FUTURE ONCOLOGY

TECHNOLOGY, PRODUCTS, MARKETS AND SERVICE OPPORTUNITIES

A NEW MEDICINE PUBLICATION | JUNE/JULY 1995 | VOLUME 1, NUMBER 2/3

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

CANCERS OF THE DIGESTIVE SYSTEM-PART II

MEETING COVERAGE

RADIOIMMUNOSCINTIGRAPHY IN CANCER DIAGNOSIS

A REPORT FROM THE TENTH INTERNATIONAL CONFERENCE ON MONOCLONAL ANTIBODY IMMUNOCONJUGATES FOR CANCER, 9-11 MARCH 1995; SAN DIEGO, CA

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

CANCERS OF THE DIGESTIVE SYSTEM-PART II

SCREENING, DIAGNOSIS, AND MONITORING

As therapeutic interventions become more successful, demand for better methods to detect and monitor disease will increase. Most desirable diagnostic methods involve *in vitro* tests that are inexpensive and can be performed repeatedly throughout the course of treatment to monitor disease status and assess effectiveness of treatment and patient prognosis. Because of new insights into chemoprevention of certain cancers and potential development of prophylactic vaccines, opportunities are also emerging in the application of *in vitro* tests to screen high risk individuals for genetic markers that may predispose them to certain types of cancer. Demand for testing patients for susceptibility to certain chemotherapeutics is also on the rise, because of the variety of regimens available and new agents in late stage clinical trials.

Esophageal Cancer

The first clinical symptom of esophageal carcinoma, i.e. dysphagia, often presents in patients with advanced disease. Consequently, few patients with this cancer are diagnosed in early stages of the disease. Routine screening of asymptomatic individuals is not cost effective except in suspected high risk cases. When symptoms such as dysphagia, odynophagia, heartburn, or regurgitation persist, diagnostic evaluation routinely includes fiberoptic upper endoscopy, barium swallow, and upper gastrointestinal radiographic evaluation. In patients without dysphagia, but in whom carcinoma is suspected or cytology is positive, endoscopic examination of the entire length of the esophagus, stomach and duodenum is indicated. In all cases tissue biopsy is essential for confirmation.

Tumor stage dictates prognosis and guides choice of therapy. The factors which predict prognosis are depth of esophageal wall penetration, number and location of lymph node metastases and presence of distant metastases. CT scanning represents the standard method to assess wall penetration; MRI scanning is under investigation. Endoscopic ultrasonography, using an ultrasound probe mounted on a flexible fiberoptic esophagoscope, a newer technology still under evaluation, promises greater accuracy in determining both wall penetration and lymph node status.

Stomach Cancer

Diagnostic evaluation involves a multidisciplinary approach for both early and invasive cancer. Roentgenography (upper GI or barium swallow) is mandatory for screening, but if negative, it is not considered sufficient in either symptomatic or healthy subjects. Double contrast x-rays and cineradiography improve the detection of curable early gastric cancer, but false-negative rates still range as high as 17% to 31%. The risk of false-negatives and the ultimate need for follow-up with endoscopy

Exhibit 1
Estimated Worldwide Chemotherapy Regimens* for the Treatment of Digestive System Cancers (1994)

Type of Cancer	Center USA Regimens (#)	Japanese Regimens (#)	European Regimens (#)	Worldwide Regimens(#)
Esophageal	12,000	11,800	29,100	52,900
Colorectal	136,800	149,900	361,300	648,000
Liver/Biliary	19,400	39,900	27,100	86,400
Gallbladder		13,600	19,900	33,500
Pancreatic	26,600	15,700	52,100	94,400
Stomach	25,200	87,400	180,800	293,400
Total	220,000	318,300	670,300	1,208,600

* Includes monotherapy, combination therapy, immunotherapy and adjuvant and neoadjuvant therapy with or without surgery or radiotherapy. Includes initial treatment, retreatment, treatment at relapse and palliation for terminal patients; does not include long term chemoprevention.

Source: New Medicine

have led to the use of endoscopy as a primary screening test; x-rays combined with endoscopy reduce false-negative rates to about 5%. Proximal lesions, still the most difficult to detect by x-rays, are more accessible with endoscopic technology.

The stage or extent of cancer invasion, as determined by the size, depth of penetration into surrounding tissue and metastatic spread, influences the extent of both lymph node dissection and organ resection. Ultrasound offers perhaps the best additional preoperative diagnostic method, and CT and MRI may improve characterization of uncertain lesions. However, lymph nodes and peritoneum remain difficult to assess and these modalities are not substitutes for surgical staging if surgery is indicated. Open or endoscopic biopsy or, if necessary, brush cytology, is paramount in making a tissue diagnosis. Surgery with curative intent is not indicated in patients with extensive local or metastatic disease. Palliative procedures, however, may provide temporary relief from such symptoms as obstruction and other tumor-related problems.

Tumor markers have no role in screening or diagnosis. Carcinoembryonic antigen (CEA) is elevated in only one-third of patients, particularly those with large metastatic tumors; fetal sulfoglycoprotein antigen is present in the gastric juice of patients with adenocarcinoma but is not specific, having a false-positive rate of 14%. Other tumor markers elevated in stomach cancer include CA 19-9, tissue polypeptide antigen (TPA), c-erbB-2 oncoprotein, CA 125 and alpha-fetoprotein (AFP). Tumor markers may have a role in monitoring disease, but by themselves are an inadequate criterion of successful treatment or residual disease or relapse. However, a significant rise in antigen titre is an indication to undertake confirmatory tests for evidence of relapse. Standards of follow-up after surgery include endoscopy and/or CT scanning and chest x-rays every six months, and routine laboratory tests and physical examination every three months.

Liver Cancer

Hepatocellular carcinoma (HCC) is diagnosed and evaluated by imaging procedures that show the number and size of liver nodules. Among imaging modalities, radionuclide imaging has low sensitivity and spatial resolution. Neoplasms may be more or less echogenic than surrounding normal hepatic parenchyma, but unless lesions are sufficiently large or inhomogeneous, they may go undetected by ultrasound; the sensitivity of sonography for neoplasms is in the range of 70%-80%. CT combines both high sensitivity for focal lesions and high specificity regarding their nature. Adding intravenous contrast improves lesion detection by increasing the attenuation difference between enhancing normal liver parenchyma and enhancing tumor. MRI has also become an important technique in the detection of focal liver lesions. Hepatic angiography provides excellent spatial resolution and identification of hepatic vascular anatomy; digital subtraction angiography has proven useful in identifying small peripheral lesions. Angiography may be combined with CT by using dynamic sequential CT scanning during infusion of intravenous contrast.

Tumors metastatic to the liver are also detected by a variety of imaging modalities depending on tumor type and the degree of vascularity. External ultrasound is a relatively inexpensive method to screen for liver metastases in the presence of right upper quadrant symptoms or abnormal serum tests. Sensitivity of external ultrasound in this regard is approximately 65%, with lesser sensitivity for lesions in posterior liver segments and those less than 2 cm in diameter; tumor vascularity has minimal effect on lesion detection. Intraoperative ultrasound of the liver has become an important tool for detection of metastases and for anatomic mapping when liver resection is contemplated. Much better resolution is possible than with external scanning because higher frequencies are used and no tissue is interposed between probe and liver surface. Although small surface lesions

abutting the probe may be missed, such lesions are easily palpated. Lesions as small as 4 mm in diameter deeper within the liver parenchyma which may escape the most meticulous attempts at palpation by the surgeon, may be detected by intraoperative ultrasound. The combination of intraoperative ultrasound and intraoperative palpation is currently the most sensitive method to detect liver metastases.

Among non-invasive tests, CT is currently the best, most widely available choice for imaging and follow-up of hepatic metastases. Metastases that are not hypervascular are best visualized by CT with intravenous contrast. With hypervascular tumors such as endocrine metastases, a non-contrast scan may be more sensitive because contrast within such metastases may image similar to normal liver. Other CT techniques, not widely used, such as delayed CT or arterial portography CT, may sometimes provide additional data about liver metastases. MRI has a similar or slightly better accuracy as compared to infusion CT but it does not allow guidance for liver biopsy, is less widely available and its overall superiority has yet to be convincingly demonstrated. Angiography can detect highly vascular lesions within the liver (such as endocrine metastases), but, for the majority of liver metastases which are not hypervascular, angiography is less accurate than CT. Radiolabeled MAb scans for detection of metastases are under investigation. Although initial results have been optimistic such scans have not matched the accuracy of CT or MRI.

Confirmation of hepatic metastases requires biopsy and histopathologic evaluation. A number of immunohistochemical stains are used to distinguish HCC from metastatic adenocarcinoma or cholangiocarcinoma. While MAbs to AFP and alpha-l-antitrypsin lack sensitivity and specificity, MAbs to CEA can discriminate well differentiated hepatomas from metastatic adenocarcinoma and cholangiocarcinoma. Also, cytokeratin-19 MAbs stain adenocarcinomas but not neoplastic or normal hepatocytes; erythropoiesis-associated antigen (Ery-1) is a specific marker for HCC.

Neoplasms of the extrahepatic bile ducts are best detected by ultrasound which images the dilated bile ducts within the parenchyma of the liver, although it will usually not show the obstructing cancer causing this dilation. Endoscopic cholangiography can demonstrate cancers of the common bile and hepatic ducts but tumors of the hepatic duct bifurcation at the liver hilum are probably better visualized by percutaneous cholangiography. Cholangiography identifies not only the site of obstruction, but also indicates whether the biliary trees in right and left lobes of the liver are still patent, or whether flow from either has been obstructed. Carcinomas of the bile ducts are rarely diagnosed early, more commonly presenting with local extension into surrounding tissues or with lymph node or distant metastases. The diagnosis of bile duct tumors arising at the bifurcation of the common hepatic duct can be problematic. More common than previously believed, these tumors are relatively inaccessible and difficult to identify and biopsy. However, diagnosis has improved with transhepatic and endoscopic cholangiography.

Those at risk for HCC are usually evaluated with ultrasonography, although AFP has been suggested for screening. AFP is normally produced by the fetal liver and yolk sac. Serum levels of AFP fall progressively after birth to less than 10 ng/ml in adults. In symptomatic cases of HCC, AFP is elevated in 50%-90% of cases and the positive predictive value of an elevated serum level of AFP in excess of 400 ng/ml exceeds 95%. However, normal levels are not uncommon, particularly in cases of small tumors. A transient, unsustained elevated AFP between 20-400 ng/ml may be present in patients with cirrhosis, active hepatitis or following partial hepatectomy. AFP is also elevated in the majority of patients with nonseminomatous testicular carcinoma. Despite all this, a persisting elevated level of AFP suggests the possibility of HCC and should be followed up with appropriate imaging studies. Immunomedics (Morris Plains, NJ) is conducting phase II clinical trials of AFP-Scan in the *in vivo* diagnosis of testicular and liver cancer. With regard to other serologic tests, fucosylated alpha-fetoprotein can distinguish chronic liver disease from HCC, and gamma glutamyl transferase (GGT) isoenzymes are frequently elevated in HCC. Other circulating tumor markers include abnormal variants of alkaline phosphatase, desgamma carboxy-prothrombin, isoferritins and other specific tumor antigens detectable by MAbs. All of these tests are at different stages of development, availability and usefulness but none has proved better than AFP.

Both ultrasound and serologic tests are also used to screen for liver metastases. Lactic dehydrogenase, alkaline phosphatase and GGT elevations correlate with the presence of liver metastases but are nonspecific. CEA levels are frequently elevated with liver metastases; in colorectal cancer, in particular, over 90% of patients with documented liver metastases have elevated CEA levels. Unfortunately, an elevated CEA level is not specific for either liver metastases or colorectal cancer, and poorly differentiated colorectal cancer may not produce CEA.

Pancreatic Cancer

Pancreatic cancer is rarely detected at an early stage; at the time of symptoms or diagnosis, most patients have liver metastases, malignant ascites, or other tumor dissemination. Establishment of a diagnosis in the past depended upon upper GI contrast radiography, superior mesenteric angiography and radionuclide imaging of the liver. Transhepatic cholangiography improved diagnostic accuracy, but often failed to define the extend of biliary obstruction. Currently, diagnosis is accomplished by ultrasonography, CT, and endoscopic retrograde cholangiopancreatography (ERCP). Ultrasonography is noninvasive but its sensitivity varies from 53%-90% and ascites or overlying gas in the intestine limit its use by

causing one-third of pancreatic scans to be non-evaluable. Abnormal scans may reveal a dilated pancreatic duct, obstruction of the common bile duct, metastatic lesions in the liver, and stones in the gallbladder. CT scanning provides better information on the tissue planes around the pancreas but gives a poor picture of the head of the pancreas and uncinate process because it is impossible to separate radiologically the duodenum from the pancreas. It is also difficult to identify tumor involvement in the hepatoduodenal ligament. Neither ultrasonography nor CT can reliably detect small lesions in the pancreas and surrounding tissues, and MRI has added nothing in this area. Angiography is used to provide the surgeon with a vascular road map prior to surgery, and may also identify large vessel encasement by cancer, tumor vessels, and major venous pathology (all of which usually suggest nonresectability). Short of biopsy, ERCP offers the best presumptive diagnosis for carcinomas of the head of the pancreas. In excluding pancreatic cancer, ECRP has an accuracy in excess of 90%, and is useful in diagnosing pancreatic cancer at a resectable stage (if carcinoma is identified that is not seen by ultrasound or CT, it is often less than 2 cm in diameter). The development of "mother-daughter" endoscopes may further enhance the accuracy of ERCP by allowing a direct biopsy of the tumor mass from within the pancreatic duct.

Given the difficulty and expense of diagnosis, reliable screening tests for pancreatic cancer would be of value. However, no test has yet survived repeated scrutiny. CEA, 5'nucleotidase, and pancreatic ribonuclease are not more accurate than serum alkaline phosphatase but are more frequently associated with false negative and false positive results. Levels of CA 19-9 are elevated primarily in patients with lesions 3 cm in diameter or larger that are usually nonresectable. Nevertheless, CA 19-9 may be of some value in monitoring disease and arriving at a prognosis after pancreatic resection. [CA 19-9 antigen has been isolated and characterized as sialylated lacto-N-fucopentose II oligosaccharide that is identical to the terminal region of the blood group antigen, Lewis^a (Le^a); CA 19-9 is found in patients who are both Le^a and Le^b positive but not in patients who are Le^a and Le^b negative because these patients lack the Lewis fucosyltransferase enzyme required to carry out the final biosynthetic step in its production.] Centocor's (Malvern, PA) CA 19-9 test for pancreatic cancer monitoring has been approved in Europe and is in review in the USA. Leukocyte adherence inhibition assay (LAI) has shown specificity, particularly in early disease and a low rate of false positive responses, but is labor intensive and technically difficult. Among tumor markers evaluated in the diagnosis of pancreatic cancer, carbohydrate antigen (CA) 50 and TPA were the best in predicting pancreatic malignancy; TPS and CA 242 exhibited marginally significant independent discriminating power while CEA did not (Pasanen PA, etal, British Journal of Cancer, 1994 Mar, 69(3):562-5). Detection of oncogenes such as mutant K-ras (which can be detected in approximately 70% of cytologic aspirates of primary pancreatic cancers and their lymph node metastases), is also being studied.

Obtaining a tissue diagnosis was previously only possible at laparotomy and, in the absence of lymph node metastases, by large needle biopsy or wedge or shaving from the tumor. Fine needle aspiration (FNA) biopsy either percutaneously or at the time of surgery, improves the yield of biopsies. Percutaneous FNA biopsy does, however, carry a small but definite risk of implantation of tumor along the needle tract. Another technique for obtaining histologic confirmation is aspiration of the pancreatic duct during ERCP (with a success rate of approximately 50%). Laparoscopy is also being used in pancreatic cancer not only for biopsy but also for preoperative staging.

Colorectal Cancer

Routine screening for colorectal cancer has not been practical to implement in asymptomatic low risk populations. Concepts concerning appropriate tests for and intervals of screening for colorectal cancer continue to evolve, with population screening studies producing conflicting results.

Occult blood. Numerous studies in asymptomatic patients have shown that testing stool samples for occult blood is neither optimally specific nor sensitive and the number of early cancers or precancers found from such screening may not justify the cost. In Germany, where a national screening program has been instituted to check for occult blood in stools of everyone over the age of 45, only 3% of tests revealed occult blood and, of those, only 10% resulted in a diagnosis of colorectal carcinoma. Also, the majority (two thirds) of these cases had spread locally at the time of diagnosis. Recently, however, screening for occult blood was shown to impact mortality. In a study of 46,551 participants 50 to 80 years old, the 13-year cumulative mortality per 1,000 from colorectal cancer was 5.88 in the annually screened group, a 33% reduction as compared to the control (unscreened) group (Mandel JS, etal, NEJM, 1993 May 13, 328(19):1365-71).

Flexible sigmoidoscopy, a more accurate screening modality, appears to lower colorectal cancer mortality but there is controversy about the frequency of screening. The American Cancer Society currently recommends that patients be screened at 3 to 5-year intervals after age 50, but studies of asymptomatic patients suggest that those with negative initial sigmoidoscopy need not be tested so frequently. One retrospective study of 866 consecutive asymptomatic patients with no previous history of polyps, revealed that polyps were discovered in only 12 patients (1.4%), ten (2.4%) of whom had not undergone prior screening sigmoidoscopy. Most previously screened patients (88%) had been screened five or more years earlier (Sakamoto MS, etal, Journal of Family Practice, 1994 Mar, 38(3):245-8;. Sakamoto MS and Schlumpberger JM, Jama, 1994 Oct 12, 272(14):1100).

Tumor markers, including CEA, TPA, GICA, colorectal carcinoma-associated antigen (CCAA) and CA 72-4, represent simple, versatile and potentially inexpensive means of detection and monitoring of colon cancer and may play an important role in prompt diagnosis of recurrence to allow immediate surgical intervention to improve survival. In a study of 90 patients, the levels of combined serum CEA-TPA and GICA were serially measured, including CA 72.4 and CA 195 levels in 71 of them. Follow up protocol was designed using such criteria as combination of several markers and dynamic evaluation of three different levels of increase, isolated elevated value (IEV), constant level of elevation (CE), and progressive increase (PI). There was a positive correlation between elevated preoperative serum tumor marker levels and disease stage. Postoperative elevated values of CEA were useful in identifying micrometastases after primary tumor resection. In 14 patients diagnosed early with recurrence during the postoperative follow-up period, the highest sensitivity (87%) was associated with TPA as a single marker and with TPA-GICA (93%) in combination, with a lead time of 4.6 +/-5.6 and 5.4 +/- 7.8 months, respectively. In nonrelapsed patients, false positive results of 25% with TPA-GICA were fewer than those of TPA-CA 195 (31%) and TPA-GICA-CA72-4 (35%). In patients with recurrences, PI was more frequently present than IEV while in those without recurrence, the opposite was true. Routine radiographic studies were ineffective whereas liver echography revealed first signs of recurrence. Three (75%) of the 4 patients with "early" diagnosis of recurrence were alive without evidence of disease 5, 18, and 20 months after last surgery (Nicolini A, etal, Cancer Detection and Prevention, 1995, 19(2):183-95). Centocor is marketing a CA 72-4 test in Europe.

Genetic testing may also become an effective noninvasive procedure to detect and stage colorectal cancer and, eventually, even *screen* patients at high risk of developing colorectal cancer (see FO V1, #1). Recently, researchers pinpointed loss of a receptor protein for transforming growth factor-β (TGF-β) which normally inhibits the growth of epithelial cells, as one contributor to colon cancer. The TGF-β receptor is composed of two proteins, RI and RII; lack of RII caused by mutation in the gene, probably a tumor-suppressor gene encoding this protein, may be associated with colon cancer. When a good copy was inserted in a colon cancer cell line lacking the gene, the cells did not form tumors (Markowitz, S, etal, Science, vol. 268, 2 June 1995, pp. 1336-8). These results link the mutation in the RII gene to tumorigenesis and may lead to development of diagnostic approaches to detect colon cancer and gene transfer techniques to restore TGF-β activity and block tumorigenesis.

Also, detection of allelic loss of the long arm of chromosome 18q has been associated with poor prognosis in stage II colon cancer that normally has a favorable 5-year survival of 78%. For instance, among 65 stage II patients, 93% of those with preserved 18q survived over 5 years, compared to 54% of those with 18q loss (Jen J, etal, NEJM 1994 Jul 28; 331:213-21; Tempero M and Anderson J, NEJM 1994 Jul 28; 331:267-8). Therefore, detection of allelic loss of 18q may be used to institute more aggressive chemotherapy normally reserved for stage III disease.

Use of voluntary genetic screening for colorectal or other cancers may also emerge as a means of disease management for populations at risk. Unlike some other hereditary diseases, such as Huntington's disease, for which there are no treatment options, those with hereditary factors that predispose them to colorectal cancer have a variety of options, including periodic diagnostic evaluations and early intervention to stave off critical illness. Researchers at the Department of Medical Genetics at the Radiumhospitalet (Oslo, Norway) have applied for approval to screen patients for genetic markers for a variety of cancers, including colon cancer. Also, several oncology centers in the USA are offering high-risk individuals prophylactic subtotal colectomy, a rather radical and somewhat controversial approach, as a means of prevention.

Screening of high risk groups generally involves digital rectal exam and endoscopic examination and, occasionally, double contrast roentgenographic mucosal surveillance. There is some controversy as to which modality, barium study, double contrast roentgenography, or endoscopic screening of the large bowel and rectum, is the most appropriate (i.e., cost effective) initial diagnostic approach in asymptomatic patients. Double contrast roentgenography of the colon has largely replaced plain barium enema because of increased resolution capacity for smaller mucosal lesions and lower cost. However, colonoscopy is more specific and sensitive and enables a biopsy for histologic diagnosis of any abnormal lesions. Quite often, it also enables definitive therapy by completely removing premalignant or early malignant polyps. Because no appropriately designed prospective studies have compared the two techniques in the same patient population, most clinicians believe that roentgenography and endoscopy, when applied together, are better than either alone.

Colon carcinomas display a specific antigen, CEA, that is released into the surrounding body fluids. Although immunohistochemical identification of CEA in tumor tissue and measurement of CEA serum levels are important in the diagnosis, prognosis and management of colorectal cancer, the biologic significance of CEA to the cancer itself is unknown. It has been proposed that CEA may function as an adhesion molecule, enhancing the metastatic potential of otherwise weakly metastatic cells. Plasma CEA levels have been used as indirect evidence of colorectal tumor presence, although it is not specific for this indication, being elevated in the plasma of some patients with breast, lung, pancreatic, ovarian and other tumors. In general, CEA correlates with stage

of disease and is most strikingly elevated in the plasma of patients with hepatic metastases. CEA elevations, either in the postoperative period or at any time in the follow-up of patients with colorectal cancer, can be an early sign of metastatic disease.

With regard to preoperative staging studies, the resolution of both CT and MRI is suboptimal. Only if there is some evidence of liver involvement (elevated serum alkaline phosphatase or CEA) or if symptoms or physical examination suggest carcinoma involving the perirectal space, is a preoperative CT or MRI scan required. Essentially, the patient with bowel cancer needs an abdominal exploration.

In November 1994, Procyon Biopharma (London, Ontario, Canada) announced that it has signed a license agreement with The Innovations Foundation (University of Toronto) for world-wide rights to a colorectal cancer screening test that reliably identifies patients with colorectal cancer or certain predisposing conditions. The technology involves a simple chemical reaction with a rectal mucin sample and can be readily performed as part of routine physical examinations. Matritech (Cambridge, MA) is also developing a diagnostic assay to monitor colorectal and other cancers, based on detection of nuclear matrix proteins (see FO, V1, #1, p. 28).

Radioimmunoscintigraphy in the detection of colorectal cancer is accomplished by using high affinity immunoconjugates. Cytogen's (Princeton, NJ) OncoScint CR/OV, an ^{111}I-labeled conjugate (satumomab pendetide) of the anti-TAG72 murine monoclonal antibody (MAb) B72.3 and the linker-chelator glycyl-tyrosyl-(N,ε-diethylenetriaminepentaacetic acid)-lysine, is the first MAb-based diagnostic radiopharmaceutical to be approved by the FDA. OncoScint CR/OV was approved in December 1992 for determining the extent and location of extrahepatic malignant disease in patients with known recurrent or previously diagnosed colorectal and ovarian cancer. In clinical studies, OncoScint CR/OV has demonstrated a sensitivity of 73% in detecting colorectal carcinoma in patients with confirmed tumor, with sensitivity rising to about 90% when used with CT (Corman ML, etal, Diseases of the Colon and Rectum, 1994, 37:129). The major safety issue concerning use of OncoScint CR/OV has been the development of human anti-mouse antibody (HAMA) response in about 40% of patients. Cytogen is pursuing FDA approval for repeat administration of OncoScint CR/OV. After receipt of FDA approval in May 1993 of Cytogen's promotional materials, the company began marketing OncoScint CR/OV in the USA through its own specialty sales force and through a co-promotion arrangement with Knoll, which was terminated in 1994. In 1989, Chiron (Emeryville, CA) was granted exclusive marketing and distribution rights in Europe for OncoScint CR/OV, which were reacquired in 1995 by the company under the terms of a disengagement agreement. OncoScint CR/OV has been approved in several Western European countries for determining the extent of malignant disease in patients with suspected carcinoma recurrence. Another immunoconjugate for *in vivo* imaging that has completed clinical trials is Immunomedic's CEA-Scan. However, the FDA has yet to approve the company's PLA, which was filed in April 1991 for colorectal cancer imaging. Additional data was submitted to the FDA in March 1995. According to the company, CEA-Scan is associated with a 60% accuracy in predicting tumor resectability in patients with known or suspected recurrent colorectal cancer compared to 47% for CT, while in one 208-patient phase III study, accuracy was 69% as compared to 31% for CT. Biomira (Edmonton, Alberta, Canada) is in phase III clinical trials with Tru-Scint-AD, a Tc-99m-labeled MAb for the detection of colorectal cancer. Another imaging agent, AES-CEA, under development by Immunotech (Marseilles, France), is in phase II clinical trials for the detection of recurrences and metastases of tumors expressing CEA. A multicenter phase III clinical trial was initiated in France. Immunotech is also investigating the use of AES technology in cancer radioimmunotherapy.

Gallbladder Cancer

Carcinoma of the gallbladder usually arises in the fundus or neck of the organ and, occasionally, in the cystic duct and tends to infiltrate locally. At the time of diagnosis, often the entire gallbladder is replaced or invaded by carcinoma. Patients with symptoms atypical of cholecystitis may undergo CT which can define irregularities within the gallbladder itself and tumor extension into the region of the common bile duct, or lymph node metastases. Ultrasonography and CT, in combination, are quite useful in evaluating patients with symptoms of right upper quadrant pain of short duration, vomiting, fever, and localized tenderness. Alternatively, patients may undergo a radionuclide scan, although gallbladder filling and emptying may be normal in early cancer while in late disease scans show an absence of gallbladder filling without indicating the reason for the abnormality. Elevation of serum alkaline phosphatase, present in a majority of cases, may be particularly helpful in suggesting tumor in the absence of jaundice; CEA and CA 19-9 antigens may also be elevated.

Most patients undergo cholecystectomy for symptoms of acute or chronic cholecystitis. In patients with early stage carcinoma, the tumor may not be noted during diagnostic evaluation, or during surgery unless there is a polypoid growth into the lumen. Even late stage cancers may not be recognized, being confused with edema and fibrosis caused by acute and chronic inflammation of the gallbladder wall that can accompany long-standing cholecysitis.

Cancer of the Small Intestine

Most tumors of the small intestine are asymptomatic until late in their course because of their relative slow

growth and the ease with which contents of the small bowel pass even a partially obstructing lesion. One-half of small bowel cancers are found only at autopsy with the remaining discovered when patients present with such symptoms as nausea and vomiting, associated with partial obstruction if the lesion is proximal, or crampy abdominal pain and other relatively non-specific findings such as weight loss.

Diagnosis of these lesions is usually accomplished with the aid of radiographic studies. Only 25% present with a palpable mass; another 25% suffer from abdominal distention, secondary to obstruction. Plain films of the abdomen are unlikely to be of use, except to demonstrate the presence of obstruction or perhaps displacement of the bowel by a mass. Contrast studies show about one-half of these tumors, although with retrospective readings, up to 75% of small bowel tumors can be found. Some studies have reported even higher rates of diagnosis by barium enema. More recently, duodenal tumors have been diagnosed using endoscopy, and the advent of CT scanning with oral contrast has led to nearly 100% recognition of small bowel tumors in some series.

Anal Cancer

Diagnosis of anal cancer is often delayed by both patient and physician because of the plethora of benign conditions which cause symptoms common to anal carcinoma, i.e., bleeding, pain, and the sensation of a mass. Physical examination, including a digital anorectal examination and inguinal node palpation, is most important in the work-up. A biopsy (incisional) must be performed to establish the diagnosis. Both transanorectal sonography and CT have been suggested as valuable tools for assessing the primary tumor, although neither has been rigorously tested for accuracy or sensitivity.

CURRENT THERAPY

Almost all digestive system cancers are treated first by surgery, either to effect a cure or to achieve a degree of palliation and extend patient lives. However, more and more patients also receive multimodality therapy. Neoadjuvant chemotherapy, i.e., chemotherapy delivered prior to local treatment with surgery and/or radiotherapy, is also under investigation in advanced disease. The primary goals of neoadjuvant chemotherapy are to reduce the size of the primary tumor, to treat disseminated microscopic tumor metastases and to allow for more conservative treatment, for example, by reducing the need for radical surgery. Chemotherapy is being used increasingly to extend the life of thousands of patients (see Exhibit 1) with advanced/ metastatic disease. Various combination therapies are being used (see Exhibit 2 and also FO, V1 #1, pp. 13-16).

Esophageal Cancer

If the tumor is localized within the esophagus, and there are no lymph node or systemic metastases (stage I and stage II disease), surgical resection is used to effect a cure, although the 5-year survival rate even in these cases is only 5% to 20%. The esophagus, stomach, jejunum, and colon are all used to replace the resected area, with the final decision often made at the time of surgery. Surgical mortality and morbidity rates range between 2% to 10%, and 25% to 40%, respectively.

In 75% to 80% of patients with dysphagia, the tumor has either invaded through the muscularis propria or adventitia, and/or regional lymph nodes (stage III) or has metastasized to both regional lymph nodes and distant sites (stage IV). For such patients, two-year survival is less than 10% regardless of therapy, and treatment is focused on palliation. Multidisciplinary therapies involving surgery, radiotherapy, and/or chemotherapy in stages III and IV disease are under clinical evaluation. Also, a self-expandable, nickel-titanium coil stent, EsophaCoil, marketed by InStent (Eden Prairie, MN), can be inserted under topical anesthesia to prevent esophageal obstruction. (For more on stents see this issue, page 73).

A number of chemotherapeutics, such as methotrexate (MTX), 5-FU and cisplatin, demonstrate activity in advanced esophageal carcinoma, particularly in the squamous cell variety; carboplatin appears to be substantially less effective. The duration of response has been short, however, and no single agent of the many studied has proven clearly superior. By comparison, combination chemotherapy for patients with advanced squamous cell carcinoma has led to response rates of up to 76%, averaging 40% to 50%. The common element in these combinations has been cisplatin, which experimentally synergizes with a number of agents, particularly 5-FU. The response rates tend to be higher for local regional disease (50%) than more advanced disease (25%). Randomized comparisons of cisplatin-based combinations have not been performed, so it is uncertain which regimen is superior. At the 1995 ASCO meeting (M Hill, etal, Abs. 465) investigators from The Institute of Cancer Research and The Royal Marsden Hospital. (Sutton, Surrey, UK) reported extended experience in a series of 235 consecutive patients treated between 1989 and 1994 using epirubicin (50 mg/m^2) and cisplatin (60 mg/m^2) thrice weekly for six to eight weeks, with protracted venous infusion (PVI) 5-FU (200 mg/m^2/d) throughout. Response was observed in 135/220 (61%) of evaluable patients (11% were CRs and 50% PRs).

Neoadjuvant chemotherapy is under investigation in advanced squamous cell carcinoma. Recent studies have suggested that combined chemotherapy and radiation therapy may result in improved survival. In a phase III prospective, randomized, and stratified trial of patients with squamous cell adenocarcinoma of the thoracic esophagus, median survival was prolonged with four courses of combined 5-FU and cisplatin plus 5,000 cGy of radiation therapy (12.5 months) compared to 6,400 cGy of radiation therapy alone (8.9 months). Survival rates at 12 and 24 months were 50% and 38%, respec-

tively, in the combined therapy group, compared to 33% and 10%, in the monotherapy group. Patients who received combined treatment had fewer local or distant recurrences (Herskovic A, etal, NEJM, 1992 Jun 11, 326(24):1593-8; comment in NEJM, 1992 Jun 11;326(24):1629-31).

In Barrett's esophagus, reflux, which is thought to be the primary pathogenetic event, can be prevented by the surgical technique known as fundiplication which prevents reflux esophagitis and may cause regression or stabilization of Barrett's process. If patients show evidence of *in situ* or invasive neoplasia in Barrett's esophagus, esophagectomy followed by adjuvant chemotherapy is indicated. Patients with high grade dysplasia are at significant risk of adenocarcinoma and are candidates for esophagectomy.

Stomach Cancer

Standard treatment for gastic cancer is surgical resection which is of true benefit in stage I (no suspected lymph node metastases, and no serosal invasion), II (metastasis to perigastric lymph nodes, and suspected serosal invasion), and III (metastasis to lymph nodes along common hepatic artery, left gastric artery, lienal artery and nodes around the celiac axis, and definitive serosal invasion). Curative surgery is performed by simple gastrectomy combined with extensive lymph node dissection, and the resection of surrounding organs. In the early 1980s it was shown by Japanese surgeons that extensive surgery in early gastric cancer resulted in five-year survival rates of 57.8%. When early gastric cancers were classified by depth of penetration, the five-year survival rate was 100% in 2,904 cases of mucosal cancer, and 94.7% in 3,006 cases of submucosal cancer; it was 96.6% in stage I, 72.0% in stage II, 44.8% in stage III, and 14.7% in stage IV cancer; 74% survived inspite of nodal disease, provided the serosa was not invaded (Imanaga H, etal, World Journal of Surgery, 1977, 1:213).

There is no established standard treatment for patients with unresectable locally advanced or metastatic disease. Surgical intervention has a palliative goal in patients found to have metastases to distant lymph nodes or organs, carcinomatous peritonitis, or direct local extension that cannot be completely resected. The types of palliative surgery include wide local excision, partial gastrectomy, total gastrectomy, simple laparotomy, gastrointestinal anastomosis, and bypass for restoration of oral intake of food, alleviation of pain, and removal of the primary tumor, insofar as possible. Resection of the primary tumor is also performed for the purpose of emergency treatment of hemorrhage, chronic blood loss, stenosis or perforation; resection to alleviate pain is not common. With palliative resection, median survival is extended from 7 to 10 months.

Chemotherapy and radiotherapy have resulted in improvement in survival. Stomach cancer is thought to be the most chemotherapy sensitive of all GI cancers. Single-agent trials have demonstrated the following response rates for drugs with established classic cytotoxic efficacy: 5-FU, 21% by brief continuous infusion, 13% by long continuous infusion; doxorubicin, 25%; epirubicin, 22-36%; mitomycin-C (MMC) 30%; and cisplatin, 19-22% (Ishibiki K, etal, Japanese Journal of Cancer Chemotherapy, 1989, 16:3185).

Radiotherapy is a poor palliative modality compared to surgery and even chemotherapy, but 30% to 40% of selected patients derive some brief benefits. Radiotherapy to the upper abdomen is technically difficult and often poorly tolerated. Radiotherapy achieves better local control and survival when it is combined with chemotherapy than when used alone. Special techniques such as intraoperative radiotherapy and hyperthermia remain promising but unproven. Investigations are shifting the focus of effort to the neoadjuvant setting and the integration of new chemotherapeutics into combined modality regimens (Dougherty JB, etal, ASCO Educational Symposia, 1993, S10).

Low grade B-cell lymphomas of mucosa-associated lymphoid tissue (MALT) may be cured by eradication of *Helicobacter pylori* (*H. pylori*) infection (see page 73 of this issue).

Primary Hepatocellular Cancer (HCC)

HCC is highly lethal. The median survival has been reported to be 11, 3, and 0.9 months, respectively, for stage I (not advanced: liver involvement less than 50%, no ascites), stage II (moderately advanced), and stage III (very advanced) disease (Olsen JH, etal, British Journal of Cancer, 1988, 58:392). Prompt surgical resection of an isolated hepatoma is the only treatment with curative potential but only 10% to 43% of patients are resectable at diagnosis. Moreover, operative mortality of patients with cirrhosis and portal hypertension is as high as 30% compared to only 10% when the remaining liver is normal.

The 5-year survival rate after successful total excision of a solitary HCC of less than 2 cm can be as high as 85%, although most series report survival rates of 16% to 46%. Of patients who fail after hepatectomy, up to 93% experience tumor recurrences in the liver, 5% in the bone, and 2% in the lung. The high rate of local failure after hepatectomy may be due to multiple tumors or close margins. In rare circumstances, localized recurrent disease has been resected for cure. Patients with resected fibrolamellar HCC survive longer because of the favorable size, location (predominantly in the left lobe) and absence of liver cirrhosis.

Systemic chemotherapy has been widely used in patients with unresectable HCC in an attempt to prolong survival or provide symptomatic relief. Unlike with other epithelial GI tumors, 5-FU has exhibited little or no activity as a single agent in treating HCC and both doxorubicin and cisplatin show minimal activity. Combination chemotherapy (5-FU + semustine or 5-FU + semustine + doxorubicin) has yielded response rates of about 12%

Exhibit 2
Combination Chemotherapy and Multimodality Approaches in Clinical Trials for the Treatment of Digestive System Cancers

ESOPHAGEAL CANCER
CHEMOTHERAPEUTIC APPROACHES
5-FU + cisplatin (advanced disease; clinical trials)
Methotrexate (MTX) + cisplatin (advanced disease; clinical trials)
Taxol + edatrexate (phase I; ASCO95, Abs. 1523)
MULTIMODALITY APPROACHES
Surgery + radiotherapy and/or chemotherapy (advanced disease; established)
Neoadjuvant chemotherapy (5-FU + cisplatin) + surgery and/or radiotherapy (advanced disease; clinical trials)
Preoperative chemotherapy (5-FU + cisplatin + leucovorin (LV) + etoposide) + surgery (advanced disease; clinical trials)
Preoperative chemotherapy [5-FU + cisplatin or mitomycin-C (MMC)] + external beam irradiation + surgery (advanced disease; phase II)
External beam irradiation + 5-FU (unresectable cancer; established)
External beam irradiation + 5-FU + cisplatin (unresectable cancer; phase III)
External beam irradiation + intraluminal brachytherapy (unresectable cancer; phase II)
External beam irradiation + 5-FU + cisplatin + high dose brachytherapy (unresectable cancer; phase II)

STOMACH CANCER
CHEMOTHERAPEUTIC APPROACHES
5-FU Combinations
Doxorubicin + MMC (advanced disease; established)
Cisplatin (advanced disease; phase II)
Cisplatin + LV (advanced disease; clinical trials)
Hydroxyurea + LV + cisplatin (unresectable disease; clinical trials)
LV + IFN-a 2b (metastatic disease; phase II)
LV + MMC (advanced disease; clinical trials)
LV + MTX (advanced disease; clinical trials)
Doxorubicin or epidoxorubicin + cisplatin (advanced disease; phase II)
LV + epirubicin + cisplatin (advanced disease; clinical trials)
LV + epidoxorubicin + cisplatin + G-CSF (advanced disease; clinical trials)
LV + etoposide (advanced disease; phase II)
Other Combinations
Doxorubicin + etoposide + cisplatin (advanced disease; clinical trials)
Glutathione + cisplatin (advanced disease; prevention of neurotoxicity; clinical trials) MMC + UFT (phase I)
MULTIMODALITY APPROACHES
Surgery with Chemotherapy
Intra-arterial 5-FU (resectable disease; phase I/II)

— continued on next page

and 21%, respectively, but median survival rates are no better than doxorubicin monotherapy.

Because HCC derives its blood supply primarily from the hepatic artery while normal hepatocytes are sustained by the portal vein, arterial infusion therapy has the theoretical advantage of increasing local drug delivery while potentially lowering systemic and hepatic toxicity. In clinical trials using intra-arterial hepatic (IAH) 5-FU therapy in patients with HCC, an overall response of 50% (range 14%-70%) with median survival of 6-15 months has been reported (Nerenstone S and Friedman M, Gastroenterology Clinics of North America, 1987, 16:603). Intra-arterial doxorubicin is especially effective in HCC patients without signs or symptoms of cirrhosis, and epirubicin, cisplatin or MMC have also been used with some success. Combination intra-arterial therapy with doxorubicin, MMC and 5-FU can be effective (60% response) in patients with good performance status. An improvement in the efficacy of IAH chemotherapy has been obtained when it is combined with transcatheter arterial embolization as well as with lipid lymphangiographic agents such as Lipiodol (Guerbet Products, Montreal, Canada) or embolizing agents such as Avitene (Alcon Laboratories; Fort Worth, TX), Gelfoam (Upjohn; Kalamazoo, MI) and Angiostat (Regional Therapeutics; Los Angeles, CA) which concentrate in hepatic tumor tissue and cause microembolization of its blood supply. Postoperative prophylactic lipiodolization (selective regional cancer chemotherapy using lipid contrast medium plus an anticancer drug) was found to reduce intrahepatic recurrence after resection in patients with HCC (Takenaka K, etal, American Journal of Surgery, 1995 Apr, 169(4):400-4; discussion 405).

Cholangiocarcinoma is a malignant primary liver tumor rising from the intrahepatic bile duct epithelium; if it arises in a peripheral location it has a pattern similar to HCC. Treatment of peripheral-type cholangiocarcinoma is unsatisfactory because it does not respond to systemic chemotherapy and it is not amenable to surgical resection. The hilar or sclerosing-type may be palliated temporarily with bypass surgery. Overall survival is about 6 months with a range of 1-44 months.

Hepatic angiosarcoma is a rare primary tumor of the liver which is associated with exposure to thorium dioxide (thorotrast) and long-term exposure to vinyl chloride monomers. Prognosis is extremely poor and very few patients are candidates for curative resection because of multiple lesions or large size and rapid growth of the tumor. These sarcomas are unresponsive to chemotherapy and are relatively radioresistant, although liver sarcomas have been treated with intra-arterial chemotherapy or chemoembolization.

Metastatic Neoplasms of the Liver

In general, surgical resection is indicated only when liver metastases originate from colorectal adenocarcinoma. Hepatic resection is occasionally performed

Tegafur (resectable disease; clinical trials)
5-FU + MMC + cisplatin (resectable disease; phase I/II)
5-FU + epidoxorubicin + MMC (resectable disease; clinical trials)
Preoperative 5-FU (clinical trials)
Preoperative 5-FU + cisplatin (advanced disease; phase II)
Preoperative intratumoral immunomodulation (streptococcal preparation) (clinical trials)

LIVER CANCER (PRIMARY)
CHEMOTHERAPEUTIC APPROACHES
5-FU + semustine (unresectable disease; clinical trials)
5-FU + semustine + doxorubicin (unresectable disease; clinical trials)
Doxorubicin + MMC + 5-FU (unresectable disease; IAH infusion; clinical trials)
Etoposide + vincristine + Adriamycin + tamoxifen (multidrug resistant disease; clinical trials)
Doxorubicin + IFN-α 2b (unresectable disease; phase I/II)
MULTIMODALITY APPROACHES
IAH chemotherapy (MMC, epirubicin, cisplatin, IFN-α, or IL-2) using transcatheter arterial embolization + lipid lymphangiographic agents (unresectable disease; phase II/III)
External beam irradiation + radiosensitizer (floxuridine (FUDR) or bromodeoxyuridine) (unresectable cancer; phase I/II)

LIVER CANCER (METASTATIC)
CHEMOTHERAPEUTIC APPROACHES
5-FU + MMC (regional infusion by hepatic arterial ligation; clinical trials)
5-FU + LV + cisplatin (hepatic arterial infusion by implantable port; clinical trials)
FUDR + LV delivered by IAH with implantable pump (Kemeny N, etal, Cancer, Feb 15, 1994, V. 73 No. 4)
rIFN-α + 5-FU delivered by IAH (ASCO94, Abs. 591)
MULTIMODALITY APPROACHES
External beam irradiation + 5-FU or FUDR (IAH; phase III)
Hepatic chemoembolization + 5-FU + LV (pilot study; SWOG-9051; closed 4/95)
Surgery + 5-FU + LV (resectable disease; clinical trials)

PANCREATIC CANCER
CHEMOTHERAPEUTIC APPROACHES
5-FU Combinations
Cyclophosphamide + MTX + vincristine, followed by 5-FU + MMC (established)
MMC (established)
Cisplatin + MMC (advanced disease; systemic and regional infusion; clinical trials)
MMC and doxorubicin or streptozotocin (clinical trials)
LV + cisplatin (advanced disease; phase II; ICCAC95, Abs. p 713)

— continued on next page

formed for endocrine tumor metastases, but usually only for symptomatic palliation, rarely for cure. Results of liver resection for metastases originating from other than colorectal or endocrine tumors are poor. Approximately 2% to 3% of patients with colorectal cancer develop resectable liver metastases. Five-year survival rates of approximately 25% have been reported after resection of colorectal metastases, rising as high as 34% in patients without involved nodes associated with the primary cancer. Survival is similar after resection of one to three liver metastases, but decreases significantly with more than three. Tumor recurrences are found in approximately 75% of patients treated by liver resection for colorectal metastases. The initial site of recurrence is the liver alone in 28% of patients; liver is involved as an isolated site or with other sites of recurrence in 35% to 65% of patients; lung is involved as an initial site of recurrence in 18% of patients and abdomen alone is involved in 4% to 13% of patients. At medical centers where liver resection is a routine procedure, mortality averages 5%, and morbidity from 12% to 25%.

There are two major drug administration options in chemotherapy concerning tumors metastatic to the liver, systemic intravenous infusion or regional (intra-arterial hepatic or IAH) infusion. Choice of delivery route is dictated by several considerations, including the sensitivity of a given tumor type to chemotherapy and the contribution of extrahepatic disease to overall morbidity and mortality. For example, regional chemotherapy would deliver a high concentration of drug to relatively refractory tumors such as colorectal cancer, while systemic chemotherapy is more appropriate for metastatic disease. A further consideration concerns the risk/benefit ratio of systemic treatment versus regional therapy. IAH requires highly skilled medical personnel and is associated with considerable morbidity.

Regional Therapy. Interest in regional therapy for metastatic neoplasms of the liver originated from the observation that liver metastases may occur as the sole metastatic site. Unlike lung cancer or breast cancer, where extrahepatic cancer invariably is present or develops rapidly, an estimated 14,000 USA colorectal cancer patients have an isolated liver relapse. A subset of these patients will have three or fewer hepatic tumor nodules and, of these, some 25% or so may be cured by resection. The rationale for IAH therapy is well founded in dose-response considerations. Approximately 95% of the blood flow to hepatic metastases is via the hepatic artery, and IAH administration when compared to a similar intravenous schedule, can provide 50 to 100-fold higher exposure to 5-FU and 5-fluorodeoxyuridine (FUDR, Roche), respectively. Exposure advantages, although present, are much less for drugs such as MMC, cisplatin, bis-chlorethylnitrosourea, and doxorubicin. IAH infusion of FUDR and 5-FU has been practiced for over 25 years in the treatment of liver metastases. Cisplatin delivered by the IAH route is also being investigated in patients with

primary or metastatic liver malignancies as a means of enhancing its antitumor activity and reducing its systemic toxicity. Mean peak serum cisplatin level with IAH delivery was found to be about half of mean peak value for IV administration. Treatment was relatively well tolerated with no symptoms of either nephro- or neurotoxicity. This may be explained by the observation that a substantial fraction of arterially-administered drug was metabolized by the liver at first passage, leading to reduced serum drug levels (Vexler AM, etal, International Journal of Cancer, 1995 Mar 3, 60(5):611-5). Also, three patients with multiple liver metastases from colorectal cancer achieved CRs when treated with a IAH of IL-2 (8 x 10^5 JRU) in combination with MMC (4 mg once weekly) and 5-FU (250 mg daily) for 3 weeks, after which they were discharged on maintenance therapy consisting of IL-2 (2 x 10^6 JRU), 5-FU (250 mg twice weekly), and MMC (4 mg once weekly). One patient relapsed with pelvic recurrence 14 months after initiation of therapy, but the other two patients were still in remission 25 and 22 months after the initiation of therapy (Okuno K, etal, Surgery Today, 1994, 24(1):80-4).

Chemoembolization. Regional chemotherapy may also be combined with interruption of the arterial component of the hepatic tumor blood supply to treat cancer metastasized to the liver. Hepatic arterial ligation has been combined with portal venous infusion of 5-FU and MMC, and biodegradable starch microspheres have been co-administered into the hepatic artery with concentrated anticancer drug solutions. Thirty-nine patients with colorectal liver metastases were treated with a monthly bolus administration of MMC (10 mg/m^2 on day 1) plus a continuous infusion of 5-FU (500 mg/m^2 daily from days 1 to 5), delivered via both portal (one-third of the dose) and arterial (two-thirds) routes to control large and small metastases. Arterially administered MMC was mixed with individualized doses of degradable starch microspheres. Among 23 patients who received two or more cycles of treatment 5 CRs and five PRs were observed and six patients had stable disease. The median time to progression and length of survival were 6 and 16 months, respectively (Civalleri D, etal, British Journal of Surgery, 1994 Sep, 81(9):1338-41). Although encouraging responses were seen with this approach, technical considerations make it unlikely it will become widely used in the near future.

Radiotherapy as a therapeutic modality for liver metastases has not been fully explored. The liver has been considered to be relatively sensitive to the effects of radiation. However, while a whole liver dose of 3,500 cGy is considered to be the upper limit of tolerance to avoid fatal radiation-induced hepatitis, the effects of higher doses of radiation to limited portions of the liver are not well documented. This may be a particularly critical issue for most liver metastases which are considered to be relatively radioresistant, requiring doses in

Etoposide + LV + IFN-α 2b + G-CSF (phase II; SWOG-9413; 5/95)
LV + MTX (advanced disease; clinical trials)
LV + MMC (advanced disease; clinical trials)
LV + streptozotocin (clinical trials)
LV + hydroxyurea + cisplatin (advanced disease; clinical trials)
LV + mitoxantrone + cisplatin (unresectable disease; regional infusion; clinical trials)
MULTIMODALITY APPROACHES
Surgery + external beam irradiation + 5-FU (established)
External beam irradiation + surgery + intraoperative electron beam irradiation (unresectable disease; clinical trials)
External beam irradiation + 5-FU (unresectable disease; phase I)
External beam irradiation + 5-FU + streptozotocin + cisplatin (unresectable disease; clinical trials)
COLORECTAL CANCER
CHEMOTHERAPEUTIC APPROACHES
5-FU Combinations
LV (advanced disease; established)
LV + cisplatin (advanced disease; clinical trials)
LV + cisplatin (advanced disease; IAH infusion; clinical trials)
Tegafur + LV (metastatic disease; phase II)
MTX (advanced disease; established)
PALA (advanced disease; clinical trials)
LV + PALA (advanced disease; phase II)
Cisplatin + MMC and/or vincristine (advanced disease; clinical trials)
Vinorelbine + LV (advanced disease; phase II)
Hydroxyurea + LV (advanced disease; clinical trials)
Oxaliplatin (advanced disease; phase II)
LV + oxaliplatin (advanced disease; phase III)
IFN-α-2A (advanced disease; phase III)
LV + IFN-α (advanced disease; phase II)
LV + MTX (advanced disease; clinical trials)
Dacarbazine + vincristine + bis-chloronitrosourea + GM-CSF (metastatic disease; clinical trials)
Edatrexate (with ice chip cryotherapy)+ doxorubicin + G-CSF (phase I; ASCO95, Abs. 1587)
Other Combinations
IL-2 + immunomodulator flavone-8-acetic acid (advanced disease; phase I)
FUDR + LV (chronomodulated infusion; phase I)
Anti-CEA and anti-TAG72 antibody-conjugated ^{131}I + IFN-α (enhanced tumor localization; metastatic disease; phase I/II)
Taxol + ifosfamide + cisplatin (TIC) (phase I, ASCO95, Abs. 103)
Oral etoposide + IV taxol (phase I. ASCO 95, Abs. 1514)

— continued on next page

excess of 6,000 cGy for control. In the palliation of pain due to liver metastases, radiation alone has been effective in 75% to 90% of patients treated, using a variety of techniques. Generally, such treatment has had little effect on survival, although a few patients have been reported to survive 1 to 2 years. Interstitial radiotherapy has been described in some small studies in the treatment of liver metastases, but few data on results with such therapy have been reported. Hepatic arterial chemotherapy (FUDR or 5-FU) has also been combined with external beam radiotherapy; however, response rates have generally been smaller to those achieved with IAH FUDR alone.

Liver Transplants. Relatively few orthotopic liver transplants have been performed for liver metastases. In many cases of hepatic resection for metastases requiring less than total hepatectomy, tumor recurrence is attributed to metastatic disease developing outside the liver. It would, therefore, be surprising if more complete hepatic regional control would contribute to survival. In addition, immunosuppression required by transplantation may permit growth of microscopic metastases. Indeed, a review of reported experience reveals dismal results for liver transplantation in metastatic disease.

Other therapies for liver metastases have been suggested which are aimed at eradication of specific lesions without the need for excisional surgery. These modalities include cryotherapy, chemical injection (ethanol), and local hyperthermia. Firm data supporting the effectiveness of these techniques are lacking, and because of difficulty in gauging the extent of tissue destruction relative to the lesion, such techniques are unlikely to demonstrate consistent benefit.

Neoplasms of the Extrahepatic Bile Ducts

For neoplasms of the extrahepatic bile ducts, it is now technically feasible to resect nearly one-half of the tumors of the upper third of the biliary tree. However, the operative mortality rate is high and survival beyond five years is rare. On the other hand, patients with tumors of the middle third of the common duct have a lower operative mortality rate, and 10% or more will survive five years.

Most tumors of the distal third of the bile duct can be resected by pancreatoduodenectomy with an operative mortality rate of less than 10% and one in four patients or more will survive five years.

While tumors arising at the bifurcation of the common hepatic duct may be difficult to diagnose, they are even more difficult to extirpate surgically. Transhepatic and endoscopic cholangiography has not only improved the ability to identify these cancers but has encouraged aggressive surgical approaches including resections of a core from the liver hilum and if, necessary, hepatic lobectomy. However, while increasingly aggressive resectional procedures have increased the proportion of resectable tumors, five-year survival rates for these patient remain between 10% and 20%. In the absence of documented effective chemotherapy for bile duct cancer, 5-FU must be considered the mainstay of treatment. However, as a single agent 5-FU is relatively ineffective, and to date there is still no reproducibly effective chemotherapeutic treatment for this disease.

MULTIMODALITY APPROACHES
Surgery Combinations
5-FU (IV) + oral levamisole (resectable cancer; established; Moertel CG, etal, Ann Intern Med 1995 Mar 1; 122:321-6)
5-FU + LV (resectable cancer; phase III)
Intraperitoneal (IP) MMC (resectable cancer; clinical trials)
5-FU + LV (resectable cancer; clinical trials)
Autologous tumor cell-BCG vaccine (resectable cancer; phase III)
Surgery + External Beam Irradiation Combinations
5-FU (resectable cancer; established)
5-FU + LV (resectable cancer; phase III)
Cryoreductive surgery + heated IP MMC (mucinous carcinoma; phase I/II)
Preoperative irradiation + radiation boost (unresectable cancer; clinical trials)
Preoperative irradiation + chemotherapy (5-FU and/or cisplatin) (unresectable cancer; clinical trials)
External Beam Irradiation Combinations
MMC (metastatic disease; phase II)

BILE DUCT CANCER
MULTIMODALITY APPROACHES
External beam irradiation + radioisotope implantation (established)
External beam irradiation + intraoperative electron beam irradiation (clinical trials)

GALLBLADDER
CHEMOTHERAPEUTIC APPROACHES
5-FU + LV + MTX (advanced disease; clinical trials)
MULTIMODALITY APPROACHES
Surgery + external beam irradiation (clinical trials)
External beam irradiation + 5-FU (clinical trials)
Surgery + 5-FU (clinical trials)

ANAL
CHEMOTHERAPEUTIC APPROACHES
5-FU + cisplatin (clinical trials)
MULTIMODALITY APPROACHES
External beam irradiation + 5-FU + MMC (established)
Interstitial hyperthermia + high-dose brachytherapy (phase I/II)

As early as 1973, radiation therapy was shown to provide local control of well differentiated adenocarcinomas of the common bile duct (Green N, etal, Radiology, 1973, 109:687). Subsequently, radiation therapy has been used for primary treatment, as part of adjuvant therapy, and, most commonly, for palliation. Both external beam radiation and implantation of radioactive isotopes have been used for the delivery of radiation therapy to bile duct cancers, and many investigators have combined the two modalities. Conventional external beam radiation therapy provides moderately high dose of radiation therapy to a relatively large volume of tissue. It is most effective in the treatment of bulky tumor masses, such as nodal disease or residual tumor after resection which is not limited to the bile duct. Typical radiation doses have been in the range of 4,500 to 5,000 cGy, although higher doses have been used. Implantation of a radioactive isotope (such as iridium) is generally accomplished by inserting it through a biliary stent which passes through the bile duct tumor so that the radioactive source traverses the affected region. The stent can be placed either surgically, or through transhepatic or endoscopic means. Radiation delivered is quite localized to a radius of approximately 1 cm from the radioactive seeds. However, since the dose delivery is so localized, one can deliver high doses to localized disease in the bile duct after resection, or in providing palliation to patients with bile ducts obstructed by tumor. Typical doses with this technique have been in the range of 2,000 to 2,500 cGy quoted at a distance of 0.5 to 1.0 cm from the source. In combination, higher external beam doses (up to 6,000 cGy) have been delivered, supplemented by interstitial implantation.

Intraoperative electron beam radiation therapy has also been used, either alone or in addition to external beam radiation, or resection. This technique can deliver a high radiation dose in a single fraction intraoperatively, with coverage which is wider than can be accomplished by an implant, and not limited to the bile duct itself. Unfortunately, local control as well as survival remain poor; there is continued difficulty in managing biliary stents and infection is a chronic and often severe problem.

Pancreatic Cancer

Long term survival of patients with pancreatic cancer is only achievable with resection of all or part of the pancreas and adjacent tissues. In general, the cure rate following resection approaches 5%, with about one in five patients being eligible for radical surgery. Operable pancreatic cancer is treated primarily by subtotal pancreatectomy or total or partial pancreato-duodenectomy (Whipple's operation); morbidity ranges from 5% to 15% and mortality from 2% to 10%; disease recurs in up to 90% of cases. Pancreatoduodenectomy includes the en bloc removal of the gastric antrum, the entire duodenum, the first jejunal loop (and its mesentery), the gall bladder and common bile duct, and the head of the pancreas to the level of the superior mesenteric vein. Total pancreatectomy also involves resection of the body and tail of the pancreas and the spleen, and is usually performed because a tumor cannot be encompassed by a standard pancreatoduodenal resection, or because pathologic examination of a frozen section taken from the line of resection of the pancreas documents a positive margin. Reconstruction after pancreatoduodenectomy requires anastomoses between the pancreatic remnant and the jejunum, and between the common hepatic duct and the jejunum, as well as re-establishment of gastrointestinal continuity through a gastrojejunostomy. Total pancreatectomy reduces by one the anastomoses that need to be constructed. Operative mortality is low (0%-6%), but overall morbidity still approximates 40%. Complications most commonly relate to the mesenteric vessels (thrombosis or hemorrhage) but sepsis and liver necrosis (the latter perhaps from ligation of an aberrant hepatic artery) remain the major causes of death after total pancreatectomy. Five-year survival after pancreatoduodenectomy approaches 20%, but long-term survival is not increased by total pancreatectomy.

Adjuvant therapy after surgery is recommended because of the high likelihood of local recurrence or peritoneal or hematogenous spread even with optimal local therapy. Local recurrence has been observed in over 50% of cases. The importance of distant metastases is obvious from the overall 90% to 95% failure rate after pancreatectomy. Postoperative radiation therapy combined with 5-FU in surgical patients has resulted in median survival of 20 months compared to 11 months in the group treated by surgery alone, with 5-year survival rates of 19% and 5%, respectively (Gastrointestinal Tumor Study Group, Cancer, 1987, 59:2006). Radiation therapy is difficult to administer because of tumor aggressiveness as well as radiosensitivity of the surrounding normal tissues. A variety of radiation techniques have been explored for primary treatment of pancreatic cancer. Neutrons offer increased radiobiologic effectiveness compared to conventional x-rays and charged particles are able to localize radiation doses better, but neither have demonstrated survival benefits compared to conventional treatment modalities. Radiation techniques which remain in use include conventional external beam radiation using high energy x-rays (10 MeV or greater) and high radiation doses (6,000 cGy delivered to the tumor), often combined with at least 5-FU chemotherapy; radioactive implantation into the tumor mass, primarily using ^{125}I; and intraoperative electron beam radiation (1,500-2,000 cGy to the tumor) after conventional external beam radiation (4,500 cGy). Dosage from radioactive implants is limited in patients with unresectable disease because of tumor extension into the portal vein or superior mesenteric vessels, structures that cannot be implanted. Intraoperative electron beam radiation therapy is limited because sufficient doses cannot be delivered without placing surrounding tissues at risk, although the boost dose can avoid substantial radiation to the most sensitive

normal structures (except for the C-loop of the duodenum), and it is biologically much more effective given in a single dose than as a fractionated regimen.

Chemotherapy may also influence survival of patients with pancreatic cancer. In a classic study involving administration of a combination of 5-FU, cyclophosphamide, MXT, and vincristine, followed in six weeks by 5-FU and MMC, median survival of treated patients was 48 weeks, compared to 12 weeks for controls (Mallinson CN, etal, British Medical Journal, 1980, 281:1589). Other drug combinations that have received considerable attention add either doxorubicin or streptozotocin to 5-FU and MMC regimens. There is some question, however, whether these and other combination programs are more effective than monotherapy. In a review of trials involving more than 290 patients, the response rate to 5-FU alone ranged from 15% to 28%, and to MMC was 27%. No other single agent has been found to be nearly as effective, although wide variations in response rates have been reported. For example, response rates to ifosfamide vary between 3% and 60%.

Gemcitabine (Gemzar, Eli Lilly), a novel nucleoside analog with a broad spectrum of preclinical activity in solid tumor models, has demonstrated marginal activity in pancreatic cancer without excessive toxicity. In a multicenter phase II clinical trial, 44 patients with measurable adenocarcinoma of the pancreas who had received no previous chemotherapy, were administered 800 mg/m^2 of gemcitabine intravenously weekly for 3 consecutive weeks, followed by one week rest, every 4 weeks; 35 patients had at least 2 cycles of therapy. PRs were observed in 5 patients (11%) with a median duration of 13 months. All responding patients had stabilization or improvement in performance status; 14 had stable disease of 4 or more months (Casper ES, etal, Investigational New Drugs, 1994, 12(1):29-34).

Colorectal Cancer

Early stage colorectal cancer is treated surgically. Endoscopic removal of polyps may obviate laparotomy, but the entire lesion must be removed with complete pathologic review of the specimen, analysis of depth of invasion of carcinoma, analysis of possible invasion into the stalk of a polypoid tumor, and pathologic definition of free margins. For those with polypoid carcinoma that is totally removed by endoscopy but with histologically confirmed penetration at or through the muscularis mucosa, the decision of subsequent bowel resection is made on an individual basis. If endoscopically removed cancers exhibit pathologically free margins and depth of penetration is confined to the mucosa or submucosa, subsequent surgery is not required. Surgical resection of colorectal cancer involves removal of the entire cancer with enough bowel proximal and distal to the tumor mass to eliminate the possibility of submucosal lymphatic tumor spread; removal of regional mesenteric draining lymph nodes (under the assumption that there is sequential spread of disease to regional mesenteric nodes prior to distant involvement); and adequate visual, tactile, and intra-operative ultrasound evaluation at the time of primary resection.

Local treatment alternatives, particularly for distal adenocarcinomas, have been available for many years. Most local treatment modalities have been performed on selected patients with severe co-morbid disease or in patients with mobile, superficial, exophytic, distal adenocarcinomas with little or no chance of microscopic nodal involvement. Treatment plans have included multiple coagulation sessions, local excision (without subsequent external beam radiation or chemotherapy), and endocavitary irradiation, in which very low energy X rays (50 KVp) are delivered through a large rectoscope inserted transanally and placed directly over the tumor. In properly selected patients (tumors equal to or less than 3 cm in maximum diameter, not poorly differentiated, not on the anal verge and without any evidence of deep ulceration or invasion), the latter approach can be delivered on an outpatient basis with minimal morbidity and with excellent long-term control (95% at five years). Another approach for similar tumors is local excision combined with 5-FU and external beam radiation therapy using doses of approximately 4,500 to 5,000 cGy.

For colorectal cancer surgery, a mortality rate (mainly caused by thromboembolic, infectious and anastomotic problems) of less than 10% is considered acceptable. Long-term results of surgery are linked to the stage of disease at the time of diagnosis. For patients with tumors confined to the mucosa or submucosal, 5-year survival following surgery (without adjuvant therapy) exceeds 90%; for patients whose tumors involve the muscularis propria but not beyond, 5-year survival is about 85%; in tumors involving all layers of the bowel wall, with or without invasion of immediately adjacent structures, 5-year survival ranges between 70% and 75%; in cases of bowel wall involvement, tumor extension beyond contiguous tissue, and/or lymph node metastasis or evidence of distal metastasis, 5-year survival ranges between 45% and 60%.

Surgical patients considered to be at high risk for recurrence may undergo adjuvant chemotherapy and/or radiation therapy. Adjuvant chemotherapy using 5-FU and oral levamisole (Ergamisol; Janssen Pharmaceutica), a veterinary antihelminthic and immunostimulatory molecule, has been evaluated in large clinical trials to result in significantly improved disease-free survival (recurrence reduced by 41%) and overall survival (mortality reduced by 33%) compared to surgery alone, with survival advantage continuing for more than 12 years; the proportion of patients with cancer that had spread to lymph nodes who were cured increased from 49% to 65% (Laurie JA, etal, Journal of Clinical Oncology, 1989, 7:1447; Moertel CG, etal, NEJM, 1990, 322:352; Lopez M, Diseases of the Colon and Rectum, 1994, 37:S86). Adjuvant therapy with levamisole and 5-FU is designed

to prevent tumor recurrence by destroying any micrometastases that remain after surgery, although the reason for the efficacy of levamisole when combined with 5-FU is unclear, particularly because levamisole alone is ineffective as adjuvant therapy. Levamisole does demonstrate evidence of immune stimulation (specifically stimulation of macrophage activity), but the clinical importance of this effect remains obscure. Alternatively, levamisole may act as a specific biochemical modulator of 5-FU cytotoxicity, as it exhibits several pharmacologic activities, including inhibition of alkaline phosphatases, augmentation of cyclic guanine monophosphate, and dimunition of cyclic AMP in lymphocytes (Stevenson HC, etal, Journal of Clinical Oncology, 1991, 9:2052)

In April 1989, on the basis of data from clinical trials, NCI issued an advisory to physicians regarding the benefits of combination levamisole and 5-FU chemotherapy as an adjuvant treatment for stage III colon cancer. In April 1990, a Consensus Development Conference sponsored by NIH concluded that adjuvant therapy with levamisole and 5-FU is effective for patients with stage III colon cancer and, on June 20, 1990, FDA approved routine use of levamisole in adjuvant therapy of colon cancer. Universal application of 5-FU plus levamisole as surgical adjuvant therapy for the over 20,000 patients in the USA diagnosed annually with stage III colon cancer would prevent about 7,000 deaths. The estimated cost-effectiveness of the combined treatment is a very favorable $2,094 per year of life saved. Under a variety of less favorable assumptions, cost-effectiveness is still less than $5,000 per year of life saved. The net value of the return to the NIH research investment ($10.84 million between 1978 and 1990) was estimated to be $1.66 billion (Brown ML, etal, Journal of NCI, 1994, 86:424).

Because a substantial percentage of patients develop local disease after resection, adjuvant radiation therapy may be beneficial when wide surgical margins cannot be implemented. Radiation doses of approximately 4,500 cGy at 180 cGy per fraction are used in most clinical situations. Studies have also demonstrated an advantage to concurrent administration of 5-FU, typically at doses of 500 mg/m^2/day for three days during week one and week five of radiation therapy, with reductions of up to 54% in local recurrence rates (Thomas PRM and Lindblad AS, Radiological Oncology, 1988, 13:245).

Although there is no standard therapy for advanced colorectal cancer, multimodality therapy is often employed. The role of radiation is currently under investigation, but data suggest that after preoperative therapy (approximately 5,000 cGy) for locally unresectable disease, approximately 40% to 70% of tumors become resectable with a portion of patients being cured. However, despite this aggressive therapy, a substantial percent of these patients develop local recurrence. Therefore, other techniques, such as the use of an external beam boost, interstitial implant, or intraoperative radiation therapy (IORT) have been developed to deliver a boost dose of radiation therapy to the tumor bed. Patients receive high dose preoperative radiation therapy followed by surgery 3-4 weeks later. The long-term results with this approach have not been established, but doses in the range of 1,500 to 2,000 cGy can be used safely, and approximately 20% of patients with locally advanced primary tumors appear to be cured.

At the time of diagnosis of colorectal cancer, 38% of patients have regional spread of disease and 21% have distant spread, indicating a need for effective treatment of advanced disease. Although, to date, there is no evidence that standard chemotherapy improves survival, new strategies have focused on 5-FU biomodulation by various agents and 5-FU-based combinations. Response rates with intravenous 5-FU monotherapy are superior to intensive oral treatment (26% vs. 19%), with comparable hematologic side effects but less gastrointestinal toxicity, and avoidance of the problem of erratic absorption. In view of the short half-life of the drug, there is a reason for regimens using prolonged low-dose infusion; although the optimal 5-FU schedule has yet to be determined, five day loading courses (12 mg/kg/day for 5 days) are the standard against which all other schedules and combinations are measured.

LV has been reported to enhance tumor toxicity of 5-FU and produce second responses after 5-FU alone failed (Bonaventura A, etal, Proceedings of ASCO, 1990, 9:111). Several clinical trials have demonstrated that the combination of LV and 5-FU results in a significantly better response rate (23%) than that reported with 5-FU alone (11%) (Sotos GA, etal, Cancer Treatment Review, 1994, 20:11; Poon MA, etal, Journal of Clinical Oncology, 1991, 9:1967; Houghton JA, etal, Cancer Research, 1993, 53:4243). On the basis of these findings, regimens of 5-FU and LV in the treatment of advanced colorectal cancer have been adopted into clinical practice (a five-day regimen of 5-FU plus low-dose LV appears to lower both toxic effects and drug costs).

MTX also enhances the effect of 5-FU when used in a sequential manner 18 to 24 hours earlier than 5-FU (Marsh JC, etal, Proceedings of the ASCO, 1989, 8:103). MTX potentiates 5-FU cytotoxicity by increasing phosphoribosylpyrophosphate (PRPP), thereby increasing biosynthesis of the 5-FU metabolite, fluorouridylate, and augmenting its incorporation into RNA. MTX may also increase fluorodeoxyuridylate binding to thymidylate synthase. MTX and 5-FU combinations may improve durability of responses and survival, but the major benefit appears to be improved quality of life (improved performance status, weight gain, and relief of symptoms) as compared to other available chemotherapeutic options. Another agent which potentiates 5-FU, N-phosphonoacetyl-L-aspartate (PALA; U.S. Bioscience), a radio/chemosensitizer, inhibits aspartate transcarbamylase and de novo pyrimidine biosynthesis, thereby depleting tissue uridine nucleotide pools which in turn leads to enhanced uptake of 5-FU as an alternate pyrimidine

metabolite (Darnowski JW and Handschumacher RE, Pharmacological Therapy, 1989, 41:381). Although neurotoxicity has been a concern in clinical trials, overall low toxicity and quality of response are competitive with other biochemical modulation regimens.

Attempts have been made to increase response rates by adding such agents as cisplatin, MMC, and/or vincristine to 5-FU. Multiday cisplatin and 5-FU continuous infusion has produced some CRs and appeared to increase overall rates of response. However, the impact on survival, if any, has been minimal, and it remains to be established if there is a critical window of dose and schedule for both drugs. In addition, toxicities reported in all studies have been substantial. MMC and vincristine in combination with cisplatin and 5-FU also produced high rates of response, but toxicity and poor overall survival make this combination investigational.

ML Laboratories (Liverpool, UK) is investigating delivery of 5-FU intraperitoneally (IP) using an icodextrin solution (Icodial) as the delivery vehicle. A phase I pharmacokinetic study was completed demonstrating a 1,000 fold concentration benefit to IP delivery as well as the possibility of a once daily regimen (McArdle, CS, etal, British Journal of Cancer 1994, Oct. 70(4):762-6). A phase II dose ranging study in colorectal patients confirmed the pharmacokinetic benefit of IP icodextrin drug delivery and the fact that patients could manage daily fluid exchanges in a homecare setting. In June 1995 ML was in the process of implementing a phase III program. Icodial was recently approved in Europe as a dialysis solution in chronic ambulatory peritoneal dialysis (CAPD).

Gallbladder Cancer

Approximately 50% of tumors are technically resectable. Currently, about 50% of these are diagnosed preoperatively, although increasing use of preoperative ultrasound as the primary diagnostic test for gallbladder disease should improve this figure. Among patients with resected tumors, 5-year survival averages between 14% and 20%. For patients with carcinoma *in situ*, the 5-year survival is 60% to 80%. Little is lost in terms of 5-year survival when the tumor penetrates the submucosa or muscularis, but when the serosa is invaded, it falls to 11%. Invasion of adjacent organs or lymphatics reduces 5-year survival to less than 3%. Gallbladder tumors grow slowly; deaths after 5-years have been noted in many series.

A large number of surgically-treated patients are re-explored and subjected to more extensive surgical procedures, but long-term survival following these procedures is almost entirely limited to patients without recurrent or residual cancer in the specimen removed at second-look laparotomy; 5-year survival is rare when the tissue removed contains residual cancer. Overall poor results of resection of the liver bed and radical lymph node dissection, and high operative mortality (far exceeding potential patient salvage), limit re-exploration. Risk of recurrence depends more on disease stage and pathologic findings in the specimen than on surgery.

Post-surgical adjuvant radiation therapy is of no proven benefit, although local failure after resection suggest a role for such therapy to treat gross or microscopic residual disease. Overall median survival of patients not administered adjuvant radiation is frequently 6 months or less, whereas those treated tend to live for more than a year. But when analyzed by extent of cancer invasion, there is no evidence to support the efficacy of radiation therapy when there is no apparent gross residual disease after surgery. Irradiation is also performed as primary therapy (without surgical resection), using either external beam alone (4,400-6,360 cGy), or external beam (2,160-5,040 cGy) plus implant (949-2,567 cGy). The median survival is improved when both modalities are used (15 months) compared to external beam alone (7 months). However, almost all patients develop local-regional failure despite this moderately aggressive local therapy. Radiation combined with chemotherapy has not been studied in any consistent manner, and it is possible that combination of radiation with 5-FU or radiosensitizers may be of benefit. Chemotherapy has not been extensively explored as a surgical adjuvant. Retrospective anecdotal data suggests that when cancer extends through the gallbladder wall, a 3-4 month increase in median survival is achievable with administration of adjuvant systemic chemotherapy (primarily 5-FU).

Cancer of the Small Intestine

The primary treatment for adenocarcinomas of the small intestine is surgical resection with wide margins and removal of draining lymph nodes and the vascular pedicle. Despite this treatment, survival rates are poor in node positive patients. If there is involvement of the proximal duodenum, pancreatoduodenectomy may be necessary and for disease in the terminal ileum, a right hemicolectomy is indicated to insure complete resection and adequate margins. For jejunal involvement a wide resection is recommended. In patients deemed resectable, only about 30% survive 5 years, with 70% of node negative patients surviving 5 years but only 13% of node positive ones. Intraoperative radiation therapy has been advocated for patients deemed unresectable at the time of operation, but lack of clinical trials supporting this therapy limits its use to specialized centers. External beam radiation may be helpful in palliation but, again, lack of experience makes formal recommendations difficult. Chemotherapy usually incorporates 5-FU and nitrosoureas; in some settings, these agents have led to tumor regression and increased survival.

Primary treatment for sarcomas is also surgical resection with wide margins. With sarcomas, however, lymph node dissection is not as critical, because lymphatic spread is rare. An aggressive approach for metastatic disease may also be warranted for solitary hepatic metastasis, although data to support this are scant. Use of adjuvant radiotherapy and chemotherapy has been pro-

posed because sarcomas elsewhere in the body are radiation sensitive. Chemotherapy focuses on doxorubicin-based regimens and is used primarily when surgical resection is incomplete, in order to slow tumor growth and metastasis.

Surgery is also used to treat lymphomas. Use of radiation therapy for large intestinal lymphomas is associated with high rates of tumor necrosis, perforation and bleeding. Surgery typically involves removal of the bowel segments with wide margins, and the involved mesenteric lymph nodes, if possible. Margins need to be completely clear of tumor since lymphomas may spread for long distances in the submucosal plane. The long term survival rate following complete resection is about 45%. The benefit of adjuvant chemotherapy, usually administered to patients resected for cure, remains unproven. Also, radiation therapy and chemotherapy are recommended for patients with non-resectable lymphoma.

The cure rate of patients with carcinoid multicentric disease in whom all visible tumor is resected, remains low. At five years of follow-up, 80% are apparently tumor free, but recurrences continue to occur up to 25 years post surgery. Even in light of the slow rate of growth and indolent course of this tumor, aggressive surgical therapy is warranted, including segmental resection of ileal lesions with right hemicolectomy, as needed. Jejunal lesions should also be widely resected, and duodenal lesions, although rare, may require pancreatoduodenectomy. In the case of advanced disease, there may be some benefit to debulking procedures, but this remains controversial because the absolute size of the tumor does not correspond well with the degree of symptoms. Some authors recommend aggressive treatment even in those patients with widely metastatic disease, including resection of all intra-abdominal tumor deposits, segmental liver resection as needed, and hepatic arterial embolization, with cholecystectomy performed to prevent gallbladder necrosis during hepatic embolization. While it is not known if this aggressive surgical approach can increase survival, it has resulted in resolution of associated biochemical abnormalities in up to 25% of patients.

Anal Cancer

Very early local lesions may be treated by local excision alone, although less than 10% of anal tumors are eligible for such an approach. Cure rates have ranged from 45% to 75% in reported studies. The majority of localized tumors with or without regional node involvement require a more aggressive approach. Standard treatment now consists of a multimodality regimen which has proven to be very effective in achieving cures with sphincter preservation and minimal toxicity. The basic regimen involves concomitant external beam irradiation (45-50 Gy) and chemotherapy (5-FU and MMC). Surgical intervention is confined to biopsy of the primary tumor and suspicious lymph nodes. Five-year survival is approximately 75% to 80%, with sphincters preserved in about 80% to 90% of patients treated.

External beam irradiation alone has been suggested as an alternative for patients who cannot tolerate chemotherapy due to age, poor renal function, AIDS, or other medical conditions. The total dose of irradiation required is high (60-75 Gy) and, as a result, complications requiring surgery occur in about 5% of patients treated. In various studies, 5-year survival has ranged from 55% to 92%, with local control rates of 66% to 77%. Interstitial radiation therapy alone has also been used for early anal cancers; both radium and ^{192}Ir have been employed. The local recurrence rates have been relatively high, however, especially in tumors greater than 5 cm in diameter (77% local recurrence). Local necrosis has also been high with this approach (15%-25%). Local control is considerably better when interstitial irradiation is combined with external irradiation, but complication rates are still high because of the magnitude of total irradiation dose necessary.

Because anal cancer is rare and few patients ever develop metastatic disease, experience with chemotherapy is limited to small series of few patients. As single agents, both Adriamycin and cisplatin have yielded responses and, as in other epithelial tumors, major responses have been noted with cisplatin and 5-FU in combination.

Melanoma comprises about 2% of anal cancers. The prognosis has been uniformly dismal despite aggressive surgical approaches involving not only abdominoperineal resection (APR) but also inguinal and pelvic lymphadenectomy. Initial symptoms, primarily rectal bleeding, are often confused with hemorrhoids, resulting in delays in diagnosis. Regional node metastases are frequent and hematogenous spread also occurs early. APR resection was the recommended surgical procedure until recently. Currently, however, local excision is being increasingly advocated since cures are extremely rare despite APR. Recent reports confirm that there is essentially no difference in survival between local excision and APR, although local recurrence is less frequent with APR. Anal melanomas may also be treated with local excision and adjuvant irradiation, utilizing at least 4 Gy per fraction. Radiation alone or post-APR may also be useful for palliation of symptoms, such as bleeding, pain or obstruction. Unfortunately, even when local control has been achieved, systemic progression has remained a serious problem. While there have been some reports of 5-year survivors when chemotherapy was added to local excision, other studies have reported no advantage. Newer adoptive immunotherapy methods and other approaches to cutaneous melanomas, currently under study, may prove useful in the treatment of mucosal melanomas.

NEW DRUGS AND ALLIED THERAPIES IN DEVELOPMENT

Numerous agents are in various stages of development for the treatment of digestive system cancer (see Exhibit 4).

Gene Therapy/Vaccines /Immunomodulating Agents/Monoclonal Antibodies

There has been a resurgence in interest in cancer vaccines that may be used both as therapeutics after cancer has been detected and, also, potentially in the prevention of selected cancers in selected populations. Newly discovered genetic markers may identify significant numbers of individuals at risk for cancer who may benefit from immunization approaches to prevent malignancies. Vaccines may give rise to both humoral and cellular immune responses and confer long lasting immunity to the host. Anticancer vaccine strategies can now target intracellular antigens that are involved in the process of malignant transformation, such as oncogene products or mutated tumor suppressor genes. Several programs in this area are summarized below. A more comprehensive review of cancer vaccines will be published in an upcoming issue of FUTURE ONCOLOGY.

MAbs may also be making a comeback both as immunoconjugates and as immunomodulators. Over the past decade various clinical trials have used MAbs as therapeutic agents against solid tumors but results have been disappointing, partly because of low tumor uptake of the administered MAb and antigenic heterogeneity of tumor cells. However, recent advances in MAb design and targeting may improve the therapeutic value of MAbs.

Aphton (Woodland, CA), a public biopharmaceutical company, is developing therapeutics using vaccine-like products called immunogens for neutralizing or blocking hormones that participate in both malignant and non-malignant disease, in the GI and reproductive systems. GI system diseases include gastroesophageal reflux disease (GERD), ulcers and colorectal, stomach and pancreatic cancers. After a June 1994 approval by UK regulatory authorities, Aphton proceeded with a phase I clinical trial, in collaboration with the Cancer Studies Unit at the University of Nottingham in the UK, of its lead product, Gastrimmune, in patients with colon cancer metastastic to the liver, measurable by CAT scan, who have a life expectancy of at least three months. Aphton also plans to start pivotal trials with Gastrimmune for GERD. Gastrimmune consists of a synthetic peptide fragment of hormone G17 which is targeted to be neutralized (i.e., prevented from reaching and binding to its receptor), a "carrier" diphtheria toxoid, to which a number of the synthetic peptides are chemically bound and an adjuvant incorporated in an oil-based vehicle. Gastrimmune, which is administered by injection, with booster shots at six-month intervals, induces antibodies in the patient which cross-react with and neutralize G17 hormone which is known to activate the growth of colorectal, stomach and pancreatic cancer.

In October 1994 Aphton announced that it demonstrated the efficacy of Gastrimmune in inhibiting the growth of stomach cancer driven by a variant of G17 hormone. Collaborative research with two universities in the UK, showed that human stomach cancer cells secrete a variant or "precursor" of G17 hormone called "glycine-extended G17" which serves as a stimulus accelerating the growth of cancer cells. Anti-G17 antibodies generated by immunization with Gastrimmune and administered into animals which carry the human stomach tumor, caused the complete neutralization of the G17 precursor, inhibiting the growth of stomach tumors.

Biomira, a public company, is clinically evaluating a vaccine, Theratope, based on synthetic carbohydrate antigens of cell surface mucins (large molecular weight glycoproteins) that appear early during malignant transformation. These antigens are strongly expressed on cancer cells and low doses of shed cancer mucin antigens can suppress T cells, and, thus, inhibit an effective immune response. Presence of one particular carbohydrate epitope, the sialyl-Tn (STn), is associated with a very poor outlook in some cancer patients and may be used as a marker of prognosis or cancer aggressiveness. Another of these epitopes (the Thomsen-Friedenreich or TF antigen), is also a common antigenic determinant on adenocarcinomas. These carbohydrate epitopes are not specific to any one particular type of cancer but, instead are expressed generally on solid tumor cancer cells. And because they are rarely seen on normal cells, it is believed that they play some functional role in cancer.

In ongoing phase II clinical trials of Theratope in colorectal cancer, as of late 1994, the median survival among 40 patients was 14.4 months, equivalent to that obtained from chemotherapy trials. In these studies, a high titer of IgM antibody reactive with the target STn epitope of the vaccine appeared to be associated with a longer survival time. Experimental animal data showed enhanced immune responses to Theratope when a newly reformulated adjuvant, Detox-B-SE, was used for emulsion. This adjuvant formulation has FDA approval for use in clinical testing and is now being used in Biomira's Theratope therapeutic vaccine clinical trials at the University of Nebraska Medical Center (Omaha, Nebraska) and The University of Texas M. D. Anderson Cancer Center (Houston, TX). The latter's breast cancer study is the first clinical trial looking at Theratope in combination treatment with interferon.

Bristol-Myers Squibb initiated in early 1995 a phase II clinical trial in breast cancer of BR96-Dox, a chimeric human IgG_1 isotype which targets epithelial cancer conjugated to eight molecules of doxorubicin. In May 1995 Genzyme Transgenics (Framingham, MA) entered into a contract with Bristol-Myers Squibb to provide transgenic goats that secrete BR96 MAb as a lower cost production alternative.

Canji (San Diego, CA) received RAC recommendation in December 1994 for its protocol for the treatment of primary and metastatic liver cancer and is awaiting NIH approval to initiate a phase I study to be conducted at the University of California, San Francisco, involving 27 patients with alterations in the tumor suppressor gene

p53. The protocol intends to introduce wild-type p53 using a recombinant adenovirus vector via an intra-hepatic catheter. Primary trial endpoint is safety, and secondary endpoints include pharmacokinetics and efficacy (see FO, V1 #1, p. 26).

Centocor received approval in January 1995 by the Paul Ehrlich Institute (PEI), the German regulatory agency for vaccines and antibodies, to market Panorex (17-1A) as adjuvant therapy following surgery for colorectal cancer. Centocor and its partner, Wellcome, had applied for marketing authorization in Germany in July 1994. Applications have also been filed in Austria, Finland and Sweden, and Wellcome is expected to begin additional phase III clinical studies with Panorex in Europe, the USA (where 6,000 patients are to be enrolled), and Japan in 1995. Germany is the first market for Panorex and represents the first approval worldwide for a MAb used in cancer therapy. In a pivotal clinical trial conducted at the University of Munich, Panorex was used to target minimal residual disease in 189 patients with Dukes' stage C colorectal cancer who had undergone curative surgery and were free of detectable residual tumor. Patients were randomly assigned to an observation regimen or to postoperative treatment with 500 mg of Panorex, followed by four 100 mg infusions each month. The effect of antibody was most pronounced in patients who had distant metastasis as first sign of a relapse, an effect that was not seen for local relapses. Toxicity was mild (Riethmuller G, etal, Lancet, 1994 May 14, 343(8907):1177-83). Treated patients who were followed for 5 years experienced a 30% reduction in mortality, compared to controls. Enrolled patients in whom the tumor had invaded nearby lymph nodes but had yet to spread to other organs, were either treated with surgery, and radiation or with the foregoing and Panorex. After five years, 51% of patients not treated with Panorex had died compared to 36% of treated patients. Recurrence rate decreased from 67% to 49%. The 17-1A MAb may also be used in the treatment of pancreatic and gastric cancer and certain types of lung, breast and ovarian cancer.

In November 1993 Centocor reached a definitive agreement to form an alliance with Wellcome (London, UK) for the latter to help finance Centocor's development and marketing of certain MAb-based anti-cancer agents such as Panorex and market and sell any approved drugs in most parts of the world. Wellcome acquired a nearly 5% stake in Centocor for $20 million, paid $10 million at the closing and will make certain future payments of up to $70 million. A year later the two companies extended their alliance to include Japan, Taiwan, South Korea, and China.

Corixa (Seattle, WA), founded in September 1994, is developing vaccines which trigger specific T cell responses. Its leading vaccine product uses Muc-1 tumor antigen to activate T cell responses in breast, pancreatic and colorectal cancer.

Cytel (San Diego, CA) is developing cancer vaccines using mixtures of peptides from various tumor-associated antigens such as MAGE, HPV, HER2/neu, p53, PSA, and CEA. Contents of each mixture vary depending on the tumor type targeted. Peptides that bind to major histocompatibility complex (MHC) are being selected that may function as epitopes to tumor-specific cytotoxic T lymphocytes (CTLs). The company is currently clinically testing vaccines to treat melanoma and cervical carcinoma and is in preclinical stage of development of therapeutic vaccines for prostate, breast, ovarian and colon carcinomas. CY 2010 (MAGE 3), designed to stimulate the immune system to recognize and reject tumor cells by inducing production of cytotoxic T cells, is in phase I/II trials in advanced melanoma.

Genetics Institute (GI; Cambridge, MA) suspended phase II clinical trials of recombinant human interleukin-12 (rhIL-12) in June 1995, after several patients under treatment for advanced kidney cancer needed to be hospitalized and one patient died. GI begun clinical safety trials of rhIL-12 in May 1994 following preclinical animal studies that showed that administration of rhIL-12, a cellular immune activator, shrank or entirely eliminated a variety of cancers including melanoma, lymphoma and lung, kidney, and colon cancer. GI has cross-licensed the IL-12 patent with Hoffmann-La Roche and is developing the agent worldwide through a joint venture with Wyeth-Ayerst, except in Japan where it will be marketed by GI-Yamanouchi.

ImClone (New York, NY) and Cancer Research Campaign Technology (London, UK) entered in an exclusive worldwide collaborative and licensing agreement for a human anti-idiotypic MAb (105AD7) targeting tumor antigen gp72, developed by the Department of Surgery, University of Nottingham, UK. The agent has been shown to induce antitumor cellular responses in animals and appears to prolong survival in patients with metastatic colorectal cancer without associated toxicity (Durrant LG, etal, Cancer Research, 1994 Sep 15, 54(18):4837-40). At the same institution, in a phase I/II clinical trial, 13 patients with advanced colorectal cancer were immunized with 105AD7 and their survival was compared with that of a group of unimmunized patients with similar disease status. Median survival following diagnosis of advanced disease of immunized patients was 12 months, compared with 4 months in unimmunized patients. There was no toxicity associated with the treatment (Denton GW, etal, International Journal of Cancer, 1994 Apr 1, 57(1):10-4).

In May 1995, ImClone also initiated two phase Ib/IIa clinical trials of C225, an epidermal growth factor receptor (EGFr) antagonist. The first study will test C225 alone in patients with EGFr positive cancers and the second will test C225 in conjunction with cisplatin or radiation, to eliminate cancerous cells through a mechanism which may involve the induction of apoptosis. In both

studies, a planned total of 24 patients will receive C225 weekly by intravenous administration in a multiple injection dose escalation protocol over a four-week period; treatment will be extended for up to eight additional weeks to further assess anticancer activity. ImClone's IND application to clinically evaluate C225 was approved in November 1994 and a phase I study was completed in March 1995 at Memorial Sloan-Kettering Cancer Center, and the Yale University School of Medicine (New Haven, CT). EGFr is expressed in certain normal tissues and was shown to be overexpressed on the cells of approximately one-third of all cancers. Antibodies directed against EGFr may inhibit the uncontrolled cancer growth associated with this receptor's activity. Indications for C225 include breast, ovarian, prostate and head and neck cancers and may also be applicable to GI cancers.

Immune Response (Carlsbad, CA) announced in July 1994, that it was granted an exclusive license by the San Diego Regional Cancer Center (San Diego, CA) to develop a therapeutic vaccine comprised of irradiated fibroblasts from a skin biopsy, genetically modified to produce IL-2, combined with irradiated cancer cells from the patient. Preclinical results in a colon cancer model demonstrated this technology may prevent tumor growth and eliminate established tumors. The clinical protocol for colon cancer has been approved by RAC and a phase I clinical trial has begun that is to enroll up to 12 patients.

Immunomedics, under a government grant, has been testing a vaccine based on an anti-idiotypic antibody of the original CEA molecule. Injection of this agent, is expected to stimulate the patient's immune system to mount an immune response to the replica of CEA and, thereby, also attack the CEA-containing cancer cells.

ImmunoTherapeutics (Fargo, ND) is developing lipophilic disaccharide peptides related to muramyl dipeptide, the minimum immunologically active unit of the mycobacterial cell wall, for the prevention and treatment of malignant and infectious disease. These compounds are active immunoadjuvants resulting in cytokine production, an increase in cellular and humoral immunity, and activated macrophages which are cytotoxic for malignant cells. In the cancer area, the company has focused on the development of a systemic macrophage activator, ImmTher, a liposome incorporated lipophilic disaccharide tripeptide that has caused significant cancer regression in patients with metastatic colorectal cancer. It is currently in phase II clinical trials in patients with metastatic colorectal and prostate cancer, and metastatic malignant melanoma. An analog of the compound which has an amide linkage between the peptide and lipophilic group is being used in a vaccine formulation, Theramide. The amide linkage is not subject to hydrolysis and prolongs the half-life of the compound. The amide-linked analog is not incorporated in a carrier liposome and is currently in phase I clinical trials in patients with malignant disease. Theramide and its non-pyrogenic threonine analog are candidates for vaccine formulations designed for preferential increase in cell mediated immunity.

Jenner Technologies (Danville, CA) is developing vaccines incorporating whole recombinant tumor associated or tumor specific antigens and an adjuvant, packaged in liposomes. The whole recombinant antigen is made up of many epitopes, thereby allowing the patient's immune system the opportunity to respond to the one compatible with its repertoire. The liposome targets to the site of the immune response and facilitates presentation of the antigen in such a way as to enhance the immune response, causing proliferation of cytotoxic T cells. The adjuvants are selected to enhance the development of cytotoxic T cells.

NeoRx (see this issue, page 69)

Seragen (Hopkinton, MA) has created $DAB_{389}EGF$ fusion toxin by genetically fusing the enzymatically-active domain of diphtheria toxin to human epidermal growth factor (EGF), to selectively target tumors overexpressing the EGF receptor (EGFr). $DAB_{389}EGF$ binds specifically to EGFr, is rapidly internalized by tumor cells and kills them by inhibiting protein synthesis. Phase I/II clinical trials in patients with various cancers, including pancreatic tumors, are ongoing. In a phase I clinical trial of 18 patients with advanced malignancies overexpressing EGFr, $DAB_{389}EGF$ was administered as a 30 minute infusion in 5 consecutive daily doses repeated every 28 days. MDT was not reached with this regimen and no serious toxicities were observed (Theodoulou, M, etal, ASCO95, Abs. 1561). Eli Lilly has the option to obtain worldwide development, distribution and marketing rights for $DAB_{389}EGF$.

Somatix Therapy (Alameda, CA) is developing GVAX, a cancer vaccine approach based on *ex vivo* transfection of a patient's (autologous) tumor cells with the gene encoding granulocyte-macrophage colony-stim-ulating factor (GM-CSF) and irradiation of these cells to prevent further division before they are re-infused into the patient post-surgery. GM-CSF modified cells were shown to elicit potent, specific and long-lasting antitumor response in animal models (Mulligan, R, etal, PNAS, April 1993). The company completed a $4.5 million agreement with a private investment group in May 1995 to finance the application of GVAX in the treatment of colorectal cancer; $2 million of this will be immediately invested to support preclinical research with the remaining $2.5 million to be paid upon filing an IND. In exchange, the investment group acquired shares of Somatix stock and will receive royalty payments of 2% of net sales, capped at $1,750,000. GVAX is also in clinical trials in kidney cancer and melanoma and trials are planned in prostate cancer.

StressGen Biotechnologies (Victoria, BC, Canada), which specializes in stress proteins as therapeutic agents, plans to develop Oncocine vaccines to treat various malignancies, including colorectal cancer. The vaccines are based on its Unigen technology, which combines myco-

bacterial stress proteins as carriers with tumor-spe-cific antigens (TSAs). A preclinical trial has demonstrated that stress proteins hsp65 and hsp71 have potential as immunostimulants.

Therion Biologics (Cambridge, MA) is collaborating with Jeffrey Schlom, PhD, Chief of NCI's Tumor Immunology & Biology Laboratory, in evaluating TBC-CEA vaccine, a live recombinant poxvirus expressing CEA in phase I trials in colorectal, breast and lung cancer. Therion also entered into a 5-year Cooperative Research & Development Agreement (CRADA) with the NCI, in November 1994, to develop cancer vaccines based on live recombinant poxvirus vectors that express specific tumor-associated antigens identified by tumor-infiltrating lymphocyte (TIL) technology. The goal of the collaboration is to develop vaccines that stimulate a full range of antibody and cell-mediated immune responses to the tumor antigens.

University of Alabama investigators at the Birmingham Comprehensive Cancer Center received RAC approval in June 1995 to initiate phase I clinical trials of an intramuscular injection of a polynucleotide vaccine consisting of a plasmid DNA encoding the full-length cDNA for CEA, driven by the cytomegalovirus early promoter/enhancer (Conry RM, etal, Gene Ther, 1995 Jan, 2(1):59-65). The study will enroll 15 patients with metastatic colorectal cancer who failed at least one chemotherapy regimen and whose tumors contain CEA. Two groups of patients will receive a single 0.5 ml vaccination injected in the bilateral deltoid muscle and two others will receive the same dose three times in three-week intervals. Patients will then be monitored for eight weeks for toxicity and humoral/cellular responses.

Vical (San Diego, CA) is pursuing clinical trials of Allovectin-7, a gene-based product intended for direct intratumoral injection. The product contains a gene that encodes a mismatched transplantation antigen (HLA-B7) which, when injected into tumors, causes malignant cells to bear the foreign antigen on their surface. The rationale is that the patient's immune system, which previously failed to recognize the tumor cells as aberrant, may now attack and destroy those cells expressing the foreign antigen. Following allowances by the FDA and RAC, Vical is pursuing phase I/II clinical trials, started in 1995, in patients with malignant melanoma, colorectal carcinoma (at the Mayo Clinic) and renal cell carcinoma. Vical recently submitted to the FDA and RAC an amendment to its Allovectin-7 clinical protocol to allow for inclusion of previously treated patients under certain circumstances. At the 86th Annual Meeting of the American Association for Cancer Research (AACR) in Toronto in March 1995, scientists from the University of Arizona (Tuscon, AZ) presented two papers on results of laboratory tests on forty patients with advanced cancers. The clinical trial was designed to test the safety of Allovectin-7 at varying dosage levels and to assess HLA-B7 gene transfer and expression in tumor lesions following administration. Data presented at the AACR meeting showed that direct gene transfer into different tumor types appeared to be safe and feasible; transfer of HLA-B7 gene and protein could be detected for several weeks in more than 75% of patients (28 of 36 available biopsies), and intratumoral T cell infiltration (10 of 11 tested) and a functional cell mediated immune response was observed (3 of 8 tested) in patient samples. During a December 1994 meeting, RAC also approved a protocol sponsored by Vical, to inject into tumors the IL-2 gene in a plasmid-lipid complex (Leuvectin) to treat solid tumors and lymphomas. A phase I/II study of 25 patients commenced in April 1995 at the Arizona Cancer Center (Tuscon, AZ) and the Scott and White Memorial Hospital (Temple, TX).

The Wistar Institute (Philadelphia, PA) is targeting the cancer associated C017-1A/GA733 antigen (Ag), bound by MAbs C017-1A and GA733, which define two different epitopes on the Ag. This Ag has proven to be a useful target in passive and active immunotherapy of colorectal carcinoma. In a recent randomized trial with MAb C017-1A, performed in patients with Dukes' C colorectal cancer, those treated survived significantly longer as compared to controls. Previous studies suggest that anti-tumor effects observed in MAb-treated patients may be mediated by idiotypic cascades. In approaches to active immunotherapy against the Ag, polyclonal goat and monoclonal rat anti-idiotypic antibodies (Ab2) directed against MAb C017-1A or GA733 were administered as alum precipitates to 54 patients with Dukes' B, C and D colorectal cancer. Approximately 30% of patients tested developed specific cellular immunity (Ag-specific T cells mediating a delayed-type hypersensitivity reaction *in vivo* or proliferating upon stimulation with the Ag *in vitro)*. Nine of the 13 patients with Dukes' B or C, treated with polyclonal goat Ab2 GA733 showed no evidence of disease after 41-88 months of observation. Two new monoclonal Ab2s which mimic C017-1A or GA733 epitopes have been developed which are superior to the previously used Ab2 in their capacity to induce humoral and cellular immunity in animals. Recently, C017-1A/GA733 Ag was molecularly cloned and expressed in baculo-, adeno-, and vaccinia viruses.

Thymidylate Synthase Inhibitors

Inhibition of thymidylate synthase (TS) by the combination of 5-FU and LV has resulted in improved treatment of metastatic colorectal cancer and adjuvant therapy of Dukes' C colon cancer. The realization that more effective inhibition of TS may be beneficial has spurred development of novel agents in this area that act independent of folate cofactors like leucovorin. According to Charles Erlichman of the Mayo Clinic (Rochester, MN) in his presentation on TS inhibitors during ASCO95, two approaches used in the development of these agents include modifications of the folate chemical

structure at the pteridine ring, para-aminobenzoic acid moiety and the glutamate end and the design of a molecule based on the x-ray crystallography structure of TS which would bind to the reduced folate binding site.

ZD1694 (Tomudex, Zeneca), a water soluble specific thymidylate synthase inhibitor, has demonstrated substantial activity in patients with advanced colorectal cancer, along with a convenient administration schedule and a manageable toxicity profile. In a phase II study, 176 patients with advanced or metastatic colorectal cancer were treated with ZD1694 at a dose of 3 mg/m², as a short IV infusion, given thrice weekly. Over 80% of the patients had liver metastases and 30% had additional extrahepatic metastases. Overall, 26% of patients (46/176) responded to treatment, with four CRs, 37 PRs, and seven minor responses. Responses were early, occurring at the first objective response assessment after two cycles of drug. More than 80% of patients completed treatment without significant delay or dosage reduction (Cutsem EV, etal, 1995 Digestive Disease Week, Abs. Pg. A155;619).

AG337 and AG331 are lipophilic TS inhibitors designed based on the crystallographic structure of the drug target. They are the first agents created in this manner to reach clinical trials. At the March 1995 annual meeting of the American Association of Cancer Research (AACR), Agouron Pharmaceuticals (La Jolla, CA) reported results from phase I clinical studies of AG337 carried out by clinical investigators in England. In one study, Dr. Hilary Calvert and colleagues at the University of Newcastle upon Tyne administered escalating doses of AG337 as a five-day infusion every three weeks in 19 patients with advanced solid tumors. The maximum tolerated dose (MTD) was determined to be 1,130 mg/m² per day. Dose limiting toxicity was myelosuppression of short duration and transient mucositis; no organ toxicity was observed. Also, at doses less than the MTD, anti-tumor effects were observed in several patients with advanced tumors. In a second phase I study, 15 patients were treated with escalating doses of an oral capsule formulation of AG337 to determine the extent of the drug's oral bioavailability. Rapid absorption of AG337 was observed and total oral bioavailability drug exceeded 80%. The pharmacokinetic profile of AG337 delivered orally appeared very similar to that of the drug delivered intravenously. A dose of 1,000 mg/m² per day tested in current phase II studies, resulted in reductions in tumor mass greater than 50% in some patients.

In October 1994, Agouron began six concurrent phase II studies at four sites (Deaconess Hospital in Boston, the University of Colorado Cancer Center in Denver, the University of Pittsburgh Medical Center, Pittsburgh Cancer Institute, and Queen's Medical Center in Honolulu) to evaluate AG337 against solid malignant tumors of the colon, lung, liver, pancreas, prostate, and head and neck. Also, AG331 is in phase I clinical trials at the University of Southern California (Los Angeles, CA) and at Fox Chase Cancer Center (Philadelphia, PA).

UFT, developed by Taiho (Japan) is a fixed-ratio combination of uracil and tegafur (Ftorafur), a prodrug that is absorbed orally and metabolized in vivo to 5-FU. Uracil potentiates 5-FU through interference with its catabolism. In May 1995 Bristol-Myers Squibb (BMS) said it obtained exclusive rights to UFT in North and Latin America, Europe, and all other countries except Japan where it is marketed by Taiho and the Philippines and other Asian countries and Spain, where it is marketed by Otsuka (Tokyo, Japan). In these countries UFT is marketed for a variety of indications, among them colorectal, gastric, breast, and other cancers. Sales of UFT in Japan were reported as $630 million in 1994, accounting for over 32% of the Japanese anticancer drugs market. The Japanese labeling for UFT was amended in 1994 to include warnings about the drug's potential association with pancreatitis. BMS will undertake completion of phase III trials in the USA and Europe for colorectal cancer and other indications that may include biliary tract, gallbladder, liver, pancreas and stomach tumors. A USA NDA is expected to be filed in 1998. BMS will have exclusive marketing rights and make milestone and royalty payments. UFT has also shown activity in lung and head and neck cancer. The UFT patent expires in 1999 in the USA but market exclusivity will be extended to 2004 under the Waxman/Hatch Act.

In 21 patients (20 evaluable) with advanced incurable colorectal cancer treated at Sloan-Kettering Cancer Center (New York, NY) with a combination of UFT (350 mg/m²/day divided every 8 hours) and LV (5 mg tablet every 8 hours) concurrent with each UFT dose, five major objective responses (one CR and four PRs) were observed. Treatment was continued for 28 consecutive days, followed by a 7-day rest. Toxicity was mild, no dose-limiting myelosuppression was observed; side effects included diarrhea and dose-limiting mucositis (Saltz LB, etal, Cancer, 1995 Feb 1, 75(3):782-5). Another clinical trial of 157 previously untreated surgical candidates with stage II and III gastric adenocarcinoma who were randomized to receive adjuvant chemotherapy with IV MMC (10 mg/m² q 28 days X 6 doses) and oral UFT (300 mg/m²/day continuously for 1 year), or no further treatment, showed no difference in survival, or in disease free survival, between control and treatment arms. Median survival of those treated was 2.3 years compared to 2.6 years for controls, after a median follow up of 3.13 years (Carrato A., etal, for the TACG Group at Alicante University Medical School, Spain, ASCO 95 Abs. #468). Similar results were reported by the Northern Kyushu Cooperative Study Group for Cancer Chemotherapy (Fukuoka, Japan). In 243 patients with stage II, III, or IV gastric cancer randomized to receive either tegafur 600 mg or UFT 600 mg orally daily beginning two weeks after operation and continuing for two years, there were no significant differences in 5-year survival although patients who were given UFT lived longer (Arima S, etal, European Journal of Surgery, 1994 Apr, 160(4):227-32).

Topoisomerase I Inhibitors

Several topoisomerase I inhibitors which block topoisomerase I and, ultimately, DNA synthesis, are in development. Among these inhibitors, various water-soluble and insoluble derivatives of the plant alkaloid camptothecin, administered parenterally or orally, have shown effectiveness in colon cancer in clinical trials. Two water-soluble derivatives, topotecan (SmithKline Beecham) and irinotecan are in late stage development for various cancers and another, the totally synthetic camptothecin analog GG-221 (Glaxo), is in phase II clinical trials in colon and lung cancer.

CPT-11 (irinotecan; Yakult Honsha), a semisynthetic analog of camptothecin, is a prodrug that changes to an active metabolite, SN-38, which is 100 fold more active than the parent compound. In January 1994, Upjohn (Kalamazoo, MI) acquired USA and Canadian marketing rights to irinotecan from Yakult Honsha, which developed CPT-11 in Japan, in collaboration with Daiichi Pharmaceutical (Tokyo, Japan). Under the agreement, Upjohn will develop, register and market irinotecan in North America while Daiichi will continue co-development and co-promotion with Upjohn in the USA. The compound was cleared for marketing in Japan in January 1994 for primary lung, cervical and ovarian cancers. As part of the agreement, Yakult Honsha will co-develop and co-market in Japan, Upjohn's anticancer drug adozelesin (formerly U-73975) which is in phase II clinical trials in the USA and phase I in Japan for the treatment of solid tumors and leukemia. Irinotecan will be marketed in Europe by Roger Bellon (Rhône-Poulenc Rorer) which submitted a PLA in its first European market, France, in late 1994, for the treatment of advanced colorectal cancer which progressed after standard treatment. Irinotecan is expected to be launched in France and submitted to the CPMP in 1995, with France as reference country. The approval of irinotecan generated controversy in Japan, where the product was reported to be associated with serious ADRs, such as reduced white blood cell counts, and a number of fatalities during clinical testing. As a result, certain use precautions have been applied in Japan.

Drug Delivery

Liposomes used to encapsulate anticancer drugs may reduce toxicities and improve acute and chronic tolerance. The increased uptake of liposomes by colon adenocarcinoma cell lines may enable them to circumvent the p-glycoprotein-mediated anthracycline resistance of colon cancer cells. Several agents, currently in development against other cancers, may be applicable in treating GI malignancies. NeXtar (Boulder, CO) filed an NDA for DaunoXome (liposome-encapsulated daunorubicin), that has completed phase III trials in AIDS-related Kaposi's sarcoma. In June 1995 FDA's Oncologic Drugs Advisory Committee recommended approval of DaunoXome as first-line therapy for Kaposi's sarcoma. Other liposomal anticancer formulations in development include two doxorubicin products, Sequus' (formerly Liposome Technology) DOX-SL, which has been submitted for approval in the USA and Europe for Kaposi's sarcoma, and The Liposome Company's TLC D-99 which is in phase III trials in breast cancer.

Matrix Pharmaceutical (Menlo Park, CA) reported results from phase I/II testing of two different intratumoral formulations of IntraDose injectable gels in five patients with nonresectable esophageal cancer at the American Gastroenterology Association meeting held in San Diego in May 1995. Four patients received four weekly injections of IntraDose-CDDP gel (cisplatin-based) and one received four bi-weekly injections of IntraDose-MTX injectable gel (MTX-based). Data presented by Dr. Lopa Mishra of the VA Medical Center and Georgetown University (Washington, DC) demonstrated the product's safety, low toxicity and palliative benefits. Treated patients had dysphagia scores ranging from 2.6 to 3.8 based on a scale of 0 (no dysphagia) to 4 (difficulty in swallowing saliva). One patient was completely relieved of dysphagia and in the remaining four patients dysphagia scores were reduced to 0.5. Patients had tumors ranging in size from 5 to 12 cm. Of the four patients treated with IntraDose-CDDP, two showed stable disease and the other two showed a decrease in tumor volume of 58% and 75%, respectively. The one patient treated with IntraDose-MTX showed no change in tumor volume. No evidence of systemic toxicity was noted. In this indication, these products are currently demonstrating a potential as palliative treatments (also see FO V1 #1, p. 20).

Sparta Pharmaceuticals (Research Triangle Park, NC), a public company, is developing an oral prodrug of 5-FU for the treatment of liver cancer. This agent is formulated using Sparta's LADD (liver-associated diseases and delivery) technology to target the product to the liver. The drug is metabolized to active 5-FU in the liver in a single-step process. The drug can act as a specific treatment for liver-associated tumors in a low dose formulation or as an orally administered systemic treatment in a high dose version.

Photodynamic Therapy (PDT)

Several companies are developing PDT approaches for the treatment of GI cancer. For a description of Quadra Logic Technologies' (QLT; Vancouver, BC, Canada) products see FO, VI,#1, p. 29. QLT's photophrin was launched in Japan in April 1995 by Lederle (Japan) for the treatment of lung, gastric, esophageal and cervical cancers. Ergo Science (Charlestown, MA), a privately held company founded in 1990, acquired exclusive patent rights in 1995 to a unique class of photochemotherapeutic dyes from the Rowland Institute (Cambridge, MA). Another PDT dye in development is Scotia Pharmaceuticals' (Guildford, Surrey, UK) EF9 (mesotetrahydroxyphenylchlorin; mTHPC), in phase II clinical trials for the treatment of head and neck cancer. Another company,

Exhibit 3
Drugs in Development for the Treatment of Digestive System Cancer

Primary Developer/ Affiliate(s)	Generic Name/ Number/Brand Name	Drug Type/ Target/ Mechanism/ Delivery	Status/ Location/ Indication	Comments
Agouron Pharmaceuticals/ Cancer Research Campaign	AG337	Thymidylate synthase inhibitor/ IV, PO, IP	Phase II/UK/solid tumors; phase II (1995)/USA/ colon liver, pancreatic cancer	At a dose of 1000 mg per m^2 per day, reductions in tumor mass greater than 50% were observed
Agouron Pharmaceuticals/ Cancer Research Campaign	AG331	Thymidylate synthase inhibitor	Phase I/USA/solid tumors	
Ajinomoto/ Roussel-Morishita; Yamanouchi	Lentinan/LC-33, YM-09222	Polysaccharide isolated from Lentinus edodesimmune/ response agonist/IV	L86/Japan/stomach cancer; clinical/Japan/ colorectal cancer	
Alfacell/NIH	Onconase/P-30	Microtubule inhibitor/15 kD protein isolated from Rana pipiens oocytes and early embryos	Phase III (1/95)/USA/ pancreatic cancer	In combination with tamoxifen
Amira (Repligen)	Cyclocreatinine/ AM-285	1-carboxymethyl-2-iminoimidazolidine/ substrate analog of creatinine kinase/ creatinine kinase inhibitor	Phase I/USA/ colon cancer	Effective in combinations, including 5-FU; no additional toxicity; increases in tumor-growth delay were 1.7- to 2.4-fold compared to monotherapy (Teicher BA, etal, Cancer Chemotherapy and Pharmacology, 1995, 35(5):411-6)
Ansan (Titan Pharmaceuticals)	AN-9	Butyric acid derivative/ apoptosis agonist	Preclin/USA/colon and pancreatic cancer	(see FO, V1 #1, pp. 30 & 31)
Aphton	Antigastrin-17 immunogen/ Gastrimmune	Gastrin inhibitor	Phase I/II/UK/ colorectal cancer (may also be effective in stomach and pancreatic cancers)	Trials are planned for gastro-esophageal reflux disease (GERD)
Asta Medica	D 22213 (RC-3095; D-21663; RC-3440)	Bombesin/gastrin releasing peptide (GRP) antagonists/SC	Preclin/Germany/ colon cancer	Licensed from Tulane U (Professor AV Schally)
Banyu Pharmaceutical	NB-506	Topoisomerase I inhibitor	Phase I/Japan/solid tumors	ASCO 95, Abs. 373
Beaufour-Ipsen	BIM-26226	Bombesin/gastrin releasing peptide (GRP) antagonist	Phase I/USA/liver and colon cancer	
Biomira	Theratope	Synthetic carbohydrate antigens of cell surface mucins	Phase II/USA/ colorectal cancer	Also in combination with Detox-B-SE adjuvant
Boehringer Mannheim/Bowman Gray School of Medicine	Limofosine/ BM-41.440; Et-16S-OEt	Ether phospholipid analog/phosphocoline acyltransferase inhibitor	Phase II/ Germany/ colon cancer	Dosage of 5 mg/kg/day was well tolerated
Bristol-Myers Squibb	Elsamitrucin; elsamicin-A/ BBM-2478A; BMS-181171; BMY-28090; NSC-369327	Chartresusin-related compound/isolated from Actinomyces/DNA topoisomerase I and II inhibitor/IV	Phase II/USA, Europe/ advanced colorectal cancer	A 25 mg/m^2/week given as a 5-10 min. infusion at least 3-6 times weekly resulted in no objective responses (Verweij J, etal, Ann Oncol, 1994, 5(4):375-6)
Bristol-Myers Squibb	BR96-Dox	Chimeric MAb conjugated to doxorubicin/IV	Phase I (12/94)/USA/ solid tumors	

— continued on next page

Bristol-Myers Squibb	Murine L6 and chimeric ChL6	Chimeric MAb conjugated to drugs or isotopes/IV	Phase II/USA/solid tumors	Nine patients with metastatic breast cancer were treated with ^{131}I-conjugated ChL6 (DeNardo SJ, etal, Cancer, 1994 Feb 1, 73 (3 Suppl):1023-32)
Bristol-Myers Squibb	Acidic and basic FGF-Pseudomonas exotoxin fusion proteins (aFGF-PE40 and aFGF-PE40 KDEL and bFGF-PE40 and bFGF-PE4E KDEL)	Fusion protein against tumor cells bearing FGF-receptors/angiogenesis inhibitor	Preclinical/US colon, hepatocellular cancer	
British Biotech/ SmithKline Beecham	Batimastat/BB-94	Matrix metalloproteinase inhibitor/IP	Phase II/USA, Europe/ colorectal, pancreatic and stomach cancer	Phase III trials were suspended in 2/95 because of adverse effects caused by manufacturing process
British Biotech/ SmithKline Beecham	Batimastat/ BB-2516	Matrix metalloproteinase inhibitor/oral formulation	Phase I/UK/ colorectal, pancreatic and stomach cancer	
Cancer Research Campaign/ImClone (ww licensee)	105AD7	Vaccine (prophylactic and therapeutic)	Phase I/UK/colon cancer	
Canji/Schering-Plough		Gene therapy/wild-type p53 insertion by recombinant adenovirus vector/IAH	Preclin (recommended by RAC 12/94)/USA/ primary and metastatic liver cancer	(See FO, V1 #1, pp. 26 & 30))
CarboMed/NCI	CM101	Polysaccharide exotoxin produced by Group B Streptococcus/ angiogenesis inhibitor	Phase I (completed 12/94)/USA/solid tumors (including colon)	Selectively binds to developing blood vessels of tumors inducing a severe inflammatory reaction leading to destruction of the neovasculature and tumor necrosis without harming healthy tissues
Cell Pathways	FGN-1	Sulfone metabolite of the NSAID sulindac/ apoptosis agonist	Phase I/II/USA/familial adenomatous polyposis (FAP); colonic polyps	(See FO, V1 #1, pp. 30 & 31)
Celltech/American Cyanamid (AHP)	CPD 833-Y yttrium 90	Azamacrocycles linked to engineered human MAb Fab fragments conjugated to ^{90}Y	Phase I/USA/ colorectal cancer	As of 6/94 preliminary studies were completed at Memorial Sloan Kettering Cancer Center
Cell Therapeutics	CT-2584	Cytotoxic, potent antimetastatic and antiangiogenic properties	Preclin/USA	Potently toxic to breast, lung, and colon cancer cells, including MDR malignancies; minimal or no toxicity to normal cells
Centocor/ Ajinomoto; Wellcome	1083-17-1A; 17-1A; C017-1A/ Panorex	Murine IgG2a MAb/chimeric	Approved (1/95)/ Germany; prereg/USA/ colorectal cancer	May also be effective in pancreatic cancer
Chemex	Masoprocol/C-205, CHX-100/Actinex	Lipoxygenase inhibitor	Preclinical/USA/ colon cancer	Launched/USA/actinic keratosis (topical)
Chugai	Picibanil/OK-432, NSC-B116209, PCB-45	Immunostimulant	L(75)/Japan/ GI cancers	
Corixa	Muc-1 tumor antigen	Activates tumor-reactive T cells	Phase I/USA/colorectal and pancreatic cancer	
Cytel	MAGE-1,-2 and -3; CEA; p53, p21 ras	Mixture of peptides from various tumor-associated antigens/immuno-stimulant; induces production of CTLs	Preclin/USA/colon stomach, pancreatic and liver cancer	CY 2010 (MAGE 3) is in phase I/II in advanced melanoma

— continued on next page

Cytogen/Sanofi	OncoRad GI103	MAb conjugated to ^{90}Y	Phase I/USA, France/GI cancer	
Debiopharm	Vapreotide/ BMY-41606; RC-160/ Octastatin	Octapeptide analog/ somatostatin analog/ epidermal growth hormone antagonist/SC	Prereg/Europe/ pancreatic exocrine solid tumors	Patent rights obtained in 1986 from Tulane U (Professor AV Schally)
Debiopharm/ Roger Bellon (Rhône-Poulenc Rorer), Sanofi	Oxaliplatin/1670RB; RP 54780/L-OHP	Trans ammine (cyclohexylamine) dichlorodihydroxo-platinum/IV	Phase III/Japan/ colorectal and stomach cancer	Administered before and after gastrectomy (see FO, V1 #1, pp. 17 & 19)
Du Pont Merck	DMP-840; XE-840	Bisnaphthalamide/binds with high affinity to DNA causing single strand breaks/ 24-hour infusion	Phase I/USA/ solid tumors	MDT is projected at 60 mg/m2 (O'Reilly S, etal, ASCO95, Abs. 1529)
Eisai	E-7010	Sulfonamide	Phase I/Japan/ colon cancer	
Eli Lilly	Gemcitabine/ LY-188011	Difluoronucleoside agent/DNA synthesis inhibitor/IV	Treatment IND (3/95)/ USA/pancreatic cancer; prereg/Spain/ pancreatic cancer	In a phase II trial of advanced gastric cancer, a regimen of 800 mg/m2, for 3 consecutive weeks, followed by a 1-week rest period had no effect (Christman K, etal,: Cancer, 1994 Jan 1, 73(1):5-7)
Eli Lilly	Sulofenur/LY-295501 and LY-186641	Diarylsulfonylurea (DSU)	Preclin (93)/USA/colon and pancreatic cancer	
Eli Lilly	LY-231514	Antifolate/thymidylate synthase inhibitor	Phase I/USA/solid tumors	
Enzon/Rhône-Poulenc Rorer (NA licensee); Research Corporation Technology (licensor)	Pegaspargase; asparaginase/ Oncaspar; Oncospar	Asparaginase stimulant/ pegylated L-asparaginase	Phase I/USA/ pancreatic cancer	Approved (2/94)/USA/ acute lymphocytic leukemia (ALL)
Enzon/NCI	Single-chain antigen-binding (SCA) protein technology/cc49	Drug delivery	IND (10/84)/USA/ colorectal cancer	SCA licensees include Oncologix (Argonex), Lilly, Baxter, Bristol-Myers Squibb, Cell Genesys
Ergo Science/ Rowland Institute		Photodynamic therapy	Research/USA/ pancreatic cancer	Integration of PDT with the company's "neuroendocrine resetting therapy" may enhance its effects
Fuji Photo Film	FJ-776	Rhodacyanine dyes/IP	Phase I/Japan/ pancreatic and colon cancer	
Genetics Institute/ Wyeth-Ayerst (ww except Japan; Yamanouchi in Japan)	Recombinant interleukin-12/rhIL-12	Enhances the immune system's killing ability and may trigger production of other immune system regulatory proteins that may initiate an adaptive immune response	Phase I/II (suspended 5/95 due to deaths)/ USA/colon cancer	In preclinical models of a variety of cancers, rhIL-12 either caused tumors to shrink or entirely eliminated them
Genta	Antisense targeted against bcl-2	Apoptosis enhancing	Phase I/II (1/95)/UK/ follicular lymphoma (also shown effective in colon cancer)	UK authorization under physician INDs at the Royal Marsden Hospital (Sutton, Surrey, UK); also see FO, V1 #1 pp. 26-27)
Glaxo	GG-211 (GI-1447211A; GI-147211C)	Topoisomerase I inhibitor	Phase II/UK/ colon cancer	
Hoffmann-La Roche	Doxifluridine	5-FU prodrug	Preclin/USA/ stomach, colon cancer	

— continued on next page

Hoffmann-La Roche	Galocitabine/ Ro-09-1390	Prodrug of doxifluridine	Preclin/USA/ stomach, colon cancer	
Hygeia (Novopharm Biotech)	SK-1/ Monopharm-C	Humanized MAb/ sialoglycoprotein antigen/ MAb-secreting human B-lymphocyte and human cell fusion partner (SHFP-1) hybrid producing SK-1	Phase I/Canada/ colorectal and pancreatic cancer	
ICN/Nucleic Acid Research	Sulfinosine/ sulfasine	DNA synthesis inhibitor/ racemic mixture of R and S enantiomers exhibit highest activity/SC, IP, PO	Preclin/ USA/ colon cancer	
ImClone Systems (ww licensee)/ Cancer Research Campaign, U Nottingham	Human anti-idiotypic MAb (105AD7) targeting tumor antigen gp72	Vaccine (prophylactic and therapeutic)/ induces antitumor cellular responses	Phase I/UK/ metastatic colon cancer	
ImClone Systems/ U California (licensor)	C225	Anti-EGFr MAb	Phase I completed 3/95/USA/solid tumors (may be useful in colorectal cancer alone or in combination)	Also obtained license (6/94) form Rhône-Poulenc Rorer for use of anti-EGFr MAbs in combination with marketed chemo-therapeutics
Immune Response/ San Diego Regional Cancer Center	Tumor vaccine	Irradiated fibroblasts genetically modified to express IL-2	Phase I (6/95))/USA/ colon cancer	Clinical protocol approved by RAC
Immunomedics	ImmuRAIT-CEA	Humanized CEA MAb hMN-14, linked to ^{90}Y or ^{138}Re or ^{131}I	Preclin/USA, Canada/ colorectal cancer	
Immunomedics	Anti-idiotypic MAb to CEA	Vaccine	Preclin/USA/ colorectal cancer	
Immunotech	AES-CEA	CEA-based immunotherapy	Preclin/Europe/ colorectal cancer	In phase III for radio-immunoscintigraphy
ImmunoTherapeutics	ImmTher	Immunomodulator/ lipophilic disaccharide peptides related to muramyl dipeptide/- liposome encapsulated	Phase II/USA/ metastatic colon cancer; adjuvant therapy in resected colon cancer (orphan drug)	
ImmunoTherapeutics	Theramide	Vaccine/induces cellular immunity	Phase I/USA/ solid tumors	Long-acting analog of ImmTher
Imutec	Virulizin	Bovine reticuloendothelium/ tumor necrosis factor α (TNF α) agonist	Phase III/Canada/ pancreatic cancer	Fast tract status; launch due 1996
Inflazyme/BioChem Therapeutic	Fitofosine	Phosphonate/inhibits signal transduction pathways involved in cancer cell growth and differentiation	Research/Canada/ colorectal cancer	
Introgen/Rhône-Poulenc Rorer; U Texas M.D. Anderson Cancer Center	(Also see FO, V1 #1, pp. 26 & 30)	Retroviral delivery of intact wild-type p53 gene	Preclin/USA/ colorectal cancer	
Japan Energy	Arinase deiminase/ CX-108	DNA synthesis inhibitor	Preclin/Japan/ colon carcinoma	
Jenner Technologies	Vaccine	Whole recombinant tumor associated or tumor specific antigens and an adjuvant packaged in liposomes/elicits CTLs	Preclin/USA/ colorectal cancer	
Kirin Brewery	KRN-5500; SPK-241	Spicamycin derivative/ DNA synthesis inhibitor	Preclin/Japan/ colon cancer	

— continued on next page

The Liposome Company/Pfizer, U British Columbia, and McGill U (Canada)	Doxorubicin/ TLC D-99; TLC-DOX99	RNA synthesis inhibitor	Phase II/USA/ metastatic liver cancer	Liposome formulation
Matrix Pharmaceutical	MPI-5010/ Intradose-CDDP	Biodegradable gel matrix containing cisplatin/ DNA antagonist	Phase II/USA/ liver cancer	(See FO, V1 #1, pp. 20-21)
Matrix Pharmaceutical	MPI-5011; 5-FU/ Intradose-FU injection	Thymidylate synthase inhibitor	Phase II/USA/liver cancer; phase III (due 1995)/USA/liver cancer	
Medac	Titanocene dichloride/MKT4	Organometal	Phase I/Germany/ solid tumors	Scheulen ME, etal, ASCO95, Abs. 1501; Berdel WE, etal, ASCO95, Abs. 1512
Medarex/NCI (clinical trials)	MDX-210	Bispecific MAb/stimulates immune response in cancers that overexpress HER-2	ND filed (11/94)/USA/ colon, gastric and pancreatic cancer	Phase I/II clinical trials in patients who failed multiple chemotherapy regimens completed 5/94; phase II initiated in breast and ovarian cancer
Medco Research	Adenosine triphosphate	Adenosine 5'-(tetrahydrogen triphosphate)/ Adenosine agonist	Preclin/USA/ advanced prostate and colorectal cancers; phase I/USA/ colon cancer	
MicroGenSys	Recombinant CEA vaccine	Vaccine	IND (95)/USA/ colon cancer	
EORTC/Kyowa Hakko; New Chemical Entities; U Amsterdam Department of Chemistry/NCI	Indoloquinones;/ EO9; EO1; EO4; EO68; EO70; EO72; NSC-382456	Mitomycin-C analog/ DNA antagonist	Phase I/USA, Europe, Japan/colon, pancreas, stomach cancer	
NCI (NIH)/ Pharmachemie	Decitabine; dezocitidine/ NSC-127716	DNA synthesis inhibitor/IV	Phase II/USA, Europe/ colon cancer	Major side-effects in patients with solid tumors include myelosuppression, nausea, vomiting, diarrhea, phlebitis
NeoRx	Avicidin	MAb-streptavidin conjugate; binds to markers on tumor cell surface; biotin-^{90}Y complex binds to streptavidin and kills tumor cells	Preclin/USA/colon	
NeXtar	Daunorubicin/ VS-103/ DaunoXome	RNA synthesis inhibitor/ liposome formulation	Phase I/II/USA/ colorectal cancer	NDA filed for Kaposi's sarcoma
NIH/Lilly	NSC-646958; NSC-646959; NSC-646960	Furocoumarinsulfonamide/ protein kinase C inhibitor	Preclin/USA/ colon cancer	
Nippon Roche Research Center	Mofarotene/ Ro 40-8757	Arotinoid	Preclin/Japan/HCC	
OncoTherapeutics/ Hoffmann-La Roche (ww licensee)	OncoLipin-2 (formerly OTx-287)	Proteo-liposome encapsulated formulation of IL-2	Preclinical (95)/USA/ colon cancer metastasized to the liver	Orphan drug for kidney and renal pelvis cancer and brain and CNS tumors
PerImmune (Organon)	Vaccine	Immunization using host tumor cells and BCG	Phase II/USA/ colorectal cancer	
Pharmacyclics/ U Texas	Gadolinium-texaphyrin/Gd-Tex	Radical formation agonist	Preclin/USA/ radiation therapy	Also liver tumor imaging
PharmaGenics/ Boehringer Mannheim Therapeutics; Xenova		Agents to restore normal function of p53	Research/UK	Collaborative agreement (3/95) with BMT to screen its compounds

— continued on next page

PharmaMar/NIH	PM-92100; PM-92102; PM-92104; PM-92105; PM-92114; PM-93107	Natural products/derived from marine life	Preclin/USA/ colon cancer	
Quadra Logic Technologies/Lederle Japan (American Cyanamid/Takeda)	Portimer sodium; dihematoporphyrin ether/CL-184116/ Photofrin	Photodynamic therapy	Recommended (9/94) (orphan drug)/USA/ refractory esophageal cancer; launched (4/95)/ Japan/ esophageal and gastric cancer; registered/ Netherlands/esophageal and gastric cancer	
Repligen/ Centocor; New York U	Recombinant platelet factor 4 (rPF4)	Factor IV agonist	Phase I/II/USA/ colon cancer	
Rhône-Poulenc Rorer/Chugai; NIH	Docetaxel; taxotere/ NSC-628503; RP-56976	Microtubule stimulant/ tubulin polymerization/IV	Phase II/France/ colon cancer	
Rotta Research/ Kaken Pharmaceutical; Tokyo Tanabe	Loxiglumide/ CR-1505/	CCK antagonist/IV	Prereg/Europe, Japan/ pancreatic cancer	
Sandoz/Sankyo; Zuellig; Italfarmaco	Octreotide/ SMS-201-995/ Longastatina; Sandostatin	Somatostatin analog/ growth hormone antagonist	Preclin/Europe/ liver metastases	
Sankyo/Fuji Chemical Industries	FO-152	Thymidylate synthase inhibitor	Phase II/Japan/stomach adenocarcinoma	
Sankyo	Palmitoylrhizoxin/ RS-1541	Rhizoxin derivative/ inhibitor of tubulin polymerization	Preclin/Japan/ stomach and colon carcinoma	
Scotia Pharmaceuticals (Efamol)	Synthetic chlorins, mTHPC, synthetic porphyrins; mesotetrahydroxyphenylchlorin/EF-9	Mesotetrahydroxyphenylchlorin/ photosensitizer	Phase I/UK/ esophageal cancer	
Scotia Pharmaceuticals (Efamol)/St. Bartholomew's Hospital	EF-13	Lithium gammalinolenate/ prostaglandin synthase stimulant/IV, PO	Prereg/UK, Denmark, Ireland/pancreatic cancer; phase II/USA, Europe/colorectal cancer	Marketing will be aimed in Europe and, possibly, the USA
Sequus (formerly Liposome Technology)	DOX-SL/Stealth	Liposomal formulation of doxorubicin	Phase II/USA/ liver cancer	Recommended (2/95) for accelerated approval for AIDS-related Kaposi's sarcoma
Seragen/Lilly (option)	EGF fusion toxin/ $DAB_{389}EGF$	Epidermal growth factor conjugate/targets EGF receptor on tumor cells	Phase I/II/USA (94)/ colon and pancreatic cancer	
Shumeido	LAC-83	Cyclic oligomers of lactic acid	Preclin/Japan/ liver cancer	No serious toxicity in animal models
SmithKline Beecham	Topotecan		Phase II/USA/ esophageal cancer	SWOG-9339 (5/95)
Somatix Therapy	GVAX	*Ex vivo* transfection of a patient's (autologous) tumor cells with the gene encoding GM-CSF	Preclin/USA/ colon cancer	In clinical trials in kidney cancer and melanoma; trials are planned in prostate cancer
Sparta Pharmaceuticals	5-FP (5-FU prodrug)	5-fluoro-2-pyrimidone/ thymidylate synthase inhibitor/PO	Preclin/USA/ liver tumors	
Sparta Pharmaceuticals	N-4-(amino-4-deoxypteroyl)-N-hemiphthaloyl-L-ornithine; PT-523	Folate antagonist	Preclin/USA/ colorectal cancer	More lipid soluble than MTX to cross cell membranes
Sparta Pharmaceuticals	IPdR prodrug of IUdR	Radiosensitizer	Preclin/USA/ liver cancer	

— continued on next page

StressGen Biotechnologies	Oncocine vaccines	Vaccine technology combines stress proteins with tumor antigens	Research/Canada/ colorectal cancer	
Sunkyong Industries	SKI 2053R	Alkylating agent/ platinum-based drug	Phase I (completed late 94)/S. Korea/solid tumors; phase II trials are planned in stomach cancer	Stable disease was seen in 3 colon cancer patients at 480 mg/m2 one-hour infusion every 4 weeks; side effects were hepatotoxicity and myelosuppression
SunPharm/Warner-Lambert (exclusive ww licensee, except Japan); Nippon Kayaku (licensee, Japan)	Diethylnorsperimine (DENSPM)	Polyamine analog/natural polyamine inhibitors	Phase I/USA/ pancreatic cancer	
Taiho/Miguel Labs (Otsuka)	Tegafur + uracil	Thymidylate synthase inhibitor/chemosensitizer/ inhibits 5-FU degradation so higher levels remain in tumor cells/oral	Phase III/USA/biliary tract, colon, gallbladder, liver, pancreas and stomach tumors; launched/Japan/solid tumors	Dose of 600 mg/day resulted in response rate of 30%; side-effects were anorexia and stomatitis
Therion Biologics/ NCI (co-developer)	Cancer vaccine/ TBC-CEA	Live recombinant pox virus vaccine	Phase I/II/USA/ colorectal cancer	The first CEA-based recombinant cancer vaccine to be tested in the USA
U Alabama/Agracetus		Polynucleotide vaccine containing cDNA for CEA/IM	Received RAC approval (6/95) to initiate phase I clinical trials/USA/ metastatic colorectal cancer	
Upjohn/Yakult Honsha (Japanese co-development and co-marketing rights)	Adozelesin; adezolin/ U-73975	Highly potent alkylating agent/synthetic analog of the antitumor antibiotic CC-1065/undergoes binding in the minor groove of double-stranded DNA (ds-DNA) at A-T-rich sequences followed by covalent bonding with N-3 of adenine in preferred sequences	Phase I/Japan; phase II/ USA/solid tumors	When given as a 24-hour continuous IV infusion, prolonged thrombocyto-penia and granulocyto-penia were dose limiting; no anti-tumor responses were observed; recom-mended dose is 100 mg/m2, every 6 weeks (Fleming GF, etal, Journal of the NCI, 1994 Mar 2, 86(5):368-72)
Upjohn/ NIH	Bizelesin/ NSC-615291; U-77779	Potent, bifunctional analog of the cyclopropylpyrrolo-indole antitumor antibiotics CC-1065 and adozelesin/ binds to and alkylates DNA at the N-3 position of adenine in a sequence-selective manner	Preclin/USA/ colon cancer	
U.S. Bioscience/ Schering Plough; SmithKline Beecham; Parke-Davis (Warner-Lambert); NCI (NIH)	N-phosphonoacetyl-L-aspartate (PALA); sparfosate sodium/ CI-882; NSC-224131	Nucleotide analog/aspartate carbamoyltransferase inhibitor	Phase III/USA/colon cancer; phase I/II/USA, Europe/colorectal and pancreatic cancer	Enhances activity of 5-FU and FU-related drugs (tumor response rate was increased from 20% to 40%)
U.S. Bioscience/ Parke-Davis (Warner-Lambert; licensor)/Schering (Latin America and Asia; licensee)	Trimetrexate/ CI-898; JB-11; NSC-249008; NSC-328564; NSC-352122/ NeuTrexin	Lipid soluble analog of MTX/dihydrofolate reductase inhibitor/IV (oral and topical formulations in research)	Phase II/USA/ metastatic colorectal, gastric and pancreatic cancer	Approved 12/93 USA and 9/94 Europe; launched 1/94 USA as alternative therapy for Pneumocystis carinii; orphan drug; USA patent expires 10/2000

— continued on next page

Vical	Allovectin-7	Gene therapy/gene encodes a mismatched transplantation antigen (HLA-B7)/intratumoral injection	Phase I/II/USA/ colon cancer	
Vical	Leuvectin	Gene therapy/IL-2 gene in a plasmid-lipid complex/ intratumoral injection	Phase I/II (4/95)/USA/ solid tumors and lymphomas	
Wellcome	5-ethynyluracil (5-EU/776C85)	Chemomodulator/potent dehydrogenase inhibitor (the enzyme that rapidly degrades 5-FU)/IP, SC, IV, PO	Preclin/UK/ advanced colon carcinoma	
Wellcome/Burroughs Wellcome	BW-1843U89	Folate analog/thymidylate synthase inhibitor	Phase I/USA/solid tumors	
Wyeth-Ayerst (AHP)	Sirolimus; rapamycin/ AY-22989; NSC-226080; NSC-606698/ Rapamune	Triene cytostatic antibiotic	Phase I/USA/ colon cancer	
Yakult Honsha/ Daiichi Pharmaceutical; Prodesfarma (Spain); Rhône-Poulenc Rorer (Europe); Upjohn (USA)	Irinotecan/CPT-11; DQ-2805; SN-38/ Campto and Topotecin (Japan)	Semisynthetic analog of camptothecin/DNA topoisomerase I inhibitor	L94/Japan; PLA (1/95)/ France; phase II/III/USA, Canada; approval sought in Spain (2/95)/colorectal cancer; phase II/Japan/ stomach and pancreatic cancer	Also see FO, V1 #1, pp. 15 & 16)
Yamanouchi/ Kayaku/Kuraray	Zinostatin stimalamer, SMA/YM-16881; YM-881/SMANCS	Enedyine; neocarzinostatin/IA	L94/Japan/liver cancer (also active against colorectal and gastric cancer)	Also see FO, V1 #1, p. 29 & 31
Zeneca/BTG	ZD-1694; ICI-D-1694/ Tomudex	Thymidylate synthase inhibitor/IV	Phase III/ USA, UK/ advanced colorectal cancer; phase II/USA/ pancreatic cancer	Phase III trials in combination with LV completed in Europe

Source: New Medicine and Report #401, Cancer Vaccines-Technology, Products, Developers and Market Opportunities Worldwide

PDT (Santa Barbara, CA), signed an exclusive licensing agreement in July 1995 with Pharmacia SpA (Milan Italy) for its dye, tin ethyl etiopurpurin (SnET2), which is in phase II clinical trials in cutaneous carcinoma and AIDS-related Kaposi's sarcoma. The first stage of funding will be approximately $25 million, including an equity investment (400,000 shares of PDT for $12 million); the potential value of the collaboration, excluding royalties, is $100 million if certain milestones are met.

MEETING COVERAGE

RADIOIMMUNOSCINTIGRAPHY IN CANCER DIAGNOSIS

A REPORT FROM THE TENTH INTERNATIONAL CONFERENCE ON MONOCLONAL ANTIBODY IMMUNOCONJUGATES FOR CANCER, 9-11 MARCH 1995; SAN DIEGO, CA

- Imaging radioimmunoconjugates such as radiolabeled monoclonal antibodies (MAbs), used to detect cancer *in vivo* by homing on cells bearing the appropriate antigenic target, have yet to find a niche in the management of cancer.
- This article reviews latest research and results of clinical trials using immunoconjugates for cancer detection, presented at the Tenth International Conference on Monoclonal Antibody Immunoconjugates for Cancer (9-11 March 1995; San Diego, CA), sponsored by the San Diego Regional Cancer Center, a Sharp HealthCare affiliate.

Radioimmunoconjugates for the *in vivo* detection/ imaging of cancer are constructed by coupling MAbs to radioactive isotopes (or possibly contrast-enhancing agents). In immunodiagnosis, the main objective with regard to malignancies is the detection of (small) solid tumors as well as micrometastases by a rapid, noninvasive method that normally does not require hospitalization. Although attempts to attach other compounds (e.g., MRI, ultrasound, or x-ray contrast agents) to MAbs have been undertaken, so far only radioimmunoconjugates have achieved clinical relevance. Clearly, immunodiagnostic methods must compete with traditional imaging techniques, such as ultrasound, x-ray, CT, and MRI. Also, some cancers, such as disseminated leukemias and lymphomas, are better detected by serological means. Nonetheless, radiolabeled MAbs may have a role in locating

tumors of small size not detectable by other techniques. Because of its complexity, requirement for IV delivery and need for highly trained staff, radioimmunoscintigraphy is expected to be used primarily in cases where there is a reasonable suspicion of malignancy that may have eluded other simpler and less expensive diagnostic procedures. In addition, radioimmunoscintigraphy may allow tumor staging and/or localization before treatment.

Despite potential advantages, the use of radiolabeled MAbs in clinical diagnosis has progressed slowly. Radioimmunoconjugates only recently gained some usefulness and acceptability in the diagnosis of cancer. A number of MAb-based cancer imaging agents have been developed and tested clinically, and several are awaiting market approval. Cytogen has already received FDA approval for OncoScint CR/OV, an ^{111}I-labeled MAb (B72.3), for the *in vivo* imaging of colorectal and ovarian cancers (see page 39 for more details). Newer and potentially improved immunoconjugate imaging agents are also in development (see Exhibit 4).

CHALLENGES FACING MAB-BASED RADIOSCINTIGRAPHY

During a workshop chaired by Hani A. Nabi of the State University of New York (Buffalo, NY), various hurdles were described that are still hampering development and utilization of radiolabeled MAbs for *in vivo* cancer detection. The detection of pathology using radioimmunoconjugates is dependent upon both the *in vivo* biodistribution of the immunoradiopharmaceutical and the physical characteristics of the radionuclide and imaging system. The main criticism of immunoscintigraphy with existing MAbs has been the relatively small differences in uptake between lesions and surrounding tissues. Although this is partly related to the relative antigen expression between normal and abnormal sites, it is also related to the choice of radionuclide and the efficiency of photon detection during the imaging procedure. Practical considerations in the application of MAbs for imaging (and radiotherapy) include, suitability of the isotope for imaging (or radiotherapy); availability of the radionuclide; labeling chemistry that meets the desirable technical performance characteristics of stable linkage to the MAb, clinically convenient procedure, and favorable clearance properties; and costs.

Radioisotope Choice

Three radionuclides, ^{99m}Tc, ^{123}I, and ^{111}In, have met the above criteria as practical isotopes for imaging with MAbs. A comparison of the practical considerations for these isotopes is given in Exhibit 5. For imaging applications, ^{99m}Tc is viewed by many as the isotope of choice, because of lower cost, availability, and favorable radiation characteristics. Primary drawbacks associated with its clinical use include a somewhat complex labeling process and a short half-life (six hours); the latter poses a particular problem when whole antibody conjugates are used that require at least 18 hours of imaging time post administration for optimal results. Regional administration or conjugation to MAb fragments is used to circumvent this problem. ^{99m}Tc is available commercially from a ^{99}Tc/^{99}Mo generator in the form of aqueous sodium [^{99m}Tc] pertechnetate. Over the past several years, major advances have been made in providing fast, convenient chemistry to label antibody fragments with ^{99m}Tc.

Development of MAbs for *in vivo* Imaging Applications

A number of MAbs against tumor-associated markers have shown sufficient tumor targeting to be potentially useful agents for imaging. Of these, anti-CEA MAbs have been the most commonly used from the very beginning of radioimmunoscintigraphy. Like CEA, most cancer associated antigens appear to be quantitatively increased in malignancy; MAbs against such antigens frequently react with a wide range of carcinomas. It is indeed rare to find a MAbs that is specifically reactive with a single organ or tissue type, whether normal or neoplastic. Although many target antigens are present, albeit in reduced quantities, in many normal tissues, a preferential localization to tumor is nonetheless achieved because the injected MAbs do not usually have access to normal structures, such as to mucosal epithelium when the basement membrane is intact. However, when tumors invade and gain access to neighboring structures and blood vessels, a new and different vasculature and permeability appear to allow greater access to injected macromolecules. This increased accretion of macromolecules in tumors, including immunoglobulins, has been recognized for over five decades.

Targeting growth factors or oncogenes. With regard to target markers, an intriguing prospect is the use of MAbs against growth factor or oncogene products for imaging (MA Bakir, etal, J Nucl Med, [1992] 33:2154; K De Santes, etal, Cancer Res, [1992] 52:1916). This has an advantage over tumor-associated differentiation antigens, which are the tumor markers most commonly used so far, since growth factor and, especially, oncogene products may not only be expressed to a greater degree in neoplasms than in normal tissue, as are tumor-associated differentiation antigens, but may be more intimately associated with the neoplastic state. In addition, overexpression of certain oncogenes, such as c-erb B2, correlate with a decreased sensitivity to treatment with chemotherapeutic drugs, suggesting that imaging of tumors using MAbs directed against products of these oncogenes may be informative with respect to how aggressive the therapy should be, thereby offering an advantage over imaging with MAbs to antigens whose expression does not correlate with prognosis.

MAb fragments. Also of importance in gaining access to the tumor is the nature of the MAb. For instance, it has been determined that bivalent F(ab')$_2$,

Exhibit 4
Immunoconjugates in Cancer Diagnosis

Developer/ Affiliate	Generic Name/ Number/Brand Name	Drug Type/ Target/ Mechanism	Status/Location/ Indication	Comments
Biomira	Tru-scint-SQ	Tc- 99m-labeled MAb	Phase II/ USA, Canada, Germany/ soft tissue sarcoma of the head & neck, lung and cervical cancer	
Biomira	Tru-Scint-AD	Tc- 99m-labeled MAb	Phase III/USA, Europe/ adenocarcinoma of breast, ovary, colon and rectum	IND submitted in the USA for breast cancer
Cytogen	Capromab pendetide/ ProstaScint (Oncoscint PR356; CYT-356; 7E11-C5. 3-GYK-DTPA)	MAb 7E11-C5.3 binds to an epitope on prostate specific membrane antigen (PSMA), conjugated to ^{111}I	PLA (1/95)/USA/ prostate cancer	
Cytogen	Satumomab pendetide/ OncoScint CR/OV	B72.3 MAb conjugated to ^{111}I	Approved (12/92)/USA/ colorectal and ovarian cancer (orphan drug); prereg/Europe/colorectal and ovarian cancer	
GEA Farmaceutisk Fabrik	COU-1	Human IgM MAb conjugated to ^{131}I	Phase I/Denmark/ colorectal cancer	Will enter phase II trials in late 1995
Immunomedics/ Pharmacia	CEA-Scan (was ImmuRAID-CEA)	Tc-99m-labeled Fab' fragment of murine anti-CEA IMMU-4	Phase III/USA/ lung cancer	
Immunomedics/ Pharmacia	CEA-Scan (was ImmuRAID-CEA)	Tc-99m-labeled Fab' fragment of murine anti-CEA IMMU-4	PLA/USA, Europe, Canada/colorectal cancer	
Immunomedics/ Pharmacia	CEA-Scan (was ImmuRAID-CEA)	Tc-99m-labeled Fab' fragment of murine anti-CEA IMMU-4	Phase II/USA/ breast cancer	
Immunomedics	AFP-Scan (was ImmuRAID-AFP)	Tc-99m-labeled anti-alpha-fetoprotein (AFP)	Phase II/USA/testicular and liver cancer	Pharmacia agreement terminated (6/94); orphan drug in the USA
Immunomedics	LymphoScan (was ImmuRAID-LL2)	Tc-99m-labeled LL2	Phase III (orphan drug)/ USA/non-Hodgkin's B cell lymphomas; leukemia	
Immunotech	AES-CEA	Unlabeled bispecific anti-CEA Fab' fragment chemically coupled to an anti-DTPA-indium fragment	Phase III (6/95)/Europe (France and Germany)/ colorectal cancer	Pretargeting; injection of unlabeled MAb is followed by injection of the bivalent DTPA hapten labeled with ^{111}In
Immunomedics/Center for Molecular Medicine and Immunology	ImmuRAID-LL1	Tc-99m-labeled LL1	Preclin/USA/ Hodgkin's Disease	
ICRT/Sanofi	PR1A3	Immunoconjugate/MAb	Phase I/UK/imaging of colorectal cancer	

Source: New Medicine, excerpted from Report #202, entitled, The USA Market for Radiopharmaceuticals

and monovalent Fab' and Fab fragments are best for imaging because they penetrate tumor better than whole immunoglobulin, demonstrating more rapid targeting and higher tumor/non-tumor localization ratios (e.g., higher tumor/background ratios) at earlier times than for whole MAbs. In addition, MAb fragments appear to be the least immunogenic, further supporting their suitability as imaging agents.

Exhibit 5
Comparisons of Characteristics for Practical Radioimmunoscintigraphy Isotopes

Characteristic	^{123}I	^{99}mTc	^{111}In
Physical half-life	13 hours	6 hours	67.5 hours
Gamma-emission, keV (%)	159 (84)	140 (90)	142 (90) 247 (94)
Availability	Cyclotron	Generator	Cyclotron
Linkage to antibody	Covalent	Chelate	Chelate
Instant kit	No	Yes	Yes
Organ retention	Thyroid	Kidney, liver	Kidney, liver
Relative cost	High	Low	High
Other potential barriers	Isotope contamination (^{124}I, ^{125}I, ^{126}I)	Labeling chemistry	Trace metal contamination; stabilization of ^{111}In (III) at labeling pHs

Source: New Medicine, excerpted from Report #202, entitled, The USA Market for Radiopharmaceuticals

MAb dosing requirements. The subject of immunoconjugate dose is complex and sometimes confusing, primarily because the effects are interpreted in terms of radionuclide uptake in tumor and other organs, which may not in fact always reflect the antibody's biodistribution in contrast to that of the radionuclide. In radioimmunoscintigraphy the absolute accretion of MAb in tumor is less relevant than the ratio of tumor to background signal. Animal and clinical studies both indicate that, at least in the case of radioiodine labels, no advantage is achieved with higher doses of MAb.

SPECT imaging. Low-energy radionuclides such as ^{99m}Tc permit use of single-photon emission computed tomography (SPECT) that produces improved image contrast as compared to planar scanning methods. However, an increase in the rate of false-positives may occur because of reconstruction artifacts associated with SPECT. Therefore, combined use of SPECT and CT imaging for comparison has attracted interest as a means of increasing the level of confidence in the interpretation of results by either imaging modality. At present, tumors as small as 0.5 cm (although usually in the range of 1.0 to 2.0 cm) can be imaged within several hours of immunoconjugate injection by using ^{99m}Tc-labeled MAb fragments and SPECT.

SELECTED DIAGNOSTIC APPLICATIONS

Clinical indications for radioimmunoscintigraphy include pre-surgical staging of extent of disease; post-surgical follow-up to disclose residual disease or recurrence; disclosure of site of recurrence in patients with rising tumor marker titers; confirmation of a viable tumor lesion revealed by CT scan or other static radiological procedures; and evaluation of suitability of patients for radioimmunotherapy with the same MAb, i.e., confirmation of tumor targeting by antibody. It is noteworthy that many studies have shown that occult tumors can be revealed by radioimmunoscintigraphy, and the disclosure of occult tumors as well as the confirmation of neoplastic sites revealed by other, more anatomic radiologic methods currently appear to be the most established indications. However, evidence in support of other applications is increasing, especially for the disclosure of disease activity and site of tumor recurrence in cancer patients with rising tumor marker titer but no confirmed lesion. David M. Goldenberg, Chairman and CEO of Immunomedics and President of the Garden State Cancer Center at the Center for Molecular Medicine and Immunology (Newark, NJ), expressed the opinion that radioimmunoscintigraphic studies have already contributed to better patient management in 25% to 50% of cancer patients in whom the procedure has been performed.

Colorectal Cancer

David Colcher and his associates at the University of Nebraska Medical Center (Omaha, NE), together with researchers at the National Cancer Institute (Bethesda, MD), are investigating ^{111}In and ^{90}Y-labeled MAbs that bind to TAG-72 (a high molecular weight tumor-associated glycoprotein with the properties of a mucin, expressed in approximately 90% of colorectal carcinomas), for imaging and therapy studies. TAG-72 antigen has been shown to be secreted by tumor cells and can be found in the circulation of some patients with colorectal cancer. In earlier work using ^{131}I-labeled anti-TAG-72 MAb B72.3, Colcher and associates correctly detected about 52% of tumor deposits. In the work reported here, the researchers used CC49, a "second generation" murine IgG MAb to TAG-72. CC49 was developed by immunizing mice with TAG-72 purified by B72.3 antibody affinity chromatography. Both B72.3 and CC49 have been shown to react with repeated carbohydrate moieties on the TAG-72 molecule; however, CC49 has been shown to have a 6.4-fold higher affinity than B72.3, and reacts to a greater percentage of tumor cells within tumor masses than B72.3.

A macrocyclic bifunctional chelating agent, PA-DOTA, was used to label CC49 with both ^{111}In for imag-

ing studies, and ^{90}Y for therapy. [PA-DOTA is a peptide-linked DOTA derivative, DOTA-Gly_3-L-(p-isothiocyanato)-Phe-amide, which forms neutral complexes with trivalent radiometals.] This agent offers the advantage that other species in the chelation reaction mixture, such as excess chelating agent, complexes containing divalent metals, and DTPA complexes, are negatively charged, and can thus be filtered out quickly using an appropriately designed anion-exchange column. In addition, by introducing a cleavable linker between chelate and MAb, accumulation of radioactivity in the liver can be reduced. Radiometal-labeled MAbs using DOTA-peptide derivatives have demonstrated excellent kinetic stability under physiological conditions, and the radiolabeled CC49 was tested for immunoreactivity, stability and efficacy in both preclinical studies and a phase I clinical trial.

Preclinical studies have shown that radiolabeled MAb CC49, conjugated to ^{111}In and ^{90}Y, is over 85% and 80% immunoreactive, respectively. The radioimmunoconjugates are stable in human serum with over 99% of ^{111}In and 97% of ^{90}Y retained by the immunoconjugates after five days. Both ^{111}In and ^{90}Y-labeled CC49-PA-DOTA were tested in athymic mice bearing human colon carcinoma (LS-174T) xenografts. Over 50% and 22% ID/g were in the tumors four days post-injection with the ^{111}In and ^{90}Y-labeled CC49, respectively. Tumor-to-blood ratios as high as 33:1 and 10:1 and tumor-to-bone ratios of 40:1 and 10:1 were obtained with the ^{111}In and ^{90}Y-labeled CC49, respectively. Clinical studies demonstrated similar blood clearance curves with T1/2α of 4.5 and 3.8 hours and T1/2β of 44.7 and 54.6 hours for the ^{111}In and ^{90}Y-labeled CC49-PA-DOTA (similar to that previously obtained with ^{131}I-labeled CC49 IgG). Bone marrow aspirates and liver biopsies were obtained from selected patients, and from 0.014% to 0.032% ID/g of ^{90}Y-labeled CC49-PA-DOTA was observed in tumor-free liver; studies of the bone marrow aspirates demonstrated that virtually all of the radioactivity (both ^{111}In and ^{90}Y) was associated with the extracellular component. A phase I study, designed to determine the maximum tolerated dose for non-hematopoietic toxicity, is continuing.

Another MAb, PR1A3, which binds to a CEA epitope, was successful in *in vivo* imaging of colorectal cancer in part because of its minimal reaction with circulating antigen in patients' sera. The epitope bound by PR1A3 was at the site of membrane attachment and involved parts of the glycosyl-phosphatidylinositol anchor and the B3 domain of CEA to form a conformational epitope. Access to this epitope, possible when the antigen was on the cell surface, appeared to be blocked when CEA was released from the cell. The nature and location of the epitope on CEA appear to be responsible for the unique properties of PR1A3 (Durbin H, etal, Proceedings of the National Academy of Sciences of the United States of America, 1994 May 10, 91(10):4313-7).

Pancreatic Cancer

At the University of Genoa (Genoa, Italy), the CNR Institute of Clinical Physiology and the University of Pisa Medical School (Pisa, Italy), and the Center for Molecular Medicine and Immunology (Newark, NJ), G. Mariani and colleagues, together with scientists at Sorin Biomedica (Saluggia, Italy), are assessing the pharmacokinetic pattern of distribution and potential usefulness for tumor immunoscintigraphy of two radiolabeled IgG_1 MAbs in patients with pancreatic cancer. MAbs being used are AR-3, which reacts with a mucin-like antigen different from CEA or CA19.9 antigen (but carried by the same mucin carrying the CA19.9, CA125 and BW494 epitopes), and PAM4, which was originally raised against mucin purified from xenografted RIPI human pancreatic carcinoma. These MAbs have been shown immunohistochemically to recognize a large fraction (over 80%) of human pancreatic cancers.

The two MAbs were labeled with ^{131}I, and were each injected into five patients with suspected primary or recurrent pancreatic cancer (confirmed in eight out of the ten patients). Dynamic sequences over the chest and abdomen (0-30 minutes post-injection) and whole-body maps (0.5-1.44 hours post-injection) were recorded by a computerized gamma camera. Nonspecific accumulation for both radiolabeled MAbs in the liver, spleen and bone marrow was found to be extremely low, and attributed only to circulating blood pool activity. The two radiolabeled MAbs also exhibited similar patterns of plasma clearance ($T_{1/2}\alpha$ 4-6 hours, $T_{1/2}\beta$ 40-60 hours). Immunoscintigraphy with ^{131}I-AR-3 was weakly positive (at 24-48 hours post-injection), while ^{131}I-PAM4 allowed primary tumor lesions to be clearly outlined, but only in late scans (48-72 hours post-injection); liver metastases appeared in the early scans with ^{131}I-PAM4 as cold defects within the liver blood pool image, filling in at later time points. In one patient with massive peritoneal metastases the tumor lesions were clearly evident in the earlier scans as well. For both MAbs, immunohistochemistry studies revealed various degrees of antigen expression in all tumor lesions, usually with stronger positivity for PAM4. These results suggest that radiolabeled PAM4 may be useful for the immunoscintigrapic detection and staging of pancreatic cancer; however, some of the imaging parameters would probably be improved by employing faster-clearing fragments of the MAb.

Hodgkin's Lymphoma

Although primary diagnosis of hematologic malignancies, e.g., leukemias and lymphomas, is more easily accomplished by serological means, radioimmunoscintigraphy may still prove to be of clinical value in determining cancer stage or location before treatment in Hodgkin's lymphoma.

At M.D. Anderson Cancer Center (Houston, TX), E.S. Delpassand and associates are evaluating the diagnostic value of ^{111}In-labeled anti-ferritin antibodies in Hodgkin's disease. A total of 69 ^{111}In-anti-ferritin (^{111}In-AF) studies were performed in 39 patients with recurrent end-stage Hodgkin's disease. Radiolabeled antibody was injected

intravenously and whole-body imaging was performed at 2, 20, 40 and 110 hours; SPECT imaging was performed at 40 hours. Pharmacokinetic studies at 2, 20, 40, and 110 hours, using blood and urine samples, were also conducted. Results of ^{111}In-AF imaging were compared with other diagnostic imaging modalities, including CT, MRI, and bone scan). A total of 103 lesions were identified by ^{111}In-AF imaging. Eight of those identified were not detected by other imaging methods; seven of these eight lesions were below the diaphragm. However, seven lesions above the diaphragm were detected by other imaging modalities but were not visualized by ^{111}In-AF scintigraphy. Pharmacokinetic studies revealed rapid and slow blood elimination phases ($T_{1/2}$ five hours and 45 hours, respectively), with an α/β ratio of 1.5; less than 3% of injected activity was detected in the urine in the first 24 hours. Only one of the 39 patients developed HAMA, and no acute side effects were noted. Pharmacokinetics were similar for ^{90}Y-labeled anti-ferritin MAb, which suggests, that ^{111}In-AF may be useful not only in the staging of Hodgkin's lymphoma but as a preliminary diagnostic approach prior to ^{90}Y-AF therapy.

CLINICAL CHALLENGES TO RADIOIMMUNOSCINTIGRAPHY

Since its first clinical use in the early 1980s, immunoscintigraphy has been performed in thousands of patients. Yet, many clinicians are still not very convinced of its efficiency. Reasons for its inconclusive status include an often only moderate target to background ratio, immunogenicity of murine MAbs, and lack of a clear demonstration of true benefit in large series of patients.

Increasing Scintigraphic Contrast

One of the advantages of radioimmunoscintigraphic imaging has been the ability to perform relatively rapid, whole-body scanning. In this regard, it would be desirable to use immunoscintigraphy to detect occult tumor foci throughout the body, but to be efficient, such systematic scanning requires high radioactive uptake by the target and the highest possible ratios between target and normal tissue activity. Unfortunately, these conditions are not usually met in immunoscintigraphic studies. The low target to non-target ratios, usually associated with MAb targeting, is attributed not only to the low absolute amount of antibody accumulating in the target tissue, but also to the relatively long blood pool survival of remaining radiolabeled material. It is unlikely that a significant increase in absolute radioactive uptake can be achieved in tumor or non-tumor targets.

Pretargeting. It is possible to reduce normal tissue background activity quite appreciably by using pre-targeting techniques. In this approach, the antibody is first administered without a radiolabel and, when it has accumulated in target tissue, but blood-pool background levels have dropped, a radiolabeled tracer capable of binding to the antibody is injected. Ideally, the labeled tracer will bind rapidly and efficiently to the pre-targeted MAb, but otherwise be cleared from the blood and catabolized more rapidly than antibody. John M. Reno, Vice President of Research and Development for NeoRx (Seattle, WA), discussed the technique of pretargeting, and in particular the strategy being taken by NeoRx which relies on the high binding affinity of avidin and biotin. Avidin is a protein of about 70,000 dalton molecular weight, found in egg white, and there is a similar material called streptavidin of microbial origin. These proteins react with extraordinarily high affinity with the small molecular size (244 dalton) vitamin biotin (the association constant of this interaction is about 1015 which is four or five orders of magnitude higher than that of antibody-antigen interactions).

With this system, one can inject the MAb conjugated to biotin, allow maximum accumulation in target tissue to take place, and then inject suitably radiolabeled avidin or streptavidin and image some time later. To prevent the labeled avidin from complexing with antibody-biotin still remaining in the circulation, unlabeled avidin can be given some time before the labeled avidin. The complexes formed between excess antibody-biotin and unlabeled avidin would be allowed to clear from the circulation before labeled avidin is given. Alternatively, biotinylated MAb could be given, followed by unlabeled avidin or streptavidin, which would partly localize to the biotin bound to the target lesion and partly facilitate clearance of circulating biotinylated antibody. Administered radiolabeled biotin would then bind to one of the three valences of avidin already bound by its fourth valence to the biotinylated antibody taken up by tumor cells. Conversely, avidin or streptavidin could be conjugated to the antibody pre-targeted to the target lesion, to be followed by an injection of radiolabeled biotin. In this case, excess antibody-avidin conjugate could be cleared from the circulation by giving biotin conjugated to another protein, such as transferrin.

It is the latter approach, originally developed by David Goodman at Stanford University (Palo Alto, CA), which is under investigation at NeoRx. In the company's AVICIDIN technique, antibody-streptavidin conjugate is administered at a dose considered sufficient to saturate the tumor target. At 20 to 28 hours post-administration, a clearing agent is injected which allows conjugate in the circulation to be removed by the liver. Three to eight hours following administration of the clearing agent, radiolabeled biotin is injected (unbound radiolabeled biotin in the circulation is rapidly cleared by the renal system); a methyl group has been added to the biotin to stabilize the radioconjugate against enzyme metabolism of the biotin component and to prevent subsequent separation of the radiolabel. Currently, NeoRx is studying dose optimization for ^{90}Y-labeled pan-carcinoma antibody NR-LU-10, in phase I therapy trials being conducted at the Virginia Mason Hospital (Seattle, WA) and the Stanford University School of Medicine; however, Reno pointed out that this system is also of interest for radioimmunoscintigraphic studies.

At Harvard Medical School (Boston, MA), A.I. Kassis and associates are studying antibody-dependent signal amplification in tumor xenografts following pretreatment with biotinylated MAb and avidin or streptavidin. The researchers have compared the uptake of ^{125}I-labeled avidin or streptavidin in tumor and normal tissues following injection of biotinylated MAb B72.3 in nude mice bearing LS174T subcutaneous tumors. The percentage ID/g of tumor remained constant over the range of injected doses for avidin, whereas that for streptavidin varied. As larger amounts of avidin or streptavidin were injected, the number of moles of each trapped within the tumor increased, with the values for streptavidin being higher, suggesting streptavidin as the preferable "second-step" reagent. At a streptavidin dose of about 2.5 mg/kg, the number of receptors available for targeting by radiolabeled biotin derivatives was approximately 18 times the number of antigen-binding sites accessible for targeting by radiolabeled antibody, with up to 45-fold target-signal amplification achieved.

Scientists at Kyoto University (Kyoto, Japan) and the Gunma University School of Medicine (Gunma, Japan) reported on the "chase" effect of avidin, streptavidin, neutravidin, and avidin-conjugated ferritin, on a radiolabeled antitumor MAb in tumor-bearing nude mice. H. Kobayashi and associates administered a radioiodine-labeled biotinylated MAb (OST7) to athymic mice bearing KT005 human osteogenic sarcomas. After 24 hours, various amounts of a "chase" of avidin, streptavidin, neutravidin or avidin-conjugated ferritin was intravenously injected, and biodistribution of the radiolabeled MAb was determined at two hours post-injection of the chase. Clearance from the blood was found to be dose dependently accelerated by avidin and its effect was l0-fold stronger than that of either neutravidin or avidin-ferritin; streptavidin did not promote clearance of the biotinylated antibody. The chase effect of avidin to the biotinylated antibody is caused by the avidity of avidin for liver cells. Repeating the avidin "chase" improved the biodistribution of radiolabeled biotinylated antibody significantly without decreasing radioactivity in the target tumor.

At Lund University (Lund, Sweden), scientists are using avidin-biotin extracoporeal whole-blood immunoabsorption to improve tumor immunotargeting for radioimmunoscintigraphy and immunotherapy. Michael Garkavij and colleagues conjugated ^{125}I-labeled anti-Le^{Y}Ag chimeric BR96 MAb to biotin and injected it into Norwegian male rats inoculated with syngeneic rat colon carcinomas expressing Le^{Y}Ag. Chimeric BR96, a human IgG$_1$ isotype, exhibits high tumor selectivity for most human carcinomas of the breast, lung, ovary, and gastrointestinal tract. At 12 hours post-injection, extracoporeal whole-blood immunoabsorption was initiated using an absorption column containing avidin covalently linked to Sepharose 6MB macrobeads; about five blood volumes were passed through the absorption column during a two-hour procedure. This approach resulted in a reduction of 48% to 62% of whole body radioactivity and a reduction in plasma activity (%/g) of 85%; target uptake ratios were enhanced in all tumor models by a factor varying from 2.2 to 4.2. No hemolysis, organ edema or other complications were observed.

Enhancing tumor access. Several physiological barriers responsible for the poor localization of immunoconjugates and other macromolecules in tumors have been identified. For instance, heterogeneous blood supply limits delivery of blood-borne molecules to well perfused regions of a tumor. But even when the target is reached, exit from the vascular system may be restricted. To gain access to extravascular sites, such as cancer cell surface antigens, an antibody must pass through the endothelial lining of a capillary or postcapillary venule. The application of certain physical (e.g., radiation, heat) or chemical (e.g., vasoactive drugs, cytokines) agents can lead to an increase in tumor blood flow or vascular permeability. At Loma Linda University School of Medicine (Loma Linda, CA) and Hybritech (San Diego, CA), researchers are studying the localization of ^{111}In-labeled MAbs ZCE025 and CYT-103 directed against secreted (CEA) and cell-bound (TAG-72) antigen, respectively, following pre-irradiation of human colon tumor xenografts. Daila S. Gridley and her associates have previously reported that enhanced antibody uptake can be achieved following a single dose of radiation (Ninth International Conference on Monoclonal Antibody Immunoconjugates for Cancer, 3-5 March 1994, San Diego, CA); in this reported work, Gridley and associates irradiated athymic nude mice bearing either T380 or LS174T human colon tumor xenografts, with a single dose of 10 Gy (0.35 Gy/min) using 60Co as the source of external radiation. The radiolabeled MAbs were injected intraperitoneally two hours post-irradiation, and biodistribution studies were conducted two days later. The ^{111}In-ZCE025 localization in the T380 tumors receiving pre-irradiation was increased by 139%, while no difference was seen between pre-irradiated and non-irradiated animals given ^{111}In-CYT-103 in either colon tumor system. These results suggest that pre-irradiation of solid tumors may not always result in enhanced MAb delivery. Another problem with approaches such as pre-irradiation is that the increase in blood flow or vascular permeability is generally short-lived and usually confined to well vascularized regions of the tumor.

Jeffrey Schlom, Chief of the Laboratory of Tumor Immunology and Biology at the NCI , discussed other methods of potential value in improving MAb-based targeting, with particular focus on the use of recombinant human interferons to augment tumor antigen expression. Recombinant human interferons are capable of altering the antigen phenotype of a variety of human cells (JW Greiner, Cancer Invest, [1986] 4:239), and have been shown to up-regulate some specific tumor-associated antigens such as TAG-72 or CEA. Schlom and his colleagues have demonstrated enhanced targeting of radiolabeled CC49 in human colon cancer xenografts in

murine models when the immunoconjugate is given in combination with IFN-γ. NCI researchers have also enhanced tumor targeting in patients with refractory breast cancer by co-administering a diagnostic dose of ^{131}I-labeled CC49 and recombinant human IFN-α; of 19 known lesions, 17 were imaged.

Reducing Antibody Immunogenicity

Virtually all MAbs used to date for immunoscintigraphy are of murine origin and, therefore, likely to elicit a HAMA response in patients. HAMA prevents repetition of immunoscintigraphy because complexes formed with any murine MAbs in subsequent injections are rapidly eliminated by the reticuloendothelial system. Under these conditions, radiolabeled MAbs cannot reach their tumor target. Another consequence of HAMA is interference with tumor marker assays and risk of false-positive results, complicating patient monitoring. Approaches to curb HAMA include use of short antibody fragments (e.g., Fab or Fv), chimeric antibodies, reshaped or CDR-grafted antibodies and human antibodies.

Human MAbs. Use of human antibodies would overcome many of the problems of immunogenicity. However, as noted by Sherie L. Morrison, a professor of microbiology and molecular genetics at the University of California at Los Angeles Molecular Biologic Institute, the production of wholly human MAbs has proven difficult. Techniques generally involve immortalizing human immune B cells with Epstein-Barr virus followed by fusion with an appropriate murine-myeloma or human-lymphoblastoid cell line. Unfortunately, development of stable antibody-producing human hybridomas has been hindered by a number of technical problems. Human B cells fused to murine myeloma cells are highly unstable and preferentially lose human chromosomes. There is also a paucity of fusion-efficient human myeloma cell lines. Finally, it is usually impossible (and unethical) to immunize humans to obtain antigen-reactive human B cells. Therefore, there is no readily available source of hyperimmunized human cells. Because of these constraints, only a few human MAbs have been available for clinical testing.

Clinical trials with human MAbs in cancer have been reported for four IgM and one IgG monoclonal reagents, four of which have been used as immunoconjugates. The first reported clinical use was a trial conducted at the Jonsson Comprehensive Cancer Center of the University of California at Los Angeles, in collaboration with the Bristol-Myers Squibb Pharmaceutical Research Institute (Seattle, WA). Eight patients with cutaneous metastatic melanoma nodules were intralesionally injected with a naked human IgMMAb, L72, directed against the GD2 ganglioside. Total dose administered ranged from three to 15 mg divided into two to four hour treatments. Five patients demonstrated either a mild or strong antibody response during the course of treatment based on a passive sheep erythrocyte agglutination assay. This work has progressed to phase II trials involving intralesional administration of human MAbs directed against GD2, GD3, and GM2 gangliosides, with 11 CRs and seven PRs reported in one series of 28 melanoma patients.

Researchers at PerImmune (was Organon Teknika Biotechnology Research Institute; Rockville, MD) have conducted phase I studies with two human IgM MAbs, 16.88 and 28A32, directed against colon cancer-associated antigens. These antibodies have been labeled with ^{131}I for the *in vivo* radioimmunodetection and radioimmunotherapy of metastatic colon cancer following intravenous administration. No evidence of antibody response to the 16.88 MAb has been observed using a latex agglutination assay; the assay did exhibit a high degree of "nonspecific" positivity with MAb 28A32, although all responses were of low titer. PerImmune scientists have also labeled a human IgG3 immunoglobulin, 88BV59-1, with ^{99m}Tc for the radioimmunodetection of colorectal carcinoma. This radiolabeled antibody has been administered to 100 patients with colorectal cancer in a phase II imaging study with no evidence of immunogenicity; this included ten patients who had significant HAMA responses preventing imaging with murine MAb. The ^{99m}Tc-labeled antibody has proven to be more sensitive than CT in detecting abdominal and pelvic lesions. Recently, investigators at PerImmune used an ^{111}In-labeled IgM human MAb for radioimmunoscintigraphic presurgical staging in primary breast cancer patients. These studies indicate that human MAbs have low immunogenicity and better pharmacokinetic characteristics than murine reagents, although patients may potentially still mount an immune response against idiotypic (antigen binding site) or allotypic (Fc) determinants of the antibody.

Efficient production continues to be one of the major problems limiting the clinical use of human MAbs. Some of the new strategies that are being employed in an effort to overcome these limitation include using plasmid vectors and yeast artificial chromosomes to produce transgenic animals capable of secreting human MAbs, creation of human phage immunoexpression libraries, immunoglobulin chain shuffling, and epitope imprinted selection.

Assessing True Benefit of Radioimmunoscintigraphy

It has been difficult to demonstrate significant benefits associated with immunoscintigraphy in view of its complexity and cost. For instance, Cytogen has reported much slower than anticipated sales of OncoScint CR/OV, the first MAb-based *in vivo* cancer imaging product approved in the USA. The company believes that this will change if the nuclear medicine community were made aware of both proper use and cost-benefit advantages of immunoscintigraphy. In the latter case, what must first be determined are the consequences of immunoscintigraphy on the patient's quality of life and survival. This requires randomized studies of sufficient numbers of patients over a period of several years under optimal protocols. Results of such studies are not available, but based on an ad hoc analysis of recently published data in peer-

reviewed medical journals, Cytogen has developed a preliminary cost-benefit profile which supports the use of OncoScint CR/OV. As presented at the company's 1994 Annual Meeting, this analysis involved data from a study by the late Charles G. Moertel of the Mayo Clinic, which described current treatment practices and associated costs across the USA involving patients at risk of recurrence of colorectal cancer. Moertel's study showed that for every 1,000 patients monitored for recurrent colorectal cancer, 95 undergo exploratory surgery. A second study authored by Marvin Corman of the Sansum Clinic (Santa Barbara, CA), involving colorectal cancer patients, went beyond Moertel's work by including use of OncoScint CR/OV. When the two studies were compared, results showed that use of OncoScint prior to exploratory surgery would eliminate 27 of the 95 procedures in the Moertel study. Cytogen is continuing to work with large managed care groups around the USA to conduct additional cost-benefit studies in order to firmly establish OncoScint CR/OV's cost-effectiveness and to ensure its inclusion in managed care.

INTRAOPERATIVE USE OF RADIOIMMUNOCONJUGATES

Detection of small tumor masses is essential to the appropriate surgical management of cancer.

Radioimmunoguided Surgery (RIGS)

One approach to localize tumors relies on use of intraoperative radiation probes, a technique commonly referred to as radioimmunoguided surgery or RIGS. RIGS is a registered trademark of Neoprobe (Columbus, OH). RIGS employs a hand-held, intraoperative gamma-ray detecting probe to locate radiolabeled MAbs (or fragments) concentrated in cancerous tissues. In RIGS radiolabeled antibody is administered prior to surgery, and after allowing for tumor uptake and blood clearance of circulating conjugate, the patient undergoes surgical exploration with the aid of a hand-held gamma detecting probe. Because the probe can be directly placed over the source of gamma emission during the surgical procedure, small lesions can be detected using lower isotope doses.

Although RIGS has successfully identified small, deep-lying lesions not otherwise detected by visual inspection and/or palpation, it has also resulted in cases of false positive signals from lymph nodes. As reported by E. Cornelius and A. West of the Yale Medical School (New Haven, CT), false lymph node positive results were observed in patients with recurrent colon or ovarian cancer, particularly when radiolabeled antibodies to CEA are used. Immunohistochemistry studies were carried out on tissues in such cases and on tissues from colon cancer patients using the tumor-associated anti-TAG-72 CC49 antibody and the CD21 antibody for follicular dendritic cells (FDCs). In the colon cancer patients, CC49 staining of primary tumors was observed as well as staining of metastases in lymph nodes, and staining of non-cellular TAG-72 antigen in lymphatic vessels near primaries and in lymph nodes; CC49 staining (not due to intact tumor cells) was also observed in lymph node follicles, in a pattern similar to that of CD21 staining for FDCs. RIGS cases demonstrated follicular positivity for CC49. Prior immunohistochemistry studies have shown CC49 immunohistochemical positivity of lymph node macrophages. These studies suggest that the problem of false positives can be explained by two mechanisms:

- shedding of antigen by tumors in transit or during pure catabolism in lymph node macrophages which could be recognized by MAb CC49
- antitumor antibody responses to tumor antigen resulting in the formation of antigen-antibody complexes in the draining lymphatics, followed by their catabolism by lymph node macrophages and attachment to FDCs of the germinal centers (representing an immunologic memory mechanism not involving macrophages)

The researchers note that ^{111}In-labeled MAb could attach to non-cellular tumor antigen in lymph nodes, and although this might be evident on immunohistochemistry, false positivity could persist in lymph nodes which become immunohistochemically negative, due to lysosomal retention of ^{111}In. Long persistence (weeks) of the ^{125}I label in lymph nodes, seen in RIGS, is due to an extracellular location, which the researchers suggest is evidence for attachment of an antigen to FDCs, accounting for the observed false positives.

Beta-ray Systems

At Memorial Sloan-Kettering Cancer Center (New York, NY), F. Daghighian and associates developed other types of hand-held intraoperative probes for radioimmunodetection during surgery, including two different cameras which are sensitive to beta rays (electron or positron) while being insensitive to gamma rays. In both cameras, thin sheets of plastic scintillator which is insensitive to gamma rays (because of its low density and low atomic number), was used to detect of beta rays. A position-sensitive photomultiplier tube (PSPMT) was used to provide the position of the scintillation light. In one camera construct, the scintillator was directly connected to the PSPMT, while in the other camera, a 90 cm long flexible optical fiber bundle was used to connect the PSPMT to a sheet of plastic scintillator. Phantom imaging studies with these cameras demonstrated a resolution of approximately 1 mm. The sensitivities of the fixed and flexible beta cameras were 5,500 and 4,000 cts/sec/microCi, respectively, as measured by imaging a 5 mm diameter piece of paper soaked with ^{131}I and covered by tape. These sensitivities are sufficient for the rapid detection of small tumors (> 50 mg). Also, the sensitivities of the cameras increase for isotopes which emit higher energy beta rays. Two possible applications for these types of cameras include detection of metastatic sites of ovarian cancer on the surface of the peritoneum and scanning of the resected tumor bed for any residual malignancy.

HIGHLIGHTS OF DIGESTIVE DISEASE WEEK SAN DIEGO, CALIFORNIA, MAY 14-17, 1995

GASTRIC MALT LYMPHOMA AND *H. PYLORI*

Helicobacter pylori (H. pylori) appears to be present in most patients with gastric MALT lymphoma and curing *H. pylori* infection with antibiotics results in full tumor regression. When 13 patients with low grade gastric MALT lymphoma were empirically treated with omeprazole, clarithromycin, bismuth subsalicylate, and amoxicillin or tetracycline plus metronidazole (for penicillin-allergic persons) for three weeks and again, at eight weeks, treated for another three weeks, 12 of the 13 patients who were positive for *H. pylori* infection (one patient had Sjogren's syndrome without *H. pylori*) were cured of their infection. Furthermore, six of nine individuals with tumor less than 2.0 cm had complete regression by endoscopy and histopathology and, in the other four patients with tumor masses larger than 10 cm, one went on to complete regression, one showed complete regression on endoscopy but had residual tumor by histopathology, one had a greater than 90% tumor reduction of a growth larger than 20 cm, and one went on to chemotherapy (Steinbach G, etal, 1995 DDW Abs. Pg. A665:2658).

Endoscopic ultrasonography (EUS) has proved to be an accurate method for the staging and follow-up of gastric MALT lymphoma treated by *H. pylori* eradication. Six patients with gastric MALT lymphoma and confirmed *H. pylori* infection, were treated with omeprazole 40 mg daily, amoxicillin 2 gm daily, and metronidazole 1.0 gm daily, for 14 days, and followed up with clinical, endoscopic (with multiple biopsies) and EUS evaluation. Four patients had stage I-1 disease and two persons had stage I-2 by EUS criteria. One month after stopping the treatment, all patients were *H. pylori* negative and complete regression of lesions was documented at endoscopy and EUS. Clinical, endoscopic, and EUS follow-up was performed every four months and no recurrence or reinfection has been detected to date (Nobre-Leitäo C, etal, 1995 DDW Abs. Pg. A766:3063).

If *H. pylori* eradication either fails or does not result in gastric MALT lymphoma resolution, continuous oral chemotherapy with a single alkylating agent is proposed as second-line therapy. Twenty-four previously untreated patients with low grade B-cell MALT lymphoma were treated with cyclophosphamide or chlorambucil orally each day for a period of 12 to 24 months. Median follow-up was 45 months (14 months to 17 years). Complete remission was obtained in 18 patients (75%) after a median duration of treatment of 12 months. Of the five individuals who relapsed, two were successfully retreated with cyclophosphamide. Chemotherapy was stopped at 24 months in six individuals who achieved only PRs. Only two of these, however, required further treatment for progressive disease (Hammel P, etal, 1995 DDW Abs. Pg. A571:2281).

ESOPHAGEAL CANCER TREATMENTS

Multimodality Therapy

Multimodality therapy with chemotherapy, radiotherapy, and surgery for esophageal adenocarcinoma, in contrast to common belief, provides significant downstaging and survival benefits compared to surgery alone. Ninety- seven patients were randomized to multimodality therapy with two courses of chemotherapy (5-FU and cisplatin) given on weeks one and six, radiotherapy (40 Gy) given on weeks one and three, followed by surgery on week eight or to surgery alone. Because of protocol violations, eight patients were withdrawn from the study. Significant downstaging occurred following multimodality therapy with 55% of patients being node negative compared to 21% in the surgery group. At two years, 44% (12/27) of those in the multimodality therapy group and 26% (8/30) of those who were treated by surgery alone were alive. Median survival of patients on multimodality therapy was 16 months versus 11 months for those treated by surgery alone (Walsh TN, etal, 1995 DDW Abs. Pg. 829:3316).

Stents

Coated, self-expanding metallic stents offer palliation in upper gastrointestinal tract malignancies, preventing tumor ingrowth in malignant esophageal strictures, sealing pulmonary fistulae in patients with or without associated strictures, and improving dysphagia in selected patients. Twenty three patients with advanced cancer and malignant strictures of the esophagus or malignant esophageal-pulmonary fistulae had prototypic versions of a new silicon-coated, self-expanding metallic stent (Wallstent, Schneider USA). These individuals initially underwent stricture dilation to a diameter wide enough to accommodate a 38 French delivery catheter, at the time of stent insertion. At 30 days, 17 patients (74%), were still alive with nine having functional stents. The mean dysphagia of eight persons evaluated with functional stents improved significantly compared to baseline, going from 4.9 to 2.75. Mean survival in the total patient population was 88 days (Axelrad AM, etal, 1995 DDW Abs. Pg.: A205:819).

MICROWAVE COAGULATION THERAPY (MCT) IN LIVER CANCER

MCT is a promising new therapeutic option in HCC. Nineteen patients with HCC involving 30 nodules (13 patients had one nodule and the remaining six had two to five) were administered MCT (Microtase, Osaka, Japan) at 70-80 watts for 30 seconds. MCT consistently created a columnar necrosis 10 mm in diameter around a needle electrode. Irradiation was repeated to create segmental necrosis, containing the main tumor and surrounding liver parenchyma, to provide tumor-free margins. CT scans proved that 27 of the 30 lesions were completed treated and no recurrences were detected in 28 lesions. Two nodules progressed due to failure to coagulate the entire lesion. Fifteen patients are still alive three to 52 months after MCT (Sato M, etal. 1995 DDW Abs. Pg. A365:1459).

MECHANISMS IN MALIGNANCY

DRUG RESISTANCE IN CANCER-PART I

- Acquired resistance to cancer chemotherapy continues to represent the single most important reason for treatment failure.
- Drug resistance related to the inhibition of apoptosis was discussed in FUTURE ONCOLOGY, V1 #1, May 1995; part II of this article will appear in V1, #4 and will incorporate a database of agents in development to combat MDR

Tumor response to chemotherapeutic regimens vary. For some intrinsically drug-sensitive tumors such as childhood acute lymphoblastic leukemia (ALL), Hodgkin's disease, large cell lymphomas, and testicular cancer, chemotherapy results in a high cure rate. However, for some patients acquired resistance to chemotherapy leads to treatment failure. Other tumors, including metastatic breast carcinomas, small cell lung cancers, and bulky ovarian carcinomas, that are also usually highly responsive to initial treatments, usually become refractory to further therapy and are rarely cured. Finally, tumors such as non-small cell lung cancers, malignant melanoma, and colon cancer are intrinsically resistant to most chemotherapeutic agents. Few anticancer agents are effective against these tumors and significant chemotherapeutic responses result in a minority of cases.

Drug resistance may be circumvented by development of new non-cross-resistant agents, by novel delivery schemes or combinations of known drugs, and by other treatments that may augment drug activity or reverse resistance to known anticancer agents. Over the past several years there has been substantial progress in understanding the molecular biology associated with mechanisms by which malignant cells adapt and survive after exposure to cytotoxic agents. It has become apparent that there are multiple mechanisms of drug resistance (see Exhibit 6) involving both general cellular and biochemical mechanisms as well as anatomic, pharmacologic, and host-drug-tumor interactions. Mechanisms of drug resistance are frequently interrelated and multiple independent mechanisms of drug resistance may coexist in a population of tumor cells.

GENERAL MECHANISMS OF DRUG RESISTANCE

For simple drug resistance, tumor cells become resistant to one drug or to the one class of drugs to which they have been repeatedly exposed. This phenomenon is explained by shared drug transport carriers, drug-metabolizing pathways, and intracellular cytotoxic targets of structurally and biochemically similar compounds. In general, resistant cells retain sensitivity to drugs of different classes with different mechanisms of cytotoxic action. Thus, as a rule, the strategy for countering simple drug resistance is to use other cytotoxic drugs, or dose intensification followed by subsequent salvage of normal cells. An exception is the emergence of cross-resistance to multiple, structurally and functionally unrelated drugs to which the patient was never exposed. The phenomenon by which tumor cells become resistance to multiple drugs of unrelated chemical structure and mechanism of action is termed multidrug resistance or MDR. However, despite apparent differences in drug families associated with MDR phenotypes, these agents often share common metabolic pathways, efflux transport systems, or sites of cytotoxic action.

Decreased Drug Accumulation

Decreased intracellular levels of cytotoxic agents represents one of the most common mechanisms of drug resistance. This can result from decreased drug influx due to a defective carrier-mediated transport system, or enhanced drug efflux. For example, decreased influx via a high-affinity folate-transport system is a well-described cause of methotrexate resistance, whereas cells which overexpress the P-glycoprotein drug efflux pump represent a classic example of drug resistance due to enhanced drug efflux.

Altered Drug Metabolism

Modified drug activation or inactivation, or altered cofactor levels can confer resistance to selected anticancer agents. Antimetabolites and alkylating agents (such as cyclophosphamide) that are administered as prodrugs, must be activated to their cytotoxic forms by the targeted tumor or other tissues. For instance, resistance to some nucleobase drugs has been associated with decreased conversion of these analogs by kinases and phosphoribosyl transferase salvage enzymes, to their cytotoxic nucleoside and nucleotide derivatives. Enhanced inactivation of pyrimidine and purine analogs by elevated deaminases has been linked to resistance toward these agents, while altered levels of cofactor 5,10-methylene tetrahydrofolate can affect the formation of inhibitory complexes between 5-fluorodeoxyuridine monophosphate (FUMP) and its target enzyme, thymidylate synthase.

Increased Repair of Drug-Induced Damage

Multiple systems are involved in the repair of cellular membrane and DNA damage, and because such damage may occur as a consequence of cytotoxic drug action, alterations in these intrinsic repair mechanisms can influence drug sensitivity. An example is resistance to cisplatin, in which increased DNA repair is presumed to affect a cytotoxic action thought to involve intrastrand DNA cross-linkages.

Altered Gene Expression

Cellular mechanisms of drug resistance depend upon altered levels or function of key gene products. Such alterations may result from changes occuring at any point along the transduction pathway of genetic information, including DNA mutation, deletion, or amplification;

altered transcriptional or post-transcriptional control of RNA levels; and altered post-translational modification of proteins. These changes reflect the genetic instability of cancer cells under the selective, and possibly mutagenic, pressure of cytotoxic drug exposure.

IN VIVO DRUG RESISTANCE FACTORS

The failure of chemotherapy to eradicate a tumor *in vivo,* despite sensitivity to drug *in vitro*, may be caused by anatomic or pharmacologic drug barriers, e.g., tumor sanctuaries. An example would be the difficulty in delivering adequate amounts of many drugs across the blood-brain and blood-testicular barriers. Chemotherapy failures are frequently attributed to decreased drug delivery in large solid tumors that have overgrown the vascular supply. Furthermore, the development of acidosis and hypoxia in poorly perfused areas of large tumors may interfere with the cytotoxicity of some drugs (BA Teicher, Cancer Metas Rev, [1994] 13:139). Some studies suggest that resistant tumors may harbor cellular resistance factors that are operative only in conjunction with host factors, thus mediating resistance by altered drug pharmacokinetics *in vivo* only (BA Teicher, etal, Science, [1990] 247:1457).

MULTIDRUG RESISTANCE

Neoplastic cells often develop resistance to cytotoxic actions of multiple, structurally and functionally unrelated chemotherapeutic agents. Several well-defined MDR patterns have been documented, of which the overexpression of the P-glycoprotein within cellular membranes has been the most extensively researched. However, the development of MDR has also been observed with the use of topoisomerase II inhibitors, and in cases apparently unrelated to P-glycoprotein or topoisomerase II functions, although the pattern of drug cross-resistance may resemble the other two groups.

Classic (P-Glycoprotein-Dependent) MDR

The first description of the MDR phenotype was published in 1970, and involved cross-resistant drug patterns in Chinese hamster cell lines (JL Biedler and H Riehm, Cancer Res, [1990] 30:1174). In 1974, others reported that the observed MDR was related to reduced intracellular drug concentrations and a 170 kDa membrane glycoprotein referred to as P-glycoprotein or P-gp (V Ling and LH Thompson, J Cell Physiol, [1974] 83:103). P-gp was subsequently shown to act as an energy-dependent efflux pump that decreases intracellular drug accumulation and diminishes drug cytotoxicity (M Inaba and Y Sakuri, Cancer Lett, [1979] 8:111). Most studies of drug accumulation in MDR cells support the hypothesis that MDR-related agents enter cells by passive diffusion and are then actively transported out of the cells by P-gp.

The gene encoding P-gp belongs to a multigene family, designated mdr; the mdr genes form a subfamily within a large superfamily of genes known as the ATP-binding cassette (ABC) superfamily (R Allikmets, etal, Leukemia, [1993] supp2:S13). The ABC superfamily is composed of a diverse set of active membrane transport proteins found in both eukaryotes and prokaryotes; these gene products transport sugars, amino acids, ions, peptides, and proteins across cellular membranes (PF Juranka, etal, FASEB J, [1989] 3:2583).

While other species may display up to six classes of mdr, humans only exhibit two classes, mdr1 and mdr2. However, only the mdr1 gene encodes P-gp responsible for conferring the MDR phenotype, although the mdr1 and mdr2 genes are closely related and highly homologous. The mdr1 gene is located on the long arm of chromosome 7 at 7q21.1; it is translated as a 140 kDa protein that subsequently undergoes post-translational modification. Within two to four hours, the precursor is converted to the final form, a 170 kDa protein. Because P-gp can not be autophosphorylated, it requires enzymatic phosphorylation by protein kinase C to achieve its active form (IB Roninson, Mutation Res, [1992] 276:151; IB Roninson, Biochem Pharmacol, [1992] 43:95). P-gp is a homodimer of 1280 amino acids, each containing six membrane-spanning regions and an ATP (adenosine 5'-triphosphate) binding site; a carbohydrate moiety is attached to the extracellular part of the molecule (V Ling, Adv Oncol, [1993] 9:3). It is thought that hydrophobic loops, involved in drug binding and transport, span the cell membrane and that ATP binding sites provide energy for the active pump process. The existence of multiple drug binding sites likely contributes to the capacity of P-gp to process the structurally diverse drugs associated with MDR. In one suggested model of P-gp action, cytotoxic agents cross the cell's lipid bilayer, entering the cell by passive diffusion, and binding to P-gp on the cytoplasmic side of the cellular membrane. Utilizing the energy of ATP hydrolysis, P-gp then actively pumps the agents out of the cell. Others have suggested that binding to P-gp occurs in the lipid bilayer rather than within the cell (P Gros, etal, Cell, [1986] 47:371), or that P-gp is localized both on plasma and intracellular membranes, with drug that enters the cell being pumped into the lumen of the intracytoplasmic membrane and then removed by exocytosis (WT Beck, Biochem Pharmacol, [1987] 36:2879).

A growing body of evidence implicates mdr1 as a key determinant of response of some forms of cancer to chemotherapy. Drugs exhibiting the cross-resistance pattern of classical MDR are listed in Exhibit 7. Generally, exposure of cells to any of the drugs related to this MDR phenotype can result in cross-resistance to all other members of the phenotype. Studies have shown that high levels of P-gp expression in cultured cell lines are often associated with mdr1 gene amplification and transcriptional activation (JA Endicott and V Ling, Annu Rev Biochem, [1989] 58:137). However, clinical tumor samples in general do not exhibit mdr1 gene amplification, and it appears that *in vivo*, mdr1 mRNA levels are probably regulated more specifically by transcriptional as well

as post-transcriptional processes (MM Gottesman, J Natl Cancer Inst, [1989] 80:1352; IB Roninson, Roninson, Mutation Res, [1992] 276:151). In addition to cytotoxic drugs, increased expression of P-gp can also be stimulated, at least *in vitro* by several types of stress-inducing treatments, including heat shock, heavy metals, toxic and ablative liver insults, differentiating agents, repeated exposure to ionizing radiation, and agents that activate protein kinase C (PM Chaudhary and IB Roninson, J Natl Cancer Inst, [1993] 85:632).

Exhibit 6
Mechanisms of Drug Resistance

General Cellular and Biochemical Mechanisms
■ Decreased drug accumulation due to decreased drug influx, increased drug efflux, and/or altered intracellular movement of drug
■ Altered drug metabolism due to decreased drug activation, increased inactivation of drug or toxic intermediate, or altered cofactor or metabolite levels
■ Increased repair of drug-induced damage to DNA, protein and/or membranes
■ Altered gene expression involving DNA mutation, amplification or deletion and/or alterations in transcription and post-transcription processing or translation
Mechanisms Unique to Tumor Cells *In Vivo*
■ Host-tumor interactions involving pharmacologic and anatomic drug barriers
■ Host-drug interactions involving increased drug inactivation or decreased drug activation by normal tissues and/or a relative increase in normal tissue drug sensitivity

P-gp are not unique to drug-resistant cells, but have also been identified in various mammalian tissues that are often characterized by a type of secretory function, particularly along the apical surface of secretory epithelium of the jejunum and colon, bile canaliculi, proximal tubular epithelium of the kidney, pancreatic small ductule epithelium, and the glandular epithelium of the pregnant uterus, as well as in the adrenal gland, capillary endothelium of the blood-brain and blood-testis barriers, and in hematopoietic precursors and lymphocytes (RJ Arceci, Blood, [1993] 81:2215). Although the normal physiologic function of the mammalian mdr1 gene remains unknown and an endogenous substrate for P-gp has not been identified, the polarized distribution of P-gp on secretory epithelia and its role in drug efflux in MDR cell lines, suggest that P-gp is involved in important cellular transport functions required by these normal tissues (F Thiebaut, etal, Proc Natl Acad Sci USA, [1987] 84:7735), probably in the excretion of naturally occurring toxins or commonly encountered xenobiotics (K Nooter and H Herweijer, Br J Cancer, [1991] 63:663). A role for P-gp in hormonal secretion in the adrenal gland or uterus or in maintenance of the blood-brain, blood-testis, or placental barriers has also been postulated (CR Leveille and AS Moore, Adv Vet Sci Comp Med, [1993] 37:31). The idea that the transport function of P-gp extends well beyond its role in the efflux of chemotherapeutic agents from cancer cells and that P-gp may play an essential role in the survival of an organism, receives support from the observation that mdr gene sequences are highly conserved between humans, mice, rats and non-human primates, suggesting a common evolutionary pathway.

Other Gene Products in MDR

Similar phenotypes of multiple resistance to antineoplastic agents have been described that are associated with the expression of membrane proteins other than P-gp, with resistance often occurring independently of P-gp expression. The mechanisms of MDR in these cell lines and whether these membrane proteins are directly involved in drug sensitivity, or are merely markers of the resistant phenotype, are subjects of current investigations.

In one form of MDR in which P-gp levels are not elevated, overexpression of a 6.5-kilobase, 190 kDa integral membrane N-glycosylated phosphoprotein referred to as MRP (for MDR-related protein) has been observed. First described by scientists at the Cancer Research Laboratories, Queen's University (Kingston, Ontario, Canada), MRP, like P-gp, belongs to the superfamily of ATP-binding cassette transport systems; the gene for MRP maps in normal cells to chromosome band 16p13.1 (SPC Cole, etal, Science, [1992] 258:1650; ML Slovak, etal, Cancer Res, [1993] 53:3221). Transfection studies with MRP expression vectors have demonstrated that the protein confers resistance to multiple natural product drugs, such as anthracyclines, vinca alkaloids, and epipodophyllotoxins (CE Grant, etal, Cancer Res, [1994] 54:357; KG Almquist, etal, Cancer Res, [1995] 55:102; DW Loe, etal, Proc Am Assoc Cancer Res, [1995] 36:322, abstract 1915).

Precisely how MRP confers multidrug resistance is not known. The amino acid identity of MRP with mdr1 is only 14%, and there is as yet no direct evidence that MRP acts in a manner analogous to P-gp; to establish whether MRP functions as a drug efflux pump, purification and reconstitution studies will be needed. Subcellular fractionation studies indicate that the majority of MRP is localized to the plasma membrane.

Several preliminary studies indicate that MRP mRNA is expressed at low levels in a broad range of normal tissues, including hematopoietic cells (GJR Zaman, etal, Cancer Res, [1993] 53:1747), but so far these studies have not provided substantial clues as to the normal function(s) of MRP. It has, however, been recently determined that MRP can function *in vitro* as an ATP-dependent transporter for the amphiphilic glutathione conjugate leukotriene C_4, a potent arachidonic acid derivative involved in host defense, intracellular communication, and signal transduction (I Leier, etal, J Biol Chem, [1994] 269:27807). Investigations of the specificity of MRP for other endogenous substrates, and identification of the specific cell types

which express this protein are in progress (I Leier, etal, Proc Am Assoc Cancer Res, [1995] 36:321, abstract 1911).

At McGill University (Montreal, Quebec, Canada), researchers have identified another membrane protein, MRAP (MDR-associated protein) in several MDR human cells which do not overexpress either P-gp or MRP. MRAP expression appears to correlate with the level of resistance to such drugs as Adriamycin and vinblastine, and studies are in progress to determine the role of MRAP in the MDR phenotype (Y Wang, etal, Proc Am Assoc Cancer Res, [1995] 36:322, abstract 1914).

Expression selection has been often used to clone mammalian genes whose mutation or overexpression results in a dominant selectable phenotype. Many phenotypes, however, stem from downregulation rather than overexpression or dominant mutations of specific genes. Scientists at the University of Illinois (Chicago, IL) have developed a general strategy for cloning mammalian genes whose downregulation results in a selectable phenotype (AV Gudkov, etal, Proc Natl Acad Sci USA, [1993] 90:3231). This strategy is based on expression selection of genetic suppressor elements (GSEs), e.g., cDNA fragments encoding either specific peptides that act as dominant inhibitors of protein function or antisense RNA segments that efficiently inhibit gene expression. Since GSEs counteract the gene from which they are derived, they can be used as dominant selectable markers for the phenotype associated with downregulation of the corresponding gene. The researchers have used GSE selection from a retroviral library of normalized cDNA fragments to identify mammalian genes whose downregulation results in drug resistance (AV Gudkov, etal, Proc Natl Acad Sci USA, [1994] 91:3744). Three GSEs have been isolated, two of which are derived from presently unknown genes and one from a member of the kinesin gene family that had not been previously associated with drug response. The kinesin-derived GSE was found to induce resistance to several DNA-damaging drugs and to immortalize senescent mouse embryo fibroblasts, indicating that kinesin is involved in a common mechanism of growth arrest induced by exposure to DNA-damaging drugs or by cellular senescence.

At the University of Maryland Cancer Center (Baltimore, MD), researchers have identified an N-linked 95 kDa membrane sialoglycoprotein, P-95, the overexpression of which appears to confer MDR on certain human tumor cell lines which do not overexpress either mdr1 or MRP gene products, suggesting the existence of another drug resistance pathway distinguishable from P-gp or MRP resistance mechanisms (LA Doyle, etal, Proc Am Assoc Cancer Res, [1995] 36:324, abstract 1927).

MDR Associated With Topoisomerase Alterations

One form of MDR, separate from P-gp mechanisms, makes cancer cells resistant to topoisomerase inhibitors, such as anthracyclines and mitoxantrone, although sensitivity to agents that interfere with tubulins is retained.

Exhibit 7
Drugs Exhibiting Cross-Resistance Patterns of Classical (P-gp) MDR

Drug Class	Drug
Anthracyclines	Doxorubicin Daunorubicin Mitoxantrone
Antibiotics	Actinomycin D Plicamycin
Antimicrotubule drugs	Vincristine Vinblastine Colchicine Taxol
Epipodophyllotoxins	Etoposide Teniposide

Exhibit 8
Clinically Important Topoisomerase II Poisons

	Drug Class	Drug
Nonintercalators	Epipodo-phyllotoxins	Etoposide Teniposide
Intercalators	Anthracyclines Acridine Anthracenedione Antibiotic Ellipticine	Doxorubicin Daunorubicin Amsacrine (m-AMSA) Mitoxantrone Actinomycin D 9-Hydro-xyellipticine

Topoisomerases are nuclear enzymes that catalyze the formation of transient single or double-stranded DNA breaks, facilitate the passage of DNA strands through these breaks, and promote rejoining of the DNA stands. Consequently, topoisomerases are thought to be critical for DNA replication transcription, and recombination. The cytotoxicity of drugs that target topoisomerases, sometimes referred to as topoisomerase poisons, is thought to depend upon the DNA cleavage activities of topoisomerases. There are two classes of these enzymes found in mammals, topoisomerase I and topoisomerase II. Topoisomerase I catalyzes the formation of single-stranded DNA breaks, whereas topoisomerase II catalyzes both single- and double-stranded breaks. During cleavage reactions, reversible DNA-topoisomerase complexes (cleavable complexes) can be stabilized by interactions with topoisomerase poisons, and formation of these stabilized DNA-topoisomerase-drug complexes is thought to initiate production of lethal DNA strand breaks. Of the chemotherapeutic agents that affect topoisomerase activities, topoisomerase II poisons have been the most important clinically; a partial list of these agents, which includes DNA intercalating and nonintercalating drugs is given in Exhibit 8.

Exhibit 9
Substrates of GSTs Related to Drug Detoxification and Repair of Drug-Mediated DNA Damage

Antineoplastic Drugs
■ Nitrogen mustards Chlorambucil Melphalan Cyclophosphamide
■ Nitrosoureas
1,3-bis(2-chloroethyl)-1-nitroso urea (BCNU)
■ Anthracenedione
Mitoxantrone
Products of Membrane and DNA Oxidation
■ Fatty acid hydroperoxides
■ 4-Hydroxy alkenals
■ DNA hydroperoxides (?)

The mechanism of resistance to topoisomerase II poisons is thought to involve altered topoisomerase II activity. Both qualitative (e.g., mutations) and quantitative (e.g., reduced levels) changes in enzyme activity have been observed in resistant tissue culture cell lines; reduced levels of topoisomerase activity has been associated with decreased drug-induced DNA strand breaks as well as reduced drug cytotoxicity. However, studies in tumor samples from patients have failed to reveal mutations in topoisomerase II, and a clear relation between enzyme levels and drug sensitivity has only been detected in a limited number of clinical samples. Hence, the actual role of altered topoisomerase II in clinical MDR is unresolved (E Schneider and KH Cowan, Med J Australia, [1994] 160:371). Other studies have implicated intrinsic changes in drug-induced catalytic properties or associated cofactors as the basis of drug resistance in some cells (LA Zwelling, etal, J Biol Chem, [1989] 264:16411), and it may well be that the normal down regulation of topoisomerase II in non-dividing cells is responsible for the relative insensitivity to topoisomerase II-poisons of some solid tumors containing a large proportion of quiescent cells (L Liu, Annu Rev Biochem, [1989] 58:351).

In the case of topoisomerase I poisons, the cytotoxic agent camptothecin has been shown to enhance topoisomerase I-mediated DNA strand breaks. However, until recently host toxicity has prohibited the clinical use of such topoisomerase I poisons. The prospect of less toxic analogs of this drug that maintain a high level of activity against topoisomerase I-rich human cancer cells has renewed interest in the clinical application of this class of compounds (BC Giovanella, etal, Science, [1989] 246:1046) and several topoisomerase I inhibitors are currently in clinical trials. Consequently, the emergence of resistance to these agents may become an increasingly important consideration.

Altered Expression of Drug-Metabolizing Enzymes and MDR

The emergence of acquired drug resistance may be considered as either an acute or chronic adaptive response of tumor cells to drug challenge or some other form of environmental stress. For instance, rapid transient induction of P-gp may result from acute exposure to a cytotoxic drug or a heavy metal exposure, or from heat shock; chronic or repeated exposure to drugs may enhance P-gp levels by complex, stable genetic changes. Alternatively, challenges with cytotoxic agents may result in alterations in the expression of drug-metabolizing enzymes (CR Fairchild, etal, Proc Natl Acad Sci USA, [1987] 84:7701). These drug-metabolizing enzymes are generally considered to be involved in the sequential oxidation of xenobiotics to more electrophilic, reactive intermediates followed by the formation of less toxic conjugated compounds, which may be further metabolized or excreted. The emergence of this phenotype appears to represent a programmed cellular stress response that might offer generalized protection from a variety of exogenous toxins. Of the drug-metabolizing enzymes, the glutathione S-transferases (GSTs) have been among the most extensively studied (D Hedley and S Chow, Meth Cell Biol, [1994] 42:31).

The GSTs are comprised of multiple soluble and membrane-associated isozymes which catalyze the conjugation of electrophilic, hydrophobic compounds with the thiol, glutathione (GSH). Another catalytic activity, selenium-independent glutathione peroxidase activity, has also been attributed to some isozymes of GST. These and other GST-mediated reactions are of interest because of their potential to detoxify oxidative damage to membranes and DNA.

Studies using cell-free preparations of GSTs have identified a limited number of antineoplastic drug substrates of these enzymes. These drugs and other substrates possibly associated with drug-mediated oxidative damage are summarized in Exhibit 9. However, whether GST levels in tumor cells are sufficient to detoxify antineoplastic drugs to a clinically significant extent is still a matter of considerable debate, and it may be that GST is more of a marker for drug resistance than a cause. Circumstantial evidence has linked the increase in specific GST isozymes or bulk GST activity in cells with resistance to alkylating agents, doxorubicin, and other drugs, but direct evidence of the action of GSTs in the alteration of drug sensitivities is limited.

Apoptosis in MDR

Recent evidence indicates that apoptosis may be an important mechanism for the lethality of cancer chemotherapeutic drugs. Interference with apoptosis caused by expression of the bcl-2 gene or p53 mutation, can confer resistance to anticancer drugs. For instance, wild-type p53 protein inhibits transformation by myc

and ras, which in turn affect gene expression. Mutational alterations in the p53 gene are among the most common genetic lesions in human cancer. In cooperation with an activated mutant ras gene, p53 leads to phenotypic transformation of cells. Inactivation of the wild-type p53 gene is considered a critical event the induction of malignant transformation (V Rotter, etal, Trends in Cell Biol, [1993] 3:46). Subsequent accumulation of the mutant product may cause disruption between the balanced normal cell proliferation and apoptosis, leading to the establishment of cells with novel characteristics - including mdr1 expression. The promoter of the human mdr1 gene is stimulated by the products of both the c-Ha-Ras-1 oncogene and the mutant p53 suppressor gene; although the effect of the ras is not specific for mdr1 alone, the wild-type p53 exerts specific mdr1 repression (RL Zastawny, etal, Oncogene, [1993] 8:1529; SW Lowe, etal, Science, [1994] 266:807; SW Lowe, etal, Cell, [1993] 74:957; KV Chin, etal, Adv Cancer Res, [1993] 60:157). For a more thorough discussion of the mechanisms of apoptosis in cancer development, the reader is referred to V1, #1 of FUTURE ONCOLOGY (May 1995 issue pp. 22-31).

Multiple Mechanisms in Drug-Resistant Tumors

Many treatment protocols designed to circumvent the problem of drug-resistant tumors involve the administration of multiple drugs with different structural properties and mechanisms of action. This approach assumes that if enough carefully selected drugs are delivered at optimal doses and intervals, individual clones of cells resistant to one class of drug will be effectively killed by another drug in the regimen. However, the rapid appearance of refractory tumors despite an initially favorable cytoreductive response suggests that the emergence of multiply resistant tumor cell clones is a common clinical occurrence. The discussion above reviewed how a single genetic change such as increased P-gp or altered topoisomerase II can mediate cross-resistance to several anticancer drugs. Yet, while these mechanisms may provide a molecular explanation for some cases of broad-spectrum resistance, it is clear that many refractory tumor clones must simultaneously develop multiple resistance mechanisms (WT Beck, Cancer Treatment Reviews, [1990] 17suppA:11). Such cells can be considered as exhibiting a "mixed" MDR phenotype. For example, several human and rodent cell lines selected for resistance to the anthracycline, doxorubicin, appear to display overexpression of P-gp as well as decreased DNA topoisomerase II activity and alterations in glutathione-metabolizing enzymes such as GST.

Next issue: *This article continues with a discussion of approaches to overcome MDR, including a database of agents in development against MDR.*

INDEX OF COMPANIES & INSTITUTIONS

INDEX OF COMPANIES & INSTITUTIONS

FUTURE ONCOLOGY

PUBLISHED BY **NEW MEDICINE, INC.**

PUBLISHER AND EDITOR: **Katie Siafaca, MS**

SENIOR EDITOR: **Tim Sharon, PhD**

RESEARCH ASSOCIATES: **Sarah Nghiem and Henry Shiau**

CIRCULATION MANAGER: **Sanjay Kumar**

DESIGN & PRODUCTION: **Jill Burch**

NEW MEDICINE, INC. MAILING ADDRESS:

P.O. Box 909
Lake Forest, California 92630
Tel: 714. 830. 0448 ■ Fax: 714. 830. 0887

SUBSCRIPTION INFORMATION:

- FUTURE ONCOLOGY (ISSN 1082-331X) is published as 12 issues per year, with a free annual index listing companies/institutions and subjects covered. One-year subscriptions, sent first class to U.S. addresses, are US $600. One-year subscriptions, sent air mail to addresses outside the U.S., are US $630.
- Back issues are available for $80 each; double issues are $146.
- Additional subscriptions mailed in the same envelope are $390 each.
- Payment must accompany your order; checks must be drawn on a U.S. bank. (A purchase order number is acceptable; however, the subscription will not begin until payment is received.) Make checks payable to New Medicine. Payment may also be made by AMERICAN EXPRESS, VISA or MASTERCARD and wire transfer; please call 714. 830. 0448.