742	358.2	36,656	326.4	233,462	276.2
Former USSR	23,402	150.8	22,708	253.4	12,693	278.7	76,157	169.9
Japan	5,877	81.6	5,703	121.9	3,187	133.6	19,125	96.7
United States[3]	40,000	375.0	44,700	494.6	26,000	480.9	141,600	373.6
Canada[3]	4,400	363.9	4,300	446.5	2,200	397.8	14,400	340.7
Total North America[3]	44,400	373.8	49,000	490.0	28,200	473.2	156,000	370.3
Triad (Europe[2], Japan, North America)	121,388	268.2	122,444	364.5	68,043	347.6	408,586	279.1

[1]Per 100,000 population [2]Excluding the former USSR [3]Estimates are for 1996

BRCA1 functions as a growth inhibitory gene; loss of the protein encoded by BRCA1 appears to contribute to the development of breast cancer. Also, the location of the protein may determine its role in breast cancer. Researchers from the University of Texas Health Science Center (San Antonio, TX) reported that BRCA1 protein is found in the nucleus of normal breast cells but in the cytoplasm of tumor cells (Chen Y, etal, Science, 3 Nov 95, 270:789-791). Scientists at Vanderbilt School of Medicine (Nashville, TN) discovered that the protein encoded by BRCA1 exhibits sequence homology and biochemical analogy to the granin protein family. Interestingly both granins and BRCA1 are responsive to hormones. Also, both are secreted by a regulated pathway; thus, BRCA1 acts through a pathway not previously associated with tumor suppressor gene products (Jensen, RA, etal, Nature Genetics, 1996 March, 12(3):303-8). Despite rapidly accumulating knowledge, the normal function of the BRCA1 gene has yet to be elucidated. Scientists at NIH's National Center of Human Genome

Research (NCHGR; Bethesda, MD) have developed a mouse knockout model to study the function of BRCA1.

BRCA2, a second breast cancer susceptibility gene, was detected on chromosome 13q12-13. BRCA2 encodes a 2339-amino-acid protein. Mutations in BRCA2 are believed to be responsible for a third of inherited breast cancers, but this gene, unlike BRCA1, does not seem to be associated with ovarian cancer. Discovery of BRCA2 was announced in December 1995 in Nature by scientists at the Institute of Cancer Research (ICR; Sutton, UK) and Duke University School of Medicine (Durham, NC). CRC Technology (London, UK), the commercial arm of Cancer Research Campaign (London, UK) which funded the BRCA2 research, filed for a patent in the UK. Myriad Genetics, in collaboration with scientists at Memorial Sloan-Kettering Cancer Center (New York, NY), the University of Utah (Salt Lake City, UT) and the International Agency for Research on Cancer reported that Jewish women of Eastern European decent have a significantly higher risk of carrying the BRCA2 mutation associated with breast cancer. In November 1994, Myriad Genetics obtained an exclusive license from the University of Utah to commercialize discoveries associated with BRCA2.

Dietary, Environmental and Other Factors

Other risk factors usually associated with most cancers, such as diet, environmental exposure and socioeconomic status, have not

Exhibit 5
Estimated Incidence of Breast Cancer Cases Caused by BRCA1 Mutations and Prevalence of BRCA1 Mutations in Selected World Populations

Country	BRCA1-related Breast Cancer Incidence[1] (#)	BRCA1 mutation Prevalence (#)	
		Low	High
Belgium	205	3,224	17,197
Denmark	114	1,638	8,737
France	923	18,571	99,043
Germany	1,627	26,113	139,267
Greece	91	3,316	17,683
Ireland	57	1,112	5,930
Italy	990	18,363	97,937
Luxembourg	8	129	687
The Netherlands	308	4,892	26,090
Portugal	92	3,174	16,930
Spain	387	12,594	67,170
United Kingdom	1,360	18,609	99,250
Total Western Europe - EEC	6,161	111,735	595,920
Austria	147	2,548	13,590
Finland	73	1,638	8,737
Iceland	4	84	447
Malta	3	116	620
Norway	64	1,369	7,300
Sweden	139	2,771	14,777
Switzerland	144	2,271	12,110
Total Western Europe - non EEC	574	10,796	57,580
Bulgaria	75	2,796	14,910
Czechoslovakia	167	5,012	26,730
Hungary	102	3,295	17,573
Poland	292	12,308	65,640
Romania	158	7,240	38,613
Yugoslavia	118	4,038	21,533
Total Eastern Europe[2]	912	34,688	185,000
Europe Total[2]	7,647	157,219	838,500
Total Former USSR	2,479	94,101	501,870
Argentina	328	11,006	58,700
Australia	212	5,662	30,197
Chile	74	4,513	24,067
Costa Rica	14	1,053	5,617
Cuba	68	3,381	18,030
Hong Kong	31	1,792	9,557
Israel	58	9,463	28,390
Japan	623	39,806	212,297
New Zealand	44	1,132	6,037
Singapore	15	879	4,690
Uruguay	41	1,021	5,443
United States[3]	4,608	168,366	448,977
Canada[3]	465	18,884	50,357
Total North America[3]	5,073	187,250	499,333
Triad (Europe[2], Japan, North America)	13,342	384,274	1,550,130

[1]Based on a rate of 2.5% of all cases [2]Excluding the former USSR [3]Estimates for 1996

been categorically shown to contribute to breast carcinogenesis. The relationship between obesity and elevated breast cancer risk appears to be limited to post-menopausal women. This relationship was not shown to be valid in pre-menopausal women and, in fact, a few studies have found a reverse effect, i.e., increased weight is associated with reducted risk. These studies appear to lack rigorous cause and effect assumptions; it should be expected that because breast cancer is more common in older women who also tend to be obese, there would be a link between obesity and breast cancer in post-menopausal women.

High intake of dietary fat has also been linked to breast cancer development. It has been shown that post-menopausal women with less than 20% daily fat intake have lower serum estradiol levels than those with a higher dietary fat consumption. A comparison of diets of 2,569 breast cancer patients and 2,588 controls did not find a correlation between diets high in fat and breast cancer (Lancet, 1996, 347:1351-1356). Conversely, low intake of fat in combination with exercise and a diet high in fiber, appear to be protective, but this also has not been well established. Ecological studies have shown a positive correlation between breast cancer mortality and consumption of dietary fat but case-control studies have not shown this to be true. Other evidence for the diet hypothesis has been supplied by migration studies which have found that migrants have similar incidence rates to that in the country of origin, but succeeding generations acquire the risks of the host country.

Among mutagenic factors, exposure to high-dose ionizing radiation has been consistently associated to an excess in breast cancer risk as indicated by a higher incidence of breast cancer among female Japanese atomic bomb survivors. However, risk associated with low-dose radiation is small although certain populations, such as ATM heterozygotes, may be at increased risk. A study of 105,382 radiologic technologists did not show increased risk for breast cancer (Boise JD, etal, JAMA, 1995, 274:394-401). Studies of the effects of occupational and environmental exposure on breast cancer risk have not been consistent. Also, alcohol consumption, even in moderate amount, has been shown to increase breast cancer risk.

Molecular Markers

In view of the lack of a convincing culprit in the etiology of breast cancer and the unreliability of risk factors, early diagnosis using either mammography or even better, molecular markers that indicate susceptibility to cancer, may be the only means of identifying women with a true likelihood that they may develop a life-threatening disease.

Next issue: Screening, diagnosis, staging, prognosis and monitoring of breast cancer, including worldwide populations by stage; markers, genetic testing, etc.

MEETING COVERAGE

RECENT DEVELOPMENTS IN NUCLEAR MEDICINE

FROM THE 43RD ANNUAL MEETING OF THE SOCIETY OF NUCLEAR MEDICINE DENVER, CO, JUNE 3-6, 1996

The role of nuclear medicine in cancer diagnosis and monitoring is rapidly expanding for all major indications. In an unprecedented situation, three *in vivo* nuclear imaging agents were recommended for approval within six months of each other and two of them were subsequently approved for marketing, one in July and the other in August 1996 (see Exhibit 6). Several others are in late stages of development. Many other technique refinements are being introduced to improve diagnosis and staging of cancer, including expanded use of positron emission tomography (PET) and dual-head single photon emission computed tomography (SPECT).

COINCIDENCE IMAGING AND EXPANDED USE OF FDG

FDG (fluorine-18 deoxyglucose) is one of the primary positron emitters used in PET studies (for a more detailed discussion of FDG and its role in tumor imaging, see FO, V1 #6, pp 164-165). Within the past year, nuclear equipment manufacturers have been successful in adapting PET coincidence imaging techniques to conventional SPECT nuclear cameras. This was facilitated by the availability of more effective digital electronic components and more powerful computers capable of the necessary 3D reconstruction within a reasonable imaging time. In the past, use of full-scale PET systems was limited because of their high cost and the fact that the procedure was not generally reimbursed. Availability of a relatively low-cost coincidence imaging technique, using a conventional nuclear camera, has added an entire new capability to nuclear medicine making it possible to produce good quality clinical images, particularly in whole-body imaging for the detection and staging of cancer. Some manufacturers also believe that coincidence imaging may produce better resolution in cardiac applications than possible with conventional techniques using thallium, but this has not been confirmed in a rigorous manner. At this point it appears that the primary application of coincidence imaging will be in cancer with cardiology assuming a lesser role.

ADAC Laboratories' MCD Camera

ADAC Laboratories (Milpitas, CA), whose Molecular Coincidence Detection (MCD) camera received FDA approval in December 1995, is the only manufacturer with 510(k) approval. ADAC is currently gathering clinical data to finalize the design of the MCD camera and properly position it on the market. In evaluating the MCD

Exhibit 6
Radioimmunoconjugates in Diagnostic Imaging-Approved or Filed for Approval

Developer/ Collaborator/ Affiliate	Generic Name/ Number/ Brand Name	Drug Type/ Mechanism/Target	Status/Location	Comments
Biomira	Tru-Scint AD	^{99m}Tc-labeled MAb-170	NDS (5/96)/Canada/ recurrent breast cancer and primary, residual or recurrent ovarian cancer	Submission is based on phase II Canadian clinical trials; in phase II/III clinical trials in the USA in recurrent breast cancer
Cytogen/Faulding and CISbio (marketing and distribution outside the USA).	OncoScint CR/OV	^{111}I-labeled B72.3 MAb	Approved (12/92)/USA/ colorectal and ovarian cancer; phase II (12/95)/ USA/breast cancer	In November 1995 the FDA approved repeat administration of OncoScint
Cytogen/C. R. Bard (co-marketing rights)	Capromab pendetide (CYT--356)ProstaScint	^{111}I-labeled Mab/ recognizes prostate specific membrane antigen (PSMA)	Rec (7/96) USA/ detection of occult metastatic prostate cancer	
Immunomedics/ Mallinckrodt Group (marketing USA, Europe)	Arcitumomab/CEA-Scan (was ImmuRAID-CEA)	^{99}mTc-labeled Fab' fragment of murine anti-CEA IMMU-4/ targets CEA	Approved (7/96)/USA; prereg/Europe, Canada/ colorectal cancer	
Neoprobe/Syncor (USA), Nordion (Europe, Africa and the Middle East)	RIGScan CR-49	^{125}I-labeled CC49 MAb/targets tumor-associated antigen TAG-72	Phase III (c4/96)/USA/ colorectal cancer; filed (5/96)/Europe/ colorectal cancer	Under review in anticipation of an imminent PLA filing with the FDA
NeoRx/DuPont Merck Pharmaceutical (NA marketing); Boehringer Ingelheim (marketing outside NA)	Nofetumomab merpentan/NR-LU-10/ Verluma (formerly OncoTrac)	^{99m}Tc-labeled Fab' fragment of NR-LU-10 MAb	PLA (3/94); rec (14/12); A (8/96)/USA/sclc staging	Boehringer Ingelheim's subsidiary, Dr. Karl Thomae, will manufacture the product for WW use

camera, the protocol used by ADAC provided a basis of comparison between standard PET, which was performed first, followed by coincidence imaging using the MCD camera. A 10 mCi dose of FDG was administered initially for the PET study and allowed to decay to about 3 mCi prior to the MCD study. ADAC found that the 3 mCi saturation level of the MCD system is adequate for imaging. The MCD camera, in order to improve imaging sensitivity, uses a thicker scintillation crystal than that employed in conventional nuclear cameras. Electronic filters were also adapted to recover resolution that is otherwise lost. Because of the camera configuration, there is better resolution along the center line (6 mm) of the detector than at the outer edge (8 mm). Although anatomic resolution is not as good as PET, it is acceptable for certain types of studies.

ADAC entered into an alliance with P.E.T.Net Pharmaceutical Services (Atlanta, GA) to promote MCD by offering up to a year's worth of free FDG to MCD buyers. This promotion is aimed at familiarizing potential users with this technique, expanding use of PET imaging and convincing payors and managed care organizations of its cost-effectiveness. Currently, only special applications of PET are reimbursed by some insurance carriers and none by Medicare.

Other companies with nuclear medicine capabilities such as Elscint (Hackensack, NJ), Picker International (Highland Heights, OH) and Toshiba America Medical Systems (Tustin, CA), are also developing systems that take advantage of the unique imaging abilities of PET and FDG.

Hot Spot Imaging

"Hot spot" imaging for the detection of tumors has been the focus of whole-body studies using FDG, because tumor uptake of FDG is 5-15 times higher than that of technetium, yielding a high contrast ratio. FDG gets dephosphorylated by an enzyme (phosphorylase) which is present in certain tissue, such as tumors. As a consequence of this metabolic reaction, it enters and remains in tumor cells. However, not all tumors exhibit high concentration of this enzyme. For instance, brain tumors have lower concentration of phosphorylase and, therefore, a larger radiation dose (in the 10 mCi range) is necessary to image these tumors. It may be that brain imaging with coincidence imaging would not provide adequate

resolution to compete with PET. However, tumors elsewhere in the body exhibit a high uptake of FDG.

Staging of Cancer

An important application of FDG-based whole-body imaging is cancer staging (for more information on whole-body imaging and cancer staging see FO, V #6, p 65). A number of outcomes studies have been performed that demonstrated the cost effectiveness of staging. It is hoped that these findings will provide the rationale for consistent reimbursement of coincidence imaging as well as conventional PET imaging.

BREAST SCINTIGRAPHY TO DIFFERENTIATE BENIGN FROM MALIGNANT LESIONS

Although mammography is very sensitive in detecting breast abnormalities, it often cannot accurately differentiate between benign and malignant disease, exposing women to needless biopsies; 75% of the women who undergo biopsy have benign lesions. Also, 33% of mammograms are uninterpretable. Another serious limitation of conventional modalities is imaging the 25% of adult women with radiologically dense breasts. Use of nuclear medicine techniques may improve diagnostic sensitivity of breast imaging, particularly in women with dense breasts or scarring from previous interventions. Despite recent improvements in mammographic equipment and techniques, radiologically dense breasts remain difficult to image and evaluate allowing many cancers to escape detection.

In the past several years, one agent, technetium sestamibi, sold as Cardiolite for cardiac imaging by DuPont Merck Pharmaceutical (North Billerica, MA), has been extensively evaluated in scintimammography under the trade name Reluma (see FO, V1 #6, pp 165-166 and #12, p 278). An important objective was to determine the sensitivity and specificity of Reluma in distinguishing malignant from benign lesions. Based on results from one of the largest multi-center clinical trials of scintimammography involving over 1,000 patients, sponsored by DuPont Merck and coordinated by UCLA (Los Angeles, CA), investigators concluded that compared to conventional mammography, scintimammography has a high sensitivity and specificity in the characterization of carcinoma of the breast. Furthermore, scintimammography resulted in correctly distinguishing benign from malignant lesions in patients with indeterminate mammographic results and may be used as an adjunct to mammography. Although scintimammography will not replace conventional mammography, there are a number of important niches it can effectively address.

CANCER IMAGING WITH MONOCLONAL ANTIBODIES AND PEPTIDES

Considerable research efforts invested in the development of monoclonal antibodies (MAbs) for cancer imaging, appear to have paid off with the imminent market launch of several new products. Research in peptide tracers has also progressed significantly and, in addition to products already on the market, a number of companies are developing novel indium- and technetium-labeled agents for the detection of endocrine tumors that will challenge currently used techniques. For a review of this area, see FO, V1 #6, pp 164-167 and #12, pp 276-279.

Monoclonal Antibody-based Agents

The first MAb-based diagnostic radiopharmaceutical, OncoScint CR/OV developed by Cytogen (Princeton, NJ), was approved in 1992 (see FO, V1 #2/3, pp 39, 71-72, #12, pp 277-278). This indium-labeled agent allows detection and presurgical staging of colorectal and ovarian cancer using tumor-specific MAbs. Immunoscintigraphy with these MAbs has the added benefit of evaluating the entire body simultaneously to detect primary tumors as well as metastatic spread and occult disease. Such scanning is particularly specific in identifying locoregional recurrences and metastases to the lymph nodes, which represents a significant advantage over computed tomography (CT). Moreover, used together, radiolabeled MAbs and CT are better at detecting tumors than either method used alone. Two other MAb-based imaging agents were also approved recently (see Exhibit 6) and several are in late stages of development.

Antisoma (London, UK), in addition to developing AS 109, a technetium-labeled peptide for the diagnosis of breast and gastrointestinal cancer, is working on modifications of peptides to increase their residence time for therapeutic applications. Because peptides do not remain in the tumor long enough to be effective as therapeutics, nor do they have the same specificity as the parent antibody, Antisoma is developing cancer therapeutics using MAbs. In conjunction with Hammersmith Hospital (London, UK), the company is also developing a method whereby antisense oligonucleotides are linked to radionuclides and targeted to cancer cells by MAbs. Antisoma also licensed a MAb, AS114, from Scripps Research Institute (La Jolla, CA) that targets squamous cell carcinoma for the treatment of head and neck cancer.

Biomira (Edmonton, Alberta, Canada) announced in May 1996 that it proceeded with a Canadian New Drug Submission (NDS) for Tru-Scint AD imaging agent for the detection of recurrent breast cancer and of primary, residual or recurrent ovarian cancer, based on data from phase II clinical trials. The Canadian Health Protection Branch (HPB) has confirmed that the product qualifies for fast-track status. Tru-Scint AD is currently in phase II/III clinical trials in the USA for the detection of recurrent breast cancer and a phase III protocol in ovarian cancer is under development (see FO, V1 #12, p 277).

Cytogen has developed ProstaScint (capromab pendetide), an imaging agent based on a MAb that targets prostate specific antigen (PSA) which is expressed on the surface of malignant prostate tumors, wherever they are in the body. The MAb is labeled with indium-111 using the

same chemistry as OncoScint CR/OV. Imaging with ProstaScint determines both the extent and location of the cancer. There is a definite medical need for an effective agent to assess the degree of spread of prostate cancer in patients with metastatic or recurrent disease. Because of inadequacies of currently used non-invasive diagnostic studies, many patients with prostate cancer must undergo surgical staging to determine the presence or absence of lymph node metastases. Because surgical intervention is only recommended in early stages of the disease, accurate preoperative evaluation of patients with prostate carcinoma is critical in identifying suitable prostatectomy candidates. Currently, there are no reliable imaging modalities for the detection of early prostate metastases, particularly lymphatic involvement. CT and MRI are of limited use in the detection of lymphatic invasion. Although both radionuclide bone scans and MRI exhibit fairly high sensitivity for detecting bony metastases, bone scans are not specific enough, and MRI is impractical for surveying the entire skeleton at one time. It is here that MAb-based imaging will play a role in the detection of lymph node involvement as well as skeletal and other visceral metastases, with a single scan.

Cytogen submitted a PLA in March 1995 for ProstaScint after completing phase III trials which were conducted in over 30 medical centers in the USA. On July 22, 1996, FDA's Medical Imaging Drug Advisory Committee (MIDAC) unanimously recommended the approval of ProstaScint for use in prostate cancer patients in whom there is a high clinical suspicion of occult metastatic disease. Cytogen believes this indication will provide both newly diagnosed patients and those with recurrent disease access to ProstaScint. In clinical trials, ProstaScint imaging was effective in detecting both presurgical and recurrent prostate cancer. At Cytogen's suggestion, MIDAC recommended that the information provided by ProstaScint should be considered in conjunction with other available diagnostic information. ProstaScint can be safely administered intravenously and produces a low incidence of HAMA (human anti-mouse antibody). Therefore, it potentially could be used for sequential injections, which might be required to maintain surveillance over patients on an ongoing basis.

Cytogen entered into a co-marketing agreement with Bard Urological (Covington, GA), a division of C. R. Bard (Murray Hills, NJ), to sell ProstaScint in the USA market. However, as has been the case in the launch of OncoScint, it is going to be difficult to market this product to the primary physician/oncologist/urologist who is unfamiliar with immunoscintigraphy and reluctant to use a technology that may be difficult to interpret or implement. A more suitable marketing partner for all of Cytogen's radiopharmaceuticals may be a company with experience in the distribution of radiopharmaceuticals. However, the fact that Cytogen's agents are linked to indium makes them less desirable than those labeled with technetium.

In August 1996 Sloan-Kettering Institute, an affiliate of Memorial Sloan-Kettering Cancer Center (New York, NY) was issued U. S. patent # 5,538,966, that claims the nucleotide sequence encoding prostate specific membrane antigen (PSMA) recognized by ProstaScint. Cytogen holds an exclusive option to license this technology. PSMA may turn out to be a more sensitive diagnostic and monitoring test for prostate cancer than prostate-specific antigen (PSA). The PSM test uses PCR (polymerase chain reaction) technology that detects prostate cancer cells circulating in the blood.

Immunomedics' (Morris Plains, NJ) CEA-Scan (see FO, V1 #2/3, p 39), a colorectal cancer diagnostic imaging agent, was approved for marketing by the FDA in July 1996. CEA-Scan comprises an antibody fragment (Fab') of a murine MAb that targets carcinoembryonic antigen (CEA), a tumor marker expressed by more than 90% of colorectal cancers as well as other carcinomas, including lung, breast, pancreas, uterus, and ovarian cancers. Use of a small antibody fragment, at a low dose, virtually eliminates HAMA reactions with CEA-Scan. In addition, linking the fragment with technetium-99 permits tumor detection within a few hours after administration of the agent using a conventional gamma camera. CEA-Scan was the first technetium-labeled immunoconjugate and the first small fragment MAb-based agent to be approved for *in vivo* imaging applications in the USA.

Results from two prospective phase III clinical trials of CEA-Scan conducted in 18 sites in 210 patients with occult colorectal cancer, demonstrated that this agent was statistically superior to conventional methods, such as CT, in detecting metastases in the abdomen and pelvis and equivalent to CT in the liver. Among a subgroup of 122 patients with established disease, CEA-Scan, used in conjunction with conventional tests, was 98% predictive compared with 68% to 70% when either method was used alone. CEA-Scan also improved the diagnostic capabilities of conventional techniques in the remaining 88 patients with suspected recurrence and/or distant disease but whose status was unknown at the time of evaluation.

In May 1996, at the the annual meeting of the American Society of Clinical Oncology, Immunomedics announced the results of a clinical study involving 84 colorectal cancer patients with suspected recurrence or spread of their disease, but with negative conventional diagnostic tests, including CT scans, who were evaluated pre-operatively with CEA-Scan. The study, performed at M.D. Anderson Cancer Center (Houston, TX), found that in a group of patients in whom colorectal cancer recurrence and spread was missed by CT and other diagnostic methods, the addition of CEA-Scan to the diagnostic work-up decreased the false-negative rate of the other diagnostic tests; out of 59 CT-negative patients who had disease, CEA-Scan correctly identified 36 patients (61%). The likelihood of incorrect diagnosis is estimated at three-fold higher with CT than with CEA-Scan. By iden-

tifying about twice as many patients whoare not candidates for surgery and about 40% more patients who are operable for potential cure, CEA-Scan may become an important tool for the management of colorectal cancer.

CEA-Scan will be marketed in the USA and, after its approval also in Europe, under a collaborative agreement with Mallinckrodt Group (St. Louis, MO. Launch of CEA-Scan is planned in the fall of 1996. In May 1996, the European Committee for Proprietary Medicinal Products (CPMP) unanimously recommended approval of CEA-Scan to the EMEA, the European agency for the evaluation of medicinal products. The CPMP recommendation permits multiple administration of CEA-Scan in patients who do not develop HAMA. In clinical trials of CEA-Scan, fewer than 1% of the 400 patients evaluated developed HAMA after one injection. Immunomedics will market CEA-Scan in Europe in collaboration with Mallinckrotd BV (Petten, The Netherlands).

Neoprobe (Dublin, OH) has completed preliminary review of data from a phase III trial with its RIGScan CR-49 system for intraoperative scanning (for more information on RIGScan see FO, V1 #2/3 p 72 and 278-279). The trial evaluated RIGScan CR-49 for surgical detection of cancer in patients with metastatic colorectal cancer. The company's analysis suggests that this product can help surgeons detect otherwise hidden tumor tissue during surgery. In pivotal phase III clinical trials RIGScan CR49 identified tumors in 9 out of 10 (89%) of evaluable patients and pathology-confirmed tumor that would not have been found without the technology in 1 of 5 (21%) of cases of localized cancers. Surgeons made major treatment decisions in 87% of the evaluable patients with hidden tumor, based only on RIGS-provided information.

RIGScan CR49 is based on Neoprobe's RIGS technology, which consists of hand-held gamma-ray detecting probes, injectable cancer-specific targeting agents such as radioactive tracer bound to a MAb or peptide selected for specific cancer types, and methods for their combined use in finding tumors during surgery. In RIGS, a targeting agent is administered by injection before surgery. This iodine-125-radiolabeled agent attaches to chemical receptors on the surface of cancer cells or to antigens produced by such cancers. Surgery is scheduled after sufficient time elapses to allow the agent to accumulate in diseased tissue. During surgery, the surgeon uses the RIGS hand-held gamma detector to locate concentrations of RIGScan agent in malignant tissue so that it can be surgically removed. The probe gives an immediate siren sound to alert the surgeon to the precise location of targeted tissue.

Based on the completed phase III study in colorectal cancer, Neoprobe announced in July 1996 that its marketing application for RIGScan CR49, filed in May 1996 with the EMEA and to two assigned "rapporteur" reviewers in the UK and Denmark, was formally accepted for review. Neoprobe is currently reviewing clinical and preclinical data with the FDA in anticipation of an imminent PLA filing. Neoprobe intends to commercialize RIGS for intraoperative detection of other indications including breast, ovarian, prostate and endocrine cancers.

In August 1996 Neoprobe announced that it entered an agreement with Biomira to obtain an exclusive worldwide license to use Biomira's MAb-170 with its RIGS technology for surgical detection of breast cancer. MAb-170 is the same antibody used in Biomira's Tru-Scint AD kit. Neoprobe plans to perform a phase I clinical trial involving up to 45 women who are undergoing breast-conserving surgery. In exchange, Biomira received an upfront fee and will receive milestone payments and royalties upon commercialization of a RIGS product using MAb-170. The agreement also allows Neoprobe to test MAb-170, under similar terms, in other RIGS indications such as ovarian cancer. In addition, Neoprobe has access to improvements to MAb-170 and an option to license another Biomira antibody, MAb-174.

NeoRx (Seattle, WA) obtained FDA approval on August 22, 1996 for Verluma (technetium-99 nofetumomab merpentan) for staging of small cell lung cancer (sclc). Verluma was recommended for approval by the Oncologic Advisory Panel of the FDA on December 14, 1995. Verluma is the first technetium-labeled product developed to stage a specific type of cancer, as opposed to merely identifying tumor deposits. It is also the first diagnostic imaging agent that has been compared, in a phase III clinical trial, to an entire traditional battery of tests, where it was shown to be nearly as accurate in staging patients as four traditional staging tests combined. Staging is very important in sclc. Patients with localized disease (approximately 30% of all sclc patients) have a 20% chance of cure, but only if they receive both chemotherapy and chest radiation therapy, an expensive and often toxic combination. By contrast, patients with more extensive disease do not benefit from the addition of radiation therapy and have a lower cure rate. Only patients with limited disease, therefore, should be exposed to the potential toxicity, extra cost and inconvenience of chest radiation therapy. Data submitted to the FDA to support product approval indicates that Verluma is the most sensitive test available to determine that a patient's disease has spread beyond the focal area. Approval of Verluma triggered a $4.5 million payment to NeoRx by DuPont Merck Pharmaceutical that has obtained marketing rights for the product in North America.

Radiolabeled Peptides

Radiolabeled peptides such as Mallinckrodt's OctreoScan, entered the market in 1995 but generally experienced a slow start. There are several reasons for this phenomenon, among them physician inexperience with the agents and their high cost. These agents are priced in the $800 range per dose, because their radiolabel, indium, costs $450 per dose compared to technetium at $5.00 per dose. Also, radiolabeled peptides are targeting

a less common group of neoplasms, referred to as neuroendocrine tumors that originate from endocrine cells or from cells that have undergone neuroendocrine differentiation. These neoplasms usually arise in the central and peripheral nervous system as well as in the GI and respiratory tracts and generally express a high density of somatostatin receptors. The binding sites of these receptors are usually sufficient to permit radiolabeled somatostatin analog imaging of even small primary and metastatic lesions (see FO, V1 #6, p 167).

Mallinckrodt is continuing to build on the base established with OctreoScan thus far, and is attempting to demonstrate its effectiveness in a broader range of applications. This would substantially increase the opportunities of OctreoScan, in particular, and of peptide imaging, in general. Additional applications under evaluation involve quantification of uptake of the radiolabeled peptide to predict the biochemical and functional characteristics of tumors. This might allow the clinician to predict response to octreotide therapy. There also appears to be a direct correlation between tumor uptake and decrease or elimination of patient symptoms that result from the increased secretion of hormones associated with these tumors. Most neuroendocrine tumors are slow growing, and their most harmful effects are often related to this hormonal overproduction. (see FO, V1 #6, p 167).

Diatide (Londonderry, NH) is developing a peptide agent, Techtide P829 (successor to P587), currently in phase III clinical trials which is similar in function to OctreoScan, but offers the advantage of a technetium radiolabel (see FO, V1 #6, p 167). Diatide's management indicated that it will attempt to file for broader indications than OctreoScan. However, Mallinckrodt also indicated its intentions to file for expanded indications. Diatide is currently evaluating Techtide P829 for lymphoma and neuroendocrine tumors, as well in staging of breast and lung cancer.

TECHNOLOGY UPDATE

CANCER VACCINES HUMORAL VERSUS CELLULAR IMMUNITY

- Lack of progress in treating cancers with standard chemotherapeutic agents has intensified research efforts to develop cancer vaccines that would augment host immune system responses to malignancy.
- Numerous cancer vaccines are currently in development based on different scientific principles and employing a variety of technologies. The products highlighted in this report are provided as illustrations of cancer vaccines designed to elicit humoral, cellular or combined immune responses.
- This article was excerpted from NEW MEDICINE report #401 entitled, Cancer Vaccines: Technology, Products, Markets and Business Opportunities. More on cancer vaccines may be found in FUTURE ONCOLOGY articles describing drug development activities for certain cancer indications, including a comprehensive review of vaccines against malignant melanoma (see FO, V1 #6, pp 142-152) and prostate cancer (see FO, V2 #2/3, pp320-324).

RATIONALE FOR CANCER VACCINE DEVELOPMENT

Development of tumor vaccines is based on the premise that:

- there are qualitative and/or quantitative differences between tumor cells and most normal cells
- the immune system can identify these differences
- the immune system can be taught to consistently recognize these differences and mediate tumor rejection through immunization

It is also postulated that the immune system mounts a response to tumors either by:

- activation of T lymphocytes to directly or indirectly kill tumor cells (cell-mediated immunity)
- elicitation of antibodies that block or inhibit tumor growth (humoral immune response)

However, any distinction between cell-mediated and humoral immunity may be oversimplistic. Although various cancer vaccines are in development based on either type of immunity or both, the issue as to which arm of the immune response is more effective in tumor resistance has not been resolved. Actually, ground-breaking research is challenging some of the basic tenants of immunology and may have a profound effect on the development of cancer vaccines (see inset).

Implicit in the concept of tumor immunology is the assumption that there are characteristics of the tumor that are sufficiently different from those of corresponding normal tissues for the host to recognize the tumor as foreign and mount an immune response against it. The foreigness recognized by immunity is the antigenicity of a tumor cell. However, although tumors are antigenic, differing from normal tissues from which they have arisen, tumor cell antigens are weakly immunogenic, making their identification and detection difficult. Also, the question of whether any antigen is truly unique to a tumor is still a matter of debate. Nonetheless, since antigenicity exists, research into the tumor-host relationship as well as into the means of using that relationship in anti-tumor strategies, rests on a solid foundation.

The classic concept of immunization is based on infectious disease vaccines that provide useful lessons for tumor vaccine development:

- Vaccines do not prevent infection, they limit it. Natural systems and local immunity then cure infec-

tion at the site of entry. If circulating tumor cells can be eliminated in a similar manner, then local treatment may cure cancer.

- Not all viral or bacterial antigens are useful vaccination targets and, in some cases, vaccination may be counterproductive. As is the case with infectious disease vaccines, selection of useful cancer-associated antigens may be assisted by identifying responses that lead to protection from subsequent exposures. However, because most tumor antigens are poorly immunogenic, it is necessary to resort to an empirical approach in antigen selection.
- Prevention of infectious diseases with vaccines is easier than therapy, suggesting that adjuvant vaccination against cancer may be more successful than vaccination of patients with advanced disease.
- Viral or bacterial antigens as they are progressively purified or prepared as single antigenic epitopes, become less immunogenic because of loss of highly immunogenic surrounding antigens that augment the immune response. This effect can often be overcome by using highly immunogenic carrier proteins, immunologic adjuvants, or recombinant viral or bacterial vectors. These approaches represent strategies which are also applicable to tumor vaccines.

Broadly speaking, tumor vaccine approaches fall into one of three categories, active, adoptive, and passive immunotherapy (see Exhibit 7), derived principally from classic immunization strategies.

HUMAN IMMUNE RESPONSE

The human immune system is thought to distinguish "self" from "non-self," permitting the detection and elimination of foreign substances and organisms. This response is mediated by different lymphoreticular cells and their products. Mononuclear phagocytes or macrophages, which are derived from pro-monocytes in the bone marrow that subsequently mature into monocytes and migrate to the liver, spleen, lymph nodes, and lung, are the first to make immunologically productive contact with foreign or antigenic substances. Tissue macrophages respond nonspecifically to intruders, phagocytizing and digesting foreign substances. Macrophages then carry foreign molecules or antigens to local lymphoid tissue, where they become associated with follicular dendritic cells. Macrophages and dendritic cells act as antigen-presenting cells to the lymphocytes, which synthesize cell-surface receptors. Macrophages and dendritic cells can also secrete cytokines such as interleukins (IL-1, IL-6), tumor necrosis factor-α (TNF-α), interferons, prostaglandins, and other monokines that can affect the function of both T cells and B cells. Responses in these situation are termed adaptive because cell replication, differentiation, and affinity maturation of antibody occur after stimulation. As a result to adaptive responses, specific immunological memory, i.e. related to a given antigen, is generated. Cancer vaccines that induce either humoral or cell-mediated immunity make use of this antigenic specificity.

The concept of immune surveillance as a mechanism to recognize and destroy nascent neoplastic cells has been discussed and researched since the turn of the century. There are many anecdotes from clinical experience suggesting that antitumor immunity exists and influences the course of neoplastic disease, as manifested by:

- spontaneous regression of tumors
- prolonged survival or cure of patients after incomplete removal of a malignant lesion
- sudden appearance of metastasis many years after successful therapy
- regression of metastasis after local treatment of the primary lesion

More recently, immune responses to tumor antigens have been rigorously documented and measured in patients with cancer. However, debate persists as to which arm of the immune system provides the best antitumor activity. There is considerable evidence supporting the notion that cell-mediated immunity may be more important than antibody-based immune response in attacking established tumors, but it is unclear how this knowledge can best be applied to tumor vaccines. Currently, the NCI and most developers are focusing on tumor vaccine strategies involving stimulation of cell-mediated immunity. However, many scientists are working with genetically engineered vaccine approaches that primarily stimulate an antibody response in the host. A definitive characterization of the roles of humoral and cell-mediated immunity in tumor resistance would facilitate the design of future generations of cancer vaccines.

It is also conceivable that these two basic forms of immune response can be effective at different stages of malignancy. For instance, humoral immune response may be exploited in preventative vaccines that are aimed at patients who are considered cured after the tumor is surgically removed while vaccines eliciting a cellular immune response may be employed as a therapeutic modality to treat minimal disease in cases not treatable by surgery alone because of distant spread.

Cancer vaccine design and evaluation is considerably more challenging than that involving chemotherapeutics. Because of its intention, immunization is primarily a preventative or prophylactic methodology. However, because of safety considerations, to date vaccines have been mostly evaluated in late-stage cancer, often after all else has failed. In such circumstances, the task of fine-tuning vaccine design, establishing adequate dosage levels and administration schedules, and assessing vaccine efficacy has been difficult because of the trial subjects' advanced disease, poor health status and short anticipated survival. Although, to date, no cancer vaccine has demonstrated truly curative and/or prophylactic effects,

no such vaccine has been tested in ideally-selected candidates under favorable conditions.

VACCINES ELICITING A HUMORAL (B CELL) IMMUNE RESPONSE

B cells are organized in follicular aggregates within lymph nodes, the spleen and gut-associated lymphoid tissue, in proximity to T cells and antigen presenting cells. Antibody production can be augmented by T cell participation and can be down-regulated by T cell or macrophage "suppression." Humoral immune responses involve recognition of circulating antigens by antibodies that are expressed on the surface of B lymphocytes, generation of circulating antibodies and mediation by B lymphocytes. B cells are capable of binding specific antigens via their antigen receptors. Mature B cells synthesize and express immunoglobulin on their cell surface; initial B cell differentiation is independent of antigen, but subsequent differentiation requires antigen and proceeds optimally in the presence of T cell-derived factors such as IL-2, IL-4, IL-5, and IL-6. After interaction with antigen and T cell products, different clones of B cells differentiate into one or more plasma cells that produce a single antibody which binds noncovalently to a particular antigen. Binding depends upon a precise complementarity between combining sites of antigen and antibody, the tertiary structure of which is determined by the amino acid sequence of each immunoglobulin. Immunoglobulin molecules consist of light (L) and heavy (H) polypeptide chains, with each L and H chain divided into an amino-terminal variable (V) region and a carboxy-terminal constant region. The V region of each L and H chain includes three complementarity determining regions (CDRs) which determine the antigen's binding site and its specificity. Antibody diversity is generated by several mechanisms, including somatic recombination of immunoglobulin gene segments, association of different H and L chains, as well as somatic mutation within the CDRs. Through these mechanisms, more than 100,000 antibody specificities can be produced.

If the site to which the antibody binds is critical for the biological function of a specific antigen, its effects can be neutralized. Antigen-B cell antibody binding also stimulates proliferation and differentiation of B cells, leading to the secretion of multiple copies of the unique antibody expressed on the surface of these B cells in a process referred to as clonal expansion. Released B cell antibodies bind with circulating antigen to form antibody/antigen complexes which trigger local effector mechanisms such as complement activation (the complement system is comprised of proteins that kill microorganisms by drilling pores in cell membranes and, thus, disrupting their cellular integrity) and opsonization (enhancement of phagocytosis by macrophages and neutrophils that express receptors for the antibody and digest the entire antigen/antibody complex), resulting in the neutralization and elimination of foreign substances or antigens. Antibodies can also cause cellular destruction by interact-

Current Concepts on the Function of the Immune System

There are three primary effectors in the induction of an immune response:

- *B cells, one type of antigen presenting cells that become activated by the presence of foreign antigens*
- *T cells whose receptors recognize cell surface antigens*
- *Dendritic cells that are antigen-presenting cells that evoke a stronger immune response than B cells; these cells transport antigens from tissues to lymph nodes*

Work performed in the 1980s pointed to a dual activation of T cells, with a second costimulatory signal emanating from dendritic cells. It is not clear what happens to T cells when this signal is not present but they disappear by dying, becoming anergic or being transformed into cells that stimulate antibody production. In this process the host's immune system becomes tolerant of the antigen that initially stimulated the T cells. Recently, however, immunologists have proposed that this second signal is delivered only after dendritic cells are activated by yet another signal, a "danger alarm", generated by distressed (damaged) cells. For instance, adjuvants may act by damaging cells that in turn send out signals that activate dendritic cells and induce an immune response.

The immune system may be modulated by the B cell/T cell or dendritic cell/T cell ratios. Immune response may also depend on the amount of antigen present; too much antigen may overwhelm and deactivate T cells. Fine tuning these variables may be the key in controlling the immune response.

In the case of cancer, in order for the immune system to mount a successive response against the tumor, dendritic cells must be activated by a danger signal. It may be that surgery is often successful in "curing" early cancer because it serves as such a danger signal. Because the cancer is in the early stages, the immune system is not overwhelmed by excessive antigen and can effectively deal with remaining tumor cells, resulting in long term remissions and even cures. In advanced disease, excessive antigen may disarm T cells, allowing the immune system to tolerate the tumor which then grows unchecked.

Although the make up of the danger signal has not been elucidated, it is suggested that it may be originating from heat shock proteins that are gene-regulating proteins produced when cells are "stressed" by adverse environmental conditions.

ing with local effector cells, such as certain T lymphocytes. The effector cell either attaches to circulating antibody and is, thus, carried to the target cell, or to the antibody after it has bound to the target cell antigen. This process is termed antibody-dependent cellular or cell-mediated cytotoxicity (ADCC). Because B cell-mediated humoral immune response recognizes and neutralizes antigens found on the surface of cells, it principally defends the body against foreign proteins. It cannot defend the host once an invader has managed to enter and replicate in a cell. Humoral immunity is, therefore, ineffective against chronic infections such as HIV and, possibly, established tumors.

Exhibit 7
Tumor Vaccine Approaches

Type of Immunotherapy	Description
Active Immunotherapy	Stimulation of the host's intrinsic antitumor immunity
Nonspecific	Activation of macrophages, NK cells and other nonspecific effectors using microbial or chemical immunomodulators; T cells activated secondarily through macrophages
Specific	Activation of specific effector cells and "armed" macrophages using antigenic tumor cells, cell lysates, or extracted/synthetic tumor antigens
Adoptive Immunotherapy	Transfer of immunological cells or informational molecules
Passive Immunotherapy	Transfer of antibodies or antisera, providing exogenous immunity

Active Specific Immunotherapy (ASI)

Biomira (Edmonton, Canada) is developing Theratope (Theratope STn-KLH), a vaccine based on a cancer-associated carbohydrate antigen which contains synthetic versions of glycoproteins (mucins) that appear on the tumor cell surface, combined with keyhole limpet hemocyanin (KLH), a carrier protein for the tumor-associated antigen, and an adjuvant. Glycoconjugate vaccines produce strong antibody responses. Theratope induces antibodies not only to synthetic STn (the active ingredient in the vaccine) but also to the natural mucin-bearing STn-like antigens. The vaccine has minimal side effects and may be administer on an outpatient basis (also see FO, V1 #2/3, p 51).

Currently, Theratope is in phase II trials involving more than 200 patients with late-stage breast, colon and ovarian cancers. As of early 1996, 147 patients with metastatic cancer evaluable for immune response were administered a series of four Theratope injections over a nine-week period. Patients were evaluated after three months and those with stable disease were kept on a maintenance therapy involving a series of Theratope vaccine injections.

At the Sixth International Congress on Anti-Cancer Treatment meeting in Paris, France, held in February 1996, investigators reported an apparent survival advantage for patients with metastatic breast or ovarian cancer who develop a strong vaccine-induced antibody response against Theratope. These patients experienced a statistically significant improvement in survival, suggesting there is an association between antibody production against the active ingredient in Theratope and extended survival. In addition, two other prognostic variables were noted. Patients with lymphocyte counts >1.125 x 10^9/l and patients with normal serum MUC-1 mucin levels (<40 units/ml), as measured by Biomira's Truquant BR blood test prior to treatment, experienced prolonged survival. Biomira plans to include these prognostic factors in the design of phase III clinical trial protocols of Theratope which the company plans to pursue with a corporate partner.

ImmunoTherapy Corporation (Tustin, CA and Seattle, WA) has exclusive rights to a cancer vaccine that elicits antibodies to the beta subunit of human chorionic gonadotropin (βhCG) that is found on the surface of all cancer cells. The role of βhCG as a unique marker of cancer cells was first postulated by Hernan Acevedo of the Allegheny-Singer Research Institute (Pittsburgh, PA) who subsequently demonstrated that βhCG is produced (expressed) by cells in every type of cancer (15) and in every cell line (74+) tested, but it is not produced in measurable quantities by cells of healthy individuals except pregnant women. Also, it was observed that presence of hCG correlates with stage of tumor; the more advanced the malignancy the higher the levels of hCG.

It is speculated that hCG, found exclusively on fetal cells in developing embryos and sperm cells, in addition to its role in sustaining pregnancy, may shield the embryo from the mother's immune system. Embryonic cells that express hCG are thought to possess a strong electric charge that repels similarly charged maternal immune system cells such as macrophages. The development of a tumor vaccine is based on the assumption that when a mature normal cell becomes malignant it reverts to an embryonic stage and produces hCG to protect itself from the immune system. However, although researchers found that genes encoding hCG were uniformly active in all tumor samples examined (Acevedo HF, etal, Cancer, 1995 Oct 15, 76(8):1467-75), neither the reason for this activation or the role of hCG in the development and/or spread of cancer has been elucidated.

Originally developed as a contraceptive, a vaccine to block hCG consists of the carboxyl-terminal peptide

sequence (residues 109-145) of βhCG conjugated to diptheria toxoid, with muramyl dipeptide (MDP) as the adjuvant and squalene/mannide monooleate as the vehicle. A phase I clinical trial involving 23 late-stage cancer patients suggests that antibodies created by three inoculations with the anti-hCG vaccine appear to significantly improve health status, extend survival and, in some cases, reduce the size of primary tumor. Antibodies to hCG may also be effective in preventing tumor metastasis. Results with pancreatic cancer were particularly encouraging. During treatment, disease stabilized in pancreatic cancer patients who had high levels of circulating antibodies. The vaccine was safe and was not associated with serious side effects beyond sterile abscesses at the inoculation site in 20% of the subjects after the third injection. The FDA approved a phase I/II clinical trial of the anti-hCG vaccine in colorectal cancer which began in December 1995. A second phase II clinical trial in pancreatic cancer is scheduled for 1996.

ImmunoTherapy's hCG vaccine is being combined with Optivax vaccine delivery system provided by Vaxcel, a subsidiary of CytRx (Norcross, GA). Optivax acts both as a delivery system, targeting antigens to immune cells, and as a vaccine adjuvant. Preclinical data with Optivax show a broad range of activity, which may result in single-dose formulations for vaccines that currently require multiple injections or in the development of oral vaccines. Also, because of its simple construction it may be less expensive than other more sophisticated vaccine delivery systems.

Progenics Pharmaceuticals (Tarrytown, NY), under a license agreement with Memorial Sloan-Kettering Cancer Center (New York, NY) entered in December 1995, obtained worldwide, exclusive rights to ganglioside technology for use in the development of cancer vaccines and for GMK, a therapeutic vaccine for the treatment of melanoma. GMK is a ganglioside conjugate vaccine comprised of ganglioside GM_2 coupled to KLH and formulated with QS-21 adjuvant (a homogeneous saponin purified from the bark of *Quillaja saponaria Molina*). GM_2 is present in approximately 95% of melanoma cells. GMK induces antibodies against GM_2 ganglioside capable of specifically killing melanomas cells. GMK is to be evaluated in two pivotal, randomized phase III clinical trials. A placebo-controlled phase III clinical trial in Stage II and III melanoma is to be conducted by the Institute of Cancer Research (ICR) of the Royal Cancer Hospital (London, UK) and the Cancer Research Campaign (CRC) in the UK, in conjunction with other hospitals and clinics throughout Europe. The other phase III clinical trial in Stage III melanoma is being conducted by the Eastern Cooperative Oncology Group (ECOG; Denver, CO) in conjunction with the NCI and other hospitals and clinics in the USA. An earlier version of this vaccine has undergone phase I and II clinical trials with promising results. Also, Progenics recently completed a phase I/II study of GMK at Memorial Sloan-Kettering Cancer Center.

A second ganglioside conjugate vaccine, multi-ganglioside vaccine (MGV), based on gangliosides GD_2 and GM_2, is also expected to enter clinical trials in 1996 at Sloan-Kettering for the treatment of other cancers including colorectal, gastric and small cell lung cancers, sarcoma, and neuroblastoma. A worldwide license and supply agreement was also signed between Progenics and Aquila Biopharmaceutical (was Cambridge Biotech; Worcester, MA) for use of QS-21 in the GMK and MGV vaccines. The company also signed a patent licensing agreement with Proteus (Macclesfield, Cheshire, UK) to use its NISV vaccine adjuvant. In return, Proteus will receive milestone payments and royalties on sales of any vaccine using technology covered by its patent.

ASI Using Anti-Idiotypic Antibodies

A novel "molecular" approach to the construction of tumor vaccines is based on the concept of idiotypic mimicry using anti-idiotypic MAbs (anti-Ids). The region of an antibody which is unique to that antibody is termed the idiotope. The idiotype contains the antigen-combining site, although it is not identical to it. Idiotypes may act as antigenic determinants and induce formation of antibodies. Anti-Ids may be directed against determinants directly involved in antigen-antibody interaction within the antigen binding site or determinants that define the structure of the variable region. Anti-Ids may mimic the structure of the epitope of the original antigen and are, thus, said to bear an "internal image" of the antigen. It may be, therefore, possible to use anti-idiotype vaccines in active specific immunotherapy as a substitute for specific tumor derived antigens, inducing antibody reactions against tumor antigens which have not otherwise been immunogenic (PB Chapman and AN Houghton, Biologic Therapy of Cancer, VT DeVita Jr, etal, eds., JB Lippincott, New York, 1992, p. 1).

Three types of anti-idiotypic antibodies have been defined (Gaulton GN, etal, J Immunol, 1986, 137:2930; Roitt IM, etal, Lancet, [1981] 1:1041):

- alpha type-the target for the anti-Id is not too close to the antigen-combining site of the original antibody
- gamma type-the target for the anti-Id is so close to the antigen-combining site that it can interfere with antigen binding
- beta type-the original antibody binds to an idiotypic determinant of the anti-Id; anti-Id-beta is believed to resemble the antigen and has vaccine potential

Immunization of a host with an anti-Id bearing the internal image of a primary antigen (anti-Id-beta) could result in the elicitation of an anti-anti-Id directed against the anti-idiotype internal image. The anti-anti-Id may bind to the original antigen as well as to the anti-Id bearing the antigen's internal image, thereby conferring immunity to the host. By using hybridoma technology to

produce anti-Ids which mimic the antigenic determinant against which an immune response is desired, the possibility exists of focusing the immune response against a relevant epitope without having to use the antigen itself. This would be particularly advantageous in situations when it is difficult to obtain adequate amounts of antigen, when the antigen in question cannot be sufficiently purified to make a good vaccine, or when a conventional inactivated vaccine has an unacceptable potential of reversion to virulent form.

Vaccines containing anti-Ids may have other advantages as well. For instance, compared to carbohydrate antigens, protein simulation of the original antigen may more readily induce T cell help and increased production of IgG antibodies because proteins are more readily processed and presented to T cells than carbohydrates. Also, because of the importance of the idiotype-anti-idiotype cascade for immune regulation, as originally proposed by Jerne in 1974, immunization with anti-Ids may induce more effective protection than immunization with the original antigen. However, while anti-idiotype vaccines may provide a different and sometimes more immunogenic form of the original antigenic epitope, they may require conjugation to immunogenic carrier proteins and potent adjuvants for optimal immunogenicity.

Anti-idiotypic vaccines have several potential drawbacks. The majority of anti-Ids studied to date have been murine in origin and, although, the human immune response to these foreign proteins may result in the endogenous production of human antibodies directed against a primary antigen target, this response will not be restricted to anti-idiotypic determinants. Initial exposure to a foreign protein may be acceptably tolerated, but repeated immunization with heterologous serum may lead to the development of serum sickness. The use of human anti-Ids cannot be expected to entirely eliminate this problem, as alloantigens located within the constant region of the antibody molecule are also immunogenic. A possible solution may be to use expression vectors encoding the variable genes as single-chain Fv, which would avoid potentially harmful responses against the constant regions of the antibodies (RE Hawkins, etal, J Immunother, [1993] 14:273). Another potential problem with anti-idiotypic vaccines is heterogeneous expression and modulation of target antigens. An anti-Id reacts with only a small proportion of the determinants which recognize the original antigen; to confer complete protection and to address the heterogeneity of antigen expression it may necessary to use a "cocktail" of anti-Ids mimicking multiple epitopes of an antigen, and directed against multiple target antigens, collectively expressed by the majority of tumor cells. Antigenic modulation may be a particularly serious problem for anti-idiotypic vaccines directed against tumor-associated antigens. Available evidence suggests that tumor cells can "escape" an anti-Id by accumulating mutations within the receptor variable region genes. This action generates new clones of cells resistant to the original anti-Id. Finally, most anti-Ids down-regulate the immune response, reflecting the ability of anti-Ids to affect essentially all of the cells involved in the immune network, including T cytotoxic or suppressor cells, in addition to B cells and T helper cells (RC Kennedy, et al, J Virol, [1984] 50:951).

ImClone Systems (New York, NY) is developing, in collaboration with Merck KGaA (Darmstadt, Germany), BEC-2, a murine anti-Id that mimics GD_3, a glycolipid (ganglioside) antigen found on the surface of a number of tumor cells of such cancers as melanoma, small cell lung cancer (sclc) and a variety of soft tissue sarcomas. A phase I/II clinical trial of BEC 2 is ongoing in melanoma, and a phase I clinical study began in February 1995 at the Memorial Sloan-Kettering Cancer Center in the treatment of other solid tumors. In May 1996, at the 32nd American Society of Clinical Oncology (ASCO) annual meeting, ImClone reported that patients with sclc treated with BEC-2 demonstrated statistically significant prolonged survival, with median survival surpassing 36 months; average median survival was 16.2 months in controls. The survival rate of patients with sclc treated with BEC-2 was 67% at 36 months.

Passive Immunotherapy

Passive immunotherapy involves transfer of MAbs against tumor-specific antigens (if they exist), against tumor-associated antigens, or against differentiation antigens (also shared by certain normal cells). Although binding of antibodies to antigens on the surface of tumor cells is generally necessary, but not sufficient, to inhibit tumor growth, MAbs with appropriate isotypes can trigger complement-dependent cytotoxicity, or participate in antibody-dependent cell-mediated cytotoxicity (ADCC) after binding to antigen. However, many human tumor cells are relatively resistant to lysis by human complement components, and effectors for ADCC may not be plentiful within the tumor compartment, and their function may be impaired by several factors, including the presence of endogenous antigen-antibody complexes.

In addition to the potency (or lack thereof) of effector mechanisms, several other factors may impede the efficacy of MAbs, including complexing with shed antigen, failure to penetrate tissue, antigenic modulation, heterogeneity of antigen expression, and the development of HAMA. Fortunately, not all cell surface antigens are shed, and not all shed antigens impede reactivity of MAbs with tumor cells. While saturation of antigenic sites on solid tumor nodules has proven difficult to achieve, a significant fraction of sites can be occupied by MAbs after intravenous injection. Of course, even when antibody gains access to a tumor, it may not attach to all tumor cells because not all such cells express any given antigen. However, use of multiple antibodies in combination may compensate for this heterogeneity. Despite many potential limitations associated with passive immunotherapy, if experience in animals holds true, it is possible that a

dispersed tumor such as leukemia or ascitic ovarian cancer will respond to therapy with passively transferred antibodies.

Centocor (Malvern, PA) has developed Panorex (17-1A) as an adjuvant therapy in the treatment of post-operative colorectal cancer. Panorex is a MAb that binds to colon cancer cells and destroys them by various immunologic mechanisms including complement and/or antibody dependent cellular cytotoxicity. Although the exact mechanism of action of Panorex has not been elucidated, it is believed that the MAb binds and destroys micrometastatic cells that break away from the primary tumor and spread to other sites such as bone marrow (see FO, V1 #2/3, p 52). In a phase III trial conducted in Germany, Panorex showed a 5-year 30% survival benefit and 27% reduction in tumor recurrences compared to controls. Glaxo Wellcome is conducting additional phase III trials in colorectal cancer, with a three-year survival endpoint, in North America, Europe and certain other countries; earlier stage trials are also underway in Japan. Other indications targeted by Panorex include pancreatic and gastric cancers, and certain types of lung, breast, and ovarian cancers.

VACCINES ELICITING A CELLULAR (T CELL) IMMUNE RESPONSE

Like B cells, T cells also originate in bone marrow, but differentiate in the thymus. A significant fraction of T cells that recognize "self" determinants are eliminated during thymic differentiation; the remaining mature T cells then travel through the peripheral circulation and return to lymph through the venous and postcapillary venules of skin and lymph nodes. T cells mediate cellular immune responses, including delayed hypersensitivity, graft rejection, and regulation of other T cells, B cells, monocytes, and marrow progenitors.

In contrast to B cells, T cell antigen receptors do not recognize intact antigenic molecules or cells, but rather recognize macrophage-processed antigen as a peptide of 12 to 18 amino acids in length. The specificity of interactions with different antigens is mediated by a large family of 90 kDa cell surface T cell receptors (TCRs). Different clones of T cells bearing distinctive TCR recognize different antigenic peptides; diversity of TCR arises through somatic recombination of gene segments analogous to that observed during generation of immunoglobulin diversity. In contrast to B cells that can recognize antigen in the fluid phase, T cells only recognize antigenic peptides when they are associated with appropriate membrane-bound major histocompatibility complex (MHC) molecules on antigen-presenting cells. In humans, MHC is a genetic locus on the short arm of chromosome 6 and is commonly referred to as the human leukocyte antigen (HLA) system, consisting of several hundred immune response (Ir) genes that control the ability to produce an immune response. MHC molecules bind processed foreign antigen (usually peptide fragments) in a membrane groove on the distal face of the molecule; T lymphocytes are able to recognize and bind to the resulting complex of processed antigen and MHC molecule. Antigen-presenting cells, including dendritic cells, macrophages, and B cells, digest exogenous proteins and display antigenic peptides on their surface in the context of Class II MHC antigens (HLA-DR, DQ and DP), while antigenic peptides from endogenous viral and cellular proteins become associated with Class I MHC antigens (HLA-A, B and C) during their synthesis and expression on the cell surface.

Mature or extrathymic T cells are typically classified according to their specialized functions and surface markers (clusters of differentiation or CDs). All mature T cells have a TCR that is responsible for MHC-restricted recognition of antigen (Weidmann E, etal, Cancer Immunol Immunother, [1994] 39:1). This receptor is associated with a set of nonpolymorphic molecules on the T cell surface collectively called CD3. There is coordinate expression of CD3 and TCR; if a cell line loses its TCR, it ceases to express CD3, and conversely, if its genes express TCR but not CD3, no TCR appears on the cell surface. In humans, T cells are also separated into two major subsets distinguished by the expression of either CD4 or CD8 receptor. CD8-positive T cells (cytolytic T cells or CTLs) interact with antigen bound to Class I determinants and often mediate suppression or cytotoxicity, whereas CD4-positive T cells recognize antigen bound to Class II MHC antigens and often exert helper/inducer function. In either case, TCR binds to antigenic peptides that are bound to MHC molecules on the cell surface, and generates a signal that is transduced through CD3, releasing intracellular calcium and activating the phosphatidyl inositol or tyrosine kinase pathways. In addition to the primary signal provided by antigen presented in an appropriate context, secondary signals are required to fully activate T cells, including soluble factors such as IL-2, as well as contact with ligands on the surface of other lymphoreticular cells. In the absence of such secondary signals, T cell activity may become down-regulated, resulting in tolerance to an otherwise foreign antigen. CD8 T cell activation is widely regarded as one of the most potent immune response mechanisms in cancer.

Evidence taken from animal experiments makes it appear unlikely that antibodies act independently of leukocytes to kill tumor cells *in vivo*; it is more likely that T cell-mediated toxicity is the major mechanism for destroying tumor cells. T cells may act directly, as CTLs or indirectly, by amplifying CTL cell responses, activating macrophages and inducing LAK cells and specific antibody production, among others (Melief CJM, Adv Cancer Res, [1992] 58:143). The precise mechanism of direct T cell-mediated cytotoxicity is not known, but perforins, phospholipase, lymphotoxins, direct membrane interactions, and induction of apoptosis have all been proposed (Young LHY, etal, Annu Rev Med, [1990] 41:45; Restifo NP, etal, J Immunol, [1995] 154:4414). However,

while T cells have been shown to kill some tumor cells *in vitro* by direct cytotoxicity within four to six hours, it is possible that their major effect *in vivo* is to recruit and activate macrophages. Cellular immunity has "memory" and the involvement of memory T cells ensures that long-lived immune recognition of the tumor will occur (Greenberg PD, Adv Immunol, [1991] 49:281).

Efforts to identify CTL-defined tumor peptides for the development of peptide-based cancer immunotherapy has intensified based on findings that class I MHC-restricted CTL recognize peptide antigens (epitopes) bound to class I MHC molecules. However, metastatic cancer cells may escape T cell recognition through divergent mechanisms of defective class I MHC assembly. Therefore, cancer immunotherapy approaches may need to focus on strategies that can circumvent sole reliance on class I MHC-mediated tumor cell recognition by CTL.

Tumor Antigen Vaccines

Cytel (San Diego) is developing Theradigm, an antigen-specific immunostimulant that is a therapeutic vaccine consisting of small "antigenic peptides" that are segments of foreign proteins normally presented to the body's immune system by Class l human leukocyte antigens (HLA-1). Presentation of these antigenic peptide/HLA-1 complexes induces a cellular immune response (see FO, V1 #2/3, p 52, and #6, p 147 and V2 #2/3 pp 321-322). Two versions of Theradigm are being clinically investigated as cancer vaccines. Theradigm-MAGE 3 entered an investigator-initiated phase I/II clinical trial in MAGE-3-associated melanoma in December 1994 under a physician's IND. It is being clinically-evaluated in NCI-sponsored phase II study for treating advanced solid tumors and in a two-year study in Stage III melanoma to prevent recurrence in patients with minimal residual disease. Theradigm-HPV is currently being evaluated in an investigator-initiated phase I/II study in the Netherlands for the treatment of advanced-stage cervical carcinoma in patients positive for HPV. A second study with Theradigm-HPV in patients with precancerous cervical lesions attributable to HPV infection, is currently being planned. Additional targets include breast, ovarian, colon, lung and prostate cancers. Vaccines for these cancers are based on such antigenic peptides as Her-2 (breast and ovarian) and PSA (prostate).

Theradigm vaccines are based on a modular design consisting of an uniform delivery and booster component and antigenic peptides that are specific to the cancer being targeted. Theradigm consists of:

- a lipopolysaccharide delivery sequence which guides the antigenic peptide to the appropriate intracellular compartment so that it can complex with HLA-1 molecules
- a booster or T-helper sequence (incomplete Freund's adjuvant in Theradigm-MAGE 3 and Theradigm-HPV); Cytel has also developed a second-generation helper epitope (a novel and proprietary composition of matter known as PADRE) which stimulates the immune system to enhance the presentation of the antigenic peptide
- a disease-specific antigenic peptide to complex with HLA-1 molecules on antigen-presenting cells to specifically stimulate only those T cells that have a receptor that recognizes that particular antigenic peptide/HLA-1 complex; appropriate antigenic peptides were selected from HLA-1 variants present in each individual (every human expresses 3-6 of the 70-80 different known forms of HLA-1 molecules); Cytel initially focused on identifying and patenting antigenic peptides that bind best with HLA-1(A)2 which is the most frequently encountered HLA-1 "isotype" found in Caucasians and Asians (more than 45% of all Caucasians and more than 40% of all Asians use the HLA-1A(2) isotype to present antigens), and HLA-A1, A11, A3 and A24 which comprise nearly 90% of the alleles expressed by Caucasians and Asians

Lidak Pharmaceuticals (La Jolla, CA) is evaluating LP2307, a cancer immunotherapeutic based on the company's Large Multivalent Immunogen (LMI) technology that stimulates the body's immune system to more aggressively and effectively destroy invading tumor cells. LMIs are created by isolating and attaching a high concentration of antigen from a tumor cell onto cell-size microspheres which are then injected into the cancer-bearing host to stimulate CTLs (see FO, V1 #6, p 147). In August 1995, Lidak obtained FDA clearance to begin a phase I/II trial of LP-2307 for the treatment of melanoma. In September 1995, Lidak began a phase I/II clinical trial at the University of California, San Diego that is to enroll 18 patients. Lidak is also experimenting with dendritic cell-based vaccines.

Heat Shock Proteins

Another alternative to purified antigen preparations which avoids prior determination of antigenic epitopes of cancer cells, is use of stress-induced, or heat-shock proteins (HSPs) (Udono H and Srivastava PK, J Exp Med, [1993] 178:1391; Srivastava PK, Experientia, [1994] 50:1054). HSPs continue to appear with intriguing regularity among antigens detected by immune response to cancers (Young RA, A Rev Immun, [1990] 8:401), and are among the most highly conserved proteins in living systems.

The cellular stress response is a powerful, universal mechanism used by all known organisms to defend against potentially life-threatening challenges. Stress proteins that are intimately involved in fundamental cell processes, are thought to mediate cellular protection. HSPs are relatively abundant components of virtually all disease-causing bacteria and parasites. In most infections, stress proteins produced by the invading pathogen, are highly immunostimulatory and are major targets of

the host's immune system. Immunostimulatory properties of stress proteins also help the immune system recognize and attack cancer cells. Stress proteins also appear to be effective vehicles for antigen presentation. These attributes make HSPs attractive candidates for a new generation of vaccine components and therapeutics. HSPs, often referred to as "molecular chaperones", also play a key role during non-stressful conditions. For instance, they are essential in helping maintain the three-dimensional folding of cellular proteins.

Recent evidence indicates that HSPs gp96, hsp90, and hsp70 associate with antigenic peptides derived from cellular proteins, suggesting that HSPs may not be immunogenic per se, but may act as carriers of antigenic peptides (Srivastava PK and Maki RG, Curr Top Microbiol Immunol, [1991] 167:109). In this scenario, HSPs are released from tumor cells *in vivo* during cell lysis by the action of antibodies or nonspecific effectors. HSPs then complex with antigenic peptides derived from cognate cells and are taken up by macrophages or other specialized antigen-presenting cells, possibly through a receptor-mediated mechanism. HSP-borne peptides are then routed to endogenous presentation pathways in an antigen-presenting cell, and are displayed in the context of that cell's MHC class I molecules where they are finally recognized by precursor CTLs (Srivastava PK, etal, Immunogenetics, [1994] 39:93). Indeed, studies have shown that homogeneous preparations of HSPs derived from any cell type contain a wide assortment of peptides (6-35 mers) noncovalently bound to, or "chaperoned" by, the HSP (Z Li and PK Srivastava, EMBO J, [1993] 12:3143). One consequence of this phenomenon is that HSP preparations contain the entire repertoire of peptides generated in a cell, a repertoire consisting not only of self but of antigenic peptides. Thus, HSPs derived from tumors are complexed with peptides derived from tumor antigens. Vaccination of animals with such HSP-peptide complexes elicits CD8-positive T cell specific responses for the antigenic peptides present in the HSP preparation, resulting in tumor-specific protective immunity (Udono H, etal, Proc Natl Acad Sci USA, [1994] 91:3077). This immunity appears to be general, since three of the major cellular HSPs, hsp70, hsp90 and gp96, have been shown to act as cancer vaccines against three antigenically distinct murine sarcomas (Srivastava PK, Adv Cancer Res, [1993] 62:153; Udono H and Srivastava PK, J Immunol, [1994] 152:5398).

Antigenics (New York, NY), founded by Dr. Pramod Srivastava in 1994, is a biotechnology company developing innovative therapies for the treatment of cancer and infectious diseases. Antigenics is a private, closely held concern structured as a limited liability company in order to eliminate double taxation and allow non-taxed flow-through of non-USA profits to foreign partners. The company conducts its business through two subsidiaries, OncoAntigenics that develops and commercializes cancer vaccines and MediAntigenics which primarily focuses in the development of vaccines for infectious diseases. Antigenics cancer vaccines rely on the activation of CD8+ T lymphocytes. Central to the production of CD8+ cells are HSP/antigen complexes. Antigenics obtained exclusive rights to the hsp70 and gp96 constructs through patents licensed from the Mount Sinai School of Medicine (New York, NY) and Fordham University (New York, NY). Antigenics is concentrating on autologous vaccines and is working with PerSeptive Biosystems (Framingham, MA) to streamline processing of autologous tissue. Antigenics will have exclusive rights to such technologies and systems developed by PerSeptive Biosystems. A phase I clinical trial was completed in May 1995 in Berlin, Germany using autologous HSP vaccines to treat various cancers.

StressGen Biotechnologies (Victoria, BC, Canada) is developing HSPs as cancer vaccines (Oncocines) using its proprietary Unigen technology. Oncocines combine stress proteins with tumor antigens to treat various cancers including melanoma and breast and colorectal cancer (also see FO, V1 #6, pp 147-148).

Adoptive (Cellular) Immunotherapy

In adoptive or cellular immunotherapy cells with anticancer properties are transfered into cancer patients in the hope that they either directly or indirectly mediate anticancer effects on growing tumors. In early studies, immunity to tissues present in highly immunized mice could be transferred to virgin mice by adoptive transfer of lymphocytes; in contrast, transfer of serum containing large amounts of antibody was relatively ineffective in transferring immunity. In general, animals treated with adoptive immunotherapy manifested systemic immunity by rejecting subsequent tumor challenges in an immunologically specific manner. These findings led to efforts to transfer lymphocytes among cross-immunized cancer patients. However, inability to generate cells from cancer patients that elicited specific immune reactivity against cancer, and that could be generated in large enough numbers, limited use of this procedure in the past. Subsequently, several novel approaches to raising immune cells designed to overcome these obstacles, such as use of lymphokine-activated killer (LAK) cells, tumor infiltrating lymphocytes (TIL), gene-modified cells, and tumor-sensitized lymph node cells, were successfully used in cellular therapy of advanced cancer in humans.

CANCER VACCINES THAT STIMULATE BOTH HUMORAL AND CELL-MEDIATED IMMUNITY

Some tumor vaccines act to elicit both humoral and cell-mediated immunity. When the relationship between humoral and cell-mediated immune responses to tumor vaccination and clinical outcome were examined concurrently in the same patients (K. Miller, etal., Proc Am Soc Clin Res, [1993) 12:1353), both types of immune responses were independently associated with enhanced

outcome and no major differences were noted in clinical outcomes. However, the most favorable clinical outcome was observed in patients with combined humoral and cell-mediated responses.

Allogeneic Tumor Cell Vaccines

Allogeneic tumor cell vaccines have been extensively investigated in the treatment of melanoma, a highly immunogenic malignancy that was one of the first cancers targeted by tumor vaccines. (For more information on treatment of melanoma using immunotherapy/vaccines see FO, V1 #6, pp 142-152). Currently, a trio of melanoma vaccines based on allogeneic tumor cells are in late stages of development. Although none has demonstrated clearcut benefit over other modalities in the treatment of advanced melanoma, their promise may lie in the management of early disease.

A polyvalent allogeneic melanoma cell vaccine (CancerVax) containing high concentrations of six melanoma-associated antigens has been tested in phase I and II clinical trials at the John Wayne Cancer Center (Santa Monica, CA) with promising results in Stage IV metastatic melanoma. A phase III clinical trial of CancerVax is planned for 1997.

A partially purified, polyvalent, melanoma antigen vaccine constructed from material shed into culture medium by a pool of four melanoma cell lines, is under clinical evaluation at Kaplan Comprehensive Cancer Center at New York University Medical Center (New York, NY). Several phase II and phase III randomized clinical trials using various adjuvants (alum, QS-21, liposomal IL-2, and others) are in progress.

Ribi ImmunoChem Research (Hamilton, MT), is developing Melacine, a therapeutic vaccine exclusively targeting melanoma. Melacine consists of fractured pieces of two different kinds of human melanoma tumor cells (lysates), containing tumor-associated antigens combined with an immunostimulant (Detox). The tumor cell lines were licensed from the University of Southern California (USC; Los Angeles, CA). Detox adjuvant consists of endotoxin (monophosphoryl lipid A, MLA) from *Salmonella minnesota* and *Microbacterium phlei* cell wall skeleton (CWS). Melacine is freeze-dried and then reformulated with sterile water and administered as a simple injection. Melacine, elicits both humoral and cellular immunity. Currently, Mealcine is being investigated in a pivotal phase III study by the Southwest Oncology Group (SWOG; San Antonio, TX), sponsored in part by the National Cancer Institute, to determine if it would prevent recurrence of disease in Stage II melanoma patients who have had primary lesions surgically removed. Approximately 650 patients are to be randomized to either Melacine or observation-only follow-up as a control. The trial's primary endpoint is disease-free interval. Based on findings from a 140-patient phase III clinical trial (see FO, V1 #6, p 147), Ribi intents to file commercial product license applications for Melacine in the USA, Canada and the Europe in 1997.

In 1992 Ribi licensed to Biomira exclusive Canadian marketing rights to Melacine. Biomira will be responsible for conducting clinical trials and obtaining regulatory approval. In addition to license fees, Ribi will receive transfer payments for supplies of Melacine and will be entitled to royalties upon commercial sale of Melacine in Canada. In August 1993 Ribi ImmunoChem and Lidak Pharmaceuticals entered into an agreement under which Ribi has granted Lidak an experimental license to use Ribi's melanoma cell lines in the clinical development of Lidak's LMI technology. In return, Lidak has granted Ribi an option for an exclusive license to use the LMI technology with Ribi's melanoma cell lines.

Gene Transfer Into Tumor Cells

Genetic modification of tumor cells is being used to produce cancer vaccines with more defined biological effects. Understanding interactions that activate lymphocytes at the molecular level has led to identification of specific molecules such as cytokines, which are critical in regulating immunologic responses. Improved strategies for efficient gene transfer have enabled investigators to express particular genes encoding such immunologically active molecules within tumor cells. The two technical approaches to genetically modified human cancer vaccines include *ex vivo* gene transfer of cytokine and other genes into tumor cells, followed by reimplantation, as well as *in vivo* gene transfer of MHC class I molecules by direct physical application or injection of the gene to transfect tumor cells and/or surrounding normal tissues.

"Naked" plasmid DNA, directly injected into muscle and tumors, enters cells where the gene product is expressed without additional manipulation. Although this method of gene transfer has to date been relatively inefficient, with a low frequency of integration into host chromosomes, intensive development efforts in this area are producing new approaches that may improve efficiency of such vaccines. Eventually, naked DNA vaccines may prove superior to their conventional counterparts because they may be able to induce high levels of both antibody (humoral) and cellular immune responses, generating a broadly directed immune response. Also, they are more straightforward to manufacture than recombinant protein vaccines and easier to deliver to the patient. Naked DNA vaccines may also induce expression of antigens that resemble native tumor epitopes more closely than do standard vaccines because the host cell manufactures the epitope. It is too early to predict if naked DNA vaccines will prove safe and effective. One concern is insertional mutagenesis that may occur if DNA plasmids insert themselves into the host genome. Also it is not clear what would be the effect of prolonged expression of an antigen by the host. Currently, several small biotechnology companies in collaboration with large pharmaceutical partners, are developing naked DNA vaccines against viral and tumor targets.

Somatix Therapy (Alameda, CA), in collaboration with Bristol-Myers Squibb, is developing GVAX, a cancer vaccine based on an *ex vivo* approach involving extraction of tumor cells from patients which are then transduced with the gene for GM-CSF, irradiated and reinfused into patients to elicit an immune response (see FO, V1 #6, p 149 and V2 #2/3 p 323).

Therion Biologics (Cambridge, MA) is developing therapeutics for melanoma by inserting melanoma-associated antigens and/or other selected immunomodulators, such as cytokines, into tumor cells using its recombinant poxvirus vectors (see FO, V1 #6, p 150 and V2 #2/3 p 323).

Vical (San Diego) is developing Allovectin-7, a cancer vaccine that contains a gene that encodes a mismatched transplantation antigen (HLA-B7) which, when intralesionally injected, is intended to cause the malignant cells to bear the foreign antigen on their surface (see FO, V1 #2/3, p 54 and #6, p 150). Vical also developed naked DNA expression vectors, maximal expressing regulatable vectors (MERVs), that produced encoded proteins that were at levels over 200 times higher than obtainable by any DNA vectors previously reported. In addition, protein production from certain of these MERVs could either be turned on or off *in vivo* by the administration of simple chemical compounds.

POTENTIAL MARKETS FOR CANCER VACCINES

The market opportunity for cancer vaccines is enormous. Cancer vaccines may be used in a variety of ways:

- as monotherapy in advanced, metastatic disease when other therapies have failed
- as adjuvant therapy following surgery, radiation or chemotherapy in advanced disease
- as maintenance therapy to ensure active immune responses against future recurrences
- as preventive agents in selected populations at high risk, identified by hereditary factors, genetic screening, etc.
- as general immunization agents (based on somewhat selected populations using such criteria as age, family history, etc.)

Each application area is associated with its own broad technologic and clinical challenges as they relate to immunization against cancer, in general, and against certain cancers, in particular. Vaccines may be cancer type-specific, based on one antigen or may comprise cocktails of multiple antigens to ensure continued effectiveness against recurrence and metastasis in cases when cancers become resistant to single antigens. Vaccines may be intended as a cure or to provide long-term disease control. For instance, new developments in genetic screening that can identify patients at risk for heritable cancers, as is the case in breast, colorectal and other cancers, or more sensitive diagnostic screening that may identify latent cancer, as is the case in prostate cancer, are shifting emphasis in prevention, thus benefiting the outlook for tumor vaccines.

Based on an arbitrary vaccine cost per regimen (see Exhibit 8), a "steady state" market (reflecting at least 5 years of continuous marketing) for cancer vaccines by indication in North America, Europe and Japan, is presented in Exhibit 9. Vaccine costs are based on current regimen costs of standard drug therapies. However, developers of vaccines have announced plans to price their cancer vaccines as high as $30,000 per regimen if they prove truly therapeutic.

Exhibit 8
Estimated Costs of Immunization Regimens

Vaccine Type	Costs ($)
Therapeutic vaccine, administered once	2,500
Annual booster	1,000
Prophylactic vaccine, administered once	500
Annual booster	350

Exhibit 9
Potential Markets* for Tumor Vaccines for Selected Cancers in North America, Europe and Japan

Cancer	Therapeutic Vaccine ($mil.)	Therapeutic Vaccine with Annual Booster ($mil.)	Prophylactic Vaccine ($mil.)	Prophylactic Vaccine with Annual Booster ($mil.)
Bladder Cancer	12.6	156.9	72.2	274.3
Breast Cancer	78.8	604.4	262.8	998.6
Cervical Cancer	14.3	84.2	35.0	132.8
Colorectal Cancer	306.7	787.7	240.5	913.9
Lung Cancer	766.6	1,286.3	259.9	987.5
Melanoma	50.0	130.0	26.4	152.0
Non-Hodgkin's Lymphoma	42.5	179.6	68.6	260.6
Ovarian Cancer	71.8	118.1	23.1	88.0
Prostate Cancer	219.0	844.6	312.8	1,188.7
Renal Cancer	68.5	153.9	42.7	162.3

Source: NEW MEDICINE report #401 entitled, Cancer Vaccines: Technology, Products, Markets and Business Opportunities, June 1996

**Based on a "steady-state" market at least five years after introduction*

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FUTURE ONCOLOGY

PUBLISHED BY **NEW MEDICINE, INC.**

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SUBSCRIPTION INFORMATION:

- FUTURE ONCOLOGY (ISSN 1082-331X) is published as 10 issues (two double issues) per year, with a free annual index listing companies/institutions and subjects covered.
- A one-year subscription, (issues V2 #1 to V2 #12), sent first class to U.S. addresses is US $720. A one-year subscription, sent air mail to addresses outside the U.S., is US $780.
- One-year's subscription plus back issues (V1, #1-#12) is $1,300 (U.S.) and $1,390 (outside the U.S.).
- Additional subscriptions sent in the same envelope are $390 each.
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