Reflection on the Discovery of Carcinoembryonic Antigen, Prostate-Specific Antigen, and Cancer Antigens CA125 and CA19-9

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One of the hottest current areas of research is the discovery and validation of novel biomarkers for many diseases, including cancer. For cancers, the reality is that no new major cancer biomarkers have entered the clinic in the last 30 years. Despite the emergence of highly powerful genomic, proteomic, epigenomic, metabolomic, microarray, and other omic technologies, which have been used intensively for the discovery of novel cancer biomarkers, the yield has been poor. In previous analyses, I pinpointed the possible reasons for such failures, and I proposed remedies for avoiding common and repetitive mistakes *(1–4)*. Other accounts have done the same, including some articles in this issue *(5, 6)*.

It is astonishing that some of the most useful cancer biomarkers were discovered in the 1960s [e.g., carcinoembryonic antigen (CEA)[8] and α-fetoprotein], the late 1970s [prostate-specific antigen (PSA)], and the early 1980s [cancer antigen 125 (CA125), CA19-9, and some others]. The discoverers of these biomarkers achieved their goals by means of classic analytical techniques, especially the more or less primitive (by today's standards) immunologic and chromatographic assays,

or by taking advantage of the then new monoclonal antibody technology, which originated in 1975. If we compare the technological tools of the 1960s to the 1980s with those we have today and the volumes of data that we can generate in the same time with the new high-throughput technologies, we can conclude that contemporary technologies have not delivered the goods promised in the arena of cancer biomarker discovery, at least to date. We should admire the pioneers of cancer biomarker research, who used more ingenuity and inventiveness and less technology to reach their goals. Every major discovery, such as the 4 representative examples described below, has a story behind it, a group of characters, and, similar to a movie, a script, actors, and a director. Obviously, we could not cite all the unsung heroes (technicians, graduate students, and postdocs) who participated in these discoveries, but we can certainly identify the "directors." Four of these directors comment on their teams and the environment associated with their discoveries, the impact of these discoveries in clinical care, and their projected future in cancer research. As others have said, we should look to the past for the important lessons we can use to shape the future. I hope these essays on the discovery of 4 major and clinically useful biomarkers will teach us some lessons that can guide us in overcoming the current difficulties with cancer biomarker discovery, and I hope this field will become more fertile in the years to come.

CA125: A Serendipitous Biomarker. Robert C. Bast, Jr.

The discovery of CA125 was a serendipitous event that arose from attempts to provide more effective therapy for patients with ovarian cancer. As a medical student at Harvard, I had spent 2 years with Dr. Hal Dvorak studying maturation of the immune response in guinea pigs *(7, 8)*. Af-

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[8] Nonstandard abbreviations: CEA, carcinoembryonic antigen; PSA, prostate-specific antigen; CA125, cancer antigen 125; CA19-9, cancer antigen 19-9; BCG, bacille Calmette–Guérin; ADCC, antibody-dependent cell-mediated cytotoxicity; OC125, ovarian cancer 125; CA125II, heterologous double-determinant CA125 assay; FDA, US Food and Drug Administration; ROMA, risk of ovarian malignancy algorithm; HE4, human epididymis protein 4; PPV, positive predictive value; PLCO, Prostate, Lung, Colon, and Ovary Cancer (Screening Trial); TVS, transvaginal sonography; UKCTOCS, UK Collaborative Trial of Ovarian Cancer Screening; ROCA, Risk of Ovarian Cancer Algorithm; SPORE, Specialized Program of Research Excellence; CEACAM, CEA cell adhesion molecule; USPSTF, US Preventive Services Task Force.

ter completing a medical internship at Johns Hopkins, I spent the 3 years between 1972 and 1975 at the Biology Branch of the National Cancer Institute in Bethesda working with Drs. Herbert Rapp and Berton Zbar to optimize intratumoral immunotherapy in a guinea pig hepatoma model that used bacille Calmette–Guérin (BCG), a live attenuated strain of *Mycobacterium bovis* widely used in the early 20th century as a vaccine against tuberculosis. Herb and Bert had found that the intense inflammatory response to local injection of BCG could produce regression of syngeneic hepatoma transplants growing on the flanks of guinea pigs, eliminate regional lymph node metastases, and induce tumor-specific immunity *(9)*. Their work paralleled ongoing clinical studies at the National Cancer Institute on intratumoral injection of cutaneous melanoma metastases and anticipated the intravesical administration of BCG to control superficial papillary carcinomas of the bladder, a treatment that is still widely used *(10)*.

After returning to Boston to complete a medical residency and fellowship in medical oncology, I joined the faculty at the Dana Farber Cancer Institute. In establishing my own laboratory, I wanted to apply the principles that I had learned in Bethesda to develop an effective immunotherapy for a visceral cancer. An ideal candidate seemed to be ovarian cancer, in which clinically important metastases developed on surfaces of the peritoneal cavity. We thought that intraperitoneal administration of an immunostimulant might induce a chronic inflammation on the peritoneal surface that would be sufficient to control metastatic disease. Dr. Robert Knapp, the head of Gynecologic Oncology at the Brigham and Women's Hospital, and his fellow, Dr. Ross Berkowitz, who now holds that position at the same institution, were pursuing a similar strategy, so we joined forces and combined our 2 laboratories. Bob and Ross had adapted a murine model for ovarian cancer developed by Dr. Stanley Order, in which murine ovarian cancer cells grew within the abdominal cavity, blocked diaphragmatic lymphatics, and induced ascites. Intraperitoneal injection of a heat-killed preparation of *Corynebacterium (Propionobacter) parvum* was able to prolong the survival of tumor-bearing mice, and adding a rabbit antimurine ovarian cancer heteroantiserum further prolonged survival. My initial studies showed that the synergistic antitumor activity of the 2 agents depended on antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by macrophages attracted into the peritoneal cavity and activated by *C. parvum (11)*.

In the days before translational research, we had translated this murine model directly to the clinic to treat ovarian cancer patients with residual peritoneal disease following conventional chemotherapy. We re-

peatedly administered *C. parvum* intraperitoneally through a peritoneal-dialysis catheter. An objective response rate of 30% was observed, and 2 complete responses lasted more than a year *(12)*. Macrophages washed from the peritoneum demonstrated enhanced activity for ADCC, suggesting that therapy might be improved by the addition of a specific antibody against human ovarian cancer. We used the then new monoclonal antibody technology developed by Kohler and Milstein *(13)* to develop the first monoclonal antibodies against human ovarian cancer. The 125th promising clone was designated OC125 (for ovarian cancer 125), and the cancer antigen recognized by this antibody became CA125. We found that CA125 was produced in normal amnion, Müllerian duct, and peritoneum during fetal development and in normal adult endometrium, lung, and cornea, but not in normal ovary *(14, 15)*. Approximately 80% of ovarian cancers produced substantial amounts of CA125, but it soon became apparent that CA125 was shed from the cancer cell surface. It was found in supernatants from cultured ovarian cancer cells, limiting its potential for serotherapy. A shed antigen, however, might prove valuable as a biomarker to monitor response to treatment and thus fill an unmet clinical need.

Working with Dr. Vince Zurowski at Centocor— then a start-up company housed in a commercial incubator facility at the University of Pennsylvania—we developed a homologous double-determinant RIA with OC125 to capture and detect CA125 by taking advantage of the fact that multiple identical peptide subunits of the high molecular weight mucin bound to OC125 *(16)*. Increased CA125 concentrations were found in sera from 90% of patients with advanced ovarian cancer and in 50% of patients with stage I disease. False-positive increases occurred with benign effusions and in gynecologic conditions such as endometriosis and uterine fibroids. Increased CA125 concentrations were found in other malignancies, including endometrial, fallopian tube, breast, and lung cancers.

Over the last 3 decades, the CA125 assay has evolved into a heterologous double-determinant assay (CA125II) that uses 2 epitopes: one recognized by OC125 *(14)* and the other by the M11 antibody developed by Tim O'Brien *(17)*. The CA125II assay has less day-to-day variation than the original CA125 assay. A number of other antibody-based assays have been shown to be equivalent to CA125 or CA125II. Because these assays have different limits for normal values and different calibration curves, consistent use of only 1 assay should be used to monitor a particular patient. Over the years, the strengths and limitations of CA125 have been defined for a number of indications.

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MONITORING RESPONSE TO TREATMENT

The CA125 assay was originally developed to monitor the response to chemotherapy. When the biomarker is increased, CA125 tracks the progression or regression of ovarian cancer with >90% accuracy. A persistent increase in CA125 after primary chemotherapy was found to be a highly specific marker (>90%) for residual ovarian cancer, which led to its approval by the US Food and Drug Administration (FDA) in 1987, four years after the initial publication of the assay. Despite its high specificity, the biomarker is not optimally sensitive for detecting persistent disease. CA125 can return to normal concentrations, and residual disease can be found at second-look operations in up to half of cases.

ESTIMATING PROGNOSIS

The rate at which CA125 decreases after surgery and during chemotherapy correlates statistically with prognosis. A short apparent half-life for CA125 is associated with prolonged survival; however, this correlation has not proved sufficiently precise to aid in the care of individual patients.

DETECTION OF RECURRENT DISEASE

Sequential monitoring of CA125 after surgery and chemotherapy for women in a complete clinical remission can detect disease recurrence in 70% of patients with a lead time of 3 to 4.8 months. Whether monitoring recurrence actually benefits patients has been debated. Only 1 study has evaluated this question directly (18), and this trial, although it produced negative results, had important limitations: CA125 increases within the reference interval were not considered, only 25% of the participating women received optimal treatment for recurrent disease, and secondary surgical cytoreduction was performed in only a small fraction of the patients (19). Although each patient must decide whether she wants to be monitored with CA125, earlier detection of disease does provide additional time for participation in clinical trials and for administration of the several drugs known to have activity against the disease.

REFERRAL TO GYNECOLOGIC ONCOLOGISTS

Several studies have documented improved outcomes when patients are referred to specially trained gynecologic oncologists for their primary operations. Despite this fact, less than half of patients receive their primary surgery from gynecologic oncologists. CA125 has aided in identifying patients with pelvic masses who are likely to have ovarian cancer. Preoperative diagnosis has depended on age, physical examination, and imaging with ultrasonography, magnetic resonance imaging, or computed tomography. Increases in serum biomarkers have also been used to increase the accuracy of differential diagnosis. Integrating biomarker, clinical, and imaging data has required mathematical analysis. The Risk of Malignancy Index, which was developed in the UK, includes menopausal status, CA125, and imaging (20). The OVA1 algorithm developed by Drs. Zhen Zhang, Dan Chan, and Eric Fung working with Vermillion, includes 5 serum biomarkers (CA125, β_2-microglobulin, transferrin, apoplipoprotein A1, and transthyretin) that are used in combination with imaging data (21). A risk of ovarian malignancy algorithm (ROMA), developed by Drs. Steven Skates and Richard Moore working with Fujirebio, uses CA125 and human epididymis protein 4 (HE4) to triage patients for operation by a specially trained surgeon (22). The ROMA has proved more sensitive than the Risk of Malignancy Index in a direct comparison (23). The OVA1 and ROMA algorithms have not been compared directly. In different trials, the 2 assessments exhibit comparable sensitivities (90%), but the ROMA is somewhat more specific (24).

EARLY DETECTION

The 5-year survival rate for ovarian cancer patients has increased substantially over the last 3 decades, but long-term survival rates have not changed, largely because diagnosis often occurs at a late stage. Up to 90% of patients can be cured when the disease is detected in stage I, whereas <30% of patients are cured when the disease is in stage III or IV. Given the prevalence of ovarian cancer in the postmenopausal population (1 in 2500), any screening strategy must have high sensitivity for early-stage disease (>75%) and very high specificity (>99.6%) to achieve a positive predictive value (PPV) of 10%, i.e., 10 operations for each case of ovarian cancer detected.

The Prostate, Lung, Colon, and Ovary Cancer (PLCO) Screening Trial in the US screened postmenopausal women with CA125 and transvaginal sonography (TVS) and found no improvement in survival (25). How these modalities are used matters, however. Although a single CA125 measurement lacks the requisite sensitivity and specificity, a greater PPV can be attained by performing TVS in the fraction of women with increasing CA125 values. CA125 increases progressively with ovarian cancer but remains stable over time with benign disease. The UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS), conducted by Drs. Usha Menon and Ian Jacobs, has randomized >200 000 postmenopausal women at average risk into 3 groups: (a) controls who receive routine care (101 359); (b) annual TVS in all women (50 639); and (c) annual CA125 measurements followed by TVS in the <2% of women with increasing values (50 640), as judged by the Risk of Ovarian Cancer Algorithm (ROCA) developed by Steven Skates (26). The entire trial is powered to detect an improvement in survival and will be completed in 2015. The only report to

appear thus far was for the prevalence phase of the trial, which observed a shift in stage, with a near doubling in the fraction of early-stage (I and II) cancers detected. In contrast to the 25% of cancers usually diagnosed in stage I or II, 48% of the cancers detected by screening were in an early stage. CA125 followed by TVS detected 89% of the ovarian cancers. CA125 followed by ultrasound prompted 2.8 operations per case, compared with 36.2 operations per case with annual ultrasound alone. Moreover, ovarian cancers appeared to develop 2 years before they were detected by conventional means, suggesting that annual screening would be effective.

With Dr. Karen Lu, the MD Anderson Cancer Center SPORE (Specialized Program of Research Excellence) has conducted a smaller trial over the last 10 years with 4543 postmenopausal women from the third arm of the UKCTOCS trial (annual CA125 screening followed by TVS) who were at average risk (27). Fewer than 0.9% of the participants have been referred for TVS after each annual screening, and 2.6% have been referred over multiple years on the study. The 11 operations prompted by the ROCA algorithm have detected 6 cases of ovarian cancer—2 borderline stage IA cases and 4 invasive high-grade cases in stages IA, IC, IC, and IIB. With a PPV of 60% for all cancers and 40% for invasive cancers, no more than 3 operations would be required with this strategy to detect each case of ovarian cancer. All invasive cases were detected during screening, and 2 of the 4 were still within the reference interval for CA125.

With serum samples from the PLCO Screening Trial performed in the US, investigators found increased CA125 concentrations in only 40% of the patients before diagnosis (28). Panels of biomarkers have been evaluated with proteomic and multiplex techniques to increase the sensitivity of CA125 for early-stage and preclinical disease (29). The most promising panel developed to date includes CA125, HE4, CA72.4, and matrix metalloproteinase 7. A new algorithm is being developed, and a new trial is being planned to determine the specificity and the PPV of this algorithm during annual screening. Each assay is being developed to be performed on a nanobiochip, which will permit rapid assay at the point of service with a drop of blood obtained by fingerstick (30).

BIOLOGY

CA125 [also known as MUC16 (mucin 16, cell surface associated)] may contribute to ovarian cancer pathogenesis (31). The MUC16 molecule, which has been cloned by Dr. Ken Lloyd (32) and Tim O'Brien (33), is a high molecular weight (M_r, 1×10^6) mucin with (a) an N-terminal domain, (b) up to 60 tandem repeat subunits containing identical sequences of 156 amino acid residues, (c) a membrane-spanning domain, and (d) a short cytoplasmic tail with a phosphorylation site. The extracellular domain is highly glycosylated and can bind to mesothelin, possibly facilitating attachment of metastatic cancer cells to mesothelial cells on the peritoneal surface. Signaling through the intracellular domain does not affect proliferation but can regulate migration, invasion, and xenograft growth. In ovarian cancer cells, overexpression of the gene encoding MUC16 appears to be related in most cases to transcriptional or posttranslational regulation rather than to amplification. In normal mice, CA125 is not required for normal development or reproduction (34) but may regulate susceptibility to neoplasia in aging animals. Much remains to be discovered regarding the role of MUC16 in health and disease.

CEA: Past, Present, and Future. Phil Gold

By the early 1960s, when the work on CEA had begun, studies had revealed little regarding unique molecular structures in human cancers that might be useful in the diagnosis and/or treatment of these diseases (35). Studies of artificially induced and transplantable tumors in inbred mice had shown, however, that tumor-specific transplantation antigens did exist, but they did not necessarily lead to tumor rejection (35). Hence, it was certainly feasible that human tumors contained comparable, unique molecular structures that would not prove adequately immunogenic to induce cancer rejection.
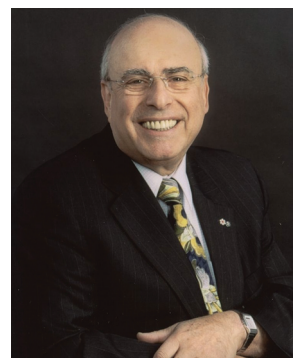
The problem with human cancer, however, was obtaining appropriate control tissue to compare with the tumor tissue under consideration (35). For this reason, colon cancer was initially chosen for study, because in its gross aspect this tumor does not extend intramurally more than 6 or 7 cm either distally or proximally from the cancerous tissue (36). The fact that the appropriate surgical technique frequently requires fairly extensive colonic resection allowed me, working with Sam Freedman, to compare the central tumor with areas of normal bowel mucosa sampled >7 cm away from either side of the tumor.

Normal tissues and cancer tissues from the same donors were compared immunologically with the techniques of immunologic tolerance and antiserum absorption (36). After a variety of analyses, we found a single distinctive antigenic entity that we initially believed to be

colon tumor specific; however, this antigenic entity was then found to be a general feature common to all endodermal-derived digestive system cancers (37).

Indeed, the same molecule was found in embryonic and fetal digestive tissues, which were obtained from spontaneous abortions in the first and second trimesters of gestation. The technology available at that time indicated the molecule had apparently disappeared by the third trimester. It did not reappear until tumor transformation had occurred, a phenomenon that was termed "derepressive dedifferentiation." Hence, the name "carcinoembryonic antigen" (i.e., CEA) was applied to the material in question (37), effectively ushering in the field of oncodevelopmental biology.

CEA, now also designated according to the international CD coding system as CD66e, was subsequently purified in our laboratories in conjunction with John Krupey and Chaim Banjo, and a virtually complete structural analysis of this glycophosphatidyl inositol–bound cell surface glycocalyceal glycoprotein followed (35). With David Thomson, then a research fellow in our laboratories, we then demonstrated that CEA was released into the circulation, where an RIA could detect it in bowel cancer patients (35). With this and other comparably sensitive techniques, CEA has been detected at low concentrations in healthy bowel and in >70% of all human cancers. This type of increasing serum CEA concentration over time was not seen either in the normal situation or under noncancerous inflammatory conditions.

The serum assay for CEA was the first clinical marker to achieve widespread use and, after some 45 years of scrutiny, remains the most widely used and most useful tumor marker assay worldwide. This assay has been the standard against which all other tumor markers of clinical importance have been measured, even though the CEA assay does not approach the perfection of complete tumor specificity that one would desire of an ideal tumor marker. The clinical importance and utility of CEA is well established and is a routine test that assists in the diagnosis and management of bowel cancer patients, as well as patients with other cancers (35).

As the only marker the American Society of Clinical Oncology recommends, its guidelines indicate serum CEA testing as a useful guide to the effectiveness of systemic therapy and as a preoperative guide for staging and surgical planning. Thus, quarterly CEA assays are recommended for 3 years for patients who have stage II or III colon or rectal cancer and who are candidates for further surgery or systemic therapy (38). In addition, the National Comprehensive Cancer Network recommends 5 years of serial CEA testing for patients with T2 disease or higher if the patient is a candidate for resection of isolated metastases. Thus, other

than for population screening, the CEA assay remains a standard for all stages of colorectal malignancy.

Further work led to elucidation of the structure of the gene that encodes the protein core of CEA (35). This protein core remains the central character of the new CEA cell adhesion molecule (CEACAM) (35) nomenclature of the 29 CEA gene family members. This family is itself a subgroup of the immunoglobulin gene superfamily (35), and studies of CEA and its family members continue undiminished (39, 40).

Although tumor markers need not have biological roles when they are initially defined or for the diagnostic roles they might play, studies of the CEA molecule and its family members—vis-à-vis their functions in embryologic life, cell differentiation, intercellular adhesion, and carcinogenesis—have been ongoing (35). CEA molecules demonstrate a unique form of intercellular reciprocal 2-point adhesion between themselves, and CEA molecules have important relationships with one or more integrins (and fibronectin) in the intercellular matrix. Hence, the role of CEA in a tumor's metastatic potential is becoming increasingly interesting (36).

The CEA system, in addition to the RIA role for which it is most frequently used, is important in tumor imaging (35) and immunopathology. Its role in the biological treatment of cancer patients continues to expand annually. The numerous clinical trials that have been initiated include trials for the naked CEA gene incorporated-DNA vaccines and for drug and isotope "homing" in conjunction with partial hepatectomy for cancers that have metastasized to the liver (35).

The advent of cancer genomics and biopharmaceuticals obviously will have enormous impacts on the areas of cancer prediction, diagnosis, and treatment. Tumor markers such as CEA will likely become a footnote in the field of cancer diagnosis and treatment, but one hopes CEA will have been of some value in moving the field forward.

PSA Discovery to Application: A Historic Journey. T. Ming Chu

Shortly after I joined Roswell Park Memorial Institute in 1970 as a new staff scientist, my department chair took me to see Dr. Gerald Murphy, institute director and urologist. Dr. Murphy warmly accepted me into the Roswell Park family and said, "Ming, you may do your tumor

marker research and any research you want, but make sure that you do prostate cancer research too." I replied, "Yes, sir." Thus began my journey to PSA!

As an active investigator of CEA at the dawn of the cancer biomarker era, I welcomed the PSA project as an addition to my research portfolio. This "marching order" from the Institute prompted me to submit an NIH grant application, entitled "Antigen Markers in Diagnosis of Prostate Cancer." I proposed, "We will search for prostate cancer-specific or associated antigens. . . . Usefulness of the prostate tumor antigen as a marker for the presence of early tumor and for the evaluation of treatment will be determined." My goal was to discover a new prostate cancer marker and to develop a simple blood test for the early detection of prostate cancer.

At that time, three-quarters of prostate cancers were detected after they had already metastasized. The commonly used blood test for diagnosis was for prostatic acid phosphatase, which had been developed in 1938. Unhappily, a result of an increase in this enzyme was always a gloomy finding.

In this article, I share some of my reflections on the discovery of PSA and the development of the PSA test. This was a team effort. Basic science and laboratory support was provided by my own group at Roswell Park. Clinical support was provided by the National Prostate Cancer Project.

In the beginning, I worked with my own technicians. A few years later, with the support from the NIH and the American Cancer Society, I greatly expanded my research project to include additional staff scientists and postdoctoral fellows. By means of immunochemical techniques, we used extracts of prostate tumor as the immunogens to prepare an antiserum reagent to differentiate prostate cancer from normal prostate.

After many years of scientifically challenging and technically difficult research, we published our first report on PSA, which described its identification and purification, in *Investigative Urology* in 1979 *(41)*. The first author, Ming Wang, was a staff scientist in my department. The report described creating a monospecific antiserum and purifying the PSA molecule (M_r, 34 000). The molecule was initially abbreviated as PA. As evidence of the importance of this new discovery, the American Urological Association centennial issue of *The Journal of Urology* in February 2002 cited this report as one of the 12 most important articles in prostate oncology. Additionally, *The Journal of Urology* republished this report in March 2002 as a Milestone in Urology.

With PSA and anti-PSA antiserum, we were able to show circulating PSA in prostate cancer patients, a finding that was published in July 1980 *(42)*. The first

author, Larry Papsidero, was a postdoctoral fellow. Shortly thereafter, we developed the PSA blood test and demonstrated its diagnostic potential; these results were published in December 1980 *(43)*. The first author, Manabu Kuriyama, was a postdoctoral fellow. Worth noting is that the American Association for Cancer Research Centennial in 2007, in referring to this report, cited our PSA work as a Landmark Scientific Discovery of the past century of cancer research.

Through the National Prostate Cancer Project, we evaluated the clinical application of PSA without delay *(44)*. Both the prognostic value and the monitoring value of PSA were evident immediately. An important finding was noted early in our clinical study: The usefulness of PSA for detecting early disease recurrence was always demonstrated in patients who had localized cancer and received curative therapy.

We undertook our study of the biological nature of PSA simultaneously. We reported the protease activity of PSA in 1984, with Yoshihito Ban, a postdoctoral fellow, as the first author. The sequence of 240 amino acid residues, which was determined by staff scientist Rueming Loor and colleagues, led to the conclusion that PSA is a chymotrypsin-like protease that forms the basis of today's antichymotrypsin "complexed" vs "free" forms of PSA. This area of investigation was pursued productively a few years later by other researchers.

Of note is that the clinical application of PSA was based on the prostate specificity of the PSA molecule. The specificity of PSA for prostate epithelial cells was established in 1981. This prostate specificity was further assured in studies with a series of monoclonal anti-PSA antibodies generated shortly thereafter, in 1983. The availability of anti-PSA monoclonal antibodies and a simplified procedure for purifying PSA from seminal plasma published in 1982 allowed large-scale production of the essential reagents for the PSA test and their standardization.

Additionally, our PSA patent, issued in 1984, greatly facilitated the transfer of our PSA technology to the biomedical industry. Our PSA patent was nonexclusively transferred to the biomedical industry, which has made PSA reagents and test kits readily available since 1986, when the FDA approved its use. Consequently, the clinical applications of PSA were investigated extensively, and these studies led to the widespread use of PSA testing in patient care around the globe.

One of the most important impacts of PSA has been the dramatic shift in the profile of newly diagnosed prostate cancer. The proportion of men with advanced cancers at the time of diagnosis was 75% before the PSA era. Today, 90% of prostate cancers are detected before the disease has spread to other organs. In

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essence, I have accomplished the research goal I proposed in my NIH grant application submitted 40 years ago. PSA has helped achieve a 99% 5-year survival rate for prostate cancer. PSA also is an important factor in the almost 50% reduction in the mortality rate of prostate cancer observed over the past 20 years.

Considering this progress, it is incomprehensible that the US Preventive Services Task Force (USPSTF) recently recommended the abandonment of the PSA test for prostate cancer screening. The sole rationale is that "screening may benefit a small number of men but will result in harm to many others." After this recommendation was announced, the American Medical Association criticized the composition of USPSTF for including neither oncologists nor urologists. Nationally recognized experts in the care of prostate cancer patients have disagreed with this recommendation by pointing out that the USPSTF report was based on flawed clinical trials and contained errors and misinterpretations *(45)*. It is important that PSA-based screening continue with an informed decision-making process. Men at average risk and with at least a 10-year life expectancy should begin conversations with their physicians at 50 years of age. Men in higher-risk groups should review the risk/benefit information at the age of 40 years.

Like any diagnostic test, this simple PSA test has its strengths and weaknesses, but it is the best test currently available. The focus of discussion should be on how to use PSA in assisting patient care. We should not turn back the clock to the time when too many men experienced a painful and unnecessary death from a prostate cancer that was detected too late.

CA19-9: From Discovery and Structural Analysis to Function and Drug Design. John L. Magnani

CA19-9, a functional cell surface carbohydrate antigen, is the only FDA-approved marker for monitoring the progression of pancreatic cancer. As a functional marker, CA19-9 is being explored both as a potential measure of a clinical end point and as a target for the development of novel therapies. I have had the good fortune and privilege to be involved in its discovery, its structural elucidation, the determination of its function, and its evaluation for drug design. These investigations have yielded a molecular mechanism that

has promoted the understanding of a variety of disease states, while offering the potential to intervene with novel therapeutic compounds.

My interest in functional carbohydrates stems from my formative years studying embryonic cell adhesion in Malcolm Steinberg's laboratory at Princeton University. Convinced of the importance of carbohydrates as recognition molecules, I started my career under the mentorship of one of the pioneers in glycobiology, Victor Ginsburg at the NIH. At that time, new methods were needed to identify carbohydrate ligands recognized by protein receptors. I developed a simple technique of binding such receptors directly to thin-layer silica gel plates that had been treated to immobilize chromatograms of separated glycolipids extracted from tissues. Soon thereafter, we were approached by Hilary Koprowski of the Wistar Institute, who was using the new exciting technology of monoclonal antibodies to distinguish tumor antigens from normal antigens on cell surfaces. This era was the early 1980s, when Cesar Milstein, Niels Jerne, and Georges Kohler received the Nobel Prize in physiology or medicine (1984) for the "discovery of the principle for the production of monoclonal antibodies." Applying this technology to cell surfaces allowed the detection of specific novel antigens on tumor cells as seen through the eyes of the immune system. Many of these antibodies revealed the existence of aberrant forms of glycosylation in tumor cells, which are missed completely in the currently restricted use of genomics to study tumor markers, because carbohydrates are secondary gene products and cannot be detected simply by a genomics approach. The Wistar Institute sent us 2 antibodies (1116-NS-19-9 and 1116-NS-52a) that displayed the best specificity for colorectal cancer and appeared to be directed against epitopes that were carbohydrate in nature, because of their resistance to proteases but sensitivity to glycosidases. Upon analysis with the new method of immunostaining thin-layer chromatograms of glycolipids extracted from colorectal cancer cells, we detected a novel monosialoganglioside as the antigen for both antibodies *(46)*. Once it was identified, we scaled up the purification of this monosialoganglioside and determined its structure via chemical techniques, including GC-MS. The monosialoganglioside detected by the 2 antibodies had a new carbohydrate structure, which we identified as sialylated lacto-*N*-fucopentaose II *(47)*, more commonly known as sialyl Le[a]. The excitement over this novel tumor-associated carbohydrate antigen prompted the founding of Centocor, which developed a diagnostic assay with one of these antibodies (1116-NS-19-9). It was thus that the carbohydrate tumor marker sialyl Le[a] became CA19-9, according to the immunologist's nomenclature. Although the structural analysis was performed with

simple gangliosides, we analyzed the major source of the CA19-9 antigen in patients' sera and to our surprise discovered that it originated mainly from mucins secreted from these adenocarcinomas into the bloodstream (48).

Although serum CA19-9 concentrations are increased in both gastrointestinal and pancreatic cancers, this biomarker shows its highest sensitivity and specificity for the detection of pancreatic cancer in symptomatic patients (approximately 80% and 90%, respectively). The core carbohydrate structure of CA19-9 contains a fucose linkage that is under the control of the Lewis blood group system, and individuals who are Lewis negative (Le^{a-b-}, representing about 5%–7% of the population) lack the fucosyltransferase needed to synthesize the CA19-9 structure. These patients test negative with this assay.

Serum CA19-9 is the most extensively studied and clinically useful biomarker for pancreatic cancer and is the only validated assay that the FDA has approved for monitoring pancreatic cancer patients. Studies have shown a significant decrease in survival rate for patients with high preoperative serum CA19-9 concentrations. Likewise, high postoperative CA19-9 values are indicative of lower survival rates for patients and can be considered a prognostic indicator of metastatic disease. More importantly, at least 8 different clinical studies have reported that pancreatic cancer patients who show a decrease in CA19-9 during chemotherapy (responders) have a significantly increased survival rate compared with treated patients with constant or increasing CA19-9 concentrations during treatment (49). These findings have prompted discussions on the potential future use of CA19-9 as a clinical end point.

Almost 10 years after discovering the structure of sialyl Le^a (CA19-9), we were approached by Eugene Butcher of Stanford University for assistance in discovering the structure of a carbohydrate receptor for an adhesion molecule expressed on blood vessel endothelial cells that functions in the extravasation of immune cells during an inflammatory response. The adhesion molecule is now known as E-selectin, and we quickly discovered that it binds CA19-9. More specifically, we described a trisaccharide domain shared by both sialyl Le^a and its isomer, sialyl Le^x (found on immune cells), as the true binding epitope for E-selectin (50). This was an exciting time in the laboratory, because we now understood the function of CA19-9 and why it was a prognostic indicator of metastatic disease. We hypothesize that pancreatic cancer cells expressing high concentrations of CA19-9 readily bind to E-selectin on vascular walls, thereby hijacking the inflammatory pathway for extravasation of cells from the bloodstream and promoting metastasis. Support for this theory also comes from studies on E-selectin. Colorectal cancer patients with a genetic variant of E-selectin (S128R) exhibit greater E-selectin–mediated cell adhesion and show a significantly decreased survival rate over an 8-year period. Other studies have shown that increased serum E-selectin concentrations are associated with a higher prevalence of metastatic disease, and combining measures of serum CA19-9 and serum E-selectin improves the prediction of metastatic spread. In addition, one interesting study has shown that inhibiting the expression of E-selectin by treatment with cimetidine significantly increased the 10-year survival rate of colon cancer patients who expressed high concentrations of CA19-9 on their tumors (51).

Thus, CA19-9 is a prognostic marker of disease for pancreatic cancer because it functions in the process of metastatic spread of cancer cells that strongly express this antigen on the cell surface. Because we have identified a small trisaccharide domain within CA19-9 that binds E-selectin and is responsible for this function, we were presented with an opportunity to design a small-molecule mimic of this domain as a novel glycomimetic drug to inhibit metastasis. Our first glycomimetic design (GMI-1070) included other domains required to inhibit all 3 selectins (E, P, and L) and showed efficacy in preclinical models of both inflammation and cancer (52, 53). GMI-1070 is now in phase II clinical trials to treat sickle cell patients in vaso-occlusive crisis, and it is the basis of a recent partnership with Pfizer, one of the largest licensing deals in the biotechnology industry for 2011. The interest in this novel glycomimetic antagonist validates this approach, and we are now focusing our new design on a more restricted glycomimetic of CA19-9 to develop an orally available, potent E-selectin–specific antagonist to be used in combination therapy with standard-of-care treatments for both solid and liquid tumors.

Reflections

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