The Carcinoembryonic Antigen (CEA): Past, Present, and Future*

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INTRODUCTION

Just as Kierkegaard observed that, "life must be understood backward [but]...lived forward" (1), so it is with science, which must be performed forward--but may be written backward. In the telling, the phenomenon of scientific revisionism unfortunately omits much of both the humanity and the enjoyment of the scientific process. "How could they have guessed that those results would be obtained?" asks the novice. With due respect to "gut" intuition, the answer is very frequently that "they" couldn't have guessed. Yet, the most exciting science is that which leads to unexpected findings and requires the eye and mind of the iconoclast to realize that new ground has been broken.

About 30 years before the writing of this article began, the first author was given the opportunity to begin a series of experiments that would lead to the discovery of CEA as a human tumor marker, and to the myriad avenues of CEA research that are being actively pursued at the present moment. As noted above, it might be very satisfying to state that these investigations were undertaken with clear insight into the potential experimental pitfalls that might be encountered and the ultimate value of the data that would be obtained. This was not quite the case, and the problems of data interpretation, confounding semantics, and personal biases were certainly not anticipated.

In the course of the work to be described, the meaning of the Kuhnian paradigm (2) and the manner in which accepted scientific constructs are defended by the "establishment" came to be appreciated first-hand. As do too many other discoveries, advances in CEA research endured the prevailing mindset that "if it's new, it's not true," "if it's true, it's not important," and "if it's new and true and important, then we knew it all the time." Yet, the controversies surrounding CEA quite fortuitously stimulated, rather than inhibited, further studies that ultimately led to some very exciting outcomes.

THE DISCOVERY OF CEA
The idea for the work on CEA devolved from two lectures that the first author attended in 1961-2, during a year of Rotating Internship at the Montreal General Hospital and McGill University. The first described the attempts and failures to detect either components or functions unique to cancer cells and not found in any normal tissues. The second lecture dealt with the then relatively recent description of Acquired Immunological Tolerance (3)—until 1953 only a theoretical concept—and the new perspectives that this phenomenon was creating in the understanding of the immune system and in various areas of clinical medicine. Hence, the experiments subsequently undertaken were predicated on the somewhat naive hope that the burgeoning tools of immunology, such as immunologic tolerance, would provide both the specificity and sensitivity that had been lacking in the previous efforts to find one or more specific tumor constituents in human cancer tissues.

In the 1950s, after perhaps a century of study in a variety of disciplines and using numerous technologies, the paradigm in the field was very simply that "tumor-specific materials" did not exist in cancer tissue and, moreover, that these would not be found (4). Nevertheless, by the early 1960s, studies of the rejection of well-defined transplantable tumors between highly inbred, or syngeneic, animals had clearly demonstrated the existence of tumor-specific transplantation antigens (TSTAs) in these animal models (5). Conventional wisdom of the day maintained that such artificially-generated data could hardly be applicable to outbred, "wild-type" humans. Notwithstanding this, a sizable field of investigation grew up around the question of how TSTA-bearing animal tumors escape the immune surveillance system, a question that has yet to be answered satisfactorily. It is to a field of tumor immunology in this state of flux that CEA research traces its origin.

Lacking a syngeneic human population, to say nothing of the moral and ethical prohibitions to human tumor transplantation, the initial approach taken was to investigate the absorption of antiserum against colon cancer by normal tissue, and to employ immunologic tolerance to compare colon cancer tissue with normal colonic tissue from the same donors (6). Colonic cancer was chosen as a model because this tumor does not extend intramurally beyond 5-6 cm, either proximally or distally, from the tumor tissue in the gross. Hence, normal and tumor tissues taken from the same individual at operation could be rationally compared without concern for the problem of alloantigenicity that had plagued previous studies comparing tumor and normal tissues taken from different individuals. The problem of obtaining fresh surgical specimens from the operating room without interfering with the critical process of appropriate determination of the tumor should be noted. Much gratitude is due to numerous colleagues at the Departments of Surgery and Pathology at the Montreal General Hospital for their fervent efforts to assist in this project without ever compromising patient priority and safety.

In one of the earliest series of experiments, then, adult male rabbits were immunized with colonic cancer tissue extract and the resulting antiserum was absorbed with 'an excess' of the corresponding normal material, while in a second series of studies an attempt was made to render neonatal rabbits tolerant to the normal tissue extract. These animals were subsequently immunized with the corresponding tumor material, as adults. The necessary tissue extracts and antisera now in hand, these two series of studies led, with the use of immunologic techniques as indicator systems, to the demonstration of a tumor component in colonic cancer tissue that was not found in the corresponding normal tissue (6).

In a subsequent series of studies (7), it was found that the same cancer antigen was also present in all endodermally-derived gastrointestinal tumors—from the lower esophagus, above, to the anorectal junction, below. The antigen was also demonstrable in primary tumors of the pancreas and the liver, both derivatives of the second stage of the duodenum during embryologic development. Tumors of gut origin that underwent extra-enteric metastasis to such tissues as lung, bone and brain, retained the antigenic activity in question, whereas tumors of all other organs that spread to the liver, for example, were devoid of such activity. Hence, the parameter that determined expression of the antigenic activity described was the site of tumor origin, and not the site of growth.
During a departmental seminar in which these findings were outlined, a good deal of interest was generated by the "embryologic Confinement" of the tumor antigenic activity in question. The substance appeared to be related to cancers that arose from tissue lying between the embryologic stomatodeum, above, to the proctodeum, below. Thus, further investigations were done with human embryo- and fetus-derived tissues. Since this was done at a time well before abortion-on-demand, it took a fair amount of time to collect tissues from spontaneous abortuses at various points in pregnancy. Nevertheless, it was found that gut-derived tissue in the first two trimesters of gestation expressed the antigenic moiety of interest but that comparable tissue in the third trimester did not. Hence, the material was named the carcinoembryonic antigen(s) of the human digestive system, subsequently abbreviated to CEA (7).

The observations described above appeared in the *Journal of Experimental Medicine* in the first two papers on CEA, published in 1965 and 1966 (6,7). Immediately, there was expression of international interest in the reported findings, and the subsequent shipment of CEA and anti-CEA antisera to colleagues around the world allowed work on CEA to be performed in a time-frame that, otherwise, would have taken years longer for our laboratory alone.

**THE BIOLOGY OF CEA**

The extensions of the two initial series of studies can primarily be divided between those focused on the basic biology (localization, purification, chemical definition, functional activity and genetic control) of the CEA molecule, and those directed at answering clinical questions. As concerns the biology of CEA, early investigations involved the purification and characterization of the molecule, utilizing anti-CEA antiserum as a guide to the recovery and concentration of CEA at each stage (8,9). These early studies would, years later, lead to the cloning of the CEA gene and to the present understanding of the functional biology of the CEA molecule and other members of its molecular family (see below).

The CEA molecule is an oncodevelopmental human tumor marker, and bears the Cluster Differentiation designation CD 66e (10), a subtype of the CD 66 group to which both the CEA molecule and other CEA family members belong (see below). CEA was initially found in adenocarcinomas of the human digestive system (6,7) and was shown to be a membrane constituent (11). In the membrane, it demonstrates redistribution, or "capping" under appropriate experimental conditions, suggesting that the CEA molecule is a surface glycoprotein that interacts with the microskeleton of the cell (12,13). From the cell surface, CEA may be released into the interstitial space and, thence, into the circulation of the tumor-bearing patient, where it can be detected by immunoassay (14).

The CEA molecule has a nominal molecular mass of 180 kDa with a protein core that makes up somewhat less than half of the molecule (9,15). As deduced from the complete sequence of the cloned CEA gene, the protein consists of a single polypeptide chain, containing a 107 amino acid NH-terminal domain followed by three highly homologous domains of 178 amino acids each (16,17). The C-terminal domain, consisting of 26 amino acids, is processed so that CEA binds to the plasma membrane through a glycosylphosphatidyl inositol (GPI) anchor (18). Carbohydrate side-chains comprise the remainder, or over half, of the molecular mass bound to the protein core via 28 potential Asn-linked glycosylation sites that have been identified on the CEA molecule (16). The molecule appears as a screw- or cruller-shaped structure with dimensions of approximately 9 x 40 nm when visualized by electron microscopy after appropriate shadow casting (19).

Even very early immunobiochemical studies had revealed a number of molecules closely related to CEA, indicating the existence of a "CEA molecular family" (20,21). Candidates for this group included the non-specific cross-reacting antigen (NCA), biliary glycoprotein (BGP I-II), the meconium antigen (MA), pregnancy-specific β-glycoproteins (PSG), and the tumor antigen denoted TEX. Until the CEA gene was cloned, the exact size of the CEA-related family and the relationship between family members, and still other known glycoproteins, was difficult to ascertain. Molecular weights varied depending upon the degree of
glycosylation, degradation, or aggregation of molecular species, and variations in immunospecificity existed among the antibody probes employed in any given laboratory. The latter consideration became rather important in the semantic dialogue dealing with the designation of CEA as tumor-specific, as discussed below. The high level of glycosylation of CEA made protein sequencing a rather difficult task (22,23). By extension, cloning of the CEA gene and its family members became a somewhat arduous undertaking. Nevertheless, once the technological obstacles had been overcome (23), the isolation of cDNA clones for CEA and related family members was achieved through the screening of genomic and cDNA libraries via two methods: (i) using synthetic oligonucleotides based on known CEA protein sequences; and (ii) using anti-CEA antibodies to identify CEA proteins expressed in suitable vectors (17,24-33).

The CEA gene family comprises 29 gene-like sequences, in two defined clusters on chromosome 19, between 19q13.1 and 19q13.3 (34-36). The PSG gene family, found in the same chromosomal region, has been defined as a sub-family of the main CEA family and is represented by 19 of the 29 gene sequences noted above (37). It is rather sobering to realize that the first author has spent the greater part of his life focused on the product of such a minuscule region of the human genome.

Complete sequence data for a number of the genes in question have been obtained and the primary structure for the peptides for which they code has been deduced for many of them (24-33). A high degree of sequence conservation between the various protein moieties of the CEA/PSG molecules suggests that they have a common genetic ancestry (38). Furthermore, protein and DNA sequence comparisons indicate that the CEA gene family is a subset of the immunoglobulin (Ig) gene superfamily with analogous variable and constant regions (39). The CEA gene itself begins with an amino terminal Ig V-like domain, followed by six Ig C2-set-like domains (17,24-29).

If one examines the known members of the CEA family, they fall into two distinct groups on the basis of membrane anchorage. Some, like CEA itself, are of the GPI-linked group, while others, like BGP, appear on the cell surface with a transmembrane linkage (40). It is of interest to note that the GPI members tend to be up-regulated in human tumors, while the transmembrane group tends to show down-regulation under these circumstances (41,42). In addition to the mode of membrane binding, the two subgroups of molecules represented, for example, by CEA and BGP, have very different properties. Hence, whereas the GPI-linked CEA molecule shows temperature- and Ca$^{2+}$- independence of the classical intercellular adhesion molecular type, BGP-mediated adhesion is both temperature- and cation-dependent, more characteristic of the cadherin group of molecules. Further, unlike the double reciprocal binding between two domains in CEA-CEA adhesion, that involving BGP requires only one domain (43). Finally, whereas the ectopic expression of CEA in myoblasts can block myogenic differentiation while leaving the cells with the ability to divide, similar expression of BGP does not effect, or may even accelerate, myogenic differentiation (44).

Comparative studies of CEA gene family members in mouse and man, and the observation that the nucleotide sequences of repeated domains in a given gene, and of domains in different genes, are more closely aligned (>80%) than the analogous amino acid sequences (>60%), suggest that the CEA gene family arose relatively recently in the evolutionary process. The gene family and its products may presently be in a state of evolutionary transition (45), which may explain some of the diverse potential functions that have been proposed for CEA and its relatives based on observations from different model systems described briefly below.

With regard to the possible functions of CEA, studies have revealed a number of fascinating possibilities. In a series of experiments employing cultured human colonic adenocarcinoma cells and rodent cells transfected with CEA cDNA, so that these cells now expressed the CEA molecule on their surfaces for the first time, it was demonstrated that CEA mediates Ca$^{2+}$- and temperature-independent aggregation, as noted above (46). Further, CEA was shown to induce the homotypic sorting of cells in heterogeneous populations of aggregating cells. Thus, CEA can be considered an intercellular adhesion molecule, in contradistinction to the
Ca\textsuperscript{2+}- and temperature-dependent cadherins (47,48).

CEA is found primarily on the lumenal aspect of epithelial cells in normal adults, albeit in very small amounts, but on basolateral cell membranes in both embryonic intestine and colonic cancer (46,49). It may be that when large amounts of CEA are produced, as is the case in the developing embryonic intestine and in colonic tumors, the normal adult single palisade layer of epithelial cells is replaced with a multilayered cellular array. This could be a consequence of weaker intercellular adhesion due to a possible interference by CEA with other intercellular adhesion systems. The tight association seen between normal adult cells may be necessary for the single-layered configuration and the ordered differentiation of adult epithelial cells, which arise from the crypt stem cells and move toward the tip of the villi, where the fully differentiated cells are extruded. Disruption of this structure during tumorigenesis by the adoption of an embryo-like multilayered configuration, as either a cause or effect of the cancerous change, could disturb normal differentiation (see below) and favor the development of overt malignancy (31,49). In an alternative theory, von Kleist et al. have proposed that CEA may act as a signal protein inhibiting further intercellular contact rather than as an adhesion molecule. This would foster cell migration and, thereby, the formation of metastases, as well as inhibit close contact between CEA-expressing target cells and cytotoxic effector cells of the immune system (50).

As concerns the relationship of CEA concentration to the metastatic potential of CEA-producing tumors, at least two possibilities must be examined. The first is the association of intercellular CEA concentration, or perhaps more critically that of free intercellular CEA or CEA peptides, to the strength of intercellular adhesion and, hence, to metastatic potential (51). It has also been proposed, though never demonstrated, that another parameter facilitating cancer cell implantation might be the interaction between circulating CEA-bearing tumor cells and blood-borne CEA trapped on the surface of the Kupffer cells of the liver. Alternatively, circulating CEA may bind to a specific receptor on Kupffer cells, causing them to produce cytokines that stimulate the growth of metastatic cells (52,53). In this regard, it is interesting to note that a serum CEA-releasing factor has recently been postulated based on the finding that a factor in human serum significantly augments the release of CEA from cultured tumor cells (54).

The molecular mechanism of CEA-CEA-mediated adhesion has been studied through the use of "designer molecules" made possible by the availability of the CEA gene. These molecules included various CEA peptides, altered CEA fragments, and hybrid molecules of CEA and the neural cell adhesion molecule (NCAM), a comparable member of the Ig gene superfamily capable of homotypic Ca\textsuperscript{2+}-independent intercellular adhesion. It was found that CEA-mediated intercellular adhesion is unique for intermolecular binding between Ig superfamily members. This adhesion involves two point reciprocal binding between the N (Ig V-like) and A3B3 (two of the Ig C2-set-like) domains, on anti-parallel molecules of apposing CEA-bearing cell surfaces (55).

To examine the possible effect of CEA on differentiation, a series of studies was performed based on the ectopic production of CEA on the myogenic differentiation of cultured L6 rat myoblasts (56). These cells differentiate and fuse into multinucleated myotubules when serum growth factors are reduced, initiating a complex myogenic differentiation program. When transfected with functional CEA cDNA, stable transfectant clones of L6 cells producing relatively modest amounts of CEA were shown to be incapable of myogenic differentiation and fusion but retained their proliferative potential. This block in differentiation was shown to be critically dependent on the double reciprocal binding between anti-parallel molecules, presumably on apposing cell surfaces, as described above. Moreover, the differentiation-block imposed by CEA could be rapidly reversed by disruption of the CEA-CEA bonds. The mechanism by which intercellular binding through CEA inhibits the differentiation pathway is presently under investigation.

It is of interest that human colonic cancer almost always develops in mucosal tissue that has already undergone multiple steps of genetic change. It has been postulated that these multiple steps create a field
effect which is characterized by morphologically normal, but biologically altered, epithelial cells. Indeed, CEA has been used as a phenotypic marker of this field effect by examining the immunohistochemical expression of CEA on morphologically normal mucosa adjacent to colonic adenocarcinomas. It has been shown very clearly that CEA expression occurs in "normal" peritumoral mucosa and that there is a gradient of CEA expression, falling off at increasing distances from the tumor (57). These data are relevant to both the biology of human colorectal cancer, and more practically, to the optimal location of surgical resection. Quite clearly, a good deal of work remains--and should be rapidly forthcoming--that promises to integrate and clarify the roles of CEA and its family members in terms of evolution, ontogeny, phylogeny, tissue architecture, invasion, and metastatic potential.

**THE CLINICAL ROLE OF CEA**

A role for CEA in clinical medicine first became a consideration with the development of a radioimmunoassay for circulating CEA by Thomson and colleagues in 1969 (14). The first series of data, derived from patients with established colonic cancer, were most exciting; despite warnings to the contrary, the news was soon abroad that "the definitive test for bowel cancer was at hand." When more extensive studies revealed the expected false-negative assays, particularly in early stage bowel cancer, and false positive results in patients with non-enteric cancer or other non-cancerous conditions (see below), the response of a subset of the medical and scientific communities was again inflammatory, this time swinging to the other extreme.

As an aside, it must be understood that the field of cancer research had been plagued for a very long time by the semantic dilemma surrounding the use of the adjectives "tumor-associated" and "tumor-specific" when applied to cancer cell constituents. In this context, it was de rigueur to believe that unless a substance would be found to be absolutely unique to tumor cells, it would not be very worthy of further study for either its biological function or its potential application in clinical medicine. It did not seem to matter if the information might be of help to clinicians in the diagnosis, or other aspects of care, of the cancer patient. Moreover, as noted earlier, the conventional wisdom at the time held that tumor-specific materials did not exist.

Notwithstanding the precedent of a variety of clinically-useful non-specific markers for tumors, such as Bence-Jones Protein (BJP) (58), serum immunoglobulins, and various hormones and enzymes (59,60), it was debatable 25 years ago that considerations of clinical utility would succeed in increasing international interest in performing basic, and even clinical, studies dealing with CEA. Indeed, much of the subsequent CEA research was directed toward resolving the issue of tumor-specificity.

Since the discovery of CEA over three decades ago, a large body of literature has accumulated describing the value of serum CEA measurements in the care of cancer patients. Information concerning the clinical usefulness of CEA has also been reviewed in two major consensus conferences held in 1977 and 1980 (61-63). In general, current clinical applications of CEA may be divided into the categories of detection (screening for cancer in an asymptomatic population), diagnosis (differentiating malignant from benign tumors), prognosis (staging and classification), treatment monitoring (therapeutic effect, tumor recurrence), special pathologic techniques (immunohistochemistry, immunocytochemistry), localization (imaging of tumors with radiolabeled antibodies), and therapy (antibody-linked cytotoxic agents, vaccine vectors carrying the CEA gene).

**CEA and Screening**

Colon cancer is the second most common fatal malignancy in North America, due in part to the late stage at which it is often diagnosed (64). In the search for tumor markers to aid in the early diagnosis of cancer, several oncodevelopmental entities have been described, most of which have limited clinical utility (65).
Currently, the most clinically useful of these oncofetal markers is CEA. As alluded to earlier, the initial high expectations for the radioimmunoassay were tempered by the finding of elevated plasma CEA levels in several benign diseases (66-73). Today, with refinements in assay technique and the accumulation of data, it is understood that the differences in CEA levels between normal and tumor-bearing individuals are primarily, but certainly not entirely, quantitative in nature. The circulating CEA level in a given patient is the end result of various factors, including the level of gene expression, the rate of CEA synthesis, its subsequent release by the tumor, the half-life of CEA in the circulation, the degree of necrosis and vascularization of the tumor, as well as the rate of CEA catabolism by the liver. Despite the complexity of the system, the CEA assay has taken an important role in the management of patients with cancer.

Concentrations of 2.5-5.0 ng/ml (depending upon the assay used) are commonly considered cut-off points for distinguishing normal from abnormal levels of serum or plasma CEA. In two large surveys of apparently healthy persons, 85 to 87% had serum antigen levels of less than 2.5 ng/ml, 95 to 98% had levels of less than 5 ng/ml and virtually no one had a level greater than 10 ng/ml (74, 75). CEA concentrations are, in general, more often raised in smokers than in non-smokers (73,76), and more frequently elevated in men than in women (77). The same is true for older subjects when compared with younger individuals (78,79). Racial differences in the frequency of serum elevations of CEA have been suggested (80) but not established.

Although guidelines for the appropriate use of CEA assays have been reported, Fletcher (81) argues that physicians in practice apparently have greater confidence in the practical value of CEA measurements than do "experts" in the field. In one study in 1979, over 50% of physicians believed that a CEA assay was worthwhile for initial detection of colonic cancer and that elevated serum levels of CEA in a non-smoking person without symptoms should prompt an aggressive search for colonic cancer. Both premises were questioned by investigators in the CEA field at that time (82).

Currently available CEA assays cannot be used as screening tests (strictly defined as procedures for detection of disease in asymptomatic individuals) for colorectal cancer, insofar as false-negative tests can be obtained. For example, in earlier stages of colorectal cancer (Dukes Stages A and B), CEA level is less likely to be elevated. By contrast, assays for CEA more readily detect advanced colorectal cancer of Dukes Stages C and D (74,81,83-85). Again due to the lack of sensitivity of existing tests, CEA assays perform no better in screening for other cancers commonly associated with elevated CEA levels (86,87). Elevated plasma levels of CEA, or CEA-reactive materials, have been described in advanced breast cancer (67,69,88-93), pancreatic cancer (67,90,94-102) lung cancer (90,103), and other non-colonic adenocarcinomas (104-106). However, the difficulty in achieving simultaneous sensitivity and specificity of CEA assays in the context of specific cancer profiles, together with the low prevalence of cancer in asymptomatic populations, results in too many false-positive and false-negative results to warrant the use of current CEA assays in true screening for early cancers. This is supported by data from the large, cohort, population-based Framingham study, which examined CEA levels in serum samples from patients with newly detected cancers (83), and is also in agreement with the recommendations of the National Institutes of Health Consensus Development Conference of 1981 (85).

CEA as a Diagnostic Test

In the strictest sense, diagnostic tests are used not in screening, but in "case-finding": i.e., in determining whether disease is present in individuals at high risk for, or suspected of, having the disease. The following discussion pertains to findings in patient populations in whom CEA was used specifically as a diagnostic test.

With respect to colonic adenocarcinoma and polyps, although 80% or more of patients with advanced colonic adenocarcinoma have circulating CEA (14), the CEA assay should not be used as the sole diagnostic test for suspected cancer. Positive CEA in symptomatic patients cannot be interpreted as indicating the presence of malignant growth, as various benign conditions are associated with elevated CEA levels (82). Foremost
among these is liver disease (107); over 90% of patients with chronic liver disease and 50% of those with acute liver disease have raised plasma levels of CEA or CEA-like substances (108). There are numerous reports of elevations of CEA or CEA-like substances in a variety of conditions. However, benign conditions are rarely a cause of substantial elevations (> 10 ng/ml), and such conditions do not give rise to the progressive increases in CEA levels seen in cancerous states. CEA levels are likely to be higher in symptomatic, rather than asymptomatic, colorectal cancer patients, but such patients are more likely to have advanced disease (109).

With increasing tumor burden, there is a rise in both the frequency of positive CEA assays and the absolute plasma CEA levels. Thus, the incidence of positive CEA assays may range from 20% in patients with Dukes Stage A to 90% in Dukes Stage D colon cancer. Holyoke et al. (110) demonstrated significant CEA elevations of 18%, 53%, 62%, 65% and 79% in patients with colon cancer of Dukes Stages A, B1, B2, C1, and C2, respectively. Similar results have been reported by Booth et al. (111) and recently by Goldberg et al. (112). Increased CEA has also been observed in fecal samples from 50% of colorectal carcinoma patients; the antigen appears as the membrane-bound form, reflecting the destruction of epithelial cells (113).

The application of monoclonal antibody (mAb) technology targeting CEA, alone or in combination with other tumor markers, has recently demonstrated high sensitivities in the diagnosis of colon adenocarcinoma. A sensitivity of 78% for Dukes Stage B and 91% for Dukes Stage C has been achieved using mAbs to the combination of CEA, CA 19-9, and TPA; Stage D disease can be diagnosed with 100% sensitivity when mAbs to a combination of only CEA and CA 19-9 are used (114).

Adenomatous colonic polyps are the precursors of invasive cancer. Such polyps are usually not associated with elevated serum CEA levels and serum CEA levels are not presently useful for distinguishing locally invasive polyps from benign lesions (112,115). The same is true of the utility of the CEA assay in distinguishing benign from early malignant lesions of the stomach and pancreas (116-119).

As alluded to earlier, sensitive radio- and enzyme-immunoassays have shown that serum CEA may be elevated in neoplastic diseases of the breast, lung, prostate, bladder and stomach as well as in various gynecological malignancies (120). As in the case of colorectal cancer, CEA measurements are not sufficient as the sole parameter for diagnosis of early breast cancer (121-132). Early diagnosis of subclinical lymph node metastases from cancer of the breast or gastrointestinal tract has recently shown promise through the use of a reverse transcriptase PCR assay detecting CEA-expressing carcinoma cells; this diagnostic modality has demonstrated a greater than two-fold increase in the incidence of positive nodes (133). In advanced breast cancer, quite interestingly, the correlation between CEA level and occurrence of metastases from the breast has been found to vary with the site of metastasis (124). Patients with bone or visceral involvement have more frequent elevations (48-100%) and higher levels than patients with soft tissue metastases (9-52%).

In lung cancer, CEA can also be a useful biomarker. CEA is elevated in approximately two-thirds of non-small cell lung cancer patients and one-third of those with small cell lung cancer (134-136). Both the frequency and levels of CEA elevation are considerably lower in patients with benign lung diseases. Determination of CEA concentration can be used as a diagnostic tool for malignant pleural effusions, in that 40-70% of the fluids from initial pleural taps give positive CEA assays (136). However, although no large randomized study has been performed to date, preliminary work has suggested limited clinical utility for serum CEA and BALF CEA in the diagnosis of lung cancer (137). The determination of CEA levels in cerebrospinal fluid, albeit less studied, seems useful in diagnosing meningeal carcinomatosis (138), while analysis of gastric juice for CEA is useful in identifying high-risk patients for gastric cancer (139). In a recent study involving 90 patients with esophageal or gastric adenocarcinoma, CEA positivity in serum (i.e., > 5 ng/ml) was found to be 20% (140). Although serum CEA has not proved satisfactory as an indicator of early gastric cancer (141), elevated CEA levels have been detected in the majority of patients with advanced carcinoma of the stomach (80,142).
Although serum CEA assays have not been as useful as had been envisaged initially in the screening and diagnosis of colonic carcinoma, they play an important role in the clinical management of patients with colorectal adenocarcinomata (143), as discussed below.

**CEA as a Prognostic Indicator**

Considerable investigation has addressed the potential utility of CEA in determining disease prognosis in patients with colorectal and various other cancers. Preoperative serum CEA levels in diagnosed colorectal cancer are elevated in 40-70% of patients (144-148), and have been found to correlate inversely with tumor grade and directly with pathological stage (84,149-161). Thus, CEA is raised in 95% of patients with well-differentiated tumors, while it is elevated in as few as 30% of those with poorly-differentiated adenocarcinomas (155,162).

A significant inverse relationship between preoperative elevated plasma CEA levels and patient survival has been observed (149,151,153,159,163-167). Despite disagreement among various investigations of the relationship between preoperative CEA and prognosis, most report that a high preoperative CEA level is indicative of a poor prognosis. This association is often as predictive of prognosis as are pathological staging and grading (91,155,156,165,168-173). On the basis of these observations, the National Institutes of Health suggested in their 1981 Consensus Statement (62) that CEA determination should be used as an adjunct to staging. However, the precise preoperative CEA value that reliably discriminates high-risk from low-risk cases for postoperative recurrence is as yet unclear (148,151,153,155,160,163,164,168).

CEA is also an important marker of prognosis for cancer of the breast. Preoperative CEA levels have been studied as prognostic factors in early breast cancer by many investigators. The results are variable and controversial (81,88,91,93,125,127,128,131,132,153,160,174-191), but generally there is sufficient evidence that the CEA level is a prognostic indicator in patients with breast cancer (81,132,178,183,186,190,192,193). In most cases, preoperatively raised CEA levels have been found to correlate with a poor prognosis, such as increased likelihood and decreased latency of tumor recurrence. By detecting a rise in plasma levels of antigen, serial postoperative CEA measurement in clinically disease-free patients appears to accurately predict the development of metastatic disease (115,186,194). An elevated or rising CEA level preceded clinical recurrence by one to 31 months in some patients (124,194,195).

Clinical evidence also indicates that both pre- and post-treatment CEA levels could serve as prognostic markers in cancers of the stomach (196) and lung (197,198), although the evidence is not as compelling and well-documented as for cancers of the colon and the breast. Further investigations are needed to establish whether changes in patient management based upon these prognostic indicators will be of clinical benefit.

**CEA in Treatment Monitoring**

At the present time, treatment monitoring is certainly the most useful area for CEA testing. A rise in the blood CEA concentration in a patient after apparently successful surgical treatment for cancer has repeatedly been shown to signal a recurrence of the tumor. After apparently complete surgical resection of colorectal cancer, the blood CEA concentration, if elevated before surgery, falls to the normal range in nearly all patients (154). The fall usually occurs within one month but sometimes takes up to four months (199). If levels fail to fall to the normal range, it is likely that the resection has been incomplete or that the cancer had already metastasized. After the initial return of CEA to normal levels, it is not uncommon for transient, small elevations in serum CEA levels to occur in the absence of recurrent tumor. However, a sustained and progressive rise has repeatedly been shown to signal tumor recurrence. Although the best evidence for this phenomenon is found in colorectal cancer, similar data have been obtained in other CEA-expressing tumors. Serial CEA monitoring is currently considered the best non-invasive technique for detecting recurrent colorectal cancer (163, 200-204). In a study of over 300 colorectal cancer patients followed post-operatively
with serial serum CEA determination, the observation of two consecutive elevations in the CEA level yielded a sensitivity of 84% and a specificity of 100% in the detection of recurrence, and in 72% of cases preceded all other clinical signs (205).

The debate concerning the merit of postoperative determinations of CEA values in monitoring patients with resected colon cancer continues to rage. At one extreme are those who suggest that cancer cures attributable to CEA monitoring and subsequent intervention are achieved too infrequently to justify the costs and stress that such testing may cause for patients (175). Nevertheless, numerous authorities maintain that intensive follow-up using CEA assays makes possible the identification of treatable recurrences at a relatively early stage; this latter view has been substantiated by a recent extensive meta-analysis (176). The literature provides various parameters for predicting tumor recurrence based on postoperative CEA monitoring for colorectal carcinomas (126,153,170,199,204,206-212). These include both CEA levels exceeding a predetermined cut-off value and progressively rising CEA levels exceeding a specific rate of change. Various cut-off values ranging from 4 to 20 ng/ml have been suggested, depending on assay sensitivity and the patient population studied. Clinical investigations based on these parameters have, expectedly, yielded a variety of sensitivity/specificity ratios. Clearly, further controlled studies are required in order to arrive at an internationally agreed upon standard of procedure.

Nevertheless, it has been suggested recently that when CEA increases more rapidly than an average of 12.6% per month, recurrence should be strongly suspected (213). Serial CEA determinations may in this way allow initiation of anti-tumor chemotherapy, radiotherapy, or second-look surgery at an earlier stage of progression of recurrent colorectal cancer and offer a better outcome for the patient. Again, a well-controlled double-blind clinical trial will be required to determine the efficacy of this approach. In the context of a dismal overall prognosis for patients with recurrent disease after surgical resection, serum CEA determination may offer the only chance of a cure for a select group of individuals.

In addition to its use in colorectal cancer, there is general agreement that the implementation of regular and sequential assays for plasma or serum CEA provides valuable information postoperatively concerning regression or progression of cancer growth in patients receiving chemotherapy and/or radiotherapy for breast cancer, lung cancer, or meningeal carcinomatosis. In breast cancer, studies utilizing serial determinations of serum CEA and CA 15-3 in combination for the postoperative follow-up of patients with primary or recurrent disease have demonstrated sensitivities ranging between 64% and 94% in the detection of metastases (214,215). In breast cancer patients who had been treated by either radical mastectomy or simple mastectomy plus local radiotherapy, the overall specificity using the combination of CEA and CA 15-3 was 99% in the detection of non-locoregional metastases (215). Interestingly, estrogen receptor (ER)-positive and progesterone receptor (PR)-positive tumors were associated with greater lead-times, higher levels of both tumor markers at the time of diagnosis of recurrence, and higher sensitivities in the early diagnosis of relapse (215), suggesting a potential for improved success of hormonal therapy via this regimen of treatment monitoring.

In addition, it has been shown that 80% of distant metastases arising in breast cancer patients followed by serial CEA and CA 15-3 in combination are detectable up to several months earlier than by standard follow-up (216). It is worthy of note here that, based on improved diagnostic sensitivity and specificity rates, the use of individual reference limits reflecting variations in marker levels for a given patient has recently been advocated in the detection of breast cancer recurrence, in lieu of standard thresholds for CEA and CA 15-3 (217). While the aforementioned results for the utility of serum CEA and CA 15-3 toward the early detection of metastatic breast cancer are encouraging, they must be tempered by the fact that studies to date have not adequately established whether this early detection provides clinical benefit.

As for lung cancer, the use of serial serum CEA determinations in the monitoring of patients with surgically-treated non-small cell lung cancer revealed a sensitivity of 58% in the detection of recurrence; this figure
increased to 88% with concomitant analysis of serum CA 125 and squamous cell carcinoma antigen (218). Serial CEA assay may also be useful in a number of other tumors that have been less well studied to date (89,117,137,138,185,172,184,187,188,195,219-229). In patients with gastric or esophageal carcinoma, for example, an elevated CEA level during serial follow-up after preoperative chemotherapy has been shown to be predictive of relapse, and has demonstrated the potential for diagnosis of recurrent disease in advance of its clinical presentation (140).

Overall, the available data indicate that the assay for CEA is useful, both preoperatively and postoperatively, in the management of patients in whom the diagnosis of cancer has already been established. Assuredly, major advances in treatment of recurrent disease will further increase the clinical utility of the CEA assay.

**Immunohistochemical Diagnosis**

Immunoperoxidase staining of neoplastic tissue is a simple, sensitive method that is widely used in modern clinical pathology laboratories. The value of tissue CEA staining in cancer diagnosis depends greatly on the antibodies used for detection. A wealth of mAbs to CEA have been generated. They exhibit various degrees of cross-reactivity to other members of the CEA molecular family but, nevertheless, several excellent anti-CEA mAbs reagents are available commercially.

Immunohistochemically, CEA has been identified in cancers of the colorectum (230-232), breast (233), lung (234) uterine cervix (235), gallbladder (236), stomach (237), pancreas (238), liver (239), prostate, urinary bladder, and uterus (240,241), and in neuroendocrine tumors associated with the larynx, lung and thyroid (242).

There have been numerous attempts to compare the incidence of immunohistochemical staining for CEA with plasma CEA levels, tumor differentiation, disease stage, histologic tumor type, CEA intensity, distribution, cellular localization and clinical prognosis for various carcinomas (118,180,187,231-237,243-249). A positive correlation between serum CEA concentrations and the intensity of tissue CEA staining has been reported for cancers of the colorectum (250, 251) breast (252,253), pancreas (243), as well as for gestational and non-gestational choriocarcinomas (254). No correlation was observed for gastric cancer (118). Overall, the incidence of positive colorectal cancer tissue staining is similar to the frequency of elevated serum CEA levels. When differences have been found, these have indicated a higher sensitivity for tissue staining than for serum CEA levels.

Conflicting results have been published with regard to the relationship between tissue CEA staining and tumor differentiation in colorectal carcinomas, with some reports indicating no correlation (143,246,250, 255-257). Certainly, study design, different antibody preparations and methods of interpretation may result in apparently differing observations.

There are good reasons to regard ulcerative colitis and certain colonic adenomas as premalignant lesions (253). Immunoperoxidase staining for CEA supports the concept of a polyp-adenoma-cancer sequence (258). Studies of the localization of CEA in colonic polyps designated histopathologically as benign showed weak to moderate staining for CEA, whereas 100% of polyps that were graded as being severely dysplastic were strongly positive for CEA. These findings indicate that immunoperoxidase staining of apparently benign polyps for CEA may be of value in indicating when there is a need for close monitoring of patients with colorectal polyps. Both chronic inflammatory bowel disease and colorectal adenomas show higher tissue CEA concentrations than normal colonic mucosa, suggesting that these situations can be regarded as precancerous conditions biochemically (249,259,260). Strong CEA-positivity has also been observed in chronic pancreatitis (238), cystic fibrosis (261), gallbladder hyperplasia and chronic cholecystitis (236).

That anti-CEA antibodies stain normal colon mucosa, albeit very weakly (6,180,262-264), is not surprising
since normal colon mucosal cells produce CEA in culture (265,266) and CEA mRNA is actively expressed in
the normal colonic mucosal cells (17,24,29,34). CEA has also been demonstrated immunohistochemically in
normal epithelial cells of the respiratory system (267). It has also been found in 77% of pancreatic tumors by
this technology but the approach has no value in discriminating between chronic pancreatitis and pancreatic
carcinoma, since a significant proportion of inflamed tissues stain positively for CEA (238,268).
Immunohistochemical demonstration of CEA in breast tumors has been attempted by several groups and it is
evident from these studies that CEA is present in mammary cancer tissue in the majority of cases, whereas
benign lesions are virtually all negative.

Results concerning the correlation between positive tumor CEA immunohistochemistry and histologic grade,
lymph node involvement, locoregional recurrence, disease-free interval and patient survival remain
controversial. Nevertheless, considerable data indicate that a significant relationship exists between
immunohistochemical CEA-positivity of mammary carcinomas on the one hand, and the presence of lymph
node metastases, five-year survival rates and histological type on the other (180,245,263,269). A nearly 90%
correlation between Grade III breast cancer with lymph node metastases and this form of CEA-positivity has
been found as opposed to 40-45% CEA-positivity in Grade I and Grade II breast cancer (253). Invasive
ductal carcinoma usually shows more intense tissue CEA staining as compared to tubular, cribriform, and
invasive lobular carcinomas. The intensity of CEA staining is also associated with a worse prognosis.
However, contrary data has also been published (270-274). In addition, no correlation has been found
between tissue CEA and estrogen receptor status (274). Thus, it appears that the role of
immunohistochemistry in determining the prognosis in breast cancer remains to be clarified.

CEA staining is strongly positive in gastric tumors and its distribution apparently correlates with histologic
type, degree of differentiation, and tumor prognosis (112,237,264,275). Staining for CEA in fine needle
aspiration biopsy specimens has been reported to differentiate between primary hepatocellular carcinoma and
CEA-producing metastatic cancer to the liver (239). Ninety percent of hepatocellular carcinomas show CEA
staining that has a predominantly biliary canalicular pattern, while metastatic lesions demonstrate a diffuse
cytoplasmic pattern, where positive. A few reports have described CEA immunostaining in small cell lung
carcinoma (234,241,276) but no correlation between
the degree of immunostaining and clinical parameters has been reported. The majority of cervical
adenocarcinomas show positive staining for CEA, but no relation to clinical or histopathological tumor
characteristics has been demonstrated (235,277). With the data available, it has been suggested that the CEA
staining status of tumor tissue, and the pattern of that staining, might be very helpful in determining if the
tumor tissue under consideration was primary to the organ or of metastatic origin. This would be very helpful,
for example, in gastrointestinal
cancer metastases (CEA-positive) to the ovary (CEA-negative) and in other comparable situations
(109,167,231,232,240,278-280). As the impact of
CEA immunohistochemistry becomes increasingly important, it must also be underlined that CEA can easily
be demonstrated, retrospectively, in routinely-fixed, paraffin-embedded sections.

Radioimmunolocalization (RIL) of Cancers Using Anti-CEA Antibodies

RIL procedures using tumor-specific antibody has the potential to differentiate between malignant and benign
tissues in vivo. RIL of tumors was pioneered by Pressman and Korngold in 1953 using osteosarcomas as the
target (281). In 1974, Reif (282) attempted to detect metastatic cancer in a patient with advanced colon
carcinoma using radiolabeled antibody to CEA. The radiolabel did not localize to the cancer tissue. The first
successful reports demonstrating specific localization of anti-CEA in xenografts of human tumors in animals
were published by Goldenberg and Primus (283,284) and Mach et al. (145). Subsequently, extensive studies
have demonstrated the RIL of antibodies against human tumor markers in both animal models and in patients.
Due to cross-reactivity of polyclonal anti-CEA antibodies with NCA and other members of the CEA family,
only affinity-purified anti-CEA polyclonal antibodies and/or monoclonal antibodies having minimal or no
cross-reactivity with normal tissue epitopes or plasma components are likely to be effective as targeting agents (285).

The pioneering work of Goldenberg et al. (286-288) and Mach et al. (289) using affinity-purified, iodine-labeled polyclonal antibodies to localize advanced neoplastic diseases in man stimulated a number of clinical studies of RIL over the past 15 years. The availability of sophisticated nuclear imaging technology and the development of high-affinity anti-tumor monoclonal antibodies has subsequently improved imaging quality.

In humans, many of the early reports of tumor imaging with labeled anti-CEA antibodies were inconsistent and rather discouraging (281-283,289-292). This was primarily due to variability in the distribution of the conjugate and free isotope between normal and tumor tissues, as well as to blood pooling in various normal tissues and organs. Residual anti-CEA antibody cross-reactivity with antigens on normal cells, either by mAbs or polyclonal antisera, might also have contributed to the problem. However, most results to date indicate that, despite the technical and biologic problems defined, primary and secondary cancers can be successfully imaged with appropriately prepared anti-CEA antibodies (86,286-288,293-315).

Studies of gastrointestinal cancers utilizing antibodies to CEA have demonstrated effective RIL of tumors in 70% to over 90% of patients. Hepatic metastases, on the other hand, appear to be much more difficult to image because of the high background uptake and the blood pool of radioactivity in normal liver tissue and relatively low uptake in the metastatic lesions, particularly with $^{111}$In. The reported sensitivities for detection of liver metastases have ranged from 0-94% in different studies, reflecting differences largely in technical ability and skill in avoiding pitfalls. To improve the uptake of antibody-bound radiolabel by hepatic metastatic lesions, intraperitoneal (302) and intra-aortic (301), rather than intravenous, injections of anti-CEA preparations have been used with some success, as has the simultaneous injection of monoclonal anti-CEA antibody in an attempt to "block" the normally high liver uptake. In the latter case, the improved scintigrams of metastatic lesions suggest that the "blocking" effect of unlabeled mAbs influences the nonspecific distribution of labeled monoclonal antibody, perhaps primarily through the reduction of liver uptake of isotope-labeled anti-CEA mAbs. This has increased the sensitivity for detecting metastases (308,314).

The use of nuclear scintiscans to detect potentially operable recurrences has met with variable success depending on the site of tumor spread and the immunoconjugate used. Other potential variables include the radiolabel employed, the route of administration of the conjugate, tumor size and location, tumor vascularity, the patient population studied, the imaging technology employed, the unavoidable parameter of subjective interpretation of scans (even in blinded situations), and the type of antibody preparation used. Direct comparisons are difficult, because different techniques for imaging tumors have been employed. As in the case of animal studies, antibody fragments rather than intact immunoglobulins have been utilized with improved imaging quality (302,303,315,316). It is worthy of note that although many of the nuclear scintiscan studies to date have utilized correlative data from other imaging modalities, such as X-rays, CT scans, nuclear scans and ultrasound studies, histopathological assessment of the imaged tissue has seldom been reported in the published investigations.

Several major problems have hindered the progression of RIL to routine medical use. The relatively low antibody accumulation of isotope-antibody conjugate in tumors when compared with the amount of antibody injected has resulted in less than desirable contrast between cancerous and normal tissues. In addition, high uptake by normal liver tissue when $^{111}$In-labeled antibodies are employed has limited the usefulness of such reagents and the high uptake of radiolabeled monoclonal anti-CEA by normal mesenteric lymph nodes (294) and testis (293) poses a challenge to the realization of full clinical utility. New technetium labeling kits, however, may well have a major impact on RIL, primarily due to low cost, simplicity of labeling, and lower liver uptake of the conjugates.

Different isotopes and different methods of imaging, such as positron emission tomography (PET) (297) and
single-photon emission computed tomography (SPECT) (295,305,306), have been employed to increase tumor/normal tissue image contrast ratio, improving the sensitivity of detection. Further advancement may stem from a recent report on the effects of hyperthermia on tumor CEA expression, which indicates that tumor tissue warming may well increase membrane CEA expression and, thereby, have clinical implications for RIL (317). In addition, preliminary studies have demonstrated that the use of combinations of antibodies to different epitopes of CEA increases the ease of detection of tumors, presumably reflecting increased total uptake of the injected preparations (318). Alternatively, increased amounts of antibody to the same epitope may produce similar results. However, it might be postulated that attempts to saturate the circulating and tissue epitopes by administering high doses of mouse anti-CEA mAbs may evoke some allergic side effects due to foreign protein or lead to the development of anti-murine antibodies, which could inactivate or eliminate the administered antibody, precluding sequential imaging procedures. The presence of antibodies against murine immunoglobulin, as well as anti-idiotypic antibodies after injection of murine mAbs, has in fact been reported in humans (319-321), although the clinical significance of this is unclear.

Circulating CEA poses another potential problem for the RIL process. The CEA antigen shed into the circulation was shown in early studies to bind to administered anti-CEA antibodies (319,322) with the possibility of antigen-antibody complex formation and the attendant possibility for development of renal and other pathologies. Fortunately, there has been no evidence to date of any such problem. Moreover, the clinical evidence described to date indicates that there is no correlation between serum CEA concentration and the quality of the image or frequency of lesion detection, while there is a direct relationship between both tumor size and tumor CEA expression and sensitivity of RIL. At present, effective imaging can be achieved for those tumors expressing membrane CEA and measuring larger than 1.5 cm.

Recently, considerable advances have been made in the clinical utility of RIL, particularly in the contexts of colorectal and breast cancer. A European multicenter trial using a $^{99m}$Tc-labeled anti-CEA monoclonal antibody for immunoscintigraphic visualization of CEA-expressing tumors showed an 80% sensitivity rate in the detection of colorectal primaries and their abdominopelvic metastases and a 90% sensitivity rate in the detection of recurrent disease (323). Importantly, in 17% of the patients studied, this imaging modality detected cancers that had eluded standard diagnostic methods, and in a considerable number of patients yielded additional clinically-relevant information. These favorable results have been corroborated by further work in which immunoscintigraphy of patients with colorectal cancer using a radiolabeled anti-CEA mAb demonstrated an overall sensitivity of 93% in tumor detection (324). In addition, in a recent study involving nine patients with metastatic colorectal cancer, $^{123}$I-labeled anti-CEA single-chain variable fragment (scFv) antibody imaging with SPECT not only demonstrated the ability to localize all tumor deposits detected by conventional methods, but also those liver metastases only confirmed by CT portography (325). Furthermore, a study of the use of $^{99}$Tc-labeled anti-CEA antibody immunoscintigraphy in combination with CT to define the extent of tumor recurrence or metastasis in colorectal cancer patients has demonstrated an increase in the identification of resectable patients by 40%, and of non-resectable patients by 100%, as compared to use of CT alone (326).

As for breast carcinoma, one series of immunolymphoscintigraphy for metastases showed remarkable success, with a sensitivity of 90% and a specificity of 88% (327). In addition, the aforementioned $^{99}$Tc-labeled anti-CEA antibody imaging modality, originally developed for colorectal cancer (326), has quite recently achieved a very high specificity in the detection of breast cancer in patients with indeterminate mammographic lesions, offering the potential for a substantial decrease in the number of such patients undergoing surgical biopsy for subsequently histopathologically-proven benign disease (328).

The future promises further success in tumor imaging through better comprehension of the pharmacokinetics of radiolabeled antibodies and a clearer Understanding of the immunobiology of CEA-producing tumors.
Immunotherapy of CEA-Producing Tumors

Given that adenocarcinoma of the colorectum is the second most prevalent cancer in the United States (329), and that relatively early diagnosis presently results in surgical cures in about 50% of patients, the potential impact of the development of effective adjuvant therapies is far-reaching. Yet, current adjuvant chemotherapy is not very successful in those patients whose tumors have been resected but have a high risk for recurrence (i.e., Dukes Stages B2 and C tumors).

The ability to detect tumors by RIL raises the exciting possibility of treating such tumors by targeting with the same technology. Passive immunotherapy for the treatment of human CEA-producing cancers may be performed in a number of ways using anti-CEA antibodies as "homing devices" to target, with improved specificity, the delivery of cytotoxic radionuclides or chemotherapeutic agents, toxins, or pro-drugs to tumor cells. Active immunization has also been attempted in model systems with purified CEA-producing autologous cancer cells, anti-CEA anti-idiotypic antibodies, and photodynamic therapy (for review, see 330).

Another intriguing approach to the treatment of CEA-producing tumors has developed through the incorporation of the recombinant CEA gene or gene segments into an appropriate vector, such as vaccinia virus. This construct, both alone and in combination with other gene products to stimulate an immune response, has been shown to enhance cell-mediated anti-CEA immunity in both animal and human studies (331), and is currently being analyzed in clinical trials. An additional vaccine prototype has shown a protective effect against tumor challenge in mice, and has recently been modified for human clinical trials (332). Yet another prospect for treatment involves the gene therapy approach. Xenografting of CEA-expressing clones into which a chimeric gene for CEA and CD-145 has been introduced has recently yielded antitumor effects in mice after treatment with 5-fluorocytosine, illustrating the potential for specific gene therapy of CEA-positive tumors (333).

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