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

MALIGNANT MELANOMA

MEETING COVERAGE

19TH INTERNATIONAL CONGRESS OF CHEMOTHERAPY, MONTREAL, QUEBEC, CANADA, JULY 16-21, 1995

MECHANISMS IN MALIGNANCY

DRUG RESISTANCE IN CANCER-PART III

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

MALIGNANT MELANOMA

Malignant melanoma is a relatively rare tumor. Although highly aggressive it is usually curable when treated in its early stages. In spite of its low prevalence and limited impact, melanoma has become the object of a disproportionally large research effort because of its immunogenic nature. Melanoma elicits both host humoral and cellular responses, and techniques developed to understand and exploit this phenomena may lead to the discovery of immunotherapy strategies to treat this and other cancers exhibiting similar behavior.

ETIOLOGY AND RISK FACTORS

The etiology of melanoma remains obscure. Risk factors associated with melanoma include hereditary predisposition, mutations in pivotal genes, occupational and/or environmental factors, exposure to ultraviolet (UV) radiation, and various precancerous and cancerous conditions. Risk for development of malignant melanoma has been reported to be greater in persons of higher socioeconomic status. According to a study sponsored by the American Cancer

Society, the risk of development of malignant melanoma in men was significantly higher in high-paying versus low-paying occupations (odds ratio = 1.58) and in white-collar versus blue-collar occupations (odds ratio = 1.33). No significant conclusions could be drawn for women. Interestingly, no significant differences in risk were observed between those with indoor versus outdoor occupations. Among specific occupational exposures, only exposure to X-rays significantly raised malignant melanoma risk (odds ratio = 1.37) (Pion IA, etal, Cancer, 1995 Jan 15, 75(2 Suppl):637-44).

Although rising incidence of all skin cancer (basal cell and squamous cell carcinoma as well as malignant melanoma) among fair-skinned Caucasians in the developed world has been attributed to increased exposure to ultraviolet (UV) radiation, the connection between such exposure and the development of skin cancer has not been elucidated. Nevertheless empirical observations indicate that UV exposure is one of the major contributing factors. For instance, the risk of contracting cutaneous and ocular melanomas is much higher among fair-skinned than dark-skinned individuals, indicating a protective effect of melanin against sun exposure. Although it is possible that sunlight exposure is an indirect risk factor, (by stimulating growth factor production, which then stimulates melanocytic proliferation, leading to melanoma), it is more likely that sunlight effect on melanoma is primarily direct. For instance, visceral melanomas, an extremely rare form of this cancer, occured with similar frequency in blacks and whites, as was shown in a study of 25,184 visceral melanoma cases (Neugut AI, etal, American Journal of Public Health, 1994 Nov, 84(11):1828-9).

Epidemiologic data suggests that it is the repeated sun tanning and sunburns that place individuals at higher risk for malignant malanoma. Exposure to shorter wavelength UVB radiation (280-320 nm in wavelength) is considered the most damaging, although UVA radiation (320-400 nm in wavelength) may also be harmful. Interestingly, sunscreens are very effective in blocking UVB but not UVA, leading to the hypothesis that exposure to UVA is the culprit for the rise in the incidence of malignant melanoma.

Skin exposure to potentially carcinogenic therapies, such as ionizing radiation or alkylating agents, may also give rise to secondary cutaneous malignancies. Six out of 164 patients with cutaneous T-cell lymphoma treated by total skin electron beam therapy between 1974 and 1990 developed malignant melanoma within 12 to 95 months of treatment. Three of the six patients had also received oral psoralen with UVA as additional therapy and two had received topical mechlorethamine (Licata AG, etal, Archives of Dermatology, 1995 Apr, 131(4):432-5).

Certain skin lesions are also markers for increased risk. The most important precursor/marker for melanoma is the atypical mole or dysplastic nevus which occur in 5-10% of the USA population. Although in some cases these nevi have been observed to evolve into cutaneous melanoma, the frequency of conversion to melanoma of any single nevus is quite low. However, in melanoma-prone families, these cancers are associated with dysplastic nevi in more than 80% of the cases. Also, giant congenital melanocytic nevi are associated with a greatly increased risk of melanoma (approximately 6% lifetime risk of melanoma development). In a study conducted to assess the degree of risk associated with such nevi, among 33 patients with a congenital nevus covering at least 5% of body area, two melanomas occurred during follow-up that were fatal. Patients with nevi 1% to 4% of body area did not exhibit increased risk. Lentigo maligna develop into invasive melanoma with a frequency reported in the literature ranging from 5-50% (Sober AJ and Burstein JM, Cancer, 1995 Jan 15, 75(2 Suppl):645-50 and Swerdlow AJ, etal, Journal of the American Academy of Dermatology, 1995 Apr, 32(4):595-9).

Patients with a history of basal cell and/or squamous cell skin cancer are at substantial increased risk for developing malignant melanoma. Ten of 290 white patients with a history of non-melanoma skin cancer but with no personal or family history of malignant melanoma developed this cancer within an average of 109 months of follow-up (range, 3-17 years). All cases were less than 0.70 mm in Breslow thickness and 80% occurred on non-exposed sites. The expected number of malignant melanoma in the control population was 0.59, resulting in a relative risk of 17 for those with a history of skin cancer (Marghoob AA, etal, Cancer, 1995 Jan 15, 75(2 Suppl):707-14).

Retinoblastoma patients (often exhibiting germline mutations in the retinoblastoma tumor suppressor gene) and their relatives also appear to have an increased risk of other cancers, especially malignant melanoma, which represents 7% of second primaries in retinoblastoma survivors. Individuals belonging to families with the atypical mole syndrome (encountered in families with a genetic susceptibility to melanoma) have a recognizable phenotype, with many atypical melanocytic nevi (Bataille V, etal, British Journal of Dermatology, 1995 Jan, 132 (1):134-8). Intraocular malignant melanoma is also linked to familial atypical multiple mole syndrome.

PATHOGENESIS, CLASSIFICATION, STAGING AND PROGNOSIS

The genesis, evolution and progression of malignant melanoma are not fully understood. Although various genetic factors have been linked to melanoma (see Exhibit 1), their role is unclear. Even the role of the generally accepted contributor to melanoma, UV radiation exposure, remains obscure. However, although the precise molecular impact of UV radiation in the pathogenesis of melanoma is not known, UV has been shown to alter expression of multiple genes in both melanocytes and melanoma cells, some of which may have a role in the initiation or progression of melanoma (AACR95, Abs.

Exhibit 1
Various Genetic Markers Associated with Malignant Melanoma

Gene/Chromosome	Product	Comments
MAGE gene family	Codes for antigens recognized by autologous cytotoxic T lymphocytes (CTL) on melanoma tumors	Not expressed in normal tissues except for testis
MAGE-1	Melanoma peptide antigen (46 kDa) recognized by cytotoxic T-lymphocytes	
MAGE-3		
BAGE	Codes for a putative protein of 43 aa; seems to belong to a family of several genes; BAGE is expressed in 22% of melanomas (Boel P, etal, Immunity, 1995 Feb, 2(2):167-75)	Like the MAGE genes, it is silent in normal tissues with the exception of testis; the antigen recognized by the autologous CTL consists of BAGE-encoded peptide AARAVFLAL bound to HLA-Cw 1601 molecule
Tyrosinase gene	Codes for differentiation antigens recognized on human melanoma by autologous CTL	
Human leukocyte antigen (HLA) genes	Linked to susceptibility to a variety of malignancies, including melanoma	
HLA class II allele DQB1*0301	The DQB1*0301 allele was present in 56% of melanoma patients vs. 27% of 200 Caucasian controls; presence of DQB1*0301 in melanoma patients was associated with advanced disease	The difference was highly significant; no other class II allele tested was present at significantly increased or decreased frequency in melanoma patients (Lee JE, etal, International Journal of Cancer, 1994 Nov 15, 59(4):510-3)
Chromosome 9p21-22	A locus for familial melanoma has been mapped on this chromosome	
p16 gene (CDNK2)	p16 was mapped in this region; frequent homozygous deletions and point mutations of p16 gene were reported in melanoma cell lines	A low frequency of p16 mutation suggests the existence of another gene(s) in 9p21 involved in development of melanoma; however, in melanoma derived cell lines, some reported deletions involved potential p16 flanking regulatory regions rather than the coding region (AACR95, Abs 3448)
Chromosome 11	Loss of heterozygosity of the long arm of chromosome 11 is detected in at least 35% cases of malignant melanoma and may play a role in this malignancy (AACR95, Abs 3266)	Chromosome 11 suppresses cell growth and tumorigenicity in human malignant melanoma cells and is the site of at least two known tumor suppressor loci; chromosome 11 may be the site of a tumor suppressor gene whose loss of function plays a role in the development of human malignant melanoma (AACR95, Abs 1167)
Chromosome 17	Presence of chromosome 17 in metastatic melanoma cell lines protected them from anti-Fas induced apoptosis (AACR95, Abs. 30)	
Chromosome 6	Introduction of normal, neo-tagged human chromosome 6 (neo6) into highly metastatic human melanoma cell line C8161 resulted in complete suppression of metastasis *in vivo*	A metastasis-suppressor gene(s) on human chromosome 6 may regulate cellular motility and thus inhibiting metastasis (You J, etal, Biochemical and Biophysical Research Communications, 1995 Mar 17, 208(2):476-84)

3655). It is also hypothesized that sunlight affects immune function perhaps by causing the release of normally hidden antigens or inducing the production of antigens that are not normally present in the host. Also, urocanic acid, a natural component of the stratum corneum may be implicated in the spread of malignancy. It is postulated that urocanic acid suppresses autoimmune responses originally triggered to remove burned skin cells and, inadvertently, also suppresses host immune responses against those skin cells that became malignant.

Melanomas are classified according to several schemes (see Exhibit 2). Although malignant melanoma is an aggressive tumor, it is highly curable if detected in its early stages. This is reflected in overall mortality and in 5-year survival rates (see Exhibit 2). However, 50% of patients with deep (> 4 mm) primary melanomas, 60-85% of those with regional lymph node metastases (stage III), and 95% of those with metastases to distant sites (stage IV) experience recurrence, which is associated with a dismal prognosis. Interestingly, malignant melanoma is the single most common tumor reported to spontaneously regress, although the incidence such regressions is less than 1%.

EPIDEMIOLOGY

Malignant melanoma is a relatively rare cancer. Worldwide, the highest rates are among whites (see

Exhibit 2
Various Classification Schemes of Malignant Melanoma

Cellular Classification	Typical Distribution*	Comment
Superficial spreading	79	Risk factors included presence of large nevi, light hair color, light complexion, and maternal Northern or Central European ancestry
Nodular	13	Risk factors included presence of large nevi, light hair color, ever being over-weight by 20 pounds (9 kg) or more, and the presence of freckles
Lentigo maligna	3	
Acral lentiginous (palmer/ plantar and subungual)		Subungual melanoma is a unique and rare melanoma subtype accounting for only 1% to 3% of all melanoma cases
Other rare types (mucosal lentiginous, desmoplastic and verrucous)		
Unclassified	5	

Staging Classification

Breslow's Classification-Thickness (mm)	Clark's Classification-Level of Invasion	TNM Classification-Primary Tumor (PT)	Clinical Stage	5-year Survival Rate
≥0.75	Level II (invasion of the papillary dermis, but does not reach the papillary-reticular dermal interface	pT1	Stage I	100% (10-year survival rate is 95%)
0.76 - 1.50	Level III (invasion fills and expands the papillary dermis, but does not penetrate the reticular dermis)	pT2	Stage I	85% (10-year survival rate is 75%
1.51 - 4.0	Level IV (invasion into the reticular dermis but not into the subcutaneous tissue)	pT3-pT3a (tumor more than 1.5 mm but not more than 3 mm in thickness) -pT3b (tumor more than 3 mm but not more than 4 mm in thickness)	Stage II	65% (10-year survival rate is 30%)
≤4.1	Level V (invasion through the reticular dermis into the subcutaneous tissue)	pT4 (and/or satellite(s) within 2 cm of the primary tumor) -pT4a (tumor more than 4 mm in thickness and/or invades the subcutaneous tissue) -pT4b (satellite(s) within 2 cm of the primary tumor)	Stage III	20% (10-year survival rate is no greater than 15%)

** Based on a total of 452 women with cutaneous malignant melanoma who participated in a population-based case-control study carried out in the San Francisco Bay Area between 1981 and 1986 (Holly EA, etal, American Journal of Epidemiology, 1995 May 15, 141(10):934-42).*

Exhibit 3) in affluent countries. In the USA approximately 34,100 new cases are expected in 1995, compared to one million cases for all skin cancer. The overall incidence rate of malignant melanoma in the USA is estimated at 12.9 per 100,000 in 1995, significantly higher than the 10.9 per 100,000 estimated for 1990. Although overall incidence is higher among males (14.5 versus 11.4 per 100,000) this does not hold true by age group. Before age 40, white females have higher rates than white males; from age 40 to 44 there is no difference; for those <45 years of age, rates are higher among white males; and by age 65 and thereafter, men develop melanoma at more than twice the rate of women. Melanoma mostly affects whites; rates among blacks are only 0.8 per 100,000.

Similar rates are reported from various European countries and other industrialized world regions. According to a study conducted by the Cancer Research Campaign (London, UK) in seven areas in England and Scotland, representing a population of 3.6 million, the average annual age-adjusted incidence rates of melanoma were 7 and 12 per 100,000 in males and females, respectively. The incidence was significantly higher in females than males and increased with age. Fifty-three percent and 65% of tumors in males and females, respectively, were thin (Breslow thickness ≤1.5 mm), similar to the national figures from Scotland (Melia J, etal, British Journal of Dermatology, 1995 Mar, 132(3):414-21). In France, approximately 5,000 new cases of malignant melanoma are estimated for 1995, representing a rate of 8.6 per 100,000 population. Total deaths are estimated at 1,000.

Melanoma is rare in children. Only 2% of melanomas occur in patients under the age of 20 years, and 0.3% to 0.4% occur in prepubertal children. As in adults, approx-

Exhibit 3
Incidence and Mortality Associated with Malignant Melanoma in Selected World Regions

Incidence (#)

	Male	Rate*	Female	Rate*	Total	Rate*
USA	18,700	14.5	15,400	11.4	34,100	12.9
Japan	1,513	2.5	1,794	2.9	3,308	2.7
Europe-EEC	15,591	9.1	17,816	10.0	33,407	9.6
Europe-non EEC	2,252	13.7	1,859	10.8	4,111	12.2
Eastern Europe**	6,212	10.4	6,050	9.8	12,262	10.1
	44,268		42,919		87,188	

Mortality (#)

	Male	Rate*	Female	Rate*	Total	Rate*
USA	4,500	3.5	2,700	2.0	7,200	2.7
Japan	364	0.6	315	0.5	679	0.5
Europe-EEC	3,752	2.2	3,124	1.8	6,875	2.0
Europe-non EEC	542	3.3	326	1.9	868	2.6
Eastern Europe**	1,495	2.5	1,061	1.7	2,556	2.1
	10,653		7,526		18,178	

** Per 100,000*
*** Excluding the former USSR*

imately 80% of children diagnosed with melanoma have stage I disease. Incidence of melanoma also appears to be rising among young adults. A study analyzing incidence and mortality over the 1974-1992 period among young adults (aged 20 to 44 years) from the Vaud Cancer Registry in Switzerland, found that in males a rise in the overall age-standardized (world population) incidence was mostly attributed to increased incidence of cancers of the oral cavity and pharynx, lung cancer, skin melanoma and colorectal cancer, while testicular cancer rates remained stable. Among females, the leading contributors to an increase in incidence were breast cancer, skin melanoma and lung cancer. Death rates rose for lung cancer in both sexes, and for skin melanoma and breast cancer in females (Levi F, etal, International Journal of Cancer, 1995 May 29, 61(5):606-10).

Incidence rates of cutaneous malignant melanoma have risen in most affluent countries over the last three or four decades. In fact, incidence of malignant melanoma among whites in the USA increased faster than that of any other cancer. Between 1973 and 1991 incidence rates increased by 94% in the USA, representing a rise in incidence among whites of 5.3% each year over the 1975-79 period, slowing to 0.1% per year in the 1987-91 period, suggesting stabilization). Rise in incidence rates was different in males and females. In the 1973-1991 period, incidence rates for white men increased 117.2% overall, whereas the rate for white women increased 74.6%. The increase in incidence has been especially pronounced among white men age 65 and over; in the 1975-79 period it rose by 171.8%.

A similar increase in the incidence of malignant melanoma has been noted among whites in most industrialized regions. In a screening campaign organized in Southern Limburg, the Netherlands, the proportion of screens with lesions suggestive of melanoma increased from 1.1% in 1990 to 1.7% during the 1993 campaign. The proportion of dysplastic nevi rose from 2.1% to 7.7% (de Rooij MJ, etal, Archives of Dermatology, 1995 Apr, 131(4):422-5). The incidence of cutaneous malignant melanoma doubled from 5.7 to 11.4 per 100,000 in south-east Scotland over the 1982-90 period, according to a study launched in 1987 by the Cancer Research Campaign. The percentage of thin tumors (Breslow thickness ≤1.5 mm) increased steadily and significantly (from 43% in 1982 to 68% in 1990), but the number of thick tumors (Breslow thickness > 3.0 mm) remained constant over the same period (22%). Five-year survival increased from 70% in the 1982-84 cohort to 84% in the 1987-89 cohort (Herd RM, etal, British Journal of Dermatology, 1995 Apr, 132(4):563-70). Increase in incidence of malignant melanoma was also reported among Jews. Incidence rates in all Jews increased throughout the 1960-1989 period, with a monotonic annual increase of 4.8% for males and 4.3% for females. Similarly to USA trends, incidence rose steeply until the mid-1970s, with an a posteriori leveling-off. Incidence was higher in females than in males. Interestingly, there were variations in incidence among Jewish subpopulations. Israel-born Jews had the highest incidence with an average age-adjusted incidence rate of 7.8 and 9.4 per 100,000 for males and females, respectively, followed by Jews born in Europe and America at 6.1 and 7.3 per 100,000 for males

and females, respectively. The lowest rate was for Jews born in Africa and Asia at 1.3 per 100,000 for both males and females (Iscovich J, etal, Public Health Reviews, 1995, 23(1):1-23).

Part of the increase in incidence rates may be attributed to better case-finding procedures resulting from both professional and public awareness about the problem but it is not sufficient to account for most of the increase. A contributor to the rise in incidence in the late 1970s may have been increases in voluntary sun exposure and the popularity of artificial tanning. Also it may have been a result of early (childhood) intermittent sun exposure which is considered a strong risk factor for melanoma. Stemming the tide in the late 1980s and beyond was increased awareness of the dangers of sun exposure and use of sunscreens or other protective measures.

Malignant melanoma of the uveal tract (iris, ciliary body and choroid) are the most common primary intraocular tumors in adults. According to the Surveillance, Epidemiology, and End Results (SEER) program, age-specific rates for ocular melanoma rose with increasing age (especially for males). Age-adjusted rates were higher for whites than for blacks and declined from 1973-1977 to 1983-1987 in whites. Among cases diagnosed in 1983-1987, 5-year relative survival rates for melanoma of the eye were 79% (Polednak AP and Flannery JT, Cancer, 1995 Jan 1, 75(1 Suppl):330-7). In Israeli Jews the average annual incidence rate of posterior uveal melanoma between 1961-1989 was 5.7 per million for both males and females (Iscovich J, etal, International Journal of Cancer, 1995 May 4, 61(3):291-5).

According to the Centers for Disease Control (CDC; Atlanta, GA), death rates from malignant melanoma rose 34.1% in the 1973-1992 period in the USA; in males the increase (47.9%) was the highest for all cancers. For white males the 1992 death rate was 5.9 times higher than that of any other races and 2.1 higher than that of women (MMWR 1995, May 5; 44-337, 346-7). The rise in mortality rates is probably attributable to the accompanying rise in incidence; otherwise, 5-year survival rates improved dramatically in the 1973-1991 period, from 79.7% in the 1974-1976 period to 85.1% in the 1983-1990 period.

DIAGNOSIS

Early diagnosis of cutaneous malignant melanoma is the most critical contributor to survival. Also, early tumors may be excised in the outpatient setting with a minimum discomfort, inconvenience and cost. Most melanomas are discovered by patients or close acquaintances and primary care providers currently find more melanomas than dermatologists. A two-pronged campaign to educate the public and the primary physician is expected to significantly increase identification and treatment of early melanomas. Various campaigns sponsored by such organizations as the American Academy of Dermatology and the American Cancer Society, promote skin awareness and self-examination and offer free examinations to detect evolving tumors. It is also recommended that a similar effort is directed at educating the primary care physician. Currently, skin cancer detection appears to be a low priority in primary care and many providers lack expertise to adequately identify high risk lesions.

Screening and early detection of melanoma rely on visual inspection. The A (asymmetry), B (border irregularity), C (nonuniform color) and D (diameter > than 6 mm) approach is recommended to assess the likelihood that a pigmented lesion or mole is malignant. The diagnosis is then made by biopsy of the suspected lesion and additional tests are required to detect local and distant metastasis and establish disease stage which is a critical step to ensure suitable treatment or management.

New findings in molecular biology may translate into screening tools to monitor patients at a particular high risk for melanoma. In 1994 researchers from the National Center for Human Genome Research of the National Institutes of Health (Bethesda, MD), Myriad Genetics (Salt Lake City, UT), the University of Utah and others reported the identification of a gene, CDNK2, that plays a crucial early role in the growth of inherited malignant melanoma and may also play a role in non-inherited melanoma. In its normal state, the gene encodes protein p16 which inhibits cell proliferation by regulating cell division. Those who inherit a defective version become unusually vulnerable to melanoma. Only about 10% of melanoma occurs in people with an inherited tendency and it is unclear what percentage of inherited cases are caused by defects in CDNK2. Defective versions of the gene may also be involved in many, or maybe even most, cases of non-inherited melanoma. In such cases, a normal gene becomes defective by unknown means. However, although defects in CDNK2 have been found in some melanoma-prone families and in melanoma patients, these defects are not consistently present in every case. Nonetheless, the discovery of a susceptibility gene may be used to screen for people at risk for melanoma.

Monitoring of patients at risk may be accomplished by topodermatograpy to perform quantitative videographic image analysis of skin-lesion changes over time. A recently described system using a high-speed processor with an onboard co-processor, a high-resolution video camera, specifically designed image processing software, and a position framework for the adjustment of the patient's standing position, obtained digitized measurements of skin-surface image parameters in 109 consecutive patients who were at risk for melanoma (98), had lesions from Kaposi's sarcoma (4), had metastatic skin deposits from melanoma (3), and had breast cancer (4). Diametric enlargement of skin lesions over time were identified reliably within a few millimeters. In addition, metastatic tumor lesions on the skin were monitored dynamically, to evaluate the impact of systemic therapy on multiple skin deposits from melanoma (Voigt H and Classen R, Cancer, 1995 Feb 15, 75(4):981-8).

Telespectrophotometry, a variation of reflectance spectroscopy which provides an objective evaluation of surface color, is being also developed as a clinical tool to discriminate cutaneous melanoma from other pigmented cutaneous lesions. Researchers at the Division of Health Physics, Instituto Nazionale per lo Studio e la Cura dei Tumori (Milano, Italy) have designed a spectrophotometric system, based on the use of a charge coupled device camera provided with a set of interference filters, that may be used to acquire images of cutaneous pigmented lesions at selected wavelengths ranging from 420 to 1040 nm. Captured images are digitized by a frame grabber and stored in a personal computer for off-line data analysis. Peliminary results suggest that telespectrophotometry may be applicable in the diagnosis of cutaneous pigmented lesions (Marchesini R, etal, Photochemistry and Photobiology, 1995 Jul, 62(1):151-4).

Diagnostic tests to assess systemic spread of malignant melanoma include chest radiography, serum lactate dehydrogenase (LDH), computed tomography (CT), magnetic resonance imaging (MRI) and whole-body positron emission tomography (PET). In 33 patients either with known metastasis or newly diagnosed melanoma, whole-body PET with 2-[fluorine-18]-fluoro-2-deoxy-d-glucose (FDG) correctly depicted 37 out of 53 lesions and correctly excluded malignancy in 10 cases identified by other imaging modalities. PET's sensitivity for the detection of malignant lesions was 92% and its specificity was 77% (Steinert, HC, etal, Radiology 1995; 195:705-709).

Radioscintigraphy may also play a role on the detection of distant metastasis. Researchers at the Nuclear Medicine Department of the National Cancer Institute in Milan, Italy are experimenting with Iodine-123-labeled benzamide, [123I]-(S)-IBZM, to image melanoma metastases. In 11 patients with proven metastatic melanoma, whole-body and planar scintigrams performed 2, 4 and 24 hours after IV injection of a mean tracer activity of 205 MBq, detected of all six cutaneous lesions, five of six superficial pathologic lymph nodes, four of five pulmonary and one of two hepatic metastases. Hepatobiliary excretion of the tracer may, however, limit detection of intra-abdominal lesions. The mechanism of radiopharmaceutical uptake in melanoma is unclear but it may be attributed to binding to membrane receptors or to interactions with intracellular structures (Maffioli L, etal, Journal of Nuclear Medicine, 1994 Nov, 35(11):1741-7).

Assays to detect circulating cancer cells may also yield invaluable information about metastatic status and tumor progression kinetics without surgical intervention or conventional radiologic diagnostic procedures. However, because the number of circulating cancer cells in the blood may be very small, techniques for their detection must be both sensitive and specific. Polymerase chain reaction (PCR) has been successful in detecting small numbers of residual tumor cells in hematologic malignancies which have consistent DNA abnormalities. Solid tumors, however, have proven more challenging. Recently, a reverse transcriptase (RT)-PCR for tissue-specific gene expression proved useful in identifying small numbers of circulating cells in melanoma and neuroblastoma patients (Burchill SA, etal, British Journal of Cancer, 1995 Feb, 71(2):278-81). RT-PCR was also useful in the identification of patients with micrometastases that are not detected by routine histopathologic examination. Among 31 sentinel nodes of melanoma patients on which RT-PCR was performed to detect tyrosinase mRNA, all were positive for micrometastases as compared to 13% found positive by routine histology. RT-PCR may thus identify patients who may benefit from more complete dissection or adjuvant therapies (AACR95, Abs. 1266).

To overcome the problem of heterogeneity of antigen expression of melanoma cells, a PCR assay was developed that uses four melanoma-associated gene markers (tyrosinase, p97, MUC18, and MAGE-3). In 119 patients with stage I to IV melanoma, evaluated for circulating melanoma cells using the four gene markers under optimal conditions, all melanoma-associated gene markers were expressed in at least 80% of the melanoma lines, whereas 37 of 39 normal peripheral blood lymphocytes (PBL) tested negative for all markers with the remaining two PBL being positive for MUC18. There was a significant correlation between the number of positive PCR markers, stage of disease, and progression of disease. In all stages, there were more PCR-positive patients with disease than without disease (Hoon DS, etal, Journal of Clinical Oncology, 1995 Aug, 13(8):2109-16).

Activation and/or differential expression of integrins that are cell receptors that mediate cell matrix interactions are implicated in primary tumor formation and in metastasis. One of these integrins, β-1, which is expressed in human malignant melanoma, may be useful as a marker of occult metastases. In a study of primary cutaneous melanomas from 51 patients with clinical stage NO melanoma who underwent elective lymph node dissection, lymph node involvement was present in 12/21 patients with β-1 integrin-positive tumors and in 1/30 with -negative tumors. Also β-1 integrin staining of >10X of the tumor area was present in 12/13 cases with positive and 3/38 with negative lymph node involvement. Therefore, β-1 integrin expression in primary cutaneous malignant melanoma may indicate occult metastases to the lymph nodes and immunostaining of more than 10X of tumor area may be a predictor of occult lymph node metastases and be used to select patients who may benefit from elective lymph node dissection (AACR95, Abs 3816).

CURRENT DISEASE MANAGEMENT APPROACHES

Melanomas diagnosed before they have spread beyond the site at which they developed are highly curable. Melanomas are classified using several different schemes, as shown in Exhibit 2. The outlook for melanoma patients by clinical stage is presented in Exhibit 3, which also describes standard treatment options. New approaches

Exhibit 4
Estimated Melanoma Incidence and Treatment Options by Disease Stage in the USA

Stage	TNM Classification	Estimated Number of New Diagnoses (#)/ Percent of Total (%) in 1995	Standard Treatment Options
Stage I	pT1, NO, MO pT2, NO, MO	27,962 (82)	May be treated by conservative (1.0 cm) re-excision; often require wider surgical excision of the primary and up to 3 cm margins of adjacent skin; skin grafting or an appropriate flap is often necessary to close the resulting defect; elective regional lymph node dissection is of unproven benefit
Stage II & Stage III	pT3, NO, MO pT4, NO, MO any pT, N1 or N2, MO	4,774 (14)	Surgical excision of the primary tumor with margins of 3 cm of adjacent skin as for stage I; elective regional lymph node dissection is optional Wide local excision of the primary tumor with up to 3 cm margins of adjacent skin, with skin grafting if necessary to close the resulting defect, and, often, regional lymph node dissection of clinically involved lymph nodes
Stage IV	Any pT, any N, M1	1,364 (4)	Treatment mostly palliative; isolated metastases to the lung, GI tract, bone, or the brain may be palliated by resection with occasional long survival; radiation therapy may provide symptomatic relief for metastases to brain, bones, and viscera; in advanced melanoma biologic response modifiers, cytotoxic agents, bolus IL-2, LAK, alpha interferon, dacarbazine, semustine, melphalan, thiotepa, paclitaxel showed response (see Exhibit 5 for experimental combination chemotherapy and multimodality therapy)

Abbreviation Key:

Lymph Node Status

NX: regional lymph nodes cannot be assessed
NO: no regional lymph node metastasis
N1: metastasis 3 cm or less in greatest dimension in any regional lymph node(s)
N2: metastasis more than 3 cm in greatest dimension in any regional lymph node(s) and/or in-transit metastasis
-N2a: metastasis more than 3 cm in greatest dimension in any regional lymph node(s)
-N2b: in-transit metastasis
-N2c: both N2a and N2b

Metastasis Status

MX: presence of distant metastasis cannot be assessed
MO: no distant metastasis
M1: distant metastasis
-M1a: metastasis in skin or subcutaneous tissue or lymph node(s) beyond the regional lymph nodes
-M1b: visceral metastasis

and agents in development for the treatment of positive-node and metastatic melanoma will be presented in Part II of this report.

Surgery

Surgery, using excisions with margins proportional to the microstage of the primary lesion, is standard treatment for localized melanoma. For most thin (smaller than 1 mm) lesions, the recommended re-excision margin is about 1.0 cm and 2 cm for lesions that are between 1 mm and 4 mm. In about 90% of patients, the soft-tissue defect created by 2 cm excisions can be closed in one procedure, so that surgery for early-stage melanoma can be performed on an outpatient basis.

Melanomas that have spread to regional lymph nodes may be curable with wide (up to 3 cm) excision of the primary tumor and removal of the involved regional lymph nodes. The role of elective lymph node dissection in higher risk primary tumors has not been adequately investigated. Selective lymphadenectomy using lymphatic mapping and sentinel node biopsy offers a rational approach to clinically negative regional lymph node basins. Although many retrospective trials have demonstrated a survival benefit for elective lymph node dissection in the treatment of clinical stage I malignant melanoma, two prospective randomized trials found none (Evans RA, Anticancer Research, 1995 Mar-Apr, 15(2):575-9).

Surgery also plays a role in the management of metastatic disease for palliation and, in selected cases, to prolong survival. In those patients with surgically inaccessible lesions, radiation therapy can provide valuable palliation. The value of hyperthermia in addition to radiation therapy in the treatment of metastatic melanoma is still under investigation (Ross MI, Current Opinion in Oncology, 1994 Mar, 6(2):197-203).

Treatment of subungual melanoma, a rare type of melanoma, may combine amputation, lymph node dissection and regional/isolated limb perfusion. Survival did not differ among 46 patients who received amputation alone or those who underwent amputation in combination with lymph node dissection or perfusion; however, the use of limb perfusion reduced the incidence of locally recurrent disease. The level of amputation did not affect patient survival or the incidence of local recurrence. Although isolated limb perfusion may reduce the incidence of local recurrence, the use of either lymph node dissection or limb perfusion in the routine management

of subungual melanoma remains controversial (Heaton KM, etal, Annals of Surgery, 1994 Feb, 219(2):197-204). In limb perfusion, the main vessels of the affected limb are surgically isolated and connected to a heart-lung machine which allows the infusion of high dose chemotherapy directly into the extracorporeal circulation. Regional perfusion therapy following resection of high risk extremity melanomas is currently being evaluated by the World Health Organization and the North American Perfusion Group in a multicenter setting.

Chemotherapy

When melanoma metastasizes to distant sites the prognosis is bleak. Unfortunately, chemotherapy is marginally effective. It is probably most appropriate when tumor burden is small. Various chemotherapeutics have been approved worldwide for the treatment of malignant melanoma and numerous combinations of approved and experimental drugs are under investigation (see Exhibit 5).

Dacarbazine [DTIC-Dome; Miles (Bayer)], approved for the treatment of melanoma in the USA and various other countries abroad (see Exhibit 6), results in tumor shrinkage in only 20% of cases. Dacarbazine is in numerous clinical trials in combination with other chemotherapeutics and with nonspecific immunotherapy, as shown in Exhibit 5. However, to date no combination regimen demonstrated significant improvement in disease-free or overall survival.

Fotemustine (S-10036, Muphoran; Servier), an aminoacid linked chloroethyl nitrosourea, has shown activity against disseminated malignant melanoma, brain metastases and primary brain tumors. It was launched in France in 1989, in Australia and Argentina in 1993 and in Portugal and has been approved in New Zealand and the UK, for the treatment of metastatic malignant melanoma. In 1993 fotemustine was approved in France for the treatment of primitive malignant cerebral tumors. Fotemustine is the only chemotherapeutic agent that has shown therapeutic efficacy in cerebral metastases from malignant melanoma because it penetrates the blood brain barrier. In a phase II clinical trial of 31 patients with histologically confirmed metastatic malignant melanoma who were administered IV fotemustine as a rapid infusion at a dose of 100 mg/m^2 on day 1, 8 and 15 every 4 to 5 weeks, there were three objective responses (CR + PR). Most common side effects were hematologic toxicity and nausea (Falkson CI, etal, Investigational New Drugs, 1994, 12(3):251-4).

Vindesine (VDS) (CMP-99094, DVA, Eldisine, Fildesin, VND) is a vinca alkaloid developed by Eli Lilly and launched in Japan in 1985 by Shionogi (Osaka, Japan) for the treatment of refractory malignant melanoma alone and in combination with other agents. The drug has also been approved for the treatment of numerous other cancers and has also been registered in

Exhibit 5
Representative Combination Chemotherapy and Multimodality Approaches for the Treatment of Melanoma

COMBINATION CHEMOTHERAPY
Dacarbazine (DTIC) Combinations
BDP [carmustine (BCNU) + cisplatin (CDDP)] (Phase III; ASCO95, Abs. 1309)
BDP + recombinant interleukin-2 (rIL-2) + interferon-α 2b (IFN-α 2b) + tamoxifen (TAM) (Phase I; Tumori, Mar.-Apr. 1995, 81(2):102-106)
BDP + TAM (Phase III; ASCO95, Abs. 1309)
BOLD (bleomycin + oncovin + lomustine) + IFN-α 2b (Phase I; ASCO95, Abs. 1297)
CDDP (Phase I; ICACC95, poster presentation 329 and ASCO95, Abs. 1298)
CDDP + AMD + VBL (Phase I; ASCO95, Abs. 1327)
CDDP + VBL + IL-2 + IFN-α (Phase II; ASCO95, Abs. 1305)
CDV [CDDP + vindesine (VDS)] (Phase II; Japanese Journal of Cancer and Chemotherapy, Jan. 1995, 22(1):23-7)
CVD [CDDP + VDS] + TAM + IFN-α 2a ± IL-2 (Phase II; ASCO95, Abs. 1315)
DAV [nidran (ACNU) + vincristine (VCR)] (Phase II; Japanese Journal of Cancer and Chemotherapy, Jan. 1995, 22(1):23-7)
BCNU + procarbazine (PCB) (Phase II; ICACC95, poster presentation 218)
BCNU + thymidine (Phase II; ASCO95, Abs. 1312)
Carboplatin (Phase III; ICACC95, poster presentation 522)
CDDP + lomustine (Phase III; ICACC95, poster presentation 522)
IFN-α 2a + TAM + IL-2 (Phase II; ASCO95, Abs. 1315)
rIL-2 (Phase I; Cancer Feb. 1995, 75(4):1038-44)
VCR + bleomycin + lomustine + IFN-α (Phase II; Journal of Cancer Research and Clinical Oncology, 1995, 121(3):175-80)
VDS (Phase II; European Journal of Cancer 1995, 29A(5); 708-711)
Faranox + VCR (Phase I; ICACC95, poster presentation 681)
Faranox + VCR + aranoza (Phase I; ICACC95, poster presentation 681)
Fotemustine (FTMU) + VDS (Phase II; ICACC95, poster presentation 764)
FTMU + IFN-α 2a (Phase II; ICACC95, oral presentation 472)
IFN-α + IL-2 + CDDP (Phase II; Recent Results in Cancer Research, 1995, 139:383-90)
Nidran (ACNU) + CDDP (Phase II; ASCO95, Abs. 1329)
VDC (VDS + CDDP) + retinoid derivatives + cantastim (Phase III; ICACC95, poster presentation 741)
Other Combinations
Actinomycin-D (AMD) + vinblastine (VBL) (Phase III; Photochemistry and Photobiology May 1995, 61(5);479-83)
CDDP + etoposide (ETOP) (Phase II; ASCO95, Abs.605)
CDDP + interleukin-2 (IL-2) + IFN-α (Phase II; ICACC95, oral presentation 754 and poster presentation 762)
CDDP + rIL-2 + IFN-α + TAM (Phase II; ICACC95, oral presentation 429)
CDDP + TAM (Phase I; ASCO95, Abs. 1300)

— continued on next page

Chimeric CH14.18 antibody + IL-2 (Phase I; ASCO95, Abs. 1326)
Docetaxel + 5-FU or cyclophosphamide (CY) or ETOP or vinorelbine or methotrexate (MTX) (Phase I; Seminars in Oncology Apr. 1995, 22(2 suppl. 4):3-16)
Docetaxel + vinca alkaloids (VDS + vinorelbine) (Phase II; AACR95, Abs 1780)
Ethyldeshyhydroxy-sparsomycin (EDSM) + CDDP or cytosar (Ara-C) or MTX or 5-fluorouracil (5-FU) (Phase II; Anti-Cancer Drugs Feb. 1994, 5(1):35-42)
EDSM + Ara-C or 5-FU or VCR (Phase II; Anti-Cancer Research Apr. 1995, 6(2):277-84)
EDSM + doxorubicin (DOX) or 5-FU or ETOP (Phase II; Anti-Cancer Drugs Feb. 1994, 5(1):35-42)
FTMU + TAM (Phase II; European Journal of Cancer 1995, 31A(3):421-2)
Human leukocyte IFN-α (Alphaferon) + 13-cis-retinoic acid (Phase I; Cancer Immunol Immunotherapy 1995; 40(3); 157-164)
IFN-α + cis-retinoic acid (cRA) (Phase II; ASCO95, Abs. 1313)
PAV (peplomycin + ACNU + VCR) (Phase II; Japanese Journal of Cancer and Chemotherapy, Jan. 1995, 22(1):23-7)
PCB + VCR + lomustine (Phase I; European Journal of Cancer 1994, 30A(14):2054-6)
Recombinant IFN-α 2a (Roferon) + 5-FU (Phase I; ASCO95, Abs. 1310)
Recombinant TNF-α + IFN-γ+ melphalan (L-PAM) (Phase I; ICACC95, oral presentation 696)
rIL-2 + cyclophosphamide (CY) (Phase II; ICACC95, poster presentation 348)
rTNF-α + rIFN-γ + L-PAM (Phase I; Chest Apr. 1995, 107(4):1074-82)
TNF-α + L-PAM (Phase I; ASCO95, Abs. 1323)
VCR + Ara-C + 5-FU + EDSM (Phase III; Anti-Cancer Drugs April 1995, 6(2):277-84)
IFN-α 2a + IL-2 (Marincola FM, etal, Journal of Clinical Oncology, 1995 May, 13(5):1110-22)
Ifosfamide + carboplatin + etoposide (Fields KK, etal, Journal of Clinical Oncology, 1994 Mar, 12(3):544-52)
IL-1 + IL-2 (Triozzi PL, etal, Journal of Clinical Oncology, 1995 Feb, 13(2):482-9)
MULTIMODALITY THERAPY
Radiation + lomustine or BCNU + CDDP + DTIC + BCG vaccine (Phase III; ICACC95, oral presentation 631)
Radiation + DTIC +ACD (Phase II; ICACC95, poster presentation 343)

several European countries. The drug is mostly used in combination therapy.

Interferon-α 2b. In July 1995, FDA's Oncologic Drugs Advisory Committee (ODAC) recommended for approval interferon-α 2b (Intron-A; Schering-Plough), a genetically engineered human protein made by Biogen (Cambridge, MA), for the treatment of malignant melanoma. Schering-Plough had submitted an PLA for this indication in August 1994 based on data from a 29-center clinical trial of 287 melanoma patients conducted from 1985-1993 by the Eastern Cooperative Oncology Group that showed that Intron-A patients lived an average 3.8 years compared with 2.8 years for controls. In addition to survival the other end-point of the trial, relapse-free survival, was 1.7 years in those treated compared to 1 year in controls.

Although results were significant in node-positive patients who survived 3.8 years compared to 2.1 years in controls and experienced relapse-free survival of 1.7 years compared to just over six months in controls, the indication recommended for approval was adjuvant treatment to surgery for all patients who have had a malignant melanoma surgically removed but were at risk for systemic recurrence. This was despite of the fact that in patients with no nodal involvement, the controls survived longer than the treated patients. Another clinical trial to confirm the agent's effectiveness that is expected to be completed in 1998 had enrolled 642 patients as of mid-summer 1995.

Treatment consisted of a 4-week induction phase during which patients were infused with 20 million IU/m^2 of Intron-A five times a week, followed by a maintenance regimen of 10 million IU/m^2, delivered by subcutaneous injection thrice weekly. Although drug therapy was associated with severe side effects, ODAC members decided that the benefits outweighed the risks. Approximately 67% of those treated experienced severe adverse events, forcing 24% to drop out. Also, two patients died of liver failure as a result of treatment.

Interferon-α 2a. In another study, sponsored by the WHO Melanoma Program (Milan, Italy), 444 patients with regional nodal involvement after radical surgery were randomized to receive either rIFN-α 2a, or placebo. The goal of the study was to evaluate the efficacy of rIFN-α 2a administered at low doses, three times a week for three years. Among 218 evaluable patients 46% achieved 2-year disease-free survival compared to 27% of controls. Moreover, the number of positive nodes and sex were independent determinants of disease free survival. Improved survival was seen in treated males with increasing age but in females the trend was reversed. Women under 50 years of age and man older than 50 benefited significantly, in terms of disease-free and overall survival.

Sequential Chemoimmunotherapy

Sequential chemoimmunotherapy shows promise but drug-related complications may make continued therapy intolerable. To evaluate the efficacy and safety of sequential chemotherapy in patients with metastatic malignant melanoma, eight melanoma patients were treated with carmustine 150 mg/m^2 IV on day one, dacarbazine 220 mg/m^2 and cisplatin 25 mg/m^2 IV on days one to three and days 22 to 24, 10 mg twice daily as an oral dose for

Exhibit 6
Profile of Approved Chemotherapeutics for the Treatment of Malignant Melanoma in the USA

Generic Name (Brand Name; Supplier), Mechanism	Approved Indication, Delivery and Dosage	WW Markets
Dacarbazine [DTIC-Dome; Miles (Bayer)]; the drug is manufactured by Ben Venue Laboratories (Bedford, OH) DTIC-Dome is an alkylating agent that may inhibit DNA synthesis via an action similar to purine analogs	Malignant melanoma/IV by either of two recommended regimens: 2-4.5 mg/kg/d for 10 days (may be repeated at 4-week intervals) 250 mg/m2/d for 5 days (may be repeated every three weeks) Toxicities and side effects include anorexia and nausea and hematopoietic depression, among others Also indicated as second-line therapy in combination with other drugs in Hodgkin's disease	Approved in the USA and abroad; worldwide sales are under $5 million In the USA the AWP of DTIC-Dome is $13.2/100 mg

six weeks, IL-2 9X10^6 IU twice daily and IFN-α 6X10^6 IU once daily subcutaneously from days four to eight and days 25 to 29. The cycle was repeated every six weeks. Two of the eight patients had a complete response, there was one partial response, two persons had disease stabilization, and three individuals had progression of disease. In all five patients with local recurrence, irrespective of response to treatment or the existence of metastatic disease, there was disappearance of local recurrence. In the majority of patients (6 of 8), treatment was abandoned after three cycles, however, because of side effects including weakness, fever, myalgias, sweating, rigor, water retention, hypotension, and dyspnea (Ginopopulos P, etal, ICC95, Can J Infect Dis, July 1995 Vol 6 Suppl C; Pg 347C:2025).

A multicenter phase II clinical trial of sequential treatment with dacarbazine and recombinant interleukin-2 (rIL-2) was undertaken to establish if there was possible synergism in this chemoimmunotherapy regimen. Fifty-seven patients with metastatic malignant melanoma received 135 treatment cycles. Treatment consisted of dacarbazine (days 1-5) at 250 mg/m^2 by a 30-minute slow infusion, and IL-2 by constant intravenous infusion (days 21-25 and 28-32) at 18 x 10^6 IU/m^2 for 24 hours. After a one-week rest patients who did not experience tumor progression or serious toxicities were retreated as originally. Maximum treatment consisted of two induction and four maintenance cycles. The objective response rate was 15.8% (one CR and eight PRs) and the disease stabilized in 14 patients. For responders, median response duration was 13.9 months (6.3-39.0+), and median survival was 19.0 months (6.3-39.0+); overall median survival was 9.3 months (0.8-39.0+). Common toxicities included fever, hypotension, nausea/vomiting, anemia, leukopenia, thrombocytopenia, an increase in serum lactic dehydrogenase levels and diarrhea. Although feasible this regimen produced long-lasting responses in only a minority of patients (Dummer R, etal, Cancer, 1995 Feb 15, 75(4):1038-44).

Next issue: *Part II of this article presents strategies and agents in development for the treatment of melanoma, including a database of over 70 agents in development, and describes 23 commercial drug development programs.*

MEETING COVERAGE

19TH INTERNATIONAL CONGRESS OF CHEMOTHERAPY, MONTREAL, QUEBEC, CANADA, JULY 16-21, 1995

NEW TREATMENT APPROACHES FOR INFECTIONS IN NEUTROPENIC PATIENTS

Piperacillin/Tazobactam

Piperacillin/tazobactam (Zosyn; Lederle/Wyeth-Ayerst International), a unique combination of an extended-spectrum penicillin and a β-lactamase inhibitor, is significantly more effective for the treatment of potentially fatal infections in neutropenic cancer patients than current standard therapy. In a recent study conducted by the International Antimicrobial Therapy Cooperative Group of the European Organization for Research and Treatment of Cancer (IATCG-EORTC), 696 cancer patients with 858 episodes of febrile neutropenia were randomly assigned to piperacillin/tazobactam at 4 g/500 mg every six hours or ceftazidime (Fortaz; Glaxo or Tazicef; SmithKline Beecham) at 2 g every eight hours, with both treatment groups also receiving amikacin (Amikin; Apothecon) at 20 mg/kg in a single daily dose. For cessation of treatment, patients were required to have four consecutive days without fever. The overall

success of treatment for 706 evaluable febrile episodes was 61% in the patients treated with piperacillin/tazobactam plus amikacin versus 54% for those on ceftazidime plus amikacin. In addition, fever subsided more quickly, the time to failure was much longer, and the probability of failure was significantly less in the piperacillin/tazobactam plus amikacin group (Klastersky J. ICC95, Can J Infec Dis, July 1995, Vol 6 Suppl C; Pg 194C:0017).

Recombinant Granulocyte-Colony Stimulating Factor Combination Therapy

Combination antibiotic therapy with recombinant granulocyte-colony stimulating factor (rhG-CSF) (Neupogen; Amgen) is a more effective empiric approach for the treatment of septicemia in neutropenic patients with hematologic malignancies than the use of antibiotics alone. Sixty-five neutropenic patients with hematologic malignancies and septicemia were treated with sulbactam (Unasyn; Roerig)/cefoperazone (Cefobid; Roerig) 2 g and piperacillin (Piperacil; Lederle/Wyeth-Ayerst International) 2 g two to three times daily and with rhG-CSF 2 to 5 mg/kg/day. Another 39 neutropenic patients with hematologic malignancies and septicemia received sulbactam/cefoperazone and piperacillin alone. The success rate (cure or clinical improvement) for patients treated with antibiotics and rhG-CSF was 81% (21/25) while that of patients on antibiotics alone was 69% (27/39). In addition, patients who received antibiotics plus rhG-CSF experienced a much more rapid return to normal body temperature than did those on antibiotics alone (Toyama K etal, ICC95, Can J Infec Dis, July 1995 Vol 6 Suppl C; Pg 265C:0493).

Meropenem

Empiric monotherapy with meropenem (Merrem; Zeneca) has been shown to be an effective and realistic alternative to combination therapy for the treatment of febrile neutropenic patients, including those with profound and persistent neutropenia. In a randomized comparison study carried out under the auspices of the EORTC, 958 evaluable patients were randomly assigned to meropenem 1 gm every eight hours IV or ceftazidime 2 g every eight hours IV plus amikacin (20 mg/kg) as a single daily dose. Treatment success was defined as resolution of fever and clinical signs of infection and eradication of the infecting organisms (where isolated) without modification of the initial empiric regimen, for a minimum of four days after stopping therapy and not recurring within one week after cessation of treatment. The overall clinical response rates were 56% (270/483) with meropenem as monotherapy and 52% (247/475) with the ceftazidime and amikacin combination. In patients with microbiologically documented infections, the overall response rates were 43% (54/125) for patients on meropenem and 32% (41/129) for those on the standard combination regimen. Furthermore, meropenem was more active against methicillin-sensitive, coagulase-negative staphylococci than the combination (90% versus 47%) but neither meropenem or ceftazidime plus amikacin was effective against methicillin-resistant strains (Glauser MP, ICC95, Can J Infec Dis, July 1995, Vol 16 Suppl C; Pg 218C:0191).

Pefloxacin with Teicoplanin

Administration of pefloxacin with teicoplanin (Hoechst Marion Roussel) once daily is as safe and effective as pefloxacin with vancomycin (Vancocin; Lilly) twice daily for the treatment of neutropenic cancer patients with fever, with the added advantage of not needing a central or peripheral venous catheter insertion. In a prospective comparative trial, 42 neutropenic cancer patients with fever were randomly assigned to either pefloxacin 400 mg twice daily plus vancomycin 10 mg/kg twice daily or pefloxacin 800 mg once a day plus teicoplanin 5 mg/kg once a day. The majority of pathogens isolated were staphylococci and enterococci. Success rates (cure and clinical improvement) were identical (81%) in both treatment groups (Studena M etal, ICC95, Can J Infec Dis, July 1995, Vol 6 Suppl C; Pg 265C:0491).

Ciprofloxacin

Ciprofloxacin [Cipro; Miles (Bayer)] has been proven to be a safe and effective antibiotic for the treatment of neutropenic infections in children with malignancies. In this study, ciprofloxacin was administered to 114 children with 151 episodes of infection, 100 of which were febrile neutropenia. The most common pathogenic agents found were *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA). Average duration of administration of ciprofloxacin was 14.38 days (3-32 days range). The quinolone antibiotic was successful in 71.5% of the episodes, with no serious adverse effects. Up until now, the pediatric use of ciprofloxacin has been restricted to life-threatening infections because of concern over chondral growth retardation but, in this study, no such drug-related side effects were seen on MRI screening of the knees (Agaoglu L etal, ICC95, Can J Infec Dis. July 1995, Vol 16 Suppl C; Pg 310C:1093).

Fluconazole

Fluconazole (Diflucan; Roerig) is equally as effective as amphotericin B (Fungizone; Apothecon) as empiric therapy for unexplained fever in neutropenic cancer patients, with the added advantage of being far less toxic. Overall, 112 neutropenic cancer patients with fever of unknown origin after at least four days of antibacterial therapy were randomized to fluconazole 6 mg/kg/day up to 400 mg daily or amphotericin B 0.8 mg/kg/day as empirical antifungal therapy. Success, measured as resolution of fever and survival at the resolution of neutropenia, was comparable in the two treatment arms. Success rates for those on fluconazole were 75% (42/56) com-

pared to 66% (37/56) for those on amphotericin B. While there was no difference in efficacy between the two antifungal agents, fluconazole was significantly less toxic than amphotericin B. No toxic adverse effects were seen in 66% of patients treated with fluconazole, compared with only 18% (10/56) of those on amphotericin B. In addition, five persons in the amphotericin B treatment group stopped therapy due to adverse drug-related events versus none on fluconazole. The study was discontinued because of excess toxicity in the amphotericin B arm (Vicoli C and Castagnola E, ICC95, Can J Infec Dis, July 1995, Vol 6 Suppl C; Pg 285C:0629).

MECHANISMS IN MALIGNANCY

DRUG RESISTANCE IN CANCER-PART III

Parts I and II of this article were presented in FO, V1, # 2/3 and # 4, respectively.

NOVEL AGENTS IN DEVELOPMENT TO OVERCOME P-GLYCOPROTEIN-MEDIATED RESISTANCE

Several novel agents designed to reverse P-gp MDR have entered into clinical trials in combination with various chemotherapeutic agents (see Exhibit 7). However, most P-gp MDR-reversing agents are in preclinicals (see Exhibit 8). Numerous agents have shown activity against P-gp MDR, but none proved feasible in the clinic as yet. Therefore, this area represents a unique opportunity for drug developers which is reflected by the stepped-up research activity.

Novel Drugs Being Evaluated in P-gp MDR

Byk Gulden (Konstanz, Germany) is clinically evaluating dexniguldipine HCl (B859-035, B895-35), a dihydropyridine that demonstrated antiproliferative effects in a mammary cancer cell line via inhibition of Ca^{2+} calmodulin. It is a potent inhibitor of mitogenic signal transduction pathways dependent on PKC activation in several small-cell and non-small-cell lung cancer cell lines while it failed to inhibit cyclic-AMP-dependent cell proliferation (Schuller HM, Journal of Cancer Research and Clinical Oncology, 1994, 120(6):354-8). Among patients with hematologic malignancies overexpressing P-gp, one daunorubicin-cytarabine-pretreated refractory AML patient treated with the oral form of dexniguldipine achieved complete remission for a duration of 7 months (Nussler V, etal, Annals of Hematology, 1994, 69 Suppl 1:S25-9). It was determined that dexniguldipine does not affect the expression of the MDR-1 gene and has no effect on a MDR resistant phenotype caused by a mutation of topoisomerase II; it only modulates MDR resistance by direct interaction with P-gp (Hofmann J, etal, Biochemical Pharmacology, 1995 Mar 1, 49(5):603-9). Dexniguldipine was also found to be a potent allosteric inhibitor of [3H]vinblastine *in vitro,* binding to P-gp of the human lymphoblastic cell line CCRF ADR 5000. It appears that P-gp possesses at least two allosterically coupled drug acceptor sites; receptor site 1 which binds vinblastine, doxorubucin, etoposide and cyclosporin A, and receptor site 2 which binds dexniguldipine and other 1,4-dihydropyridines (Malkhandi J, etal, European Journal of Pharmacology, 1994 Dec 15, 288(1):105-14). In phase I trials involving 37 cancer patients treated with oral dexniguldipine in increasing doses for up to 7 days, no major toxicities were observed. Despite the lack of dose-limiting toxicity, higher doses of dexniguldipine did not appear to be useful for clinical evaluation because of the pharmacokinetic properties of the compound and, therefore, 2,500 mg/day was recommended for phase II trials (Ukena D, etal, Cancer Chemotherapy and Pharmacology, 1995, 36(2):160-4).

Cell Therapeutics (Seattle, WA) has developed CT-2584, a synthetic small molecule which regulates phospholipase-D (PLD) activity in tumor cells. PLD is a family of related enzymes that control metabolism of intracellular lipid second messenger phosphaditic acids (PAs) that have been implicated in various abnormal cellular responses, including the unregulated growth and spread (metastasis) of cancer cells. CT-2584 inhibits PA and also interferes with the action of P-gp in a variety of human and animal tumor cell lines (Rice GC, etal, Proc Am Assoc Cancer Res, [1995] 36:383, Abs. 2282). In preclinical animal studies CT-2584 demonstrated potent cytotoxicity against breast, lung, and colon cancer (including human cancers which exhibit MDR), without being toxic to normal cells. CT-2584 is under review as a NCI phase I joint development candidate. It is in phase Ib clinical trials at Christie Hospital (Manchester, UK) under sponsorship of the Cancer Research Campaign.

CytRx (Norcross, GA) has an active program in the development of chemosensitizers to restore effectiveness of chemotherapeutics in MDR cancers. The company's agents are synthetic analogs of isolates of the active species from commercial surfactants (solubilizing agents) such as Cremophor EL (CRL-1336), Solutol HS 15 (CRL-1095) and Tween 80 (CRL-1605). These analogs are significantly more active than the original solubilizers. The company filed an IND in May 1994 for CRL-1336, its first generation agent believed to inhibit the activity of the P-gp pump. A phase I clinical trial was carried out in refractory breast cancer in collaboration with the Peter MacCallum Cancer Institute in Australia. In preclinical studies these second generation agents demonstrated superior activity against P-gp MDR than verapamil or cyclosporine. Also, in higher concentrations they inhibit other drug efflux pumps such as MRP.

Knoll Pharmaceuticals (Whippany, NJ) is clinically evaluating dexverapamil, a competitive P-gp inhibitor. Patients with lymphomas refractory to etoposide, pred-

nisone, vincristine, cyclophosphamide, and doxorubicin (EPOCH) chemotherapy, received escalated doses of verapamil (eight dose levels, from 240 to 1,200 mg/m²/d). The trial involved 154 patients, 109 with non-Hodgkin's lymphoma (NHL) and 45 with Hodgkin's disease (HD). The maximum-tolerated dose of dexverapamil was 900 mg/m²/d. Among 41 evaluable NHL patients (excluding mycosis fungoides), three experienced complete responses (CRs), two partial responses (PRs) and five minor responses (MRs); two of 10 HD patients achieved PRs. EPOCH and dexverapamil were well tolerated but, compared with EPOCH alone, the combination caused higher hematologic toxicity. The phase II recommended dose of dexverapamil with EPOCH is 150 mg/m² every 4 hours. Dosage was well tolerated when administered on an outpatient basis and plasma concentrations of dexverapamil and nor-dexverapamil were within the effective range for P-gp inhibition *in vitro* (Wilson WH, etal, Journal of Clinical Oncology, 1995 Aug, 13(8): 1985-94 and 1995-2004). Dexverapamil, in combination with vinblastine (VBL), was also evaluated in a phase I/II clinical trial involving 23 patients with advanced renal cell carcinoma. VBL was administered as a 0.11 mg/kg IV bolus injection on days 1 and 2 every 21 days and dexverapamil was begun 18 hours before day 1 of VBL administration and was given orally every 6 hours (either as a 120 mg/m² dose or a 180 mg/m² dose plus dexamethasone) for 12 doses in patients who exhibited resistance to VBL. No CRs or PRs were observed (Motzer RJ, etal, Journal of Clinical Oncology, 1995 Aug, 13(8):1958-65). In another clinical trial the combination of VBL given at 1.4 mg/m²/d plus dexverapamil given at 3000 mg/d was considered safe and well tolerated in the treatment of VBL-resistant renal cell carcinoma. Among 9 heavily pretreated patients one PR and three MRs were noted (Mickisch GH, etal, World Journal of Urology, 1994, 12(4):214-23).

Vertex Pharmaceuticals (Cambridge, MA) begun phase I clinical testing of VX-710, a proprietary MDR-reversing drug, in November 1994 in cancer patients undergoing treatment with paclitaxel (Taxol; Bristol-Myers Squibb). The study will involve up to 45 patients with solid tumors. A phase I/II study with VX-710 in cancer patients undergoing treatment with doxorubicin was initiated in early 1995. The company's ultimate goal is to evaluate VX-710 in a broad range of solid tumor types and hematologic malignancies in combination with a number of different chemotherapy agents. Currently, VX-710 is administered intravenously but, ultimately, the company intends to test an oral formulation of the drug. VX-710, a small molecule drug, was selected by Vertex for clinical evaluation from a series of novel compounds designed to restore the effectiveness of a variety of chemotherapy agents based on potent MDR-1 blocking activity. In January 1995 Vertex scientists reported that VX-710 may also block MRP resistance.

Xenova (Slough, Berks, UK) is conducting preclinical trials with series of novel small molecule P-gp inhibitors. It has isolated more than 170 chemical analogues in the XR1500 lead series, based on a microbial source, of which a number have demonstrated activity in *in vitro* models in restoring drug sensitivity to MDR-resistant cell lines. In June 1995 Xenova announced that it will drop several discovery programs and reduce its staff to stem its burn rate. However, the company said it plans to continue preclinical development of XR1500. Xenova aims to develop XR1500 for use in combination with other drugs, such as paclitaxel, vincristine, and doxorubicin, for indications including ovarian and breast cancer, acute myeloid leukemia, and multiple myeloma.

Oligonucleotide-based agents/ribozymes may be used to interfere at the transcriptional level to provide an effective means of reversing MDR. Strategies involve direct inhibition of mdr-1 or blocking activities of selected signal transduction pathways. Oligonucleotide-directed intermolecular triple helix formation allows site-specific targeting within megabase DNA to inhibit mdr-1 mRNA transcription. A natural phosphodiester 27-mer triple helix-forming oligonucleotide, synthesized as an inhibitor of mdr-1 transcription in human MDR cell lines, caused partial reduction of MDR1 mRNA levels in MDR cells (Scaggiante B, etal, FEBS Letters 352 (1994) 380-384).

Ribozymes have also been designed to reverse P-gp-mediated drug resistance in a specific manner. In a cell-free system, two hammerhead ribozymes designed to cleave the GUC sequence in codon 179 and 196 of MDR1 (PGY1) mRNA, cleaved a target piece of MDR1 RNA into 2 fragments at the specific sites at a physiological pH and temperature. The cleavage reaction was dependent on time, ribozyme:substrate ratio, and magnesium concentration. The 196 MDR1 ribozyme which was the more active of the two was then cloned into a human expression vector used to transfect vincristine-resistant cells. The level of resistance and the amount of MDR1 RNA expressed appeared to correlate inversely with the level of ribozyme expression. A disabled 196 MDR1 ribozyme did not cause specific cleavage *in vitro* nor lower MDR1 expression in transfectant cells. These results indicate that it was the ribozyme activity and not antisense activity which was responsible for decreased MDR1 RNA (Kobayashi H, etal, Cancer Research, 1994 Mar 1, 54(5):1271-5.)

Another hammerhead ribozyme, possessing catalytic activity that cleaves the 3'-end of the GUC sequence in codon 880 of the mdr-1 mRNA, was able to cleave a reduced substrate mdr-1 mRNA at the GUC position under physiological conditions in a cell-free system. A DNA sequence encoding the ribozyme gene was incorporated into a mammalian expression vector and transfected into a daunorubicin-resistant human pancreatic carcinoma cell line expressing the MDR phenotype. The expressed ribozyme decreased the level of mdr-1 mRNA

expression, inhibited the formation of P-gp and reduced the cell's resistance to daunorubicin dramatically (Holm PS, etal, British Journal of Cancer, 1994 Aug, 70(2):239-43).

Hammerhead ribozymes have also been designed that suppress c-fos oncogene expression to reverse MDR. Human ovarian carcinoma cells resistant to actinomycin D exhibiting the MDR phenotype were transfected with a hammerhead ribozyme (cloned into the pMAMneo plasmid) designed to cleave fos RNA. Induction of the ribozyme resulted in decreased expression of c-fos, mdr-1, c-jun, and mutant p53. The transformed cells displayed altered morphology and restored sensitivity to MDR-susceptible chemotherapeutics. An anti-mdr ribozyme, separately expressed in the same cancer cell line; efficiently degraded mdr-1 mRNA but only one-fourth as rapidly as that induced by the anti-fos ribozyme. These results appear to confirm the central role played by c-fos in MDR through its participation in signal transduction pathways (Scanlon KJ, etal, PNAS USA, 1994 Nov 8, 91(23):11123-7).

Other agents in development. Ether phospholipids have shown activity against a variety of tumor cell lines, including drug-resistant sublines. Three such compounds, ET-18-OCH3 (edelfosine), BM 41.440 (ilmofosine; Boehringer Mannheim) and the aza-derivative (BN 52205; Beaufour-Ipsen), when tested *in vitro* against three leukemic sublines expressing the P-gp phenotype, caused accumulation of anthracyclines. Ether phospholipid action is closely linked with the membrane biochemical composition and these drugs are able to change the dynamic structural organization of the tumor cell membrane (Principe P, etal, Anti-Cancer Drugs, 1994 Jun, 5(3):329-35). Racemic and nearly optically pure ether lipids were also synthesized and evaluated *in vitro* regarding any antineoplastic activities (Duclos RI Jr, etal, Journal of Medicinal Chemistry, 1994 Nov 25, 37(24):4147-54).

RS33295-198 is a high affinity P-gp modulator being co-developed by Lilly Research Laboratories (Indianapolis, IN) and Roche Bioscience (was Syntex; Palo Alto, CA) (Shepard RL, etal, Proc Am Assoc Cancer Res, [1995] 36:344, Abs. 2051). Lilly acquired an exclusive worldwide license for this and related MDR-reversing compounds under development by Roche Bioscience in September 1994.

S-9788, an MDR-reversing agent developed at Servier (Courbevoie-Cedex, France) and the Institut Gustave Roussy (Villejuif, France), that has demonstrated high activity in overcoming MDR to doxorubicin and vinca-alkaloids in animal and human tumor cell lines, has entered phase II testing in combination with doxorubicin in patients with refractory cancer (Goncalves E, etal, Proc ASCO, [1995] 14:182, Abs. 411; Tueni E, etal, Proc ASCO, [1995] 14:182, Abs. 413).

Mitotane (Lysodren; Bristol-Myers Squibb), an oral chemotherapeutic agent approved in the USA for the treatment of inoperable adrenal cortical carcinoma, has entered phase I trials in combination with vinblastine in patients with refractory cancers at the University of Maryland Cancer Center (Baltimore, MD) (Gutheil JC, etal, Proc ASCO95, 14:183, Abs. 415).

SDZ 280-446, a hydrophobic cyclopeptide derivative is under development by Sandoz Pharma as an MDR-reversing agent and chemosensitizer (Boesch D and Loor F, Anti-Cancer Drugs, [1994] 5:229).

Various anthracycline analogs are in development that may be poor substrates for P-gp MDR because of such attributes as increased polarity and/or hydrophilicity and, thus, avoid tumor cell resistance. One such analog, the 14-O-hemiadipate of doxorubicin (H-DOX), accumulated in doxorubicin-resistant cell lines *in vitro* indicating that such analogs may prove effective in treating MDR tumors (Leontiva OV, AACR95, Abs. 2335). Two other lipophilic anthracycline derivatives, idarubicin and iododoxorubicin also circumvented P-gp mediated MDR in drug resistant tumor cells *in vitro*. Differently modified novel anthracycline analogs in which the -NH2 group in C-3' position is substituted with a morpholino, methoxymorpholino (morpholinyl-anthracycline), or an alkylating moiety, showed equivalent efficacy in drug-sensitive human leukemic cells lines and their drug-resistant sublines. These results indicate that such molecules may exert their cytotoxic effect through a mode of action different from that of "classical" anthracyclines and is not mediated through topoisomerase II inhibition (Mariani M, etal, Investigational New Drugs, 1994, 12(2):93-7). Another anthracycline analog, methoxymorpholino doxorubicin (MXMF, FCE 237620), under development by Pharmacia, (Milan, Italy), has shown activity *in vitro* and *in vivo* in P-g and MDR associated protein positive systems; *in vitro* MXMF is 3-15 fold more potent than doxorubicin. MTD was established at 1.5 mg/m^2 for 3 weeks in pretreated patients. A phase I study using this dosage for 4 weeks administered as an IV bolus injection in 48 chemotherapy naive patients resulted in one PR and two mixed responses (ASCO95, Abs. 1515).

Intracellular histamine antagonist N,N-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine-HCl (DPPE) may increase the cytotoxicity of certain chemotherapeutic agents. In a phase I/II trial patients with advanced refractory cancer received a weekly infusion of a maximally tolerated dose of DPPE (240 mg/m^2) over 80 or 440 minutes in combination with various single agents to which, in most cases, the patient's tumor was previously resistant. Of 48 patients monitored for a minimum of four DPPE/chemotherapy treatment cycles, 16 (33%) progressed, 12 (25%) stabilized, 12 (25%) improved, and eight (17%) responded (one CR and seven PRs). Four of 11 subjects who did not respond to the 80-minute infusion regimen improved with the 440-minute infusion; one had a partial remission of melanoma (Brandes LJ, etal,

Journal of Clinical Oncology, 1994 Jun, 12(6):1281-90). DPPE and histamine also caused a reduction of tumor size in human colorectal cancer in the subrenal capsule assay. When pooled by their growth potential, as assessed by the growth of saline-treated controls, DPPE caused distinct tumor reduction in rapidly growing tumors (Suonio E, etal, Agents and Actions, 1994 Jun, 41 Spec No:C118-20).

Rifampicin (Rifadin; Hoechst Marion Roussel), a semi-synthetic antibiotic derivative of rifamycin B used in the treatment of tuberculosis, was also shown to down-modulate P-gp MDR and enhance vinblastine accumulation in both rodent and human tumor cells *in vitro*. The drug may be effective *in vivo* in non-toxic concentrations (Fardel O, etal, Biochemical Pharmacology, Vol. 49, No. 9, pp 1255-1260, 1995).

The riminophenazines clofazimine and B669 also reversed P-gp MDR against vinblastine, doxorubicin and mitomycin C in a human lung cancer line *in vitro* (Constance, EJ, etal, Cancer Letters 85, (1994) 59-63) . Riminophenazines are relatively nontoxic, non-carcinogenic and non-myelosuppressive agents. Clofazimine (Lamprene; Ciba-Geigy) is approved in the USA for the treatment of leprosy.

Drug Delivery Methodologies Used to Reverse P-gp MDR

Liposomal formulations of antineoplastic drugs have demonstrated ability to reverse MDR *in vitro* (Lum BL, etal, Cancer, [1993] 72supp:3502), possibly by alteration of the fluidity of the cell membrane and conformal changes that interfere with the function of the P-gp pump (Cuvier C, etal, Biochem Pharmacol, [1992] 44:509).

Aronex (was Argonex/Argus Pharmaceuticals; The Woodlands, TX) scientists have produced a liposomal formulation of lipophilic anthracycline antibiotic annamycin, Annamycin^{LF}, for the treatment of cancers resistant to anthracycline antibiotics such as doxorubicin. Originally developed at The University of Texas M. D. Anderson Cancer Center (Houston, TX), Annamycin^{LF} entered phase I clinical testing in May 1995 at M. D. Anderson in cancer patients treated with anthracyclines. Annamycin was entrapped in liposomes of different size (median diameter of 1.64 microns for multilamellar liposomal annamycin (L-Ann) and 0.030 micron for small unilamellar annamycin (S-Ann)] with > 90% entrapment efficiency. Partial circumvention of MDR resistance by annamycin is associated with comparable inhibition of DNA synthesis in the nuclear matrix of sensitive and resistant cells (Ling YH, etal, International Journal of Cancer, 1995 May 4, 61(3):402-8).

In contrast, a liposomal formulation of daunorubicin (DaunoXome; NeXstar), recommended for approval by FDA's oncology advisory committee in June 1995 as first-line treatment for Kaposi's sarcoma, did not exhibit significant clinical activity in patients with adenocarcinoma of the colon who failed treatment with a 5-FU containing regimen. In a Phase II trial, 16 patients with metastatic adenocarcinoma of the colon, whose disease has progressed after receiving one 5-FU-containing regimen, were treated with DaunoXome 100 mg/m^2 repeated every 3 weeks. There were no objective responses (Eckardt JR, etal, American Journal of Clinical Oncology, 1994 Dec, 17(6):498-501).

Combination of MDR Modulators in Cancer Therapy

It has been suggested that all MDR modulators do not share the same targets, and that different mechanisms of action can be hypothesized to explain their effect (Muller C, etal, Bull Cancer, [1994] 81:386). Indeed, in screening and comparing the reversing properties of several modulators, researchers at the Foundation Bergonie and Université de Bordeaux II (Bordeaux-Cedex, France) have found some evidence that the mechanisms of action of these compounds on MDR are diverse (Huet S, etal, Eur J Cancer, [1993] 85:632). For instance, a compound like verapamil is able to restore in resistant cells the level of accumulation of doxorubicin reached in sensitive cells, but even when this effect has been reached, the resistance to the drug is not fully overcome. In contrast a compound like quinine has only a modest effect on doxorubicin accumulation in resistant cells; its effect on MDR reversal is, however, similar to that obtained with verapamil. In addition, verapamil does not modify the intracellular amount of drug associated with 50% inhibition of cell growth, which is much higher in resistant cells than in sensitive cells. Quinine, though, considerably lowers this parameter in resistant cells, almost to the level presented by sensitive cells. These observations favor the existence of different targets for the two compounds; verapamil interfering strongly with P-gp and governing drug accumulation and quinine having intracellular targets involved in drug redistribution within the cell rather than in global drug accumulation. If MDR modulators act on distinct targets, then their simultaneous use might lead to synergistic actions.

A number of studies have examined the utility of combining MDR reversal drugs which may inhibit different binding sites on P-gp. In one study, quinine and verapamil used together demonstrated synergy in blocking P-gp function *in vitro* at concentrations readily achievable *in vivo* (Lenhart M, etal, Blood, [1991] 77:348). Sensitivity to daunorubicin (DNR) and to its 4-demethoxy derivative idarubicin (IDA) was modulated in MDR-resistant cells *in vitro* by introduction of the D-isomer of verapamil (DVRP), cyclosporin A (CyA) and SDZ PSC 833. Down-modulation of resistance with MDR modifiers was greater for DNR than for IDA in MDR cells. However, restoration of full sensitivity could only be achieved for IDA, not for DNR. DVRP and CyA in combination were more effective than either compound alone and could abolish P-gp-related resistance to IDA at concentrations of 1-2 microM and 1.6 microM, respectively.

SDZ PSC 833 alone was even more effective and set MDR to zero at a concentration ranging between 0.8 and 1.6 microM (Michieli M, etal, Haematologica, 1994 Mar-Apr, 79(2):119-26).

A phase I study, using BCNU as the primary chemotherapeutic agent, combined four modulators, interferon-α 2b (IFN-α 2b), hydroxyurea (HU), tamoxifen and streptozocin (STZ), to concurrently block different resistance mechanisms. STZ was used to block O^6-alkylguanine-DNA alkyltransferase, HU to inhibit excision repair, and TAM and IFN-α 2b and tamoxifen were used to block other non-specific alkylator resistance mechanisms. One CR was observed in a patient with breast cancer using this regimen (Panella T, etal, ASCO95, Abs. 402).

Monoclonal Antibodies

In another line of investigation, anti-P-gp monoclonal antibodies have been used to specifically reduce the burden of P-gp-positive tumor cells (Rittmann-Grauer LS, etal, Cancer Res, [1992] 52:1810; WT Beck, J Natl Cancer Inst, [1991] 83:1364). In two recently reported studies, novel use was made of an anti-P-gp antibody in combination with a cytokine or liposomes to circumvent P-gp-associated MDR. The core of both studies was the enhancement of anti-P-gp monoclonal antibody MRK-16 (which recognizes an external epitope of human P-gp) activity, either by recombinant human IFN-α (Fogler WE, etal, J Natl Cancer Inst, [1995] 87:94) or by liposome encapsulated vincristine (Sela S, etal, J Natl Cancer Inst, [1995] 87:123), in augmenting drug efficacy against a human colon carcinoma cell line expressing P-gp.

Ingenex (Menlo Park, CA), an operating affiliate of Titan Pharmaceuticals, in a commercial approach to MDR reversal using MAbs, has received notice of allowance for a USA patent covering the company's IGX anti-P-gp MAb.

OVERCOMING OTHER TYPES OF MDR

Overcoming Resistance to Topoisomerase II Poisons

The DNA topoisomerases are important enzymes involved in resolving topological constraints in DNA in association with such cellular functions as transcription, translation, and chromatid separation. As noted above, resistance to topoisomerase II poisons may occur as a consequence of P-gp overexpression or altered topoisomerase II activities. However, neither of these mechanisms necessarily result in cross resistance to all topoisomerase II-directed drugs. For instance, resistance to epipodophyllotoxins and anthracyclines associated with increased expression of P-gp is not usually associated with resistance to the acridine derivative amsacrine. Similarly, resistance to amsacrine and other intercalating drugs due to alterations in topoisomerase II protein is not always associated with resistance to the nonintercalating epipodophyllotoxin class of topoisomerase II poisons. These findings suggest the administration of an alternative class of topoisomerase II poison in selected cases of clinical resistance to a different class of topoisomerase II-directed drug.

Overcoming Resistance to Free Radical-Mediated Drug Cytotoxicity

Several anticancer drugs, of which the anthracyclines are the most important, form free radical intermediates that are thought to contribute to drug cytotoxicity, along with the mechanisms of nucleic acid synthesis inhibition, induction of topoisomerase II-mediated DNA strand breaks, and perturbation of cell membranes. Doxorubicin and related drugs can undergo one-electron reductions in reactions catalyzed by a variety of enzymes (Sinha BK, Chem Biol Interact, [1989] 69:293). The semiquinone radicals so generated may either form covalently binding free radical derivatives, or in the presence of oxygen may be reoxidized to the quinone species in a reaction producing superoxide anions. Decomposition of hydrogen peroxide formed by dismutation of the superoxide anions produces a highly reactive hydroxyl radical, which may directly damage DNA, lipid, and protein. Thus, cellular factors that limit hydrogen peroxide production or repair peroxidative damage to macromolecules could in theory confer some resistance to anthracyclines.

In this regard, increased intracellular levels of catalase and glutathione peroxidase (GSHPx) can deplete hydrogen peroxide, reducing the formation of toxic hydroxyl radicals; researchers have reported an association between increased GSHPx activity and reduced doxorubicin-stimulated hydroxyl radical formation in MDR cells (Sinha BK, etal, Biochemistry, [1987] 26:3776). While such results are consistent with the importance of hydrogen peroxide and hydroxyl radical formation in anthracycline cytotoxicity, other studies have revealed that increased catalase, GSH, and GSHPx levels are not always protective of doxorubicin-mediated damage (Keizer HG, etal, Cancer Res, [1988] 48:4493). Thus, the relative importance of free radical generation in tumor cell kill is unknown and the protective mechanisms outlined above are still speculative. Nevertheless, the GSH-dependent detoxification pathways are of some interest as they are amenable subject to pharmacologic manipulation.

Overcoming Resistance to Alkylating Agents and Platinum Compounds

Resistance to alkylating agents and platinum compounds can be described by at least three broad mechanistic categories, decreased drug accumulation, increased drug inactivation, and enhanced repair of DNA damage (also see FO, V1, #1, p18).

Recent reports have suggested that the ATP-dependent glutathione S-conjugate export pump (GS-X pump) is involved in cell lines with acquired cisplatin resistance

in vitro (Fujii R, etal, J Natl Cancer Inst, [1994] 86:1781; Ishikawa T, etal, J Biol Chem, [1994] 269:29085). This hypothesis has been supported by the analyses of cell lines that have acquired doxorubicin resistance *in vitro* and that overexpress the MDR-associated protein (MRP), as well as cell lines transfected with an MRP-expression vector, which indicate that overexpression of MRP results in an increased activity of the GS-X pump (Mueller M, etal, Proc Natl Acad Sci USA, [1994] 91:13033; Jedlitschky G, etal, Cancer Res, [1994] 54:4833; Leier I, etal, J Biol Chem, [1994] 269:27807). However, more recent studies conducted at the University Hospital Groningen (Groningen, The Netherlands) demonstrate that overexpression of the GS-X pump does not necessarily have to result in cisplatin resistance (de Vries EGE, etal, J Natl Cancer Inst, [1995] 87:537). The fact that overexpression does not necessarily result in cisplatin resistance suggests that the ultimate effect of the GS-X pump depends on other factors, such as the glutathione system (glutathione, glutathione S-transferase activity, or glutathione peroxidase activity).

Reactions of electrophilic alkylating agents with thiol-containing compounds represent a relatively general mechanism of antineoplastic inactivation or detoxification. For example, GSH forms conjugates with a variety of alkylating agents in both non-enzymatic and in GST-dependent reactions. Several laboratories have demonstrated an association between increased bulk GST levels or specific GST isozymes and resistance to drugs such as nitrosoureas, chlorambucil and other nitrogen mustards.

Terrapin Technologies (South San Francisco, CA) is attempting to exploit this phenomenon by constructing inhibitors of GST isoenzymes to enhance the cytotoxicity of anticancer drugs of these types; TER199, an orally available small molecule which inhibits the PS-1, a GST isoenzyme elevated at least two-fold in certain solid tumors over normal tissues, has demonstrated chemosensitizing activity *in vitro* (see FO, V1, #1 pp 18 & 20).

The correlations between GST levels and drug resistance are, however, variable, and some studies have failed to demonstrate a relationship between the overexpression of multiple isozymes of GST and antineoplastic resistance (Fairchild CR, etal, Mol Pharmacol, [1990] 37:801; Leyland-Jones BR, etal, Cancer Res, [1991] 51:587), while in other studies that have compared paired parental and resistant cell lines, the magnitude of alkylating agent resistance associated with increased GST activity is often modest. Thus, the clinical importance of GST and GSH in alkylating resistance is still the subject of debate.

Cisplatin toxicity is thought to be mediated primarily by the formation of lethal intrastrand DNA cross links. Several reports have suggested that increased DNA repair is associated with resistance to this compound, i.e., unscheduled DNA synthesis, which is thought to be indicative of DNA repair, is relatively increased in response to cisplatin treatment in cisplatin-resistant ovarian cancer cells when compared with drug-sensitive parental cells (Masuda H, etal, Cancer Res, [1988] 48:1988), and in a murine leukemia model, cells selected for cisplatin resistance showed enhanced ability to repair cisplatin-induced intrastrand DNA cross links (Sheibani N, etal, Biochemistry, [1989] 28:3120). Intracytoplasmic binding of metallothionein, a small molecule synthesized in the liver and kidney, prevents active molecules of cisplatin from reaching nuclear DNA in tumor cells, and in so doing, exhibits a protective action against cisplatin-induced cytotoxicity (Goncharova EI, etal, ICACC [31 January-3 February 1995; Paris, France], Abs. P239).

Institute Pasteur (Paris, France), and Hôpital de la Salpétriére (Paris, France) researchers are attempting to overcome metallothionein-mediated cisplatin resistance by using a liposomal transfection system to insert the thymidine kinase suicide gene into the human metallothionein promoter. Ganciclovir-mediated cytotoxicity to the liposome-metallothionein-thymidine kinase complexes was demonstrated *in vitro*, and may prove a clinically applicable strategy for overcoming cisplatin resistance (Rixe O, etal, ICACC [31 January-3 February 1995; Paris, France], Abs. 0757).

L-S, R-buthionine sulfoximine depleted GSH (10%-20% of controls) in tumor cells resistant to alkylating agents. In a phase I clinical trial using continuous infusion of BSO and melphalan (L-PAM), GSH depletion to <10% baseline was achieved in tumors but only 20%-40% of baseline in peripheral blood lymphocytes. Clinical activity was noted in patients with refractory ovarian cancer (Bailey HH, etal, ASCO95, Abs. 408). Preincubation of etoposide-resistant human breast cancer cells with BSO sensitized them to etoposide and vincristine, resulting in elevated intracellular drug levels. In contrast, simultaneous exposure to BSO did not result in increased drug accumulation. Also, no clear effects of BSO on drug efflux were observed and drug retention was only minimally increased after BSO treatment, indicating that chemosensitization by BSO may be mediated through increased intracellular drug concentrations and/or protein binding (Schneider E, etal, British Journal of Cancer, 1995 Apr, 71(4):738-43).

Overcoming Resistance to Antimetabolites

Antimetabolites are a clinically important group of cancer drugs used in the treatment of a variety of solid tumors and hematologic malignancies. Traditionally, this group is divided into folate antagonists, pyrimidine analogs, and purine analogs. Strategies designed to overcome the multiple described mechanisms of cellular resistance to these compounds include dose escalation, pharmacologic manipulation of drug metabolism, and rational design of new antimetabolites (Skovsgaard T, etal, Int Rev Cytology, [1994] 156:77).

Among the antifolates, methotrexate is the most important drug, and probably one of the most well-understood antineoplastic compounds. Methotrexate (MTX), an analog of folic acid, displays significant tumoricidal activity against a variety of human neoplasms, including acute leukemia, osteogenic sarcoma, choriocarcinoma, breast cancer, and head and neck cancers, and others. Following uptake by the folate transport system, MTX can bind avidly to and inhibit its primary enzyme target dihydrofolate reductase (DHFR). In the presence of adequate thymidylate synthase activity, inhibition of DHFR results in depletion of the reduced folate pools essential for thymidylate and de novo purine synthesis. The cytotoxicity of MTX is significantly influenced by intracellular polyglutamation. MTX polyglutamates are retained preferentially by cells and bind more effectively to DHFR; polyglutamyl derivatives can also inhibit other folate-dependent enzymes including thymidylate synthase and 5-aminoimidazole carboxamide ribotide (AICAR) transformylase, two enzymes involved in thymidylate and de novo purine synthesis, respectively. Thus, resistance to MTX can result from a several alternative mechanisms, including reduced MTX uptake via a defective folate transport system (Sirotnak FM, etal, Cancer Res, [1981] 41:4447), reduced polyglutamation leading to decreased drug retention as well as reduced inhibition of thymidylate synthase and AICAR transformylase (Cowan KH and Jolivet JA, J Biol Chem, [1984] 259:10793), and either elevated levels of DHFR or reduced affinity of DHFR for MTX (Meilera PW, etal, J Biol Chem, [1980] 255:7024; Goldie JH, etal, Eur J Cancer, [1980] 16:1539). However, while all of these mechanisms have been described in examples of resistance of cultured cells to MTX, increased DHFR levels secondary to gene amplification is the only mechanism identified to date that has been associated with clinical MTX resistance (Trent, JM etal, J Clin Oncol, [1984] 2:8; Borst P, Acta Oncol, [1991] 30:87; Skovsgaard T, etal, Int Rev Cytology, [1994] 156:77).

Resistance to MTX occurs in about 50-60% of patients after three-to-four treatment cycles. The use of high-dose MTX combined with subsequent rescue of normal tissues by administration of the reduced folate leucovorin (5-formyltetrahydrofolate) has been advocated as an approach to circumvent most mechanisms of MTX resistance. At high systemic drug concentrations, MTX penetrates the membrane by passive diffusion. In addition, prolonged exposure of cells to high extracellular concentrations of drug can maintain cytotoxic intracellular drug levels in the face of a drug retention defect secondary to decreased polyglutamation, and increased intracellular MTX delivered by high dose therapy can saturate DHFR in cells whose resistance is due to amplification of the DHFR gene or due to lowered affinity of DHFR for MTX.

In other efforts designed to improve drug efficacy, analogs of MTX which are not substrates for the known mechanisms of resistance have been developed (Bertino JR, J Clin Pharmacol, [1990] 30:291). Trimetrexate (NeuTrexin; U.S. Bioscience), a "non-classical" folate antagonist, represents such an analog. Although binding to DHFR like MTX, because of its high lipophilicity, trimetrexate is taken up by cells independently of the folate-carrier system. And unlike MTX, trimetrexate is unable to formate polyglutamates, so that if resistance to MTX is a result of impaired transport or reduced formation of polyglutamate, there is no cross-resistance to trimetrexate (Bertino JR, etal, NCI Monogr, [1987] 5:87). On the other hand, cells that are resistant to MTX on the basis of amplified DHFR will be cross-resistant to trimetrexate. The utility of trimetrexate is also limited by the association of classical MDR with cross-resistance to trimetrexate, suggesting that trimetrexate and drugs of the MDR phenotype share the same P-gp efflux pump (Assaraf YG, etal, J Natl Cancer Inst, [1989] 81:290).

Sparta Pharmaceuticals (RTP, NC) is developing PT523, licensed from the Dana-Faber Cancer Institute (Boston, MA), as an alternative to methotrexate (MTX) for use in cancer, rheumatoid arthritis and psoriasis. PT523 is a potent new antifolate that cannot be polyglutamated and, therefore, may not cause liver toxicity associated with MTX. Animal studies with PT523 suggest that the compound will be less likely to produce MTX resistance. In addition, its increased lipophilicity may give it a higher efficacy. In murine models the combinations of PT523 with etoposide or novobiocin (a veterinary antibacterial agent) were significantly more effective than when these drugs were combined with MTX, producing tumor growth delays of 8.4 days and 6.9 days, respectively. Overall, the antifolate/topoisomerase II inhibitor treatment combinations produced tumor growth delays that were apparently additive to greater than additive (Holden SA, etal, Cancer Chemotherapy and Pharmacology, 1995, 36(2):165-71).

Several strategies to improve fluoropyrimidine efficacy and overcome resistance have been advanced, including prolonged or continuous exposure to drug (Aschele C, etal, Cancer Res, [1992] 52:1855), and co-administration with 5-FU of the reduced folate leucovorin (Peters GJ, etal, Cancer, [1991] 68:1903). The latter approach results in increases in intracellular 5,10-methylene tetrahydrofolate (5,10-meTHF), a cofactor that stabilizes the FdUMP-thymidylate synthase inhibitor complex. Pretreatment of cells with methotrexate enhances the toxicity of 5-FU by increasing the level of phosphoribosyl pyrophosphate (PRPP); the expanded pool of PRPP is available for conversion of 5-FU to FUMP and FUTP (Peters GJ and van Groeningen CJ, Ann Oncol, [1991] 2:469). Phosphonacetyl-L-aspartate (PALA, U.S. Bioscience), another inhibitor of de novo pyrimidine synthesis, has also been used with 5-FU in an effort to reduce pyrimidine metabolites that compete for the targets of fluoropyrimidine toxicity (Grem JL, etal, Cancer Res, [1988] 48:4441).

Cytosine arabinoside, or Ara-C (Cytosar; Upjohn), an analog of the physiologic nucleoside deoxycytidine, is an important antineoplastic agent effective in the treatment of acute leukemias. Ara-C enters the cell by the nucleoside carrier transport system. It is then activated by phosphorylation to Ara-cytidine monophosphate (CMP) by deoxycytidine kinase (the rate limiting step). Ara-CMP is further phosphorylated to Ara-cytidine diphosphate (CDP) (by dCMP kinase) and to Ara-cytidine triphosphate (CTP) (by nucleoside diphosphokinase), which is the active form of the drug. Ara-CTP inhibits DNA synthesis by competitive inhibition of DNA polymerase alpha (physiological substrate dCTP).

Since Ara-CTP is the active metabolite of Ara-C, the concentration and duration of exposure to this compound determine cells killed. Thus, all mechanisms which tend to reduce the intracellular level of Ara-CTP may result in resistance to Ara-C. A decrease in activation by deoxycytidine kinase is probably the most important mechanism of resistance to Ara-C, and has been demonstrated in animal and human tumor cell lines (Momparle RL and Oretto-Pothier N, in Resistance to Antineoplastic Drugs, Kessel D, ed., CRC Press, Boca Raton, 1989, p. 353); however, other mechanisms of resistance may be relevant, including diminished nucleoside transport of Ara-C (Wiley JS, etal, J Clin Invest, [1985] 75:632), accelerated inactivation of Ara-C by increases in cytidine deaminase (Stewart CD and Burke PJ, Nature New Biol, [1971] 233:109), altered DNA polymerase affinity for Ara-C (Tanaka M and Yoshida S, Cancer Res, [1982] 42:649), increased dCTP pool (due to increased CTP synthetase activity or increased ribonucleotide reductase) in competition with Ara-C (Weinberg G, etal, Proc Natl Acad Sci USA, [1981] 78:2447; Meuth M, etal, Eur J Biochem, [1976] 71:39), reduction of the fraction of tumor cells in the S phase of the cell cycle (Ara-C is a specific inhibitor of cells in the S phase) (Pallavicini MG, Pharmacol Ther, [1984] 25:207), or tumor cells which escape the cytotoxic action of Ara-C through localization in pharmacologic or anatomic sanctuaries (Capizzi R, etal, Semin Oncol, [1985] 12supp3:65).

Administration of high-dose Ara-C represents one approach to overcoming drug resistance and has been clinically useful in the treatment of some leukemias refractory to conventional doses of Ara-C. In cases of resistance secondary to increased drug inactivation by cytidine deaminase, co-administration of Ara-C and a cytidine deaminase inhibitor such as tetrahydrouridine may reverse this mode of drug resistance (Ho DH, etal, Cancer Res, [1980] 40:2441). An alternative pyrimidine analog, Ara-AC (arabinofuranosyl-5-azacytosine or fazarabine), has demonstrated activity against a broad range of tumor cells (Surbone A, etal, Cancer Res, [1990] 50:1220); Ara-AC contains a triazine ring that is resistant to deamination.

Clinically relevant purine antimetabolites include 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG), both of which are widely used in the treatment of acute leukemias; 6-MP and 6-TG are substituted analogs of the physiologic purines hypoxanthine and guanine, respectively. The initial step in the mechanism of action of both compounds is a conversion to their respective ribonucleoside 5-monophosphate derivatives by hypoxanthine-guanine phosphoriribosyltransferase (HGPRT). Subsequently, they are further activated to deoxyribonucleotides; in mammalian cells, 6-MP may be converted to 6-TG deoxyribonucleotide. The cytotoxicity of 6-MP and 6-TG is probably correlated with the incorporation of the nucleotide derivatives in DNA; however, 6-TG-derivatives may also be incorporated in RNA.

An important mechanism of resistance to 6-MP and 6-TG appears to be an inability to perform the initial step of the activation, i.e., the formation of the respective monophosphates by HGPRT (Van Diggelen O, etal, J Cell Physiol, [1979] 98:59; Wolpert MK, etal, Cancer Res, [1971] 31:1620). Another important mechanism may also be an increase in the cellular catabolism of these compounds, due to increased deaminase activity (LePage GA, Can J Biochem, [1968] 46:655) or increased alkaline phosphatase (Scholar EM and Calabresi P, Biochem Pharmacol, [1979] 28:445). In addition, since PRPP is an important factor in both the purine nucleotide synthesis and activation of 6-MP and 6-TG, reduced PRPP synthesis could be a factor contributing to drug resistance (Higuchi T, etal, Antimicrob Agents Chemother, [1977] 12:518).

Reversal of Apoptosis-Mediated Chemoresistance

Because most anticancer agents can induce apoptosis in sensitive cells (JA Hickman, Cancer Metastasis Rev, [1992] 11:121), drug effectiveness may depend on metabolic events leading to apoptosis as well as specific interaction with biochemical targets (KV Chin, et al, Adv Cancer Res, [1993] 60:157). For instance, the bcl-2 gene is expressed in many types of human tumors and becomes transcriptionally deregulated in a majority of non-Hodgkin's lymphomas as the result of t(14;18) chromosomal translocations that move the bcl-2 gene at 18q21 into juxtaposition with the Ig heavy chain locus at 14q32, thereby leading to overproduction of bcl-2 mRNAs and the p26 integral membrane protein of bcl-2 (J Reed, et al, Oncogene Res, [1989] 4:271); the 26 kDa bcl-2 protein has been shown to block apoptosis induced by many types of stimuli, including a wide variety of chemotherapeutic drugs (JC Reed, et al, Ann Oncol, [1994] 5 supp1:S61). The bcl-2 gene is also expressed in a variety of solid tumors and leukemias (L Campos, et al, Blood, [1993] 81:3091; J Reed, et al, Cancer Res, [1991] 51:6529; TJ McDonnell, et al, Cancer Res, [1992] 52:6940). The mechanism by which bcl-2 protects cells from drug-induced death remains obscure, but appears to involve interference with events that occur downstream of drug-mediated DNA damage.

For a comprehensive review on apoptosis-related drug resistance see FO, V1, #1, pp 22-31.

Exhibit 7
Combination Approaches in Clinical Trials Against MDR Cancers

MDR-reversing Agent	Chemotherapeutic Agent	Status/Location/Indication
SDZ PSC 833 (5 mg/kg, PO qid)	Paclitaxel (60-80 mg/m2, IV)	Phase I/USA/solid tumors (ASCO95, Abs. 1585 and 406)
SDZ PSC 833 (12.5 mg/kg PO,q 12 hours for 8 days)	Vinblastine (0.9 mg/m2 continuous IV q day for 5 days)	Phase I/USA/metastatic cancer, renal cancer (ASCO95, Abs. 1558)
SDZ PSC 833 (4 mg/kg q 8 hours 8 times)	Doxorubicin (20 mg/m2) + vincristine (0.5 mg/m2) +cyclophosphamide (750 mg/m2) + prednisone (100 mg/m2)	Phase I/USA/refractory non-Hodgkin's lymphoma (ASCO95, Abs. #1578)
SDZ PSC 833 (5 mg/kg q 6 hours or 20 mg/kg/d)	Etoposide (60-70 mg/m2/d x 3, IV)	Phase I/USA/ASCO95, Abs. 407)
S 9788 (escalating continuous IV infusion of 160, 240, 320, 400, 480 mg over 6 hours)	Doxorubicin (50 mg/m2 bolus 3 hours after S 9788 infusion onset, q 3 weeks)	Phase I/France/refractory malignancies (ASCO 1995, Abs. 411)
S 9788 (MTD was 96-104 mg/m2 as a 30 minute IV infusion)	Doxorubicin(50 mg/m2 over 5 minutes)	Phase Ib/Europe/colorectal cancer (ASCO95, Abs. 413)
VX-710	Paclitaxel	Phase I/USA/solid tumors
VX-710	Doxorubicin	Phase I/II/USA/solid tumors
L-S, R-buthionine sulfoximine (BSO) (0.75 g/m2/h x 24 to 1.5 g/m2/h x 48, (Continuous infusion)	Melphalan (15 mg/m2, IV)	Phase I/USA/tumors resistant to alkylating agents via GSH (ASCO95, Abs. 408)
Dexniguldipine (oral)	Daunorubicin	Phase I/Germany/hematologic malignancies (cytarabine-pretreated refractory AML)
Mitotane (escalating; starting at 4 gm, 5 gm and 6 gm, PO, q week 3 hours prior to vinblastine)	Vinblastine (6 mg/m2 IV, q week)	Phase I/USA/refractory malignancies (ASCO 1995, Abs. 415)
IFN-α 2b (escalating dose from 0.5 mu/m2 to 1 mu/m2 on days 5-9) + hydroxyurea (500 mg q 8 hours, days 5-9) + tamoxifen (40 mg/m2, days 1-10) + streptozocin (500 mg/m2, days 6-9)	BCNU (125-150 mg/m2 on day 8)	Phase I/USA/advanced malignancies (ASCO95, Abs. 402)
Intracellular histamine antagonist N, N-diethyl-2-[4-(phenylmethyl)phenoxy] ethanamine-HCl (DPPE) (240 mg/m2 over 80 or 440 minutes weekly)	Various single agents to which, in most cases, the patient's tumor was previously resistant	Phase I/II/Canada/advanced refractory cancer (Brandes LJ, etal, Journal of Clinical Oncology, 1994 Jun, 12(6):1281-90).
L-verapamil (125 mg/m2 PO q 4 hours beginning 24 hours before and continuing for 24-hours after paclitaxel infusion)	Paclitaxel (140 mg/m2 as a 96-hour infusion)	Phase I/USA/anthracycline resistant metastatic breast cancer (ASCO95, Abs. 403)
Dexverapamil (240-1200 mg/m2/day)	Etoposide (200 mg/m2) + vincristine (1.6 mg/m2) + doxorubicin (40 mg/m2) continuous IV x 96-hours, days 1-4 and cyclophosphamide (750 mg/m2 day 6) + prednisone (60 mg/m2, days 1-6)	Phase I/II/USA/relapsed lymphoma patients (ASCO95, Abs. 404 and Wilson WH, etal, Journal of Clinical Oncology, 1995 Aug, 13(8):1995-2004)
Dexverapamil (3000 mg/d)	Vinblastine (1.4 mg/m2/d)	Phase I/II/The Netherlands/ renal cell carcinoma (Mickisch GH, etal, World Journal of Urology, 1994, 12(4):214-23)
Dexverapamil (begun 18 hours before day 1 of vinblastine administration and given orally every 6 hours (either as a 120 mg/m2 dose or a 180 mg/m2 dose plus dexamethasone) for 12 doses)	Vinblastine (0.11 mg/kg IV bolus injection on days 1 and 2 every 21 days	Phase I/II/USA/advanced renal cell carcinoma (Motzer RJ, etal, Journal of Clinical Oncology, 1995 Aug, 13(8):1958-65)
Etanidazole (16 g/m2 by 96-hour continuous infusion)	Ifosfamide (16 g/m2) + carboplatin (1.6-1.8 g/m2) + etoposide (1.2 g/m2) + autologous stem cell support	Phase I/USA/advanced malignancies (ASCO95, Abs. 405)

Exhibit 8
Selected Agents in Development for the Treatment of Lung Cancer

Primary Developer/ Affiliate(s)	Generic Name/ Number/Brand Name	Drug Type/Target/ Mechanism/Delivery	Status/Location/ Indication	Comments
Aronex (was Argonex/ Argus)/ U Texas, M. D. Anderson Cancer Center	Annamycin/AR-522	Lipophilic anthracyline analog/RNA synthesis inhibitor/liposomal formulation/injectable	Phase I (5/95)/USA/ cancers treated with anthracyclines	
Beaufour-Ipsen	BN 52205, BN 52207 and BN 52211	Ether phospholipids	Preclin/Europe	
Block Drug	Masoprocol/C-205; CHX-100; CHX-2053; CHX-2054/Actinex (USA), Gebrentil	Natural product derived from Larrea tridendata/ P-gp inhibitor/ lipoxygenase inhibitor	Preclin/USA/lung, breast, colon, ovarian cancer	L92/USA/actinic keratosis (topical); Chemex sold rights to the drug to Block Drug in June 95
BioChem Pharma/ Glaxo Wellcome	BCH-1184	Heteroanthracycline	Preclin/Canada	Exhibits activity equivalent to doxorubicin but avoids P-gp MDR
BioChem Pharma/ Glaxo Wellcome	BCH-2050	Heteroanthracycline	Preclin/Canada	Exhibits activity equivalent to doxorubicin but avoids P-gp MDR
Boehringer Ingelheim	BIBW22BS	Phenylpteridine analog of dipyridamole/bifunctional modulator of P-gp and nucleoside transport	Preclin/Europe	
Boehringer Mannheim	Ilmofosine; Et-16S-OEt/ BM 41.440	Ether phospholipid analog (thio-derivative)/selective inhibitor of protein kinase C (PKC)/may change the dynamic structural organization of tumor cell membrane	Phase II/Germany	
Byk Gulden	Dexniguldipine-HCl/B859-035, B895-35	Dihydropyridine compound, R-enantiomer of the calcium antagonist niguldipine/Ca^{2+} calmodulin inhibitor; may inhibit PKC/oral	Phase II/Germany; USA/ renal cell carcinoma, acute myeloid leukemia resistant to chemotherapy, refractory multiple myeloma	
Cell Therapeutics	CT-2584	Synthetic small molecule/ regulates phospholipase-D (PLD) activity in tumor cells/ anti-angiogenic and anti-metastatic activity, reverses P-gp MDR	Phase Ib/UK	Minimal or no toxicity to normal cells (AACR95, Abs. 2301)
CytRx/Rush-Presbyterian St. Luke's Hospital	Cremophor EL/ CRL-1336	Solubilizing agent; chemosensitizer/reversible inhibitors of P-gp	Phase I/Australia/ advanced cancer	
CytRx	Solutol HS 15/ CRL-1095	Solubilizing agent; chemosensitizer/ reversible inhibitors of P-gp	Preclin/USA	
CytRx	Tween/80CRL-1605	Solubilizing agent; chemosensitizer/reversible inhibitors of P-gp	Preclin/USA	
Glaxo Wellcome	GF-120918	Radio and chemosensitzer/ P-gp inhibitor	Preclin/UK/solid tumors	
Glaxo Wellcome/ Cancer Research Campaign	1069C85	Synthetic tubulin binder/ circumvents resistance associated with vinca alkaloids/oral	Phase I/USA/solid tumors	
Ingenex (Titan Pharmaceuticals)	IGX	MAb/reverses P-gp MDR/ injectable	Preclin/USA/ leukemia and lymphoma	
Isis Pharmaceuticals		Antisense compounds/ MRP inhibitors	Research/USA	
Knoll (BASF)/ Mitsui Pharmaceuticals (licensee, Japan)	Dexverapamil	Chemosensitizer	Phase II/Europe	See Exhibit 7

— continued on next page

Mitsui Pharmaceuticals (Mitsui Toatsu Chemicals)/ Knoll AG (licensee in Europe and NA)	MS-209	Quinilone derivative/ MDR modulator	Phase II/Japan	In combination with vincristine or doxorubicin
Proter/Knoll (semi-exclusive licensee)	Ambamustine/ PTT-119	Tripeptide analogs of L-PAM/DNA synthesis inhibitor	Reg/Italy/myeloma, lymphoma, relapsed or resistant non-Hodgkin's lymphoma	May be used in clinical trials as a non-cross-resistant agent in multi-drug protocol (Manna A, etal, Experimental Hematology, 1994 Jun, 22(6):517-20)
Roche Bioscience (was Syntex)/Eli Lilly (licensee)	RS-33295	Radio/chemosensitizer for naturally-derived anticancers	Preclin/USA	
Sandoz	SDZ PSC-833	A non-immunosuppressive, non nephrotoxic analog of cyclosporine A	Phase I/USA, Europe/ prostate cancer; breast; lung; ovarian	See Exhibit 7
Sandoz	SDZ 280-446	Hydrophobic cyclopeptide derivative		
Servier/Institut Gustave Roussy	S-9788	Radio/chemosensitizer; MDR-reversing agent/ doxorubicin and vinca-alkaloids	Phase II/France/colon carcinoma, breast cancer (in combination with doxorubicin in refractory cancer)	See Exhibit 7
Sparta Pharmaceuticals/ Dana-Faber Cancer Institute (licensor)	PT523	Antifolate that cannot be polyglutamated	Preclin/USA	
Sphinx Pharmaceutical (Eli Lilly)	Safingol/ Kynacyte	Protein kinase C (PKC) inhibitor/injectable	Phase I/USA	Also in combination with doxorubicin
Sphinx Pharmaceutical (Eli Lilly)	SPC-10406	Analog of protein kinase C (PKC) inhibitor balanol (SPC-100840)/ non-specific PKC inhibitor	Preclin/USA	Increased VBL accumulation compared to verapamil, was synergistic with DOX in cytotoxicity assays and at nontoxic doses potentiated DOX in mice bearing B16F1 melanoma (AACR95, Abs. 1747)
SRI/Roberts Pharmaceutical (licensee); HN Pharma (licensee-Europe, except UK and Ireland, Middle East and Africa); DuPont Merck (licensee-USA), Taiho (licensee-Japan)	Etanidazole/ DuP-453, SR-2508/ Radinyl	Radio/chemosensitizer/ hypoxic tumor cells/IV	Phase III/USA/head & neck cancer; phase II/USA/lung, bladder and prostate cancer	
SunPharm/Warner-Lambert (worldwide rights); Nippon Kayaku (licensee); Johns Hopkins U; Roswell Park Cancer Institute; U Florida	Diethylnorspermine (DENSPM)	Synthetic polyamine analog/ inhibits natural polyamine synthesis and depleting cell of existing polyamines causing tumor cells to die	Phase I/USA/melanoma; pancreatic cancer; ovarian; lung cancer	
Thorax Hospital (Heidelberg, Germany)	Edelfosine/ET-18-OCH3; ET-18-OME; NSC-324	Ether lipid analog, (methoxy-substituted)/ phospholipase C inhibitor/PO	Phase II/ Germany/ non-small cell lung cancer	Dosage used is 300 mg/day
Vertex Pharmaceuticals	VX-710	MDR-1 (P-gp) inhibitor/ iv, oral	Phase I/II/USA	
Xenova	XR1500	Small molecule inhibitors of the P-gp; isolated from a microbial source/parenteral	Preclin/UK/breast, lung and ovarian cancer	
Xenova (exclusive licensee)/Cancer Research Campaign	XR5000	Topoisomerase inhibitor/ synthetic anticancer active in cancer cells exhibiting various types of MDR including P-g	Phase I/UK/solid tumors (colon carcinoma, melanoma, breast and sclc)	

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