Questions and Answers: OvaCheck ™ and NCI/FDA Ovarian Cancer Clinical Trials Using Proteomics Technology

1. What is the National Cancer Institute/U.S. Food and Drug Administration Clinical Proteomics Program?

The collaboration between the National Cancer Institute (NCI) and the U.S. Food and Drug Administration (FDA) began in 1997, and is led by Lance Liotta, M.D. Ph.D., of NCI's Center for Cancer Research and Emanuel Petricoin, Ph.D., of FDA's Center for Biologics Evaluation and Research (CBER).

The NCI/FDA clinical proteomics program ties the study of all proteins in living cells (or proteomics) to the clinical care of patients. Specific technologies developed in this program are at an early stage of application to diagnosis and treatment of cancer patients.

The scientific goal of proteomics is to capture the information flow within the cell and the organism. Petricoin and Liotta have created new technologies to measure protein fingerprints. Potential benefits of clinical proteomics include developing individualized therapies using targeted treatments that have been predetermined to be effective for each patient; determining the toxic and beneficial effects of treatments in the lab before using them in patients; diagnosing cancer earlier than is now possible; and improving the understanding of tumors at the protein level, leading to better treatments.

Petricoin and Liotta have identified hundreds of proteins in cancer cells and tissues of the breast, ovary, prostate, and esophagus that change in amount as the cells in these tissues grow abnormally. This abnormal complement of proteins may provide new means of diagnosing and treating cancers earlier.

Through the Clinical Center at the National Institutes of Health, Elise Kohn, M.D., of NCI, uses a special Laser Capture Microdissection Microscope (LCMM) to analyze biopsied cells from cancer patients (before and after treatment). LCMM was invented in Liotta's laboratory. The microscope allows the isolation of pure normal cells, pre-cancerous cells, and tumor cells from the same patient. By capturing cells directly from the tissue, the original protein pattern of the cells is maintained, which is not the case with traditional methods of isolating cells.

The scientists are also analyzing the patterns of proteins in the extracted tumor cells following treatment. For example, researchers are studying how a particular treatment changes the network or circuitry of proteins in a cell and are also looking for protein patterns changes if the tumor returns after treatment.

NCI has recently begun the world's first clinical trials using proteomic technology to conduct research on biopsies of tumor metastasis before, during, and after administration of experimental therapies. The hope is that proteomic profiling of cancer cells will provide information useful to study how a candidate drug works, the mechanism of drug resistance, and how to reduce treatment side-effects.

One such example is a NCI phase I trial of Gleevec for relapsed ovarian cancer patients that is open for accrual. Interested patients can call the Clinical Studies Support Center at 1-888-624-1937.

2. What type of research have you conducted to develop a test for the detection of ovarian cancer?

In 2002, scientists from the FDA, NCI and Correlogic Systems, Inc. (a bioinformatics company) described patterns of proteins found in patients' serum that may reflect the presence of disease. In this study reported in *Lancet* (359: 572-577, 2002), scientists used serum proteins to detect ovarian cancer, even at early stages. They reported that this new diagnostic concept might be applicable to nearly any type of disease.

The research, conducted under a Cooperative Research and Development Agreement between the FDA/NCI Clinical Proteomics Program and Correlogic Systems Inc., unites two exciting disciplines: proteomics - the study of the proteins inside cells - and artificial intelligence computer programs. Using blood from a finger stick in a test that is completed in 30 minutes, researchers were able to differentiate between serum samples taken from patients with ovarian cancer vs. normal individuals. The approach relied on software that is able to detect patterns of key proteins in the blood. Using a sophisticated artificial intelligence computer program developed by Correlogic, scientists were able to "train" the computer to distinguish between patterns of small proteins found in the blood of cancer patients vs. control samples. The artificial intelligence program identified a pattern consisting of only a handful of proteins, among thousands, that could be used to distinguish between women with ovarian cancer and women with non-cancerous conditions.

The scientists used serum samples from known cancer patients and unaffected individuals to establish proteomic patterns that were present at different levels in the cancer and normal (unaffected) groups. Once these patterns were identified, the researchers compared them with the patterns of the same proteins in serum samples from other patients with and without cancer. The researchers correctly identified 50 out of 50 cancers and 63 of 66 non-cancer samples.

The researchers analyzed serum proteins with mass spectroscopy, a technique used to sort proteins and other molecules based on their weight and electrical charge. The identity of the key proteins and the role they may play in cancer is unknown, but is currently being investigated.

Currently, more than 80 percent of ovarian cancer patients are diagnosed at a late clinical stage when these patients have only a 20 percent chance of survival at five years. In contrast, the 20 percent of women diagnosed with early-stage disease have an excellent

prognosis, with over 95 percent alive five years after diagnosis. It is encouraging that these investigators were able to correctly identify the disease in a small sample of patients that were all stage I ovarian cancer cases. The results of this study indicate that proteomic technology, once validated in large trials, may help clinicians diagnose the disease much earlier than current methods.

Ongoing Questions and Focus

There is still a need to confirm the sensitivity and accuracy of this technique as a diagnostic tool. For example, the investigators hope that by combining the proteomic approach with other methods of ovarian cancer diagnosis, such as ultrasound, its precision can be further improved.

3. What type of research have you conducted since the 2002 *Lancet* publication?

The *Lancet* publication focused on using patterns of proteins in the blood as early biomarkers for detecting ovarian cancer. The NCI/FDA scientists are continuing this work by conducting a clinical trial to determine if this proteomics tool is comparable to the single protein test, CA125, which is presently used to detect ovarian cancer recurrence. Elise Kohn, M.D., the principal investigator of the trial, is currently recruiting ovarian cancer patients in remission after treatment at both the National Institutes of Health in Bethesda, Md., and later in 2004 at multiple institutions across the United States. Interested patients can call the Clinical Studies Support Center at 1-888-624-1937.

The NCI/FDA researchers are also continuing to improve the performance of the proteomic analysis for ovarian cancer. The *Lancet* report concluded that the proteomic analysis performed with a sensitivity of 100 percent (95% Confidence Interval [CI], 93%-100%), specificity of 95 percent (95% CI, 87%-99%). Results from a recently accepted paper authored by scientists from NCI, FDA, SAIC-Frederick (the operations and technical support contractor for the NCI at its Frederick, Md., research center, and a subsidiary of Science Applications International Corporation,) and Correlogic Systems, Inc., shows that both the sensitivity and specificity for blinded samples were 100 percent with a larger group of participants - using a higher resolution instrument and entirely different patterns compared to the one pattern reported earlier in the *Lancet* paper (accepted for publication in *Endocrine and Related Cancers*, June 2004).

Despite this promise, validation in a very large clinical group is needed before a commercial test for this technique is available. Sensitivity measures the proportion of people with the disease that test positive; specificity measures the proportion of the people without the disease who test negative. A specificity of 99 percent means that 1 percent of those who did not have cancer would test positive, which is far too high a rate for commercial use. For a rare disease such as ovarian cancer, which has an approximate prevalence of 1 in 2500 in the general population, a 99 percent specificity and 100

percent sensitivity translates into 25 women falsely identified for every one true cancer found.

Following their studies of ovarian cancer recurrence, the NCI/FDA scientists plan to extend the technology to two other indications: a) distinguishing benign conditions from ovarian cancer in women presenting with an undiagnosed pelvic mass; and b) confirm the sensitivity and specificity of proteomic analysis for stage I ovarian cancer in trials of high-risk and low-risk women. Kohn, working with the Gynecologic Oncology Group (a cooperative group of NCI-supported research centers), is collecting blood samples for this early-stage study. The proteomic tool will be tested alone or in combination with current screening options (e.g., CA125). The NCI/FDA scientists are exploring the use of many pattern recognition approaches and mass spectrometry instrumentation to determine the most robust and significant features of each approach for clinical implementation.

4. What additional proteomics research done by Drs. Liotta and Petricoin contributed to the current proteomics technology platform and what is planned next?

Pivotal to the ongoing and most recent development of the proteomics pattern technology reported in various manuscripts by Liotta and Petricoin was the discovery that the low-molecular protein, metabolites and peptides useful for early detection of ovarian cancer accumulate on larger carrier proteins in the blood such as albumin. This piggy-backing ensures the smaller molecules a longer life in the circulating blood (Liotta L, et al, *Nature,* Oct. 2003, "Written in Blood" and Mehta AI, et al., *Disease Markers,* Dec 2003, "Biomarker amplification by serum carrier protein binding"). Knowing this, the scientists can obtain a greater concentration of potential biomarker proteins by extracting the carrier protein fraction from the blood. Scientists from the NCI/FDA Clinical Proteomics Program are working with Mauro Ferrari, Ph.D., a world-renowned nanotechnology expert, to create synthetic nanoparticles that can act like carrier proteins that could be used to further enrich diagnostic protein patterns.

Although it is not necessary in theory to know the identify of the proteins that prove to be useful to detect early disease or response to treatment, many of these proteins have been identified and are leading to an understanding of the molecular pathways involved in disease states. These efforts are also leading to the discovery of many new protein biomarkers in the blood.

Besides ovarian cancer, similar techniques are being applied to other cancers. The researchers are looking for protein patterns in the blood that are diagnostic for early-stage aggressive prostate, lung, and breast cancers, as well as patterns that can predict risk for prostate, colon, skin, and pancreatic cancers.

In addition to analyzing proteins in the blood, another thrust of the proteomics program is to compare the proteins in tumor tissue vs. healthy tissue. Using this approach, researchers are probing tissues for phosphorylated proteins known to be important in

carcinogenesis and are looking for useful diagnostic patterns. The work is yielding new insights about which molecular pathways are altered in tumor development.

The general strategy of the proteomics program is to extract proteins from the blood or tissue, analyze them with mass spectrometry to create patterns of protein fragments, sort through the patterns with a variety of pattern recognition programs, and ultimately discover differences that will help distinguish, for example, cancer patients vs. healthy controls or patients that respond to therapy vs. those who do not. NCI/FDA experts are continuing to test alternative mass spectrometry platforms and computer algorithms that they hope will yield clinically useful patterns.

5. Do you plan to submit the NCI/FDA proteomic methodology and a diagnostic test to the FDA for their approval?

Yes. The collaborative NCI/FDA group has begun working with an independent pre-market review group at FDA (the Office of In Vitro Diagnostics) to prepare a submission to obtain pre-market marketing clearance for ovarian cancer recurrence monitoring.

6. How does your work relate to the OvaCheck™ test being developed by private industry?

According to Correlogic Systems, Inc., "OvaCheck™, a blood test for the early detection of ovarian cancer, is the first diagnostic test using Correlogic's approach and technology. It will be offered to women nationwide through LabCorp and Quest Diagnostics."

The OvaCheck™ test is being independently developed by Correlogic Systems in conjunction with Quest Diagnostics and LabCorp, two non-governmental, private companies. This test is unrelated to previous published work with the NCI and FDA, and utilizes different mass spectrometry instrumentation and detection methods, as well as different sample handling and processing methods. The OvaCheck™ test employs electrospray ionization (ESI) type of mass spectrometry using highly diluted denatured sera, whereas the NCI/FDA group uses matrix-assisted laser desorption ionization (MALDI) analysis of undiluted native sera samples. The class of molecules analyzed by these two approaches, and thus the molecules that constitute the diagnostic patterns, are entirely different. Neither the NCI nor FDA has been involved in the design of OvaCheck™ methodology, or its validation.

Scientists from FDA, NCI, and Correlogic Systems are collaborating on research studies to identify MALDI-based patterns of protein expression indicative of specific disease states, including ovarian cancer, using Correlogic's proprietary software. These studies are distinct from the development of the OvaCheck™ test.

7. What other early detection research is being done at NCI and FDA?

NCI supports a program called the Early Detection Research Network (EDRN), which is focused on finding biomarkers for cancer. Biomarkers are proteins, genes, etc. that are related to the presence of cancer. The goal of the EDRN is to discover and validate biomarkers.

Biomarker research consists of: initial discovery of biological markers, evaluation of the most promising biomarkers, and validation to determine that they can work in a clinical setting.

The EDRN has a straightforward mission: to ultimately translate newly emerging molecular knowledge of validated biomarkers into practical clinical tests to detect cancer and cancer risk. For most cancers, successful treatment depends on early detection and successful prevention depends on the accurate evaluation of risk.

The power of bioinformatics and computer-assisted programs are being put to full use to analyze EDRN data and to facilitate faster answers to key questions. The EDRN was conceived on the premise that a "vertical" approach to biomarker research—that is, an established integrated, multidisciplinary environment—will facilitate collaboration among technology developers, basic scientists, clinicians, epidemiologists, biostatisticians, and other health professionals, and therefore expedite clinical applications of validated biomarkers.

8. What are biomarkers and how are they developed?

Successfully translating research on biomarker applications from the laboratory to patients involves five phases:
> Phase 1 includes exploratory studies to identify potentially useful biomarkers -- this is called the "discovery" phase.
> Phase 2 is where biomarkers are studied to determine their capacity for distinguishing between people with cancer and those without -- the validation phase.
> Phase 3 determines the capacity of a biomarker to detect pre-clinical disease by testing the marker against tissues collected longitudinally from research cohorts.
> Phase 4 includes prospective screening studies.
> Phase 5 is when large-scale population studies evaluate not just the role of the biomarker for detection of cancer, but the overall impact of screening on the population.

9. Is NCI sponsoring other research in the area of ovarian cancer diagnosis?

NCI investigators are developing other novel approaches in their goal to identify ovarian cancer at the earliest stage. In addition to the NCI/FDA proteomics program, two other examples are:
• Investigators from the Brigham and Women's Hospital in Boston have successfully used cDNA (complementary DNA) microarray analysis to compare ovarian cancer epithelial cell lines and normal surface epithelial cells to identify genes that are up-regulated in cancer. Two genes, *prostasin* and *osteopointin*, are the newest candidate markers for ovarian cancer and may prove complementary to CA125, an ovarian cancer biomarker that is used primarily in managing treatment of the disease, but is also being evaluated as a cancer screening test.
• Investigators at Northwestern University, Chicago, have found that lysophosphatidic acid (LPA) is elevated in the plasma of women with ovarian cancer, including 90 percent of women with stage I disease. The presence of LPA in early-stage disease may suggest that it is produced by the cancer itself and plays a role in allowing the cancer to spread.

10. What are the mortality and survival rates associated with ovarian cancer?

Ovarian cancer is the leading cause of death from gynecological malignances and is the fifth most common cancer in women. Unfortunately, only 25 percent of patients are diagnosed when ovarian cancer is still localized to the ovary. Up to 90 percent of these very early cancers can be successfully treated, while only 30 percent of the patients with more advanced cancers will survive five years.

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