

New Frontiers in Ovarian Cancer Diagnosis and Management*

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Ovarian carcinoma is now the leading cause of death among women. Surgery has reached its limits, and further aggressive surgery will result in an inordinate morbidity and mortality.

Ovarian carcinoma is ideally treated by complete surgical removal of the cancer, followed by anti-cancer chemotherapy. Since it is often impossible to remove all of the cancer, adjunctive chemotherapy is playing an increasingly important role in the management of the cancer. New anti-cancer drugs must be found or synthesized, and new combinations of current anti-cancer drugs with mechanisms to protect the bone marrow must be explored.

The field of genetics and the identification of the patient at high risk because of a familial history of ovarian cancer must be expanded.

The role of tumor markers and oncogenes requires more in-depth study so that these signs can play a greater role in monitoring and identifying the patient with early ovarian cancer.

The emerging fields of genetic engineering and biologic response modifiers are opening up new avenues for additional modalities of therapy.

The expanding areas of research in cancer are starting to dispel the doom and gloom of the last three decades with a spirit of optimism for the diagnosis and treatment of ovarian cancer, as the new century approaches.

Great strides have been made, in the last two decades, in understanding basic cellular functions. This knowledge is the keystone in structuring a program to identify the patient at high risk, formulating tests for making an early diagnosis, and treating the cancer on guidelines established through this research.

It is estimated that there will be 20,700 new cases of carcinoma of the ovary diagnosed in 1991, with approximately 12,500 deaths [1,2]. This fact means that approximately one woman in 70 at some time in her life will develop an ovarian tumor. The tragedy is that in each decade more than 100,000 women at the height of their economic and social productivity will die from this dread disease. Although progress has been made in diagnosing and treating ovarian cancer, the discouraging

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Abbreviations: AFP: alpha-fetoprotein CA125: cancer antigen 125 CCD: cell cycle distribution CEA: carcinoembryonic antigen DCT: Division of Cancer Treatment, National Cancer Institute DDTC: diethyldithiocarbamate DI: DNA index DM/70K or NB/70K: Dianon marker NB/70K EGFR: epidermal growth factor receptor ER: estrogen receptor FCM: flow cytometry GSH: glutathione HCG: human chorionic gonadotropin H2n: HER-2/neu oncoprotein LASA-P: lipid-associated sialic acid in plasma PR: progesterone receptor SI: synthesis index TPA: tissue polypeptide antigen UGF: urinary gonadotropin fragment

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reality is that, although the treated patients are living longer and more comfortably, the five-year survival rate has not been appreciably improved in the last two decades.

Carcinoma of the ovary is not a surgical disease. In the present state of our knowledge and treatment modalities, however, surgery is the backbone of treatment, and the prognosis depends upon the size of the residual cancer following surgery. In addition, surgery has obviously reached its limits, and any additional aggressive surgery will create an inordinate morbidity and mortality. It is becoming obvious that ovarian cancer, like many others, is really a systemic disease. Until 1970, it was extremely rare to find carcinoma of the ovary metastasizing to the liver parenchyma, to the bone marrow, lung, or brain. Now that chemotherapy has changed the longevity of these patients, however, late recurrences are beginning to be found, with increasing frequency, in these organs. It is obvious that more attention must be given to the long-term systemic treatment of ovarian cancer. It is time to explore the new frontiers for ovarian cancer.

NATIONAL PROGRAM FOR THE CONQUEST OF CANCER: REPORT OF THE NATIONAL PANEL OF CONSULTANTS ON THE CONQUEST OF CANCER

Ralph W. Yarborough, Chairman of the Committee on Labor and Public Welfare of the U.S. Senate, on November 27, 1970, stated, "Cancer is a disease which can be conquered. Our advances in the field of cancer research have brought us to the verge of important and exciting developments in the early detection and control of this dread disease, but as a nation we have not put forth the effort necessary to exploit the full potential of these gains, nor have we made the proper effort to ascertain what additional avenues of research should be opened. The National Program for the Conquest of Cancer had been initiated by President Nixon who committed millions of dollars to this project over the next decade." A great deal has been accomplished, but much more must be done. As scientists and clinicians look to the next decade, they see certain areas that have been identified but need more development.

TUMOR MARKERS

Tumor markers have made a significant contribution to stimulating interest in cell metabolism and pathophysiology. They have been particularly useful in monitoring treatment; however, the markers have and will probably always have a disadvantage in identifying an early tumor. To date, only two, human chorionic gonadotropin (HCG) and alpha-fetoprotein, are known to be specific. They are also sensitive. The others are not only not specific, but they are not very sensitive. It is, however, important to review their background and what they represent.

The problem with tumor markers as a means of making a diagnosis is that a tumor marker is developed from a certain volume of tumor. By that time, it is no longer an early but rather a biologically late tumor.

Serum assays for tumor markers, most notably cancer antigen 125 (CA125), have been evaluated as a means of decreasing the need for second-look laparotomy. There is now abundant evidence that when the CA125 level is normal, many patients still harbor occult tumor. Almost all patients have a significant amount of tumor when the CA125 level is elevated.

By the time a tumor has reached the size of one cubic centimeter, there are a billion cells present. That size requires 30 population doublings. Although it is not

directly known how long the doubling time for ovarian cancer is, since this type is a heterogeneous tumor with different clones of cells present, it may be assumed that the doubling time might encompass 30 to 200 or more days to reach the size of a cubic centimeter. It may be regarded clinically as an early tumor, but, in reality, it is biologically a late tumor. A question that remains to be answered, but which may offer an explanation for some unanswered questions, is that some of these multiple clones present in ovarian cancer may contain metastasizing phenotypes; if so, it is conceivable that a metastasis may occur in the first ten to 20 doubling times. The author therefore believes that the role of the tumor marker will be for monitoring treatment and unfortunately will not be able to serve as a predictable means for making an early diagnosis.

Alpha-Fetoprotein (AFP)

AFP is an enzyme immunoassay measuring protein associated with fetal tissue and malignancy. It is elevated in primary tumors of the liver and non-seminomatous testicular germ-cell tumors; it is particularly important to the gynecologic oncologist when dealing with an endodermal sinus tumor of the ovary.

Cancer Antigen 125 (CA125)

CA125 is an immunoradiometric assay using the monoclonal antibody OC125, with 88–90 percent sensitivity in detecting non-mucinous epithelial ovarian carcinoma and 60 percent sensitivity in detecting uterine cancer. It is an assay developed by Bast et al. [3], who began to use monoclonal antibodies to identify antigens circulating in the blood of patients with ovarian cancer. The one hundred and twenty-fifth monoclonal antibody screened was designated OC125, and its antigenic determinant was called CA125. The presence of CA125 antigenic determinant in serum can be identified only by the OC125 antibody and is measured by immunoassay. The surface epithelium of normal fetal and adult ovaries does not express the CA125 antigenic determinant, except in the presence of an inclusion cyst, areas of metaplasia, and papillary excrescences. This finding makes the CA125 test valuable in the diagnosis of ovarian cancer, particularly in monitoring therapy for this malignancy.

Carcinoembryonic Antigen (CEA)

CEA is an enzyme immunoassay, using a monoclonal antibody against glycoprotein produced by immature and/or malignant cells that originate in the gut. Elevated values are associated with carcinomas of the rectum, colon, lung, and breast. It probably has its greatest value in monitoring therapy for gastrointestinal tract tumors.

Dianon Marker NB/70K (DM/70K or NB/70K)

This test is a radioimmunoassay for detecting human ovarian tumor-associated antigen NB/70K, using the monoclonal antibody NB 12123. A sensitivity of 70 percent is found in ovarian carcinoma [4]. Elevated levels have also been associated with other gynecologic malignancies, as well as with carcinoma of the lung and breast.

Human Chorionic Gonadotropin, Beta Sub-Unit (HCG)

HCG is an enzyme immunoassay measuring hormone ordinarily made by the placenta and used as an indicator of pregnancy. HCG is also produced by tumors of germ-cell origin, such as testicular and ovarian tumors, as well as some lung cancers. It is particularly important in evaluating gestational trophoblastic neoplasias.

Lipid-Associated Sialic Acid in Plasma (LASA-P Test)

LASA-P test is a biomarker, useful in a wide range of malignancies, that reflects alteration in the surface membrane of malignant cells. The LASA-P test measures total gangliosides and glycoproteins by the biochemical extraction and partition method of Katopodis and Stock [5]. Sensitivities range between 77–97 percent, depending on the cell of origin of the neoplasm. Studies have shown improved predictive value when the LASA-P test is combined with other biomarkers and biomarker profiles.

Tissue Polypeptide Antigen (TPA)

TPA is an immunoradiometric assay utilizing molecules derived from the cytoskeleton of epithelial cells. TPA is useful in the management of many cancers. In prostate cancer, TPA has been shown to be a reliable marker for estimating prognosis. Patients with normal TPA values at the time of diagnosis have a longer survival than those with elevated values. The assay's value is being explored for the study of ovarian malignancy.

Urinary Gonadotropin Fragment (UGF)

UGF is a small peptide present in the urine and tissues of patients with gynecologic cancers. It is used as a tumor marker, particularly in the diagnosis of ovarian cancer. Clinical observations were reflected at each clinic visit by UGF alone in 67 percent, by CA125 alone in 57 percent, and by UGF and CA125 together in 87 percent of cases. When evaluated separately, UGF and CA125 levels predicted 71 percent and 57 percent, respectively; together they forecast 86 percent of recurrent cancers prior to clinical manifestations. UGF and CA125 should be used together in the detection and management of ovarian cancers. This marker adds another dimension to monitoring therapy for patients with ovarian cancer.

Dianon Systems Incorporated [6] has proposed a breast cancer profile that can also be used for studying the prognosis for ovarian cancer. This profile consists of the DNA index (DI), cell cycle distribution (CCD), and synthesis index (SI), as well as estrogen and progesterone receptors, epidermal growth factor receptor, and HER-2/neu oncoprotein (H2n).

ONCOGENES

Oncogenes traditionally have been defined as genes able to confer on cells the property of unregulated growth. All known oncogenes are derived from “proto-oncogenes,” their genetic counterparts, which are normally found in all mammalian cells and generally play a role in normal processes of cell growth and differentiation. In the past, oncogenes have been considered to be good genes that have gone bad. Recently this view has been challenged. It is reported that oncogenes are not “bad”

genes, but “good” genes that have been altered, inappropriately expressed, or inappropriately inactivated [7].

Oncogenes fall into a few general categories: those involved in phosphorylation, those that regulate growth, those that play a role in protein transcription, and those involved in DNA replication.

The protein product of each is found in that part of the cell best suited to its activity. The *src* protein, a protein kinase, spans the cell membrane; the *myc* protein, which controls messenger RNA, operates in the nucleus.

When proto-oncogenes undergo certain genetic alterations (including point mutations, insertions, deletions, and translocations), the normal control of growth and differentiation may change to the promotion of neoplastic development. The presence of oncogenes was originally assessed on the basis of their ability to induce transformation to a malignant phenotype in tissue culture (elevated through morphologic changes) or tumorigenicity when cells expressing those genes were introduced into animals.

Although most studies have been directed to the ability of oncogenes to cause uncontrolled growth of cells, and thereby the development of tumors, it is now accepted that uncontrolled growth, taken alone, does not confer on a tumor the property of being either benign or malignant. Other properties of malignant cells, including the ability to metastasize and resistance to chemotherapy and radiotherapy, are more important than the simple growth properties in determining whether therapy of a patient with cancer will be successful. The development of the changes in tumor cells that lead to a more malignant phenotype is known as tumor progression.

In recent years, investigators have studied the capacity of oncogenes to lead to two properties of malignant cells: (1) the ability to metastasize, and (2) the development of radiation resistance. Parallel to this research, there have been cytogenetic changes in the transformed cells that have been investigated in the sites of oncogene integration into the host genome, and there have been attempts to correlate such genetic events of the biologic properties of the transformed cells.

HER-2/neu oncoprotein (H2n) is an investigational assay for a protein product of the HER-2/neu oncogene. Amplification in this gene is significantly greater in patients with early relapse and poor survival. In node-positive breast disease, this test may predict outcome better than either estrogen or progesterone receptors. The standard range for HER-2/neu-positive is greater than 5 femtomoles/mg protein.

The HER-2/neu proto-oncogene is amplified in 25 to 30 percent of human primary breast cancers, and this alteration is associated with disease behavior. In studies [8], several similarities were found in the biology of HER-2/neu in breast and ovarian cancer, including similar incidents of amplification and direct correlation between amplification and overexpression, evidence of tumors in which overexpression occurs without amplification, and the association between gene alteration and clinical outcome.

Berchuck and his colleagues have recently reported [9] on the overexpression of HER-2/neu associated with poor survival and advanced epithelial ovarian cancer. They used the immunohistochemical technique involving a monoclonal antibody specifically reactive with the external domain of HER-2/neu to study expression of the HER-2/neu in frozen sections of normal ovary and advanced epithelial ovarian cancer. They found that survival with a high HER-2/neu expression was significantly

less than that of a control group and, in addition, was less likely to have a complete response to primary therapy. It is obvious from all of these findings that HER-2/neu deserves further evaluation as a prognostic marker in epithelial ovarian cancer.

FLOW CYTOMETRY (FCM)

This technique has provided investigators with a reproducible method for measuring single-cell fluorescence. Flow cytometry uses an instrument which consists of a source of light, usually a laser; light detectors or photomultiplier tubes; and a computer, which serves as a signal-processing system. Chemical stains for cellular components are utilized in order to make specific measurements.

Flow cytometry uses a focused beam of light from a laser to strike the cells as they pass, in single file, through the sample chamber. The scattered light is measured on the photodetector, and, after the light passes through a series of filters and lenses, the fluorescent signal is picked up by photomultiplier tubes. The electrical signal from the photomultiplier tubes is then processed by the computer.

Fluorescence is used because the blue-excited yellow-green fluorescence of fluorescein is easier to discriminate from autofluorescence. The focused laser beam provides illumination.

Among the properties of cells that can be measured using flow cytometry are the size, internal structure (such as granularity), DNA and RNA content, viability, cell shape, protein content, and a variety of internal and surface biomarkers. The data from the computer are usually presented as a frequency histogram, which serves to render comprehensible the data from measurements on thousands of individual cells or nuclei.

The results obtained by the flow cytometry can be measured using other methods. The advantage of flow cytometry, however, lies in its ease of measurement, reproducibility, and in the fact that it provides multi-parameter single-cell analysis, which permits direct assessment of cellular heterogeneity.

Since it is possible to provide multiple analyses on single cells, flow cytometry allows the description of cellular heterogeneity, establishing the biologic counterpart of clinical heterogeneity common to most tumors. With the ability to analyze DNA content, which is a reflection of cell ploidy, there is now a method for establishing the prognosis of many solid tumors. Many useful flow cytometry assays exist, and the important ones in oncology include:

1. Markers of malignancy, useful as an adjunct in the diagnosis of cancer and the detection of minimal disease
2. Cytokinetic markers, which can be used to describe tumor growth and hence tumor aggressiveness
3. Markers of cellular differentiation, which have been used to characterize tumor cells—at this time, predominantly in the hematologic malignancies

Following work using the Feulgen technique for staining DNA, studies have been directed to identify the close relationship between chromosome number and nuclear DNA content, as measured by flow cytometry. It is now accepted that the best evidence for the diagnosis of malignancy is the description of the clonal chromosomal abnormality. The relationship between DNA content and chromosomal abnormalities is the keystone for many studies in the field of oncology.

Flow cytometry provides a reproducible quantitation of single-cell DNA content and the description of a population of cells based on modal DNA content, usually in the G1/0 phase of the cell cycle. This finding can be compared to the DNA content of normal cells (usually a contaminant of tumor samples) and expressed as the DNA index, which may be equal to one (diploid), greater than one (hyperdiploid), or less than one (hypodiploid).

Abnormal DNA content is referred to as aneuploidy, and this condition is associated with an aggressive tumor. Currently, about 70 percent of human solid tumors display aneuploid DNA content.

At this time, flow cytometry has provided an easy and reproducible analysis of DNA content that directly reflects cell ploidy. Aneuploidy, a stable marker of malignancy, is present in more than 70 percent of solid tumors. Its value as an independent marker for prognosis is suggestive, but more work needs to be done to produce hard data on this observation. Flow cytometry's great contribution is that it provides multiple analyses of single cells and permits the description of cellular heterogeneity, which forms the biological counterpart of the clinical heterogeneity that clinicians deal with in practice.

FAMILIAL OVARIAN CANCER

For many years, there has been an awareness that ovarian cancers seem to occur in families with more frequency than chance alone would indicate.

F.P. Li, A.H. Rapoport, J.F. Fraumeni, Jr., and R.D. Jensen reported on familial ovarian carcinoma in *JAMA* in 1970 [10]. Since then, there have been many articles on this subject. Recently, Piver and his colleagues at the Roswell Park Memorial Institute in Buffalo, New York, have established the Familial Ovarian Cancer Registry. Piver has reported on the familial ovarian cancer syndrome, stating that it includes an earlier than usual age at diagnosis, as compared to the sporadic cases (average age, 47 versus 53 years); a histologic diagnosis of poorly differentiated serous adenocarcinoma; an autosomal dominant pattern of inheritance with variable penetrance; transmission in a vertical fashion from either parent; and a uniformly poor prognosis, with less than 10 percent of the women surviving for more than five years. Lynch has also contributed a great deal to research on the problem of genetics and the cancer patient.

Lynch and his colleagues [11] have described three different genetic and hereditary syndromes in ovarian cancer patients:

1. Women at risk for developing only ovarian cancer
2. Women at risk for developing breast and ovarian cancer
3. The cancer family syndrome, in which men are at risk for developing adenocarcinoma of the colon and women are at risk for developing adenocarcinoma of the colon, ovary, and uterus

Because of the autosomal dominant nature of the inheritance of these familial syndromes, the risk for female first-degree relatives of affected patients can be as high as 50 percent. When only one first-degree blood relative has been treated for common epithelial ovarian cancer, it is the author's opinion that the risk factor for such a patient would be in the range of 5 percent and no greater than 10 percent. The 50 percent figure relates to at least two first-degree blood relatives, who are mother, sister, or daughter. If, however, any of these patients has a cancer in her late 30s or

early 40s, the author believes that the occurrence of cancer in these first-degree blood relatives in the premenopausal years increases the risk factor, but that it is not as high as 50 percent.

A Familial Ovarian Cancer Registry, established in 1981, continues to add families which contain two or more relatives with ovarian cancer [12]. As of August 1989, the newsletter of the Familial Ovarian Cancer Registry states that there are 222 families that have been listed in the Registry, accounting for more than 539 patients with ovarian cancer. In addition, there are 95 additional families, for whom the Registry is verifying that there are two or more first-degree relatives with ovarian cancer. The Registry concludes that familial ovarian cancer should no longer be regarded as a rare occurrence.

The newsletter also states that genetic counseling for a prophylactic oophorectomy should be done at the time when a woman has completed her family but no later than age 35. This stage is crucial to all women with a familial history of ovarian cancer, because the disease occurs most commonly in sister-sister and mother-daughter pedigrees. Sisters and daughters in families with a history of familial ovarian cancer (two or more first-degree relatives) have a 50 percent chance of developing the disease. This rate compares to a 1.4 percent chance in women without such family history, or only one out of every 70 newborn females in the United States. In view of these statistics, genetic counseling should start in the early twenties, and actual physical surveillance should begin in the early thirties. Physical surveillance consists of pelvic and abdominal examinations and a CA125 assay every six months and pelvic or vaginal ultrasound every year. Similar genetic counseling and surveillance should include women whose mothers and grandmothers, mothers and maternal aunts, two or more aunts, or an aunt and a daughter developed ovarian cancer.

In addition to the CA125 marker, another serum marker reported is measurement of the serum level of alpha-L-fucosidase. Fucosidosis is an autosomal recessive storage disease due to a deficiency of alpha-L-fucosidase activity in tissues and body fluids. The mechanism controlling levels of the enzyme in serum is unknown. The quantity of alpha-L-fucosidase activity in the serum of humans apparently is determined by heredity. An individual may inherit either low, intermediate, or high activity of alpha-L-fucosidase in serum. Low enzyme activity (<100 U alpha-L-fucosidase/ml serum), intermediate activity (100–274 U alpha-L-fucosidase/ml), and high activity (≥ 275 U/ml) are the levels usually reported. About 8 percent of the general population have low enzyme activity in serum. It has been reported that females with low activity of alpha-L-fucosidase in serum were three times more common among ovarian cancer patients than among healthy females. This finding suggested that low activity of alpha-L-fucosidase in serum of females may be a hereditary condition, associated with increased risk of developing ovarian cancer. A study of this condition may contribute to understanding of the clinical features of the familial ovarian cancer syndrome. Wench and his colleagues have found that, even though several family members with ovarian cancer did manifest a decrease in enzyme activity, the association was not specific enough to assign risk based on this marker alone. Therefore, great caution must be exercised in interpreting the results.

SEX STEROID RECEPTORS

Sex steroid hormone receptors provide the means by which estrogen and progesterone influence their target tissues. Receptor assays have proved clinically useful in

the management of breast cancer. Although much research had been done on receptors, it was Jensen et al. [13], working with breast tissue, who brought this research to fruition. Receptor assays, as they apply to the study of ovarian cancer, are currently receiving a great deal of intensive investigation. Although estrogen and progesterone receptors have been widely used clinically to identify tissues sensitive to estrogen and progesterone, new applications are being explored.

The ovaries are identified with producing sex steroid hormones, particularly estrogen and progesterone, and their production is regulated by the pituitary. Studies have shown that ovarian epithelial malignancies do contain all classes of steroid hormone receptors. Since these receptors have been found in relatively low concentrations, it is more difficult to work with them than it is to work with breast tissue. Reports have shown that ovarian carcinoma grown in long-term tissue culture has proved to contain both estrogen and progesterone receptors and may become a model for studying the action of steroid hormones in ovarian cancer. It may soon be possible to conduct a formal trial of endocrine therapy in order to explore the correlation of receptor data with response to therapy.

Research has shown that normal lymphocytes can be stimulated to produce glucocorticoid receptors and that the induction of these receptors correlates with increasing sensitivity to glucocorticoid therapy. This finding could add another modality to the therapy of ovarian cancer if it is possible to produce estrogen and progesterone receptors on ovarian cancer cells. Studies now under way are investigating the coupling of cytotoxic agents to steroid hormones, and the administration of this new endocrine-chemotherapy molecule to patients whose tumors contain the appropriate steroid hormone receptor. If this process can be developed, it has the potential for delivering large quantities of cytotoxic drug directly to the hormone-sensitive tumor. It would also have the potential for imaging with radioactive isotopes. Granted that it is in its infancy, bringing it to fruition would be a great advance in therapy.

The estrogen receptor (ER) test measures receptor expression and reports the tumor as estrogen receptor-positive or estrogen receptor-negative. Estrogen receptor-negative tumors are aggressive and rarely respond to endocrine therapy. Estrogen receptor status is a strong, independent predictor of recurrence and survival. The reference range for an estrogen receptor-negative patient is less than 10 femtomoles ER/mg cytosol protein. Progesterone receptor (PR) expression is also reported as positive or negative. Some researchers report that progesterone receptors have greater predictive value than estrogen receptors and are better correlated with the outcome of endocrine therapy. Progesterone receptor-positive findings may indicate false-negative estrogen receptor results. The reference range for progesterone receptor-negative is less than 10 femtomoles PR/mg cytosol protein.

Epidermal growth factor receptor (EGFR) is an investigational assay. EGFR-negative tumors carry a prognosis similar to that of ER-positive disease, even in ER-negative tumors. Ninety percent of EGFR-positive tumors are ER-negative. EGFR-negative status may thus define a subgroup of ER-negative patients with better survival rates. The reference range for epidermal growth factor receptor is EGFR-negative less than 5 femtomoles/mg protein.

CHEMOTHERAPY

In contrast to surgery and radiation therapy, chemotherapy, which is the treatment of cancer with drugs, hormones, and anti-hormones, can be used effectively for

disseminated as well as localized cancer. Chemotherapy has become a reality only in the past 30 to 40 years. Each of these decades has seen some important advances in the number of compounds available and in the spectrum of their usefulness. Within the last two decades, it has become clear that, although chemotherapy had long been considered largely a palliative procedure, capable of extending but not saving lives, certain kinds of cancer can now be cured by chemical treatment. A major goal of current cancer chemotherapy is to achieve cures by prompt and vigorous treatment of such cancers.

Combinations of chemotherapeutic agents have been used with substantial success, particularly when each of the drugs used acts on the cancer cell in a different way. Major improvements in the treatment of certain types of cancer have been achieved both by using several of the active drugs simultaneously and by using different drugs in sequence.

Chemotherapy now constitutes a major and indispensable therapeutic approach, capable of producing cures, in particular kinds of metastatic cancer. The problem with chemotherapy is that it is a systemic treatment that affects normal as well as cancer cells. Organs such as the hematopoietic system and the gastrointestinal system, with a rapid turnover of cells, receive the greatest damage to normal cells from chemotherapeutic agents.

Chemotherapy can occasionally produce cures in a broad spectrum of cancer types. The cancers that can be cured by chemotherapy are not always those which grow most rapidly. Not all derive from the same primitive embryonic tissue; many organ classes are represented. Furthermore, curative chemotherapy is not always a product of a single drug nor a single technique of drug use. Rather, many different drugs are involved, sometimes in combination with each other, sometimes in combination with surgery and radiation. This high degree of specificity between a particular tumor and a particular drug or drug combination implies that no universal chemotherapeutic cure is to be anticipated. The complexity of special drugs and regimens for special tumors can also be construed to mean that a drug that fails in the treatment of one kind of cancer may exhibit major activity against another.

The sensitivity of cancer cells to drug action is dependent on a variety of possible factors, not all of which are known. The drug must reach the cancer cell surface, enter the cell, remain active or undergo necessary activation, reach a critical target site, and combine with it at a time when chemical processes on which the cell depends for its viability or reproduction are in progress. Furthermore, competing and bypass pathways in the cell must not be able to compensate for the chemical injury which the drug inflicts. Failure of the whole chain of events can result from the failure of any one step. Thus, any drug given by the wrong regimen to a mass of cancer cells which are biologically unsusceptible to its action at that time is a failure of chemotherapy. Better drug design and better understanding of the biological characteristics of each cancer type, and, indeed, of each cancer, will advance the effectiveness of chemotherapy.

Although the platinum compounds were introduced in 1965, it was not until 1979 that they became the keystone of treatment for carcinoma of the ovary. Since the initial compound cis-platinum, another platinum compound, carboplatin, has recently been developed; it produces less nausea and vomiting but still maintains its therapeutic value.

An interesting new analogue of cisplatin that is being evaluated is ormaplatin,

which was formerly termed tetraplatinum. It is a second-generation platinum analogue, which has less nephro- and neurotoxicity than cisplatin. Ormaplatin has been found to be more potent than cisplatin when tested against various human ovarian cell lines that have become resistant to cisplatin.

Ifosfamide, too, has been introduced, and, although it is mainly indicated for third-line chemotherapy of germ-cell testicular tumors, it is being used for the management of ovarian cancer, particularly when other first-line agents have failed. It should ordinarily be used in combination with a prophylactic agent for hemorrhagic cystitis, such as mesna. Ifosfamide is a drug that has marked toxic effects, but it is hoped that, as the scientists and clinicians learn to use it, particularly in combinations with other drugs, its anticipated response against ovarian cancer will probably be achieved.

Etoposide has been introduced for the management of refractory testicular tumors and small-cell lung cancers. It is, however, being used in combination with other drugs for the management of ovarian cancer. Etoposide has a marked hematologic toxicity as well as gastrointestinal toxicity.

A new regime called the *VIP* regime has been introduced for the treatment of persistent and recurrent carcinoma of the ovary. The *V* is Vepesid[®], which is etoposide, also known as VP-16; the *I* is Ifex[®], which is ifosfamide; and the *P* is Platinol[®], which is cisplatin. Although this combination is highly toxic, in the hands of qualified oncologists it is adding a new dimension to the treatment of persistent or recurrent ovarian cancer.

Taxol is a new drug that is undergoing clinical trial and is sponsored by the Division of Cancer Treatment (DCT), of the National Cancer Institute. It is a novel diterpene compound derived from the bark of the western yew, *Taxus brevifolia*. In 1977, taxol was chosen for development as an anti-neoplastic agent because of its unique mechanism of action and good cytotoxic effect against the intraperitoneally implanted B16 melanoma and the human MX-1 mammary tumor xenograft. Taxol inhibits normal cellular replication *in vitro* by promoting microtubule assembly and stabilizing tubulin polymeres against depolymerization. Clinical trials began in 1983 and have recently been expanded. A limiting factor may be that the western yew is becoming an endangered species.

Currently, taxol is not produced by any of the pharmaceutical companies, and the centers that have been approved for its use are producing their own drug. Since the drug is available in limited quantities and is being evaluated for various tumors, there are very few projects, but it is being used against ovarian cancer. There are, however, some projects, and the anticipated reports will, it is hoped, add another dimension to the management of ovarian cancer.

The cost of research directed to finding new drugs, the problem of litigation, and the resistance by the FDA to release drugs until they have been put through a long series of phase 1, 2, and 3 trials before authorizing their use has made anti-cancer drug research less desirable for the large pharmaceutical companies. Perhaps some of these problems can be solved. Until that time, scientists will direct attention to fitting the ideal combination of available anti-cancer drugs to produce additional cures in the management of carcinoma of the ovary.

Modulators of Toxicity

Drugs are being evaluated to reduce toxicity of the current chemotherapy agents. Diethyldithiocarbamate (DDTC) has been demonstrated to protect against many of

the toxic effects of platinum-based compounds without interference in their anti-cancer effect. WR 2721, an aminothiols, is another protective agent being evaluated with platinum compounds. An analogue of ACTH (Org 2766) is being tested for the prevention of cis-platinum-induced neurotoxicity. Ondansetron is widely used in Europe and has recently become available in the U.S. It has the potential to eliminate cisplatin-induced nausea and vomiting.

Modulation of Drug Resistance

Glutathione (GSH) has been identified as a mediator of cellular resistance to alkylating agents and platinum-based compounds. A synthetic amino acid, buthionine sulfoximine, blocks glutathione synthesis. Amphicodin is an inhibitor of DNA polymerases alpha and gamma; it inhibits the ability of the cell to repair DNA damage by anti-cancer drugs.

Multiple Drug Resistance in Cancer

Kartner and Ling [14] have suggested that an ancient pump protein that flushes toxin out of cells may be the reason that cancer chemotherapy fails. They have identified a mechanism and have suggested that multi-drug-resistant cancers may be made vulnerable again. Kartner and Ling suggested that, if there were not some sort of pump that could flush toxins out of cells, human beings would have vanished from this earth ages ago. The work of many researchers slowly accumulated to enable them to formulate their theory. A series of experiments led them to believe that multiple drug resistance appeared to result from a single mutation. Their theory was that a single gene could account for the multiple resistance to unrelated drugs. Working with various systems, they found that cells which were resistant to a drug somehow excluded it. This observation suggested a mechanism for the drug resistance; that is, there appeared to be some barrier that kept the drug from reaching the interior of the cell, where it would produce its lethal effect. Two possible theories were explored. One theory proposed that a permeability area prevented drugs from entering the cells. The other suggested that an efflux pump (a mechanism that actively pumped drug out of the cell once it got inside) was at work in the resistant cells; this model was based on the observation of the kinetics of drug flow into and out of the cells. It was found that when a resistant cell was temporarily poisoned with cyanide to inhibit energy production, the cell behaved like a drug-sensitive cell; it could not keep out the drug. When the cyanide was washed out and normal metabolism was restored, the cell could once again exclude the drug. Furthermore, the cell was then able to pump out the drug that had accumulated while it was poisoned. Hence, an energy-dependent drug-efflux pump seemed to be the simplest explanation.

The expression of 170,000 dalton plasma membrane protein designated P-glycoprotein, or P 170, seems to be associated with multi-drug resistance of cells to anti-cancer chemotherapy. P-glycoprotein is the gene product of the *mdr 1* gene, which has been found to be amplified in many cases of multiple-drug resistance. P-glycoprotein has been found to increase drug resistance by serving as a pump, actively removing drugs from the cell. Drug resistance may be partially blocked by calcium channel blockers, such as Verapamil[®], which bind to P-glycoprotein.

Although this is a highly logical explanation as to how drugs become resistant to anti-cancer chemotherapy, in actuality it has been extremely difficult to prove;

however, since the underlying logic is acceptable, more work must be done in this area. There is hope that the theory may be refined, and ways to block the efflux pump may be found.

BIOLOGICAL RESPONSE MODIFICATION [15]

The term “biotechnology” encompasses many activities which have in common the fact that they all harness the fundamental abilities of living organisms. One of the most impressive achievements of science has been the explosion of knowledge about the chemical composition of organisms and the way these chemicals interact to create the phenomenon scientists recognize as life. Organisms are sometimes compared to chemical factories. The strength of this analogy lies in its emphasis on the chemical nature of life—the fact that growth, development, and reproduction all depend on chemical reactions. The analogy does, however, obscure some of the most fundamental characteristics of organisms, many of which are directly related to biotechnology.

Genetic Engineering

In genetic engineering, there is a reweaving of the threads of life. The possibility of transferring genes from one organism to another is an alluring prospect, since genetic engineering can reduce the cost and increase the supply of an enormous range of materials now used in medicine, agriculture, and industry. Furthermore, there are many substances that occur naturally in only small quantities, which might well prove invaluable if they were available in sufficiently large amounts for their potential to be examined.

The biotechnological application of genetic engineering consists of four main stages: obtaining the gene which codes for the product that the microbial factory is to manufacture; inserting the gene into the microbes; inducing the microbes to start synthesizing the foreign product; and collecting that product.

The body’s inherent biochemical capacity for killing cancer cells plus the new genetic technology is called biologic response modification, or biomodulation, for short. Biologic response modification is the new wave in cancer treatment, generating excitement among scientists, patients, and the public.

The use of biologics and biologic response modifiers in the treatment of cancer is of recent origin. Biologics may be defined as any product of a mammalian organism, and biologic response modifiers are those agents and approaches that alter biological response in the host-tumor interaction. The field includes traditional immunotherapy but also encompasses the use of molecular biology, recombinant genetics, and hybridoma technology, all of which produce highly purified biologic substances with anti-cancer activity. The recognition of growth, differentiation, and maturation factors, as well as the possibility of making antagonist or competitive inhibitors to factors that support neoplastic growth, infectious disease, and hereditary disorders provides an additional biologic approach in the area.

Gene Therapy

Gene therapy is medicine’s next frontier. It is now possible to locate a single gene, of the organism’s total DNA, which can be recombined with a carrier molecule of DNA. It is now possible to introduce recombinant DNA into a bacterial cell in order to produce an altered organism. Recombinant DNA technology, commonly referred

to as genetic engineering, has provided science with the tools for the biosynthesis and subsequent mass production of a significant number of biologicals. This work should revolutionize the treatment of cancer over the next ten years. The process involves incorporation (recombination) of a segment of a DNA molecule containing a desired gene into a vector, usually a plasmid, which is, in turn, inserted into a host organism, usually *Escherichia coli*, although other bacteria, yeast, fungi, sex cells, and mammalian cells have been used. *E. coli* are cloned, and the organism producing the desired protein or polypeptide is selected. This clone is mass-produced by fermentation techniques, and the protein molecule is harvested and purified. The resultant product is a highly purified protein solution, generally with greater than 95 percent purity, and with a highly specific activity, containing the greatest possible amount of biologic activity per milligram of protein.

There are a number of biologic response modifiers that are currently being used. These include interleukins, thymic hormone, tumor necrosis factor, macrophage activating factor, lymphotoxins, growth and transforming factors, interferons, colony-stimulating factors, and monoclonal antibodies.

Monoclonal antibodies are receiving a great deal of attention. They can function as naked antibodies, as drug-carrying antibodies, and as radioactive antibodies. Their potential is great, and more work needs to be done to make the monoclonal antibody a diagnostic and therapeutic predictive modality.

No one has actually been cured by biologic response modifiers; however, metastatic disease is not often cured by any means. Medical ethics dictate that new treatments be tested on advanced cases when established treatments offer no hope. Thus, the most difficult cases are used to test these substances. The future of biologic response modifiers rests upon the answer to a crucial and fundamental question: What causes the body to perceive harmful tumor antigen as self? Once the body has decided that the tumor antigen is part of itself, any attempt to destroy that balance will have only limited success. Initially, the body recognizes a tumor antigen as harmful. It then changes its decision and accepts that antigen as self. Thus, suppressive cells are produced which inhibit the immune system's attempt to attack the tumor cells. Drugs can be used to reduce this suppression, but the effects are short-term. When it is possible to understand why the body sees the tumor antigen as part of itself, it would then be possible to use biomodulation to intervene at that critical point. Until scientists understand what is involved in the determination of self, they will not reach their potential in treating patients by modulating the immune response.

The author presents the sequential treatment plan for the management of cancer:

Restore immunity to normal by treating the patient with a thymic hormone or low-dose cyclophosphamide (has the ability to suppress the action of suppressor T cells).

Give a vaccine to stimulate the patient's own cells.

Activate the patient's immune cells to a greater degree with interleukin 2.

Monoclonal antibodies could then be used to target killer cells to their target more effectively or to act as carriers for killer molecules (toxins, drugs, or radioactive isotopes).

SUMMARY

The problem of whether ovarian cancer produces metastasis or spread throughout the abdomen or whether what appears to be carcinomatosis represents multiple

primaries must be clarified. Woodruff has stated [16] that the ovary and the entire pelvic and peritoneal cavities are mesothelial structures. If it can be determined that carcinomatosis of the ovary represents multiple primaries, there is little chance for producing an early diagnosis, and attention must then be directed entirely to the treatment of carcinoma of the ovary [17].

The research in genetics shows promise for the potential of making an early diagnosis and suggestive but not conclusive promise at this time for identifying the high-risk patient.

Flow cytometry has furnished a relatively easy and inexpensive method of providing a profile for prognostic indicators in ovarian cancer. These include a DNA index (DI), cell cycle distribution (CCD), synthesis index (SI), estrogen and progesterone receptors (ER and PR), epidermal growth factor receptor (EGFR), and HER-2/neu oncoprotein (H2n).

Gene therapy, genetic engineering, and the biologic response modifiers are in the forefront of the new frontiers in ovarian cancer diagnosis and management.

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