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

OVARIAN CANCER — PART II SCREENING, DIAGNOSIS, STAGING, AND PROGNOSIS

The most troublesome aspect in the management of ovarian cancer is the difficulty in detecting it at an early stage when intervention is curative. Lack of definite symptoms associated with early disease, inaccessible location, and the relative rarity of malignancy in those complaining of symptoms, makes early detection unlikely; over 75% of ovarian cancers are detected in advanced stage.

SCREENING

It is unclear if the management of ovarian cancer would benefit from screening asymptomatic populations to detect localized disease and, hence, achieve a higher cure rate. Although ovarian cancer meets one of the criteria used to determine if a disease is suitable for screening, namely that it is associated with serious health consequences or a high mortality rate (ovarian cancer has a 5-year survival of under 45%), there is insufficient information about this cancer to establish screening guidelines. Ovarian cancer does not fulfill other criteria regarding suitability such as being treatable in early or pre-symptomatic stage and having a detectable preclinical phase. For instance, although the natural course of progression from localized to disseminated stages is unknown, it is assumed that there is an orderly progression from Stage I to IV of an indeterminate time interval. However, individual cases may not present this way. For instance, there are case reports of women who develop peritoneal carcinomatosis following oophorectomy (Tobacman JK, etal, Lancet, 1982 Oct 9, 2(8302):795-7), suggesting that the peritoneum can undergo malignant transformation as a primary cancer site or simultaneously with the ovary. In addition, it is not known whether a premalignant stage exists for ovarian cancer. Lastly, the preclinical phase should have a high prevalence rate to justify the cost of screening. Although ovarian cancer is an important cause of mortality, its incidence and prevalence are relatively low (Mackey SE and Creasman WT, Journal of Clinical Oncology, 1995 Mar, 13(3):783-93).

Currently there is no valid screening methodology that can be used in ovarian cancer. Validity is judged by the degree of specificity, sensitivity and predictive value. Ideally, a screening test should be both highly sensitive and specific. A high rate of false positives is a serious problem in mass screening, particularly when, as is the case in ovarian cancer, the only way to definitively verify the findings is via a surgical procedure (i.e. laparotomy). The positive predictive value is dependent on the prevalence of the disease in the study population. The more prevalent the disease the higher the probability that it is actually present when the test is positive. Based on its prevalence, it has been calculated that a specificity of 99.6% is required of any ovarian cancer screening test to have a true impact on disease management.

Several screening techniques have been examined for ovarian cancer, including pelvic examination, various tumor markers and radiologic imaging using transabdominal ultrasound and transvaginal ultrasound with doppler. A 16-year, multi-center randomized screening/prevention clinical trial for prostate, lung, colorectal, and ovarian cancer (PLCO) involving 74,000 women which began in 1993, is currently ongoing to determine whether screening using physical examination of the ovaries, CA 125, and transvaginal ultrasound, can reduce mortality from ovarian cancer in women aged 55-74, stratified by age (55-59, 60-64, 65-69 and 70-74). The study is also assessing various screening variables including sensitivity, specificity, and positive predictive value. End points include cancer-specific mortality, cancer incidence, stage shift, survival, and predictive value of biologic and/or prognostic characterizations of tumor tissue.

A prospective analysis of published studies that provided estimates of ovarian cancer risk, tested operating characteristics (based on observational studies and meta-analyses), assessed effectiveness of treatment according to stage of disease (based on randomized trials) and devised mathematical models simulating screening for ovarian cancer in specific populations, was used to critically review available evidence for screening asymptomatic women for ovarian cancer with ultrasonography or CA 125 radioimmunoassay, or both. Death from ovarian cancer and morbidity from surgical procedures were the principal outcomes considered. Results indicated that annual screening with CA 125 or ultrasound in women >50 years without a family history of ovarian cancer would result in more than 30 false-positive findings for every ovarian cancer detected (Carlson KJ, etal, Annals of Internal Medicine, 1994 Jul 15, 121(2):124-32).

Based on evaluations to date, there is no direct evidence that mortality from ovarian cancer would be decreased by screening. Available evidence does not support either screening of pre-or post-menopausal women without a family history of ovarian cancer or routine screening of women with a family history of ovarian cancer in one or more relatives without evidence of a hereditary cancer syndrome. Only women with a family history of hereditary ovarian cancer syndrome who are at high risk for the disease would benefit from thorough diagnostic work-up (Carlson KJ, etal, Annals of Internal Medicine, 1994 Jul 15, 121(2):124-32).

Pelvic Examination

Although useful in early detection of other gynecologic cancers, pelvic examination is of limited value in screening asymptomatic women for ovarian cancer. In general, ovarian malignancies have disseminated by the time they are palpable.

Tumor Markers

Several serum tumor markers have been evaluated to determine their role in the early diagnosis of ovarian cancer but, with the exception of CA 125, most of these markers are of insufficient sensitivity or specificity for epithelial ovarian tumors, correlate poorly with clinical status, are elevated in advance disease only, and lack complementarity with CA 125. Various markers associated with ovarian cancer are listed in Exhibit 1 to illustrate the complexity of identifying and evaluating such entities. The majority of genomic changes are not unique to ovarian cancer and are probably attributable to genetic alterations found in most tumor cells in advanced disease.

CA 125, an antigenic determinant on a glycoprotein that is shed into the bloodstream by malignant cells derived from coelomic epithelium, was shown to correlate with ovarian cancer; serum levels of CA 125 are increased in approximately 80% of patients with epithelial ovarian cancers. However, it is important to note that levels of CA 125 are increased in patients with a number of other malignancies such as advanced endometrial cancer and pancreatic cancer (60%) and benign gynecologic conditions such as endometriosis, uterine leiomyomas, pelvic inflammatory disease, early pregnancy and ovarian cysts. Elevations of this marker are also seen in cirrhosis and pericarditis (Jacobs I and Bast RC Jr, Human Reprod, Jan, 4(1):1-12).

CA 125 has been developed for monitoring ovarian cancer treatment, detecting relapse and distinguishing benign from malignant conditions. Several studies have illustrated its utility:

- In 1983, it was reported that increasing or decreasing levels of CA 125 correlated with progression or regression of disease 93% of the time, confirming its appliability as a noninvasive test to monitor response to therapy (Bast RC Jr, etal, NEJM, 1983 Oct 13, 309(15):883-71).
- Using a serum sample obtained before a patient's condition was diagnosed, it was shown that a one-year lead time existed between the first elevation of CA 125 and clinical presentation of tumor (Bast RC Jr, etal, Gynecol Onc 1985 Sep, 22(1):115-20).
- Using a serum bank from Norway, elevations of CA 125 were noted as early as 18 months before clinical detection (Zurawski VR Jr, etal, Int J Cancer, 1988 Nov 15, 42(5):677-80).

A clinical trial to assess cumulative and relative risk of developing an index cancer (invasive epithelial cancer of the ovary or fallopian tube) after a specified CA 125 result, recruited, between June 1, 1986 and May 1 , 1990, 22,000 volunteers, all asymptomatic post-menopausal women ≥45 years of age. Serum CA 125 concentration was measured annually in the study participants for one to four years. Those with a concentration ≥30 U/ml were recalled for abdominal ultrasonography. Follow up was by annual mail questionnaire. Laparotomy was performed if the ultrasound examination was abnormal. Forty-nine index cancers developed in the study population during a mean follow up of 6.76 years. The overall cumulative risk of developing an index cancer was 0.0022 for the entire study population. It was lower for women with a serum CA 125 concentration <30 U/ml (cumulative risk 0.0012) but was appreciably higher for women with a concentration ≥30 U/ml (0.030) and >100 U/ml (0.149). Compared with the entire study population, the relative risk of developing an index cancer within one year and five years was increased 35.9-fold and 14.3-fold, respectively, after a serum CA 125 concentration ≥30 U/ml, and 204.8-fold and 74.5-fold, respectively, after a concentration ≥100 U/ml. It was, therefore, concluded that CA 125 is a powerful index of ovarian and fallopian tube cancer risk in asymptomatic post-menopausal women (Jacobs IJ, etal, BMJ (Clinical Research Ed.), 1996 Nov 30, 313(7069):1355-8).

In earlier reports from this same trial (Jacobs IJ, etal, Bmj (Clinical Research Ed.), 1993 Apr 17, 306(6884): 1030-4), apparent sensitivity ranged from 53% to 89%, too low for a primary screening test. CA 125 specificity ranges from 98.6% to 99.4%, depending on which study is reviewed. In a study of 1,082 Swedish women screened with a single CA 125 assay, specificity was 99.4% in post-menopausal women (reference used was 35 U/ml) (Zurawski VR Jr, etal, Gynecologic Oncology, 1988 May, 30(1):7-14). A study of 2,544 healthy women showed that menopausal status, a previous hysterectomy and age, significantly influence serum CA 125 levels, suggesting that the reference limit of 35 U/ml should be adjusted (Obstet Gynecol 1990;79:511-514).

Another approach to assess ovarian cancer risk is by obtaining longitudinal tumor marker levels. CA 125 tends to go up progressively in the presence of malignancy while remaining horizontal in benign disease. In order to develop and validate a screening test based on longitudinal marker levels, stored samples from women in the Stockholm screening study were re-assayed using CA 125 II (Centocor; Malvern, PA) and OVX1. Based on a CA 125 II specificity of 99.7% and sensitivity of 83%, the estimated positive predictive value was 16%, substantially greater than that based on a single assay (Skates SJ, etal, Cancer, 1995 Nov 15, 76(10 Suppl):2004-10).

Complementarity markers such as macrophage colony stimulating factor (M-CSF) and OVX1, may also be used in screening applications. Combination of two serum tests appears to improve sensitivity of early detection. Specificity of serial measurement of these two markers approaches that of transvaginal ultrasound. M-CSF is measurable in the serum of 68% of patients with clinically detectable disease (Ramakrishnan S, etal, J Clin Invest, 1989 Mar, 83(3):921-6) and demonstrates complementarity with CA 125. Among 25 patients with

Exhibit 1
Selected Ovarian Cancer Markers

Marker	Description	Comments ❑ References
67LR	Laminin receptor (67 kDa) that correlated to ovarian and endometrial tumor progression	May serve as a prognostic indicator (van den Brüle FA, etal, AACR96, Abs. 467:67)
AKT2	Putative oncogene, encodes a protein-serine/ threonine kinase containing a pleckstrin homology domain characteristic of many signaling molecules	AKT2 is amplified and overexpressed in 10%-15% of ovarian carcinomas (Altomare DA, etal, AACR96, Abs. 3942:575)
Bax	Apoptotic protein which mediates paclitaxel-induced apoptosis of ovarian cancer cells through a p53-independent mechanism	Strobel T, etal, AACR96, Abs. 118:17; see FO, p 23
Bcl-2	Bcl-2 is an integral membrane protein which represses apoptosis by an unknown mechanism; see FO, p 23	Results do not support the hypothesis that Bcl-2 expression confers resistance to carboplatin in ovarian cancer (Al-Azraqi A, etal, AACR96, Abs. 1333:195)
Bcl-x_L	Anti-apoptotic protein is overexpressed in a derivative of the ovarian carcinoma line A2780 with resistance to chlorambucil	Roy G, etal, AACR97, Abs. 909:136
Bfl-1	cDNA clone which is homologous to Bcl-2; Bfl-1 was isolated from a human fetal liver at 22-week gestation	Elevated Bfl-1 gene expression was detected in 40% of ovarian cancer (Seok-Il H, etal, AACR96, Abs. 3884:566)
BRCA1	Tumor suppressor gene implicated in hereditary breast-ovarian cancer (HBOC); may also be present in at least 10% of sporadic ovarian tumors	See FO, pp 361-362
	Mutational analysis for BRCA1 in familial and sporadic ovarian cancers	Results showed that BRCA1 played an important role in most familial breast-ovarian cancer syndrome and in a part of sporadic ovarian cancer (Aida H, etal, AACR97, Abs. 824:123)
	Expression of BRCA1 in breast and ovarian cancers is cell cycle regulated	Vaughn J, etal, AACR96, Abs. 1691:247
	Estrogen regulates expression of BRCA1 in breast MCF-7 and ovarian BG-1 cancer cells	Data supports role of the steroid estrogen and involvement of the estrogen receptor in regulation of expression of BRCA1 (Romagnolo D, etal, AACR96, Abs. 3532:516)
BRCA2	Tumor suppressor gene implicated in hereditary breast and ovarian cancer	See FO, pp 361-362
CA 19-9	Elevated in 17%-25% of patients with epithelial malignancies	Schwartz PE, etal, Cancer, 1987 Aug 1, 60(3):353-361
Carcinoembryonic antigen (CEA)	Elevation of CEA, is encountered in 30% to 65% of epithelial tumors	Mostly seen in advanced disease; does not correlate with clinical status
c-Ki-ras	Proto-oncogene; amplified in 31% of ovarian cancer, mostly in well-differentiated early-stage cancer	Meilu B, etal, Chinese Medical Journal, 1995, 108:844-848
	Mutations in Ki-ras, originating from ovarian tumors may be detected in the circulation (plasma or serum) of a significant percent of ovarian cancer patients	Costa J, etal, Keystone Symposia, 27 Jan-2 Feb 1997, (late abstracts):26
Ki-ras p21	Protein resulting from expression of Ki-ras cellular oncogene	A subset of human ovarian epithelial adenocarcinomas exist with an amplified expression of cellular Ki-ras p21 protein (Palejwala S, etal, AACR96, Abs. 1429:209)
c-myc	Proto-oncogene; amplified in 50% of ovarian cancer, primarily in advanced stage (above Stage III) and poorly-differentiated tumors	Meilu B, etal, Chinese Medical Journal, 1995, 108:844-848
c-N-ras	Proto-oncogene; amplified in 44% of ovarian cancer, mostly in well-differentiated early-stage cancer but also in advanced stage cancer	Meilu B, etal, Chinese Medical Journal, 1995, 108:844-848

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CA 125	Antigenic determinant on a glycoprotein that is shed into the bloodstream by malignant cells derived from coelomic epithelium	Serum levels of CA 125 are increased in approximately 80% of patients with epithelial ovarian cancers; also elevated in advanced endometrial and pancreatic cancer (60%), benign gynecologic conditions such as endometriosis, uterine leiomyomas, pelvic inflammatory disease, early pregnancy, ovarian cysts, cirrhosis and pericarditis
CAK1 or mesothelin	Differentiation antigen present on cell surface in mesothelium and on many mesotheliomas and ovarian cancers; may be implicated in adhesion and dissemination of malignancy	Gene cloned and sequenced and patent is available from the NIH (serial # 60/010,166, filed 5/1/96)
CD40	A member of the tumor necrosis factor-receptor superfamily CD40 is expressed on epithelial ovarian cancer (EOC) cells and modulates growth	Activation of the receptor can induce growth inhibition and sensitize EOC cells to apoptosis induced by a variety of agents (Gallagher NJ, etal, AACR97, Abs. 3357:500)
CD44	CD44 is a family of widely distributed cell adhesion molecules with multiple functions in cell-matrix interactions; different variants of CD44 protein are expressed in human ovarian carcinoma cell lines	Stickeler E, etal, AACR97, Abs. 1944:289 and AACR96, Abs. 440:63
Chromosome 6	Allelic losses on chromosome 6 in ovarian carcinomas	Results suggest that there may be several genes on chromosome 6 involved in the development of ovarian cancer (Cass I, etal, AACR97, Abs. 3448:514)
Chromosome 6q	Region 6q is one of the three distinct regions of chromosome 6 which are frequently affected by LOH in primary human ovarian carcinomas	It is hypothesized that the middle portion of chromosome 6q contains a tumor suppressor gene important for ovarian carcinoma (Wan M, etal, AACR96, Abs. 4052:591)
	The minimal region which may contain a putative tumor suppressor gene on 6q27 is 1.1cM in epithelial ovarian cancer	Cooke I, etal, AACR96, Abs. 3751:548
Chromosome 7q31.1-q31.2	A tumor suppressor gene may exist at chromosome 7q31.1-q31.2	LOH at one or more loci on 7q31.1-q31.2 were found in 55% of epithelial ovarian carcinomas (Edelson MI, etal, AACR96, Abs. 3775:551)
Chromosome 8p12-21	Allelic loss was noted on chromosome 8p12-21 in BRCA1 mutation-positive familial breast-ovarian cancer	A tumor suppressor on these loci may be important in the development of BRCA1 mutation-positive familial breast-ovarian cancer (Weiss RA, etal, AACR96, Abs. 3774:551)
Chromosome 9q34	Frequency of LOH detected in patients from ovarian cancer families in Japan at chromosome 9q34 and 17q21 was 75% and 65%, respectively	A tumor suppressor gene responsible for familial site-specific ovarian cancer may exist on chromosome region 9q34 (Takano M, etal, AACR96, Abs. 726:105)
Chromosome 12 (12p13.2-13.3)	Multiple genetic alterations, including high frequency of LOH was detected at certain loci on chromosome 12 in ovarian carcinomas	Cass I, etal, AACR97, Abs. 3444:513
Chromosomes 12p13 (region of TEL and KIP1 loci) and 12q23	LOH was detected at chromosome 12p13 and 12q23	Evidence of two new tumor suppressor genes (Hatta Y, etal, AACR96, Abs. 3781:552)
Chromosome 14q	LOH on chromosome 14 is common in ovarian cancer, and tumor suppressor genes contributing to the disease process may exist in regions 14q12-13, 14q21-23 and 14q32.1-32.2.	Bandera C, etal, AACR96, Abs. 4053.
Chromosome 17	Age-related increase of LOH on chromosome 17 in ovarian cancer	Pieretti M, etal, AACR97, Abs. 3449:514
Chromosome 17q	Commonly deleted area in sporadic ovarian cancer	A tumor suppressor gene located on distal 17q is inactivated in a high proportion of malignant ovarian tumors (Russell H, AACR97, Abs. 3445:513)
Chromosome 17p 13.3 (HIC-1); also see OVCA1 and OVCA2 below	Tumor suppressor gene cloned from chromosomal region 17p13.3 that is hypermethylated or deleted in human cancer	Pieretti M, etal, AACR96, Abs. 4029:587

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Chromosome 17q25	Allelic loss of tumor suppressor gene at 17q25	ICAM2 and TIMP2 are being investigated (Kalikin LM, etal, AACR96, Abs. 3785:552)
Chromosome 22q	LOH in a distinct region of distal 22q is present in advanced-stage epithelial ovarian carcinoma	The 2c M region may contain a tumor suppressor gene important for initiation and/or progression of ovarian cancer (Silverman M, etal, AACR97, Abs. 1018:152)
Cripto-1	Newly discovered protein of the epidermal growth factor family	Results suggest that Cripto-1 is a novel tumor marker for gynecological tumors (Pritze W, etal, AACR96, Abs. 1036:150)
Cyclin A	Sequential activation of a series of cyclins and cyclin-dependent kinases controls transition between cell cycle phases	Dysregulated cyclin A expression in ovarian surface epithelia may play a role in the development of carcinoma (Alkan S, etal, AACR96, Abs. 3482:509)
Cyclin D1 (CD 1)	Cell cycle regulator of G1/S progression which seems to play a relevant role in the pathogenesis of a variety of tumors	Increased or altered expression of CD 1 might be relevant in the development of ovarian malignancy (Alama A, etal, AACR96, Abs. 1403:205)
DOC-2	Identification of a differentially expressed gene, DOC-2, in human ovarian cancer	Down regulation of DOC-2 may play an important role in the development of both borderline and invasive ovarian tumors (Lau CC, etal, AACR97, Abs. 486:72)
Epidermal growth factor receptor (EGFR)	EGFR is overexpressed in a variety of human tumors	Moscatello D, etal, AACR96, Abs. 363:52
Glutathione S-transferase mu (GSTM1)	GSTM1 is a polymorphic member of the mu class gene family of the glutathione S-transferases	Initial survival comparisons indicate that GSTM1 null individuals may have a poorer response to therapy than those who express the gene (Lallas TA and Buller RE, AACR97, Abs. 1628:242)
HER-2/neu (c-erbB-2)	HER-2/neu protein is overexpressed in a variety of human tumors and is associated with aggressive disease in breast and ovarian cancers	Nistico P, etal, AACR96, Abs. 3277:480; however, measurement of markers associated with HER 2/neu has not proven useful in ovarian cancer (Berek JS and Martinez-Maza O, J Reprod Med, 1994 Apr; 39(4):241-8)
	Proto-oncogene c-erbB-2 is amplified in 25% of ovarian cancer, primarily in advanced stage (above Stage III) and poorly-differentiated tumors	Meilu B, etal, Chinese Medical Journal, 1995, 108:844-848
$p185^{c\text{-}erbB\text{-}2}$	Encoded by c-erbB-2, $p185^{c\text{-}erbB\text{-}2}$ is a 185 kDa tyrosine kinase type receptor overexpressed in 20%-30% of breast cancer cells	Suzuki T, etal, AACR97, Abs. 55:9; anti-p185 immunotoxins and radioconjugates may be used in the treatment of ovarian and breast cancers that overexpress p185 (Xu F, etal, AACR96, Abs. 3216:471)
Human progesterone receptor gene (HPR)	Mutations in HPR gene associated with increased cancer risk of non familial breast and ovarian cancers	Agoulnik I, etal, AACR96, Abs. 1514:222
L27a	Human ribosomal protein gene	Elevated expression of L27a may represent one of the tumor-specific genes altered in ovarian carcinomas (Park W, etal, AACR97, Abs. 705:105)
Lost on Transformation-1 (LOT-1)	Located on the short arm of chromosome 1, LOT-1 expression was diminished in tumor cell lines; LOT-1 exhibits little homology to known genes but its sequence contains 19 contiguous near-perfect repeats, 58 nucleotides in length, and a pattern of bases which is characteristic of those encoding a zinc-finger functional protein motif	This gene's transcript, a 6.4 kb protein, was expressed in ovary, testes, uterus, pancreas, brain, heart and kidney but not in liver and spleen (Hamilton TC, etal, Symposium 2, Ovarian Cancer-From the Laboratory to the Clinic, AACR96, p 623)
Lysophosphatidic acid (LPA)	Various species of LPA are part of an ovarian cancer activating factor (OCAF) receptor (2 receptors have been identified) which stimulates proliferation of ovarian cancer cells	Measurement of plasma LPA levels may be useful as a diagnostic marker for early detection of ovarian and other gynecologic cancers (Xu Y, etal, AACR97, Abs. 710:106)
Maspin	Member of serpin family of proteinase inhibitors; its expression is less common in adenocarcinoma but is overexpressed in squamous cell carcinoma	Ding I, etal, AACR96, Abs. 627:90
MMP1, MMP2, TIMP 1, TIMP 2, tPA, and uPA	Differences in expression of matrix metalloproteinases, metalloproteinase inhibitors, and plasminogen activators in cultured ovarian cystadenomas, tumors of low malignant potential, and carcinomas	Results support a role for plasminogen activator activity in modulating invasive and metastatic abilities in ovarian epithelial tumors (Luo M, etal, AACR97, Abs. 2764:412)

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mos	Expression and activation of mos oncogene product is associated with paclitaxel-induced apoptosis in human ovarian cancer SKOV3 cells	Results suggest that induction of M phase arrest and apoptosis caused by paclitaxel are at least in part associated with mos gene expression and activation (Ling YH, etal, AACR97, Abs. 1906:284)
M_r	7-kDa plasma membrane protein overexpressed in ovarian MDR cancer cells; confers resistance independent of P-gp or MRP	MAb 1D7 which recognizes M_r inhibits growth of ovarian drug-resistant cells (Yang X and Paglé M, Cancer Letters 88 (1995) 171-178
Motility-related protein-1 (MRP-1)/ CD9 and C33/KAI1/ CD82	Proteins, members of transmembrane 4 superfamily present in a variety of solid tumors	Huang C, etal, AACR96, Abs. 512:74
mdr-1	Gene found to play a role in the multidrug resistance phenotype of a number of human tumors	Kavallaris M, etal, AACR96, Abs. 2130:313
MUC2	Over expression or ectopic expression of MUC2 is the common property of mucinous carcinomas of the colon, pancreas, breast and ovary	Overexpression indicates a common genetic lesion associated with the mucinous tumor phenotype (Hanski C, etal, AACR97, Abs. 2822:421)
Multidrug resistance-associated protein (MRP)	Plays a role in the multidrug resistance phenotype of a number of human tumors	Kavallaris M, etal, AACR96, Abs. 2130:313
MSH2	Member of a gene family involved in DNA repair; mutations trigger cancer causing genes	Mutations found in hematologic malignancies and breast and ovarian cancers (Lowsky R, Blood, 1 Apr 1997)
NF-κB	Transcription factor which does not regulate p53 expression in breast and ovarian cancer cell lines	Cirisano FD, etal, AACR96, Abs. 1174:171
	Paclitaxel directly affects transcription of NF-κB	Lee L-F, etal, AACR97, Abs. 1245:186
OVarian CAncer 1 (OVCA1) and OVCA2	Novel genes on chromosome 17p13.3; OVCA1 and OVCA2 mRNAs are expressed in normal surface epithelial cells of the ovary but are reduced or undetectable in ovarian tumors and tumor cell lines	Mutational analysis of the OVCA1 gene in carcinomas of the ovary and ovarian carcinoma cell lines identified several sequence alterations indicating that OVCA1 and potentially OVCA2 may be human tumor suppressor genes (Schultz DC, etal, Cancer Research, 1 May 1996, 56(9):1997-2002)
OVX1	Antigenic determinant raised from sequential immunization of mice with different human ovarian cancer cell lines	Elevated in 67% of patients with evident ovarian cancers who are CA 125 negative (Xu FJ, etal, Cancer Research, 1991 Aug 1, 51:4012-9)
p15, p16 and p18	Genes characterized in tumor and normal samples in sporadic ovarian cancer (SOC), and p16 gene in patients with familial ovarian cancer (FOC)	p16, p15 and p18 genes appear to play a minor role in ovarian cancer (Marioli E, etal, AACR96, Abs. 4049:590)
p15/MTS2	Tumor suppressor gene	Inactivation of this gene is a potential indicator of chemoresistance in ovarian cancer (Kudoh K, etal, AACR97, Abs. 712:106)
p16/CDKN2	Tumor suppressor gene	Inactivation of this gene is a potential indicator of chemoresistance in ovarian cancer (Kudoh K, etal, AACR97, Abs. 712:106
p53	Tumor suppressor gene; p53 mutations appear to occur in as high as 52% of cases ovarian carcinomas	Zheng J, etal, JNCI, 2 Aug 1995, 87(15):1146-53; DiCioccio RA, etal, AACR96, Abs. 3983:581
	Mutations in p53 originating from ovarian tumors may be detected in the circulation (plasma or serum) of a significant percent of ovarian cancer patients	Costa J, etal, Keystone Symposia, 27 Jan-2 Feb 1997, (late abstracts):26
	Restoration of wild-type p53 gene increases sensitivity to cisplatin in an ovarian cancer cell line	Husain A, etal, AACR97, Abs. 2848:425
	Identification of p53 gene mutations in epithelial ovarian cancer before and after anti-cancer chemotherapy	p53 gene mutations may be the result of chemotherapy (Kigawa J, etal, AACR97, Abs. 702:105)
bcl-2 and p53	Proteins which regulate apoptosis and outcome in advanced epithelial ovarian cancer	Bozorgi K, etal, AACR97, Abs. 894:134
	Correlations exist between p53 status, bcl-2 expression, apoptosis and chemosensitivity in human cancer cells *in vitro*	Wu GS, etal, AACR96, Abs. 2879:422

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p53PIN3	Insertional tandem repeat in intron 3 of the p53 gene	P53PIN3 may indicate an increased relative risk of non-familial ovarian cancer but not breast cancer (Wang S, etal, AACR96, Abs. 1785:262)
p53 and $p21^{waf1}$	$p21^{waf1}$ is a cyclin-dependent kinase inhibitor, the gene for which is transcriptionally regulated by p53	Results support hypothesis that $p21^{waf1}$ expression has a clinically relevant and central role in p53-mediated response to DNA damaging agents (Al-Azraqi A, etal, AACR96, Abs. 1334:195)
p53 and mdm-2	Latent p53 protein can transactivate the mdm-2 gene in cisplatin-sensitive ovarian carcinoma cells	Wetzel CC and Berberich SJ, AACR97, Abs. 1288:192
RARα and RAXα	Retinoid nuclear receptors	RARa and RAXα play a clear role in regulating growth response of ovarian tumor cells to retinoids (Wu S, etal, AACR96, Abs. 1572:230)
Rb2/p130	A member of the retinoblastoma gene family of tumor suppressor genes	Preliminary data suggests Rb2/p130 may have a prognostic significance in human epithelial ovarian cancer (DePasquale SE, etal, AACR97, Abs. 727:109)
RIα	RIα is the functional subunit of the cAMP-dependent protein kinase A (PKA) which is overexpressed in human tumors	Data suggests that RIα overexpression is associated with the serous subtype of ovarian cancer; inhibition of RIα expression results in decreased cell growth (Mc Daid H, etal, AACR96, Abs. 3527:516)
Stratum corneum chymotryptic enzyme (SCCE) and metallo-protease pump-1	The serine proteases hepsin and stratum corneum chymotryptic enzyme (SCCE) and the metallo-protease pump-1 are overexpressed in ovarian cancer	Data confirms that proteases may play a central role in tumor invasion and metastasis of ovarian carcinoma cells (Tanimoto H, etal, AACR97, Abs. 2765:413)
Telomerase	RNA-dependent DNA polymerize that directs synthesis of telomeric DNA repeats onto the ends of eukaryotic chromosomes	Repressed in most normal cells after fetal life but reactivation is thought to be essential for continuous cell division (Wan M, etal, AACR96, Abs. 3856:562)
Transforming growth factor β (TGFβ)	TGFβ differentially inhibits epithelial ovarian cancer cells from metastatic sites without upregulation of WAF-1	WAF-1 does not seem to be involved in TGFβ regulation of tumor growth in primary ovarian cancer cells (Hurteau JA, etal, AACR97, Abs. 4267:636)
tpr and transcription factor IIB (TFIIB)	Genes which may play a role in the development of platinum resistance	Gilmore P, etal, AACR97, Abs. 3249:485
WAF-1 and mdm-2	WAF-1 and mdm-2 genes may be associated with the apoptotic response to cisplatin and pcclitaxel in ovarian cancer cells	Findings support the role of WAF-1 in the induction of apoptosis and chemosensitivity to certain drugs (Gibb R, etal, AACR96, Abs. 161:23)

Legend
LOH-Loss of heterozygosity

evidence of tumor and a negative CA 125, 56% had elevated M-CSF levels. Also, in 29 patients with a positive second look laparotomy and a negative CA 125, 31% had elevated M-CSF (Amer J Obstet Gynecol, 1991; 165:1356-62). OVX1, an antigenic determinant raised by sequential immunization of mice with different human ovarian cancer cell lines, is elevated in 67% of patients with evident ovarian cancers who are CA 125 negative (Xu FJ, etal, Cancer Research, 1991 Aug 1, 51:4012-9). A retrospective analysis of serum samples from 39 asymptomatic women with normal CA 125 levels who subsequently developed ovarian cancer over the following 2 years, concluded that 21% had elevated OVX1 measurements prior to diagnosis (Int J Gynecol Obstet, 1994;46:83). Measuring complementary serum markers over time may be used as a primary screening technique.

Other tumor markers with marginal usefulness in ovarian cancer screening have also been studied. Evaluation of carcinoembryonic antigen (CEA), encountered in 30% to 65% of epithelial tumors, is mostly seen in advanced disease and does not correlate with clinical status. Another marker, CA 19-9, is elevated in 17%-25% of patients with epithelial malignancies (Schwartz PE, etal, Cancer, 1987 Aug 1, 60(3):353-61). However, addition of CA 19-9 or CEA to CA 125 measurements does not improve either sensitivity or specificity for early detection. Lipid-associated sialic acid (LSA) is measured in the serum of 60% of patients with advanced stage disease. Combination of LSA and CA 125 improves sensitivity of detection of advanced disease (Cancer, 1987; 60: 353-61). Although IL-6 and IL-10 are present in very high amounts in ascites and serum of women with advanced disease (Berek JS, etal, Amer J Obstet Gynecol, 1991 Apr; 164 (4):1038-43), their complementarity with CA 125 is only modest. In addition, measurement of markers associated with HER 2/neu has not proven useful (Berek JS and Martinez-Maza O, J Reprod Med, 1994 Apr; 39(4):241-8).

Ultrasonography

Transabdominal ultrasonography (TAU) was proposed as a potential screening method for ovarian cancer as early as 1982 (Campbell S, etal, Lancet, 1982 Feb 20; 1(8269):425-426). Among more than 5,000 asymptomatic women who underwent three annual ultrasound procedures, 338 had abnormal screens and 326 underwent surgery. Six primary ovarian cancers and 6 metastatic ovarian cancers were detected for an overall specificity of 97.7%, a false positive rate of 2.3% and an odds ratio of 1:67 for primary ovarian cancer (Campbell S, etal, Bmj (Clinical Research Ed.), 1989 Dec 2, 299(6712):1363-7).

Transvaginal ultrasound (TVU) was explored to overcome the disadvantages of TAU. Advantages of this technique include lack of need of a full bladder, ability to scan obese patients more easily and ability of the transducer to be placed closer to the pelvic organs allowing higher ultrasound frequency and, therefore, better resolution (Mackey SE and Creasman WT, Journal of Clinical Oncology, 1995 Mar, 13(3):783-93). In evaluation of a cohort of women with a positive family history of ovarian cancer, transvaginal ultrasound was used to screen 776 who had at least one first- or second-degree relative with ovarian cancer. Three primary ovarian cancers were identified in 39 women who underwent laparotomy. This was consistent with a false positive rate of 5.2%, a positive predictive value of 7.7% and an odds ratio of 1:12 (Bourne TH, etal, Gynecol Onc, 1991 Nov; 43(2):92-97).

Transvaginal color doppler imaging, a more innovative modality is at present the most useful imaging technique. In the largest study using this technique, 56 ovarian malignancies were discovered among 14,317 asymptomatic or minimally symptomatic women. These findings were consistent with a sensitivity of 96.4% and a specificity of 99.8% (Kurjak A, etal, J Ultrasound Med, 1991 Jun; 10(6):295-297). Despite the promise of this technique, it has not been shown to decrease mortality associated with ovarian cancer.

Combination Approaches

Combinations of various screening methodologies are expected to produce better results. An effective approach combines longitudinal CA 125 measurements with ultrasonography. In this case, screening is estimated to result in two operations for every cancer detected, an acceptable rate. Based on a model using annual/semiannual CA 125 and annual ultrasonography, it was estimated that screening cost would be $64,000 per year of life saved which is in line with other screening approaches. However, a decision analysis model to estimate the effectiveness of ovarian cancer screening with CA 125 and TVU (one time screening) calculated an increase in average life expectancy in the population of less than 1 day (Schapira MM, etal, Annuals Int Med, 1993 Jun 1, 118(11):838-43).

HISTOLOGY

Histologic classification of ovarian tumors include common epithelial tumors, sex cord and stromal tumors and germ cell tumors (see Exhibit 2). Epithelial tumors comprise 85%-90% of malignant ovarian tumors. Within this category, types of tumors encountered include serous cystadenocarcinoma, mucinous cystadenocarcinoma, endometroid carcinoma, undifferentiated carcinoma, and clear cell carcinoma. Epithelial ovarian carcinomas are classified as benign, malignant or of low malignant potential. Low malignant potential tumors, also known as borderline tumors, contain neoplastic epithelial cells, increased mitotic activity and nuclear abnormalities, but lack invasion of the supporting stroma. Five-year survival rates for borderline malignancy approach 93% (Bjorkholm E, etal, ACTA Rad Oncol, 1982; 21(6):413-419).

Epithelial cancer occurs mainly in post-menopausal women, at a median age between 60 and 65 years. In women under 30 years of age, germ cell tumors are the most predominant histologic form whereas epithelial cancers are rare (less than 1%). About 20%-30% of ovarian epithelial neoplasms found in post-menopausal women are malignant, compared with only 7% in pre-menopausal women.

For invasive epithelial carcinomas, survival has not correlated with histologic type which has limited prognostic significance independent of clinical stage, extent of residual disease and histologic grade (Devita, principles and Practices of Oncology, 4th edition, 1993). The degree of cellular differentiation of epithelial cancers however, is an important independent prognostic factor that helps predict survival and response (Ozols RF, etal, Cancer, 1980 Feb; 45(3):572-581).

Stromal tumors account for about 8% of all ovarian tumors. They contain granulosa, theca, Sertoli and Leydig and collagen-containing stromal cells. The only stromal tumor seen with significant frequency is the granulosa cell tumor which is associated with feminizing effects and precocious puberty. These tumors tend to be discovered at earlier stages and have an indolent course. Stromal tumors are managed by surgery (Devita, Principles and Practice of Oncology, 4th edition, 1993).

Germ cell tumors comprise about 5% of all ovarian malignancies. They occur in young women and are treated differently than common epithelial ovarian cancers. Combination chemotherapy and limited surgery are curative in most cases. Dysgerminomas, the most commonly encountered germ cell tumors, are similar to seminomas and are very radiosensitive. These tumors are also effectively treated by multidrug chemotherapy (Williams SD, etal, Journal of Clinical Oncology, 1991 Nov, 9(11):1950-5). Endodermal sinus tumors and embryonal carcinomas that are highly aggressive and metastasize hematogenously, are effectively treated with chemotherapy (Devita, Principles and Practices of Oncology, 4th edition, 1993).

CLINICAL FEATURES

Unfortunately, in early stage disease, ovarian cancer has no specific signs or symptoms. Because, the ovary lies in a rather spacious pelvic cavity, an ovarian mass can become quite large before symptoms such as nausea, pain, dyspepsia, lower abdominal discomfort, weight loss, anorexia, constipation or obstipation and urinary frequency, are manifested. As the tumor enlarges, abdominal swelling, pain and fatigue are the most common complaints. Irregular vaginal bleeding may accompany any type of ovarian neoplasm, primarily in premenopausal women. Signs of advanced disease may include abdominal distention and a fluid wave consistent with ascites. These symptoms, however, could also be caused by other conditions or malignancies of other primary sites or carcinomatosis from metastatic gastrointestinal and breast tumors.

Exhibit 2
Ovarian Cancer Incidence by Histologic Type[1]

Histologic Type	Total (%)	North America Incidence[2] (#)	All Others Incidence[3] (#)	Total Incidence (#)
Epithelial tumors	88.0	25,344	76,549	101,893
Serous cystadenocarcinoma	45.6	11,557	34,906	46,463
Undifferentiated carcinoma	18.5	4,689	14,162	18,850
Endometroid carcinoma	16.3	4,131	12,477	16,609
Mucinous cystadenocarcinoma	13.1	3,320	10,028	13,348
Clear cell carcinoma	6.5	1,647	4,976	6,623
Other tumor types	12.0	3,456	10,438	13,894
Sex cord and stromal tumors	66.7	2,305	6,962	9,268
Germ cell tumors	33.3	1,151	3,476	4,626

Note: *Differentiated epithelial ovarian tumors are classified as benign, borderline and malignant*
[1]Based on incidence as reported in FO, p449
[2]1996
[3]1995

DIAGNOSIS AND DISEASE MONITORING

Definitive diagnosis of ovarian cancer is performed by laparotomy. Pelvic examination is recommended for all women, particularly peri-menopausal or post-menopausal females with pelvic or abdominal symptoms. However, the vast majority of palpable adnexal masses (95% in premenopausal and 70-80% in post-menopausal women) are benign. Exploratory laparotomy is indicated in the case of an adnexal mass in pre-menarchal or post-menopausal women because functional ovarian cysts which are common in women during reproductive years and regress in one to three menstrual cycles, should not occur in these age groups.

Of note Pap smears are rarely positive in ovarian cancer. Also, laparoscopic biopsy or needle aspiration of ovarian mass is not advocated because such procedures may cause malignant cells to spill into the peritoneal cavity. Aspiration of cyst fluid for cytologic examination is not a reliable method of detecting ovarian cancer (66% rate of positive cytology) (Rubin SC, etal, Obstet Gynecol, 1988 Jun, 71(6 Pt 1):851-3).

In vitro Diagnostics

Tumor markers helpful in the diagnosis of ovarian cancer include CA 125, alpha fetal-protein (AFP) forendodermal sinus tumors, and human chorionic gonadotropin β-subunit (hCG-β-subunit) for post-surgical evaluation of choriocarcinoma or embryonal carcinoma. CEA, although elevated in 58% of Stage III epithelial ovarian cancers (DiSaia PJ, etal, Cancer, 1977 Jun, 39(6):2365-70), is also elevated in many nonmalignant medical conditions including post-obstructive pulmonary disease, cirrhosis and in those with a history of smoking.

The pre-operative evaluation of patients can be aided by CA 125. Elevated CA 125 levels are most likely associated with malignant adnexal masses in post-menopausal women. In women >50 years-of-age, a serum CA 125 level >35 U/ml indicates that the adnexal mass is likely to be malignant in about 80% of cases. A CA 125 of >95 U/ml is associated with a positive predictive value of 96% in this setting.

Centocor (Malvern, PA) markets a CA 125 radioimmunoassay test, CA 125 II, in the USA and abroad (certain European countries and Japan). Biomira (Edmonton, Alberta, Canada) is marketing the CA 125 Truquant radioimmunoassay kit for detection of ovarian cancer outside the USA. In July 1995, Tosoh (Toyko, Japan), Biomira's licensee obtained regulatory approval to market, in Japan, a diagnostic kit for ovarian cancer that uses Biomira's proprietary technology. The kit is used in Japan to screen or monitor patients with active ovarian cancer or to detect disease recurrence. Tosoh, an affiliate of Industrial Bank of Japan, was granted rights by Biomira to develop and commercialize assays for use with its automated and semiautomated immunoassay systems incorporating murine MAbs against CA 125 antigen. In addition to an initial license fee, Biomira will receive royalties on future sales of Tosoh's assays incor-

porating CA 125. In 1995, Biomira entered into similar non-exclusive licensing agreements regarding CA 125, with Chiron Diagnostics (formerly Ciba Corning Diagnostics; Walpole, MA) and Behringwerke (Liederbach, Germany).

Various other tests are in development in the ovarian cancer setting that may have both diagnostic and screening utility. In the genetic susceptibility area, Myriad Genetics (Salt Lake City, UT), through its wholly-owned subsidiary, Myriad Genetic Laboratory, has obtained a permit to conduct BRCA1 testing, under the brand name BRACAone, in all 50 states and is also offering a combination BRCA1/BRCA2 test. Myriad is collaborating with Eli Lilly in this area (see FO, V2 #4, pp 337). OncorMed (Gaithersburg, MD) has been offering BRCA1 testing at its reference laboratories for the past couple of years. The company combines testing with its own counseling service. Visible Genetics (Toronto, Canada) is using its DNA sequencer to conduct fragment analysis and to locate point mutations in BRCA1 in order to develop an automatic testing method to screen for the gene.

Among companies concentrating on diagnostics, Abbott Laboratories is reported to be developing tests based on the tumor marker OVX1 and complementarity markers. Horus Therapeutics (Rochester, NY) is developing OophorAsure, a serum-based neural network-derived test combining several biomarkers for the diagnosis of early ovarian cancer. The sample analysis is based on the company's computer assisted deductive reasoning system (CADRS) that teaches a neural network to identify discrete changes in complex patterns of biochemical activities.

In vivo Imaging

Radiographic procedures useful in the diagnosis of ovarian cancer include transabdominal ultrasound and transvaginal ultrasound with color doppler imaging, discussed above. Doppler color flow imaging enhances accuracy of sonography and reduces false positive rates (BMJ, 1989; 299:1367). Computer tomography (CT) that is helpful in delineating liver and pulmonary nodules, large abdominal and pelvic masses and retroperitoneal nodes, is useful in ovarian carcinoma if bowel gas makes an ultrasound difficult to interpret (Cancer Treat Rep. 1977; 61:1537-1560). Mammography is performed to exclude primary breast cancer, which can co-exist with ovarian cancer or spread to the ovaries. Another approach to diagnose primary disease, monitor treatment and locate metastases is radioisotope-based imaging.

Ultrasonography approaches, described above, identify malignancy by detecting adnexal pelvic masses with areas of complexity such as irregular borders, multiple echogenic patterns within the mass, and dense, multiple irregular septae (Lancet, 1:425, 1982).

Radioisotope imaging of ovarian cancer is being pursued by various companies that are also deveveloping *in vivo* imaging approaches to diagnose, monitor and evaluate the spread of many different cancers (see FO, pp 276-279).

Biomira's Tru-Scint AD imaging kit, consisting of technetium 99m-labeled MAb 170 which reacts with most adenocarcinomas, in development for breast cancer imaging (see FO, pp 389-340), has also completed phase II clinical trials in ovarian cancer. Tru-Scint AD detected primary ovarian cancer, residual ovarian cancer following surgery, and recurrences, in 77 patients previously diagnosed with ovarian cancer, with a sensitivity of 89%, specificity of 81% and accuracy of 86%. Among 49 women >40 years of age suspected of having ovarian cancer, Tru-Scint AD detected ovarian cancer with an accuracy of 92%, for all three indicators. In early 1996, Biomira filed a New Drug Submission with the Canadian Health Protection Branch (HPB) for Tru-Scint AD for the detection of recurrent breast cancer and of primary, residual or recurrent ovarian cancer, based upon results from the phase II clinical trial.

Laparotomy/Laparoscopy

Diagnosis of ovarian cancer is made definitively by laparotomy. Laparoscopy, when feasible, produces similar results as laparotomy in detecting persistent or recurrent malignant disease but is associated with less blood loss, fewer days spent in the hospital and lower cost. Among 154 patients diagnosed with ovarian, primary peritoneal, or fallopian tube carcinoma who underwent 181 reassessment procedures to detect persistent or recurrent disease between January 1, 1989 and December 31, 1994 at Cedars-Sinai Medical Center, 104 laparoscopic procedures were performed (11 of these were converted to laparotomy due to severe adhesions). Second-look procedures involved 57 of 93 laparoscopies and 69 of 88 laparotomies. There was no significant difference between the two patient groups with respect to age, tumor histology, degree of primary cytoreduction, and tumor stage or grade. There were significant differences between laparoscopy and laparotomy in the following outcome variables evaluated: estimated blood loss (33.9 ml versus 164.9 ml), operative time (81.3 minutes versus 130.4 minutes), days of hospitalization (0.3 days versus 6.8 days), and direct cost/case ($2,765 versus $5,420). Despite obtaining 50% fewer biopsies with laparoscopy than laparotomy, disease was detected at a similar rate that was 47.3% versus 55.7% for all procedures and 52.6% versus 53.6% in second-look procedures (Casey AC, etal, Gynecologic Oncology, 1996 Mar, 60(3):454-61).

Intra-operative Detection/Scanning

Neoprobe (Dublin, OH), as of April 1996, completed a clinical trial using RIGScan CR49 as the targeting agent in ovarian cancer. Although results of the trial showed promise for finding additional occult tumors and better assessment of extent of disease, it was concluded that a targeting agent with a shorter clearing time will be required

to optimize the clinical value of this approach. For this purpose, Neoprobe has acquired from NeoRx (Seattle, WA) global rights to MAb NR-LU-10, a targeting agent with a reduced clearing time.

STAGING

Ovarian cancer is staged by the FIGO classification revised in 1985 (see Exhibit 3) which is based on the extent and location of disease found at surgical exploration. Inherent in the failure of ovarian cancer treatment is the inability to consistently detect disease until it has disseminated. Seventy-five percent of women will present with advanced disease resulting in a five-year survival of 30%-35% (see Exhibits 4 and 5). Adding to this frustration is lack of highly specific and sensitive screening tests, lack of complete resection in all but a few percentages of cases and short lived histopathologic remissions induced by chemotherapy.

Survival from ovarian cancer is highly stage-dependent. Ovarian cancer is usually asymptomatic until it has disseminated. In 75% of patients the tumor has spread beyond the ovary resulting in a 5-year survival of 28%-35%. The 5-year survival in patients with Stage I disease (localized to the ovary) ranges from 66% to 80% (Annals Int Med 1994; 121:124-32) to as high as 90% in a single case report (Cancer 1989;63:1070-3).

PROGNOSIS

Established prognostic factors for survival in ovarian cancer include tumor stage and grade, histologic type, DNA ploidy, patient age and performance status, amount of residual tumor after first laparotomy, and presence of ascites. One factor that confers a poor prognosis in ovarian cancer treatment is chemoresistance to standard first-line platinum-based therapy. However, chemosensitivity testing, performed *in vitro* to individualize chemotherapy, has not proven successful because responses of isolated tumor cells to chemotherapeutics are not, in most cases, reproducible *in vivo*.

A number of the markers listed in Exhibit 1 may eventually prove useful as prognostic indicators. For instance, mutations in p53, a tumor suppressor being extensively studied in all solid tumors, were detected in 15% of Stage I ovarian tumors and in 50% of cases of metasticized ovarian cancer but the significance of this finding is obscured by the fact that it has not been possible to sequence these events. Oncogene c-erbB-2 is dramatically over-expressed in about 30% of ovarian carcinomas and is associated with dire prognosis. Also, *in vitro* studies in ovarian cancer cell lines have shown that blocking of c-erbB-2 over expression with anti-c-erbB2 MAbs results in enhanced cisplatin cytotoxicity.

Exhibit 3
FIGO Stage for Primary Carcinoma of the Ovary at Diagnosis

Stage	Description
Stage I	Tumor limited to the ovaries
Stage Ia	Limited to one ovary; no ascites; no tumor on external surface; capsule intact
Stage Ib	Limited to both ovaries; no ascites; no tumor on external surfaces; capsules intact
Stage Ic[2]	Stage Ia or Ib with tumor on surface of one or both ovaries; or with ruptured capsule; or with ascites present containing malignant cells or with positive peritoneal washings
Stage II	Tumor involving one or both ovaries with pelvic extension
Stage IIa	Extension and/or metastases to uterus and/or tubes
Stage IIb	Extension to other pelvic tissues
Stage IIc[2]	Stage IIa or IIb with tumor on surface of one or both ovaries; or with ruptured capsule; or with ascites present containing malignant cells or with positive peritoneal washings
Stage III	Tumor involving one or both ovaries with peritoneal implants outside pelvis and/or positive retroperitoneal or inguinal nodes; superficial liver metastasis; tumor limited to true pelvis, with histologically proven malignant extension to small bowel or omentum
Stage IIIa	Tumor grossly limited to true pelvis with negative nodes but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces
Stage IIIb	Tumor involving one or both ovaries with histologically confirmed implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter; nodes negative
Stage IIIc	Abdominal implants >2 cm diameter and/or positive retroperitoneal or inguinal nodes
Stage IV	Tumor involving one or both ovaries with distant metastases; pleural effusion must have positive cytologic test to classify as Stage IV; parenchymal liver metastasis

Source: *International Federation of Gynecology and Obstetrics (FIGO) system, 1987*

Note: *Categories are based on clinical examination and/or surgical exploration; histologic characteristics and cytologic tests (if effusions) should be considered; prognostic evaluation of criteria for Stage Ic or IIc should assess if capsule rupture was spontaneous, or caused by the surgeon, and if the source of malignant cells detected was peritoneal washings, or ascites*

Exhibit 4
Estimated Stage Distribution of Ovarian Cancer at Time of Diagnosis in Selected World Regions in 1995

Stage	USA[1]		N. America[1]		Europe		Former USSR		Japan		Triad[2]	
	#	%	#	%	#	%	#	%	#	%	#	%
Localized	6,141	23.0	6,624	23.0	10,317	21.5	4,774	19.0	1,527	22.0	18,468	22.1
Regional	4,005	15.0	4,320	15.0	6,718	14.0	3,392	13.5	1,076	15.5	12,114	14.5
Distant	14,952	56.0	16,128	56.0	27,831	58.0	15,202	60.5	3,922	56.5	47,882	57.2
Unstaged	1,602	6.0	1,728	6.0	3,119	6.5	1,759	7.0	417	6.0	5,264	6.3
All Stages	26,700	100.0	28,800	100.0	47,985	100.0	25,127	100.0	6,942	100.0	83,727	100.0

[1] 1996, [2] N.America, Europe (excluding the former USSR) and Japan

Exhibit 5
Estimated Five-Year Survival of Ovarian Cancer by Stage in Selected World Regions in 1995

Stage	USA[1]		N. America[1]		Europe		Former USSR		Japan		Triad[2]	
	#	%	#	%	#	%	#	%	#	%	#	%
Localized	5,619	91.5	6,028	91.0	9,285	90.0	4,058	85.0	1,390	91.0	16,703	90.4
Regional	2,023	50.5	2,160	50.0	3,225	48.0	1,526	45.0	538	50.0	5,923	48.9
Distant	3,663	24.5	3,871	24.0	6,123	22.0	3,040	20.0	922	23.5	10,915	22.8
Unstaged	433	27.0	467	27.0	780	25.0	369	21.0	112	27.0	1,359	25.8
All Stages	11,737	44.0	12,525	43.5	19,412	40.5	8,994	35.8	2,962	42.7	34,899	41.7

[1] 1996, [2] N.America, Europe (excluding the former USSR) and Japan

ANTI-CANCER DRUG DEVELOPMENT

TOPOISOMERASE I INHIBITORS

The nuclear topoisomerases catalyze topological changes of the double strand DNA, participating in processes of cell metabolism that are critical for cell survival. Topoisomerases alter DNA topology during cell reproduction and DNA replication. To date, four human DNA topoisomerases have been identified, including topo I, topo IIα, topo IIβ and topo III. Both topo I and II ease the torsion created during DNA replication, transcription and recombination by producing a transient cut in one DNA strand (topo I) or both DNA strands (topo II), allowing either topo I or topo II strands to pass through before the cut is rejoined. In contrast to topo II, topo I is expressed throughout the cell cycle and in quiescent cells. Because each topoisomerase is regulated differently, it is expected that each will exhibit different effectiveness as an anti-tumor target. Targeting any or all of these topoisomerases and others that may be identified in the future may provide unique opportunities for drug development.

Topo I inhibitors exhibit broad anti-cancer activity *in vitro* and in animal models which is also being gradually demonstrated in human clinical trials. Although to date all topo I inhibitors in late stages of development are camptothecin analogs, other compounds, currently in research, preclinical and early clinical stages, have also demonstrated similar anti-tumor activities as those seen with camptothecins. Exhibit 6 lists selected topo I inhibitors in development that may prove either more efficacious or less toxic or both.

Exhibit 7 identifies Camptothecin analogs approved in various world markets. Currently, topo I inhibitors are targeting as broad or even broader indication opportunity than taxanes. In view of the latter representing a nearly $1 billion worldwide market, the outlook for topo I inhibitors is even brighter if clinical trials continue to produce positive results. There are, however, a number of problems with the currently commercialized camptothecin analogs. As second-line therapy, their activity is often marginal. Also, although manageable, their toxicity is severe. Nevertheless, in many cases, these agents represent the only option in metastatic disease refractory to standard treatments.

Camptothecin analogs are currently being evaluated in numerous clinical trials, in many cancer types, and in a variety of settings as monotherapy and combination chemotherapy (see Exhibits 8, 9 and 10). Effectiveness of combinations of topo I inhibitors will vary based on the antineoplastic mechanism of the combined chemotherapeutic and the cancer cell type, as has been the case in *in vitro* studies (Kaufmann SH, etal, JNCI, 1996 Jun 5, 88(11):734-41). Topo I inhibitors may also exhibit radiosensitizing effects, as was recently demonstrated in CNS malignancies (Lamond JP, etal, Journal of Neuro-Oncology, 1996 Oct, 30(1):1-6), particularly with

Exhibit 6
Topoisomerase I Inhibitors in Development

Primary Developer ❑ Affiliate(s)	Generic Name ❑ (Composition) ❑ Number ❑ Brand Name	Drug Type ❑ Mechanism ❑ Target ❑ Delivery	Status➢Location ❑ Indication	Comments
	Quercetin	Natural flavone ❑ catalyzes DNA re-ligation	Phase I (95)➢USA, Europe ❑ chemo-prevention, sensitization	
Banyu Tsukuba Research Institute (Merck) and Banyu	NB-506	Indolocarbazole derivative; topo I and DNA and RNA polymerase inhibitor	Phase I (11/96) ➢Japan ❑ various tumors	
BioNumerik Pharmaceuticals ❑ Johns Hopkins Oncology Center; Roswell Park Cancer Institute; Free U (Amsterdam)	Karenitecin ❑ BNP 1350	Supercomputer-engineered highly lipophilic semisynthetic camptothecin ❑ exhibits superior potency and delivery relative to water soluble camptothecins ❑ insensitive to MDR/MRP- or esterase-mediated drug resistance ❑ IV, PO	Preclin➢USA and Europe ❑ solid tumors	
Biotech Research Laboratories ❑ U North Carolina School of Pharmacy	Series of 5,6,7,8-substituted-2-phenylthiochromen-4-ones	Topo I and II inhibitors	Research➢USA ❑ ileocecal carcinoma, leukemia, melanoma, CNS cancer	Wang HK, etal, Journal of Medicinal Chemistry, 1996 May 10, 39(10):1975-80
Daiichi Pharmaceutical	DX-8951, DX-8951f	Camptothecin derivative	Preclin➢USA, Japan ❑ pediatric solid tumors	Weitman S, etal, AACR96, Abs. 2970:435; Mitsui I, etal, Japanese Journal of Cancer Research, 1995 Aug, 86(8):776-82
Glaxo Wellcome	GG211, GI-147211A, GI-147211C	Semi-synthetic camptothecin analogs ❑ bolus, IV	Phase I/II (8/96) ➢USA ❑ refractory solid tumors	Warner D, etal, AACR96, Abs. 2531:371
Glaxo Wellcome Research Institute		Water soluble 7-substituted quaternary ammonium salt derivatives of camptothecin	Research (2/96) ➢USA ❑ ovarian and colon cancer	Two (17 and 18) quaternary salts were more efficacious than topotecan in delaying tumor growth (Lakey K, etal, J Medicinal Chem, 1996 Feb 2, 39(3); 713-9)
Harrier ❑ Institute for Drug Development ❑ U Michigan		Novel series of glycosylated camptothecin analogs	Research (3/96) ➢USA ❑ leukemia, breast cancer and melanoma	Wajima M, etal, AACR96, Abs. 2963:434
IDEC Pharmaceuticals (acquired WW rights in 2/97 from Pharmacia & Upjohn); NCI (co-developer)	9-aminocamptothecin ❑ NSC 603071	Aminocamptothecin derivative ❑ PO	Phase I➢USA ❑ leukemia, prostate cancer	de Souza P, etal, AACR96, Abs. 2936:430; a new colloidal dispersion formulation is being studied (Liang MD, AACR96 Abs. 2954:432)
Imperial Cancer Research Fund (ICRF)	NU/ICRF 505 and NU/ICRF 505/M (metabolite of NU/ICRF 505)	Topo I and II inhibitor; tyrosine conjugate of anthraquinone modified at the C terminus of the amino acid as an ethyl ester	Preclin➢UK ❑ colon and ovarian cancer and nsclc	Cummings J, etal, AACR96, Abs. 2444:358
Imperial Cancer Research Fund (ICRF)	NU/ICRF 600-602	Topo I and II inhibitors	Research➢UK	Meikle I, etal, Anti-Cancer Drug Design, 1995 Oct, 10(7):515-27; NU/ICRF 602 shown to be most active topo I inhibitor

— *continued on next page*

Imperial Cancer Research Fund (ICRF)	NU/ICRF 506	Topo I and II inhibitor	Preclin➢USA ❑ ovarian cancer	Cummings J, etal, Biochemical Pharmacology, 1996 Oct 11, 52(7):979-90; Meikle I, etal, Biochemical Pharmacology, 1995 Jun 16, 49(12):1747-57
Kyowa Hakko	Epiberberine, groenlandicine	Non-camptothecins, natural products isolated from *Coptis* rhizomes	Research➢Japan	Kobayashi Y, etal, Planta Medica, 1995 Oct 6, 61(5) 414-8
Kyowa Hakko Kogyo (Tokyo Research Laboratories)	Saintopin	Antibiotic isolated from *Paecilomyces* sp.; topo I and II inhibitor	Preclin➢Japan ❑ epidermoid cancer	Taniguchi K, etal, AACR96, Abs. 2920:428; Cancer Research, 1996 May 15, 56(10):2348-54; Leteurte F, etal, NCI, Journal of Biological Chemistry, 1994 Nov 18, 269(46):28702-7
NCI	Pyrazoloacridine (PZA) (9-methoxy-acridine) ❑ NSC 366140	Topo I and II inhibitor ❑ DNA intercalator	Phase II (2/97) ➢USA ❑ metastatic colorectal cancer; preclin➢USA ❑ breast cancer	Pelley RJ, etal, AACR96, Abs. 1244:182
NCI	NSC 100880 ❑ camptothecin	Topo I inhibitor, NA salt		
NCI	NSC 95382, 107124, 176323, 249910, 295500, 295501, 364830, 374028, 606172, 606173, 606497 606499, 606985, 610456, 610457, 610459, 610458, 618939, 629971, 643833	Camptothecin derivatives	Preclin (3/97)➢USA	http://www.ncifcrf.gov/DTP/dbs/stslist.html#topo1
NCI	Camptothecin ❑ NSC 94600	Camptothecin analog	Preclin (3/97)➢USA	
NCI	Morpholino-doxorubicin ❑ NSC 354646	Topo I and II inhibitor	Preclin (3/97)➢USA	
NCI	NSC 314622	Camptothecin analog	Research➢USA	Kohlhagen G, etal, AACR96, Abs. 2945:431
NCI	NSC 665517	Camptothecin analog; topo I and II inhibitor	Preclin➢USA	Gupta M, etal, Molecular Pharmacology, 1995 Oct, 48(4):658-65
Novartis	β-lapachone	Novel topo I inhibitor; isolated from *Tabebuia avellanedae* ❑ action differs from camptothecin and chemical structure is distinct from that of current anti-cancer drugs	Preclin➢USA ❑ promyelocytic leukemia and prostate cancer	Planchon SM, etal, AACR96, Abs. 2933:429; Wuerzberger SM, etal, AACR96, Abs. 2934:430; Frydman B, etal, AACR96, Abs. 2671:391; Planchon SM, etal, Cancer Research, 1995 Sep 1, 55(17):3706-11; Li CJ, etal, Cancer Research, 1995 Sep 1, 55 (17):3712-5
Pharmacia & Upjohn	Methoxy-morpholinyl DOX (MMDX)	Anthracycline ❑ doxorubicin analog; topo I (predominant) and II inhibitor	Research➢USA ❑ various cancers	Duran GE, etal, Cancer Chemotherapy and Pharmacology, 1996, 38(3):210-6
Pharmacia & Upjohn	FCE 28536; FCE 29006; FCE 29272	9-aminocamptothecin analogs ❑ IV, PO	Preclin➢USA ❑ leukemias and solid tumors	Bedeschi A, etal, AACR96, Abs. 2692:394

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Rhône-Poulenc Rorer	Intoplicine ❑ RP 60475; NSC 645008	Topo I and II inhibitor ❑ IV	Phase I➢France ❑ solid tumors; preclin ➢USA ❑ pediatric solid tumors	
Rhône-Poulenc Rorer ❑ CNRS (Paris)	5-hydroxy-7-methoxyflavone compounds variously substituted on the B ring including velutin, genkwanin, and lethedocin (7,3´,4´-tri-O-methyltricetin)	Natural product; extract isolated from *L. tannaensis* ❑ flavone	Preclin➢France USA ❑ nasopharyngeal carcinoma and leukemia	Zahir A, etal, Journal of Natural Products, 1996 Jul, 59(7):701-3
SynPhar Laboratories ❑ U Alberta	Series of oligopeptide-substituted bisindolylmaleimides 7-12	Bisindolylmaleimides 7-12 conjugated to lexitropins (novel DNA minor groove binders)	Research➢Canada	Xie G, etal, Journal of Medicinal Chemistry, 1996 Mar 1, 39(5):1049-55
Taiho Pharmaceutical	TAS-103	Topo I and II inhibitor ❑ IV	Phase I (b8/96) ➢USA ❑ refractory solid tumors	Utsugi T, etal, AACR96, Abs. 2915:427; Ishida T, etal, AACR96, Abs. 2922:428
Xenova ❑ CRC Technology, U Auckland	XR5000/DACA	Acridine carboxamide; topo I and II inhibitor	Phase I (c9/96)➢ UK, New Zealand ❑ metastatic cancer (glioblastoma, melanoma, nsclc and breast and colorectal cancer)	Finlay GJ, etal, European Journal of Cancer, 1996 Apr, 32A(4):708-14

9-aminocamptothecin (Lamond JP, etal, International Journal of Radiation Oncology, Biology, Physics, 1996 Sep 1, 36(2):369-76).

CAMPTOTHECIN ANALOGS

Most topo I inhibitors in development are analogs of camptothecin, a natural product that was shown to have anti-cancer activity in the late 1960s (Wall M, etal, J Am Chem Soc, 1966,88:3888-90). Camptothecin was isolated from the *Camptotheca accuminata* tree indigen in China and Japan. Camptothecins are selective topo I inhibitors that act by trapping topo I cleavable complexes and preventing rejoining of the DNA. However, early clinical trials with camptothecin were abandoned because of unacceptable toxicity, notably hemorrhagic cystitis and myelosuppression (Sinha BK, Drugs, 1995, 49(1):11-19). Subsequently, clinical trials were resumed with camptothecin analogs that were created primarily by modification of the A- and B- ring of the five ring structure to increase water solubility and diminish side effects.

Mechanism of Action

To relieve the torsion that develops during DNA replication, topo I produces a transient cut in one strand of the DNA, forms a covalent bond with the cut strand called a cleavable complex, and after the intact strand passes through the cut, reseals the cut DNA strand (Sinha BK, Drugs. 1995, 49(1):11-19). Topo I inhibitors prevent religation (strand transfer) by stabilizing the complex formed between the cut section of DNA and topo I, causing accumulation of the covalent enzyme-DNA complex. During DNA replication, the interaction between the advancing replication system and the stabilized cleavable complex results in a irreversible double-stranded DNA break (Chen AY, etal, Ann Rev Pharmacol Toxicol, 1994, 34: 191-218, Creemers GJ, etal, Cancer Treat Rev, 1994, 20:73-96).

Camptothecin also acts on cells by additional mechanisms. Evidence that camptothecin analogs could induce apoptotic cell death in non-replicating cells (in the absence of DNA synthesis) could provide valuable information for the treatment of slowly growing tumors (Morris EJ, etal, AACR96, Vol. 37, Abs. 150:21). Treatment of cells with topotecan has the potential to increase or decrease topo II expression, and this could be reflected in sensitivity or resistance to topo II inhibitors (Whitacre CM, etal, AACR96, Vol. 37, p 434, #2965). Ubiquitination of topo I is independent of DNA synthesis suggesting that chromatin disassembly is an early step in DNA damage response (Duann P, etal, AACR96, Vol. 37, Abs. 2485:364).

Comparing results obtained during clinical trials with those from animal ones, it became evident that camptothecins are much less active in humans than in rodents against human tumors. This is probably attributed to the fact that in humans the lactone ring of camptothecins opens much faster than in mice. Under physiological conditions the lactone moiety of topotecan

undergoes a rapid and reversible pH-dependent conversion to a carboxylated open-ring form lacking topo I inhibitory activity. This open-ring form predominates at equilibrium (pH 7.4). For instance, under comparable conditions of administration of 9-NC, the area under the curve (AUC) results in values of 3% closed lactone for man versus 55% in mice. It may be that this is a crucial limitation that must be overcome to improve camptothecin anti-cancer efficacy.

Drug Resistance

Although camptothecin is a plant extract, it and most of its derivatives do not appear to be directly affected by the classic P-gp mdr-1 mechanism of resistance. A different type of drug resistance in camptothecin analogs may allow design of novel combination chemotherapeutic regimens. Commonly, resistance to topo I inhibitors is manifested as decreases in the cellular levels of topo I or qualitative changes in the enzyme. Cross-resistance to all camptothecins has been reported but the degree of resistance is far less, with about a tenfold difference (Pommier Y, 5th International Congress on Anti-cancer Chemotherapy, 1995, CPT 11: From DNA Topology to Clinical Activity; p 1).

Various mechanisms of topo I drug resistance have been identified. DNA analysis of five lung cancer cell lines with differing sensitivities to CPT-11 and topotecan and tumor samples from 22 lung cancer patients showed no mutations in the topo I gene, but varying levels of topo I, from high to low (Takatani H, etal, AACR96, Vol. 37, Abs. 2912:426). *In vitro* studies with a human lymphoblastic leukemia cell line and a human nsclc cell line resistant to camptothecin, demonstrated decreases in the cellular levels of topo I. Analysis of complementary DNA of these cell lines demonstrated a point mutation that produced a camptothecin-insensitive form of the enzyme. For CPT-11, another possible mechanism for drug resistance was reduction of its rate of conversion to SN-38, its active metabolite required for anti-tumor activity (Lavelle F, etal, Sem Oncol, 1996;23(suppl 3):11-20).

DNA sequencing demonstrated that topo I of a cell line made resistant to camptothecin had altered amino acid residues that accounted for the resistance (Wang LF, etal, AACR96, Vol. 37, Abs. 3011:P441). Resistance to topotecan and SN-38 was also shown to be mediated by a novel mechanism involving reduced energy-dependent accumulation (Schellens JHM, etal, AACR96, Vol. 37, Abs. 2938:430). Certain resistant cell lines express a novel mechanism of metabolism or efflux for CPT-11 or SN-38 (Sawa N, etal, AACR96, Vol. 37, Abs. 3012:441).

In a human breast cancer cell line (MCF-7/C4) resistance was not found to be caused by alterations in topo I but by changes in the downstream pathways from topo I-induced damage, involving increased p53 levels and increased overall DNA repair activity (Fujimori A, AACR96, Vol. 37, Abs. 2485:364 and Molecular Pharmacology, 1996 Dec, 50(6): 1472-8). Also, diminished p53 activity makes cells more sensitive to camptothecin, and cell cycle checkpoints play a role in camptothecin cytotoxicity (Gupta M, etal, AACR96, Vol. 37, 3014:441). Nucleolar topo I de-localization may be part of the response to the drug, and its modification may represent a novel mechanism of resistance (Wolverton JS, etal, AACR96, Vol. 37, Abs. 2940:430). Another novel mechanism of resistance to camptothecin involves bypass of the camptothecin induced S-phase delay and G2/M-cycle block, independent of the level of cleavable complex formation (Sorensen M, etal, AACR96, Vol. 37, Abs. 3008:440).

Toxicity

Generally, camptothecin analogs are very toxic. However, experience with these drugs has helped anticipate and treat serious side effects allowing more and more patients to complete regimens. In the case of CPT-11, the most common side effect and dose-limiting toxicity (DLT) encountered in weekly dosing schedule is Grade III-IV diarrhea (24% of treatment courses), while Grade III-IV diarrhea (12% of treatment courses) and Grade III-IV neutropenia (21%) are the most common DLT with the every 3-week dosing schedule. Use of intensive loperamide initiated at the earliest change in bowel habits, reduces incidence of severe diarrhea from 24% to 9% of treatment courses. Other toxicities include Grade III-IV neutropenia (12% of treatment courses) and Grade III-IV vomiting (4% and 5% encountered in the two dosing regimens, respectively). Pulmonary toxicity, encountered in several lung cancer patients treated in Japan, appears to be a rare (<1 %) and idiosyncratic side effect of irinotecan (Rothenberg, ML, Chemotherapy Foundation Symposium XIV, November 6-8, 1996, Abs. 18:20).

Myelosuppression (neutropenia, thrombocytopenia, and anemia) is the primary DLT associated with topotecan treatment. Neutropenia was responsive to administration of colony stimulating factors (Burris H, etal ASCO Ed Book, May 1995, 104-11) and anemia can respond to erythropoietin (Mitchell S, etal, ASC96, Vol. 15, Abs. 735:275). In phase II and III clinical trials involving 445 patients with refractory metastatic ovarian cancer, all patients experienced serious hematologic toxicities that were predictable, of short duration and manageable; severe neutropenia occurred in 81% of patients and 56% of patients needed blood transfusions. There were four deaths, three attributable to neutropenia and one to pulmonary embolism. Most common severe toxicities (Grade III/IV) were related to the gastrointestinal system, including nausea (9%), vomiting (7%), diarrhea (4.9%), abdominal pain (4.5%), and constipation (3.1 %); Grade II alopecia was experienced by 42% of patients and headache by 21%.

COMMERCIALLY AVAILABLE TOPO I INHIBITORS

Camptothecin analogs appear to be active against human tumors at every site including the brain. In preclinical trials camptothecins analogs, such as irinotecan

Exhibit 7
Commercially Available Topoisomerase I Inhibitors

Supplier	Brand Name ❑ Location where Approved	Delivery ❑ Regimen ❑ AWP ❑ Treatment Costs	Status ❑ Indication	Market
Irinotecan (CPT-11)				WW 1996 sales were $59 million
Pharmacia & Upjohn (licensee for USA, Canada, Central and South America, Australia and New Zealand)	Camptosar ❑ USA	IV ❑ 30-minute infusion (125 mg/m^2) q week times 4, q 6 weeks ❑ available in 20 mg/ml vials for $98.75 per mg ❑ $1,051 per cycle for 6 cycles ($6,308)	A (6/96), L (96)➢USA ❑ second-line treatment of advanced metastatic colorectal cancer in patients who failed 5-FU-based regimens	USA 1996 sales were $22 million (3,488 courses)
Rhône-Poulenc Rorer (licensee about 100 countries WW including Europe with the exception of Portugal and Spain, Africa, the Middle East, India, etc.)	Campto ❑ France, UK	IV ❑ 30-minute infusion (350 mg/m^2) once q 3 weeks ❑ available in 40 mg (2 ml) and 100 mg (5 ml) vials ❑ $1,363 (UK)-$1,450 (F) per cycle for 6 cycles ($8,176-8,700)	L (9/95)➢France; L (3/97) ➢UK ❑ second-line treatment of advanced metastatic colorectal cancer in patients who failed 5-FU-based regimens	
Yakult Honsha and Daiichi Pharmaceutical (Japan)	Campo (Yakult) and Topotecin (Daiichi)	IV	A and L (4/94)➢Japan ❑ sclc, ncslc and cervical and ovarian cancer	
Topotecan				
SmithKline Beecham	Topotecan (NSC-609699; SKF 104864-A) ❑ Hycamtin	IV ❑ 1.5 mg/m^2 as a 30-minute IV infusion daily for five days q 21 days ❑ $510 for 4 mg (UK, USA) ❑ $1,627 per cycle for 4 cycles ($6,508)	A (5/96), L (6/96)➢USA; L (1/97)➢UK ❑ refractory metastatic ovarian cancer	USA 1996 sales were $23.5 million (3,611 courses)

and topotecan, demonstrated broad activity against a variety of solid tumors which was confirmed in phase II clinical trials and served as the basis for approvals for various indications, including ovarian, colorectal, cervical and non-small cell lung cancer. Other camptothecin derivatives such as 9-aminocamptothecin (9-AC) and GI 147211C also appear promising in early phase trials. Although it is too early to assess the opportunity for camptothecin analogs, their broad applicability and their potential as both first-line and second-line monotherapy and integration into combination and multimodality regimens, augurs well for their future.

Irinotecan (CPT- 11)

Originally isolated from plant material collected by Monroe E. Wall in China in 1957, camptothecin was tested and abandoned by the NCI because of unmanageable toxicity. Synthetic versions of camptothecin were subsequently tested by Yakult Honsha (Tokyo, Japan) in Japan in the early 1980s and CPT-11 (7-ethyl-10-[4-(1-piperidyl)-1-piperidyl]carbonyloxy-camptothecin), the most promising of these, was entered into clinical trials in 1990, in collaboration with Daiichi Pharmaceutical (Tokyo, Japan). Since then CPT-11 has been approved and launched in France, the UK and the USA for treatment of advanced colorectal cancer refractory to 5-fluorouracil (5-FU)-based chemotherapy and in Japan for treatment of non-small cell lung, cervical, and ovarian cancer.

Yakult licensed CPT-11 to Rhône-Poulenc Rorer in Europe, with the exception of Portugal and Spain, which is selling the drug under the trade name Campto. Pharmacia & Upjohn (P&U) obtained a license in 1993 covering Canada, Central and South America, Australia and New Zealand and it is marketing the drug as Camptosar in the USA. CPT-11 is sold as Campo by Yakult Honsha or Topotecin by Daiichi in Japan.

The NDA for Camptosar was filed by P&U in December, 1996 and the drug was unanimously recommended for accelerated approval as second-line treatment of metastatic colorectal cancer that has recurred or progressed following a 5-FU-based regimen by the Oncologic Drugs Advisory Committee of the FDA in June 1997. Recommendation for approval was based on results from phase II clinical trials involving 5-FU pretreated patients. The currently ongoing phase III/IV trial to establish irinotecan's role in the treatment of metastatic colorectal cancer is enrolling chemotherapy-naive patients (see below) and is designed to compare 5-FU and irinotecan regimens in this setting. Because the phase III/IV trial

does not involve similar populations as those evaluated in phase II clinical trials it is feared that poor results as compared to 5-FU regimens may necessitate re-evaluation of irinotecan as second-line treatment of metastatic colorectal cancer. The Committee recommended that P&U undertake a randomized phase III/IV crossover clinical trial to confirm irinotecan's benefit in refractory colorectal cancer and establish optimal treatment regimens.

The drug was launched in France in September 1995 for the same indication as in the USA and in Japan in 1994 for sclc, nsclc and cervical and ovarian cancer. It was filed in France in December 1994 and approved in May 1995. Recommended administration schedules differ in France where the drug is administered as a 30-minute intravenous infusion at an initial dose of 350 mg/m^2, once every three weeks for six weeks and in the USA where the recommended dose of 125 mg/m^2 is administered as a 30-minute intravenous infusion once a week for four weeks followed by a two-week rest period. In Japan, irinotecan is administered on a weekly schedule with breaks only for toxicity. Despite differences in drug administration schedules, irinotecan's anti-tumor activity in the metastatic colorectal cancer setting has been remarkably consistent.

Based on single-agent clinical data, colorectal cancer and nsclc are expected to be the focus for future clinical studies of irinotecan, but the drug is expected to be useful in nearly all major malignancies when administered at different disease stages and in combination with other agents. In addition, use of high-dose irinotecan regimens may broaden the spectrum of tumors sensitive to this agent. A phase I multi-center clinical trial involving 35 patients (26 men and 9 women) with incurable advanced cancer, was undertaken to test the feasibility of treating such patients with high-dose CPT-11. Primary tumor sites included colon, head and neck, unknown primary, kidney, liver, and others. CPT-11 was administered at 600 mg/m^2 (MTD) or 500 mg/m^2 as a 30-minute infusion once every 3 weeks. At the start of the study, 77 cycles (median 1 cycle, range 1-9) were administered to 18 patients at MTD; 14 patients (78%) developed Grade III-IV neutropenia, with febrile episodes in 11 patients (61%), 9 (50%) developed Grade III-IV diarrhea and there was one toxic death. Using a dose of 500 mg/m^2 to treat 17 patients who had not been heavily pretreated, in 84 cycles (median 3, range 1-9) Grade III-IV neutropenia occurred in 7 (41%) and Grade III-IV diarrhea in 4 (24%). There were no cases of febrile neutropenia or toxic deaths. The safety of the 500 mg/m^2 dose was, therefore, considered acceptable. In the study as a whole, there were 6 PR in previously-treated metastatic colorectal cancer, including 4 in patients who had progressed under 5-FU-based chemotherapy (Merrouche Y, etal, ESMO96, Abs. 612P:127).

Colorectal cancer, a key irinotecan target, represents one of the first approved indications of this drug. Approval as second-line therapy was based on the drug's activity in colorectal cancer that relapsed or progressed following 5-FU-based therapy. Colorectal cancer patients who were unresponsive to 5-FU had response rates of 14% to 33% with a median response rate of 25% in phase II clinical trials of irinotecan conducted in the USA, France, and Japan (Burris H, etal ASCO Ed Book, May 1995, 104-11).

Results were significantly poorer in recurrent colorectal cancer. According to ML Rothenberg from the Division of Medical Oncology, The University of Texas Health Science Center (San Antonio, TX), in a presentation at the Chemotherapy Foundation Symposium XIV, November 6-8, 1996 (Abs. 18:20-22), in recurrent colorectal cancer, irinotecan has a response rate of 16%, below the 20% that is considered minimum requirement as evidence of activity of a drug against a particular disease. However, in view of current therapies that have a response rate of less than 10% (actually less than 3%), irinotecan's 16% response rate represents an extraordinary level of activity as second-line treatment. Also, irinotecan's effects are relatively long-lasting, with a median duration of response of 6 months or greater in most studies. In all studies disease stabilized for at least 2 months in a substantial proportion of patients (39% to 70%), and in 25% of patients lasted in excess of 6 months. Because in most of these trials, patients had clear evidence of progressive disease at the time of entry, stabilization of disease, even without objective response, indicates drug activity against the tumor.

Irinotecan also appears promising as monotherapy or combination therapy as first-line treatment of metastatic colorectal cancer (see FO, pp 542-543 this issue). A phase III/IV multi-center trial is underway at Memorial Sloan-Kettering Cancer Center (New York, NY) to further define the activity of irinotecan as first-line chemotherapy in metastatic colorectal cancer. This 3-armed trial, led by Len Saltz, MD, also fulfills the requirement for a phase III/IV post-approval trial mandated by the new FDA regulations for accelerated approvals of oncologic drugs and compares irinotecan alone (125 mg/m^2) every week times 4, every 6 weeks, to 5-FU (425 mg/m^2) and leucovorin (20 mg/m^2), every day times 5, every 4 weeks, and to a regimen of all 3 drugs (irinotecan at 125 mg/m^2 + 5-FU at 500 mg/m^2 + leucovorin at 20 mg/m^2) administered every week times 4, every 6 weeks. The combination arm is supported by results from a phase I clinical trial that demonstrated that all three drugs could be administered according to this regimen. The trial's primary endpoint is time to tumor progression; secondary endpoints include objective response rate, quality of life, and survival. Accrual for this trial that will enroll 660 patients is anticipated to be completed by the end of 1997.

Lung cancer, both sclc and nsclc, also appear to respond to CPT-11. Monotherapy resulted in response rates ranging from 20% to 35% and 33% to 50% in patients with nsclc and sclc, respectively. When used in combination with cisplatin response rates ranged from 43%

to 54%, respectively (Burris H, etal ASCO Ed Book, May 1995, 104-111). More recent studies using this combination have reported response rates of 75% in patients with sclc (Ueoka H, etal, ASCO96, Vol. 15, Abs. 1154:385) and 71% in patients with nsclc (Kobayashi K, etal, ASCO96, Vol. 15, Abs. 169:388). Combinations of CPT-11 and thoracic radiation in nsclc produced a response rate of 76% (Kudoh S, etal, ASCO96, Vol. 15, Abs. 1102:385) and a combination of CPT-11, cisplatin, and thoracic radiation has been used with acceptable toxicity (Yokoyama A, ASCO96, Vol. 15, Abs. 1242:407). Depending on the particular schedule, most effective regimens have been variously defined as CPT-11 (60 mg/m^2) on days 1, 8, and 15 plus cisplatin (60 mg/m^2) on day 1 every four weeks in patients with sclc (Kudoh S, etal, Lung Cancer, 1994, Abstract, 11(suppl 1):104), or CPT-11 (60 mg/m^2) and cisplatin (33 mg/m^2), both on days 1, 8, and 15, every 4 weeks in patients with nsclc (Kobayashi K, etal, ASCO96, Vol. 15, Abs. 1169:388). CPT-11 in combination with etoposide and granulocyte colony-stimulating factor (G-CSF) to minimize neutropenia, resulted in a 42% response in patients with sclc and nsclc (Negoro S, etal, ASCO93, Vol. 12, Abs. 133:l2).

Hematologic malignancies such as refractory leukemia and lymphoma also respond to irinotecan. A late phase II multi-institutional cooperative study of irinotecan was conducted to evaluate its anti-tumor effects and toxicity in refractory leukemia and lymphoma, including adult T-cell leukemia (ATL)-lymphoma. All patients with ATL were refractory or had relapsed after being treated with various conventional combination chemotherapies. CPT-11 (40 mg/m^2) was administered on day 1 for three consecutive days, repeated weekly, until evidence of disease progression. One CR (7.7%) and four PR (30.8%) were noted in 13 assessable patients with ATL. Median total dose to achieve remission was 240 mg/m^2 and median duration of response was 31 days. Major toxicities included leucopenia (83%), diarrhea (62%) and nausea and vomiting (69%) that were relatively severe, but were generally tolerable and reversible. However, one patient died probably as a result of this therapy (Tsuda H, etal, Br J Cancer 70:771-4, 1994).

CNS cancer may also be a target of CPT-11. The effectiveness of CPT-11 was evaluated against a panel of human CNS tumor xenografts growing subcutaneously and intracranially in athymic nude mice. Tumors included childhood high-grade gliomas, adult high grade gliomas, medulloblastoma, ependymomas, and rhabdomyosarcoma and sublines resistant to busulfan, cyclophosphamide, procarbazine, or melphalan. CPT-11 produced statistically significant growth delays of 21 to 90 days in all subcutaneous xenografts tested, there wastumor regression in subcutaneous tumors in every treated animal and increases in survival were demonstrated in two animals with intracranial xenografts. Of over 40 drugs evaluated, CPT-11 was the most active against CNS xenografts (Hare CB, etal, AACR96, Vol. 37, Abs. 2959:433).

Other cancers responding to CPT-11 include squamous cell cervical cancer refractory to platinum-based chemotherapy, that resulted in a response rate of 23% (Kavanagh JJ, etal, ASCO96, Vol. 15, Abs. 758:281) and recurrent and metastatic adenocarcinoma of the ovary treated with a combination of CPT-11 and cisplatin that produced a response rate of 54.5% (Sugiyama T, etal, ASCO96, Vol. 15, Abs. 796:291). A significantly increased median survival in responders when compared to non-responders was seen in patients with clear cell adenocarcinoma of the ovary treated with CPT-11 and mitomycin-C (Shimizu Y, etal, ASCO96, Vol. 15, 761:282). CPT-11 has also demonstrated effectiveness in patients with pancreatic, breast, and gastric cancer (Burris H, etal, ASCO Ed Book, May 1995, 104-111, Ohno R, etal, J Clin Oncol, 1990; 8:1907-10).

Topotecan

Topotecan (9-dimethylaminomethyl-10-hydroxy-camptothecin) was synthesized and evaluated concurrently with irinotecan by researchers at SmithKline Beecham (SKB). Topotecan was the first topo I inhibitor approved in the USA where it is sold as Hycamptin by SKB. The NDA for topotecan was submitted in December 1994, approval was recommended in April 1996 by the Oncologic Drugs Advisory Committee, and was subsequently approved by the FDA in May 1996 as second- or third-line treatment of metastatic ovarian cancer. In this setting topotecan will be competing with the taxanes paclitaxel and docetaxel. However, recent positive clinical trial results using paclitaxel and cisplatin combinations as first-line treatment of metastatic ovarian cancer may position topotecan as the only option as second-line treatment. Topotecan was also authorized for use in the 15 European Union member states as second-line treatment of metastatic ovarian carcinoma in November 1996, and was launched in the UK in January 1997.

Approval was based on phase II clinical trials of topotecan as second- or third-line treatment of metastatic ovarian cancer refractory to platinum-based therapy and a comparison study of topotecan and paclitaxel as second-line treatment. In the latter randomized multi-center trial involving 226 women, topotecan (1.5 mg/m^2) was administered as a 30-minute IV infusion daily for five days (the recommended dose) and paclitaxel (175 mg/m^2) as a three-hour infusion, both every 21 days. Overall response rate in the paclitaxel group was 12.3% (3.5% CR and 8.8% PR) and in the topotecan group 19.6% (5.3% CR and 14.3% PR); disease stabilized in 34.2% and 30.4% and progressed in 49.1% and 34.2%, respectively. There were significantly more (15.2%) non evaluable patients in the topotecan than the paclitaxel (4.4%) group. Median duration of response was 32.1 weeks with topotecan compared to 23.1 weeks with pacli-

Exhibit 8
Selected Irinotecan (CPT-11) Monotherapy Trial Results

Tumor Type	Type of Patient	Dose	Response Rate (CR+PR)	Reference
Colorectal cancer	Prior 5-FU	30-minute IV infusion of 350 mg/m^2, q 3 weeks	20.5%	Bugat R, etal, ASCO94, Vol 13, Abs. 585:200
Colorectal cancer	Prior 5-FU	90-minute IV infusion of a starting dose of 150 mg/m^2 or 125 mg/m^2, weekly for 4 weeks, q 6 weeks	23%	Rothenberg ML, J Clin Oncol, 1996, 14(4):1128-35
Colorectal cancer		350 mg/m^2, q 3 weeks	20%	Rougier P, etal, ASCO94, Vol 13, Abs. 587:200
Colorectal cancer	Naive or prior 5-FU	125 mg/m^2 weekly for 4 weeks, q 6 weeks	Naive 25%; prior 15%	Pitot HC, etal, ASCO94, Vol 13, Abs. 573:97
Colorectal cancer		125 mg/m^2 weekly for 4 weeks, q 6 weeks	32%	Conti JA, etal, ASCO94, Vol 13, Abs. 565:195
Colorectal cancer	Prior 5-FU	100 mg/m^2 weekly or 150 mg/m^2 biweekly	24%	Shimada Y, etal, J Clin Oncol, 1993;11:909-13
Colorectal cancer	Prior 5-FU	350 mg/m^2 as a 90-minute IV infusion, q 3 weeks x 6 or more cycles	14% (44% SD)	Van Cutsem E, etal, Ann Oncology 7 (Suppl 5), 1996, Abs. 156P:34
Breast cancer		100 mg/m^2 weekly, or 150 mg/m^2 biweekly, or 200 mg/m^2, q 3 or 4 weeks	16%	Taguchi T, etal, Jpn J Cancer Chemo, 1944, 21:91
Breast cancer	Prior chemotherapy	350 mg/m^2, q 3 weeks	5%	Bonneterre J, etal, ASCO93, Vol 12, Abs. 179:94
Nsclc		100 mg/m^2 weekly	32%	Fukota M, etal, J Clin Oncol, 1992, 10:16-20
Nsclc	Naive and prior chemotherapy	200 mg/m^2, q 3 or 4 weeks	20%	Nitani H, etal, Lung Cancer, 1994, 11(Suppl 2)30-31
Nsclc	Naive and prior chemotherapy	100 mg/m^2 weekly	Naive 34%; prior 0%	Nitani H, etal, Lung Cancer, 1994, 11(Suppl 2)30-31
Nsclc	73 patients with nsclc, most with advanced disease	Phase II trial; 100 mg/m^2 weekly; Grade 3/4 leukopenia and diarrhea occurred in 25% and 21% of patients, respectively	31.9% ORR (32.5% RR in 40 patients with stage IV disease; median survival was 40 weeks)	Fukuoka M, etal, J Clin Oncol, 1992, 10:16-20
Sclc		100 mg/m^2 weekly	47%	Masuda N, etal, J Clin Oncol, 1992;10:1225-29
Sclc	Naive and prior chemotherapy	100 mg/m^2 weekly	Naive 33%; prior 50%	Negoro S, etal, ASCO91, Vol 10, Abs. 822:241
Ovarian cancer	Prior chemotherapy	100 mg/m^2 weekly or 150 mg/m^2 biweekly or 200 mg/m^2, q 3 or 4 weeks	21%	Takeuchi S, etal. ASCO91, Vol 10, 617:189
Cervical cancer	Refractory disease	125 mg/m^2 weekly for 4 weeks, q 6 weeks	23%	Kavanaugh JJ, etal, ASCO96, Vol 15, Abs. 758:281
Pancreatic cancer		30-minute IV infusion of 350 mg/m^2, q 3 weeks	9%	Wagner DJ, etal, Ann Oncol, 1995, 6:129-32
Advanced solid tumors	Pretreated	30-minute IV infusion of 600 mg/m^2 (MDT) or 500 mg/m^2, q 3 weeks	15.8% PR	Merrouche Y, etal, Ann Oncology 7 (Suppl 5), 1996, Abs. 612P:127

taxel, median time to disease progression was 23.1 weeks and 14.0 weeks and median survival period was 61.3 weeks and 42.6 weeks, respectively. A 400-patient phase III clinical trial of a combination of topotecan and cisplatin as first-line treatment of ovarian cancer is under way. Post-approval studies are expected to investigate different dosing schedules.

In animal studies, topotecan demonstrated broad anti-tumor activity. Significant activity was seen in mice bearing L1210 leukemia and P388 leukemia as well as Lewis lung carcinoma and B16 melanoma. Activity was also seen in clonogenic assays of human breast, ovarian, and colorectal cancer. As a consequence of its effectiveness in these models, phase II trials of topotecan are underway in several different human malignancies. SmithKline Beecham intends to file an NDA for topotecan in recurrent sclc in 1997.

Topotecan is also in phase III clinical trials as an oral formulation. The apparent bioavailability of orally administered topotecan was evaluated in 12 patients in a two-part crossover study. An oral dose of 1.5 mg/m^2 was administered as a 200 ml drinking solution on day 1 and an intravenous dose of 1.5 mg/m^2 as a 30-minute continuous infusion on day 2. Bioavailability, calculated as the ratio of the area under the curve (AUC) of the oral compared to that of the intravenous dose, was 30% with a range of 21% to 45%. The oral dose was well tolerated. AUC ratio of lactone to open ring was similar following the oral dose (0.34 to 1.13) and the intravenous dose (0.47 to 0.98). Median time to maximum concentration was 0.78 hours. Bioavailability of oral topotecan demonstrates significant systemic exposure, which may permit chronic oral treatment (Schellens JH, etal, Br J Cancer, 1996 May, 73(10):1267-71).

Oral dose levels of 1.2, 1.8, 2.3, 2.7 mg/m^2/day for five days every 3 weeks were studied in a phase I clinical trial in 29 patients (14 females and 15 males) with such tumor types as colorectal cancer and ovarian, among others. Among 22 evaluable patients (median age 51 years, performance score 1) of whom 7 were chemotherapy naive, based on 61 treatment courses, DLT, manifested as Grade IV neutropenia, was reached at 2.7 mg/m^2/day. A regimen of 2.3 mg/m^2/day was feasible with (uncomplicated) Grade II-III neutropenia. Non-hematologic toxicity consisted of mild nausea, vomiting, fatigue, and mild alopecia. Blood transfusions were necessary in 5 of 22 patients. Among 6 patients administered a flat dose of 4 mg/day based on 13 evaluable courses, uncomplicated Grade III granulopenia occurred in 1 course (Gerrits CJH, etal, ESMO96, Abs. 597O:124).

A longer duration, oral regimen of topotecan was tested on the observation during *in vitro* experiments that prolonged exposure to topotecan yielded the highest anti-tumor efficacy. Oral topotecan was administered to 20 patients at 0.5, 0.6, 0.7 and 0.8 mg/m^2 twice daily and to 19 patients at 1.0, 1.4 and 1.6 mg/m^2 once daily for 10 days every 3 weeks. Among 33 evaluable patients (6 were not pretreated) with such tumor types as colorectal cancer (15), ovarian cancer (6) and others (12), a total of 61 courses were evaluable with twice daily and 32 courses with once daily topotecan. DLT consisted of a combination of Grade IV neutropenia or Grade IV thrombocytopenia and Grade III-IV diarrhea in both dose schedules. MTD for twice daily oral topotecan was 0.7 mg/m^2 that was associated with mild nausea, vomiting, and diarrhea. For the once daily regimen the feasible oral dose was 1.4 mg/m^2 associated with mild nausea, vomiting, diarrhea and Grade II leucopenia (Burris H, etal, ESMO96, Abs. 605P:126).

Ovarian cancer is the first approved indication for topotecan which produced a response rate of 13% in patients with advanced ovarian cancer who had failed first-line chemotherapy and 14.3% in patients who had failed second-line chemotherapy with cisplatin or carboplatin plus paclitaxel (Gordon A, etal, ASCO96, Vol. 15, Abs. 763: 282). When topotecan was compared with paclitaxel for the treatment of patients with advanced epithelial ovarian carcinoma, it produced a higher median response rate (23% vs. 14%) and longer median time to disease progression (23 weeks versus 14 weeks) than did paclitaxel (Carmichael J, etal, ASCO96, Vol. 15, Abs. 765: 283). Response rates noted in three other studies of topotecan in patients who had failed or were nonresponsive to platinum-based chemotherapy, were 10%, 16.3%, and 37%, respectively (ten Bokkel W, etal, ASCO96, Vol. 15, Abs. 768:284, Speyer J, etal, Abs. 775:285, and Sorbe H, etal, Abs. 828:299). The most common regimen was 1.5 mg/m^2 as a 30-minute infusion on days 1 to 5 every three weeks.

Based on the recommended treatment regimen for ovarian cancer, topotecan, mostly as third-line treatment in patients who fail both cisplatin- and paclitaxel-based regimens, is addressing a limited opportunity estimated at about 12,300 cases in the USA representing a potential market of about $61.5 million. Ultimately, the European opportunity (excluding the former USSR) would represent about 21,800 cases for a potential market of $131 million. However, topotecan is expected to move to second-line treatment status once paclitaxel is approved as first-line treatment, improving its market outlook.

Hematologic malignancies also seem to respond to topotecan; anti-tumor responses have been observed in patients with leukemia (Helley D, etal ASCO96, Vol. 15, p 357,#1050), myelodysplastic syndrome (MDS), and non-Hodgkin's lymphoma (NHL). In a phase II trial of topotecan (1.25 mg/m^2) in advanced NHL being conducted at Ohio State University (Columbus, OH), involving 23 patients (18 males and 5 females), including 10 with low-grade lymphoma and 13 with intermediate-grade lymphoma (median age was 62 years, ranging from 33 to 79), 3 PR (15%) were noted among 20 patients evaluable for response. One patient in CR died in a drug-related death but his response was of insufficient duration to be included

Exhibit 9
Selected Topotecan Monotherapy Trial Results

Tumor Type	Patient Type	Dose	Response Rate (CR+PR)	Reference
Ovarian cancer	Failed (cispaltin-refractory, resistant and sensitive)	Phase II (Europe); 1.5 mg/m^2 30-minute IV infusion on days 1-5, q 3 weeks	16.3% ORR (5.9%, 17.8%, and 26.7%), 1% CR, 15.2% PR	Creemers GJ, etal, Journal of Clinical Oncology, 1996 Dec, 14(12):3056-61
Ovarian cancer	Failed	1.5 mg/m^2 days 1-5, 30-minute IV infusion, q 3 weeks	23%	Carmichael J, etal, ASCO96,Vol 15, Abs. 765:282
Ovarian cancer (epithelial)	Refractory to cisplatin	Phase II; 1.5 mg/m^2 30-minute IV infusion on days 1-5, q 3 weeks (dose reductions at 1.5, 1.25, and 1.0 mg/m^2, were required by 61%, 31%, and 25% of patients, respectively)	14% PR, 61% SD; overall median survival time was 10 months	Kudelka AP, etal, Journal of Clinical Oncology, 1996 May, 14(5):1552-7
Ovarian cancer	Failed	1.5 mg/m^2 on days 1-5, q 21 days	14%	Kudelka A, etal, ASCO93, Vol 12, Abs 821:259
Ovarian cancer	Failed or paclitaxel-treated	1.5 mg/m^2 30-minute IV infusion on days 1-5, q 3 weeks	1st-line failure 13.0%; 2nd-line failure 14.3%	Gordon A, etal, ASCO96, Vol 15, 763:282
Ovarian cancer (advanced epithelial)	Failed cisplatin	Comparison study; 1.5 mg/m^2 30-minute IV infusion of topotecan on days 1-5, q 3 weeks versus 175 mg/m^2 3-hour IV infusion of paclitaxel, q 21 days	Topotecan (n=112): 21% PR, 26 weeks median response duration and 19 weeks median PFS; paclitaxel (n=124): 14%, 22 and 15, respectively	ten Bokkel Huinink W, Ann Oncology 7 (Suppl 5), 1996, Abs. 320O:68
Ovarian cancer (advanced epithelial)	62 first-line failures (FL) and 77 second-(SL) failures	Phase II; 1.5 mg/m^2 30-minute IV infusion of topotecan on days 1-5, q 3 weeks	12.9% FL and 16.9% SL; median response duration and PFS were 24 weeks and 17 weeks and 14 weeks and 11 weeks, respectively	Bolis G, Ann Oncology 7 (Suppl 5), 1996, Abs. 321O:68, International Topotecan Study Group
Sclc	48 chemotherapy-naive patients	2.0 mg/m^2 days 1-5, q 21 days (maximum x 4); some patients were administered G-CSF	39.6% PR of median duration of 4.8 months and median survival of 10 months	Schiller JH, etal, Ann Oncology 7 (Suppl 5), 1996, Abs. 512P:107; an ECOG study
Sclc		1.5 mg/m^2 days 1-5, q 21 days	35.%	Adizzoni A, etal, ASCO94, Vol 13, Abs. 1116:336; also see p ??, this issue
Sclc	36 sensitive (S) and 38 refractory (R) cases based on one prior chemotherapy regimen	1.5 mg/m^2 30-minute IV infusion on days 1-5, q 21 days	22% [(S)-8.3% CR and 11.1% PR and 36% SD; (R)-3% PR and 45% SD (median survival was 26.6 weeks and 20.4 weeks, respectively)]	Eckardt J, etal, Ann Oncology 7 (Suppl 5), 1996,Abs. 513P:107
Nsclc	Untreated patients with stage IIIB or IV disease	Phase II; 30-minute IV infusion of 1.5 mg/m^2 days 1-5, q 21 days	15% PR, 10% MR and 25% SD; overall median survival time was 38 weeks and 30% of patients were alive at 1 year	Perez-Soler R, etal, Journal of Clinical Oncology, 1996 Feb, 14(2):503-13
Nsclc		1.5 mg/m^2 days 1-5, q 21 days	0%	Lynch JJ Jr, etal, J Clin Oncol, 1994, 12 (2):347-52
Colorectal cancer		Phase II; initial dose of 0.6 mg/m^2/d, administered as a continuous infusion via an ambulatory pump for 21 days repeated every 4 weeks; starting dose was reduced to 0.5 mg/m^2/d, because of prolonged myelosuppression	10% ORR, 2.4% CR and 7.3% PR; median response duration was 7 months	Creemers GJ, etal, Journal of Clinical Oncology, 1996 Sep, 14(9):2540-5

— *continued on next page*

Cervical cancer		1.2 mg/m^2 days 1-5 30-minute IV infusion	18%	Noda K, etal, ASCO96, 1996,Vol 15, Abs. 754:280
Prostate cancer		1.5 mg/m^2 days 1-5, 30-minute IV infusion, q 21 days	7.1%	Giantonio R, etal, ASCO93,Vol 12, Abs. 774:274
Renal cell carcinoma		1.5 mg/m^2 days 1-5, q 28 days	0%	Ilson D, etal, ASCO93, Vol 12, Abs. 779:248
Recurrent glioblastoma and anaplastic astrocytoma	Minimally pretreated (radiation or chemotherapy)	Phase II; 1.5 mg/m^2 days 1-5, q 21 days	6% (2/31) PR (1 died and one survived >97 weeks; 68% SD for a median duration of 19 months	Macdonald D, etal, Annals of Oncology, 1996 Feb, 7(2):205-7
CNS malignancies (pediatric)	Either previously treated primary brain tumors refractory to standard therapy, or untreated brain stem glioma or glioblastoma multiforme	24-hour IV infusion every 21 days at an initial dose of 5.5 mg/m^2 with escalation to 7.5 mg/m^2 on the second and subsequent doses in patients who did not experience DLT	Regimen was inactive in high grade gliomas, medulloblastomas, and brain stem tumors; 1 of 2 patients with low grade glioma had a PR lasting >17 months, SD was seen in 3 with brain stem glioma, 1 with malignant neuroepithelial tumor and 1 with optic glioma, lasting 12 to 28 weeks, 41 weeks and 22 weeks, respectively	Blaney SM, etal, Cancer, 1996 Aug 1, 78(3):527-31
Soft tissue sarcoma (STS)		1.5 mg/m^2 days 1-5, q 21 days		Eisenhauer EA, etal, ASCO94,Vol 13, Abs. 488:175
Sarcoma		1.5 mg/m^2 days 1-5, q 3 weeks	10.3%	Bromwell VH, etal, Ann Oncol, 1995, 6:847-9
Pancreatic cancer	Naive	Phase II; 1.25 mg/m^2 daily for 5 days IV, q 21 days	10% PR, 36% SD for at least 8 weeks; median survival was 19 weeks	Scher RM, etal, Investigational New Drugs, 1996, 13(4):347-54
Myelodysplastic syndrome (MDS) and chronic myelomonocytic leukemia (CMML)		2 mg/m^2 by continuous infusion over 24 hours daily for 5 days (10 mg/m^2 per course), q 3 to 4 weeks until remission, then once every month for a maximum of 12 courses	MDS-27% CR, CMML-28% CR; with a median follow-up duration of 8 months, survival rate was 38% at 12 months, median survival time was 10.5 months, and median remission duration 7.5 months	Beran M, etal, Blood, 1996 Oct 1, 88(7):2473-9
Non Hodgkin's lymphoma		Phase II; 1.25 mg/m^2 30-minute IV infusion	15% PR	Kraut EH, etal, Chemotherapy Foundation Symposium XIV, November 6-8, 1996, Abs. 59:67)
Multiple myeloma (resistant/relapsing)	Patients who had prior chemotherapy	Phase II; 1.25 mg/m^2 30-minute IV infusion daily x5, q 3 weeks; G-CSF (5 μg/m^2) after first dose	25% ORR (4.2% CR + 20.8% PR); median PFS and overall survival is 13 and 21 months, respectively	Kraut E, etal, Blood, 86(10) (Suppl 1), #726:185a, 1995; SWOG study
Head and neck cancer		1.5 mg/m^2 days 1-5, 30-minute IV infusion, q 21 days	25%	Robert F, etal, ASCO94, Vol 13, Abs. 905:281

Legend

DLT: dose-limiting toxicity, MR: minor response, ORR: overall response rate PFS: progression-free survival SD: stable disease

in the total. Myelosuppression was the major toxicity with five patients with Grade II or greater neutropenia, including Grade IV neutropenia and thrombocytopenia in the patient who died (Kraut EH, etal, Chemotherapy Foundation Symposium XIV, November 6-8, 1996, Abs. 59:67).

In a similar phase II clinical trial of topotecan being conducted at M.D. Anderson Cancer Center in patients with relapsing lymphoma after treatment with a doxorubicin-containing regimen, topotecan at a higher dose (2 mg/m^2) was administered by intravenous bolus injection

daily for 5 consecutive days every 4 weeks with growth factor support starting on day 6. Among 34 patients (15 males and 19 females) who had entered the study up to the date of presentation, there were 11 (32%) low-grade and 23 (67%) intermediate-grade lymphomas. Among 18 patients evaluable for response (median age was 63.5 years ranging from 27 to 83), 7 (39%) experienced PR lasting 2.7, 4, 5.5, 6+, 7+ and 9+ months and 2 (11 %) CR lasting 3 months and 7+ months, for an overall response rate of 9/18 (50%). DLT was thrombocytopenia with 16 patients (47%) experiencing Grade III and 15 (44%) Grade IV thrombocytopenia; also, 12% developed Grade III anemia, 15% Grade III granulocytopenia with 8% having neutropenic fever episodes. Two patients developed Grade III skin reactions requiring discontinuation of treatment and two others had Grade III hyperbilirubinemia. Only one patient experienced Grade III stomatitis and one Grade III diarrhea. Response rate was similar in patients treated with one or more prior chemotherapy regimens but hematologic toxicity, mainly thrombocytopenia, appears to increase proportionally to the degree of pre-treatment. Current dose schedule is 1.5 mg/m^2 daily for 5 days without growth factor support (Preti HA, etal, ASH96, Abs. 3268:820a).

Refractory or relapsed acute leukemia, is also a topotecan target. In a phase I study conducted at M.D. Anderson Cancer Center, 27 patients with refractory or relapsed acute leukemia, including 17 patients with acute myelogenous or undifferentiated leukemia, 7 with acute lymphocytic leukemia (ALL), and 3 with chronic myelogenous leukemia (CML) in blastic phase, were treated with topotecan (3.5 mg/m^2 to 18 mg/m^2 per course), delivered by a 5-day continuous infusion every 3 to 4 weeks. DLT was severe mucositis occurring in 2 of 5 patients treated with 11.8 mg/m^2 per course; a third patient had prolonged myelosuppression. MTD was defined as 10 mg/m^2 per course. At that dose level 1 of 12 patients had severe and 5 mild to moderate mucositis. Nausea, vomiting, diarrhea, and prolonged myelosuppression were uncommon. Three patients (11%) achieved CR, two (7%) PR, and one (4%) experienced hematologic improvement. Overall response rate (CR+PR) was 19%, and 24% in AML or undifferentiated leukemia. However, in a phase II clinical trial of topotecan (10 mg/m^2) administered as monotherapy by continuous infusion over 5 days every 3-4 weeks, none of the 14 enrolled refractory or relapsed AML patients experienced objective CR. Combination studies of topotecan and cytosine arabinoside (ara-C), or two different schedules of etoposide (VPI6) were also undertaken in AML.

Single-agent activity of topotecan in myelodysplastic syndrome (MDS) and chronic myelomonocytic leukemia (CMML) was evaluated in a clinical trial of 47 patients with MDS (22 patients) or CMML (25 patients). Median patient age was 66 years (81% were 60 years or older). Topotecan (2 mg/m^2) was administered as a daily continuous 24-hour infusion for 5 days every 3-4 weeks until remission, and then once every month for a maximum of 12 courses. Thirteen patients (28%) achieved CR [6 (27%) of 22 patients with MDS and 7 (28%) of 25 with CMML], and 6 (13%) had a hematologic improvement. With a median follow-up of 8 months, 12-month survival rate was 38%, median survival was 10.5 months, and median remission duration 7.5 months. Current studies are investigating topotecan in combination with ara-C in this setting.

In a feasibility study of high-doses topotecan as a continuous infusion for 5 days every 21 days in adults with refractory acute leukemia, severe mucositis precluded dose escalation above 2.1 mg/m^2/day on the 5-day schedule. Various severe toxicities, including hyperpyrexia, rigors, and precipitous anemia, occurred in all 3 patients treated at 5.75 mg/m^2/day by a daily 30-minute infusion for 5 days every 21 days. At MTD of 4.5 mg/m^2/day, severe, albeit brief, mucositis occurred in 2 of 7 patients. Complete tumor clearance in the peripheral blood occurred during most courses with either schedule, and major responses occurred in 2 patients (CML-blast crisis and AML) on the continuous infusion schedule (Rowinsky EK, etal, ESMO96, Abs. 600P:125).

Although chronic lymphocytic leukemia (CLL) appears a reasonable target for topo I inhibitors based on the high topo I levels in CLL cells, a clinical study of topotecan (2 mg/m^2) as a daily 30-minute infusion for 5 days, repeated monthly in 12 previously-treated CLL patients, produced no response. Although DNA-protein cross-linking was detected in all nine patients whose cells were assessed *in vitro*, with levels of cleavable complex ranging from 2-fold to 7.5-fold that of controls, in only two of eight evaluable patients increased cross-linking was detectable in circulating cells after the first dose of topotecan, which was consistent with the drug's lack of effect *in vivo*.

Studies in other lymphoproliferative disorders (multiple myeloma, Waldenstrom's disease) are in progress, as are studies with 9-AC using different infusion schedules (3 days, 7 days, 21 days) and with oral 9-NC. These analyses will hopefully result in studies of optimal dose schedules and combinations based on preclinical studies and early phase I-II clinical trials (Hagop M, etal, Chemotherapy Foundation Symposium XIV, November 6-8, 1996, Abs. 58:65-67).

Progressive high grade gliomas may also respond to single agent topotecan treatment. A phase II clinical trial of topotecan administered as a continuous 21-day infusion every four weeks, starting at 0.4 mg/m^2/day and increasing to 0.5 mg/m^2/day, if no toxicity is observed in the first 2 cycles, enrolled 13 patients (10 male); 9 were pre-treated with chemotherapy. Among 10 evaluable patients, there were 2 objective responses, disease stabilized in 1 and progressed in 7. Grade III/IV toxicities included two cases of anemia and one each of neutropenia and vomiting (Brock CS, ESMO96, Abs. 635P:133).

Exhibit 10
Selected Topoisomerase I Inhibitor-based Combination Chemotherapy Trial Results

Tumor Type	Dose	Response Rate (CR+PR)	Reference
Topotecan (TPT)			
Nsclc	TPT (0.75 mg/m^2) on days 1-5 and cisplatin (75 mg/m^2) on day 1, both q 3 weeks	16.6%	Rothenberg MI, etal, ASCO93,Vol 12, Abs. 423:156
Nsclc	TPT (1.0 mg/m^2) on days 1-5 and paclitaxel (80 mg/m^2) on day 1, both q 21 to 28 days	6.25%	Lilenbaum RC, etal, ASCO94, Vol 13, Abs. 319:131
Nsclc and colon and gastric cancer	TPT (0.17, 0.34, 0.51, 0.68, or 1.05 mg/m^2) on days 1-3 and etoposide (100 mg/m^2) on days 7-9	Not reported	Eckardt JR, etal, ASCO93, Vol 12, Abs. 349:131
Advanced solid tumors	TPT (1.0 mg/m^2) on days 1-5, cisplatin (25, 50, or 75 mg/m^2) on day 1, both q 21 to 28 days	5.9%	Miller AA, etal, ASCO93, Vol 12, Abs. 1367:399
Solid tumors	TPT* (0.35, 0.50, 0.75, or 1.0 mg/m^2) on days 1-3 and doxorubicin (45 mg/m^2) on day 5, both q 3-4 weeks	8.3%	Tolcher AW, etal, ASCO93, Vol 12, Abs. 422:157
Irinotecan (CPT-11)			
Ovarian (naive and failed on cisplatin)	Irinotecan (140 mg/m^2) and mitomycin C (7 mg/m2) both on days 1, 15 and 29	60%	Shimizu Y, etal, ASCO96, Vol 15, Abs. 761:282
Ovarian cancer	Irinotecan (50 or 60 mg/m^2) and cisplatin (50 or 60 mg/m^2), both on days 1, 8 and 15	54.5%	Sugiyama TY, etal, ASCO96, Vol 15, Abs. 796:291
Nsclc	Irinotecan (30, 40, or 60 mg/m^2) weekly x 6 and thoracic radiotherapy (2 Gy) x 30	76%	Kudoh S, etal, ASCO96, Vol 15, Abs. 1102:372
Nsclc	Irinotecan (100 mg/m^2) on day 1 escalated by 20 mg/m^2 increments, cisplatin (20 mg/m^2) continuous infusion on days 1-5 and rG-CSF (2 mg/kg) daily on days 6-21 (recommended dose is 160 mg/m^2 for irinotecan and 20 mg/m^2 for cisplatin)	55%	Mori KY, etal, ASCO96, Vol 15, Abs. 1153:384
Nsclc, sclc	Irinotecan (40 mg/m^2) escalated by 10 mg/m^2 increments and cisplatin (60 mg/m^2), both on days 1 and 8, q 4 weeks (recommended dose is 50 mg/m^2 of irinotecan and 60 mg/m^2 of cisplatin)	Nsclc 75%; sclc 100%	Ueoka H, etal, ASCO96, Vol 15, Abs. 1154:385
Nsclc	Irinotecan (60 mg/m^2) and cisplatin (27, 35, or 44 mg/m^2), both on days 1, 8 and 15, q 4 weeks (recommended dose is 60 mg/m^2 of irinotecan and 33 mg/m^2 of cisplatin)	71%	Kobayashi K, etal, ASCO96, Vol 15, Abs. 1169:388
Nsclc	Irinotecan (30, 40, 50, or 60 mg/m^2) on days 1, 8 and 15 and cisplatin (80 mg/m^2) on day 1, both q 4 weeks (recommended dose is 60 mg/m^2 of irinotecan and 80 mg/m^2 of cisplatin)	54%	Masuda M, etal, J Clin Oncol, 1992;10:1775-80
Nsclc	Irinotecan (70, 80, or 90 mg/m^2) on days 1, 8 and 15, cisplatin (80 mg/m^2) on day 1 and rhG-CSF (2 mg/kg) daily, on days 4-21, except 8, 15 (recommended dose is 80 mg/m^2 of irinotecan and 80 mg/m^2 of cisplatin)	50%	Masuda M, etal, J Clin Oncol, 1994; 12:90-6
Nsclc, advanced (mostly Stage IV)	Irinotecan (60 mg/m^2) as a 90-minute IV infusion on days 1, 8 and 15 followed by cisplatin (80 mg/m^2) on day 1 only, q 28 days	4.2% CR and 25% PR (preliminary 48 patients)	DeVore R, etal, Chemotherapy Foundation Symposium XIV, Nov 1996, pp 3-4

**Some patients received granulocyte colony stimulating factor (G-CSF)*

Other cancers, such as cervical and uterine, may also respond to topotecan; in cervical cancer, topotecan produced an overall response rate of 18% (Noda K, etal, ASCO96, Vol. 15, Abs. 754:280). Anti-tumor responses have also been observed in relapsed gliomas (Burch PA, etal, ASCO96, Vol. 15, Abs. 1164:169), and sclc, nsclc, gastric cancer, hepatoma, colon cancer, and soft tissue sarcoma. Topotecan in combination with cisplatin has also been shown to be effective in nsclc, colon, and breast cancer (Burris H, etal ASCO Educational Book, May 1995, 104-11).

NOVEL CAMPTOTHECIN ANALOGS/DERIVATIVES

Three topo I inhibitors currently in early clinical trials are GI 147211, which has produced objective responses in phase I trials in patients with colon and lung cancer, 9-aminocamptothecin, and DX-8951.

9-Aminocamptothecin

A more water soluble camptothecin analog, 9-aminocamptothecin (9-AC), is currently in phase I/II clinical trials. Pharmacia & Upjohn, the original developer of 9-AC, was asked to divest the drug under a consent degree issued by the FTC as a result of the merger of Pharmacia and Upjohn. IDEC Pharmaceuticals (San Diego, CA) acquired a worldwide license from Pharmacia & Upjohn (P&U) in February 1997, in return of reimbursement of part of P&U's development costs associated with 9-AC, involving an initial fee of $3 million plus milestone payments but no royalties.

9-AC has demonstrated greater potency in animal studies than other clinically available topo I inhibitors. The drug was found to be more effective than camptothecin in studies with tumor xenografts, and to be active against tumors expressing increased levels of P-gp, indicating a lack of cross resistance in mdr-1-positive cells. 9-AC was found to inhibit *in vitro* growth of prostate tumor cell lines PC3, PC3M, DU145, and LNCaP. PC3 cells were implanted in nude mice, and the mice treated by gavage with 9-AC for five to seven days for three weeks or with subcutaneous 9-AC twice weekly. Tumor shrinkage was observed at an oral dose of 0.75 mg/kg/day and at a subcutaneous dose of 4 mg/kg/week. Stable disease was observed at an oral dose of 0.35 mg/kg/day. Plasma and tumor 9-AC levels suggested that 9-AC has a high bioavailability. 9-AC has significant activity against prostate cancer cells *in vivo* and *in vitro* at clinically relevant concentrations (de Sousa P, etal, AACR96, Vol. 37, p 434, #2965).

In a preclinical evaluation of anti-cancer activity of camptothecin and derivatives, administered by different routes (subcutaneous, intramuscular, intravenous, intrastomach, and transdermal), tested in 35 human tumors of various histologic types xenografted at various sites into nude mice and rats, the best anti-cancer/toxicity ratio was observed with 9-nitrocamptothecin (9-NC) and 9-AC into which 9-NC converts in the body of mammals (Giovanella BC, etal, Annals of the New York Academy of Sciences, 1996 Dec 13, 803:181-7).

9-AC is being developed in both IV and oral formulations. In a phase I clinical trial of IV 9-AC, DLT was myelosuppression. The most effective dosage regimen was continuous infusion of up to 72 hours (Sinha BK, Drugs, 1995, 49(l):11-19). In a phase I clinical trial, 52 and 29 patients with refractory cancers were treated either with native camptothecin or 9-NC, respectively. Favorable responses occurred with both compounds (11% with camptothecin and 24% with 9-NC). Diarrhea was the major toxicity with camptothecin, and myelosuppression with 9-NC (Natelson EA, etal, Annals of the New York Academy of Sciences, 1996 Dec 13, 803:224-30). In a phase I clinical trial of escalating dose of oral 9-NC, starting at 1 mg/m^2 daily for 5 consecutive days every week, ORR was 20% (1 CR, 4 PR, 14 NC, 6 PD); 9 patients continued treatment for 3 to 9 months (Verschragen, CF, etal, ASCO96, Abs. 1532:482).

GI 147211

GI 147211 [7-(4-methylpiperazinomethylene)-10,11-ethylenedioxy-20(S)-camptothecin] is a semi-synthetic water soluble camptothecin analog in phase I/II clinical trials in refractory solid tumors. In *in vitro* studies GI 147211 was slightly more potent than topotecan in inhibiting topo I in the cleavable complex assay and was 1.5-2 times more soluble. Tumor cell cytotoxicity assays using 5 separate cell lines demonstrated that GI147211 was 5-10 times more potent than topotecan but drug-induced toxicity appeared comparable (Emerson DL, etal, Cancer Research, 1995 Feb 1, 55(3):603-9).

GI 147211, administered at 0.3 to 0.5 mg/m^2 (MDT) daily by continuous infusion on days 7, 14 and 21, in 38 patients, produced a PR in breast and ovarian cancer. The recommended phase II dose is 0.4 mg/m^2 daily with provision for dose escalation; DLT is thrombocytopenia (Khater, C, etal, ASCO96, Abs. 1536:483); also see FO V1 #2/3, p56; Weitman S, etal, AACR96, Abs. 2970:435; Tong WQ, etal, PDA Journal of Pharmaceutical Science and Technology, 1996 Sept-Oct, 50(5):326-9; Gerrits CJ, etal, British Journal of Cancer, 1996 Mar, 73(6):744-50).

GI 147211 was administered as a daily 30-minute intravenous infusion for 5 consecutive days every three weeks, to patients with solid tumors that were refractory to standard forms of therapy. Doses were escalated in subsequent patient groups from 0.3 to 1.5 mg/m^2/day. In 18 evaluable patients, dose limiting toxicity was neutropenia and thrombocytopenia at 1.5 mg/ml/day with the lowest levels occurring for both on day 15. MDT was 1.2 mg/m^2/day. A partial response was observed in a patient with colorectal cancer (Gerrits C-J, etal, Br Med. J, 1996 March, 73(6):744-50).

Karenitecins

Karenitecins (KTC), under development by BioNumerik Pharmaceuticals (San Antonio, TX), are a novel class

of supercomputer-engineered, highly lipophilic semisynthetic camptothecins exhibiting superior potency and delivery relative to water soluble camptothecins and analogs. BioNumerik believes that these lipophilic compounds will avoid the reduced anti-tumor activity experienced with the water soluble camptothecin analogs, attributed to reduced tissue diffusion and exhibit superior drug delivery and bioavailability. Unlike CPT-11 that as a pro-drug requires activation and undergoes glucurodination and deglucurodination that may cause interpatient variability, KTCs were designed not to undergo such metabolic conversions. KTCs are also insensitive to MDR/MRP- or carboxyl esterase-mediated drug resistance. KTCs exhibit picomolar potency *in vitro* against various human solid tumors including prostate, pancreas, colon, lung, breast and ovarian tumors and melanoma. KTCs exhibit superior oral and parenteral *in vivo* efficacy and potency to other camptothecin derivatives and are engineered for oral use and to exhibit reduced toxicity, particularly diarrhea, superior pharmacologic control and to effectively cross the blood brain barrier (Hausheer F, AACR97, Abs. 1526:227). BNP 1350, under development by BioNumerik is a representative KTC. An important and consistent laboratory observation is that KTCs exhibit anti-tumor activity at picomolar concentrations against common human solid tumors; nearly all of these tumors have p53 mutations or abnormalities. Accordingly, it appears that KTCs may selectively kill p53 mutant cancer cells with great potency and offer a potential practical therapeutic advantage over gene therapy. Orally administered KTCs have recently demonstrated curative activity in human tumor xenografts without toxicity.

NOVEL TOPOISOMERASE I INHIBITORS

Although numerous topo I inhibitors, unrelated to camptothecin, have been synthesized, few have been tested in humans to date. Many of these are dual inhibitors of topo I and II, the benefits of which have not been demonstrated in the clinic.

Flavonoids

In view of their anti-tumor activity and relatively low toxicity, quercetin and related flavonoids (acacetin, apigenin, kaempferol, and morin) appear to be reasonable candidates for development as anti-cancers. Based on the structural features of these compounds it may be possible to identify more potent topoisomerase inhibitors. Moreover, the flavone structure may provide a template for the creation of a second class of topoisomerase-targeted drugs that do not inhibit the DNA cleavage reaction itself, but interfere with the binding of the enzyme to DNA (Boege F, etal, Journal of Biological Chemistry, 1996 Jan 26, 271(4):2262-70). Quercetin is already known to act on topo II religation activity in a similar way as on topo I, apparently via a different mechanism of action. In a phase I trial conducted at Queen Elizabeth Hospital (Birmingham, UK), there was some evidence that quercetin also inhibits signal transduction mechanisms and acts synergistically with carboplatin. Based on results from this trial MDT was established at about 945 mg/m^2.

NU/ICRF 505

NU/ICRF 505, a tyrosine conjugate of anthroquinone, is one of a new class of topo I inhibitors, the anthracenyl-amino acid conjugates. NU/ICRF 505 stabilizes topo I cleavable complexes at micromolar concentrations. NU/ICRF 505 has been found to be active against a panel of human cancer cell lines, including drug-resistant variants, and in animal models. In nude mice it was rapidly metabo-lized to a product that was the only detectable form in plasma and the human HT-29 colon cancer xenograft. This metabolite, found to be the free amino acid produced by cleavage of the ethyl ester bond, stabilized topo I cleavable complexes in assays. Although active against the HT-29 xenograft, it was not active *in vitro* against a panel of human tumor cell lines which included HT-29 and low intracellular levels were found in drug-uptake studies. These data suggest that NU/ICRF 505 is the inactive prodrug in nude mice for the active metabolite, NU/ICRF 505/M (Cummings J, etal, Anti-Cancer Drug Design, 1996 July, 11 (5):367-82). Induction of apoptosis may play an important role in cell death induced by NU/ICRF 505 (Meikle I, etal, Brit J Cancer, 1996 Aug, 74(3):374-9 and Biochem Pharmacology, 1995 Jun 16, 49(12):1747-57).

NB-506

NB-506 (6-N-formylamino-12,13-dihydro-1,11-dihydroxy-13-(beta-D-glucopyranosyl)-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione) was found not only to inhibit growth of the original tumor in xenografts, but also the manifestation of micro-metastasized tumors. In addition, the growth-inhibiting activity of NB-506 against micro-metastases was more potent than against the primary tumor (Arakawa H, etal, Jpn J Cancer Res, 1996 May, 87(5):518-23). In *in vitro* tests combinations of NB-506 and cisplatin demonstrated enhanced topo I inhibitory activity of NB-506 as determined by relaxation of supercoiled *E. coli* DNA (Fukuda M, etal, Cancer Research, 1996 Feb 15, 56(4):789-93). Suggested dose for phase II clinical trials is 350-450 mg/m^2 (Kanzawa F, etal, Cancer Research, 1995 Jul 1, 55(13):2806-13; Yoshinari T, etal, Cancer Research, 1995 Mar 15, 55(6):1310-5; Arakawa H, etal, Jpn J Cancer Res, 1996 May, 87(5):518-23; Fukuda M, etal, Anticancer Research, 1995 Mar-Apr, 15(2):393-8).

NSC 314662

NSC 314662 is a novel topo I inhibitor with a cytotoxicity profile comparable to camptothecin and saintopin in the NCI Drug Discovery Screen. *In vitro* data show that NSC 314662 induces DNA cleavage in a 400 base-pair segment of the c-myc gene at micro-molar concentrations in the presence of topo I. NSC 314662 cleaves some but not all sites induced by camptothecin

and does not affect SV40 DNA unwinding by topo I, indicating that it is not a DNA intercalator. NSC 314662-induced single-stranded DNA breaks in MCF7 cells were protein linked and were more slowly reversed than those induced by camptothecin. Camptothecin resistant cell line CEM/C2 (caused by a topo I point mutation) was cross resistant to NSC 314662 (Kohlhagen G, etal, AACR96, Vol. 37, Abs. 2945:431).

Pyrazoloacridine

Pyrazoloacridine (PZA) (NSC 366140), a 9-methoxy acridine compound, inhibits both topo I and II. Under development by the NCI, PZA is in phase II clinical trials in metastatic colorectal cancer but may also have utility in breast cancer (Pelley RJ, etal, AACR96, Abs. 1244:182; Grem JL, etal, Biochemical Pharmacology, 1996 Jun 28, 51(12):1649-59). In preclinical evaluations PZA interfered with topo I- and II-mediated relaxation of plasmid DNA in a cell-free system, but its cytotoxic effects did not appear to involve a direct interaction with topo I or II (stabilization of the topo I- or II-DNA cleavable complex). Rather, PZA-mediated cytotoxicity correlated strongly with inhibition of DNA and RNA synthesis, and damage to both nascent and parental DNA. Also, PZA-mediated lethality occurred in the absence of DNA replication. PZA appeared to bind avidly to DNA by interfering with the access of replication, repair, and transcription enzyme complexes (Grem JL, etal, Biochemical Pharmacology, 1996 Jun 28, 51(12):1649-59).

In a phase I clinical trial, conducted at Johns Hopkins Oncology Center (Baltimore, MD), PZA was administered on a single-dosing schedule as a 1- to 3-hour infusion at doses ranging from 400 to 935 mg/m^2 and on a multiple-dosing schedule as a 1-hour infusion daily for 5 days at doses ranging from 40 to 180 mg/m^2, every 3 weeks. On the single-dosing 1-hour schedule, CNS toxicity, characterized by neuropsychiatric and neuromotor effects, prompted prolongation of the infusion duration to 3 hours and led to a study of PZA on a multiple-dosing schedule. Both measures resulted in lower incidence of CNS toxicity. Neutropenia was the principal toxicity and precluded dose escalation to levels greater than 750 mg/m^2 on the single-dosing 3-hour infusion schedule and 150 mg/m^2 on the daily times 5 multiple-dosing schedule. Thrombocytopenia, anemia, and nonhematologic effects occurred less frequently. Responses were observed in several patients with platinum-and taxane-refractory ovarian carcinoma and anti-tumor activity was also noted in cervical and colorectal carcinomas. Generally, toxicities were manageable. Neutropenia is the DLT on either schedule. Recommended starting doses for phase II studies of PZA, on single- and multiple-dosing schedules, are 750 mg/m^2 and 150 mg/m^2/d, respectively, for minimally pretreated patients; slightly lower doses are recommended for more heavily pretreated subjects (Rowinsky EK, etal, Journal of Clinical Oncology, 1995 Aug, 13(8):1975-84).

TAS-103

TAS-103, 6[[2-(dimethylarnino)ethyl]amino]-3-hydroxy-7H-indeno[2,1-c]quinolin-7-one dihydrochloride], is a novel dual topo I and II inhibitor developed by Taiho Pharmaceutical (Tokyo, Japan). *In vitro*, TAS-103 exhibited similar topo I inhibitory ability as SN-38, and stronger topo II inhibitory ability than etoposide and was not cross-resistant to several phenotypes such as cisplatin-resistance, MDR and topo inhibitor-resistance (Ishida T, etal, AACR97, Abs. 137:21). In orthotopic implantation models including a colon, two gastric, a renal and three pancreatic cancers, TAS-103 was active in 6 of 7 tumors (86%), whereas CPT-11, VP-16 and cisplatin were active in 71%, 33%, and 57%, respectively (Utsugi T, etal, AACR97, Vol. 38, Abs. 2044:305). TAS-103 strong and broad anti-tumor activity against various tumor models may be associated with inhibition of both topo I and topo II activity. Dose limiting toxicity in mouse, rat, and monkey was leucopenia. No specific organ toxicity was encountered (Utsugi T, etal, AACR96, Vol. 37, Abs. 2915:427).

Other Agents

Mono- bi- and terbenzimidazoles were recently identified as topo I poisons. Terbenzimidazoles substituted with a 5-aryl substituent were active in mdr-1-expressing cell lines (Kim JS, etal, AACR96, Vol. 37, Abs. 2982:436; Sun Q, etal, Journal of Medicinal Chemistry, 1995 Sep 1, 38(18):3638-44 and Kim JS, etal, Bioorganic and Medicinal Chemistry, 1996 Apr, 4(4):621-30).

Intoplicine (RP 60475; NSC 645008), a novel 7H-benzo[e]pyrido[4,3-b]indole derivative that interacts with both topoisomerases I and II, demonstrated high activity in preclinical cancer models, an original mechanism of action and acceptable toxicity profile. In a phase I clinical trial 33 patients (31 patients were pretreated with radiotherapy and/or chemotherapy) with various solid tumors, intoplicine was administered as a 1-hour IV infusion at dose levels ranging from 12 to 360 mg/m^2 (Abigerdes D, etal, Anti-Cancer Drugs, 1996 Feb, 7(2):166-74). Recommended dose for phase II clinical trials is 270 mg/m^2, every 3 weeks (Weitman S, etal, AACR96, Abs. 2970:435).

Protoberberine alkaloids belong to a chemical family of compounds some of which can cause formation of topo I or topo II cleavable complexes. Two protoberberine alkaloids, epiberberine and groenlandicine, obtained from water extracts of *Coptis chenesis* were found to stabilize the mammalian DNA-topo I cleavable complex. Neither induced topo II-DNA complex cleavage. A third compound, berberrubine, produced during the processing of the rhizome, induced formation of topo II cleavable complexes (Kobayashi Y, etal, Planta Medica, 1995 Oct 6, 61(5)414-8). Another protoberberine alkaloid, the antibiotic coralyne that exhibits antileukemic activity in animal models, was shown to be a potent inducer

of topoisomerase (topo) I-DNA cleavable complexes (Makhey D, etal, AACR96, Abs. 2981:436; Wang LK, etal, Chemical Research in Toxicology, 1996 Jan-Feb, 9(1):75-83; Gatto B, etal, Cancer Research, 1996 Jun 15, 56(12):2795-800).

Morpholinyl analogs of doxorubicin such as morpholinyl DOX (MRA) and methoxy- morpholinyl DOX (MMDX) cause DNA breaks by interacting with both topo I and II with predominant inhibition of topo I (Duran GE, etal, Cancer Chemotherapy and Pharmacology, 1996, 38(3):210-6).

MEETING COVERAGE

TOPOISOMERASE I INHIBITORS

FROM THE 21ST CONGRESS OF THE EUROPEAN SOCIETY FOR MEDICAL ONCOLOGY (ESMO), VIENNA, AUSTRIA, NOVEMBER 1-5, 1996

IRINOTECAN

Colorectal Cancer

Results from American and European clinical trials agree that irinotecan is both safe and effective as second-line monotherapy in patients with metastatic colorectal cancer.

Monotherapy as second-line therapy was employed in three studies carried out in the USA in which 304 patients with advanced colorectal cancer, who had failed 5-FU therapy, were treated with irinotecan (100 to 125 mg/m^2) weekly, for four weeks, followed by a two-week rest period (six week cycle). In similar European trials, 455 patients with metastatic colorectal cancer who also had failed 5-FU therapy, were treated with irinotecan (350 mg/m^2), once every three weeks. Despite use of different dosage regimens and slightly different approaches in the American and European studies, irinotecan produced consistent results with regard to response rates, median time to disease progression, and overall survival in all clinical trials. Overall response rates were 13%, with a comparable median time to disease progression of four months. Overall survival was nine months, ranging from 8.0 to 10.5 months. Interestingly, 55% of those treated in the American trials experienced a longer interval to disease progression than when treated with first-line 5-FU (Rothenberg M, CPT-11 in Colorectal Cancer: Debate on Value and Potential, ESMO96, Pg 10).

Irinotecan's efficacy was confirmed as second-line therapy in patients with 5-FU-resistant colorectal cancer, resulting in disease control in about 50% of cases. To evaluate the efficacy of irinotecan as second-line therapy in metastatic colorectal cancer that had progressed within four weeks of prior 5-FU, 107 patients with measurable disease were treated with irinotecan (350 mg/m^2) as a 90-minute infusion once every three weeks. The median number of cycles administered per patient was six (range 1-12) and the relative dose intensity was 0.97 (range 0.62-1.08). Among 95 eligible patients, there were 13 (14%) PRs, disease stabilized in 42 (44%) and progressed in 34 (36%); 6 (6%) enrollees were non-evaluable. Median duration of response had not been reached at the time of presentation, but 11 of 13 patients were still responding beyond 37 weeks. Overall, 54% of responses occurred at cycle six or later. Median duration of stable disease was 20 weeks and median time to disease progression was 17 weeks. Disease was controlled in 50% of patients after six cycles and the probability of being free from progression of disease at six months was 27% (Van Cutsem E, etal, ESMO96, Vol 17, Suppl 5, Abs. 156P:34).

Combination therapy with raltitrexed (Tomudex, Zeneca) was attempted after *in vitro* results demonstrated synergistic effects between SN-38 and raltitrexed, supporting clinical use of this combination in advanced colon cancer. Based on comparisons of different administration schedules (simultaneous or sequential seven-day, 24-hour, and four-hour exposures) and different dose ratios of the two agents, it was deemed that sequential irinotecan to Tomudex short-term exposures should be used in initial phase I/II trials in order to achieve potentiation. Simultaneous long-term (seven-day) exposure to Tomudex and SN-38 produced less than additive cell-kill, independent of the dose ratio used. Also, simultaneous 24-hour exposures failed to produce synergistic cell-kill. In contrast, sequential 24-hour exposures in a five-to-one dosage ratio of Tomudex to SN-38 produces synergistic cytotoxic effects at high levels of cell-kill; greater values were achieved with the sequence of SN-38 followed by Tomudex compared to when Tomudex was administered first. A reduction in the relative dose of Tomudex in the combination resulted in loss of synergism. Sequential short-term (four-hour) exposures produced synergistic cytotoxicity at both low and high levels of cell kill. The synergism occurred at all dose ratios tested and with any sequence of administration. The magnitude of potentiation was greater, however, when SN-38 was given first and a higher relative dose (10:1 ratio) of Tomudex was used (Aschele C, etal, ESMO96, Vol 17, Suppl 5, Abs. 621P:129).

First-line combination therapy with irinotecan is currently being studied to establish its efficacy in combination with the few other drugs active in colorectal cancer, based on its promising results as a single agent in chemotherapy-naive patients with advanced colorectal cancer. At present, in preliminary results from seven phase I/II clinical trials ongoing in Europe to evaluate combinations of irinotecan and oxaliplatin, irinotecan and 5-FU, or irinotecan and Tomudex, all are showing clinical activity. Because different 5-FU regimens are used in clinical practice, trials combining irinotecan and 5-FU use a variety of regimens such as 5-FU plus leucovorin

as a five-day bolus, a 14-day or a 48-hour continuous infusion, and a high-dose weekly schedule. For example, in studies being conducted at the Gustav Roussy Institute (Villejuif, France), Dr. Phillipe M. Rougier and colleagues are combining irinotecan (100 to 220 mg/m^2) with a combination of leucovorin (200 mg/m^2) and 5-FU (400 mg/m^2) as a bolus injection administered one hour after irinotecan, followed by 5-FU (600 mg/m^2) as a 22-hour continuous infusion, every two weeks. Activity has been seen at every level, with one CR and two PRs out of six patients at even the lowest doses of irinotecan (100 mg/m^2), suggesting an effective combination.

At the Paul Brousse Hospital (Villejuif, France), Dr. Jean-Louis Misset and collaborators are evaluating the combination of irinotecan (150 to 350 mg/m^2) and oxaliplatin (30 to 130 mg/m^2) every three weeks, as first line treatment for advanced colorectal cancer. In 12 trial enrollees to date, dose limiting toxicities have not been reached at irinotecan dose of 200 mg/m^2 and oxaliplatin dose of 100 mg/m^2. Several objective responses were seen at these preliminary stages.

A combination study of irinotecan and Tomudex is ongoing under the auspices of Dr. David Cunningham at the Royal Marsden Hospital (London, UK). In this phase I/II clinical trial, patients with advanced colorectal cancer are being treated with irinotecan (175 to 350 mg/m^2) and Tomudex (2.6 mg/m^2 to 3.0 mg/m^2), every three weeks. The study has just begun, but minor responses already have been seen in patients with reported 5-FU resistant colorectal cancer (van Oosterom AT, CPT-11 in Colorectal Cancer: Debate on Value and Potential, ESMO96, Pg 18).

Lymphoma

Combination chemotherapy with irinotecan and Adriamycin was attempted as salvage therapy for refractory or relapsed malignant lymphoma based on the observation that irinotecan is effective against malignant lymphoma and may overcome Adriamycin resistance. In a pilot study, a combination of irinotecan and Adriamycin was tested on 12 patients with relapsed (3) or refractory (9) lymphoma. Irinotecan (25 mg/m^2) was administered intravenously on days one and two, followed sequentially by Adriamycin (40 mg/m^2) on day three. The overall response rate was 41.6%, including four CRs and one PR. Duration of response ranged from 11 to 20 weeks. Major drug-related adverse effects included leukopenia (8 patients), thrombocytopenia (3 patients), and diarrhea (1 patient) (Saotome, T etal, ESMO96, Vol 17, Suppl 5, Abs 540P:113).

TOPOTECAN

Ovarian Cancer

Topotecan was evaluated as second-line treatment in an open-label, multinational comparative study in the treatment of recurrent ovarian cancer. Treatment with topotecan resulted in a higher response rate and a longer duration of response compared to paclitaxel, an agent approved for the treatment of advanced ovarian cancer. This is of major importance because advanced ovarian cancer is extremely difficult to treat. In this study, 226 women with recurrent ovarian cancer were randomized to either topotecan (1.5 mg/m^2) as a 30-minute infusion, administered daily for five days every 21 weeks, or paclitaxel (175 mg/m^2) as a three-hour infusion, administered every 21 days. Treatment continued until disease progression or drug intolerance. The overall response rate, all PRs, was 1% (24/112) in the topotecan-treated group compared with 14% (16/114) in the paclitaxel-treated group. Median duration of response was 26 weeks with topotecan and 22 weeks with paclitaxel. Progression-free survival was also longer in the topotecan group (21 weeks) as compared to 15 weeks in the paclitaxel group. Overall survival was 65 weeks in the topotecan group and 59 weeks in the paclitaxel group (ten Bokkel Huinink W, etal, ESMO96, Vol 17, Suppl 5, Abs. 320O:68).

Topotecan was also shown effective in the treatment of advanced ovarian cancer resistant to prior therapy with paclitaxel and cisplatin or carboplatin. In an open-label multi-center, phase II clinical trial, 139 women with advanced ovarian cancer were treated with a daily 30-minute intravenous infusion of topotecan (1.5 mg/m^2), for five days, every 21 days. Among patients in this group, 62 had failed first-line therapy and 77 second-line therapy; 81% were refractory or had relapsed within six months of first-line therapy. The response rate among first-line failures was 12.9% (8/62), with a median duration of response of 24 weeks and a progression-free survival interval of 17 weeks. In second-line failures, the response rate was 16.9% (13/77), with a median duration of response of 14 weeks and a progression-free survival interval of 11 weeks. Regarding side effects, hematologic toxicities were reversible, non-cumulative, and generally not associated with significant clinical sequelae. Fever and/or infection associated with Grade 4 neutropenia was encountered in 5% of treatment courses. Non-hematologic toxicities, including nausea, vomiting, alopecia and fatigue, were generally mild (Bolis G, etal, ESMO96, Vol 17, Suppl 5, Abs. 3210:68).

Small Cell Lung Cancer

A multi-center European clinical trial demonstrated that topotecan exhibits significant anti-tumor activity in previously-treated sclc. Two groups of sclc patients, 51 refractory to first-line chemotherapy and 50 sensitive to first-line therapy who had relapsed, were treated with topotecan (1.5 mg/m^2), delivered as a 30-minute IV infusion for five consecutive days, every three weeks, until disease progressed or until patients grew intolerant of treatment-related side effects. An average of three to five courses of topotecan were delivered. Of 86 evaluable patients, the overall response rate was 39% (six CR and 11 PR) among the 44 patients who were sensitive to first-line chemotherapy, and 7.0% (one CR and two PR), among the 42 patients who were refractory to first-line chemother-

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apy; overall duration of response was 7.6 months and 6 months, respectively. Median survival of responders was 12.4 months. The major dose-limiting toxicity was hematologic, with universal leukopenia (short-lived) and Grade 3/4 neutropenia occurring in 3/4 of patients. Hematologic toxicity, however, was predictable, non-cumulative, and manageable. Non-hematological toxicity was mild, with fatigue reported in a third of patients (Adizzoni A, etal, ESMO96, Vol. 17, Suppl 5, Abs. 509O:106).

Metastatic Brain Cancer

Topotecan effectively reduces the size of tumors that have metastasized to the brain in patients with sclc after failure of first-line therapy. In this study, 29 sclc patients with brain metastases were administered topotecan (1.5 mg/m^2) as a daily 30-minute intravenous infusion, for five days, every three weeks. Three patients were treated by a continuous 21-day intravenous infusion of topotecan (0.4 mg/m^2) delivered every 28 days. Status of brain metastases was evaluated bimonthly by CT scanning. In 19 evaluable patients with asymptomatic brain metastases, overall response rate was 63%, with 4 CR and 8 PR. Disease stabilized in five patients and progressed in two. The fact that topotecan appears to penetrate the intact blood-brain barrier suggests that it may be useful in treating other types of brain metastases as well as primary brain tumors (Staab H-J, etal, ESMO96, Vol 17, Suppl 5, Abs. 510O:106).

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