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

CANCERS OF THE DIGESTIVE SYSTEM-PART I

MEETING COVERAGE

5TH INTERNATIONAL CONGRESS ON ANTI-CANCER CHEMOTHERAPY (ICACC), PARIS, FRANCE, JANUARY 31 TO FEBRUARY 3, 1995

MECHANISMS IN MALIGNANCY

APOPTOSIS

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

CANCERS OF THE DIGESTIVE SYSTEM-PART I

Digestive system cancers are comprised of malignancies of the digestive tract, including the esophagus, stomach, liver and intrahepatic passages, gallbladder and extrahepatic passages, pancreas, small intestine, colon and rectum and anus.

EPIDEMIOLOGY, ETIOLOGY, PATHOGENESIS BY TYPE OF CANCER

Digestive system cancers are prevalent worldwide; in 1993, an estimated 1,241,761 cases were diagnosed for the first time in the USA, Europe and Japan and were the cause of 638,087 deaths (Exhibit 1). In the USA, these cancers mostly occur in the aged; from 1986 to 1990, the median age was 71 years at diagnosis and 72 years at death. Changes in both incidence and mortality in the USA have been generally favorable (Exhibit 2). In contrast, mortality from these cancers increased substantially in Japan (Exhibit 3) where these cancers are particularly prevalent. Incidence and mortality statistics of digestive system cancer for the USA, Japan and Europe (excluding the countries of the former USSR) are presented in Exhibits 4, 5 and 6, respectively.

Exhibit 1
Digestive System Cancer Incidence and Deaths in the USA, Europe* and Japan in 1993

Cancer Type	Incidence (#)	Deaths (#)
Colorectal	644,487	232,046
Stomach	279,406	162,013
Esophagus, small intestine & other	120,993	63,203
Liver/biliary	101,084	90,522
Pancreas	95,791	90,303
Total	1,241,761	638,087

* Excludes the countries of the former USSR

Source: Compiled from individual region estimates, as shown in subsequent exhibits

Digestive system cancer is usually diagnosed after it has spread locally or distally; in the USA about 70% of newly diagnosed digestive system cancer has spread (Exhibit 7). Five-year survival rates in the USA range from 92.5% for those diagnosed with early stage colon cancer to 1.5% for those with metastatic pancreatic cancer (Exhibit 8); however, 5-year relative survival rates are rising (Exhibit 9).

Direct costs associated with the care of newly-diagnosed and terminal patients with digestive system cancer are estimated at about $5 billion in the USA (Exhibit 10). Approximately 253,000 hospital admissions in the USA in 1993 were for digestive system cancer (Exhibit 11).

Esophageal Cancer

Epidemiology. Esophageal cancer is a malignancy well known for its marked variation by geographic area (Exhibits 4, 5 and 6) and by race and gender in the USA (Exhibit 12). Esophageal cancer remains particularly lethal, and among white men in the USA mortality rates continue to climb. The overall 5-year survival rate, however, has increased to 9.8% for cases diagnosed between 1983 and 1990. This improvement is seen especially in the one- and two-year survival rates.

Squamous cell carcinoma is the most common malignant neoplasm of the esophagus worldwide. However, adenocarcinoma, the other major esophageal malignancy which disproportionately affects white men and rarely occurs in women, increased steadily from 1976 to 1987 at a rate greater than any other type of cancer. In contrast, there were relatively stable trends for squamous cell carcinoma of the esophagus and slight declines for adenocarcinoma elsewhere in the stomach (Blot WJ, etal, JAMA, 1991 Mar 13, 265(10):1287-9; comment JAMA, 1991 Jun 12;265 (22):2960).

Etiology and Pathogenesis. The etiology of esophageal cancer remains obscure. In the USA, alcohol and tobacco are recognized as the primary determinants of esophageal cancer, implicated in 80% to 90% of cases. Poor nutrition, in general, and reduced consumption of certain basic food groups, notably fruits and vegetables, in particular, have also been implicated in esophageal cancer. Differences in smoking, drinking, and dietary patterns among whites and blacks and males and females, as well as among socioeconomic groups, may partially explain the differences in incidence and mortality rates of esophageal cancer in these groups.

Both heavy alcohol ingestion and smoking are strongly and independently associated with an increased risk of squamous cell carcinoma; the effects of the two addictions are additive. However, neither were proven to increase the risk of adenocarcinoma, although recent data suggest a moderately increased risk. In a recent study comparing smoking and drinking habits of 173 hospitalized males with adenocarcinoma of the distal esophagus/cardia, the odds ratio (OR) for adenocarcinoma of the distal esophagus/ cardia for current smokers was 2.3 and that for ex-smokers was 1.9 relative to never-smokers; the OR for drinkers of four or more ounces of alcohol per day (relative to those consuming less than one drink per week) was 2.3. Intakes of total fat and low dietary vitamin A have also been identified as significant risk factors, and fiber intake was inversely associated with adenocarcinoma of the distal esophagus/cardia (Kabat GC, etal, Cancer Causes and Control, 1993 Mar, 4(2):123-32).

Esophageal cancer metastasizes by direct invasion through the layers of the esophagus and by spread to local and regional lymph nodes; in advanced cases the tumor may metastasize systemically to a variety of organs

including lung, liver and bone. Esophageal malignancies are known to occur with increased frequency among patients with other diseases, but the predisposing conditions vary for squamous cell carcinoma and adenocarcinoma. Squamous cell carcinoma is more common in patients with tylosis and Plummer-Vinson syndrome. In addition, the risk of squamous cell carcinoma increases after a long latent period of 30-50 years following ingestion of caustic substances, such as lye, that cause esophageal stricture. For example, in certain countries of Latin America, esophageal cancer has been linked to the carcinogenic effect of hot mate drinking. Also, people who have had other foregut cancers such as carcinoma of the tonsil or oral cavity, or those with carcinoma of the lung, have an increased risk of developing a second primary squamous cell carcinoma in the esophagus.

Exhibit 2
Changes in Incidence and Mortality of Selected Digestive System Cancers in the USA (1973-1991)

Cancer Type	Incidence (%)	Mortality (%)
Liver and intrahepatic passages	45.4	24.0
Esophageal	14.8	15.6
Colorectal	0.3	(15.5)
Pancreatic	(9.8)	(1.4)
Stomach	(26.0)	(32.1)

Source: The Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute (NCI) and the National Center for Health Statistics (NCHS)

Incidence of adenocarcinoma is particularly increased in patients who develop Barrett's esophagus, an acquired condition in which columnar (stomach-type) epithelium replaces squamous epithelium in the distal esophagus. Barrett's esophagus occurs almost exclusively in individuals with severe gastroesophageal reflux which exposes the esophagus to acidic stomach contents. In a study of 241 patients with Barrett's esophagus treated at the Lahey Clinic Medical Center (Burlington, MA) between January 1973 and January 1989, 65 presented with adenocarcinoma of the esophagus for a prevalence rate of 27%. Of 176 patients followed up for a total of 497 patient-years, adenocarcinoma developed in five patients for an incidence of one per 99 patient-years. (Williamson WA, etal, Archives of Internal Medicine, 1991 Nov, 151(11):2212-6).

Stomach Cancer

Today, adenocarcinoma of the stomach is the second most common cancer worldwide. However, it lends itself well to efforts directed at prevention, early detection, and intensive therapy.

Epidemiology. In the USA stomach cancer, ranks seventh in mortality and at least tenth in incidence, but in many countries it is the most common type of cancer. Worldwide, there are currently more cases of stomach cancer than any other except lung cancer; it is estimated that over 750,000 die annually from this disease worldwide. In particular, elevated stomach cancer mortality rates are encountered in Costa Rica and several other Latin American countries, Japan, the former Soviet Union and several Eastern European countries.

Rates of cancer occurring in the upper part of the stomach (the gastric cardia or gastro-esophageal junction) are increasing in the USA and western Europe (see esophageal cancer above). Reasons for the rising rates of gastric cardia cancer and for its occurrence more often in white men than in black men or in women are unknown.

Etiology and Pathogenesis (see Exhibit 13). The decline in stomach cancer rates over the past 60 years is credited in part to changes in methods of food preservation, refrigeration and freezing which replaced salting and smoking, reducing the nitrite content of food. The consumption of large quantities of fresh fruits and vegetables which contain vitamins and other compounds, shown experimentally to diminish the effects of carcinogens, may protect against stomach cancer. In addition, the decline in *Helicobacter pylori* infection also contributes to lower rates of stomach cancer in the developed countries.

Exhibit 3
Changes in Mortality Rate of Selected Digestive System Cancers in Japan (1975-1992)

Cancer Type	Mortality Rate in 1975	Mortality Rate in 1992
Stomach	44.8	38.9
Liver and intrahepatic passages	9.5	21.9
Small intestine	5.3	14.5
Colon	5.0	14.0
Gallbladder/ extrahepatic passages	4.0	10.5
Rectum	5.3	8.2
Esophagus	4.5	6.4
Pancreas	5.1	11.5

Source: Health and Welfare Statistics in Japan, Health and Welfare Statistics Association, 1994

A genetic influence in stomach cancer is suggested by an increased incidence in some families. Oncogenes associated with gastric carcinoma include c-Ha-ras, hst, v-raf, erb-b2, l-myc, and k-sam. Deranged DNA methylation, a dysfunction of the DNA regulatory process which may lead to decreased production of pepsinogen mRNA, also occurs in some stomach tumors and premalignant cells (Ichinose M, et al, Cancer Research, 1988, 48:1603). An association between gastric ulcers and cancer has been postulated but remains unproven.

Exhibit 4
Digestive System Cancer-Incidence and Deaths by Type in the USA in 1994

	Incidence					
Cancer Type	**Number (#)**	**Rate***	**Male (#)**	**Rate***	**Female (#)**	**Rate***
Total	233,300	90.0	123,100	97.7	110,200	82.7
Esophagus	11,000	4.2	8,000	6.3	3,000	2.3
Colon-Rectum	149,000	57.5	75,000	59.5	74,000	55.5
Colon	107,000	41.3	52,000	41.3	55,000	41.3
Rectum	42,000	16.2	23,000	18.3	19,000	14.3
Liver/ Biliary	16,100	6.2	8,800	7.0	7,300	5.5
Pancreas	27,000	10.4	13,000	10.3	14,000	10.5
Stomach	24,000	9.3	15,000	11.9	9,000	6.8
Small Intestine	3,600	1.4	2,000	1.6	1,600	1.2
Other	2,600	1.0	1,300		1,300	
	Deaths					
Cancer Type	**Number (#)**	**Rate ***	**Male (#)**	**Rate***	**Female (#)**	**Rate***
Total	121,450	46.8	64,550	51.2	56,900	42.7
Esophagus	10,400	4.0	7,800	6.2	2,600	2.0
Colon-Rectum	56,000	21.6	27,800	22.1	28,200	21.2
Colon	49,000	18.9	24,000	19.0	25,000	18.8
Rectum	7,000	2.7	3,800	3.0	3,200	2.4
Liver/ Biliary	13,200	5.1	7,200	5.7	6,000	4.5
Pancreas	25,900	10.0	2,400	9.8	13,500	10.1
Stomach	14,000	5.4	8,400	6.7	5,600	4.2
Small Intestine	950	0.4	500	0.4	450	0.3
Other	1,000	0.4	450	0.2	550	

* per 100,000

Source: Cancer Facts & Figures-1994, American Cancer Society and New Medicine

Over 90% of primary malignant gastric neoplasms are adenocarcinomas (ACS) which are divided into two major types, intestinal and diffuse. Both originate in the gastric mucosal lining. Prognosis is based on the most unfavorable microscopic element, in order of worse case scenario: tubular, papillary, mucinous, signet ring cell, and undifferentiated lesions. The pattern and frequency of spread is highly predictable. Local extension is common, with invasion through the wall and into adjacent organs (spleen, pancreas, bile duct, liver, and transverse colon). Direct extension into the duodenum accompanies 25% of distal tumors. Gastric cancer metastasizes to the liver via the portal vein frequently (about 40%) and is carried to various other organs such as lungs (20%), bone (10%), and rarely brain, kidney, and adrenals; the likelihood of metastasis correlates with the depth of invasion into the gastric wall and the extent of invasion into veins. Lymph node metastases occur when a tumor invades lymphatics within the gastric wall and spreads to regional lymph nodes and then to more distant lymph nodes, often involving the left supraclavicular and umbilical nodes. The rates of distant lymphatic metastasis for the intestinal versus diffuse neoplasms are equivalent (36% and 44%, respectively).

Liver/Biliary Cancer

It was generally believed that the incidence of secondary (metastatic) liver cancer far exceeded that of primary neoplasms. However, epidemiologic studies, originating from South Africa and Asia, indicate that primary neoplasms of the liver are in fact a major cause of cancer mortality among the population of these areas.

Epidemiology. Although relatively rare in the USA, cancer of the liver and intrahepatic bile duct is one of the leading causes of death in other parts of the world. This cancer is particularly common in Asia; extremely high mortality rates have been encountered in Hong Kong. A strong correlation between liver cancer and hepatitis B virus (HBV) infection may explain this cancer's frequency in Asia. However, because the liver is a common site of metastasis, it is often listed as the primary cause of death when in fact the primary cancer was of a different origin, resulting in inflated mortality rates for liver cancer worldwide. In the USA, mortality rates for cancer of the

Exhibit 5
Estimated Incidence and Mortality of Cancers of the Digestive System by Type in Japan in 1993*

	Incidence		Deaths			
Digestive System Cancers	**Number (#)**	**Rate***	**Number (#)**	**Male Rate***	**Female Rate***	**Total Rate***
Esophagus	8,405	6.7	7,947	10.8	2.1	6.4
Stomach	83,184	66.7	48,524	50.3	27.9	38.9
Small intestine	68,743	55.1	18,140	15.1	14.0	14.5
Colon-rectum						
Large intestine	38,256	30.7	17,519	14.5	13.6	14.0
Rectum	61,488	49.3	10,248	10.2	6.3	8.2
Liver	33,266	26.7	27,274	32.3	11.8	21.9
Gallbladder/extrahepatic	13,619	10.9	13,064	9.3	11.6	10.5
Pancreas	14,924	12.0	14,316	12.9	10.1	11.5
	TOTAL	321,885	258.0	157,032	47.7	125.9

* Death estimates are based on actual 1992 death rates applied to 1993 population numbers; incidence estimates were obtained based on USA death/incidence ratios.
* per 100,000

Source: Health and Welfare Statistics in Japan, Health and Welfare Statistics Association, 1994 and New Medicine

liver and intrahepatic bile duct have often exceeded incidence rates because metastatic liver cancer was erroneously reported as primary cause of death.

Biliary cancer is more common among females in most countries except Japan. High mortality rates are encountered in Japan and Central Europe; the lowest are in Britain and the USA. Of the approximately 16,000 new cases of cancer of liver and biliary passages discovered each year in the USA, only 2,000-3,000 are tumors of the extrahepatic bile ducts.

Etiology and Pathogenesis. While "primary liver cancer" is often used synonymously with primary hepatocellular carcinoma (HCC), there are a number of types of both benign and malignant primary liver tumors. Benign tumors include hepatocellular adenoma, hemangioma, infantile hemangioendothelioma, mixed hamartoma, and mesenchymal hamartoma. Malignant tumors include HCC as well as cholangiocarcinoma (cancer of the bile duct), angiosarcoma and, in children, hepatoblastoma. Among malignant tumors HCC is by far the most prevalent worldwide.

HCC is associated with a number of risk factors for hepatic damage, the most important being chronic HBV and hepatitis C virus (HCV) infection, alcoholic cirrhosis, exposure to aflatoxins, Thorotrast (a contrast medium used in radiologic procedures through the mid 1950s), and vinyl chloride, and use of steroid hormones (see Exhibit 14). The relationship between chronic HBV infection and HCC has been substantiated by over twenty years of investigation, including epidemiologic prospective and retrospective studies, perinatal transmission studies, studies of HBV marker expression in tissue and serum, studies of integration of HBV DNA in the host genome, and animal model studies. In high incidence areas where the predominant risk factor is HBV infection, the most effective way to prevent HCC is to prevent viral transmission.

The precise mechanism of carcinogenesis in HCC is unclear, but in experimentally induced liver tumors both an initiating event, affecting the cellular genome and a promoting event, affecting cell division, appear necessary (Farber E and Cameron R, Advances in Cancer Research, 1980, 31:125). It is clear that chemical carcinogens and chronic HBV infection are each capable of accomplishing both events. In the case of HBV, the virus can integrate into the host genome and also elicit a host response; viral integration near cellular proto-oncogenes in some tumors has suggested that the virus may "turn on" oncogenes (dominant oncogenesis). However, other studies have shown that viral integration in HCC shows no consistent pattern but rather occurs in random chromosomal locations in different tumors. Integrated viral DNA is often rearranged with nonfunctional viral genes. A "recessive oncogenesis" model has also been postulated in which tumor suppressor genes are inactivated. There is some evidence supporting the existence of such a mechanism in HCC (Buetow KH, Proceedings of the National Academy of Sciences of the USA, 1989, 86:8852). A heterogeneous collection of HCCs has shown loss of heterozygosity on chromosome 4q postulated to occur during integration and/or excision of HBV DNA. Alternatively, chronic HBV infection could increase the cell turnover rate, thereby increasing the likelihood of a point mutation or chromosomal recombination.

In addition to being a site for primary tumor development, the liver is frequently the site of metastatic cancer from virtually any part of the body in which cancer can arise. In fact, most common neoplasms spread to the

liver by time of death. In an autopsy series performed at the Roswell Park Memorial Institute (Buffalo, NY) in which 10,736 extrahepatic primary tumors were identified, metastases to the liver were present in 41% of cases (Pickren JW, et al, in Liver Metastases, Weiss L and Gilbert HA eds, GK Medical Publishers, Boston, 1982, p2).

Exhibit 6
Incidence and Mortality of Digestive System Cancer in Europe (1993)*

Cancer Type	Incidence (#)	Rate**	Deaths	Rate**
Esophagus	26,445	2.5	24,991	0.5
Stomach	172,222	9.9	99,887	3.4
Colorectal	392,743	14.7	147,279	7.8
Liver	22,614	1.8	18,521	0.5
Gallbladder & bile ducts	19,878	1.9	19,063	0.4
Pancreas	53,167	5.1	50,987	1.1
Total	687,069	35.9	360,728	13.7

* Based on 1989 death rate data and USA incidence to death ratios; European population is estimated at 502,500,000, excluding the countries that made up the former USSR.

** per 100,000

Cholangiocarcinoma is commonly associated with oriental liver fluke infestations in China and parts of southeastern Asia. Liver flukes may cause an inflammatory reaction within the bile duct which is then sensitized to carcinogens excreted by flukes (Bismuth H and Malt RA, New England Journal of Medicine, 1979, 301:704). Also, approximately 10%-20% of patients with symptomatic bile duct cysts will develop carcinoma of the bile duct. Again, an inflammatory process is believed to precede the development of cancer, perhaps from reflux of pancreatic juice into the biliary tree and cyst (Suda K, et al, Cancer, 1983, 52:2086). Although the incidence of cholangiocarcinoma is more frequent among patients with ulcerative colitis, most patients with this condition do not develop bile duct cancer. Potential carcinogens may leak through the inflamed colon wall into the portal circulation and be excreted through the biliary tract. This mechanism may also be implicated in sclerosing cholangitis, which has a well documented association with both ulcerative colitis and bile duct cancer.

Pancreatic Cancer

Ductal adenocarcinoma, comprising 90% of pancreatic cancers, is a relentlessly progressive and fatal disease.

Epidemiology. Cancer of the pancreas accounts for only two percent of all cancer diagnosed in the USA annually, but is responsible for close to 5% of all cancer deaths. This cancer is often difficult to diagnose in its early stages because of a lack of early symptoms or signs of disease. As a result, in about one-half of all cases, the cancer has already spread to other organs at the time of diagnosis. The five-year survival rate of 3% remains the poorest of any malignancy. In recent years overall incidence rates for cancer of the pancreas appear to have declined slightly (1.7% per year), driven largely by rate declines among white men. Reasons for this decline remain obscure.

Etiology and Pathogenesis. The etiology of pancreatic cancer is uncertain but smoking, exposure to chemical agents, and dietary factors seem to play a role (see Exhibit 15). The importance of this cancer is magnified by the fact that fewer than 5% of patients are cured, and over 90% die within 2 years of diagnosis. Problems of obstructive jaundice, cholangitis, pain, weight loss, and malnutrition are common in the latter stages of the disease.

Gallbladder Cancer

Epidemiology. The 6,000 new cases of gallbladder cancer diagnosed each year in the USA represent slightly fewer than 3% of all patients who are explored for anticipated benign disease of the gallbladder and biliary tree.

Exhibit 7
Stage of Digestive System Cancer by Type at Time of Diagnosis in the USA

	Early/ Localized		Regional		Metastatic		Unstaged	
Cancer Type	Number (#)	Total (%)	Number (#)	Total (%)	Number (#)	Total (%)	Number (#)	Total (%)
Esophagus	2,750	25.0	2,530	23.0	2,970	27.0	2,750	25.0
Colon	37,450	35.0	41,730	39.0	22,470	21.0	5,350	5.0
Rectum	17,220	41.0	14,700	35.0	6,720	16.0	3,360	8.0
Liver/biliary	3,381	21.0	3,703	23.0	4,025	25.0	4,991	31.0
Pancreas	2,430	9.0	5,940	22.0	13,230	49.0	5,400	20.0
Stomach	4,080	17.0	7,920	33.0	8,640	36.0	3,360	14.0
Total	67,311	29.6	76,523	33.7	58,055	25.6	25,211	11.1

Source: The Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute (NCI) and New Medicine

Exhibit 8
Estimated Five-Year Survival Rates of Digestive System Cancer by Type in the USA

	Early/ Localized		Regional		Metastatic		Unstaged		Total	
Cancer Type	Number (#)	Total (%)	Number (#)	Total (%)	Number (#)	Total (%)	Number (#)	Total (%)	Number (#)	Total (%)
Esophagus	556	20.2	185	7.3	65	2.2	223	8.1	1,028	9.3
Colon	34,641	92.5	26,248	62.9	1,528	6.8	1,621	30.3	64,038	59.8
Rectum	14,913	86.6	7,718	52.5	356	5.3	1,223	36.4	24,209	57.6
Liver/ Biliary	494	14.6	248	6.7	101	2.5	135	2.7	977	6.1
Pancreas	209	8.6	244	4.1	198	1.5	243	4.5	894	3.3
Stomach	2,391	58.6	1,568	19.8	207	2.4	336	10.0	4,502	18.8
Total	53,204	79.0	36,211	47.3	2,455	4.2	3,781	15.0	95,648	42.1

* based on cancers diagnosed in 1990

Source: The Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute (NCI) and New Medicine

As with benign gallbladder disease, there is a predominance of female cancer patients with a female:male ratio of about 2:1. Gallbladder cancer usually occurs in the seventh decade of life (median age 62-66 years); males tend to be slightly older than females.

Etiology and Pathogenesis. The origins of gallbladder carcinoma remain obscure. Approximately half of patients have a previous history of chronic cholecystitis. Although chronic cholecystitis is associated with gallstones, the presence of gallstones is not required for the development of adenocarcinoma. For instance, carcinoma of the gallbladder is quite common in Hong Kong where cholesterol gallstones are relatively uncommon and may be linked to the high incidence of pyogenic and parasitic biliary tract diseases among the indigenous Chinese population.

Over 90% of the carcinomas of the gallbladder are columnar cell carcinomas with a pattern similar to that in bile ducts. Papillary adenocarcinomas and tumors with an acinar tubular pattern, containing varying degrees of connective tissue stroma, are also seen. Occasionally, columnar cells produce mucin in abundance or produce a signet ring appearance. Other forms of carcinomas include squamous cell carcinoma or a mixed adenosquamous tumor sometimes called an adenoacanthoma.

Colorectal Cancer

Epidemiology. Colorectal cancer is a major cause of death in developed countries. It is diagnosed in more than 149,000 Americans annually, making it the fourth most common cancer in the USA (Exhibit 4). Incidence rates do not vary significantly by gender but rise with increasing age; rates increase from 0.2 per 100,000 for ages 15 to 19 to 518.3 for those 85 and over. Even though colorectal cancer increased significantly for both white and black men and black women between 1973 and 1990, from 1986 to 1990 there was a significant drop in the rates for white men and women, possibly attributed to the implementation of endoscopic screening and removal of benign polyps. According to the NCHS, inpatient endoscopic polypectomy procedures increased dramatically in the USA from 48,000 in 1989 to 85,000 in 1993. Mortality rates also declined in the 1973-1990 period and survival rates have increased for both men and women. About 90% of colorectal cancer patients diagnosed while the cancer is still confined to the colon or rectum are alive 5 years post-diagnosis.

Exhibit 9
Changes in Five-Year Survival of Patients with Digestive System Cancer in the USA

	5-year Survival Rates - All stages		
Type of Cancer	1983-1990 (%)	1983-1989 (%)	Change (%)
Esophagus	9.4	8.5	9.8
Colon	59.8	58.7	2.0
Rectum	57.6	56.4	2.2
Liver/Biliary	6.1	5.9	2.2
Pancreas	3.3	3.3	(1.1)
Stomach	18.8	17.5	7.1

Source: The Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute (NCI)

Mortality rates from colon cancer are elevated in both sexes in most developed countries. The highest mortality rates, over 25 per 100,000 in males and over 15 per 100,000 in females, have been observed in Central and Northern Europe and in New Zealand. In contrast, rates up to tenfold lower were encountered in Latin America and in some Asian and African countries. Although part of this discrepancy may be attributed to problems in diagnosis and certification, the rates are consistent with a role for dietary factors such as high fat consumption in the etiology of colorectal cancer.

Etiology and Pathogenesis (see Exhibit 16). Environmental and dietary factors are thought to contribute to 85% to 90% of all colorectal cancers. Diet affects the biochemical composition of fecal content, thereby altering the milieu for colonic mucosal cells and changing the rate and pattern of their proliferation. Increased dietary fat may cause the secretion of increased amounts of secondary bile acids, which may act as tumor promoters or co-carcinogens. Alternatively, they may raise intraluminal pH, which is also postulated to be a factor in increased carcinogenesis. Dietary fiber may exert its beneficial effect by reversing the adverse biochemical consequences of saturated fat and secondary bile acids, by reducing both fecal bile acid levels and colonic pH. Dietary fiber also reduces colon transit time, resulting in decreased contact time between putative carcinogens in fecal content and the colon, and in decreased time for gut microflora to generate possible carcinogens. Lower-fat diets in the USA may account for part of the recent decline in colorectal cancer incidence.

The inherited proclivity for colon cancer may be divided into polyposis and nonpolyposis types. The polyposis type is infrequent and manifests at an earlier age than the non-polyposis type. Examples include familial adenomatous polyposis (FAP), Gardner's syndrome (GS), Turcot's syndrome (TS), Peutz-Jegher's syndrome, and juvenile polyposis. The first three of these syndromes are associated with an inherited defect in a genetic locus on chromosome 5, the APC (adenomatosis polyposis coli) locus, while the genetic basis of the other two syndromes is unknown. The APC locus itself appears to be central to the development of colon cancer. Not only are inherited mutations in APC associated with FAP, GS, TS, but sporadic mutations are also seen in 90% of all colon cancers. Further, APC mutations are the earliest genetic changes identified in the development of sporadic colon cancers. The frequency of mutations in the APC gene is equally high in (early) benign adenomas and in advanced colon cancers. Together, these observations suggest that APC acts as the "gate keeper" of colon cancer; defects in APC allow for the development of premalignant colorectal tumors.

Exhibit 10
Estimated Direct Expenses for Initial and Terminal Care of Colon Cancer and Digestive System Cancer Patients in the USA in 1994

Stage of Colon Cancer	Cost per Case (6 mos. duration)	# of Colon Cases	# of All Digestive System Cancer	Direct Costs of Colon Cancer ($ 000)	Direct Costs of All Digestive System Cancer* ($ 000)
Local	13,848	37,450	66,001	518,607.6	913,981.8
Regional	15,398	41,700	77,453	642,096.6	1,192,621.3
Distant	17,233	22,470	57,796	387,225.5	995,998.5
Unstaged	15,398	5,350	25,850	82,379.3	398,038.3
Subtotal	15,241	106,970	227,100	1,630,309.0	3,500,639.9
Terminal	12,110	17,519	121,450	212,155.1	1,470,759.5
Total				1,842,464.1	4,971,399.4

* based on direct costs estimated for colon cancer

Source: Taplin, SH, etal, Journal of the National Cancer Institute, Vol. 87, N0. 6, March 15, 1995 and New Medicine

Exhibit 11
Hospitalizations for Digestive System Cancer in the USA (1989-1993)

ICD-9-CM	Cancer Type	1989	1990	1991	1992	1993
150.	Esophageal	14,000	17,000	20,000	21,000	18,000
151.	Stomach	29,000	23,000	26,000	24,000	26,000
153.	Colon	114,000	115,000	113,000	108,000	111,000
154.	Anorectal	47,000	55,000	52,000	48,000	43,000
155.	Liver/intrahepatic	8,000	10,000	12,000	12,000	16,000
155.0	Primary liver	N/A	N/A	N/A	N/A	12,000
156.	Gallbladder/ extrahepatic	10,000	11,000	8,000	8,000	8,000
157.	Pancreatic	31,000	27,000	41,000	35,000	29,000
	Total	254,989	259,990	273,991	257,992	252,993

Source: The National Hospital Discharge Survey of the National Center for Health Statistics (NCHS)

Studies of familial cancer led to the identification of non-polyposis-type of hereditary colon cancer, now known as hereditary nonpolyposis colorectal cancer (HNPCC). HNPCC may account for as much as 6 to 15% of colorectal cancers (Lynch HT, et al, Gastroenterology, 1993, 104: 1535). HNPCC is divided into a site-specific type (Lynch syndrome I) and a cancer family syndrome type (Lynch syndrome II). Lynch syndrome I is characterized by autosomal dominant inheritance, early age of onset, predilection to the proximal colon, and multiple primary (metachronous and synchronous) colon tumors. Lynch syndrome II, in addition to all the characteristics of syndrome I, includes multiple primary adenocarcinomas in the stomach, colon, endometrium, ovary, and most strikingly (because its occurrence is otherwise rare), adenocarcinoma of the small bowel.

Exhibit 12
Profile of Esophageal Cancer

Type and Site	**Comments**
Squamous cell	
Middle third of the esophagus	50% of cases
Lower third of the esophagus	25% of cases
Adenocarcinoma	The majority of adenocarcinomas are located in the distal esophagus and may arise in Barrett's esophagus
Sex & Race	**Incidence**
Males	6.4 per 100,000
Females	1.9 per 100,000
Whites	4.8 per 100,000
Males	5.4 per 100,000; 44% adenocarcinomas
Females	1.7 per 100,000; 19% adenocarcinomas
Blacks	10.8 per 100,000
Males	18.4 per 100,000; <3% adenocarcinomas
Females	4.9 per 100,000; 3% adenocarcinomas

A phenomenon that characterizes HNPCC tumors is microsatellite instability, associated with DNA replication error (RER). Recently, *in vitro* studies on colon cancer cell lines with and without RER have shown that the DNA mutation rate is at least 100-fold greater in RER than in non-RER cells (Parsons R, et al, Cell, 1993, 75:1227). The defect in RER cells is in the repair of DNA replicating errors (mismatch repair). It is noteworthy that homologs of the HNPCC-associated genes are involved in DNA mismatch in bacteria. Since defective mismatch repair was not observed in the lymphoblasts of individuals with a germline mutation in only one copy of MSH2, the observed mutator phenotype of HNPCC tumor cells results from a defect in mismatch repair brought about by homozygosity, but not heterozygosity, of mutations in these genes. In 1994, a second HNPCC gene, MLH1, was identified which normally encodes proteins that correct mistakes in new DNA; a faulty version of this gene may account for 25% of HNPCC cases.

Recent evidence indicates that most if not all of adenocarcinomas begin as adenomatous polyps, and that the progression from adenomas to carcinoma occurs by the sequential accumulation of genetic changes (Fearon E, et al, Cell, 1990, 61: 759; Ende DA, et al, 37th Annual Clinical Conference and 26th Annual Special Pathology Program, 4-7 November 1993, Houston, TX). This model implies that alterations occur at several critical genetic loci and lead to the expression of the transformed state. These genetic loci encode for either tumor suppressor genes or proto-oncogenes. Tumor suppressor genes generally provide normal regulatory control of development and growth. Loss or mutation of such a gene, which usually must occur in both alleles (homozygous loss), leads to disregulated growth and cancer. This can occur in both sporadic and inherited forms of the same cancer. Proto-oncogenes, on the other hand, have a normal physiologic role in promoting cell growth. Unrestrained expression and eventual progression to cancer may occur if a proto-oncogene is altered either by environmental factors or by mutation.

Activation of one gene leading to increased proliferation (i.e., proto-oncogene activation) may be insufficient, by itself, to lead to cancer. Colon cancer often results from both oncogene activation and tumor suppressor gene loss or inactivation, acting in concert. For example, the proto-oncogene ras is frequently activated through point mutations in colon cancer, and inactivation of the tumor suppressor gene at the APC locus is observed in both familial and sporadic colon cancer, as discussed above. Similar allelic loss of the DCC (delected in colon cancer) locus on chromosome 18 and the p53 locus on chromosome 17 has been described in a spectrum of tumor states. [The product of the p53 gene appears both to prevent genetic instability in the cell and to cause apoptosis in response to DNA damage. It is likely that loss of one or both of these functions play a role in the development of colon cancer.] In addition, altered DNA methylation is seen in the earliest forms of colon cancer, although it is not clear if this is a cause, rather than a result, of cancerous transformation. Experiments in mice genetically predisposed to polyposis have shown that decreased DNA methylation is associated with a lower number of precancerous polyps (Laird PW, et al., Cell, 1995, 81:1).

Mutations in the DNA which occur during progression from normal tissue to colon cancer are postulated to occur through a series of steps. First, homozygous loss of the tumor suppressor genes on chromosome 5 (APC) leads to the development of polyps. This is followed by activation of the ras proto-oncogene, and subsequent homozygous loss of tumor suppressor genes on chromosomes 18 (DCC) and 17 (p53). Persons with familial HNPCC have defects in genes that repair DNA, as described above, and defective DNA repair may lead to an

increased mutation rate and to faster progression through the sequential steps of colon carcinogenesis. Approximately one American in 200 inherits faulty versions of either MSH2 or MLH1. These people face a 70 to 90% chance of developing colon cancer during their lifetime.

Prevention. Minimizing exposure to predisposing elements in the diet and environment is the first step in colorectal cancer prevention. Considerable evidence suggests a protective effect of dietary fiber on colon carcinogenesis, although further research is needed to understand this effect. Fiber is a term used to describe a complex mixture of substances with different chemical compositions, and there exists the possibility that dietary fiber - depending on the type of fiber - might actually promote colon tumor growth (Potter JD, et al, Journal of the National Cancer Institute, 1986, 76:557). Dietary fibers are polysaccharides that may be classified according to solubility (soluble vs. insoluble in the small intestine). Insoluble fiber passes through the digestive tract largely unmetabolized and is excreted in the feces. Because this type of fiber binds water, it enhances fecal bulking, laxation, absorption of colonic fluid and electrolytes, and it reduces fecal transit time. Thus, insoluble fiber (e.g., wheat bran and cellulose) may be more effective than other fibers in protecting against chemically induced colon cancer. In contrast to insoluble-fiber, soluble-fiber polysaccharides tend to slow upper intestinal tract transit and do not increase stool mass. This alteration in transit time appears to enhance steroid excretion and interfere with lipid absorption, making them effective for lowering serum cholesterol in some cases. Soluble fiber is also 50% to 80% fermented in the colon, producing short-chain fatty acids (SCFAs). These acids, in turn, lower intestinal pH and increase intestinal cell proliferation. Foods rich primarily in soluble-fiber polysaccharides (pectin and gum) have been reported to increase the concentration of bile acids in feces. These conditions are believed to favor carcinogenesis.

Cancer of the Small Intestine

Although the small intestine accounts for almost 90% of the mucosal surface of the digestive system, fewer intestinal neoplasms develop there. The reasons for this

Exhibit 13
Profile of Stomach Cancer

Types	Description
Intestinal type	Associated with chronic atrophic gastritis and intestinal metaplasia of the gastric mucosa; incidence is declining among whites in the USA and in Canada, Australia, and New Zealand
Diffuse type	Often associated with cells of signet ring morphology; incidence increasing in both low and high risk regions
Etiology	
Intestinal	Diet and environment are important factors; postulated to be the end result of a complex multiple-hit process proceeding from gastritis to atrophy to dysplasia
Initial stages of gastritis and atrophy	Linked to excessive salt intake, use of cured and smoked foods and infection with *H. pylori*
Intermediate stages	Ingestion of ascorbic acid and nitrite, determinants of intragastric nitrosation (nitrosating agents are candidate carcinogens; may originate in the gastric cavity or in the inflammatory infiltrate)
Final stages	Linked with β-carotene and with excessive salt intake from food
Diffuse	Diet has little, if any, influence on incidence
Tobacco and alcohol	Consumption of alcoholic beverages and tobacco are high risk factors, especially in older persons; smokers experience a 40% higher risk compared to nonsmokers
Occupational exposure	Increased risk with exposure to high doses of ionizing radiation and occupational hazards (probably account for a small percentage of cases)
Genetic factors	In young patients, 94% of the cases are of the diffuse type and, in 13%, there is evidence of genetic predisposition; also a strong association with blood group A (Mecklin JP, Scandinavian Journal of Gastroenterology, 1988, 23:307); however, the inter-relationship between genetic factors, immune pathology and cancer is poorly understood
Site	**Incidence**
Entire organ (diffuse involvement)	10%-20%
Lower	30%-65%
Middle (half body and half fundus)	39%
Upper	16%; cardia cancers accounted for about 50% of all stomach cancers with specified subsites

Sources: Correa P., Cancer Research, 1992 Dec 15, 52(24):6735-40 and New Medicine

Exhibit 14
Profile of Liver Cancer

Etiology	Comments
Hepatitis B (HBV) and C (HCV) virus	HBV infection is the etiologic agent for 75%-90% of HCC on a worldwide basis; HCV also contributes to the incidence of HCC but its impact remains unclear
Cirrhosis	An estimated 60-90% of HCC occurs in cirrhotic livers
Low-incidence areas	HCC also occurs as an end result of chronic alcoholic micronodular cirrhosis; in the USA, alcoholic cirrhosis is the major risk factor for HCC
High-incidence areas	The majority of HCC cases are related to HBV; macronodular cirrhosis is often discovered during HCC diagnosis but is not alcohol related
Aflatoxin B1	Epidemiologic evidence supports aflatoxin B1, a naturally occurring toxin and potent carcinogen, as a cofactor in causation of HCC
Thorotrast (colloidal 232 thorium dioxide)	An x-ray radiopaque contrast medium used for cerebral angiography and liver-spleen scans in Japan and Europe from 1930 to 1955; latency period from exposure to tumor development is 20-25 years; those exposed are 47 times more likely to develop liver cancer
Polyvinyl chloride (PVC)	A carcinogen used in industry; induces angiosarcoma of the liver
Drugs	
Contraceptives	Oral contraceptive (OC) use is associated with benign hepatic adenomas; the association between OC and HCC is less clear; OC use of more than five years may pose a higher risk, as may use of OCs containing mestranol (replaced by other types of OCs in the late 1970s)
Anabolic steroids	Anabolic steroids, principally oxymetholone, are associated with HCC as well as peliosis hepatitis and cholangiocarcinoma; tumors develop after prolonged use and often are not very aggressive
Excess iron stores (siderosis)	Siderosis may predispose to the development of HCC, particularly in persons infected with HBV (London WT, et al, 1990 International Symposium on Viral Hepatitis and Liver Diseases, 220:629)

may include rapid transit of content through the small bowel, local protective mechanisms, or the relative lack of carcinogens in contact with mucosal surfaces. Hallmarks of cancer of the small intestine are summarized in Exhibit 17.

Epidemiology. Fewer than 5% of all digestive system tumors and only 1% of all malignancies arise in the small intestine; in 1994, it is estimated that 3,600 new cancers of the small intestine were diagnosed in the USA. Benign tumors of the small intestine include leiomyoma, adenoma, and lipoma, with rare tumors including fibromas, fibromyxomas, neurofibromas, ganglioneuromas, hemangiomas, and lymphangiomas. Malignant tumors include adenocarcinomas, carcinoids, lymphomas, sarcomas and their subtypes, and other rare tumor types such as Kaposi's sarcoma, seen primarily in patients with end-stage HIV infection. Adenocarcinomas are primarily found in the proximal small intestine. Some have postulated that the richness of IgA secreting cells in the ileum that neutralize luminal carcinogens, account for its relative sparing from adenocarcinoma. Others have noted that the abundance of the enzyme benzopyrene hydroxylase may play a protective role by detoxifying potential carcinogens, or perhaps the alkaline content of the small intestine and the rapid turnover time of its mucosal cells may help prevent carcinogenesis.

Etiology and Pathogenesis. The etiology of adenocarcinoma of the small intestine is uncertain, although it has been associated with nontropical (celiac) sprue and Crohn's disease; unlike colon cancer, there exists less evidence to indict Crohn's disease as a cause of adenocarcinoma of the small intestine. Celiac sprue predisposes to intestinal lymphoma and has also been associated with adenocarcinoma. Gluten-mediated jejunoileitis is also an independent risk factor for the development of adenocarcinoma and lymphoma of the small intestine.

Adenocarcinoma can spread via lymphatics, blood circulation (to liver, lungs, and bone most commonly) or by direct extension through the serosal surfaces and into the peritoneal cavity. Because of their insidious nature, adenocarcinomas are diagnosed in late stages when either lymph node involvement or distant metastases are present. In one series, up to 70% of cases had spread beyond local sites (Brophy C and Cahow CE, American Surgeon, 1989, 55:408). One other series noted 67% node involvement and 22% distant metastases (Ouriel K and Adams JT, American Journal of Surgery, 1984, 147:66). Sarcomas, on the other hand, tend to spread primarily by local extension growing into the mesentery or surrounding serosal surfaces. Vascular metastases are common but lymphatic spread is unusual. Histologic types include leiomyosarcoma, which is by far the most common sarcoma of the small intestine; angiosarcoma, a rapidly growing tumor with a poor prognosis; fibrosarcoma and liposarcoma, both of which carry an intermediate prognosis; and sarcomas of the neural tissue, which are rare except in patients with neurofibromatosis (von Recklinghausen's disease).

Exhibit 15
Profile of Cancer of the Pancreas

Parameter	Incidence
Staging By Type (primary neoplasms)	50% metastatic
Ductal adenocarcinoma	90%
Carcinomas of the exocrine or endocrine parenchyma	10%
Epidemiology	
Race	Incidence in blacks was 56% higher than in whites (13.8 versus 8.8 per 100,000) in the 1986-90 period
Gender	Mortality is higher in men (male:female ratio 1.4:1.0 in the 1986-90 period)
Age	Median age at diagnosis is 71 years
Etiology	**Comments**
Tobacco	Relative risk for smokers (more than a pack a day) four times that of nonsmokers; may account for 27% of pancreatic cancers in the USA
Alcohol	No correlation
Coffee	Correlation weak
Nitrosamines nitrosoureas and other industrial contaminants (benzidine, betanapthylamine derivatives, and metal dusts)	Although causal in animals, relationship has been difficult to establish in humans; increased proportion of deaths due to pancreatic cancer among members of the American Chemical Society
Diet	
Consumption of fresh fruits and vegetables	Associated with decreased risk in a number of studies (Warshaw, AL and Castillo, CF, NEJM 1992; 326:455-465
Fat and carbohydrate consumption	May increase the risk of developing pancreatic cancer
Medical conditions	Diabetes, pancreatitis, and gallbladder disease have been linked to pancreatic cancer, although associations not consistent in all studies

Anal Cancer

Cancer arising in the anal canal formerly was treated with colostomy and loss of the anal sphincter, and had an uncertain prognosis. The advent of successful multimodality therapy has allowed sphincter preservation, even for large primary tumors.

Epidemiology. Cancer in the anal region is rare, with only 1-2% of large bowel tumors arising in this area. Most commonly seen in middle age (50-60 years), historically, it has been more common in females than in males (M:F ratio about 2:1). However, during the last decade, there has been an increase in incidence in men under 45 years in the USA, reversing the gender ratio in this younger group.

Etiology and Pathogenesis. Cancers in the anal region may arise either in the anal margin (perianal cancer) or in the anal canal. Tumors involving the dentate line are considered anal canal tumors. Approximately 1-1.5 cm superior to the dentate line lies the anorectal ring; tumors above this level are considered rectal tumors. Between the dentate line and the anal verge tumors have been considered either canal or margin tumors depending on institutional preference. In the majority of patients with carcinoma of the anus no etiologic factor has been identified, although a history of anal-receptive intercourse in men (but not in women) appears to be strongly associated with anal cancer (relative risk 33.1 compared with controls). This association as well as the increased incidence in young men argue that anal sexual activity is a risk factor for anal cancer.

One recently identified potential causative agent is the human papilloma virus (HPV). There is a relationship between HPV and the development of genital warts (condylomata acuminata), which can evolve into anal cancer within 5-40 years. Specifically, DNA from HPV type 16 has been found most frequently in studies of anal squamous cell carcinoma. Immunosuppression may also contribute to both HPV infection, and anal intraepithelial neoplasia, a premalignant condition. A dramatic 100-fold increase in anogenital tumors has been observed in kidney transplant patients (who also have a high incidence of condylomata and herpes genitalis) compared with the non-transplanted population. Other weaker associations include herpes simplex virus type 2 and chlamydia trachomatis in women, and gonorrhea in men. Anal canal carcinomas have also been associated with prior irradiation and many benign conditions such as fistulae, fissures, abscesses, hemorrhoids, and lymphogranuloma venereum, but rigorous studies of relative risk have not been reported. Smoking has also been pinpointed as a major risk factor, with a relative risk of 7.7 in women and 9.4 in men. HIV as a causative agent has been suggested in homosexual men, but large studies of HIV-associated tumors suggest that anal tumors are extremely rare.

Histologically, there are many rare malignant cell types that occur in the anal canal, but the majority, approximately two-thirds, are variants of squamous cell carcinoma. Anal canal tumors spread locally in the submucosa. They may also extend deeply into the sphincter muscles, and spread beyond into adjacent organs such as the vagina, urethra, or bladder. The perianal and pelvic areas are rich in lymphatic channels, and regional lymph node metastases may occur even with small primary tumors. Overall, mesenteric nodes are involved in one-third to one-half of patients with anal canal cancers; and pelvic and inguinal node metastases each occur in one-third. Hematogenous spread is uncommon, but when it does occur, is equally likely to metastasize to liver and lung.

Next issue: *Diagnostic and therapeutic modalities for each major digestive system cancer, approved chemotherapeutics in combination, new drugs in development for digestive system cancers (including a comprehensive database of agents in development and profiles of leading commercial developers), adjunct therapies, novel delivery systems, and much more.*

MEETING COVERAGE

5TH INTERNATIONAL CONGRESS ON ANTI-CANCER CHEMOTHERAPY (ICACC)

PARIS, FRANCE, JANUARY 31 TO FEBRUARY 3, 1995

- To date, chemotherapy for the treatment of gastrointestinal (GI) cancers has not been very effective. Only a few drugs, such as 5-fluorouracil (5-FU) for all GI cancers and cisplatin, anthracyclines, and methotrexate (MTX) for gastric cancer, have demonstrated a limited efficacy. There is a clear need, therefore, for effective new drugs in the management of GI cancers.

- Platinum-based chemotherapeutics are being used increasingly in combination therapy for a variety of solid tumors. Newer platinum complexes in clinical trials and preclinical development promise to overcome some of the problems of older drugs, such as toxic side effects, need for parenteral delivery and development of tumor drug resistance [based on a presentation by K. R. Harrap, CRC Centre for Cancer Therapeutics at the Institute of Cancer Research (Sutton, Surrey, UK)].

NEW CHEMOTHERAPEUTIC APPROACHES IN THE TREATMENT OF GASTROINTESTINAL CANCER

Esophageal Cancer

Traditional unimodality therapy cures very few patients with advanced disease. A number of multimodality trials have now been completed in esophageal cancer in an effort to improve upon single modality results. These include chemotherapy followed by surgery and, more recently, the preoperative use of concurrent chemotherapy and radiation therapy.

Use of 5-FU and cisplatin followed by surgery in patients with locally advanced esophageal squamous cell cancer (LAESCC) results in five-year survival rates comparable to those generally obtainable in early stage disease. Over a five year period, 154 patients with LAESCC (mediastinal spread—58%, tracheo-bronchial tree involvement—56%, laryngeal nerve palsy—15%) were treated with cisplatin (Platinol, Bristol-Myers Squibb) 100 mg/m^2 on day 1 and 5-FU (Fluorouracil, Roche) 1 gm/m^2 as a 24 hour infusion on days 1-5, every 22 days. In 144 patients, there were 10 (6.9%) complete responses (CRs), 46 (31.9%) partial responses (PRs) and 27 minor responses (18.7%). Overall, 38.8% of patients with LAESCC treated with combination chemotherapy had a significant clinical response. After chemotherapy, 78 individuals with resectable disease underwent surgery (50 radical and 28 palliative procedures). Postsurgery mortality was 10.3%. Patients who underwent surgery had a five-year survival rate of 32.9% versus 5.02% in those with unresected disease. The 5-year survival rate was 40.6% in radically resected individuals and 19.2% in those whose surgery was palliative (Chiarion-Sileni V, etal, Abstracts of the 5th ICACC, Pg. 128:O 397).

Concomitant chemo-radiotherapy followed by surgery led to a 3-year survival of 48%, suggesting that this aggressive approach may be effective in young patients with a good performance status who are responders to chemo-radiotherapy. A total of 119 patients with locally advanced esophageal carcinomas were treated with a combination of 5-FU and cisplatin and radiotherapy. After evaluation, patients underwent surgery or received a last cycle of chemo-radiotherapy. Total radiation dose was 60 to 65 Gy. Clinically, dysphagia was improved in 66% of patients, weight gain was evident in 45%, and there was an objective response rate of 79%. Thirty eight individuals (37%) went on to surgery. Complete histologic remissions were seen in nine persons (24%) and eight patients died (21%) post-operatively. Median survival for the non-surgical group was 12 months compared to 32 months for the surgical group. At three years, survival for the non-surgical and surgical groups were 19% and 48%, respectively (Hannequin C, etal, Abstracts of the 5th ICACC, Pg. 123:O 334).

Stomach Cancer

In a recent study carried out under the auspices of the European Organization for Research and Treatment of Cancer (EORTC), docetaxel (Taxotere, Rhône-Poulenc Rorer) appeared promising as palliative therapy in patients with advanced gastric cancer. Final results are available in 42 patients, 37 of them being eligible and 33 evaluable for efficacy. Docetaxel was given intravenously over one hour at a dose of 100 mg/m^2, once

Exhibit 16
Profile of Colorectal Cancer

Characteristics	Comments
Age	Patients 65 years or older account for 35% of cases; (percentage of patients ≥85 years old treated surgically for colorectal cancer rose from 57% to 80% from 1973 to 1990)
Type	
Adenocarcinomas	The majority of cancers in the large bowel are adenocarcinomas; two-thirds are located in the rectum, rectosigmoid or sigmoid colon, with the remainder are distributed throughout the colon
Origin	
Hereditary nonpolyposis colorectal cancer (HNPCC).	6%-15% of cases
Familial adenomatous polyposis	>5% of cases; APC locus can be tested for mutations allowing confident prediction of disease inheritance
Etiology/Prevention	
Diet	Dietary saturated fat and total caloric intake, as well as mutagenic byproducts of high temperature cooking, may increase risk (polyunsaturated omega-3 fish oils seem to have a protective effect); natural dietary fiber from fruits and vegetables may be protective
Physical activity	May be beneficial
Dietary calcium intake	May be protective, at least partly because of fecal bile or free fatty acid metabolism; a mechanism independent of fat involving vitamin D metabolism may also occur
Aspirin	May have antineoplastic effect in the large bowel (Greenberg ER, et al, J NCI, 1993, 85:912; Paganini-Hill A, Semin Surg Oncol, 1994, 10:158); regular use of aspirin reduced the risk of colorectal cancer by 40%-50% (Rosenberg L, et al, J NCI, 1991, 83:355; Kune GA, et al, Can Res, 1988, 48:4399) and reduced the risk of dying from colon cancer by 40% (Thun M, et al, NEJM, 1991, 325:1593)
Antioxidants	Possible agents for chemoprevention; prevent carcinogen-induced oxidative damage to DNA; vitamins C and E were shown to inhibit nitrosamine formation, reduce fecapentaene production and have a beneficial effect in experimental colon cancer studies; prospective controlled clinical trials have not shown a benefit
Nonsteroidal anti-inflammatory drugs (NSAIDs) such as piroxicam and sulindac	Inhibit experimental colon tumors; may be related to reduction in endogenous prostaglandin generation (Reddy BS, et al, Cancer Research, 1990, 50:2562)
Medical conditions	Ulcerative colitis has been implicated as a predisposing condition for colon cancer, a smaller risk also seen in Crohn's disease; duration of disease (early age of onset) and stricture formation are associated with higher incidence of dysplastic and malignant change

every three weeks without premedication. In 18 patients (49%), the primary tumor had not been removed surgically, while in 51% the median interval between surgery and docetaxel chemotherapy was eight months. Liver metastases were present in 12 persons, and 16 patients had metastatic sites in retroperitoneal lymph nodes. Eight of the 33 evaluable patients (24%) experienced a partial remission, with a median duration of 7.5 months; disease stabilized in another 11 individuals for a median of four months and progressed in the remaining patients. Responses occurred more frequently in patients who had undergone resection of the primary tumor (seven of 19 or 37%), than in those who did not (one of 18 or 5%). (Rougier P, Docetaxel: An Advance in Cancer Management, Satellite Symposium at 5th ICACC, Pg. 12-13).

Combination chemotherapy with hydroxyurea (Hydrea, Bristol-Myers Squibb), leucovorin (Immunex, Burroughs Wellcome, Chiron), 5-FU, and cisplatin (HLFP regimen) is effective in advanced gastric cancer, with a low degree of toxicity. A total of 51 patients with metastatic or non resectable locally advanced gastric cancer with no previous chemotherapy were treated by HLFP and $5HT_3$ antagonist-based antiemetic therapy. Treatment was repeated every 28 days. Eleven of the patients were not evaluable for response (1 too early, 10 nonmeasurable peritoneal carcinomatosis or local disease) but were followed in the study for survival analysis. In the 40 evaluable persons, there were five CRs (12.5%) and 23 PRs (57.5%), for an objective response rate of 70%. More than half of these individuals gained weight and in 77.7% symptoms disappeared rapidly. Median progression-free survival and overall survival in the 51 patients were 9 and 12 months, respectively (Louvet C, etal, Abstracts of the 5th ICACC, Pg. 114:O 271).

Pancreatic Cancer

Regional treatment using alternating modalities of total abdominal perfusion (TAP) and selective celiac artery infusion (CAI) with a combination of 5-FU, cisplatin, and

mitomycin C (Mutamycin, Bristol-Myers Squibb) appears to be of value in the treatment of locally advanced pancreatic cancer. Over a two-year period, 37 patients with locally advanced pancreatic cancer (11 females, 26 males) were treated according to a protocol using a combination of 5-FU, cisplatin and mitomycin C administered via selective CAI alternating with TAP. Ten of these patients had evidence of extrapancreatic spread prior to therapy. The complication rate was 18.5% (12.5% minor, 6% major), including two treatment-related deaths. Analysis of survival data showed two distinct groups; long-term survivors (more than 12 months) and short-term survivors (mean 6.6 months). There were 13 long-term survivors with a mean survival of 19.3 months. Five out of 32 patients, followed up to 30 months, are alive. Local disease (no evidence of distal spread) and multiple treatments correlated with longer survival. Response to treatment varied between the two survival groups. In the long-term survivors, 15.4% experienced PR, 61% stable disease and 23% disease progression. In the short-term survivors, 42% had stable disease and the rest experienced disease progression (Klein ES, etal, Abstracts of the 5th ICACC, Pg. 120:O 304).

Results from a single-center study suggest that docetaxel is a promising drug for the treatment of pancreatic cancer in local, regional and metastatic disease. Overall, 43 patients with pancreatic cancer were enrolled into the study to receive docetaxel 100 mg/m^2 as a one-hour infusion once every three weeks, without premedication. Forty-two persons were eligible for the study (one with non-measurable disease), and 39 individuals were evaluable for efficacy. Six of the 27 evaluable patients with metastatic disease (22%) experienced a PR for seven months and disease stabilized in another eight patients. Median survival in this group was 212 days. In the 12 evaluable patients with local regional disease, three experienced a PR (25%), disease stabilized in three and progressed in six. The overall response rate was 23%, with a median duration of 11 months and an overall median survival of 442 days (Rougier P, Docetaxel: An Advance in Cancer Management, Satellite Symposium at 5th ICACC, Pg. 12-13).

Combined radiotherapy and chemotherapy offers effective local control in stage II and III unresectable pancreatic cancer. Ninety-six patients with unresectable pancreatic cancer were treated with a combination of radiotherapy and chemotherapy with 5-FU, streptozotocin (Zanosar, Upjohn), and cisplatin. Survival, adjusted for statistically significant prognostic factors (laparotomy, stage, tumor markers, evidence of significant clinical response produced by treatment, and successful restaging), was two times better than anticipated. Treatment produced sufficient response to allow for successful resection for 20 to 40% of patients, and mean survival time was 33 months from the date of first symptoms. Treatment was also effective for incompletely resected and recurrent post-resection tumors, with a mean survival time of 18 months (Bruckner HW, etal, Abstracts of the 5th ICACC, Pg. 125:O 354).

Colorectal Cancer

Irinotecan, a semi-synthetic analogue of camptothecin which acts by inhibiting DNA topoisomerase I, has been shown to be highly active in cases of inoperable advanced colorectal cancer previously treated with chemotherapy. This agent is particularly effective in cancers refractory to 5-FU treatment. In a large multicenter phase II study conducted in France, 213 patients with inoperable advanced colorectal cancer received irinotecan 350 mg/m^2 as a 30-minute infusion once every three weeks (one cycle), with treatment administered until disease progression. The majority of patients (165) had

Exhibit 17
Profile of Cancer of the Small Intestine

Type	Comments
Adenocarcinomas	Most common tumors of the small intestine (75%-80%); median age at diagnosis in the seventh decade of life; slightly more common in men
Site	
	Duodenum 45%
	Jejunum 45%
	Ileum 10%
Sarcomas	Derived from embryonic mesoderm; constitute 15%-20% of malignant tumors of the small intestine; age at diagnosis usually over 50 years
Primary lymphomas of the GI tract	Account for 20%-25% of primary small intestinal tumors but only 5% of all lymphomas; male:female ratio is 1.5-1.0; median age at diagnosis is 49 years; most common tumor of the small intestine in children; also particularly common in the Middle East, especially in Southern Iran ("Mediterranean lymphoma" occurs in children and young adults)
Carcinoid	Much less frequent than adenocarcinoma; occurs in the appendix, stomach, and rectum; 95% of tumors are in the digestive tract, mostly in the small intestine or the appendix; median age at diagnosis is 60 years

been pretreated with 5-FU. Metastatic sites included liver, lung, abdominal nodes, and peritoneum, in order of prevalence. Seventy percent of the patients had more than one metastatic site (40% two sites, 28% more than three sites, 2% unknown). In 115 evaluable patients, there were two CRs and 21 PRs, for an overall objective response rate of 20%. Of particular interest was the fact that one CR and nine PRs occurred in patients who had progressed on 5-FU therapy. Median duration of response was 9.1 months, and median time to disease progression was 4.2 months; 32% of patients were progression-free at six months (Bugat H, etal, Abstracts of the 5th ICACC, Pg. 284:P761). Irinotecan, jointly developed by Yakult Honsha and Daiichi Pharmaceutical, was launched in Japan as Campto and Topotecin in 1994 for the treatment of solid tumors with certain restrictions because of reports of adverse reactions. The drug is licensed to Rhône-Poulenc Rorer in Europe and Upjohn in the USA with Daiichi retaining co-marketing rights.

Combined chemotherapy with 5-FU, leucovorin, and cisplatin through an implantable hepatic artery infusion (HAI) port was shown to be an effective therapy for patients with colorectal cancer and liver metastases. It also may prolong survival, especially in those previously untreated. Over a three-and-a-half-year period, 28 individuals with advanced liver metastases from colorectal cancer received 5-FU 500 mg/m^2 and leucovorin 100 mg/m^2 weekly, and cisplatin 50 mg/m^2 once every three weeks, as intra-arterial boluses through an HAI port. Before the administration of HAI, 74% of the patients were treated with systemic chemotherapy. A retrospective analysis of 19 of these patients revealed three CRs and six PRs, for an overall response rate of 47.4%. During this period, four persons (21%) died from disease progression. Median survival for non-responders was 13 months, while responders did not achieve median survival during 33 months of follow-up (Lyass O, etal, Abstracts of the 5th ICACC, Pg. 157:O 690).

A combination of vinorelbine (Navelbine^R, Burroughs Wellcome) with 5-FU and folinic acid (leucovorin; FA) demonstrated good activity with moderate toxicity as second-line treatment in patients with refractory advanced colorectal cancer. Forty-three patients with refractory advanced colorectal cancer, all pretreated with schedules containing 5-FU and leucovorin, received a new combination treatment including 375 mg/m^2 of 5-FU on days 1-5, 200 mg/m^2 of FA on days 1-5, and 30 mg/m^2 of vinorelbine on days 1-8. Overall, 104 courses of combination therapy were administered, with grade III-IV neutropenia seen in 39.5% of the cycles. Non-hematologic adverse effects were well tolerated, with the most common side effect being vascular toxicity at the site of injection. Among forty patients evaluable for response, eight experienced PRs and three experienced minor responses, for an overall response rate of 27.5%. The median duration of response was seven months (Iaffaioli RV, etal, Abstracts of the 5th ICACC, Pg. 145:O 551).

Editor's Note: *A detailed report on drug development for digestive system cancer indications, including a database of over 75 chemotherapeutic agents, and descriptions of other therapies and profiles of their developers will be presented in the next issue of FUTURE ONCOLOGY.*

PLATINUM-BASED DRUGS

Cisplatin and Carboplatin

The two platinum-based drugs approved in the USA for the treatment of advanced/ metastatic cancer, cisplatin (Platinol) and carboplatin (Paraplatin), both supplied by Bristol-Myers Squibb, generated estimated worldwide sales of $430 million in 1994 (Exhibit 18). Cisplatin, cis-diamminedichloroplatinum, was the first platinum-based drug to be approved. It is one of the most widely used chemotherapeutics, often in combination therapies. Cisplatin has been particularly effective in increasing long term survival (by more than 10%) in ovarian cancer. However, its highly toxic profile has limited its use. Worldwide sales have plateaued at about $145 million in 1994 and will probably decline in the future, especially after its patent runs out in December 1996. Carboplatin, cis-diammine(1,1-cyclobutanedicarboxylato)platinum (II), first approved in Japan in 1991, is a newer platinum drug that exhibits a comparable spectrum of antitumor activity with cisplatin without its major toxicities. However, this drug did not expand the indications of platinum-based agents, nor did it overcome the problem of cisplatin-resistant cancers. In the USA carboplatin has been approved for the treatment of advanced ovarian carcinoma (initial and secondary), and cisplatin is approved for the treatment of metastatic testicular, ovarian and bladder cancer. Both drugs are also used off-label in a variety of combination therapies. Worldwide sales of carboplatin, estimated at $285 million in 1994, are expected to continue to rise at least until its patent runs out in August 1998.

New Platinum Agents

Because of the strong demand for platinum-based anticancer agents, particularly in combination therapies, considerable development efforts are ongoing to identify novel platinum-based complexes (or possibly alternative metals) to overcome two major problems associated with the currently approved platinum-based drugs, i.e. their mode of delivery (both drugs are injectable) and their limited activity against resistant tumors. Three major mechanisms of tumor resistance to cisplatin have been identified: reduced drug transport, enhanced intracellular detoxification (through glutathione and/or metallothioneins) and enhanced removal (or tolerance) of platinum-DNA adducts in the cell (critical lesions leading to cell kill). The development of cisplatin-acquired resistant cell lines, characterized with respect to these major biochemical mechanisms, is aiding *in vitro* identification of analogues that exhibit more favorable treatment profiles.

Exhibit 18
Profile of Approved Platinum-based Drugs

Cisplatin/ Platinol-AQ/ Bristol-Myers Squibb

Indications	Side Effects	Treatment Regimens	AWP per Treatment/ USA Sales / USA Cases Treated	Estimated WW Market ($ mil.)
Metastatic testicular (curative), ovarian (achieves durable remissions) and bladder cancer alone or in combination; also used off-label in the treatment of small cell lung, head and neck, cervix and breast cancer	Prolonged emetogenia, tinnitus, hearing loss, peripheral neuropathy, myelosuppression; nephrotoxicity is dose limiting but higher doses can be given with hydration	20 mg/m^2 IV daily for 5 days (alone or in combination) for testicular cancer; 75 mg/m^2 with cyclophosphamide (Cytoxan, Bristol-Myers Squibb) or 100 mg/m^2 (alone) IV in ovarian cancer once every 4 weeks; 50-70 mg/m^2 IV once every 3-4 weeks	Testicular cancer, $531.5 per treatment; ovarian cancer, $2,391.8/$116 million/ 48,000-50,000 regimens delivered	1993 $145.0 1994 $145.0 Patent expires 12/96

Carboplatin/ Paraplatin/ Bristol-Myers Squibb

Advanced ovarian cancer; also used off-label in the treatment of small cell lung, head and neck, bladder, cervix and breast cancer	Less emetogenic than cisplatin; myelosuppression, mostly thrombocytopenia, is dose limiting	360 mg/m^2 IV once every 4 weeks as monotherapy or 300 mg/m^2 once every 4 weeks in combination	Ovarian cancer, $5,831 per treatment/ $131 million/22,000 regimens delivered	1993 $265.0 1994 $285.0 Patent expires 8/98; 2004

Source: New Medicine

Platinum complexes based on the structure of carboplatin demonstrated no advantage. One series of compounds containing a 1,2-diaminocyclohexane (dach) carrier ligand (e.g., oxaliplatin, tetraplatin), that demonstrated circumvention of acquired cisplatin resistance in some preclinical tumor models (mainly murine leukemia), is currently in clinical trials. However, clinical results have been disappointing, because the drugs have shown little evidence of activity in cisplatin-resistant disease and appear to cause severe neurotoxicity. Exhibit 19 lists selected platinum-based drugs and related agents in development.

Oxaliplatin. Debiopharm's (Lausanne, Switzerland) oxaliplatin (L-OHP) is currently awaiting registration in Europe. L-OHP has been evaluated in patients with advanced gastric cancer (Enguchi, M, etal, ICACC95, Abs. # P299). Drug was intravenously administered as bolus, continuous or chronomodulated infusion (circadian delivery); a dose of 130 mg/m^2 q^3 weeks did not produce untoward side effects (Krikorian, A, etal, ICCAC95 Abs. P 562). In a phase II trial, chronomodulated delivery of 5-FU, FA and oxaliplatin produced objective response rate of 58% in 93 patients with metastatic colorectal cancer. In another trial, seven European centers enrolled 92 patients with previously untreated metastatic colorectal cancer, to compare chronomodulated with constant-rate drug delivery to see how they affect therapeutic outcome. A regimen of daily administration of 5-FU (600 mg/m^2 per day), FA (300 mg/m^2 per day), and 1-OHP (20 mg/m^2 per day) for 5 days was repeated every 21 days (16-day intermission) in ambulatory patients with the use of a programmable in-time pump. In one group (47 patients) drug delivery was kept constant over a 5-day period while it was chronomodulated (maximum delivery of 5-FU and FA infusions at 0400 hours and maximum delivery of 1-OHP at 1600 hours) in the other group (45 patients). Twenty-four of 45 patients (53%) in the group receiving chronomodulated therapy exhibited an objective response compared with 15 of 47 patients (32%) in the constant infusion group. The median progression-free survival was 11 and 8 months, respectively, and the median survival was 19 months and 14.9 months. Chronomodulated delivery was both more effective and less toxic (Levi FA, etal, J NCI, 1994 Nov 2, 86(21):1608-17). Debiopharm has signed a license agreement with Elf Sanofi (Paris, France) in Europe and is negotiating with a licensee in Japan.

Tetraplatin. Upjohn's tetraplatin was administered in 26 patients at a dose range of 4.4-60.8 mg/m^2 IV, given over 30 minutes on a day 1 and day 8 schedule, every 28 days. Nausea/vomiting occurred in 40% of patients but was well controlled with standard antiemetic therapy. However, renal toxicity, hepatotoxicity and severe neurotoxicity precluded further use of his regimen (Schilder RJ, etal, Cancer Research, 1994 Feb 1, 54(3):709-17).

JM-216 is one of a novel class of platinum-containing ammine/amine platinum(IV) dicarboxylates (mixed amines), synthesized at the Drug Development Section, Institute of Cancer Research (Belmont, Sutton, UK), in collaboration with the Johnson Matthey Technology Centre

(Reading, Berks, UK) and the Johnson Matthey Biomedical Research Center (West Chester, PA). These compounds are orally active and absorbed well from the gastrointestinal tract. They are generally more potent than cisplatin *in vitro* and retain activity in cells which are intrinsically resistant to cisplatin. In addition, oral administration of JM-216 may further reduce patient morbidity associated with platinum-based chemotherapy. JM-216 is currently in phase II clinical trials (Kelland, LR and McKeage, MJ, Drugs and Aging, 1994 Aug, 5(2):85-95). It has displayed dose-limiting myelosuppression, but a lack of nephrotoxicity and neurotoxicity in animal studies (McKeage, MJ, etal, Cancer Research, 1994 Aug 1, 54 (15):4118-22) and in clinical trials.

Overcoming Drug Resistance

Modifications of metallothionein levels. Resistance to platinum drugs is multifactorial and includes such molecular alterations as overexpression of metallothionein (MT), a small protein synthesized in the liver and kidney, of importance in ion transport and detoxification. MT was shown to be one of the mechanisms of resistance to several clinically important anticancer drugs, including cisplatin. Cisplatin's killing activity is mediated mainly by production of DNA cross-links. Intra-cytoplasmic binding of MT to platinum prevents the active molecules of cisplatin from reaching the nuclear DNA of tumor cells, protecting against cisplatin-induced cytotoxicity and mutagenicity. Modification of MT levels could circumvent MT-mediated resistance to treatment with cisplatin and other platinum-based agents (Goncharova, EI, etal, ICACC95, Abs. # P 239).

In another attempt to overcome MT-mediated resistance to cisplatin, researchers at Hôpital de la Salpétriére and Institut Pasteur (Paris, France) used a liposomal transfection system to insert the thymidine kinase (tk) suicide gene in the human MT promoter. Transactivation of this promoter results in tk gene expression and acquired sensitivity to ganciclovir. Successful cytotoxicity of the liposome/MK-tk complexes was conferred by ganciclovir *in vitro,* indicating that this method may be clinically useful in overcoming cisplatin resistance (Rixe O, etal, ICACC95, Abs. O 757)

Exploitation of metabolic defects of tumors. Another strategy to combat tumor resistance to platinum-based agents is to use metabolic defects found in tumors that may influence the tumor's reaction to these drugs. For instance, most solid and hematologic tumors have an absolute growth requirement for the essential amino acid methionine, involved in methylation reactions within the cell; normal cells can grow when homocysteine replaces methionine AntiCancer (San Diego, CA), a private company established in 1984, discovered that deprivation of methionine selectively and synchronously arrests the growth of methionine-dependent tumors before mitosis (cell division). Tumors that enter mitosis synchronously in the absence of methionine become more susceptible to cell cycle-specific drugs. According to AntiCancer, *in vitro* and animal studies have shown that most human tumors, including those of the pancreas, colon, breast and ovary, are methionine-dependent. At the 1994 8th NCI-EORTC meeting in Amsterdam, researchers from the company and Keio University (Tokyo, Japan) said that they isolated an enzyme, methioninase, from the bacterium Pseudomonas putida, which lowered plasma methionine levels in nude mice, arresting the growth of tumors. The agent (AC9301-Methionase; ONCase) appears to be of low toxicity and antigenicity. ONCase has been conjugated to polyethylene glycol (PEG) to reduce any potential allergic reaction and to increase the agent's serum half-life. It has a long serum half life and lowers methionine levels 100-fold after intraperitoneal or intravenous injection. In addition, ethionine, an inhibitor of the enzyme methionine adenosyltransferase, was found to inhibit tumor growth only under conditions of methionine depletion, both *in vitro* and in nude mice.

Modulation of glutathione. Lowering intracellular glutathione (GSH) concentration enhances the toxicity of alkylating agents *in vitro,* resulting in cellular necrosis (Fernandes RS and Cotter TG, Biochemical Pharmacology, 1994 Aug 17, 48(4):675-81). Extracellular GSH can modulate the biological activity of tetraplatin, and the combination may prove useful in such clinical applications as intracavitary platinum therapy (Kido Y, etal, Biochemical Pharmacology, 1994 Apr 29, 47(9):1635-42).

Glutathione S-transferase (GST) isoenzyme inhibitors. GST isoenzymes are elevated in tumors, a phenomenon that may be exploited to selectively destroy tissues with excess GST. Terrapin Technologies (South San Francisco, CA) has used two approaches to achieve this end result:

- TER286: glutathione is conjugated to a latent cytotoxin via an unstable link that is cleaved in an isoenzyme-selective fashion to release the toxin (in this case an analog of cyclophosphamide).
- TER199: inhibitors of certain GST isoenzymes are constructed that enhance cytotoxicity of drugs whose toxic effects are adversely impacted by GST. TER199 inhibits P1-1, an isoenzyme that is found in both normal and malignant tissues, but is elevated at least two-fold in a majority of specimens from human solid tumors of colon, lung and breast. TER199, an orally available small molecule therapeutic, has demonstrated chemosensitizing and myelostimulative effects *in vitro.* The agent was shown to potentiate the antitumor effects of cytotoxins and its use as a single treatment resulted in a two-fold increase in the numbers of granulocyte/macrophage progenitor cells in bone marrow.

Exhibit 19
Selected Platinum-based Chemotherapeutics and Related Agents in Development

Developer/ Collaborator/ Affiliate	Generic Name/ Number/ Brand Name	Chemical Name	Status/Location	Comments
Alkermes/ Alkermes Clinical Partners	RMP-7 in combination with carboplatin		Phase II (2/95)/USA and Europe/ recurrent malignant brain gliomas	Enhances permeability of the blood brain barrier
Andrulis	Polyplat/NSC-608916	Trans-1, 2-dach platinum complex with carboxyamylose	Preclin/USA/renal and lung cancer	
AntiCancer	AC9301-Methionase/ ONCase	Methioninase, an enzyme isolated from the bacterium *Pseudomonas putida*	Preclin/USA/ breast, pancreatic, colon, ovarian cancer	Conjugated to polyethylene glycol (PEG)
Argonex (was Argus)/ MD Anderson Medical Center (licensor)	AR-726	Lipophilic platinum compound	Preclin/USA/ solid tumors	
Cancer Research Center, Moscow	Cycloplatam	S(-)malatoamine (cyclopentylamine)-platinum(II)	Phase I/Russia	No cross resistance with cisplatin; no nephrotoxicity; emetogenia, myelosuppression (Syrkin, AB, etal, ICACC95, Abs. O 186)
Chugai	Loboplatin/ DWA-2114R/ Miboplatin	Trans platinum complexes	Prereg (92); NDA withdrawn/ Japan	
Debiopharm/ Roger Bellon (Rhône-Poulenc Rorer), Sanofi Winthrop	Oxaliplatin/ 1670RB; RP/54780/ L-OHP	Trans amine (cyclohexylamine) dichlorodihydroxo platinum (IV)	Prereg/France/ advanced malignant melanoma, non-Hodgkin's lymphoma	
Johnson Matthey/Institute for Cancer Research (UK)/Bristol-Myers Squibb	JM-216 (JM-118; JM-219; JM-225; JM-251; JM-269)	Bis-acetato-amine dichloro (cyclohexylamine) platinum (IV)	Phase II/USA	
Johnson Matthey/ Institute for Cancer of Research (UK)	JM-335	Trans amine (cyclohexylamine-dichlorodihydroxo platinum (IV)	Preclin/UK	Did not exhibit cross resistance in a panel of cell lines exhibiting acquired resistance to cisplatin (Mellish KJ, etal, International Journal of Cancer, 1994 Oct 1, 59(1): 65-70)
Johnson Matthey/ Institute for Cancer Research (UK)	JM-74	1, 2-dach platinum (II)	Discontinued	Did not achieve a maximum tolerated dose due to poor aqueous solubility
Lederle (American Home Products)	Enloplatin/CL-287110		Phase I/USA/ leukemia, lymphoma; phase II/USA/ advanced ovarian cancer	Minimal toxicity was observed; neutropenia, nausea and vomiting most common side-are effects; dosage of 375 mg/m2 was well tolerated
Lederle (American Home) Products)	Zeniplatin		Discontinued in phase II due to nephrotoxicity	

— *continued*

Exhibit 19
Selected Platinum-based Chemotherapeutics and Related Agents in Development

Developer/ Collaborator/ Affiliate	Generic Name/ Number/ Brand Name	Chemical Name	Status/Location	Comments
Matrix Pharmaceuticals	MPI-5010/ Intradose-CDDP	Biodegradable gel matrix containing cisplatin	Phase II/USA/ liver cancer	
Nippon Kayaku	Sebriplatin/CI-973 (NK-121)		Phase II (discontinued)/Japan	
Shionogi	Nedaplatin/245-S, S-254), NSC-375101D/Aqupla		Prereg/Japan	
SRI International/Elf Sanofi (co-developers)	Tirapazamine/ SR-4233, Win-59075		Phase II/USA	
Sunkyong Pharmaceuticals	SKI-2053R	Alkylating agent/platinum-based drug	Phase I (completed late 94)/S. Korea/ solid tumors	Dose limiting reactions were hepatotoxicity and myelosuppression
Terrapin Technologies	TER286	Glutathione conjugated to an analog of cyclophosphamide	Preclin/USA	
Terrapin Technologies	TER199	Inhibits P1-1, an isoenzyme elevated in solid tumors/ oral	Preclin/USA/ colon, lung and breast cancer	Chemosensitizer and myelostimulative
Toray	TRK-710	1-1,2-dach platinum (II)(α-acetyl-γ-methyltetronate)$_2$	Phase I/ Japan/lung cancer	Mechanism may differ from cisplatin (Inoue, S and Mizuno, S, ICAAC95, Abs. P 241)
U. S. Bioscience/ Lombardi Cancer Research Center/ Teva (option to license)		Third generation platinum agents	Preclin/USA/ ovarian, testicular, head and neck and lung cancer	Major toxicity was bone marrow suppression
U. S. Bioscience/ Southern Research Institute (licensor)/ Schering-Plough (licensee)	Amifostine/ WR-2721/ Ethyol	Injectable	NDA (9/91), ammended (1/95)/USA/ cumulative renal and hematologic toxicities associated with cisplatin; approved (94)/ Europe; launched (4/95)/Germany	Protect normal tissues from toxicities associated with radiation therapy and cisplatin and cyclophosphamide
Upjohn/NIH	Tetraplatin (D-ormaplatin), ormaplatin/NSC-363812, U-77223	Trans 1-1,2-dach tetrachloro platinum (II)	Phase I/USA	Reversible dose-limiting peripheral neurotoxicity, emetogenia and mild hematologic effects; no nephrotoxicity

Improving Drug Delivery

Matrix Pharmaceutical (Menlo Park, CA) is developing site-specific, injectable cancer therapies (IntraDose) designed to provide localized, sustained release of high concentrations of chemotherapeutic agents directly within a primary or metastatic tumor. IntraDose products are comprised of a biodegradable protein matrix, a chemotherapeutic agent and, typically, a chemical modifier. IntraDose-CDDP is an injectable cisplatin gel, for the local treatment of head and neck cancer and other accessible tumors. Preliminary results from a phase I/II clinical trial of IntraDose-CDDP in 31 patients (30 of whom had previously failed other treatments) with recurrent or refractory disease, showed that 48% of the total evaluable tumors exhibited a CR; higher CR rates were seen in adenocarcinoma (81%) and squamous cell carcinoma (59%). An additional five tumors demonstrated PRs, resulting in an overall response rate of 55%. Patients averaged 2.5 tumors each, including metastatic adenocarcinoma of the breast, lung or ovary; squamous cell carcinoma of the head and neck; melanoma; and sarcoma.

If a previously untreated patient with 12 Kaposi's sarcoma lesions who did not respond to treatment was excluded, the CR and PR rate rose to 66%, with a CR rate of 58%. A treatment regimen consisting of up to four weekly treatments of IntraDose-CDDP, ranging from 1 to 6 mg cisplatin per cm^2 of tumor, was well tolerated without clinically significant toxicities (reported by Howard Burris III, MD, of the Cancer Therapy and Research Center (San Antonio, TX) at the 1994 American Society of Clinical Oncology meeting). Data presented at the American Association for Cancer Research meeting in Toronto in March 1995 showed that IntraDose-CDDP enhanced intratumoral drug retention and lowered systemic exposure to cisplatin in a murine model of squamous cell carcinoma and fibrosarcoma. Phase III clinical trials are being planned for the treatment of head and neck cancer and superficially accessible tumors, such as melanoma, recurrent breast cancer, and squamous cell carcinoma. A phase II trial of IntraDose-CDDP was also initiated in 15 patients with surgically-treated prostate cancer.

Alkermes (Cambridge, MA) began phase II clinical trials in the USA and Europe in February 1995 with RMP-7, administered in combination with carboplatin, for the treatment of recurrent malignant brain gliomas. RMP-7 when administered intravenously or intra-arterially, transiently increases the permeability of the blood brain barrier (BBB). It is believed that RMP-7 binds to receptors located on the BBB and triggers a brief relaxation of tight cellular junctions. RMP-7 does not bind or serve as a carrier for carboplatin but rather acts independently on blood vessel walls as a permeabilizer. The drug is being developed by Alkermes for Alkermes Clinical Partners, a limited partnership, that raised $42 million in 1992 to develop RMPs.

Reducing Side Effects

Amifostine. U.S. Bioscience (USB; West Conshohocken, PA) has been developing amifostine (Ethyol; WR-2721), an injectable agent designed to protect normal tissues from toxicities associated with radiation therapy and certain chemotherapeutics such as cisplatin, without reducing the antitumor effects of these modalities. Amifostine was licensed from the Southern Research Institute (Birmingham, AL). Although the original USA composition of matter patent on Ethyol expired in July 1992, the drug has been given orphan status for use as a chemoprotective agent for cisplatin and cyclophosphamide in the treatment of advanced ovarian carcinoma and for cisplatin in the treatment of metastatic melanoma. USB filed an NDA in September 1991 for the use of intravenously administered Ethyol to reduce serious side effects, in particular hematologic toxicity, associated with cyclophosphamide and cisplatin chemotherapy. This NDA was reviewed and rejected in January 1992. An amended NDA, submitted in August 1993, incorporating results from a 200-patient ovarian cancer trial, was resubmitted in July 1994 but again failed to meet committee approval in December 1994. An additional amendment was filed in January 1995, focusing on Ethyol's ability to protect against the cumulative renal toxicities associated with cisplatin and cumulative hematologic toxicities associated with cyclophosphamide and cisplatin. In September 1994 Ethyol was recommended for approval by 9 European countries (Belgium, France, Germany, Greece, Italy, Luxembourg, Portugal, Spain, UK) to reduce neutropenia-related risk of infection (e.g. neutropenic fever) associated with cyclophosphamide and cisplatin combination regimens in advanced ovarian cancer. To date Ethyol has received approvals from the local health regulatory authorities in the UK, Germany, France, Spain and Luxembourg and was launched in Germany, its first market, in April 1995 by Schering-Plough, USB's licensee for the European market.

Tirapazamine. Platinum agents' cytotoxicity may be enhanced by the use of chemosensitizers. Tirapazamine (SR-4233, Win-59075), originally co-developed as a radiosentizer by SRI International (Menlo Park, CA) and Sanofi Winthrop/Elf Sanofi, increased tumor cell kill in murine and human xenograft tumor models when administered before treatment with cisplatin (Dorie, MJ and Martin, J, ICACC95, Abs. O 424).

Next issue: *Update on immunoconjugates in the diagnosis of cancer from the Tenth International Conference on Monoclonal Antibody Immunoconjugates for Cancer, March 9-11, sponsored by the San Diego Regional Cancer Center (San Diego, CA).*

MECHANISMS IN MALIGNANCY

APOPTOSIS

- Inhibition of apoptosis plays a critical role in oncogenesis and cytotoxic drug resistance in tumor cells but the mechanism of apoptosis remains to be elucidated
- Research programs exploiting apoptosis to develop anticancer agents are being pursued by numerous entities but challenges remain
- For a comprehensive review of the mechanism of apoptosis and its role in cancer and other human diseases, see Science, Vol. 267, March 10, 1995, pages 1445-1462; for a review of apoptosis in cancer see Cell, Vol. 78, 539-542, August 26, 1994, pages 539-542

Apoptosis refers to an intrinsic cell suicide phenomenon whose activation is regulated by a variety of cellularly or extracellularly-originating signals. The mechanism necessary to trigger apoptosis is present in all mammalian cells. Apoptosis is the end result of a "housekeeping" function to remove unwanted or damaged cells, such as tumor cells or those infected by viruses, and to maintain cell homeostasis. Although the terms apoptosis and programed cell death are often used

interchangeably, they are not equivalent. In some instances death by apoptosis, which is characterized by distinct morphologic features, is indeed programmed, but in others, it is the result of a variety of environmental stimuli.

There has been a rapid accumulation of knowledge on apoptosis, based on the observation that the process has been conserved throughout evolution allowing findings regarding apoptosis in invertebrate models such as the nematode *Caenorhabditis elegans,* to be transferred to mammalian systems. However, the similarity is limited and scientists are toiling to understand the mechanism of apoptosis in mammalian systems and its activation, inhibition and role in normal cell function and in disease.

DEATH BY APOPTOSIS

In the Constant Shadow of Death

Cell death effector molecules responsible for apoptosis are present at all times, and no new gene transcription is required to initiate the process The actions of these effector molecules are continuously suppressed in order for the cell to survive. Suppression may involve survival factors and their receptors and/or protective proteins. External stimuli that may activate apoptosis include ionizing radiation, viral infection, cytotoxic drugs and temperature changes. Endogenous regulators include genes encoding proteins that directly act as promoters or inhibitors of apoptosis; nuclear proteins that regulate gene expression, DNA replication and cell cycle progression; intracellular mediators of signal transduction; various cytokines; and other factors.

How Cells Die

How cells die during apoptosis has not been fully elucidated. Both the molecular origin and pathway of apoptosis and programmed cell death remain unresolved. When cells die by apoptosis, DNA fragmentation and chromatin condensation are first steps in a cascade of events that results in the break up of the nucleus, blebbing of the cell membrane, and fragmentation of the cell into apoptotic bodies (discrete vesicles with intact membrane). These morphologic changes are the hallmark of apoptosis. It has been observed that in apoptosis DNA degradation involves two distinct endonucleolytic activities, with only one being essential for cell death. The first is initiated by a unique endonucleolytic activity that cleaves DNA into 50- to 300-kb fragments, a degree of cleavage sufficient to cause the chromatin to undergo condensation. The second stage of fragmentation is catalyzed by calcium-magnesium endonuclease (Walker PR, etal, Exp Cell Res, 1994 Jul, 213(1):100-6). Chromosome condensation occurs normally during cell division. Chromosomal DNA is released from the nuclear matrix, a fibrous-like scaffold within the nucleus which anchors the chromatin and provides a three-dimensional support for the long DNA molecule. The nuclear matrix also appears to be the site of DNA replication, RNA synthesis, and hormone binding. During apoptosis irreversible proteolytic destruction of the nuclear matrix precedes chromatin condensation. Enzymes participating in the destruction of the nuclear matrix and DNA may be initially localized to the nuclear matrix itself.

Influx of extracellular free Ca^{2+} ions and increases in intracellular Ca^{2+} have also been observed in apoptosis triggered by a variety of agents; it is also possible that Ca^{2+} influx may in itself be a trigger for apoptosis. Also, enzymes necessary in DNA cleavage are Ca^{2+}-dependent. However, Ca^{2+} is not always involved in apoptosis.

Removal of apoptotic bodies does not involve the immune system but, rather, is accomplished by phagocytosis, mostly by neighboring cells. Membranes of apoptotic cells undergo certain changes that facilitate their recognition and speedy phagocytosis to prevent rupture and avoid an inflammatory response.

Ανοικια (anoikis, homelessness) Leads to Death

Apoptosis may also be the fate of cells denied anchorage. Normal cells require attachment to a substrate in order to grow, and die when detached. Because integrins regulate adhesion to extracellular matrices, integrin signaling may also be implicated in the regulation of apoptosis. Furthermore, integrins have been implicated in positive regulation of cell proliferation by stimulating cells to spread.

APOPTOSIS AND MALIGNANCY

The process of carcinogenesis is not well understood, although it is generally accepted that DNA damage leads to the anomalous accumulation of cells. Many carcinogens directly damage DNA or interfere with enzymes necessary to accurately replicate DNA. Subsequent "fixation" of genetic damage and amplification of clones of intermediary and neoplastic cells give rise to malignancy. Apoptosis prevents malignant transformation by removing cells with damaged DNA. For instance, apoptotic mechanisms eliminate 80% to 90% of initiated cells formed after a high dose of genotoxic carcinogens.

Apoptosis Deregulation May Participate in Oncogenesis and Induces Drug Resistance

Apoptosis appears to directly regulate oncogenesis and also interfere with the killing action of cytotoxic drugs. Apoptosis inducers may be particularly effective in improving the killing capacity of many chemotherapeutics. Tumor-specific cytotoxic drugs or radiation kill actively proliferating malignant cells primarily by necrosis. However, these agents also induce apoptosis, which may play a vital role in completing the cell killing effectiveness of these agents by removing damaged cells. It is, therefore, likely that lesions in the apoptosis pathway play a role in both carcinogenesis and tumor drug resistance, and that manipulation of apoptotic pathways and signals may prove a powerful way of treating cancer.

Cancer — a Paradigm for Apoptosis-based Interventions

Because of their acute nature and poor prognosis most cancers must be treated early and aggressively to effect a cure. Since treatment is often of short duration, far higher toxicities are tolerated than possible in treating non-life-threatening chronic diseases. Therefore, cancer presents a very attractive first target for agents inducing apoptosis. Initially, such agents may be used to enhance the effect of traditional therapy (drugs and/or radiation) by inducing apoptosis throughout the course of treatment and by reducing drug resistance. Eventually, they may prove independently useful as a way of arresting early disease. At any rate, accumulated knowledge in this area is likely to have a profound effect on the practice of oncology in terms of drug and radiation therapy. However, the mechanics of apoptosis are extremely complex and initial approaches to manipulate this phenomenon may be unsuccessful *in vivo.*

Apoptosis Thresholds May Determine Drug Toxicity

Interestingly, susceptibility to apoptosis varies in different cell types. The relative apoptosis thresholds of normal cells, for instance, may determine how they are affected by radiation therapy and chemotherapy and may explain the varying toxicity of these treatments.

Regulators, Inducers and Inhibitors of Apoptosis

Recent studies with oncogenes and tumor suppressor genes, such as bcl-2, c-myc and p53, have demonstrated that the deregulation of apoptosis may be of general significance in the development of many different types of cancer. This deregulation appears to be a critical event during multistep carcinogenesis. The selective induction of apoptosis in tumor cell populations is now being considered in the design of novel therapeutic interventions (McDonnell TJ, etal, Radiation Research, 1993 Dec, 136(3):307-12) but successes in the laboratory may not transfer to the clinical setting. Of the many effectors linked to apoptosis, the following mediators have been shown to play a role in malignancy, and are the targets of various drug development programs.

bcl-2 is a human oncogene encoding a 26-kDa protein (Bcl-2), a single chain polypeptide with a predicted transmembrane segment located at the carboxyl terminus. Expression of Bcl-2 inhibits apoptosis in humans and, remarkably, exerts a similar action on cells from worms and insects, as well as other mammals. Overexpression of Bcl-2 does not influence rates of cellular proliferation; rather, it extends cellular viability by blocking apoptosis. For instance, in mouse lymphoma cells, Bcl-2 either directly or indirectly regulates the flux of Ca^{2+} across the endoplasmic reticulum membrane, thereby abrogating Ca^{2+} signaling of apoptosis. Bcl-2 is normally expressed in many tissues (lymphoid and myeloid cells, neurons and several types of epithelial cells) and exhibits remarkable structural and functional conservation. Dysregulation of Bcl-2 expression by the t(14;18) translocation in lymphoid malignancies induces inappropriate cell survival. It appears that bcl-2 is regulated in trans through the joint action of genes on different chromosomes (Gourdeau, H and Walker, PR, Mol. Cell. Biol., 14:6125-6134, 1994). Bcl-2 has been shown to block apoptosis induced by many types of stimuli, including a wide variety of chemotherapeutic drugs and radiation. Bcl-2 expression which blocks both spontaneous and drug-induced cell death, thus conferring a selective survival advantage on malignant cells, has been observed in a wide variety of human cancers. For example, the bcl-2 gene becomes transcriptionally deregulated in the majority of low-grade non-Hodgkin's lymphomas (NHL) as the result of t(14;18) chromosomal translocations. Presence of Bcl-2 in NHL tumor cells has been correlated with poor responses to therapy. Bcl-2 is also expressed at high levels in the absence of gene rearrangements in a high proportion of B-cell chronic lymphocytic leukemias (B-CLLs). The relative levels of Bcl-2 oncoprotein is one of the key determinants of the sensitivity of lymphocytic cells to killing by most currently available anticancer agents (Reed JC, etal, Annals of Oncology, 1994, 5 Suppl 1:S61-5). Bcl-2 is also expressed at high levels in renal neoplasms potentially implicating the deregulation of apoptosis in the development and progression of these tumors (Chandler D, etal, Human Pathology, 1994 Aug, 25(8):789-96). Bcl-2 expression is also associated with poor prognosis in prostatic and colon cancer and in neuroblastoma. A Bcl-2 related protein, Bcl-xL, encoded by bcl-x, also inhibits apoptotic cell death, independently of Bcl-2. *In vivo* patterns of bcl-x gene expression in human tissues were strikingly different from those reported for Bcl-2, suggesting that Bcl-x and Bcl-2 regulate cell life and death at different stages of cell differentiation through tissue-specific control of their expression (Krajewski S, etal, Cancer Research, 1994 Nov 1, 54(21):5501-7).

c-abl may be another negative regulator of apoptosis, and its expression also seems to contribute to the chemoresistance of some tumor cell lines. In chronic myelogenous leukemia (CML), the c-abl gene is translocated from chromosome 7 to 22, leading to an up-regulation in its activity. CML cell lines expressing the Bcr-Abl fusion protein are resistant to the induction of apoptosis by a number of agents and conditions, suggesting that bcr-abl acts as an anti-apoptosis gene in CML cells. This effect appears to be dependent on the abl kinase activity of the chimeric protein Bcr-Abl. Inhibition of Bcr-Abl combined with drugs and/or treatment capable of inducing apoptosis, may prove an effective treatment strategy in CML (McGahon A, Blood, 1994 Mar 1, 83(5):1179-87).

Bax, a homologous protein, by forming heterodimers with Bcl-2 opposes the latter's cell survival-promoting activity and accelerates rates of cell death. Recently, it has been suggested that the ratio of Bcl-2 and Bax controls

the relative susceptibility of cells to death stimuli. Interestingly, the expression of Bax in mice was more widespread than Bcl-2 (Krajewski S, etal, American Journal of Pathology, 1994 Dec, 145(6):1323-36).

Bak, a newly identified Bcl-2 homologue, encoded by the bak (Bcl-2-homologous antagonist/killer) gene, primarily enhances apoptotic cell death after a stimulus to initiate the process. For instance, it is speculated that Bak acts indirectly to mitigate the direct action of Bcl-2 and Bax. However, it is also possible that Bak directly activates the apoptosis pathway or functions as part of a cell's death machinery. Bak is broadly distributed in terminally differentiated cell types. Related findings in this area were recently reported by LXR Biotechnology (Richmond, CA) (Kiefer, MC, etal, Nature, Vol. 374, 20 April 1995, pp736-739) and Apoptosis Technology (Cambridge, MA), in collaboration with Imperial Cancer Research Fund Laboratories (London, UK) (Chittenden, T, etal, Nature, Vol. 374, 20 April 1995, pp733-736).

p53, a tumor suppressor gene whose product induces apoptosis through unknown mechanisms, is also involved in modulating the cytotoxicity of anticancer agents by promoting apoptosis. It appears that p53 exerts a significant and dose-dependent effect in the initiation of apoptosis, but only when it is induced by drugs or radiation that cause DNA-strand breakage (Clarke, AR, etal, Nature, [1993] 362:849; Lowe, S, etal, Nature, [1993] 362:847). p53 is also directly linked with the regulation of tumorigenesis and may induce apoptosis in the presence of oncogenic triggers. Loss of p53 function has been linked to increased tumor aggressiveness. Mutations or deletions in p53 are among the most common genetic alterations in human cancers, having been associated with lung, colorectal, breast, stomach, brain and hematologic cancers, all common malignancies with poor prognosis. Mutations of p53 produce proteins that either complex with the wild type version, preventing its function (evidenced in cervical carcinoma and sarcomas), or become the dominant product resulting in aberrant behavior. Also, proteins encoded by oncogenic viruses, including human papillomaviruses and adenoviruses, can bind and inactivate p53 protein. These malfunctions of p53 may promote cancer development by permitting cells with DNA damage to replicate their DNA before repair is complete. Loss of p53 function has also been shown to enhance cellular resistance to a variety of cytotoxic agents and is associated with poor prognosis (Lowe, SW, et al, Science, Vol. 266, 4 Nov 1994, pp. 807-810).

c-myc, a human oncogene, is the cellular homologue of v-myc, a viral oncogene which is found in a number of avian and feline retroviruses that induce leukemia and carcinoma. In particular, c-myc is one of the immediate early growth response genes that are rapidly induced in quiescent cells upon mitogenic induction, suggesting that the gene plays some part in mediating the transition from quiescence to proliferation (B Amati, et al, Cell, [1993] 72:233). The Myc oncoprotein dimerizes with its partner, Max, to bind DNA, activate transcription, and promote cell proliferation, as well as apoptosis. Max also forms homodimers or heterodimers with its alternative partners, Mad and Mxi-1 which behave as antagonists of Myc/Max through competition for common DNA targets, and perhaps permit cellular differentiation (Amati B and Land H, Current Opinion in Genetics and Development, 1994 Feb, 4(1):102-8). In quiescent murine cells activation of c-myc in the absence of other growth signals results in the accumulation of p53 protein (Hermeking, H and Eick, D, Science, Vol. 265, 30 Sep 1994, pp 2091-2093). In normal cells, c-Myc expression is tightly regulated, but in tumor cells it is elevated or deregulated indicating a critical role for c-myc activation in multistage carcinogenesis.

Interleukin-1β converting enzyme (ICE), a cysteine protease, induces apoptosis when overexpressed in animal models. Conserved through evolution (ICE is a homolog of Ced-3 in *C. elegans*), this protein and related ICE-like proteins are parts of the omnipresent apoptosis machinery. When inert, they are sequestered in a lyosome-like organelle and, when released, cause apoptosis by triggering a protease cascade. In 1994 Vertex (Cambridge, MA) published the three dimensional structure of ICE and is collaborating with Roussel Uclaf to develope ICE inhibitors for the treatment of inflammation.

Hormones. In hormone-dependent tumors, sex steroids and other trophic hormones stimulate cell proliferation and inhibit cell death, causing rapid tumor growth. In these tumors the induction of apoptosis by chemical or surgical hormonal ablation is accompanied by tumor regression. For instance, tamoxifen (Nolvadex, Zeneca Pharmaceuticals), an anti-estrogen, flutamide (Eulexin, Schering-Plough), an anti-androgen, or Roussell-Uclaf's RU 486, an anti-progestin, block the action of trophic hormones and cause apoptotsis in susceptible cancers. One class of hormones, derivatives of Vitamin A, are the retinoids which are natural signals that modulate apoptosis; drugs which stimulate certain intracellular receptors (IRs) for retinoids induce apoptosis in susceptible tumor cells. Retinoid agonists may also be used in combination with sex steroid antagonists to prevent or treat cancer by exploiting the phenomenon of apoptosis, as confirmed in cell culture and animal studies. Two naturally occurring endogenous, biologically active retinoids are all-trans-retinoic acid and 9-cis-retinoic acid. Biologically active chemical analogues of retinoids have also been synthesized. Clinical trials are planned and underway in the USA and Italy to evaluate prophylactic treatment of individuals who exhibit high risk factors for certain malignancies such as breast and prostate cancer using a combination of approved anti-hormonal drugs and a retinoid.

Cytokines. The role of interleukins, various growth factors and tumor necrosis factor (TNF) in the regulation of apoptosis is not well understood. Peptide growth factors, initially characterized as stimulators of cell proliferation, have now been shown to inhibit death in many cell types. Deprivation of growth factors leads to the induction of apoptosis (Collins MK, Bioessays, 1994 Feb, 16(2):133-8). For instance, murine bone marrow-derived hematopoietic cells, dependent on interleukin (IL)-3 for their growth in culture, undergo apoptosis, upon cytokine withdrawal. Etoposide, a topoisomerase II inhibitor, causes a more rapid onset of apoptosis in the IL-3-dependent cell line BAF3, deprived of IL-3. This acceleration of apoptosis by etoposide is prevented by inhibitors of RNA and protein synthesis and by the nucleases inhibitor aurintricarboxylic acid. The presence of IL-3 or overexpression of the oncogene bcl-2 causes a marked delay in the induction of apoptosis by etoposide, acting in a cooperative manner (Ascaso R, European Journal of Immunology, 1994 Mar, 24(3):537-41). Two stimuli found in bone marrow, differentiation-inducing cytokines, which induce terminal differentiation associated with growth arrest, ultimately culminating in apoptosis, and transforming growth factor-β1 (TGF-β1), which induces rapid growth arrest and apoptosis of hematopoietic cells, induce apoptosis at different rates in malignant blood cells (Selvakumaran M, etal, Blood, 1994 Aug 15, 84(4):1036-42).

Παραδοξα (PARADOX)

There is no simple elegant description of the phenomenon of apoptosis, no central switch that turns the process on or off. Increasingly, new findings demonstrate that the mechanisms of apoptosis differ by cell type and between organisms. Like with all other biological phenomena, complexity and paradox rule the process which involves numerous participants but as yet claims no ultimate single and unique origin.

Dominant Oncogenes Inhibit and Promote Apoptosis

A number of dominant oncogenes act both as inhibitors and inducers of apoptosis. Oncoproteins E1A and Myc, when overexpressed, under different conditions, may either transform cells or sensitize them to apoptosis. Such contradictory functions implicate other gene products acting in concert as regulators of the outcome of oncoprotein expression. In a "two signal" model of cell growth and death, the first signal activates a common pathway that can lead to proliferation or apoptosis and a second signal determines the final outcome. Alternatively, it may be that the first stimulus initiates a sequence of metabolic events common to both apoptosis and replication, and the second stimulus acts as only a progression signal, e.g., to divert the cell toward replication, if present, otherwise apoptosis supervenes (DL Vaux, Proc Natl Acad Sci USA, [1993] 90:786). In this latter model, apoptosis is the default pathway. One such two signal system may be represented by the interaction between c-myc and bcl-2. The latter gene is a strong candidate for the source of a second signal, that blocks the apoptotic pathway and prolongs tumor cell survival.

The Ultimate Apoptosis Signals May Reside in the Cytoplasm

It was originally believed that the ultimate regulators of apoptosis reside in the nucleus and gene expression determined when and how the process was activated. However, it has been shown that molecular instructions for apoptosis may reside in the cytoplasm, because even enucleated cells go through apoptosis when exposed to inducers. Therefore, it appears that apoptosis is not always transcriptionally activated but rather triggered by cytoplasmic effector proteins which are probably synthesized in advance and remain inactive until activated by appropriate signals. However, in some models of apoptosis, these proteins may need to be turned on by a transcriptionally produced regulatory protein to activate the apoptotic pathway, which is not possible in enucleated cells.

Apoptosis May Help Malignant Cells Avoid Host Immune Mechanisms

Although inducing apoptosis may enhance the killing action of cytotoxic drugs it may also interfere with the immune response to malignancy. For instance, dysregulation of bcl-2 gene expression that promotes apoptosis may play a role in the avoidance of host immune surveillance mechanisms by cancer cells (Torigoe T, etal, Cancer Research, 1994 Sep 15, 54(18):4851-4). Such a scenario may diminish the long-term effectiveness of apoptosis-based therapy.

Anticancer Drugs May Induce Mutations in Key Apoptosis Regulating Genes

Mutations in p53 have been shown to promote carcinogenesis. Cancer treatment, in causing DNA-damage, may induce mutations in p53 that could contribute to rapid progression of disease. This may be more likely to occur in cells with a mutant p53 allele.

Apoptosis May Play a Role in Spontaneous Remissions

Spontaneous remissions in patients with advance malignancies may be caused by promotion of apoptosis, either as a delayed reaction to treatment or as a result of undetermined endogenous stimuli.

APOPTOSIS IN ANTICANCER DRUG DEVELOPMENT

Various stimuli are positive or negative regulators of apoptosis. In malignancy it is the inducers of apoptosis that are beneficial. Strategies to exploit apoptosis to treat malignancies include blocking endogenous molecular targets that inhibit apoptosis, enhancing endogenous signals or pathways that induce apoptosis, or introducing exogenous agents capable of similar actions. Manipulat-

ing intrinsic regulators at the molecular level, although attractive, may be difficult to accomplish based on limited understanding of the mechanisms of apoptosis. For instance, gene manipulation techniques offer a fundamental approach to enhancing apoptosis, but are also the most challenging to apply clinically. However, genes promoting apoptosis may also be activated using less direct methods such as depriving dependent cells of growth factors or hormones. Also, apoptosis-resistant cancer cells may be killed by cytotoxic lymphocytes (CTLs) or apoptosis-inducing cytokines.

Enhancement of the action of current anticancer treatment options may offer a more achievable short-term goal than using apoptosis pathways alone. For instance, radiation and many chemotherapeutic agents, such as docetaxel/paclitaxel, etoposide, methotrexate, doxorubicin, vinblastine, 5-FU, cisplatin, enediynes and retinoids, among others, are particularly effective in inducing apoptosis (interestingly, certain chemotherapeutics, because of their mode of action may, inadvertently, inhibit apoptosis). It may be possible to develop techniques to enhance the initial effectiveness of these drugs, and also prevent subsequent resistance by concurrently administrating agents that promote apoptosis. Exhibit 20 lists apoptosis-based agents in development for the treatment of cancer.

One of the problems in drug development in this area is that a large portion of current understanding is derived from *in vitro* experiments that may not translate *in vivo,* because of the complexity of apoptotic pathways and signaling mechanisms.

Gene Transfer

Gene therapy may be used to restore wild type activity of mutated genes such as p53 in tumor cells. Furthermore, if gene transfer can be selective for tumor cells, death of these cells will prevent overexpression of the target gene in healthy tissue. Although lung cancer and other carcinoma cells with mutant p53 have been successfully transfected with wild-type p53 using a retroviral expression vector *in vitro* (T Fujiwara, et al, Cancer Res, [1993] 53:4129), such transfection may be difficult to accomplish *in vivo.* Transfection with retroviral plasmid vectors works well *in vitro,* but lacks specificity *in vivo;* more specific targeting might be possible with recombinant parvovirus strains, harboring p53 or other apoptosis-inducing genes, because these vectors are expected to infect replicating neoplastic cells exclusively. Selective tumor cell targeting *in vivo* may be accomplished by intra-arterial or direct intratumoral injection of liposomes incorporating apoptosis-inducer genes. Tumor-selective targeting of p53 may also be possible with antibody-liposome carrier conjugates, if the cells of interest specifically express a target antigen to which the immunoglobulin can bind.

Canji, (San Diego, CA), a private company established in 1990, is investigating the use of tumor suppressor genes to treat lung, breast, prostate, liver and colorectal cancer and leukemia. Canji's first products target the retinoblastoma (Rb) and p53 genes, in which alterations occur in at least 50% of all malignancies. In one program Canji aims to replace the defective p53 gene in cancer cells with a wild-type version using an adenovirus vector. The company has been given RAC approval to treat hepatocellular carcinoma and metastatic liver cancer originating from colorectal cancer. In October 1994 Schering-Plough and Canji signed an agreement to develop cancer treatments based on Canji's p53 gene-therapy technology. The agreement, which provides Canji with an initial cash payment, annual performance and milestone payments, and royalty payments on the sales of future products, may result in a total Schering-Plough outlay of over $50 million over several years. Canji will conduct preclinical studies and Schering-Plough will be responsible for clinical trials, manufacturing, and marketing. An IND to begin clinical trials is planned in 1995.

Introgen Therapeutics (Austin, TX), a private company, is developing several gene therapy products in collaboration with RPR Gencell to treat cancer by retroviral delivery of intact wild-type p53, among others. [In late 1994, Rhône-Poulenc Rorer formed RPR Gencell, a network of alliances funded by a $100 million annual budget, to gain access to R&D carried out by various highly specialized biotechnology companies.] Introgen holds several RAC approvals obtained through M.D. Anderson Cancer Center (Houston, TX), one of the company's founders, to treat cancer by gene therapy using retroviral and adenoviral vectors.

Ingenex (Menlo Park, CA), a Titan Pharmaceuticals operating company, is using a variant of the Rb gene (SG-94), licensed from Baylor College of Medicine (Houston, TX), as gene therapy for solid tumors. When inserted into target cells *in vitro,* this variant gene expresses a protein that is a potent inducer of apoptosis and senescence.

Oligonucleotide-based Strategies

At the La Jolla Cancer Institute (La Jolla, CA), Dr. John C. Reed and associates have used antisense (AS) approaches to achieve reductions in the levels of steady state Bcl-2 protein in t(14;18)-containing human lymphoma cell lines. Both synthetic bcl-2-AS oligonucleotides and inducible expression plasmids that produce bcl-2-AS transcripts led to reductions in bcl-2 expression and a marked enhancement in the sensitivity of neoplastic cells to conventional chemotherapeutic drugs such as cytosine arabinoside (ara-C) and MTX. Reductions in bcl-2 expression may, therefore, improve the effectiveness of cytotoxic drugs (Kitada S, etal, Antisense Research and Development, 1994 Summer, 4(2):71-9).

Genta (San Diego, CA), is constructing antisense oligonucleotides targeted against Bcl-2 to reverse the chemoresistance observed in cancer cells with activated bcl-2. Oligonucleotides targeted against this target were

found to decrease the level of Bcl-2 in human cultured cells in preclinical studies. In animal models, one of these oligonucleotides was found to inhibit the growth of drug-resistant human colon tumors in nude mice, while a control oligonucleotide had no effect. Similar studies have also been conducted by Finbarr Cotter, MD, at the Institute of Child Health (London, UK). In these studies, an anti-Bcl-2 oligonucleotide was shown to cure lymphoma-like disease induced by the injection of human B-cell lymphoma cells in severe combined immunodeficient (SCID) mice (Cotter, FE, etal, Oncogene, 1994 Oct, 9(10):3049-55). Following completion of a toxicology study, Genta plans to begin human clinical trials in patients with drug resistant follicular lymphoma at the Royal Marsden Hospital (UK) under a physician's IND. Chugai Pharmaceutical (Tokyo, Japan) has the option to license worldwide marketing rights to this therapy if it decides to fund its further development.

Lynx Therapeutics (Hayward, CA), a private company established in 1992, is developing antisense-based cancer therapies targeting p53 and myc. Lynx has completed a phase I clinical trial of LR-3523, a 20-mer phosphorothioate oligodeoxynucleotide analogue targeting p53 and is planning to begin a phase II trial in June 1995. Lynx is also conducting a phase I dose-escalation study of LR-3001, a 24-mer phosphorothioate antisense to c-myb for treatment of CML in accelerated phase or blast crisis. This study of repeated courses of 7-day IV infusion is being conducted at the University of Pennsylvania (Philadelphia, PA). This agent was evaluated preclinically in SCID mouse models of human CML and malignant melanoma and is also under investigation for use with autologous bone marrow transplantation in CML. Lynx is also studying the *in vivo* efficacy of a 26-mer phosphorothioate oligonucleotide targeted against a bcr-abl breakpoint junction in the treatment of CML (Skorski, T, etal, Proc. Natl. Acad. Sci. USA, Vol. 91, pp. 4504-4508, May 1994). These experiments are being extended to include combination therapies with bcr-abl and either conventional drugs or other antisense agents.

Small Molecule Drugs

Apoptosis Technology (ATI), a subsidiary of ImmunoGen (Cambridge, MA) established in 1993, is collaborating with Paul Anderson's laboratory at Dana-Farber Cancer Institute (Boston, MA) to identify proteins involved in apoptosis and, by combining ImmunoGen's MAb-based toxin delivery system with such proteins, to deliver an apoptotic signal to tumor cells. In August 1994, ATI also announced a research agreement with the Imperial Cancer Research Fund (ICRF; London, UK) to identify compounds involved in apoptosis, using ICRF's proprietary screening system and to develop small molecule drugs to modulate the action of bcl-2 and insulin-like growth factor I (IGF-I).

Mitotix (Cambridge, MA), a private company established in 1992, is developing anticancer agents that target enzymes involved in cell cycle regulation and cell proliferation. Using small molecules, this approach offers an alternative to gene therapy, and may yield therapeutic compounds possessing both selectivity and ease of delivery (e.g., orally active, capable of penetrating cell membranes). Mitotix is also developing cancer therapeutics based on compounds which interfere with the ubiquitin-mediated breakdown of disease related proteins. (Ubiquitin is a signaling molecule present in all living cells that acts to induce the breakdown of specific proteins; most short-lived proteins which regulate key cellular processes, including p53, are degraded by a ubiquitin dependent biochemical pathway. The ubiquitin degradation pathway requires a family of highly specific ubiquitin conjugating enzymes, which target ubiquitin to its intracellular substrates.) The substrates of several new enzymes have been identified, including key regulatory proteins involved in p53 degradation. Drug development is focused on inhibiting the degradation of p53 in specific clinical conditions. For example, a protein produced by human papillomavirus (HPV) binds to p53, activating its degradation through the ubiquitin system. This accelerated degradation is a key event in inducing the transformation of cervical cells to cancer cells (90% of patients with cervical cancer are infected with HPV). Restoration of p53 levels in HPV-infected cervical cancer cell lines results in sudden, quantitative tumor cell death while normal cells are unaffected by the elevation of p53 levels. Mitotix has developed proprietary assays to identify small molecular weight drugs that prevent p53 degradation and block the proliferation of cancer cells. Using these assays the company has identified initial active compounds. In addition to cervical cancer, Mitotix is evaluating the potential for treating cancers with normal p53, but a defective retinoblastoma pathway. Blocking intracellular proteolysis of key proteins may allow the development of drugs that induce cell death in these cancer cells, but not in normal cells. Mitotix has filed three patent applications involving its intracellular proteolysis technologies.

Onyx Pharmaceuticals (Richmond, CA), a private company spun off from Chiron in 1992, is focusing its efforts on elucidating the role of intracellular signalling pathways in cancer and abnormal cell growth. The Onyx apoptosis program centers on survival factors, p53 and Bcl-2 and has developed assays to screen for small molecules that regulate these pathways. The survival factor program exploits the fact that a variety of cancers, including lung, breast, and pancreatic cancer, overexpress the receptors for the survival factor IGF I or IGF I itself. When deprived of IGF I cancer cells undergo apoptosis whereas normal cells simply cease to grow. Onyx intends to develop small molecules that intervene in the survival factor signaling pathways, targeting the cancer cells for apoptosis. The company has also developed novel strategies to identify the molecular partners of both mutant and normal p53. It has established a research collaboration focusing on p53 with Arnold J.

Levine, PhD, of Princeton University (Princeton, NJ), a co-discoverer of p53. Onyx has also identified several molecular partners of Bcl-2, among them the signal transduction protein, R-ras (Fernandez-Sarabia, MJ, and Bischoff, JR, Nature, vol. 366, 18 Nov 1993, pp 274-275). In collaboration with Bayer (was Miles; Pittsburgh, PA), Onyx is working to identify small molecule drugs that turn off inappropriate ras activity or reduce it to normal levels. Based on several molecular targets associated with the ras pathway, Onyx has developed biochemical- and cell-based assays and has identified potential ras inhibitors; one drug candidate is expected to enter clinical evaluation by the end of 1996.

IDUN (Menlo Park, CA), established in 1993, is developing small molecule anticancer drugs identified through drug screening, that inhibit bcl-2.

Nuclear Matrix Proteins

Nuclear matrix proteins (NMPs) are nonhistone proteins found in the nucleus of many eukaryotic cells. Some NMPs have been reported to be highly specific by cell type and to be expressed differentially in malignant cells. Consequently, changes in the cell nucleus at the morphologic level (observed by pathologists in the clinical diagnosis of cancer), may be reflected as changes in NMPs at the molecular level. Certain apoptosis-inducing chemotherapeutic agents such as etoposide and camptothecin bind to enzymes in the nuclear matrix (topoisomerases) causing apoptosis by enzyme inhibition. Similarly, antimetabolites such as 5-FU and 5-fluorodeoxyuracil, which also induce apoptosis, target the DNA polymerase complex which is attached to the nuclear matrix. Bcl-2 also routinely associates with the nucleus during certain phases of the cell cycle. Nuclear matrix proteins which bind Bcl-2 may also prove suitable targets for chemotherapy.

Matritech (Cambridge, MA), which was established in 1987 and went public in 1992, is using MAbs to NMPs to detect the possible presence of cancer, monitor for recurrence, or assess the effectiveness of treatment. Matritech scientists have found that NMPs can be released from dying cells in a soluble form, and that the solubilization and release of NMPs are normal events in the apoptotic pathway. Assuming that changes in the nuclear matrix are potentially observable events in the cell death program, Matritech, using immunological techniques, has developed assays capable of detecting NMPs in a quantitative fashion. NMPs are elevated in supernatants of dying cell and tissue cultures as well as in the serum of cancer patients (T Miller, et al, BioTechniques, [1993] 15:1042). Matritech is developing various diagnostics based on NMPs such as serum assays to monitor/detect recurrence of various cancers (breast, colorectal, prostate and lung), a urine assay to diagnose bladder cancer (a PLA was filed in the USA; marketing in Europe began in early 1995) and an assay of cervical Pap smears to identify precancerous cells.

In January 1995, the company announced that it received a Small Business Innovative Research (SBIR) grant of $88,000 from the National Cancer Institute to clone the gene that expresses PC-1, a prostate cancer-specific NMP. PC-1 was present in every prostate tumor examined but not present in any normal or benign prostate specimens (Partin, AW, etal, Cancer Research, 1993 Feb 15, 53(4):744-6). The objective of the funded project is to identify the gene that expresses PC-1 in order to develop a therapeutic product to block its activity and, as a result, prevent the progression of malignancy. Matritech, which has obtained exclusive rights from Johns Hopkins for the commercial, diagnostic and therapeutic use of PC-1, has been developing novel *in vivo* diagnostic products to more accurately detect prostate cancer. To support that work, Matritech was awarded an NCI SBIR grant in December 1993 to develop antibodies suitable for *in vitro* diagnostic tests.

Telomerase Inhibition

Telomeres, consisting of thousands of tandem repeats of a six-nucleotide DNA sequence, are structures at the end of eukaryotic chromosomes that confer protection, provide positioning and participate in replication. Loss of telomere length in dividing normal somatic cells is considered to represent a measure of aging, a mitotic clock that registers the number of times a normal cell undergoes division until it reaches senescence. Malignant cells, in contrast, do not lose telomere length or sequence with cell division. Telomerase, a protein that maintains telomere length by synthesizing telomeric DNA using RNA as a template, is present in carcinoma cell lines but not in normal somatic cells. It is, however, present in germ cells. Inhibition of telomerase activity may play a role in apoptosis, as both appear to act against unrestrained cell growth.

Geron (Menlo Park, CA), established in 1992, is developing telomerase inhibitors for cancer diagnosis and therapy. In cooperation with scientists at the University of Texas Southwestern Medical Center (Dallas, TX), the company developed a highly selective PCR-based assay that measured telomerase activity in 90 out of 101 biopsies representing 12 human tumor types but none in 50 normal tissue samples (Kim, NW, etal, Science, 23 Dec 1994, Vol. 266, pp 2011-2015). Because telomerase is not found in normal somatic cells, its may provide a tumor-selective target for cancer therapy. However, many challenges remain including heterogeneity of telomere length which may result in a population of tumor cells with varying replicating capacity and sensitivity to anti-telomerase therapies.

Angiogenesis Inhibitors

(A comprehensive review of the role of the extracellular matrix and angiogenesis in cancer will be presented in an upcoming issue of FUTURE ONCOLOGY).

Ixsys (San Diego, CA) is developing a humanized/optimized version of MAb LM609, first described by Dr. David Cheresh of the Scripps Research Institute (La Jolla, CA), as an inhibitor of angiogenesis via apoptotic mechanisms. LM609 is an antagonist of integrin $\alpha v\beta_3$. A single IV injection of this MAb disrupted angiogenesis in animal models and led to rapid regression of tumor xenografts (Brooks, PC, etal, Cell, Vol. 79, 1157-1164, Dec 30, 1994) Ixsys humanized/optimized murine LM609 using its proprietary Codon-Based Mutagenesis design technologies. A mammalian cell line expressing high levels of the humanized/optimized MAb has been generated at Celltech Biologics (Slough, England) and Ixsys is currently finalizing the selection of cell-line clones to proceed with the manufacturing of this MAb, trademarked Vitaxin, at Celltech. *In vitro* ligand binding and cell adhesion assays suggest that Vitaxin possesses similar characteristics as the original LM609. Clinical trials for the treatment of solid tumors with this antibody are expected to begin in 14-18 months.

Photodynamic Therapy (PDT)

PDT uses photosensitive hydrophobic dyes to sensitize malignant cells to visible light. Photosensitizing dye, injected intravenously, accumulates in tumors but is cleared rapidly from healthy tissue. The tumor site is then exposed to light, commonly a laser beam, using a fiberoptic probe. Treatment lasts between 5-20 minutes depending on the affected area. Dye is selectively taken up by tumor cells because it attaches to lipoproteins; vessels feeding tumors contain a higher than normal concentration of lipoprotein receptors. Dyes localize on membranes of tumor cells and photoactivation results in oxidation of membrane lipids and proteins. It has been shown *in vitro* that PDT induces apoptosis. As observed in murine lymphoblastic leukemia cells, PDT causes apoptosis manifested by extensive DNA fragmentation, within 30 minutes of treatment (Oleinick, NL, etal, Proceedings of ACCR, Vol. 36, March 1995, pp. 710-711). Various light sensitive PDT dyes have been identified. The most extensively tested is Photofrin, developed by Quadra Logic Technologies (QLT; Vancouver, BC, Canada), originally in collaboration with American Cyanamid (now part of American Home Products) which still retains rights to the drug in overseas markets. Photofrin was approved in Canada for the treatment of superficial bladder cancer in April 1993, in Japan for the treatment of lung, gastric, esophageal and cervical cancer in September 1994 and the Netherlands for esophageal and lung cancer in April 1994. In September 1994, FDA's Oncology Drugs Advisory Committee recommended approval of an NDA submitted in April 1994 for the use of Photofrin as a palliative measure in obstructing tumors of the esophagus. Another QLT dye, benzoporphyrin derivative (BPD) is in phase II clinical trials in cutaneous cancer and psoriasis. In addition, QLT will develop Ciba Pharma's (Ciba-Geigy's; Basel, Switzerland) proprietary PDT dye, zinc phthalocyanine (ZnPc), for cancer indications under an exclusive worldwide license granted in April 1995. Another PDT dye in development is Scotia's (Guildford, Surrey, UK) EF9 (mesotetrahydroxyphenylchlorin; mTHPC), in phase II clinical trials for the treatment of head and neck cancer.

Enediynes

Enediynes, first reported on in the late 1980s, belong to a family of naturally occurring antitumor antibiotics that include the neocarzinostatin (NCS) chromophore, calicheamicins, kedarcidin, esperamicins (A1 and C), and dynemicins. These substances can undergo aromatization to produce cytotoxic biradicals and can result in phosphodiester bond breakage of DNA. Another macromolecular antitumor antibiotic, C1027, produced by a Streptomyces strain, shows highly potent cytotoxicity to cultured cancer cells and marked DNA cleaving ability. The structure of its chromophore, responsible for most of its biological activities, was found to contain a nine-membered enediyne. In contrast to other enediynes, C1027 damages duplex DNA even in the absence of thiols (Xu YJ, etal, Biochemistry, 1994 May 17, 33(19):5947-54). Enediynes can be modulated to a fine degree through structural modification, taking advantage of differences between normal and tumor cells such as cell permeability and enzymatic activity. Synthetic enediynes, designed with low molecular complexity are also highly cytotoxic to human leukemic cells by induction of apoptosis (Hiatt A, etal, Bioorganic and Medicinal Chemistry, 1994 May, 2(5):315-22). Enediynes such as NCS may be useful in *ex vivo* purging regimens and in *in vivo* treatment of microscopic residual disease in patients with neuroblastoma (Will P, etal, Cancer Chemotherapy and Pharmacology, 1994, 35(2):115-20). Treatment of Kaposi's sarcoma (KS) cells with enediynes induced apoptosis in up to 80% of the cells (Corbeil J, etal, Cancer Research, 1994 Aug 15, 54(16):4270-3).

Other Apoptosis-based Anticancer Drug Development

LXR Biotechnology, a public company, is developing LXR023 (maspin), for the treatment of metastatic breast cancer under an exclusive license from Dana-Farber Cancer Institute (Boston, MA). LXR is also developing three proprietary technologies to assist its scientists in screening for new therapeutics based on the modulation of apoptosis. These include an *in vitro* apoptosis screening assay (ASA) to screen potential agents for apoptosis modulating activity in a non-tumorous cell strain; scanning laser digital imaging (SLDI) technology to permit rapid, computer-controlled, high-throughput screening of multiple agents for apoptosis modulating activity; and an *in vivo* serum apoptosis marker (SAM) assay to determine the level of apoptosis in various parts of the body by measuring protein associated nucleic acids (PANAs) in the blood. LXR is actively developing apoptosis-related agents for indications other than cancer.

Exhibit 20
Selected Drugs in Development to Regulate Apoptosis in Malignancy

Developer/ Affiliate	Indications	Generic Name/ Number/ Brand Name	Description	Status/ Location
Ansan (Titan Pharmaceuticals)	Solid tumors	AN-9, AN-10	Synthetic derivative of butyric acid	Phase I/USA
Bristol-Myers Squibb	Cancer	Kedarcidin	Enedyine	Preclin/USA
Canji/Schering-Plough	Hepatocellular carcinoma and metastatic liver cancer originating from colorectal cancer		Replacement of defective p53 gene in cancer cells with a wild-type gene using an adenovirus vector	Preclin (RAC approved)/USA
Cell Pathways	APC (may also be applicable to other precancerous conditions)	FGN-1	Sulfone metabolite of the NSAID sulindac	Phase I/II (95)/USA
Genta/Chugai (has option for exclusive license)	Drug resistant follicular lymphoma	Anticode	Oligonucleotide inhibitor of Bcl-2	Preclin/USA, UK
Geron	Cancer		Telomerase inhibition	Research/ USA
IDUN	Cancer		Small molecules inhibitors of bcl-2	Research/ USA
Ingenex (Titan Pharmaceuticals)/ Baylor College of Medicine (licensor)	Solid tumors		Variant of the Rb gene (SG-94); expresses a protein that is a potent inducer of apoptosis and senescence	Research/USA
Introgen Therapeutics/ RPR Gencell (Rhône-Poulenc Rorer)	Solid tumors		Retroviral delivery of intact wild-type p53 gene	Preclin/USA
Ixsys/ Scripps Research Institute (licensor)	Solid tumors	LM609/ Vitaxin	Humanized MAb against integrin $\alpha v\beta_3$; angiogenesis inhibitor	Preclin\USA
Ligand Pharmaceuticals/ Allergan	Acute promyelocytic leukemia (APL)	9cRA/LGD1057	Synthetic 9-cis retinoic acid, orally administered	Phase I/IIA
Ligand Pharmaceuticals/ Allergan	Skin cancer (Kaposi's sarcoma, cutaneous T-cell lymphoma, squamous and basal cell carcinoma)	9cRA/LGD1057	Synthetic 9-cis retinoic acid, topically administered	Phase I/II
Ligand Pharmaceuticals	Advanced cancer	LGD1069	Chemical retinoid, orally administered	Phase I/IIA
Ligand Pharmaceuticals	Skin cancer (Kaposi's sarcoma, mycosis fungoides, squamous and basal cell carcinoma)	LGD1069	Chemical retinoid, topically administered	Phase I/IIA
LXR/Dana-Farber Institute	Drug-resistant breast cancer	Maspin		Preclin/USA
Lynx Therapeutics	CML	LR-3523	20-mer phosphorothioate oligo analogue targeting p53	Phase I completed/ USA
Lynx Therapeutics	CML in accelerated phase or blast crisis	LR-3001	24-mer phosphorothioate antisense targeting c-myb	Phase I/ USA
Matritech	Prostate cancer		Gene that expresses PC-1, a prostate cancer-specific nuclear matrix protein (NMP)	Preclin/USA
Mitotix	Cervical cancer		Restoration of p53 in HPV-infected cells	Research/ USA

— *continued*

Exhibit 20
Selected Drugs in Development to Regulate Apoptosis in Malignancy

Developer/ Affiliate	Indications	Generic Name/ Number/ Brand Name	Description	Status/ Location
Onyx Pharmaceuticals	Cancer		Small molecule IGF I inhibitors	Research/USA
Onyx Pharmaceuticals/ Princeton U	Cancer		p53 targeting	Research/USA
Onyx Pharmaceuticals/ Bayer (Miles)	Cancer		Small molecule drugs that modulate signal transduction protein R-ras	Research/ USA
Receptagen	Cancer		B12 depletion via MAb inhibitor of receptor binding site on the B12 carrier protein	Research/ USA
Sennes Drug Innovation (was AGIS Pharmaceuticals)	Cancer		Regulation of cell senescence genes	Research/ USA
Yamanouchi/ Kayaku/ Kuraray	Liver cancer; also active against colorectal and gastric cancer	Zinostatin stimalamer, SMA/YM-16881; YM-881	Enedyine; neocarzinostatin (NCS)	Launched in Japan in 1994 as IA; in clinicals as IV

Source: New Medicine

Ligand Pharmaceuticals (San Diego, CA) has formed a joint venture with Allergan (Irvine, CA), Allergan Ligand Retinoid Therapeutics (ALRT), to discover and develop novel retinoids for the treatment of cancer and other diseases. The lead drug under development by ALRT is LGD1057, a synthetically-produced 9-cis-retinoic acid which appears to be a natural ligand for the RAR and RXR subfamilies of retinoid IRs. ALRT began a phase I/IIA trial of an oral formulation of LGD1057 in December 1993. The company has obtained orphan drug status for LGD1057 in the treatment of acute promyelocytic leukemia (APL) and plans to also study the agent in kidney cancer in combination with interferon-α. Topical LGD1057 is also undergoing phase I/II clinical trials in skin cancer. Ligand has also identified chemical retinoids and is developing one, LGD1069, outside the ALRT joint venture. Oral LGD1069 is in phase I/IIA in patients with advanced cancer and its topical formulation is being evaluated for certain skin cancers. Ligand is also seeking blockers of the male and female sex steroids for use in cancer.

Ansan (Menlo Park, CA), a Titan Pharmaceuticals operating company, is studying the effects of a derivative of butyric acid, pivaloyloxymethyl butyrate (AN-9), on cell proliferation, death and differentiation in C6 glioma cells and HL-60 leukemic cells in culture. At various concentrations AN-9 inhibited cell growth and induced apoptosis. The company has obtained an IND and is planning to begin phase I trials in patients with solid tumors.

Cell Pathways (CPI; Denver, CO), a private company established in 1988, is developing FGN-1 (the sulfone metabolite of sulindac), an inducer of apoptosis, for the treatment of familial adenomatous polyposis (FAP) associated with adenomatosis polyposis coli (APC). Sulindac is a prodrug that is converted *in vivo* to sulfide (SS) and sulfone (FGN-1) metabolites. SS is an active non-steroidal anti-inflammatory drug (NSAID) which inhibits prostaglandin synthesis whereas FGN-1, which is not an NSAID, does not. NSAIDs have been shown to be chemopreventive in colon cancer but the mechanism for this action has not been elucidated. In *in vitro* studies FGN-1 induced apoptosis in colonic tumors in rats. The drug is in phase I clinical trials in the USA under the auspices of the NCI for the treatment of APC and has been granted orphan drug status for this application. Phase I/II trials are expected to begin in mid-1995. The agent may also have utility in the treatment of sporadic polyps associated with colon cancer. FGN-1 is orally administered and is intended for chronic use to eliminate small lesions and prevent development of new ones. CPI is also developing FGN-1 as a treatment for cervical dysplasia and other premalignant conditions.

Receptagen (Vancouver, BC, Canada and Edmonds, WA), established in 1992 and a public company, is sponsoring research on anticancer agents that induce apoptosis using outside collaborators. In one approach, a MAb is used that reacts with the receptor binding site on the B12 carrier protein, preventing it from delivering B12, an essential nutrient and an enzyme co-factor for the production of cellular folates and nucleotides. Vitamin B12 depletion induces apoptosis in tumor cells, especially when combined with folate withdrawal. Receptagen is also sponsoring research to clone the vitamin B12 receptor so that a small molecule ligand may be identified that blocks its activity.

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