Clinical Study Report
Protocol FDI-03A2

IDE NUMBER:	NA; Non-significant risk study
STUDY NUMBER:	FDI-03
INVESTIGATIONAL DEVICE:	CA 125 and HE4 TEST KIT
FDI NUMBER:	FDI-03
STUDY TITLE:	Evaluation of multiple biomarker assays to estimate risk of ovarian cancer in patients presenting with a pelvic mass
ORIGINAL PROTOCOL DATE:	November 7, 2005
REVISED PROTOCOL DATE:	January 17, 2006 August 18, 2006
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INTENDED USE	The HE4 EIA is an enzyme immunometric assay for the quantitative determination of HE4 in human serum. The HE4 EIA used in conjunction with the ARCHITECT CA 125 II assay creates a predictive probability of epithelial ovarian cancer using a mathematical function referred to as the Risk of Ovarian Malignancy Algorithm (ROMA™), for use in premenopausal and post-menopausal women presenting with an adnexal mass who are candidates for surgical intervention. Subjects categorized as low risk for epithelial ovarian cancer using the ROMA value may have surgical intervention performed by a non-oncology specialist. The results must be interpreted in conjunction with other clinical findings in accordance with standard clinical management guidelines. The assay is not indicated as an aid in a decision to proceed to surgery.
TRIAL DESIGN	Multi-center, prospective, double-blind
DATE STUDY INITIATED	November 7, 2005
REPORT DATE	August 7, 2008

Compliance: The study described in this report was performed according to the principles of Good Clinical Practice (GCP).

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DOCUMENT APPROVALS

Documents for the acceptance of the Clinical Study Report for the FDI-03 Study titled: Evaluation of multiple biomarker assays to estimate risk of ovarian cancer in patients presenting with a pelvic mass are subject to joint approval of the following department management representatives at Fujirebio Diagnostics, Inc. approval signatures indicate acceptance of the protocols, results and conclusions described in the following document sections.

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1. EXECUTIVE SUMMARY

INTRODUCTION AND BACKGROUND

It is estimated that 5 to 10 percent of women in the United States (US) will undergo a surgical procedure for a suspected ovarian neoplasm during their lifetime, and 13 to 21 percent of these women will be found to have an ovarian malignancy. (1) Clinical impression, serum CA 125, and imaging have been the standards in the determination of whether an adnexal mass is suspicious for malignancy. (2) In the US, women with adnexal masses present primarily to gynecologists, primary care physicians or general surgeons, at the time of their initial diagnosis of a pelvic mass or ovarian cyst. However, according to a Practice Bulletin from the American College of Obstetrics and Gynecology (ACOG) (3), an important dilemma is faced by these physicians as to which patients are appropriate to refer to a gynecologic oncologist, and/or to an institution experienced in gynecologic cancer surgery. Several recent studies have demonstrated that ovarian cancer patients managed by gynecologic oncologists and at high volume institutions are more likely to undergo complete surgical staging, and optimal cytoreductive surgery with fewer complications and better survival rates than patients treated by surgeons less familiar with the management of ovarian cancer. (4-7) The ACOG Practice Bulletin published in 2007 states the following:

"*Women with ovarian cancer whose care is managed by physicians who have advanced training and expertise in the treatment of women with ovarian cancer, such as gynecologic oncologists, have improved overall survival rates compared with those treated without such collaboration*". (3)

Conversely, women receiving surgical intervention for a benign pelvic mass may be treated successfully in a community hospital by a gynecologist or general surgeon.

To improve the triage of patients with pelvic masses, we studied eight potential biomarkers in sera drawn from women with pelvic mass prior to surgery. We found that only one biomarker, HE4, was able to increase the sensitivity of CA 125 measurement alone. Therefore, we developed an algorithm that combines CA 125 and HE4 concentrations in a logistic model, and provides a probability of finding cancer in a given patient. This algorithm was developed using a Training Set obtained by pooling data across two separate Pilot Studies at Women's and Infants Hospital of Rhode Island (WIHRI) in Providence, RI, and Massachusetts General Hospital (MGH) in Boston, MA. The purpose of the current trial is to

serve as a Validation Set, or Pivotal Trial, of the algorithm previously developed in the Pilot Study. We tested the combination of CA 125II and HE4 in women with pelvic mass to demonstrate that the algorithm developed in the Pilot Study is valid in a prospective, multi-center, statistically powered, double-blind clinical trial.

INTENDED USE OF THE HE4 ASSAY

The HE4 EIA is an enzyme immunometric assay for the quantitative determination of HE4 in human serum.

The HE4 EIA used in conjunction with the ARCHITECT CA 125 II assay creates a predictive probability of epithelial ovarian cancer using a mathematical function referred to as the Risk of Ovarian Malignancy Algorithm (ROMA™), for use in premenopausal and post-menopausal women presenting with an adnexal mass who are candidates for surgical intervention. Subjects categorized as low risk for epithelial ovarian cancer using the ROMA value may have surgical intervention performed by a non-oncology specialist. The results must be interpreted in conjunction with other clinical findings in accordance with standard clinical management guidelines. The assay is not indicated as an aid in a decision to proceed to surgery.

NOTE: In the labeling, the mathematical function determining Predictive Probability (PP) will be referred to as the ROMA.

Trial Primary endpoint: To estimate the risk of finding ovarian cancer at the time of surgery in pre- and post-menopausal women presenting with a pelvic mass. Multiple serum biomarkers (CA 125 and HE4) were evaluated to estimate the risk that the patient was harboring an ovarian cancer at the time of presentation.

Trial Secondary endpoint: Comparison of a multivariate predictive algorithm combining CA 125 and HE4 with radiological imaging results, and patient risk factors (such as age, menopausal status and ethnicity) to estimate the risk of ovarian cancer, including low malignant potential (borderline) tumors, at the time of surgery in patients presenting with a pelvic mass.

METHODS

The FDI-03 Pelvic Mass study was a prospective, multi-center, double-blind, statistically powered clinical trial that enrolled female subjects ≥18 years of age presenting to a gynecologist or gynecological oncologist with a pelvic mass that were scheduled to undergo surgery. The study was conducted from December 2005 until February 2007 at 14 clinical geographically-disperse clinical sites across the US. Serum samples were drawn from all subjects and tested in the HE4 EIA at three sites including Fujirebio Diagnostics AB (FDAB), MGH, and M.D. Anderson Cancer Center, and sera were tested in the ARCHITECT CA 125II assay at Fujirebio Diagnostics, Inc. (FDI) in Malvern, PA. The Study Protocol was reviewed and approved by local or central Institutional Review Boards (IRBs). Written informed consent was obtained from each subject prior to the collection of blood or urine for this study. All patients in this study underwent laparoscopic surgery or laparotomy, and all tissues were examined by local pathologists and centrally reviewed by Johns Hopkins University School of Medicine, Departments of Pathology and Gynecology and Obstetrics (JH). Final review of Histopathology results from site and Central Review for final consensus determination was performed by two gynecologic oncologists blinded to laboratory values. The minimum number of patients enrolled was set at 500 total patients, and a minimum of 100 cases of ovarian cancer.

The FDI-03 Pelvic Mass Protocol specified combining CA 125 and HE4 concentrations into an algorithm to develop a predictive probability (PP) based on interim results from a Pilot Study conducted at WIHRI ("Protocol Analysis"). Using the pre-operative CA 125 and HE4 serum levels from the N=201 WIHRI dataset, the following logistic regression model and optimal threshold values for the resulting predicted probabilities were determined that separated them into low, moderate, and high risk groups:

- Predictive Index (PI) = 0.00307086*CA 125 + 0.01604288*HE4 – 3.195731
- PP = exp(PI) / [1 + exp(PI)]
    - Low Risk PP: < 7.7%
    - Moderate Risk PP: 7.8% to 15.0%
    - High Risk PP: > 15.1%

All patients with benign diseases were included in both analyses, but the Primary Endpoint evaluated cancer patients with invasive epithelial ovarian cancer only (N=474), whereas for

the Secondary Endpoint patients with low malignant potential tumors were included (N=495).

Subsequently, the complete dataset from WIHRI was combined with data from a similar study conducted at MGH (Boston Study) and showed that the biomarkers provide different results for premenopausal and postmenopausal women. Therefore, we conducted "Additional Analyses" based on the following logistic regression equations for premenopausal and postmenopausal women:

<u>Premenopausal women</u>

PI = -12.0 + 2.38*LN(HE4) + 0.0626*LN(CA 125)

<u>Postmenopausal women</u>

PI = -8.09 + 1.04*LN(HE4) + 0.732*LN(CA 125)

PP = exp(PI) / [1 + exp(PI)]

For both statistical and medical reasons, we chose cut-points for the "Additional Analyses" that provided a specificity of 75%. These cut-points were 13.1% PP for premenopausal patients and 27.7% PP for postmenopausal patients. In addition, we set 80% sensitivity as the required minimum sensitivity for the PP for premenopausal and postmenopausal women combined as the threshold for acceptance of this analysis. We further defined that the lower bound of the 95% Confidence Interval for Sensitivity must be >80% when Specificity is held constant at 75% for premenopausal and postmenopausal women. We included all evaluable patients with known menopausal status or determined menopausal status through FSH testing, and included all patients with invasive epithelial ovarian cancer and low malignant potential tumors in the Additional Analyses as an "Intent to Evaluate" population (N=502).

RESULTS

A total of 566 patients were enrolled of which 43 patients were non-evaluable for various reasons. All of the major racial groups were represented with approximately 15% representing minority groups. Of the 523 evaluable subjects, 280 (54%) were classified as postmenopausal and 243 (46%) were premenopausal. A total of 176 (34%) women were found to have cancer; 127 (72%) of the cancers were invasive epithelial ovarian cancers

and 21 (12%) were low malignant potential (borderline) tumors. Of 123 staged epithelial ovarian cancers, 34 (28%) were Stage I/II, and 89 (72%) were Stage III/IV. The proportion of patients in each of the demographic classifications of menopausal status, specimen type (i.e., benign disease or cancer), diagnosis of benign disease, cancer diagnosis, and stage of disease, grade of disease and histology for patients with epithelial ovarian cancer, were equivalent with respect to demographic characteristics. Therefore, pooling the data across all site pools was justified.

PRIMARY AND SECONDARY PROTOCOL ENDPOINTS

We first evaluated the Primary Protocol Endpoint using only patients with benign diseases or invasive epithelial ovarian cancer. The Chi-square p-values were < 0.05 signifying that the proportion of cancer and benign disease patients in each of the risk subgroups is statistically significantly different. We next evaluated the Secondary Protocol Endpoint in which we included patients with low malignant potential (borderline) tumors. Again, the Chi-square p-values were < 0.05 signifying that the proportion of patients with cancers and benign diseases were significantly different among the three risk groups for both the Primary and Secondary Endpoints. The data thus meet both the Primary and Secondary Endpoints.

ADDITIONAL ANALYSIS

Results of the Additional Analysis showed that at 75% specificity, the sensitivity for all patients combined (N=502) was 89% with a lower bound for the 95% confidence interval of 82.6% which serves to demonstrate that the data meet the predetermined endpoint for this analysis. In addition, the Negative Predictive Value (NPV) was 95%, 93%, and 94% for premenopausal, postmenopausal, and all patients combined, respectively. Data are summarized in Tables 1 and 2 below.

Table 1 Risk stratification of patients with pelvic mass based upon CA 125II and HE4 concentrations using a two-equation algorithm for premenopausal and postmenopausal women. Results of premenopausal and postmenopausal women combined.

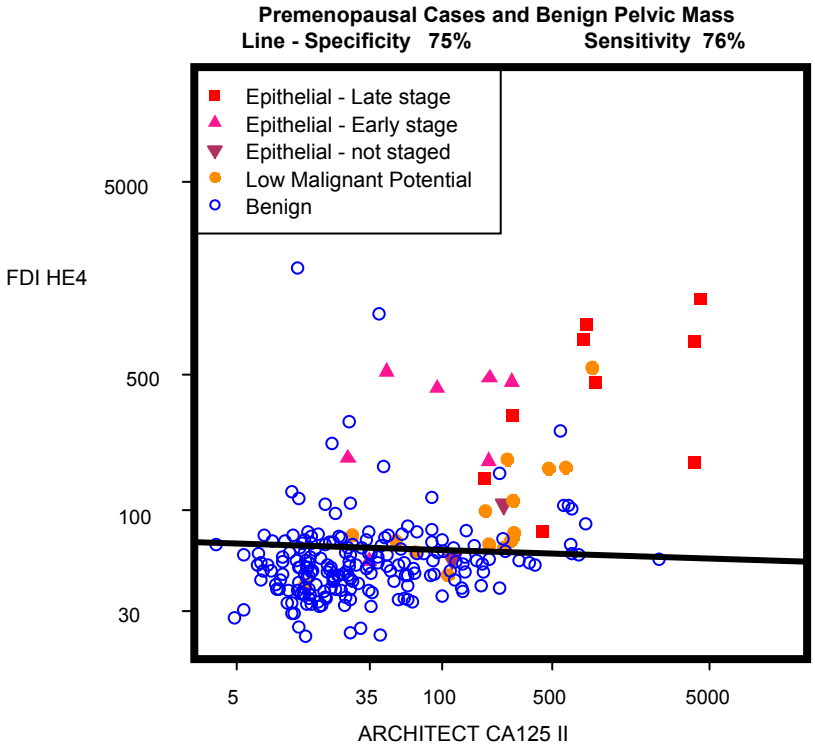
ROMA (Premenopausal & Postmenopausal Women Combined)	Low Risk	High Risk	Total
Benign	262 (75%)	89 (25%)	351 (70%)
Epithelial Ovarian Cancer / Low Malignant Potential tumors	17 (11%)	134 (89%)	151 (30%)
Total	279 (56%)	223 (44%)	502 (100%)

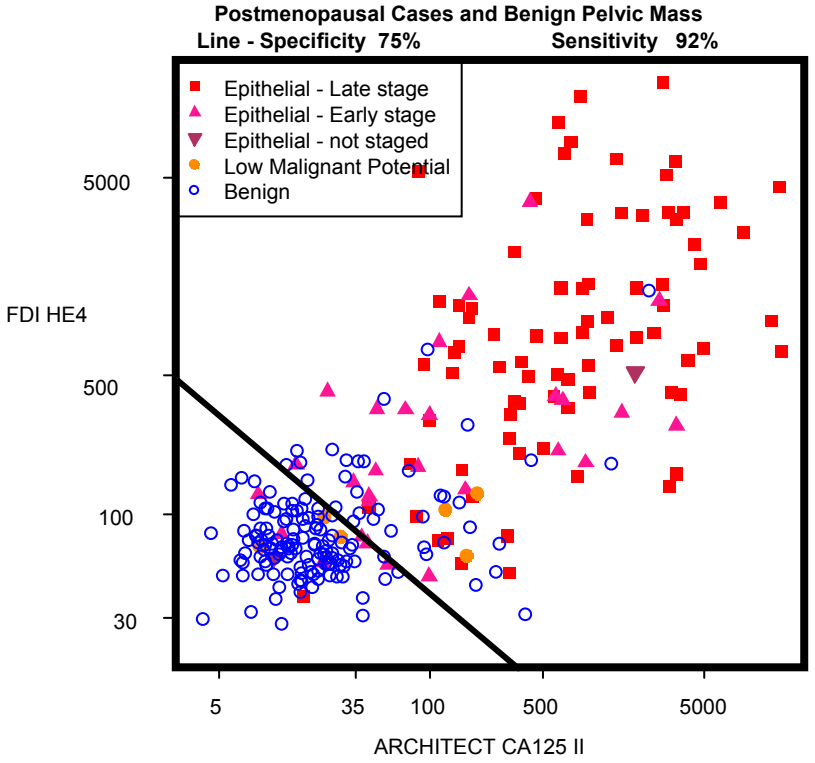
Table 2 Risk stratification of patients with pelvic mass based upon CA 125II and HE4 concentrations using a two equation algorithm, subdivided by histological subtype and Stage. Results of premenopausal and postmenopausal women combined.

ROMA (Premenopausal & Postmenopausal Women Combined)	Low Risk	High Risk	Total
Benign	262	89	351
Low Malignant Potential	9	13	22
Invasive Epithelial Ovarian Ca (I/II)	5	30	35
Invasive Epithelial Ovarian Ca (III/IV)	1	89	90
Invasive Epithelial Ovarian Ca (Unstaged)	2	2	4
Total	279	223	502

When CA 125II and HE4 were plotted as an X-Y scatter plot, the line described by the equations shown above for premenopausal and postmenopausal women demonstrate graphically the stratification of patients into low and high risk groups (Figures 1, 2).

Figure 1 and 2 **X-Y Scatter plot of CA 125II concentrations and HE4 concentrations for premenopausal (top) and postmenopausal (bottom) women.**





CONCLUSIONS

1. The Intended Use for the HE4 EIA is "The HE4 EIA is an enzyme immunometric assay for the quantitative determination of HE4 in human serum. The HE4 EIA used in conjunction with the ARCHITECT CA 125 II assay creates a predictive probability of epithelial ovarian cancer using a mathematical function referred to as the Risk of Ovarian Malignancy Algorithm (ROMA™), for use in premenopausal and post-menopausal women presenting with an adnexal mass who are candidates for surgical intervention. Subjects categorized as low risk for epithelial ovarian cancer using the ROMA value may have surgical intervention performed by a non-oncology specialist. The results must be interpreted in conjunction with other clinical findings in accordance with standard clinical management guidelines. The assay is not indicated as an aid in a decision to proceed to surgery."

   NOTE: In the labeling, the mathematical function determining Predictive Probability (PP) will be referred to as the ROMA.

   The population of patients that support the Intended Use from this study includes the "Intent-to-Evaluate" population of women with known menopausal status and invasive epithelial ovarian cancers and low malignant potential tumors.

   Based on results found with this population, the triage of women with pelvic mass into populations with Low Risk and High Risk determined by PP represents a significant improvement in the management of these patients. By defining a Low Risk population, half of women referred to gynecologic oncologists with pelvic masses using the current clinical standard of care could remain in the community with their gynecologist for surgery. This Low Risk population includes 75% of all benign gynecologic cases. The proportions of women with cancer in the two groups are appropriate for improved management of these patient groups.

2. At a set specificity of 75%, the combined use of CA 125II and HE4 in a logistic algorithm provides 89% sensitivity for premenopausal and postmenopausal patients combined. The algorithm also provides a 95%, 93%, and 94% NPV for premenopausal women and for postmenopausal women, and all women combined,

respectively when considering both invasive epithelial ovarian cancers and low malignant potential tumors together.

3. If this algorithm were applied in clinical practice, over 50% (56% in this study) of patients referred to gynecologic oncologists using the current standard of care could have their surgery performed safely by gynecologists in community health care settings. Only 6% (17/279) of patients that remain under the gynecologist's care would be expected to harbor an invasive epithelial ovarian cancer or low malignant potential tumor. Of the 6% of patients found to have cancer, only 6% (1/17, Table 2) would be expected to harbor late stage invasive epithelial ovarian cancer.

4. Of the 44% of patients in the High Risk subgroup triaged to the gynecologic oncologist, only 40% (89/223) would be found to have benign diseases. A total of 89% (134/151) of all invasive epithelial ovarian cancers and low malignant potential tumors would be included in this High Risk subgroup, and 66% (89/134) would have late stage (Stages III/IV) invasive epithelial ovarian cancer. In addition, 94% (121/129) of all invasive epithelial ovarian cancer would be classified to the High Risk subgroup.

5. The CA 125II and HE4 biomarkers behave differently in premenopausal and postmenopausal women with pelvic mass (Figures 1 and 2). For this reason, the PP should be determined separately for each population. This provides cut-points for PP of 13% for premenopausal women and 28% for postmenopausal women, rounded for convenience in implementation.

6. The stratification of pelvic mass patients into groups with low and high risk of ovarian cancer is maximized by the use of a PP compared with individual concentrations of CA 125II or HE4 alone.

7. The data presented in this report supports the intended use for the HE4 EIA because the low and high risk subgroups for premenopausal and postmenopausal women provide excellent stratification of women with benign pelvic masses from those with cancerous pelvic masses. This improves the current standard of care by decreasing

the number of women referred unnecessarily to a gynecologic oncologist for treatment of their pelvic mass.

2. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

2.1. LIST OF ABBREVIATIONS

ACOG	American College of Obstetrics and Gynecology
AE	Adverse Event
ANOVA	Analysis of Variance
AUC	Area under the Curve
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Clinical Research Organization
CT	Computed Tomography
CV	Coefficient of Variation
FDA	Food and Drug Administration
FDAB	Fujirebio Diagnostics AB
FDI	Fujirebio Diagnostics, Inc.
FSH	Follicle-stimulating Hormone
FIGO	International Federation of Gynecology and Obstetrics
HIPAA	Health Insurance Portability and Accountability Act
IRB	Institutional Review Board
JH	Johns Hopkins University School of Medicine, Departments of Pathology and Gynecology and Obstetrics
LMP	Last Menstrual Period
MAb	Monoclonal Antibody
MDCI	Medical Device Consultants, Inc.
MGH	Massachusetts General Hospital
MRI	Magnetic Resonance Imaging
NPV	Negative Predictive Value
PET	Positron Emission Tomography
PI	Predictive Index
PP	Predictive Probability
RMI	Risk of Malignancy Index

ROC	Receiver Operator Characteristic
ROMA	Risk of Ovarian Malignancy Algorithm
SST	Serum Separator Tubes
SD	Standard Deviation
SE	Standard Error
SGO	Society of Gynecologic Oncologists
SMRP	Soluble Mesothelin-Related Peptide
US	Unite States
WIHRI	Women and Infants' Hospital of Rhode Island, Brown University

2.2. DEFINITION OF TERMS

Pelvic mass

A mass in the pelvis by physical examination and confirmed by diagnostic imaging studies (i.e., ultrasound, computed tomography [CT] scan or magnetic resonance imaging [MRI]) or found incidentally during diagnostic imaging studies. The mass may be cystic or solid, occur in any age group, and may originate from any gynecologic or non-gynecologic pelvic organ or structure such as but not limited to ovary, fallopian tube, uterus, cervix, bowel, bladder and pelvic kidney.

Adnexal mass

A growth in tissue adjacent to the uterus, usually in the ovary or fallopian tube or uterine leiomyoma. Adnexal masses most often include ovarian cysts, ectopic pregnancies, and benign (non-cancerous) or malignant (cancerous) tumors arising from any of the pelvic organs or structures.

Invasive Epithelial Ovarian Cancer

The invasive epithelial ovarian cancers are characterized pathologically as tumors that have neoplastic epithelial cells, detached cellular clusters from sites of origin, increased mitotic activity, and nuclear abnormalities. They invade the surrounding stroma, and the basement membrane is no longer intact. Invasive ovarian cancer is classified histologically according to the cell type, and grade of the tumor. The most common mechanism of spread is by continuity and intraperitoneal dissemination. They

disseminate primarily through surface implantation or lymphatic spread and rarely metastasize through the blood stream.

Low Malignant Potential (Borderline) Tumor

Ovarian tumors of low malignant potential are characterized pathologically as tumors that have neoplastic epithelial cells, detached cellular clusters from sites of origin, increased mitotic activity, and nuclear abnormalities. They do not invade the surrounding stroma, and the basement membrane remains intact. Histologically they may be serous, mucinous, or endometrioid.

(Note that the tables in this report, “epithelial ovarian cancer” refers to “Invasive epithelial ovarian cancer”. Tumors of low malignant potential will be referred to as such. Borderline tumors are considered synonymous with tumors of low malignant potential in this study.)

Ovarian cyst

A tumor that arises within the ovary that can be fluid filled, a combination of fluid and solid elements, or completely solid.

3. ETHICS AND BIAS CONTROL

3.1. ETHICS

3.1.1. INSTITUTIONAL REVIEW BOARD REVIEW

The Study Protocol was reviewed and approved by the Independent IIRB for one community practice, Northwest Gynecologic Oncology in Eugene, OR. All other sites obtained IRB approvals from their own institutions. Written documentation of IRB approval was maintained by the Sponsor for each site. The study was conducted in compliance with 21 CFR Part 56 (Institutional Review Board) and CFR Part 812 (Investigational Device Exemptions).

3.1.2. SUBJECT INFORMED CONSENT

Written informed consent was obtained from each subject prior to the collection of blood and urine for this study. Subjects were told of the purpose of the study in clear laymen's terms. They were informed of their rights as subjects, that their participation was voluntary, and that the results of the tests will not be made available to them or their physician for treatment purposes. The consent form notified subjects that their serum, plasma, and urine would be stored for the purpose of conducting future research on diagnostic tests for detection of gynecologic cancer. The subjects were also notified that if they chose to withdraw from the study at any time, the stored samples will be destroyed if they notified the Sponsor in writing. The consent form also specified whom to contact if there were injuries as a result of the study, and whom to contact with general questions about the trial. The consent form specified that representatives of the Sponsor and employees of Federal Agencies may review medical records of the subject.

A sample Informed Consent is presented in Attachment 16.6 and this was made available to each participating center. Each IRB reviewed the Informed Consent and modified as they felt necessary. Compliance with Informed Consent was monitored at each site by Study Monitors. The clinical study was conducted according to 21 CFR Part 50 (Protection of Human Subjects).

3.1.3. DATA PRIVACY

The Sponsor, each participating center, and all individuals that participated in this study adhered to the principles required by the Health Insurance Portability and Accountability Act (HIPAA) privacy requirements. HIPAA requirements were contained within the Informed Consent document which allowed sharing of protected health information. All reasonable effort was made to ensure confidentiality of subject information and records were maintained in a secure location.

3.1.4. ETHICAL CONDUCT OF THE STUDY

This clinical study was conducted according to 21 CFR Part 50 (Informed Consent of Human Subjects) and the principles outlined in the "Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects" contained in the 1989 version of the Declaration of Helsinki. Any instances of nonconformance are documented and did not impact the conclusions of the study.

3.1.5. ADVERSE EVENT REPORTING

No adverse events (AEs) were expected during the course of this study because the only direct contact with patients involved drawing blood and obtaining a urine sample. However, syncope or hematoma could occur, and patients were informed of this risk in the Informed Consent. All other invasive procedures and evaluations included in this protocol (imaging, surgery, etc.) are considered to be standard of care in the evaluation of patients with a pelvic mass and would have been performed as part of routine clinical work-up of patients with a pelvic mass.

A Case Report Form (CRF) was provided to all sites for "Unanticipated Device Related Adverse Events." Sites were trained to report any AEs to the Sponsor.

3.1.6. FINANCIAL DISCLOSURE

Financial Disclosure was obtained from each site investigator and sub-investigators pursuant to 21 CFR Part 54 (Financial Disclosure by Clinical Investigators). Financial Disclosure forms are maintained in the Sponsors Study Master file.

3.2. BIAS CONTROL

3.2.1. SUBJECT ENROLLMENT AND IDENTIFIERS

All subjects enrolled into this study were identified by an eight-digit identification number. The first two digits were always 03 to denote the FDI-03 Protocol Number. The next three digits denoted the Site Number, and the next three digits denoted the

subject number for that site. Thus, Patient was the first patient enrolled into the FDI-03 Protocol at Site 36. This unique identifier was used for subject identification throughout the study. All subjects were enrolled on a "first-come, first-served" basis. Subjects were enrolled strictly according to eligibility criteria without regard to other factors.

3.2.2. STUDY BLINDING AND USE OF LABORATORY RESULTS

All laboratory testing was done by operators blinded to subject clinical status. All patient management was done by clinicians blinded to laboratory results. Laboratory results were not shared with clinical sites and clinical data was maintained unknown to laboratory operators by the Sponsor. In this way, the study remained double-blind until the final data analysis. No laboratory results were used for patient care.

3.2.3. CENTRAL REVIEW OF HISTOPATHOLOGY

All patients in this study underwent laparoscopic surgery or laparotomy. Thus, tissues were taken from all patients (several patients had no tissue removed because their masses resolved prior to surgery). All tissues were examined by local pathologists (Site Pathology Departments). In addition, slides were sent to JH to be examined by one of three independent pathologists with a sub-specialty in gynecologic pathology. If the Site Pathologist and the first pathologist from JH disagreed, then a second pathologist at JH examined the slides. In rare cases, a fourth reading was done at JH to resolve specific discrepancies. In this way, a systematic approach to histopathology was implemented to minimize differences across sites.

4. STUDY STRUCTURE AND INVESTIGATORS

The FDI-03 Pelvic Mass study was conducted from December 2005 until October 2007. Enrollment into the study was concluded in February 2007. Table 3 summarizes the Study administrative personnel and their responsibilities.

The Sponsor was responsible for the planning, protocol design, overall management and execution of the trial. Clinical consultants and a clinical research organization (CRO) were used for site management, monitoring functions and database management. The Principal Investigator participated in the study design, provided overall medical guidance in the performance of the trial, assisted with decisions in inclusion or exclusion of subjects, assisted with histological classifications, and provided medical support in all phases of the work. The Project Director and Project Manager were responsible for the overall planning and management of the study including development of the clinical protocol, the laboratory testing protocol, protocol amendments and the study materials as well as identification of study sites, collection of required site regulatory documents, training and initiation of clinical and laboratory testing sites and the Central Pathology Review process. The Sponsor and contract clinical research associates (CRAs) performed site management and monitoring functions such as verification of source documents and data monitoring. The Sponsor was responsible for the management of laboratory testing sites and data collection. The clinical report was developed by the Project Director and Project Manager in conjunction with the Principal Investigator, and was reviewed by a Project Team at FDI.

Table 3 Study Administrative Personnel

Responsibility	Name	Institution
Principal Investigator	**Richard Moore, MD, FACOG, FACS**	**Women and Infants' Hospital of Rhode Island**
Project Directors	**W. Jeffrey Allard, PhD** **Olle Nilsson, PhD**	**Fujirebio Diagnostics, Inc**
Project Managers	**Elizabeth Somers, BS** **Rebecca Simamora**	**Fujirebio Diagnostics, Inc**

Table 4 lists those CROs and consultants that were involved in the trial. Their responsibilities are included. Medical Device Consultants, Inc (MDCI) was contracted to perform data management including database construction, data entry from CRFs, and validation of the data entered into the database. CRAs listed in the table below were contracted to perform site management functions including training, completion of site regulatory documents, and monitoring to verify data reflects source documentation and to ensure site adherence to good clinical practice guidelines and Food and Drug Administration (FDA) regulations. Some monitoring functions and data collection were also performed by the Sponsor. Central review of histopathology results were performed at JH.

Final review of histopathology results from sites and Central Pathology Review for final consensus determination was performed by two gynecologic oncologists as listed below. Histopathology determinations were accomplished according to a written standard under the direction of two study personnel from the Sponsor.

Two clinical laboratories were contracted to perform laboratory testing of the serum samples collected in the study using the HE4 assay test kits which were manufactured and provided by the Sponsor.

Table 4 CROs and Consultants

CROs and Consultants	Type of Organization	General Role in the Study
Robert C. Knapp, M.D.,D.Sc. William H. Baker Professor of Gynecology (Emeritus) Harvard Medical School Visiting Scholar, Weill Cornell Medical College	Medical Advisor, FDI	Medical and Scientific Advisor, FDI
Medical Device Consultants, Inc (MDCI)	Contract Clinical Research Organization	Data management, data entry and database construction and validation
Steven Skates, PhD Assistant Professor Harvard University Mass General Hospital	Biostatistics Department Academic Research Harvard University	Statistical analysis and Algorithm development
M. Craig Miller, BS	Statistical consultant	Statistical analysis Database management
Kimberlee Borden, M.S. V.P. and Sr. Biostatistician Research Oriented Biostatistics, Inc (ROB, Inc)	Statistical consultant	Statistical analysis Ancillary Studies
Robert J. Kurman, MD TeLinde Professor of Gynecologic Pathology Departments of Pathology and Gynecology & Obstetrics Johns Hopkins University School of Medicine	Academic Institution and Investigator in field of Gynecologic Pathology	Central Pathology Review: Blinded review of permanent section slides for study subjects
Russell Vang, MD Assistant Professor Departments of Pathology and Gynecology & Obstetrics Johns Hopkins University School of Medicine	Academic Institution and Investigator in field of Gynecologic Pathology	Central Pathology Review: Blinded review of permanent section slides for study subjects
Brigitte M. Ronnett, M.D. Professor Departments of Pathology and Gynecology & Obstetrics The Johns Hopkins University School of Medicine	Academic Institution and Investigator in field of Gynecologic Pathology	Central Pathology Review: Blinded review of permanent section slides for study subjects
Herbert Fritsche, PhD Professor and Chief Clinical Chemistry, Division of Laboratory Medicine MD Anderson Cancer Center	Clinical Chemistry Laboratory of the MD Anderson Cancer Center	Testing of serum samples using the HE4 EIA assay. Complete analytical study testing for ancillary studies.

Table 4 con't CROs and Consultants

CROs and Consultants	Type of Organization	General Role in the Study
Pat Sluss, Ph.D. MGH Reproductive Endocrinology lab Mass General Hospital	Clinical Chemistry Laboratory of the Dept of Reproductive Endocrinology, Mass General Hospital	Testing of serum samples using the HE4 EIA assay. Complete analytical study testing for ancillary studies.
Suzan Allen Clinical Consulting	Independent Clinical Research Consultant	Clinical Consultant Source document verification and monitoring
Carl Chelle Pharmaceutical Consulting Bedminster, NJ	Independent Clinical Research Consultant	Clinical Consultant, Protocol Development, Site and data management, Source document verification and monitoring functions
Diane Bott, RN, MS, CCRA Kansas Research Consulting, Inc.	Independent Clinical Research Consultant	Site and data management Source document verification and monitoring functions
Bonnie Hein Clinical Consultant	Independent Clinical Research Consultant	Site and data management Source document verification and monitoring functions
Amy Brown, MD Gynecologic Oncologist	Dept Gyn/Oncology Hartford Hospital	Central Pathology Review: Consensus Determination
Katina Robison, MD Gynecologic Oncology Fellow Dept Gyn/Oncology	Dept Gyn/Oncology Women and Infants Hospital of Rhode Island	Central Pathology Review: Consensus Determination
Rachel Radwan	Independent Clinical Research Consultant	Data Review

CLINICAL SITES

Table 5 lists the clinical sites participating in the study. The study was conducted at 14 clinical sites geographically dispersed throughout the US to enable a diverse subject population. Site selection was based on the sites' ability to enroll adequate numbers of evaluable subjects, relevant clinical expertise and staff, and experience of Investigators and the Institution. Investigators were chosen for participation in the study based on their past experience with clinical research and expertise in the field. A detailed list of Investigators, Sub-investigators' Curriculum Vitae and Financial Disclosure Forms are included in Attachments 16.8 and 16.9.

Table 5 Participating Clinical Sites

Site Number	Investigator	Institution	Subjects Enrolled
036	Richard Moore, MD Principal investigator	**Women and Infants' Hospital of Rhode Island** Providence, RI	**100**
037	Dan Schlitzer, MD	**Healthcare for Women, Inc.** New Bedford, MA	**21**
038	Steven DePasquale, MD	**Chattanooga Gyn-Oncology** Chattanooga, TN	**24**
039	Walter Gajewski, MD	**New Hanover Regional Medical Center** Wilmington, NC	**87**
040	Laura Havrilesky, MD	**Duke University Medical Center** Durham, NC	**58**
041	Donald Chamberlain, MD	**Chattanooga Gyn-Oncology** Chattanooga, TN	**4**
042	Amy Kirkpatrick Brown, MD, MPH	**Hartford Hospital** Hartford, CT	**5**
043	Amy Kirkpatrick Brown, MD, MPH	**New Britain General Hospital of Central Connecticut** New Britain, CT	**2**
044	Alan Gordon, MD, FACOG	**Arizona Gyn-Oncology** Phoenix, AZ	**22**
045	Scott McMeekin, MD	**Oklahoma University Health Science Center** Oklahoma City, OK	**100**
046	Howard Homesley, MD	**Brody School of Medicine** Leo Jenkins Cancer Center, Greenville, NC	**30**
048	Elizabeth Swisher, MD	**University of Washington Medical Center** Seattle, WA	**8**
062	Audrey Garrett, MD	**Northwest Gynecologic Oncology** Eugene, OR	**75**
063	Alexander Burnett, MD	**University of Arkansas for Medical Sciences** Little Rock, AR	**30**

5. INTRODUCTION AND BACKGROUND

Ovarian cancer is the leading cause of death from gynecologic malignancy (1) and the fifth leading cause of cancer death in American women (8). The American Cancer Society estimates that 22,430 women will be diagnosed with ovarian cancer in 2007, with 15,280 deaths as a result of ovarian cancer (9) which provides a lifetime risk of ovarian cancer of 1 in 70 women (10). The incidence of ovarian cancer is 1.4 per 100,000 women under age 40 increasing to 45 - 50 per 100,000 for women over age 60. Over 70% of cases are diagnosed with advanced stage disease.

It is estimated that 5 to 10 percent of women in the US will undergo a surgical procedure for a suspected ovarian neoplasm during their lifetime, and 13 to 21 percent of these women will be found to have an ovarian malignancy (1). Since the majority of adnexal masses will be determined on final pathology to be benign, it is important to determine preoperatively whether a patient is considered to have a lesion that is at high risk for an ovarian malignancy, in order to ensure proper management (1).

A complete history and physical examination is essential in the evaluation of a patient with an adnexal mass. (1) The history should include the age of the patient, prior surgical procedures, menopausal status, menstrual history, pelvic pain and abnormal vaginal, urinary or rectal bleeding. A bimanual and rectovaginal examination is carefully performed, determining size, nodularity, and consistency of the adnexal mass. In the postmenopausal woman, an enlarged, nodular or fixed mass is highly suspicious for malignancy. In the premenopausal woman it may be indicative of endometriosis or pelvic inflammatory disease. Non-adnexal conditions must also be considered such as diverticulitis, pelvic kidney and a pediculated subserous uterine fibroid. Metastatic cancers such as breast, colon or stomach may also present as an adnexal mass.

Since the initial report in 1988, clinical impression, serum CA 125 and ultrasound along with CT scan, MRI and CT/Positron Emission Tomography (PET) have been the standards in the determination of whether an adnexal mass is suspicious for malignancy. (2) Although the literature is replete with papers describing which modality is the more accurate, the combination of physical examination, CA 125 and imaging affords the highest positive predicative value. (11-13) An algorithm called “risk of malignancy index” (RMI) reported by

Jacobs et al. 1990 incorporated the serum CA 125 level, menopausal status, and ultrasound morphologic features. (14) Studies have reported on the utility of the RMI. (15-17) Bailey et al. 2006 confirmed the effectiveness of the RMI algorithm in clinical practice for the identification and subsequent referral to cancer centers of potential ovarian malignancy. (18) The use of Doppler Sonography in addition to transvaginal ultrasound, CT scan, MRI and PET have all been utilized as imaging tools in the differentiation of a benign and malignant adnexal mass. (19, 20) Regardless of the imaging tool selected, the size of the mass, consistency of the mass (cystic, solid or mixed), bilaterality and excrescences within the tumor are all indications of possible malignancy. An international multi-center study evaluated a logistic model combining physical examination, ultrasound, and serum tumor markers. The logistic model gave a correct prediction in 87% of all cases. (21)

Once an adnexal mass has been documented, management depends upon a combination of many predictive factors including age, menopausal status, presence or absence of symptoms, level of CA 125, and imaging features including size of the mass and bilaterality. Although age is the most significant risk factor for epithelial ovarian cancer, it can occur in young women as well. In the premenopausal woman, CA 125 may be elevated in many common benign conditions such as endometriosis, functional cysts, benign ovarian neoplasms, pelvic inflammatory disease and fibroids. Imaging may also give a false impression of malignancy due to the radiographic appearance of an endometrioma or other benign complex cysts. In these women, expectant management may be appropriate and should include a repeat physical and pelvic examination, imaging and repeat CA 125 testing. Changes in clinical or radiographic findings, and a rising CA 125 level or persistence of a significant mass, are indications for surgery. Benign conditions (i.e., endometriosis, functional cysts) causing adnexal masses in the premenopausal woman are not commonly present in postmenopausal women. Therefore, surgical intervention is usually required, although studies have evaluated the place for expectant management in postmenopausal women. (22)

Gostout and Brewer from the Mayo Clinic described guidelines for referral of the patient with an adnexal mass. (4) They reviewed the evidence supporting the specialty care of a gynecologic oncologist on the basis of the physical examination, imaging studies, family history and CA 125 determination. They recommend adherence to the ACOG/Society of Gynecologic Oncologists (SGO) Joint Opinion guidelines. “Referral to a gynecologic

oncologist seems warranted for postmenopausal women with elevated CA 125, nodular or fixed pelvic mass, metastatic disease, ascites, or family history of breast or ovarian cancer. Premenopausal women should be referred if the CA 125 is elevated above 200 U/mL, there is an evidence of metastatic disease or ascites, or strong family history of breast or ovarian cancer."

In the US, patients with adnexal masses present primarily to gynecologists, either directly or indirectly via referral from family physicians or internists. A Practice Bulletin from the ACOG describes the management of patients with adnexal masses. (3) According to this Bulletin, most patients with adnexal masses are candidates for surgery. However, an important dilemma faced by gynecologists is whether or not to refer the patient with an adnexal mass to a gynecologic oncologist, and/or to an institution experienced in gynecologic cancer surgery. This is because several recent studies have demonstrated that ovarian cancer patients that are managed at institutions with multidisciplinary teams, and by surgeons that specialize in ovarian cancer, have fewer complications and better survival rates (4-7, 23). Bristow and associates examined the distribution of primary ovarian cancer surgery with regard to surgeon and hospital case volume in Maryland between 1990 and 2000 (24). They concluded that low-volume surgeons carry out the majority of surgery and half are being performed at low-volume hospitals with both factors believed to impair surgical outcome. They recommend, "In light of the positive volume-outcomes data for malignancies treated with technically complex operative procedures, increased efforts to concentrate the surgical care of women with ovarian cancer are warranted." A prospective nation-wide study of surgical treatment of ovarian cancer in different hospital categories was carried out in Finland. (25) The results of this study demonstrated differences in surgical treatment of ovarian cancer in different hospital categories in favor of the university hospitals.

The surgical management of ovarian cancer currently includes surgical staging and cytoreductive surgery. Appropriate staging is important to accurately select the next modality of treatment; whether chemotherapy is necessary, the type of chemotherapy and duration of treatment. Surgical stage and ability to optimally cytoreduce patients are prognostic factors that predict overall survival in patients with ovarian cancer. (25, 26) Studies have shown that patients treated by a gynecologic oncologist are more likely to undergo a complete surgical staging and to have optimal cytoreductive surgery, compared with patients treated by gynecologists or general surgeons (27, 28). Despite these findings,

in the US, less than 50% of women with ovarian cancer will receive initial operative treatment by a specialist trained in the surgical management of this disease (29). The ACOG Practice Bulletin published in 2007 states the following

"*Women with ovarian cancer whose care is managed by physicians who have advanced training and expertise in the treatment of women with ovarian cancer, such as gynecologic oncologists, have improved overall survival rates compared with those treated without such collaboration*." (3)

Conversely, approximately 80% of women receiving surgical intervention for an ovarian cyst or pelvic mass will be treated successfully in a community hospital by a gynecologist or general surgeon. This is appropriate for patients with non-malignant diseases.

Currently, the clinical algorithm for the triage of patients with a pelvic mass to tertiary centers, as recommended by ACOG (3), includes:

<u>Premenopausal (younger than 50 years)</u>
CA 125 levels > 200 U/mL
Ascites
Evidence of abdominal or distant metastases (by results of examination or imaging study)
Family history of breast or ovarian cancer (in a first-degree relative)

<u>Postmenopausal (older than 50 years)</u>
Elevated CA 125 levels
Ascites
Nodular or fixed pelvic mass
Evidence of abdominal or distant metastases (by results of examination or imaging study)
Family history of breast or ovarian cancer (in a first-degree relative)

These guidelines include the combined use of imaging, serum biomarker testing with CA 125, physical examination and family history. The serum biomarker CA 125 has been utilized in combination with imaging, physical exam and family history to help predict the presence of malignancy in patients with a pelvic mass. However, the specificity and

sensitivity for CA 125 alone in both premenopausal and postmenopausal women is low. (30) The lack of specificity of CA 125 is secondary to its levels being elevated in many benign gynecologic and non-gynecologic conditions. The ACOG Practice Bulletin (3) notes the following:

"*The value of elevated CA 125 levels is in distinguishing between benign and malignant masses in postmenopausal women. Few studies evaluate the predictive value of CA 125 levels stratified by menopausal status but, of those that do, specificity and positive predictive value are consistently higher in postmenopausal patients…..*

Typically CA 125 values will increase over time when a cancer is present, whereas this is not necessarily true for benign masses."

While CA 125 measurement is considered an important component in the work-up of patients with pelvic mass, there is universal agreement that CA 125 is hindered in its utility by low specificity, especially in premenopausal women. As noted in the 2007 ACOG Practice Bulletin: "*The low specificity occurs because the CA 125 level frequently is elevated in many commonly encountered clinical conditions including uterine leiomyomata, endometriosis, chronic or acute pelvic inflammatory disease, ascites of any etiology, and even inflammatory conditions*…" (3)

In an effort to improve the triage of patients with adnexal masses, we sought to improve the sensitivity and specificity of currently available biomarkers, notably CA 125. Several novel markers have been evaluated in patients with ovarian cancer. Although their sensitivities and specificities for predicting ovarian cancer were no better than those of CA 125 alone, the combination of multiple markers is promising. (31) Therefore, we studied eight potential biomarkers in sera drawn from women with pelvic mass prior to surgery. We measured increased sensitivity at fixed levels of specificity and found that only one biomarker, HE4, was able to increase the sensitivity of CA 125 measurement alone. (32) In addition, we found that HE4 showed improved specificity compared with CA 125, and that HE4 had better specificity than CA 125 in premenopausal women with benign diseases.

While the Upper Limit of CA 125 is generally accepted as 35 U/mL, the concept of an Upper Limit becomes problematic with multiple biomarkers. Therefore, we developed an algorithm that combines CA 125 and HE4 concentrations in a logistic model, and provides a probability of finding cancer in a given patient. This algorithm was developed using a

Training Set obtained by pooling data across two separate Pilot Studies at the WIHRI in Providence, RI, and MGH in Boston, MA. The purpose of the current trial is to serve as a Validation Set, or Pivotal Trial, of the algorithm previously developed in the Pilot Study. We tested the combination of CA 125II and HE4 in women with pelvic mass to demonstrate that the algorithm developed in the Pilot Study is valid in a prospective, multi-center, statistically powered, double-blind clinical trial.

6. STUDY OBJECTIVES AND INTENDED USE

6.1. STUDY OBJECTIVES

The purpose of this clinical study was to evaluate multiple serum biomarkers to estimate the risk of ovarian cancer in women presenting with pelvic mass (defined as a simple, complex or a solid ovarian / pelvic mass). The primary purpose was to provide data to support claims of Safety and Effectiveness for the HE4 EIA in combination with the ARCHITECT CA 125II Assay to predict the probability that women presenting with pelvic mass harbor epithelial ovarian cancer.

6.2. STUDY ENDPOINTS

6.2.1. PRIMARY ENDPOINT:

To estimate the risk of finding ovarian cancer at the time of surgery in premenopausal and postmenopausal women presenting with a pelvic mass. Multiple serum biomarkers (CA 125 and HE4) were evaluated to estimate the risk that the patient was harboring an ovarian cancer at the time of presentation.

6.2.2. SECONDARY ENDPOINT:

Comparison of a multivariate predictive algorithm combining CA 125 and HE4 with radiological imaging results, and patient risk factors (such as age, menopausal status and ethnicity) to estimate the risk of ovarian cancer, including low malignant potential (borderline) tumors at the time of surgery in patients presenting with a pelvic mass.

6.2.3. EXPLORATORY ENDPOINTS:

Evaluation of serum, plasma and/or urine levels of CA 125, HE4 and other additional novel biomarkers such as soluble mesothelin-related peptide (SMRP), alone and in combination with radiological imaging results and subject risk factors to determine their ability to estimate the risk of cancer at the time of surgery in patients presenting with a pelvic mass.

Results of analyses testing the Primary and Secondary Endpoints are presented in this clinical report. Analyses of Exploratory Endpoints are ongoing and will not be summarized in this document.

6.3. INTENDED USE OF THE HE4 ASSAY

"The HE4 EIA is an enzyme immunometric assay for the quantitative determination of HE4 in human serum. The HE4 EIA used in conjunction with the ARCHITECT CA 125 II assay creates a predictive probability of epithelial ovarian cancer using a mathematical function referred to as the Risk of Ovarian Malignancy Algorithm (ROMA™), for use in premenopausal and post-menopausal women presenting with an adnexal mass who are candidates for surgical intervention. Subjects categorized as low risk for epithelial ovarian cancer using the ROMA value may have surgical intervention performed by a non-oncology specialist. The results must be interpreted in conjunction with other clinical findings in accordance with standard clinical management guidelines. The assay is not indicated as an aid in a decision to proceed to surgery."

NOTE: In the labeling, the mathematical function determining Predictive Probability (PP) will be referred to as the ROMA.

7. INVESTIGATIONAL DESIGN

7.1. OVERALL STUDY DESIGN AND PLAN

This study was a prospective, multi-center, double-blind, statistically powered clinical trial that enrolled female subjects ≥18 years of age presenting to a gynecologist or

gynecological oncologist with a pelvic mass (defined as a simple, complex or a solid ovarian / pelvic mass) that were scheduled to undergo surgery. Enrollment into this study opened in December of 2005 and the study closed to enrollment in February 2007.

7.2. DESCRIPTION OF THE INVESTIGATIONAL DEVICE

The HE4 EIA is a solid phase, non-competitive immunoassay based upon the direct sandwich technique as described in Attachment 16.1. Calibrators, controls and patient samples are incubated together with biotinylated anti-HE4 monoclonal antibody 2H5 (derived from mice) in streptavidin-coated microstrips. HE4 present in calibrators or samples is adsorbed to the streptavidin-coated microstrips by the biotinylated anti-HE4 monoclonal antibody (MAb) during the incubation. The strips are then washed and incubated with HRP labeled anti-HE4 MAb 3D8. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3,3',5,5' tetra-methyl-benzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue color will develop if antigen is present. The intensity of the color is proportional to the amount of HE4 present in the samples. The color intensity is determined in a microplate spectrophotometer at 620 nm (or optionally at 405 nm after addition of Stop Solution). Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The HE4 concentrations of patient samples are then read from the calibration curve.

The Abbott ARCHITECT CA 125II assay was used in conjunction with the HE4 EIA assay to complete the analysis described in this protocol. The ARCHITECT CA 125II assay is legally marketed under 510k K042731, approval date, November 19, 2004. The ARCHITECT CA 125II assay is described in detail in the package insert included in Attachment 16.2.

7.3. STUDY POPULATION

This study was designed to enroll premenopausal and postmenopausal women scheduled to undergo surgery for a pelvic mass. The reasons for surgery were not prescribed in the protocol as it was desired to obtain a broad cross-section of patients undergoing surgery based on prevailing guidelines and current standards of practice. All

subjects were asked to provide blood for serum and plasma preparation, and a sample of urine. The minimum number of patients enrolled was set at 500 total patients, and a minimum of 100 cases of ovarian cancer. If more than 500 patients were required to detect 100 cases of ovarian cancer, then enrollment would have continued.

7.4. INCLUSION/EXCLUSION CRITERIA

Inclusion Criteria

- Females selected to undergo laparotomy or laparoscopy based on a finding of pelvic mass (defined as a simple, complex or a solid ovarian / pelvic mass);
- Premenopausal and postmenopausal women greater than or equal to 18 years of age;
- Pelvic mass demonstrated by ultrasound, CT scan or MRI;
- Able to understand and sign Informed Consent.

Exclusion Criteria

- Treatment for any malignancy (with the exception of non-melanoma skin cancer) within the last five years;
- Subjects receiving cytotoxic chemotherapies, such as cyclophosphamide or methotrexate;
- Subjects with previous bilateral oophorectomy;
- Any subject known to be pregnant.

7.5. SAMPLE SIZE

Assumptions

- Approximately 20% of the subjects presenting with a pelvic mass will be found to have ovarian cancer at the time of surgery;
- Serum CA 125 and HE4 levels are associated with the probability of detecting ovarian cancer at the time of surgery;
- Greater than 30% of the total number of subjects will fall into the low risk group;

- For the group of subjects without evidence of cancer (~80% of the evaluable subjects), ~45% will fall into the low risk group, ~45% will fall into the moderate risk group, and ~10% will fall into the high risk group;
- For the group of subjects with ovarian cancer (~20% of the evaluable subjects), ~5% will fall into the low risk group, ~15% will fall into the moderate risk group, and ~80% will fall into the high risk group.

Sample Size Determination

When the total sample size is 500, distributed over the groups as specified in the assumptions above, a 0.05 level Chi-square test will have >99% power to distinguish between the groups when the proportions in the three risk groups are characterized by an effect size (Δ^2) of 0.4328, where $\Delta^2 = \Sigma\{r_i\ \Sigma(\pi_{ij}-\pi_j)^2/\pi_j\}/\Sigma r_i$. The effect size is (1/N) times the value of the Chi-square statistic computed using the expected true proportions. Multiplying the effect size by the total sample size, N, yields the non-centrality parameter (33). A minimum of 500 evaluable subjects were enrolled into the study which provided sufficient statistical power for evaluation of the primary and secondary endpoints. A definition of formula parameters are as follows:

- Group 1 = Benign
- Group 2 = Cancer
- Category 1 = Low Risk
- Category 2 = Moderate Risk
- Category 3 = High Risk
- ni = number of patients in Group i
- n1 = number of patients in Group 1
- ri = ni / n1
- Sri = (n1/ n1) + (n2 / n1)
- pij = Proportion of patients in Group i which fall into Category j pj = Proportion of patients in Category j
- D2 = effect size

7.6. SUBJECT WITHDRAWAL OR EXCLUSION FROM STUDY

Subjects were able to withdraw voluntarily from the study at any time. 43 Subjects were withdrawn or excluded from the study for the following reasons:

1. They were inappropriately enrolled because they did not meet inclusion/exclusion criteria

2. Subjects did not provide required specimens or complete all required procedures

Details of subject disposition are outlined in Tables 7 -10.

7.7. BLINDING

All laboratory testing was done by operators blinded to subject clinical status. This blind was maintained by the Sponsor such that laboratory technicians had no access to patient clinical information at any time prior to testing of patient samples in the ARCHITECT CA 125II Assay and the HE4 EIA. All patient management was done by clinicians blinded to laboratory results. This was managed in two ways. Clinical sites had no access to laboratory data because laboratory testing was done at the Sponsor's laboratory and two laboratories at sites that did not enroll patients into the study. Second, laboratory testing was done in batch on frozen samples after completion of patient enrollment. In this way, the study remained double-blind until the final data analysis, and no laboratory results were used for patient care.

7.8. SPECIMEN HANDLING

7.8.1. SPECIMEN COLLECTION

At the time of surgery, or prior to the induction of anesthesia, 40mL of whole blood was collected into three 10mL serum separator tubes (SST), one 10mL EDTA tube, and a 10mL urine sample.

Whole Blood: Blood collected into the SST tubes was allowed to clot in a vertical position for at least 30 minutes prior to centrifugation. The SST and EDTA tubes were centrifuged for 10 minutes at 1100 - 1300g. The serum or plasma was transferred from the SST or EDTA tubes into transportation tubes and frozen on the day of collection at $\leq$ -20°C. The tubes were then stored frozen at $\leq$ -20°C at the site and shipped to FDI on dry ice in batches.

Urine: A urine sample was collected in a plastic urine collection container. Urine was transferred into a properly labeled plastic urine collection tube provided by the Sponsor and frozen at $\leq$ -20°C.

7.8.2. SPECIMEN TRANSPORT AND STORAGE

The serum, plasma, and urine samples were shipped to the Sponsor for aliquoting and storage. Detailed instructions on the shipping and short-term storage of serum, plasma and urine specimens were provided to each clinical site.

Samples were shipped in batches frozen on dry ice for overnight delivery to FDI for further processing, aliquoting, and long term storage.

Blood Serum, Plasma and Urine Allocation: Subject samples were shipped in batches to FDI. They were frozen and stored at ≤ -70ºC. At the conclusion of the study, all samples were thawed, aliquoted and refrozen. Serum aliquots were sent to testing laboratories to measure concentrations of CA 125 and HE4.

All shipping of specimens was done on dry ice and shipments were verified to have been received frozen.

7.8.3. SPECIMEN TESTING IN THE HE4 EIA AND ARCHITECT CA 125II ASSAYS

7.8.3.1. LABORATORY SITES

Serum samples were tested in the HE4 EIA at three sites including FDAB, MGH, and M.D. Anderson Cancer Center. The testing of all samples in the ARCHITECT CA 125II assay was done at FDI in Malvern, PA. The Laboratory Sites are summarized in Table 6 below.

Table 6 Central Laboratory Testing Sites

#1 Fujirebio Diagnostics, Inc. (FDI) 201 Great Valley Parkway Malvern, PA 19355 Investigator: Tim Kettlety, M.S. Operator: Edward Schmidt (610) 240-3800
#2 Fujirebio Diagnostics AB (FDAB) Majnabbeterminalen 414 55 Göteborg SWEDEN Investigator: Christina Hall, Ph. D Operator: Karin Majnesjo +46 31 85 70 30
#3 Massachusetts General Hospital (MGH) Reproductive Endocrinology laboratory 70 Blossom Street Bulfinch Basement Room 051 Boston, MA 02114 Investigator: Patrick Sluss, Ph.D. Operator: Samir Aleryani, Ph.D, Beng Yi, Sheila Mallette (617) 726-4352
#4 M. D. Anderson Cancer Center Division of Pathology and Laboratory Medicine Section of Clinical Chemistry 1515 Holcomb Boulevard Houston, TX 77030 Investigator: Herbert Fritsche, Ph.D. Operator: Terry Gornet (713) 792-6321

7.8.4. OPERATOR TRAINING

Operators were trained on the procedures outlined in the protocol and on the assay procedures according to the package insert in each laboratory participating in the study. The training was performed by a trained FDI or FDAB representative. Training was documented and all sites completed a familiarization procedure that was reviewed and approved by the sponsor prior to beginning testing on patient serum samples.

7.8.5. LABORATORY DATA COLLECTION

HE4 EIA assays were performed at the MGH, the MD Anderson Laboratory and the FDAB Laboratory. HE4 data printouts were printed using the Soft Max Pro

application. Printouts were reviewed and approved by FDI personnel to ensure assay performance and validity criteria were met. Data was transferred to CRFs by laboratory personnel and submitted with the raw data to FDI for review.

ARCHITECT CA 125II assays were performed at FDI using Abbott ARCHITECT Instruments by laboratory personnel trained on the ARCHITECT Instruments and performance of the CA 125II assays. Raw data printouts from the ARCHITECT Instrument were reviewed and approved by trained FDI personnel to ensure assay performance and validity criteria were met. Data was transferred to CRFs by laboratory personnel and submitted with the raw data for review.

CRFs were reviewed against the source documents by FDI personnel and copies of the CRFs were transferred to MDCI for inclusion into the database.

7.8.6. SPECIMEN RETESTING

HE4 assay data was reviewed by FDI personnel to ensure that the results met assay validity criteria as specified in the product Package Insert. The mean kit control values were required to be within the range specified on the vial. If out-of-range control values were obtained, a complete check of reagents and the plate reader was performed and the analysis was repeated. Duplicate replicates of calibrators and kit controls could not exceed a coefficient of variation (CV) of 15.0%. The values of the “A” (Zero) calibrator replicates could exceed 15% CV, but could not be more than 0.06 OD Units different from each other. No replicate of the A Calibrator could be higher than OD 0.1 at 620 nm. If these criteria were not met, the assays were repeated. In addition, all samples were tested in duplicate. If the % CV for any duplicate sample results exceeded 15%, the sample had to be retested.

Similarly, ARCHITECT testing included control preparations and the concentrations of control samples had to be within pre-specified limits. If the control values were not within range, the assay was repeated.

8. STATISTICAL PLAN

8.1. STUDY ENDPOINTS

8.1.1. PRIMARY ENDPOINT:

The primary objective of this trial was to estimate the risk of finding ovarian cancer at the time of surgery in premenopausal and postmenopausal women presenting with pelvic mass. Multiple serum biomarkers (CA 125 and HE4) were evaluated to estimate the risk that the patient is harboring an ovarian cancer at the time of presentation.

8.1.2. SECONDARY ENDPOINT:

The secondary objective of this trial was for comparison of a multivariate predictive algorithm combining CA 125 and HE4 with radiological imaging results, and patient risk factors (such as age, menopausal status and ethnicity) to estimate the risk of ovarian cancer, including low malignant potential (borderline) tumors, at the time of surgery in patients presenting with a pelvic mass.

8.1.3. EXPLORATORY ENDPOINTS:

Evaluation of serum, plasma and/or urine levels of CA 125, HE4 and other additional novel biomarkers such as SMRP, alone and in combination with radiological imaging results and subject risk factors to determine their ability to estimate the risk of cancer at the time of surgery in patients presenting with a pelvic mass.

8.1.4. ASSUMPTIONS

The assumptions for the primary endpoint were as follows:

- Approximately 20% of the subjects presenting with a pelvic mass will be found to have ovarian cancer at the time of surgery;
- Serum CA 125 and HE4 levels are associated with the probability of detecting ovarian cancer at the time of surgery;

- Greater than 30% of the total number of subjects fall into the low risk group;
- For the group of subjects without evidence of cancer (~80% of the evaluable subjects), ~45% fall into the low risk group, ~45% fall into the moderate risk group, and ~10% fall into the high risk group;
- For the group of subjects with ovarian cancer (~20% of the evaluable subjects), ~5% fall into the low risk group, ~15% fall into the moderate risk group, and ~80% fall into the high risk group.

Results obtained from evaluation of the primary and/or secondary endpoints from this study were intended for inclusion in one or more submissions to the Office of In-Vitro Devices in the Center for Devices and Radiological Health of the FDA and other Regulatory Agencies. The analyses were intended to be carried out at FDI or with other contract organizations.

8.2. STATISTICAL DESIGN

Steven Skates, PhD, Assistant Professor, Harvard Medical School, was responsible for the statistical analysis presented in this report and for the development of the statistical algorithm used to perform the analysis described below. Details of his credentials are located in Attachment 16.8.

M. Craig Miller, BS was contracted as an independent consultant to assist with database development and prepare data for compilation and statistical analyses. Details of his credentials are located in Attachment 16.8

Kimberlee Borden, M.S., V.P. and Sr. Biostatistician, of Research Oriented Biostatistics, Inc., was contracted as an independent consultant to assist with statistical analysis of the Ancillary Analyses described in this report. Details of her credentials are located in Attachment 16.8.

Three sets of analyses were run on the data from the FDI-03 trial. The first analysis was the analysis defined in the FDI-03 Protocol. This analysis was carried out as described in the original version of the Protocol dated November 7, 2005. Both the Primary and Secondary Endpoints were evaluated in the Protocol Analysis.

The second set of analyses was termed "Additional Analyses." In June of 2007, we completed analysis of data derived from two Pilot Studies; one completed at WIHRI, Brown University, and one completed at MGH in Boston. These are described in detail below. The salient finding from this study was that the CA 125 and HE4 biomarkers showed different behavior in premenopausal compared with postmenopausal women with pelvic mass. This prompted a separate analysis of the Pivotal (FDI-03) trial data where premenopausal and postmenopausal women were evaluated separately.

The third set of analyses was termed "Ancillary Analyses" and included dot plots and receiver operator characteristic (ROC) curves. These analyses serve as supporting data to the Primary and Secondary endpoint analyses.

8.2.1. PROTOCOL ANALYSIS: PRIMARY ENDPOINT:

For evaluation of the primary endpoint, the subjects were placed into groups having high, moderate, and low risks of ovarian cancer at the time of surgery based on their serum CA 125 and HE4 levels (both in U/mL). A pilot study conducted at WIHRI, Brown University enrolled 201 women with a pelvic mass who underwent surgery (146 benign conditions and 55 ovarian cancers). Using the pre-operative CA 125 and HE4 serum levels from these women, a logistic regression model and optimal threshold values for the resulting predicted probabilities were determined that separated them into low, moderate, and high risk groups.

CA 125 + HE4 Predictive Probabilities	**Low Risk*** ≤7.7%	**Mod Risk** 7.8 - 15.0%	**High Risk** ≥15.1%	**Total**
Benign	70 (97%)	60 (88%)	16 (26%)	146 (73%)
Epithelial Ovarian Cancer	2 (3%)	8 (12%)	45 (74%)	55 (27%)
Total	72 (36%)	68 (34%)	61 (30%)	201 (100%)

*The percentages in this table represent the number of subjects with benign disease and cancer relative to the total number in each risk group.

For the clinical trial, a PP was calculated for each subject using the following logistic regression formulas:

- *PI = 0.00307086*CA 125 + 0.01604288*HE4 – 3.195731*
- *PP = exp(PI) / [1 + exp(PI)]*
  - *Low Risk PP:* *≤ 7.7%*
  - *Moderate Risk PP:* *7.8% to 15.0%*
  - *High Risk PP:* *≥ 15.1%*

We used this logistic regression model and the thresholds to calculate a PP for each subject and categorize them as low, moderate, or high risk. Chi-square testing was utilized to compare the proportion of subjects with and without ovarian cancer across the three risk groups.

Poolability of subject results across geographically disperse clinical centers was determined. Sites within the same geographic region that enroll ≤ 10 subjects were combined prior to the poolability analysis.

8.2.2. PROTOCOL ANALYSIS: SECONDARY ENDPOINT:

The secondary endpoint was designed as a multivariate predictive algorithm combining multiple serum biomarkers (CA 125 and HE4), radiologic imaging results, and patient risk factors (such as age, menopausal status and ethnicity) for estimation of the risk of ovarian cancer, including low malignant potential (borderline) tumors, at the time of surgery in patients presenting with a pelvic mass to the use of only CA 125 and HE4. Inclusion of imaging results is an ongoing study and will not be described in this report. Ethnicity was not included in this analysis due to the small numbers in the study and age was assessed and provided no definitive conclusion so it was not included in this analyses. However, we did evaluate the effect of menopausal status and inclusion of low malignant potential (borderline) tumors.

ROC analyses were utilized to test for significant differences in the area under the curves (AUCs) for the multivariate model to that of the CA 125 and HE4 only model. Chi-square testing was utilized to compare the proportion of subjects with and

without ovarian cancer across the three risk groups as determined by the multivariate model as well as to compare the proportions of subjects in each risk group with ovarian cancer between the two models.

8.2.3. EXPLORATORY ENDPOINTS:

Analyses of Exploratory Endpoints are ongoing and will not be completed for presentation in this clinical report.

8.3. ADDITIONAL ANALYSIS

The original Protocol analysis outlined in this protocol was developed based on data generated in a pilot study completed at WIHRI. Subsequent to the development of the analysis described in the protocol and initiation of the FDI-03 Study, additional data generated based on a study completed at the MGH (Boston dataset) became available for analysis. The additional data generated at MGH was combined with the data generated in the pilot study performed at WIHRI. A statistical algorithm was developed based on the combined dataset. It was determined during the analysis of the combined dataset that menopausal status was an important factor to incorporate in the algorithm and incorporation of this factor as an independent variable in the analysis resulted in an improvement in the ability to assess the probability of risk of ovarian cancer in women presenting with a pelvic mass. The analysis described is presented in Attachment 16.13.

A decision was made by FDI to use the Fujirebio HE4 EIA values with CA 125II values derived from the Abbott ARCHITECT CA 125II platform. Sera collected at WIHRI were retested using the ARCHITECT CA 125II Assay. However, samples from the Boston dataset were initially evaluated using the Roche Elecsys 2010 CA 125II assay, and additional sera from the Boston samples were not available for this analysis. Data was available, however, from 98 serum samples measured previously in both the ARCHITECT and Elecsys CA 125 II assays. This data was used to multiply impute ARCHITECT CA 125 II values in the Boston data. With multiply imputed ARCHITECT data for each Elecsys value, the results from multiple logistic regression analysis were combined to form the correctly adjusted linear equation that provided the risk of having a

malignant pelvic mass based on the combined WIHRI and Boston studies, using the HE4 EIA and ARCHITECT CA 125II assay values. Details of this analysis are described in Attachment 16.12.

Menopausal status was determined in the study based on the date of the last menstrual period (LMP). Postmenopausal status was defined as one year past the LMP or hysterectomy with bilateral oophorectomy. In the case that the LMP was unknown, premenopausal status was defined as 48 yrs and younger with no prior history of a hysterectomy with bilateral oophorectomy and postmenopausal status was defined as 56 years and older. Women between the ages of 49 and 55 years of age who did not have a date for their LMP were considered unknown menopausal status and were not included in the original Additional Analysis (FDI-03A2, December 13, 2007).

In this report revision, the following clarifications are appended to the above menopausal determination:

In women 55 years and younger with prior history of a hysterectomy and at least one intact ovary, the menopausal status was based upon ovarian function.

In women 49 – 55 years with unknown LMP, the menopausal status was also determined based on ovarian function. This now allows the 7 women previously classified as Unknown menopausal status to be included in the Additional Analysis as described in Section 13.3 of this report.

8.3.1 RATIONALE FOR THE USE OF FOLLICLE-STIMULATING HORMONE (FSH) FOR DETERMINATION OF OVARIAN FUNCTION AND MENOPAUSAL STATUS

The World Health Organization has defined menopause as “the permanent cessation of menstruation resulting from the loss of ovarian follicular activity.” (34) The ovarian follicular activity is under the control of ovarian steroids and negative feedback regulators of FSH secretion. (35, 36) The loss of ovarian follicular activity is the result of exhaustion of the pool of primary follicles, which results in decreased negative feedback regulation of FSH secretion (35, 36) and as a consequence the serum and plasma levels of FSH increase. (37-39)

In clinical practice, physicians use ovarian function as an aid in the determination of menopausal status for women with inadequate information regarding their LMP, i.e., it is not available or cannot be obtained. Additionally, women with a prior history of hysterectomy may reach menopause earlier than commonly seen in women with an intact uterus. (39) Therefore, neither age nor LMP is the most suitable method for classification of menopausal status in this population. By measuring FSH levels, which is an accepted diagnostic tool to measure ovarian function, menopausal status can be better ascertained.

8.3.1.1 FSH ASSAY AND CUT-POINT FOR DETERMINATION OF MENOPAUSAL STATUS

To more accurately classify women less than 56 years of age with a prior hysterectomy or with unknown LMP into the premenopausal or postmenopausal groups, FSH was measured in an aliquot of each patient's specimen. The Abbot Laboratories FSH assay kit was used on an ARCHITECT platform to determine the FSH level for each of these 54 patients. The study protocol is shown in Attachment 16.4.

The package insert (Attachment 16.3) provided 95% central ranges for normally menstruating females in three stages of their cycle and for postmenopausal women. However, no definite cut-point for discriminating between pre- and postmenopausal status was provided. Therefore the data provided by the package insert was used to estimate the most appropriate FSH cut-point that would minimize the misclassification rate for determining premenopausal or postmenopausal status. The closest integer value is 22 mIU/mL.

The detailed statistical analyses for establishment of the optimal FSH cut-point using the ARCHITECT FSH assay are described in Attachment 16.15.

MGH Reproductive Endocrinology Laboratory has established and uses a reference level, or cut-point, of 22 mIU/mL using the ARCHITECT FSH assay for determination of menopausal status for their patients.

The MGH clinical standard of care utilizes a cut point of 22 mIU/mL and the statistical analysis both supports the choice of 22 mIU/mL for the FSH cut-point using the ARCHITECT FSH assay. Therefore the ARCHITECT FSH cut-point chosen for discrimination between pre and postmenopausal women is 22 mIU/mL.

8.3.1.2 FSH TESTING AND DATA MANAGEMENT

Determination of FSH levels was performed at the Sponsor laboratory by a trained operator who was blinded to the subject clinical status. The operator was trained on the protocol (Attachment 16.4) and on the assay procedures according to the package insert (16.3). Training was documented. Raw data printouts from the ARCHITECT Instrument were reviewed. Data was transferred to a CRF (Attachment 16.5) by the operator and submitted with the raw data for review. FDI was responsible for data management, which included database development, double data entry and validation of the data generated. FDI then transferred the database to an independent statistical consultant for statistical analyses.

9. CENTRAL PATHOLOGY REVIEW

9.1. CENTRAL REVIEW SITE

Central Review of pathology slides was conducted at JH, under the direction of Robert Kurman, MD, for all subjects enrolled in the Pelvic Mass Study. Three pathologists were responsible for performing the Central Pathology review: Robert Kurman, MD, Brigitte Ronnett, MD and Russell Vang, MD. The Study Manager initiated the site via a visit to JH, and reviewed the slide review process and CRF completion procedures prior to initiation of the review process.

9.2. CENTRAL REVIEW PROCESS

Representative tissue samples were taken from each subject during pelvic mass surgery and were evaluated by institutional pathology departments using institutional guidelines. A CRF was completed at the site based on information contained in the Site Pathology Report. Representative slide(s) and a copy of the Pathology Report was obtained for each subject and provided to JH for Central Pathology Review.

Three pathologists performed Central Pathology Review. Each of the three pathologists reviewed approximately one third of the total number of the subject slides for the initial review. All reviews were performed in a blinded manner. A minimum of one slide from each subject was sent to JH along with a blank CRF. A CRF was completed by the reviewing pathologist and returned to FDI. FDI performed an initial review to determine if the review was concordant or discrepant with the site's pathology review. If the review was determined to be discrepant, the slides(s) and a new CRF were returned to JH for adjudication by a second pathologist. The adjudicated results were recorded on a CRF and returned to FDI along with the slides for comparison to prior reviews. Upon review of results at FDI, discrepant reviews were returned to JH for further adjudication by a third pathologist. The slides and CRF were returned to FDI. There were a total of one to three pathology reviews completed centrally at JH for each subject with tissue slides available. Discrepancies in review results were adjudicated based on a set of rules developed prior to the review process as outlined below.

9.2.1. CONSENSUS DETERMINATION

A consensus determination was made to establish the presence or absence of cancer and major histopathology groups of cancer. The major histopathology groups included benign tumors, epithelial cell cancers, low malignant potential (borderline) tumors, non-epithelial cell cancers, metastatic cancers, other gynecologic cancers, and other cancers. The final histopathology determination was based on review of site pathology results and Central Pathology Review results. The final consensus determination was made by two gynecologic oncologists completing the final review of CRFs and Pathology Reports for each individual subject. This determination was made using rules outlined below. Results were entered onto CRFs, prior to being

entered into an electronic database using double data entry. The FDI Database was 100% reviewed for accuracy as compared to the CRFs using the services of an independent consultant.

9.2.2. CENTRAL REVIEW RULES:

- Cancer vs Benign vs Low Malignant Potential (Borderline) Tumors
  - Discrepancies were adjudicated as agreement among a minimum of 2 pathologists.
- Cancer: Major Histopathology Type Groups
  - Discrepancies were adjudicated as agreement among a minimum of 2 pathologists.
    - Epithelial ovarian cancer
    - Non-epithelial ovarian cancer
    - Low malignant potential (borderline) tumors
    - Metastatic cancer
    - Other gynecologic cancers
    - Other cancers
- Grade: International Federation of Gynecology and Obstetrics (FIGO) grading criteria used for cancer Grades 1, 2, or 3
  - Discrepancies were adjudicated if possible.
  - Discrepancies between Grade 1 and 3 were returned to JH for further review.
  - If grade was not assessed at JH, or if not in agreement, the site grade was used.
- Histopathology Sub-types for Benign and Cancer Groups
  - Discrepancies were not adjudicated.
  - If discrepancies exist between JH and site, the site pathology subtype was used.
- Stage
  - Assigned by the site and not centrally reviewed.

- Slide/tissue adequacy
  - If there was inadequate tissue or the slide could not be reviewed centrally, the site review was used.
  - New slides were obtained when possible.

The major histopathology groups and subtypes are as follows:

Epithelial Cancer Subtypes (EC):
Serous
Mucinous
Endometrioid
Clear Cell
Undifferentiated
Mixed Epithelial Cell Tumors
Other: (as indicated)

Non-Epithelial Cell Cancer, Subtypes (NEC):
Sarcoma
Malignant Germ Cell tumor
Granulosa Cell Tumor

Low Malignant Potential (Borderline) tumors:
Serous
Mucinous
Endometrioid

Malignant Metastatic Tumors (MC):
Tumors identified as originating from sites other than ovarian, fallopian tube, or primary peritoneal and non-gynecologic in origin. Example: colon, rectal or pancreatic in origin.

Other Cancers (OC):
Cancers identified as a pelvic mass, not categorized as a gynecologic cancer or metastatic to the ovary, fallopian tube or peritoneum, such as renal cell cancer.

Other Gyn Cancers (OGC):
Gynecologic cancers identified as originating other than ovarian, fallopian tube or primary peritoneal, for example: uterine, endometrial or cervical.

Benign Conditions, Common Subtypes:
Serous or Mucinous Cystadenoma or Adenofibroma
Endometriotic Cyst or Endometriosis
Simple Cyst
Mature Cystic Teratoma, Dermoid
Thecoma, Fibrothecoma
Pelvic inflammatory disease (abscesses)

10. ADVERSE EVENTS

No AEs were expected during the course of this study because the only direct contact with patients involved drawing blood and obtaining a urine sample. However, syncope or hematoma could occur, and patients were informed of this risk in the Informed Consent. All other invasive procedures and evaluations included in this protocol (imaging, surgery, etc.) are considered to be standard of care in the evaluation of patients with a pelvic mass and would have been performed as part of routine clinical work-up of patients with a pelvic mass.

A CRF was provided to all sites for "Unanticipated Device Related Adverse Events." Sites were trained to report any AEs to the Sponsor.

11. DATA INTEGRITY

11.1. PROTOCOL DEVELOPMENT AND VERSION CONTROL

The FDI-03 protocol and CRFs were designed by the Sponsor in collaboration with the Principal Investigator of the study after completion of a pilot study at the Principal Investigator's site. The Protocol and CRFs were reviewed by all sites to ensure consistency across sites in the conduct of this protocol. The sites, with one exception, utilized local IRBs for review and approval of the protocol. The one additional site utilized a Central IRB (Independent IRB) for protocol IRB approval. All amendments to the original protocol were submitted to the respective IRBs for review and approval. A letter outlining changes to the protocol was submitted to sites for each amendment, and copies of the approved versions of the amended protocols were maintained at the site and FDI.

11.2. SITE INITIATION AND MONITORING

Once IRB approval was obtained at a site, an initiation visit was performed. During this visit the protocol and CRFs were reviewed. Additional study staff was present for the review and site specific problems were addressed. Routine monitoring visits were also conducted at all sites for review of regulatory and source documents. Preprinted three part CRFs were provided to each study site. Once a patient was enrolled, data were

transcribed from the medical records and entered onto the CRF pages. FDI contracted with three independent CRAs to perform site monitoring and data retrieval functions. Monitoring functions were also performed by the sponsor. Subject CRFs were source documented verified against the medical record to ensure accuracy and completeness of data. Copies of the CRF were then collected and sent to FDI. FDI maintained one copy and sent a second copy to MDCI for database entry.

11.3. DATABASE MANAGEMENT

11.3.1. DATABASE CRO: MDCI

FDI utilized the services of MDCI located in North Attleboro, Massachusetts for database management for the study. MDCI was responsible for database development, data entry, and validation of the data generated in the FDI-03 Study in compliance with regulations under 21 CFR Part 11.

11.3.2. DATA ENTRY

The data from each patient were double data entered into the NetRegulus database. Double data entry is a process whereby all data collected on the CRFs are entered by two separate data entry people. The first entry updates the actual database and the second entry is for comparison purposes only. The separate entries were then compared and verified by the NetRegulus software. Discrepancies in entry were flagged by the software, and rectified by the data manager(s) by referring to the source document (CRF). Changes were then made to the final database through double entry.

The data were reviewed by the data manager(s) prior to and following data entry, and queries were written for data transcription errors, blanks, unclear or medically incorrect information. Guidelines for queries were agreed upon between FDI and MDCI and queries were reviewed periodically by FDI. Queries for data used in endpoint analyses have been resolved.

11.4. DATABASE INTEGRITY

MDCI utilized a NetRegulus database, which is 21 CFR Part 11 compliant for the FDI-03 Study. MDCI maintained this database in North Attleboro, MA. The study database, entry screens and management reports were created and managed by MDCI data managers. The software utilized by the data management system is programmed to record the user, the date and time of data entry, data modification or deletion, providing an audit trial through history tables in SQL. The software contains features that restrict access to database entry and modification to only those personnel with the required permission. Log-on and password protected restrictions ensure that database functions are performed by the appropriate personnel. The data manager(s) are assigned study-specific access rights.

11.5. DATA VALIDATION

With the creation of a new study database, validation steps were performed at MDCI to ensure the accuracy of data entry and data edits performed on each specific data point. Validation consists of entering test data into the data entry form through double entry, saving the data entered into the database, ensuring that the verification works correctly, printing the contents of the database, and then examining the output to ensure that the data are stored correctly. This validation procedure documents the storage of test data in proper data tables and associated tracking tables. It also documents the audit trail for edited data and ensures that changes to the database can be tracked and verified.

11.6. DATA ANALYSIS

After validation of the NetRegulus database, the entry page was locked and changes were not made. MDCI delivered the data to the sponsor. The sponsor transferred the files to an independent statistical consultant to be exported into Microsoft Excel files for compilation, addition of Histopathology Consensus Review data and statistical analyses. The sponsor then conducted an internal review of the database prior to completion of the statistical analysis.

12. ENDPOINT EVALUATION

12.1. SUBJECT DISPOSITION

A total of 566 subjects were enrolled into the study and 523 subjects were evaluable for the protocol analysis as summarized in Table 7 and Table 8. There was one subject number that was never assigned to a subject.

Table 7 Subject Disposition by Site- Protocol Analysis

Site Number	Subjects Enrolled	Subjects Excluded	Subjects Evaluable
036	100	9	91
037	21	2	19
038	24	1	23
039	87	4	83
040	58	1	57
041	4	1	3
042	5	2	3
043	2	0	2
044	22	1	21
045	100	7	93
046	30	9	21
048	8	0	8
062	75	4	71
063	30	2	28
Total	**566**	**43 (7.6 %)**	**523**

Table 8 Summary of Subject Exclusions from Protocol Analysis

Reason for Exclusion from Protocol Analysis	Number of Subjects
Subject was treated for malignancy within past 5 years	5
Subject treated with cytotoxic chemotherapy (methotrexate)	3
Subject did not undergo Surgery	15
Subject did not provide laboratory samples	4
Subject did not meet inclusion criteria based on age	1
Subject died prior to surgery	1
Subject had no assay results available	2
Subject Surgery performed at other institution, no information	1
Subject had previous bilateral oophorectomy	2
Subject withdrew consent	3
Subject had discordant pathology results	6
Total Subjects excluded from Protocol Analysis	**43**

There were 28 subjects in addition to the 43 subjects listed in Table 8 and Table 10 that were not included in the Protocol and the Additional Analysis because they had malignancies other than invasive epithelial ovarian cancer or low malignant potential (borderline) tumors such as metastatic, other cancers, other gyn cancers, and non-epithelial ovarian cancers. These subjects are listed in Table 9b.

Those subjects with previous malignancies and treatment with toxic chemotherapies that were not evaluable for the Protocol Analysis were evaluable in the Additional Analysis.

Subjects with discordant histopathology results, documented to receive treatment for a malignancy in recent years, or treatment with a toxic chemotherapeutic agent were evaluable in the Additional Analysis but not evaluable in the Protocol Analysis (n=7). They are:

Subject was not evaluable in both Protocol and Additional Analyses for multiple reasons, which include treatment for a prior malignancy and lack of sample collection. Subject was not evaluable in both Protocol and Additional Analyses for multiple reasons including treatment with a toxic chemotherapy as well as lack of a final histopathology review.

A summary comparison of subjects included in the Protocol and Additional Analyses is included in Table 9a.

Table 9a Subject Disposition- Protocol Analysis VS Additional Analysis

566 Subjects Enrolled			
Protocol Analysis		**Additional Analysis**	
Subjects Enrolled	**566**	**Subjects Enrolled**	**566**
Subjects Excluded (See Table 10 details)	**43**	**Subjects Excluded (See Table 11 details)**	**36**
Subjects with other cancers not included in Protocol Analysis (metastatic, non-epithelial and other cancers) See Table 9b	**28**	**Subjects with other cancers not included in Additional Analysis (metastatic, non-epithelial and other cancers) See Table 9b**	**28**
Total	**495**	**Total**	**502**

Table 9b Subjects (N=28) not evaluated in Protocol Analysis and Additional Analysis due to histopathology subtype: Non-Epithelial, Other cancers or Metastatic cancers.

Subjects w/ Metastatic Cancers	Subjects w l Cancers
	Subject ncers
	Subjects n Cancers

There were 43 (7.6%) subjects that were excluded from the Protocol Analysis due to reasons outlined in detail by subject in Table 10.

Table 10 Listing of Subject Exclusions – Protocol Analysis

Patient ID	Site #	Patient #	# of Patients	Detailed Other Reason Off Study
	36		5	Treated for Malignancy in Past Five Years
	36			Treated for Malignancy in Past Five Years
	36			Treated for Malignancy in Past Five Years
	45			Treated for Malignancy in Past Five Years and Lab Samples Not Tested
	46			Treated for Malignancy in Past Five Years
	36		3	Currently Being Treated with Chemotherapy (Methotrexate)
	45			Currently Being Treated with Chemotherapy (Methotrexate)
	62			Currently Being Treated with Chemotherapy (Methotrexate) and No Consensus Histopathology Results
	46		1	Female <18 Years of Age
	37		3	Subject Withdrew Consent
	39			Subject Withdrew Consent
	39			Subject Withdrew Consent
	36		15	Surgery Not Performed
	37			Surgery Not Performed
	38			Surgery Not Performed
	39			Surgery Not Performed
	41			Surgery Not Performed
	42			Surgery Not Performed
	44			Surgery Not Performed
	45			Surgery Not Performed
	45			Surgery Not Performed
	46			Surgery Not Performed
	46			Surgery Not Performed
	46			Surgery Not Performed
	46			Surgery Not Performed
	62			Surgery Not Performed
	63			Surgery Not Performed
	36		1	Surgery Performed at Another Institution - No Records Provided
	40		1	Patient Died Prior to Surgery
	36		4	Lab Samples Not Obtained
	45			Lab Samples Not Obtained
	45			Lab Samples Not Obtained
	46			Lab Samples Not Obtained
	62		2	No Assay Results Available
	62			No Assay Results Available
	46		2	Previous Bilateral Salpingo-Oopherectomy
	63			Previous Bilateral Salpingo-Oopherectomy
	36		6	Discordant Histopathology Results
	36			Discordant Histopathology Results
	39			Discordant Histopathology Results
	42			Discordant Histopathology Results
	45			Discordant Histopathology Results
	46			Discordant Histopathology Results
ber of Patients Not Evaluable for Protocol Analysis			**43**	

There were 36 (6.4%) subjects that were excluded from the Additional Analysis for reasons detailed by Subject in Table 11.

Table 11 Listing of Subject Exclusions: Additional Analysis

Patient ID	Site #	Patient #	# of Patients	Detailed Other Reason Off Study
	45	1		Treated for Malignancy in Past Five Years and Lab Samples Not Tested
	62		1	Currently Being Treated with Chemotherapy (Methotrexate) and No Consensus Histopathology Results
	46	1		Female <18 Years of Age
	37			Subject Withdrew Consent
	39		3	Subject Withdrew Consent
	39			Subject Withdrew Consent
	36			Surgery Not Performed
	37			Surgery Not Performed
	38			Surgery Not Performed
	39			Surgery Not Performed
	41			Surgery Not Performed
	42			Surgery Not Performed
	44			Surgery Not Performed
	45		15	Surgery Not Performed
	45			Surgery Not Performed
	46			Surgery Not Performed
	46			Surgery Not Performed
	46			Surgery Not Performed
	46			Surgery Not Performed
	62			Surgery Not Performed
	63			Surgery Not Performed
	36	1		Surgery Performed at Another Institution - No Records Provided
	40	1		Patient Died Prior to Surgery
	36			Lab Samples Not Obtained
	45		4	Lab Samples Not Obtained
	45			Lab Samples Not Obtained
	46			Lab Samples Not Obtained
	62		2	No Assay Results Available
	62			No Assay Results Available
	46		2	Previous Bilateral Salpingo-Oopherectomy
	63			Previous Bilateral Salpingo-Oopherectomy
	36			Discordant Histopathology Results
	39			Discordant Histopathology Results
	42		5	Discordant Histopathology Results
	45			Discordant Histopathology Results
	46			Discordant Histopathology Results
ber of Pati Not Evaluable for Additional Analysis			**36**	

12.2. PROTOCOL DEVIATIONS

Protocol deviations are summarized in Table 12. Some of the deviations included in Table 12 were prospectively approved by the Sponsor. Other deviations were retrospectively acknowledged by the Sponsor and some deviations resulted in exclusion of the subject from Protocol Analysis as described previously in Tables 8, 10 and 11. Subsequent to the initiation of the study, it was determined that the standard imaging method used by clinicians to assess a pelvic mass is not limited to the use of ultrasound, but that CT scans and MRI methods are commonly in use. Protocol deviations associated with inclusion of a subject based on the substitution of CT scan or MRI results in place of Ultrasound results were granted based on the understanding that a protocol amendment would be completed to include other forms of imaging as acceptable and equivalent based on the suggestions of multiple investigators and institution practices. This amendment was completed on August 18, 2006. Protocol deviations occurring as a result of samples and imaging being completed outside protocol specified time windows were granted as well based on the suggestion of investigators with the understanding that an amendment would eliminate the requirement for these time windows. A detailed list of deviations by site and subject are located in Attachment 16.11. This amendment was completed on August 18, 2006. Protocol amendments are included in Attachment 16.4.

Table 12 Summary of Protocol Deviations

Deviation Category	Total Number	Number Excluded from analysis
Imaging not performed as per protocol instructions or within specified window	**58**	**1**
Lab samples or slides not obtained as per protocol instructions or within specified window	**24**	**7**
Subject did not meet inclusion criteria	**30**	**15**
Other	**43**	**4**
Total	**155**	**27**

12.3. SUBJECT DEMOGRAPHICS

A total of 566 subjects were enrolled into the study, with 43 subjects excluded from the protocol analysis, leaving 523 subjects evaluable. The demographic characteristics of the evaluable protocol patient population are summarized in Tables 13, 14 and 15. All of the major racial groups were represented and approximately 15% were minority groups. This is an acceptable proportion of subjects for a study of this type. Two hundred eighty subjects (54%) of the total number of subjects enrolled were postmenopausal and 243 subjects (46%) were premenopausal. This proportion of premenopausal and postmenopausal subjects is expected with this type of patient population. A total of 176 of the evaluable subjects were diagnosed with cancer, 127 (72%) of which were Epithelial Ovarian and 21 (12%) of which were low malignant potential (borderline) tumors. Out of the 123 staged Epithelial Ovarian Cancers, 34 (28%) were Stages I/II and 89 (72%) were Stages III/IV as listed in Table 15. These proportions were expected based on published literature.

Table 13 Subject Demographics: Race

Race	All Subjects		Cancers		Benigns		Pre Menopausal		Post Menopausal	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
White	445	85.09%	157	89.20%	288	83.00%	195	80.25%	250	89.29%
Black	42	8.03%	11	6.25%	31	8.93%	23	9.47%	19	6.79%
Hispanic	16	3.06%	3	1.70%	13	3.75%	12	4.94%	4	1.43%
Asian	10	1.91%	3	1.70%	7	2.02%	7	2.88%	3	1.07%
Native American	5	0.96%	1	0.57%	4	1.15%	3	1.23%	2	0.71%
Other (Cape Verdean)	2	0.38%	0	0.00%	2	0.58%	2	0.82%	0	0.00%
Other (East Indian)	1	0.19%	0	0.00%	1	0.29%	0	0.00%	1	0.36%
Unknown	2	0.38%	1	0.57%	1	0.29%	1	0.41%	1	0.36%
Total	**523**	**100%**	**176**	**100%**	**347**	**100%**	**243**	**100%**	**280**	**100%**

Table 14 Subject Demographics: Menopausal Status and Disease Status

Menopausal Status	N	(%)
Pre-Menopausal	243	46.46%
Post-Menopausal	280	53.54%
Subtotal	**523**	**100%**

Disease Status

Cancer	N	(%)
Epithelial Ovarian	127	72.16%
Low Malignant Potential / Borderline	21	11.93%
Non-Epithelial	6	3.41%
Metastatic	14	7.95%
Other GYN	3	1.70%
Other	5	2.84%
Subtotal	**176**	**100%**

Benign	N	(%)
Normal	6	1.73%
Cystademonas, Adenofibromas, or Cystadenofibromas	113	32.56%
Cysts (All Types)	70	20.17%
Sex Cord Stromal Tumors (Thecomas, Fibroma, Fibrothecomas)	19	5.48%
Germ Cell Tumors (Teratomas and Dermoid Cysts)	29	8.36%
Endometriosis / Endometriomas	45	12.97%
Other (Abscess, adhesions, myomas, fibroids Brenner's tumor, hydrosalpinx, cystic mesothelioma, etc.)	34	9.80%
Unknown Disease Type	31	8.93%
Subtotal	**347**	**100%**

Table 15 Subject Demographics: Epithelial Cancer Grade and Stage

Epithelial Cancer Grade	N	(%)
Grade 1	11	8.66%
Grade 2	31	24.41%
Grade 3	83	65.35%
Unknown	2	1.58%
Subtotal	**127**	**100.00%**

Epithelial Cancer Stage	N	(%)
Stage IA	11	8.66%
Stage IB	1	0.78%
Stage IC	5	3.94%
Stage IIA	3	2.36%
Stage IIB	5	3.94%
Stage IIC	9	7.09%
Stage IIIA	1	0.79%
Stage IIIB	5	3.94%
Stage IIIC	77	60.63%
Stage IV	6	4.72%
Unstaged	4	3.15%
Subtotal	**127**	**100.00%**

12.4. JUSTIFICATION FOR POOLING DATA ACROSS SITES

12.4.1. SITE POOLABILITY – STATISTICAL METHODS

To justify pooling the 523 protocol evaluable patients from the 14 FDI-03 clinical sites, ten clinical site pools were developed. Sites with less than 10 subjects per site were pooled with sites in the same demographic area, as described in the FDI-03 protocol. This resulted in a total of ten clinical site pools as outlined in Table 16. Comparisons of various demographic characteristics were performed. These characteristics included age of the patient at the time of consent, menopausal status, specimen type (i.e., benign disease or cancer). For those patients with epithelial ovarian cancer, the additional characteristics of stage of disease, grade of disease and histology were also evaluated.

Ages across site pools were compared using a one-way analysis of variance (ANOVA) for unequal sample sizes (Analyze-it for Microsoft Excel, v 1.63). This statistical procedure provides an F-test of the null hypothesis that the population age means, $\mu_1 \ldots \mu_{12}$ are equivalent (i.e., H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_7 = \mu_8 = \mu_9 = \mu_{11} = \mu_{12}$). It also assumes that the values for each of the populations are normally distributed with equal variances. The mean age, standard deviation (SD), standard error (SE), and sample size for each site pool along with the results from the one-way ANOVA are presented below.

Table 16 Comparison of Ages Across Site Pools- (p= 0.0736)

Site Pool p = 0.0736	Clinical Site #s pooled	n	Mean	SD	SE
1	**36**	91	54.1	14.4	1.51
2	**37, 42, 43**	24	***44.3***	***8.8***	1.80
3	**38, 41**	26	55.0	15.9	3.11
4	**39**	83	53.1	15.5	1.70
5	**40**	57	53.4	14.2	1.88
7	**44**	21	55.5	17.9	3.90
8	**45**	93	54.3	15.2	1.58
9	**46**	21	59.8	14.4	3.13
11	**48, 62**	79	55.2	14.6	1.64
12	**63**	28	50.9	16.1	3.03

Since the p-value (p=0.0736) associated with this F-statistic is considered to be borderline significant (i.e., $0.05 < p < 0.10$), the Least Significant Difference multiple comparisons procedure was used to determine which site pools had different mean age values. Upon visual examination of the mean ages and their respective SDs, it appears that Site Pool 2 has a lower mean age and lower SD as compared to the other nine site pools. This is expected because Site Pool 2 included Site 37 which was the only site in the study that was an Obstetrics and Gynecologic practice, and would typically handle more premenopausal patients with benign conditions than the other Gynecologic Oncology sites.

From these results, it is apparent that the mean age for Site Pool 2 is significantly lower as compared to the other nine site pools. In addition, the mean age for Site Pool 12 (50.9 yrs) is also significantly lower as compared to the mean age for Site

Pool 9 (59.8 yrs) which has the highest mean age across all ten site pools. All of the remaining comparisons have p-values > 0.05 thus indicating acceptance of the null hypothesis that the mean ages across the site pools are equal.

The proportion of patients in each of the demographic classifications of menopausal status, specimen type (i.e., benign disease or cancer), diagnosis of benign disease, cancer diagnosis, and stage of disease, grade of disease and histology for patients with epithelial ovarian cancer, was also evaluated for poolability using a test of proportions for multiple samples (i.e., test for homogeneity of binomial samples) for each comparison. This statistical procedure utilizes the binomial distribution in formulating the null hypothesis that the proportion of values compared for an attribute is the same for all site pools. The null hypothesis is then tested by the following Chi-square statistic:

$X^2 = \Sigma\, n_i\, (p_i - \bar{p})^2 / (p \cdot q)$ with n-1 degress of freedom

where n_i = sample size for the ith site pool

n = total number of site pools being compared

p_i = proportion for the ith site pool

$\bar{p}$ = overall proportion for all site pools combined

$\bar{q} = 1 - \bar{p}$

$\alpha = 0.05$

Frequency results for Disease Type and Menopausal Status comparisons are presented below in Table 17.

Table 17 Disease Type and Menopausal Status

		Disease Type		Menopausal Status	
	Total # of	Frequency		Frequency	
Site Pool	Samples	Benign Disease	Cancer	Pre	Post
1	91	54	37	39	52
2	24	22	2	18	6
3	26	18	8	12	14
4	83	61	22	41	42
5	57	42	15	29	28
7	21	13	8	9	12
8	93	56	37	39	54
9	21	16	5	7	14
11	79	50	29	33	46
12	28	15	13	16	12
Total:	523	347	176	243	280

A summary of the results of the poolability analysis across sites is presented in Table 18.

Table 18 Summary of Poolability Across Sites

Poolable Across Sites

Category	Frequency	Chi-Square Statistic	p-value
Premenopausal	201	13.85	0.1279
Postmenopausal	315	12.23	0.2006
Benign - Normal	6	9.92	0.3568
Benign - Cystaden, Adenfibr. C	113	9.35	0.4060
Benign - Cysts	77	5.45	0.7932
Benign - Sex Cord Stromal	19	11.23	0.2601
Benign - Germ Cell Tumor	29	12.23	0.2005
Benign - Other	34	14.53	0.1046
Epith.Ovar. Cancer - Stage I	17	5.24	0.8131
Epith.Ovar. Cancer - Stage II	17	5.47	0.7915
Epith.Ovar. Cancer - Stage III	83	4.31	0.8898
Epith.Ovar. Cancer - Stage IV	6	8.46	0.4883
Epith.Ovar. Cancer - Grade 1	11	6.47	0.6918
Epith.Ovar. Cancer - Grade 3	83	11.36	0.2520
Epith.Ovar. Cancer - Grade N/A	2	9.57	0.3861
Epith.Ovar. Cancer - Serous	82	10.17	0.3372
Epith.Ovar. Cancer - Mucinous	6	11.21	0.2614
Epith.Ovar. Cancer - Endometri	16	1.37	0.9980
Epith.Ovar. Cancer - Clear Cell	6	8.14	0.5202
Epith.Ovar. Cancer - Mixed	7	9.67	0.3776
Epith.Ovar. Cancer - Other	10	13.31	0.1490

These results indicate that the populations used in these evaluations are equivalent with respect to demographic characteristics, except for Site 2 as discussed above; therefore, pooling the data across all site pools is justified. These results are outlined in detail in the Site Poolability Report in Attachment 16.13.

Based on the updated menopausal status, the poolability across sites was reanalyzed for menopausal status only. The reanalysis did not show any significant differences

between the sites. The result of the poolability analysis of the updated menopausal status is shown in Table 18a and Attachment 16.14.

Table 18a Summary of Poolability Across Sites for Menopausal Status

Category	Frequency	Chi-Square Statistic	p-value
Premenopausal	243	13.82	0.1461
Postmenopausal	280	13.82	0.1461
Total	523		

13. EFFECTIVENESS EVALUATION

13.1 BACKGROUND

The purpose of this section is to describe the development of a mathematical algorithm to stratify women with pelvic mass into groups with varying risk of ovarian cancer. Data from two pilot studies conducted at WIHRI, Brown University and MGH, Boston, MA, were used to generate the mathematical equations.

The FDI-03 Protocol was written in 2005. At that time, a partial dataset was available from the WIHRI study. That dataset was used to develop a logistic model that stratified women with pelvic mass into groups with low, intermediate and high risks of ovarian cancer. This database was used as the Training Set for the Protocol Analysis of the Pivotal Trial (FDI-03 Trial).

Subsequent to the analysis of 2005 that was included in the FDI-03 Protocol, the complete dataset from the WIHRI Pilot Study was combined with data from the MGH Pilot Study. During the analysis of that combined dataset, it became clear that the CA 125 and HE4 biomarkers show differences in behavior in premenopausal women compared with postmenopausal women. This led to the development of an algorithm that included menopausal status as a variable. We concluded that inclusion of

menopausal status in the risk stratification algorithm provides significant benefit to patient management. This combined database from WIHRI and MGH was used, therefore, as the Training Set for an Additional Analysis that incorporated menopausal status. The algorithm was then validated during the Validation Phase of this project using data derived from the FDI-03 prospective, multi-center trial.

In this way, we first validated that the FDI-03 Trial met the Primary and Secondary Endpoints using the analysis specified in the Protocol. Secondarily, we demonstrated that the FDI-03 Pivotal Trial data provides improved stratification of patients into risk groups when menopausal status is included in the algorithm. Development of both the Protocol algorithm and the algorithm that incorporated menopausal status (Additional Analysis) were completed prior to breaking the blind on the FDI-03 Pivotal Trial data. Thus, the development of both algorithms was done blinded to the results of the FDI-03 Pivotal Trial.

13.2 PROTOCOL ANALYSIS - SINGLE EQUATION ALGORITHM

Patient Population

The WIHRI pilot study began 1 Jan 2004. The purpose of the trial was to evaluate multiple serum biomarkers for their ability to predict the probability of cancer in women presenting for surgery with a pelvic mass. The inclusion criteria specified women presenting for surgery for removal of their ovaries. The protocol was approved by the Brown University IRB, and all patients signed Informed Consent to participate in the trial. Participation required the donation of blood samples for serum and urine samples. Tissue removed from all patients at the time of surgery was used to determine if the patient harbored benign or malignant gynecologic disease. The endpoint of the trial was to identify the most promising biomarkers for prediction of ovarian cancer in women presenting for gynecological surgery. This trial and the final analysis are described in detail in Reference 32.

Methods and Results

For evaluation of the primary endpoint, we utilized the results of the CA 125 and HE4 assays to segment the subjects into mutually exclusive low, moderate, and high risk groups. The pilot study conducted at The Program in Women's Oncology at WIHRI enrolled 201 women with a pelvic mass who underwent surgery. A total of 146 benign conditions and 55 ovarian cancers were confirmed by histopathological analysis of tissues obtained at surgery. Using the pre-operative CA 125 and HE4 serum levels from these women, a logistic regression model and optimal threshold values for the resulting predicted probabilities were determined that separated them into low, moderate, and high risk groups. The results of this analysis are shown in Table 19 below.

Table19 Risk subgroups and predicted probabilities in WIHRI Pilot study dataset.

CA 125 + HE4 Predictive Probabilities	Low Risk* ≤7.7%	Mod Risk 7.8 - 15.0%	High Risk ≥15.1%	Total
Benign	70 (97%)	60 (88%)	16 (26%)	146 (73%)
Epithelial Ovarian Cancer	2 (3%)	8 (12%)	45 (74%)	55 (27%)
Total	72 (36%)	68 (34%)	61 (30%)	201 (100%)

*The percentages in this table represent the number of subjects with benign disease and cancer relative to the total number in each risk group.

For analysis of data from the FDI-03 pivotal trial, a PP was calculated for each subject using the following logistic regression formulas:

- PI = 0.00307086*CA 125 + 0.01604288*HE4 – 3.195731
- PP = exp(PI) / [1 + exp(PI)]
  - Low Risk PP: < 7.7%
  - Moderate Risk PP: 7.8% to 15.0%
  - High Risk PP: > 15.1%

We used this logistic regression model and the thresholds to calculate a PP for each subject and categorize them as low, moderate, or high risk. Chi-square testing was

utilized to compare the proportion of subjects with and without ovarian cancer across the three risk groups.

We first evaluated the Primary Endpoint using only patients with benign diseases or invasive epithelial ovarian cancer. Results are given in Table 20 below. The Chi-square p-values are less than 0.05 signifying that the proportion of cancer and benign disease patients in each of the risk subgroups is statistically significantly different. This difference demonstrates that the data from the FDI-03 Pivotal trial meet the Primary Endpoint. Note that in Tables 20-21, and 23-31, the percentages of patients were calculated to determine sensitivity and specificity for the PPs. Thus, the percentage of patients for each disease type and risk subgroup was determined using the total number of patients for each disease type.

Table 20 Primary Endpoint analysis using the single equation algorithm from the Protocol Analysis. Invasive epithelial ovarian cancer patients only.

CA 125 + HE4 (FDI-03 Protocol)	Low Risk ≤7.7%	Mod Risk 7.8 - 15.0%	High Risk ≥15.1%	Total
Benign	53	208	86	347
Invasive Epithelial Ovarian Ca (I/II)	0	7	27	34
Invasive Epithelial Ovarian Ca (III/IV)	1	1	87	89
Invasive Epithelial Ovarian Ca (Unstaged)	0	2	2	4
Total	54	218	202	474

CA 125 + HE4 (FDI-03 Protocol)	Low Risk ≤7.7%	Mod Risk 7.8 - 15.0%	High Risk ≥15.1%	Total
Benign	53 (15%)	208 (60%)	86 (25%)	347 (73%)
Epithelial Ovarian Cancer	1 (1%)	10 (8%)	116 (91%)	127 (27%)
Total	54 (11%)	218 (46%)	202 (43%)	474 (100%)

Pearson's chi^2 =	**168.5678**	**p = 0.0000**
Likelihood-ratio chi^2 =	**184.2785**	**p = 0.0000**
Fisher's Exact Test	---	**p = 0.000**

Note: The percentages in this table represent the distribution of subjects in a disease category in each risk group.

We next evaluated the Secondary Protocol Endpoint in which we included patients with low malignant potential (borderline) tumors. Again, the Chi-square p-values are < 0.05 signifying that the proportion of patients with cancers and benign diseases is significantly different among the three risk groups. These data demonstrate that the data from the FDI-03 Trial meet the Secondary Endpoint including patients with low malignant potential (borderline) tumors as indicated in Table 21.

Table 21 Secondary Endpoint analysis using the single equation algorithm from the Protocol Analysis. Invasive epithelial ovarian cancer patients and patients with low malignant potential (borderline) tumors.

CA 125 + HE4 (FDI-03 Protocol)	Low Risk ≤7.7%	Mod Risk 7.8 - 15.0%	High Risk ≥15.1%	Total
Benign	53	208	86	347
Low Malignant Potential Tumors	1	7	13	21
Invasive Epithelial Ovarian Ca (I-II)	0	7	27	34
Invasive Epithelial Ovarian Ca (III-IV)	1	1	87	89
Invasive Epithelial Ovarian Ca (Unstaged)	0	2	2	4
Total	55	225	215	495

CA 125 + HE4 (FDI-03 Protocol)	Low Risk ≤7.7%	Mod Risk 7.8 - 15.0%	High Risk ≥15.1%	Total
Benign	53 (15%)	208 (60%)	86 (25%)	347 (70%)
Epithelial Ovarian Cancer / Low Malignant Potential Tumors	2 (1%)	17 (12%)	129 (87%)	148 (30%)
Total	55 (11%)	225 (46%)	215 (43%)	495 (100%)

Pearson's chi^2 =	**164.6350**	**p = 0.0000**
Likelihood-ratio chi^2 =	**176.8277**	**p = 0.0000**
Fisher's Exact Test	---	**p = 0.000**

Note: The percentages in this table represent the distribution of subjects in a disease category in each risk group.

13.1. ADDITIONAL ANALYSES INCLUDING PREMENOPAUSAL AND POSTMENOPAUSAL STATUS

Patient Population

The original analysis outlined in this protocol was developed based on data generated in a Pilot Study completed at the WIHRI. The WIHRI Pilot Study began January 1, 2004 and the Boston pilot study began January 1, 2003. The purpose of the trials was the same in both cases, and was to evaluate multiple serum biomarkers for their ability to predict the probability of cancer in women presenting for surgery with a pelvic mass. The inclusion criteria specified women presenting for surgery for removal of their ovaries. The protocols were approved by the Brown University IRB (WIHRI), and Partners IRB (Boston), and all patients signed Informed Consent to participate in the trial. Participation required the donation of blood samples for serum and urine samples. Tissue removed from all patients at the time of surgery was used to determine if the patient harbored benign or malignant gynecologic disease. The endpoint of the trial was to identify the most promising biomarkers for prediction of ovarian cancer in women presenting for gynecological surgery.

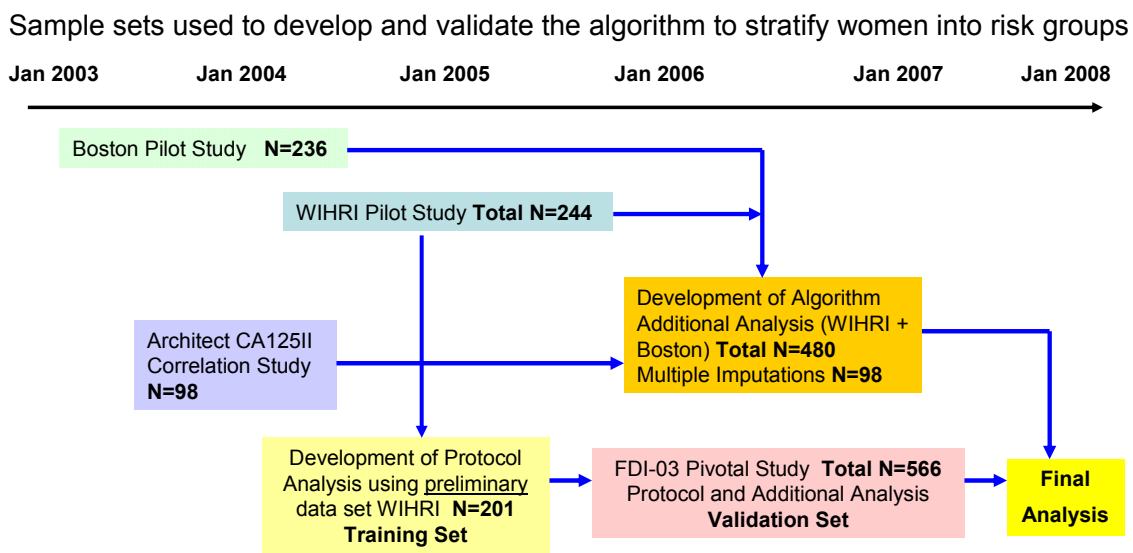
The WIHRI Pilot Study was concluded in January 2006, and included data from 166 women found to have benign gynecologic disease on surgery, 67 women with invasive epithelial ovarian cancers, 2 women with non-epithelial ovarian cancers, 9 women with low malignant potential (borderline) tumors and 166 subjects with benign disease (total N=244) (32). The Boston study contributed a total of 236 subjects in the analysis including 124 invasive epithelial ovarian cancers, 42 subjects with low malignant potential (borderline) tumors and 70 subjects with benign disease. Thus, a total of 480 patients provided evaluable data for the combined datasets, as shown in Table 22.

Table 22 Distribution of Disease Type by Menopausal Status

Disease Group	Premenopausal		Postmenopausal		Total
	MGH	WIHRI	MGH	WIHRI	
Benign	38	82	32	84	**236**
Low Malignant Potential	14	3	28	6	**51**
Early Stage Epithelial	13	3	38	12	**66**
Late Stage Epithelial	33	6	40	46	**125**
Non-Epithelial	0	0	0	2	**2**

The datasets utilized to develop the logistic regression model defined as the Protocol Analysis and the combined datasets used to develop the logistic regression model defined as the Additional Analysis were separate studies performed at different institutions. The dataset used to develop the Protocol Analysis was different than the combined dataset used to develop the training set for the Additional Analysis. The Pivotal Trial is a separate dataset and will be used as the validation set for the Protocol Analysis and the Additional Analysis. Figure 3 demonstrates that the patient populations used in the training and validation sets did not overlap.

Figure 3 Sample sets used to develop and validate the algorithm



Methods and Results

Analysis of the combined datasets indicated that the distribution of CA 125 differed significantly between the 192 premenopausal women and the 288 postmenopausal women. In particular, the CA 125 distribution differed between the 120 premenopausal and the 116 postmenopausal women with benign diseases (data not shown).

For the analysis described above, the WIHRI samples were assayed for CA 125 concentrations using the Fujirebio CA 125II RIA, and the MGH samples were assayed for CA 125II concentrations using the Roche Elecsys CA 125II assay. Because neither of these were the CA 125II assay that FDI plans to commercialize for the pelvic mass application, we retested all samples from the WIHRI dataset using the Abbott ARCHITECT CA 125II assay. However, the MGH samples were not available for further analysis. Data from a separate study were available from 98 serum samples measured previously in both the ARCHITECT and Elecsys CA 125 II assays. A tight linear regression with constant variance accurately describes the relationship between the two CA 125II measurements on a logarithmic scale. This relationship was used to multiply impute ARCHITECT CA 125II values in the Boston data. With multiply imputed ARCHITECT data for each Elecsys value, the results from multiple logistic regression analyses were combined to form the correctly adjusted linear equation that provides the risk of having a malignant pelvic mass based on the combined WIHRI and Boston studies. The logistic regression equations are (note that log refers to natural log):

Premenopausal women

PI = -12.0 + 2.38*LN(HE4) + 0.0626*LN(CA 125)

Postmenopausal women

PI = -8.09 + 1.04*LN(HE4) + 0.732*LN(CA 125)

PP = exp(PI) / [1 + exp(PI)]

The cut-points for Specificity and Sensitivity chosen for the Additional Analyses were based on results of the “Protocol Analysis” which specified a model based on a logistic regression derived from HE4 and CA 125 without transforming to natural logarithms.

The cut-point for this analysis was p = 15% for the low/moderate risk subgroups compared with the high risk subgroup. Applying this equation to the Pivotal Trial data resulted in a specificity of 75% for the low/moderate risk subgroups (described in detail below), such that 75% of women with a benign pelvic mass were classified correctly. For both statistical and medical reasons, we chose cut-points for the "Additional Analysis" that provided a specificity of 75%. We chose 75% specificity because we felt that if 75% of all patients that are currently referred to gynecologic oncologists using the current standard of care were to remain in the community then this test would provide significant medical benefit.

In addition, we set 80% sensitivity as the required minimum sensitivity for the PP for premenopausal and postmenopausal women combined as the threshold for acceptance of this analysis. To demonstrate this, we defined that the lower bound of the 95% Confidence Interval for Sensitivity must be > 80% when specificity is held constant at 75% for premenopausal and postmenopausal women. The cut-points defined by 75% specificity were 13.1% PP for premenopausal patients and 27.7% PP for postmenopausal patients.

The patient population used for the Additional Analysis was the population described in more detail in Section 12.1. This population included all evaluable patients with known menopausal status. In addition to the patients described in Section 12.1, 7 women 49 – 55 years of age with unknown LMP were classified as pre- or postmenopausal based on FSH determination as described in Section 8.3.1.1 and were, therefore, included in the Additional Analyses. The patients are described in Table 23.

Table 23 Patients with Previous Unknown Menopausal Status

Patent ID	Site	Patient	Menopause status	Pathology category	Diagnosis	Grade	Stage
	36		Pre	Epithelial Ovarian Cancer	Endometrioid	2	IIC
	38		Post	Benign	Serous cystadenofibroma	-	-
	39		Post	Epithelial Ovarian Cancer	Serous	3	IIIC
	46		Post	Other Gyn Cancer	Endometrial	3	IC
	46		Post	Benign	Serous cystadenoma	-	-
	46		Pre	Benign	Leiomyoma	-	-
	63		Pre	Benign	Endometriosis	-	-

Cancer patients were evaluable if they were found to have epithelial ovarian cancer or low malignant potential (borderline) tumors. Subjects were evaluable in the Additional Analysis and non-evaluable in the Protocol Analysis included those subjects with previous malignancies and treatment with toxic chemotherapies. These patients were included in the Additional Analysis because while they violated the inclusion/exclusion criteria in the Protocol, they were enrolled into the study and provided evaluable data; thus they qualify as an Intent-to-Evaluate population. There were 7 subjects with either discordant histopathology results, that received treatment for a malignancy in recent years or received chemotherapy that were evaluable in the Additional Analysis but not included in the Protocol Analysis. The total N for this population was 502.

For the Additional Analyses we set cut-points that divided the patient population into only two subgroups: Low Risk and High Risk. This was for two reasons. First, when we combined the Low and Moderate Risk subgroups using the Protocol Analysis, the stratification was excellent and provided appropriate populations for triage to gynecologists or gynecologic oncologists. Second, since elimination of the Moderate Risk subgroup was shown to be feasible, only two subgroups with low or high risk will simplify implementation of the test in clinical practice.

The statistical methods used to multiply impute the ARCHITECT CA 125II values, and the methods used to derive the logistic equations used in the Additional Analyses are described in detail in the Report on HE4 and CA 125 Algorithm for Pelvic Masses, Attachment 16.12.

The results of the Additional Analysis showed that at 75% specificity, the sensitivity for all patients combined (N=502) was 89% (134/151 total cancers, Table 24) with a lower bound for the 95% confidence interval of 82.6% which was in fact > 80% and serves to demonstrate that the data meet the predetermined endpoint for this analysis. An important result is ruling out sensitivity levels less than 80% at a specificity of 75%. The exact 95% confidence interval for the sensitivity of 89% is (82.6%, 93.3%) which clearly rules out any sensitivity less than 82% including 80%. In addition, the NPV was 95%, 93% and 94% for premenopausal, postmenopausal, and all patients combined, respectively. Results of these analyses are shown in Tables 24 – 29 below. The percentages in the following tables represent the distribution of subjects in a disease category in each risk group.

Table 24 Risk stratification of patients with pelvic mass based upon CA 125II and HE4 concentrations using a two-equation algorithm for premenopausal and postmenopausal women. Results of premenopausal and postmenopausal women combined.

ROMA (Premenopausal & Postmenopausal Women Combined)	Low Risk (<13.1%) (<27.7%)	High Risk (≥13.1%) (≥27.7%)	Total
Benign	262 (75%)	89 (25%)	351 (70%)
Epithelial Ovarian Cancer / Low Malignant Potential Tumors	17 (11%)	134 (89%)	151 (30%)
Total	279 (56%)	223 (44%)	502 (100%)

Pearson's chi^2 =	**171.8145**	**p = 0.0000**
Likelihood-ratio chi^2 =	**185.9094**	**p = 0.0000**
Fisher's Exact Test	---	**p = 0.000**

Measurement	Estimate	Lower 95% CI	Upper 95% CI
Positive % Agreement (Sensitivity)	88.7%	82.6%	93.3%
Negative % Agreement (Specificity)	74.6%	69.8%	79.1%
Positive Predictive Value	60.1%	53.3%	66.6%
Negative Predictive Value	93.9%	90.4%	96.4%
Overall Agreement	78.9%	75.0%	82.4%
Odds Ratio	23.2	13.3	40.6

Table 25 Risk stratification of patients with pelvic mass based upon CA 125II and HE4 concentrations using a two equation algorithm, subdivided by histological subtype and Stage. Results of premenopausal and postmenopausal women combined.

ROMA (Premenopausal & Postmenopausal Women Combined)	Low Risk (<13.1%) (<27.7%)	High Risk (≥13.1%) (≥27.7%)	Total
Benign	262	89	351
Low Malignant Potential Tumors	9	13	22
Invasive Epithelial Ovarian Ca (I-II)	5	30	35
Invasive Epithelial Ovarian Ca (III-IV)	1	89	90
Invasive Epithelial Ovarian Ca (Unstaged)	2	2	4
Total	279	223	502

Table 26 Risk stratification of patients with pelvic mass based upon CA 125II and HE4 concentrations using a two-equation for premenopausal and postmenopausal women. Results for premenopausal women only.

ROMA (Premenopausal Women Only)	Low Risk (<13.1%)	High Risk (≥13.1%)	Total
Benign	149 (75%)	51 (25%)	200 (85%)
Epithelial Ovarian Cancer / Low Malignant Potential Tumors	8 (24%)	26 (76%)	34 (15%)
Total	157 (67%)	77 (33%)	234 (100%)

Pearson's chi^2 =	**34.1959**	**p = 0.0000**
Likelihood-ratio chi^2 =	**32.2779**	**p = 0.0000**
Fisher's Exact Test	---	**p = 0.000**

Measurement	Estimate	Lower 95% CI	Upper 95% CI
Positive % Agreement (Sensitivity)	76.5%	58.8%	89.3%
Negative % Agreement (Specificity)	74.5%	67.9%	80.4%
Positive Predictive Value	33.8%	23.4%	45.4%
Negative Predictive Value	94.9%	90.2%	97.8%
Overall Agreement	74.8%	68.7%	80.2%
Odds Ratio	9.5	4.0	22.3

Table 27 Risk stratification of patients with pelvic mass based upon CA 125II and HE4 concentrations using a two equation algorithm, subdivided by histological subtype and Stage. Results of premenopausal women only.

ROMA (Premenopausal Women Only)	Low Risk (<13.1%)	High Risk (≥13.1%)	Total
Benign	149	51	200
Low Malignant Potential Tumors	6	10	16
Invasive Epithelial Ovarian Ca (I-II)	1	6	7
Invasive Epithelial Ovarian Ca (III-IV)	0	9	9
Invasive Epithelial Ovarian Ca (Unstaged)	1	1	2
Total	157	77	234

Table 28 Risk stratification of patients with pelvic mass based upon CA 125II and HE4 concentrations using a two-equation algorithm for premenopausal and postmenopausal women. Results for postmenopausal women only.

ROMA (Postmenopausal Women Only)	Low Risk (<27.7%)	High Risk (≥27.7%)	Total
Benign	113 (75%)	38 (25%)	151 (56%)
Epithelial Ovarian Cancer / Low Malignant Potential Tumors	9 (8%)	108 (92%)	117 (44%)
Total	122 (46%)	146 (54%)	268 (100%)

Pearson's chi^2 =	**119.8326**	**p = 0.0000**
Likelihood-ratio chi^2 =	**135.5441**	**p = 0.0000**
Fisher's Exact Test	---	**p = 0.000**

Measurement	Estimate	Lower 95% CI	Upper 95% CI
Positive % Agreement (Sensitivity)	92.3%	85.9%	96.4%
Negative % Agreement (Specificity)	74.8%	67.1%	81.5%
Positive Predictive Value	74.0%	66.1%	80.9%
Negative Predictive Value	92.6%	86.5%	96.6%
Overall Agreement	82.5%	77.4%	86.8%
Odds Ratio	35.7	16.5	77.3

Table 29 Risk stratification of patients with pelvic mass based upon CA 125II and HE4 concentrations using a two equation algorithm, subdivided by histological subtype and Stage. Results of postmenopausal women only.

ROMA (Postmenopausal Women Only)	Low Risk (<27.7%)	High Risk (≥27.7%)	Total
Benign	113	38	151
Low Malignant Potential Tumors	3	3	6
Invasive Epithelial Ovarian Ca (I-II)	4	24	28
Invasive Epithelial Ovarian Ca (III-IV)	1	80	81
Invasive Epithelial Ovarian Ca (Unstaged)	1	1	2
Total	122	146	268

The population included in the Additional Analysis described in Tables 24-29 did not include non-epithelial, metastatic, other gynecologic and other cancers. These cancers were combined with the epithelial ovarian cancers and low malignant potential (borderline) tumors to assess the performance of the two-equation algorithm using all cancers. The analyses were performed with premenopausal and postmenopausal women and all women and the results are described in Tables 30-32.

Table 30 Risk stratification of patients with pelvic mass based upon CA 125II and HE4 concentrations using a two-equation algorithm for all cancers. Results for premenopausal and postmenopausal women combined.

ROMA (Premenopausal & Postmenopausal Women Combined)	Low Risk (<13.1%) (<27.7%)	High Risk (≥13.1%) (≥27.7%)	Total
Benign	262 (75%)	89 (25%)	351 (66%)
All Cancers	25 (14%)	154 (86%)	179 (34%)
Total	287 (54%)	243 (46%)	530 (100%)

Pearson's chi^2 =	**175.7930**	**p = 0.0000**
Likelihood-ratio chi^2 =	**188.8379**	**p = 0.0000**
Fisher's Exact Test	**---**	**p = 0.000**

Measurement	Estimate	Lower 95% CI	Upper 95% CI
Positive % Agreement (Sensitivity)	86.0%	80.1%	90.8%
Negative % Agreement (Specificity)	74.6%	69.8%	79.1%
Positive Predictive Value	63.4%	57.0%	69.4%
Negative Predictive Value	91.3%	87.4%	94.3%
Overall Agreement	78.5%	74.7%	81.9%
Odds Ratio	18.1	11.2	29.5

ROMA (Premenopausal & Postmenopausal Women Combined)	Low Risk (<13.1%) (<27.7%)	High Risk (≥13.1%) (≥27.7%)	Total
Benign	262	89	351
Low Malignant Potential Tumors	9	13	22
Epithelial Ovarian Ca (I/II)	5	30	35
Epithelial Ovarian Ca (III/IV)	1	89	90
Epithelial Ovarian Ca (Unstaged)	2	2	4
Non-Epithelial Ovarian Ca	3	3	6
Other Gynecological Ca	0	3	3
Other Cancer	2	3	5
Metastatic Cancer	3	11	14
Total	287	243	530

Table 31 Risk stratification of patients with pelvic mass based upon CA 125II and HE4 concentrations using a two-equation algorithm for all cancers. Results for premenopausal women only.

ROMA (Premenopausal Women Only)	Low Risk (<13.1%)	High Risk (≥13.1%)	Total
Benign	149 (75%)	51 (25%)	200 (81%)
All Cancers	15 (33%)	31 (67%)	46 (19%)
Total	164 (67%)	82 (33%)	246 (100%)

Pearson's chi^2 =	**29.5334**	**p = 0.0000**
Likelihood-ratio chi^2 =	**27.9739**	**p = 0.0000**
Fisher's Exact Test	**---**	**p = 0.000**

Measurement	Estimate	Lower 95% CI	Upper 95% CI
Positive % Agreement (Sensitivity)	67.4%	52.0%	80.5%
Negative % Agreement (Specificity)	74.5%	67.9%	80.4%
Positive Predictive Value	37.8%	27.3%	49.2%
Negative Predictive Value	90.9%	85.4%	94.8%
Overall Agreement	73.2%	67.2%	78.6%
Odds Ratio	6.0	3.0	12.1

ROMA (Premenopausal Women Only)	Low Risk (<13.1%)	High Risk (≥13.1%)	Total
Benign	149	51	200
Low Malignant Potential Tumors	6	10	16
Epithelial Ovarian Ca (I/II)	1	6	7
Epithelial Ovarian Ca (III/IV)	0	9	9
Epithelial Ovarian Ca (Unstaged)	1	1	2
Non-Epithelial Ovarian Ca	3	3	6
Other Gynecological Ca	0	0	0
Other Cancer	1	0	1
Metastatic Cancer	3	2	5
Total	164	82	246

Table 32 Risk stratification of patients with pelvic mass based upon CA 125II and HE4 concentrations using a two-equation algorithm for all cancers. Results for postmenopausal women only.

ROMA (Postmenopausal Women Only)	Low Risk (<27.7%)	High Risk (≥27.7%)	Total
Benign	113 (75%)	38 (25%)	151 (53%)
All Cancers	10 (8%)	123 (92%)	133 (47%)
Total	123 (43%)	161 (57%)	284 (100%)

Pearson's chi^2 =	**130.5112**	**p = 0.0000**
Likelihood-ratio chi^2 =	**147.2517**	**p = 0.0000**
Fisher's Exact Test	**---**	**p = 0.000**

Measurement	Estimate	Lower 95% CI	Upper 95% CI
Positive % Agreement (Sensitivity)	92.5%	86.6%	96.3%
Negative % Agreement (Specificity)	74.8%	67.1%	81.5%
Positive Predictive Value	76.4%	69.1%	82.7%
Negative Predictive Value	91.9%	85.6%	96.0%
Overall Agreement	83.1%	78.2%	87.3%
Odds Ratio	36.6	17.4	76.8

ROMA (Postmenopausal Women Only)	Low Risk (<27.7%)	High Risk (≥27.7%)	Total
Benign	113	38	151
Low Malignant Potential Tumors	3	3	6
Epithelial Ovarian Ca (I/II)	4	24	28
Epithelial Ovarian Ca (III/IV)	1	80	81
Epithelial Ovarian Ca (Unstaged)	1	1	2
Non-Epithelial Ovarian Ca	0	0	0
Other Gynecological Ca	0	3	3
Other Cancer	1	3	4
Metastatic Cancer	0	9	9
Total	123	161	284

The results in Tables 30-32 demonstrate that the PP is effective in stratifying women with non-epithelial ovarian cancer, other gyn cancers, other cancers and metastatic cancers. However, the sample sizes for these subgroups were too small to draw meaningful conclusions.

We also calculated the NPVs for all evaluable patients using logistic equations derived using the Protocol Analysis, and for premenopausal, postmenopausal, and both

combined using logistic equations derived using the Additional Analysis. The data are shown below in Table 33, and demonstrate that the NPVs are > 90% in all cases. In particular, the NPVs are between 92 and 95% for all groups using the equations defined in the Additional Analyses. Thus, the algorithm defined by the Additional Analysis provides for 56% of patients currently referred to gynecologic oncologists to remain in the community setting and undergo surgery by a gynecologist. Included among these patients that would remain in the community to be treated by a gynecologist are 75% of all patients with benign disease. Additionally, only 6% of these women have been found to have cancer on surgery (Table 24). And, within this 6% of patients that will be found to have cancer by the gynecologist, our data suggests that only 6% (1/17, Table 24) will be found to have late stage disease (Stages III/IV).

Table 33 NPVs and their respective 95% confidence intervals for the various patient populations using epithelial and low malignant potential tumors combined.

Analysis	Menopausal Status	NPV	95% Confidence Interval
Protocol	Combined	93.2%	89.6%-95.9%
Additional	Premenopausal	94.9%	90.2%-97.8%
	Postmenopausal	92.6%	86.5%-96.6%
	Combined	93.9%	90.4%-96.4%

These data were also drawn graphically as X-Y Scatterplots and the results are shown below in Figures 4 and 5.

Figure 4 X-Y Scatterplot of CA 125II concentrations (X axis, log scale) and HE4 concentrations (Y axis, log scale) for premenopausal women only

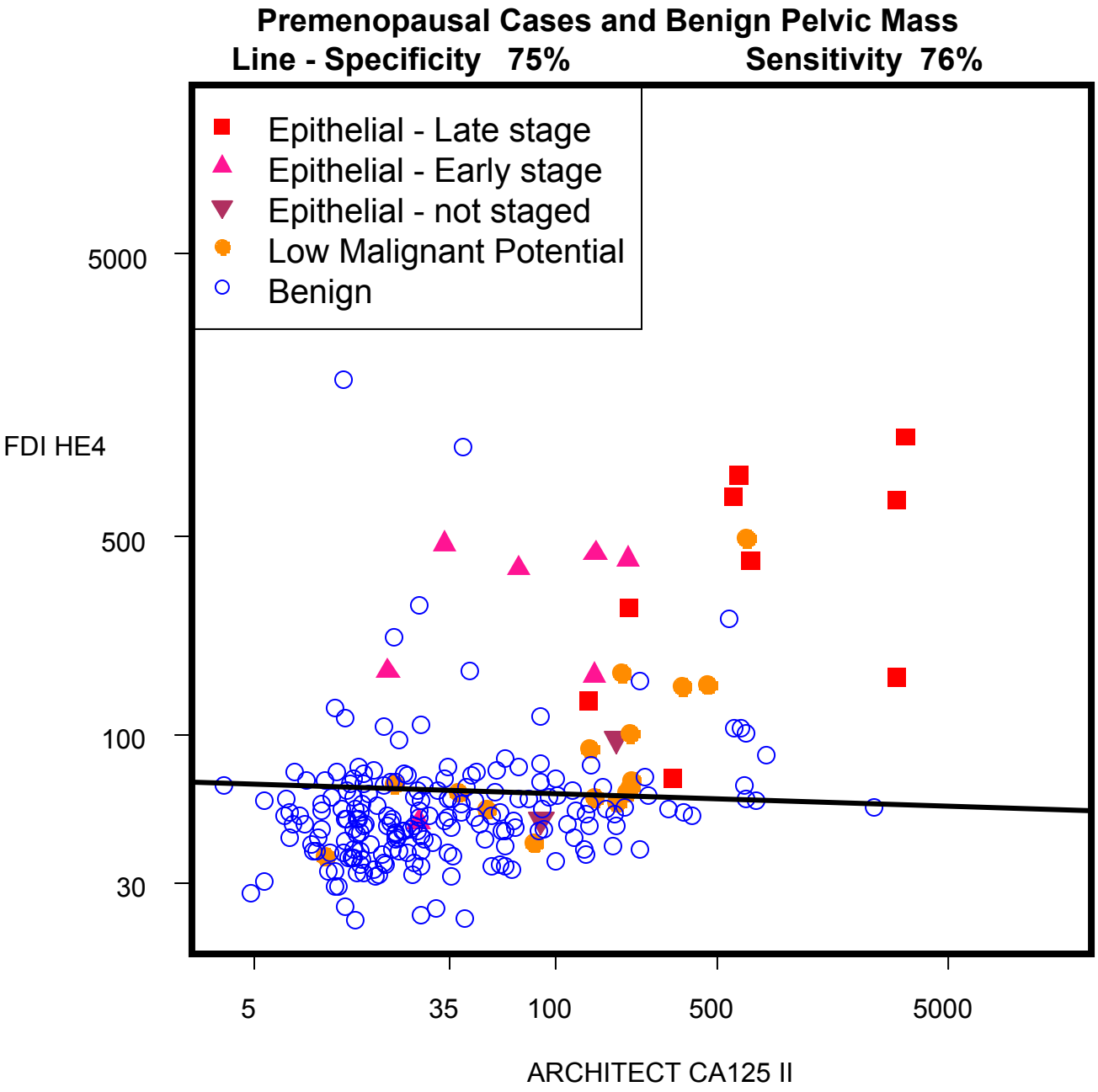
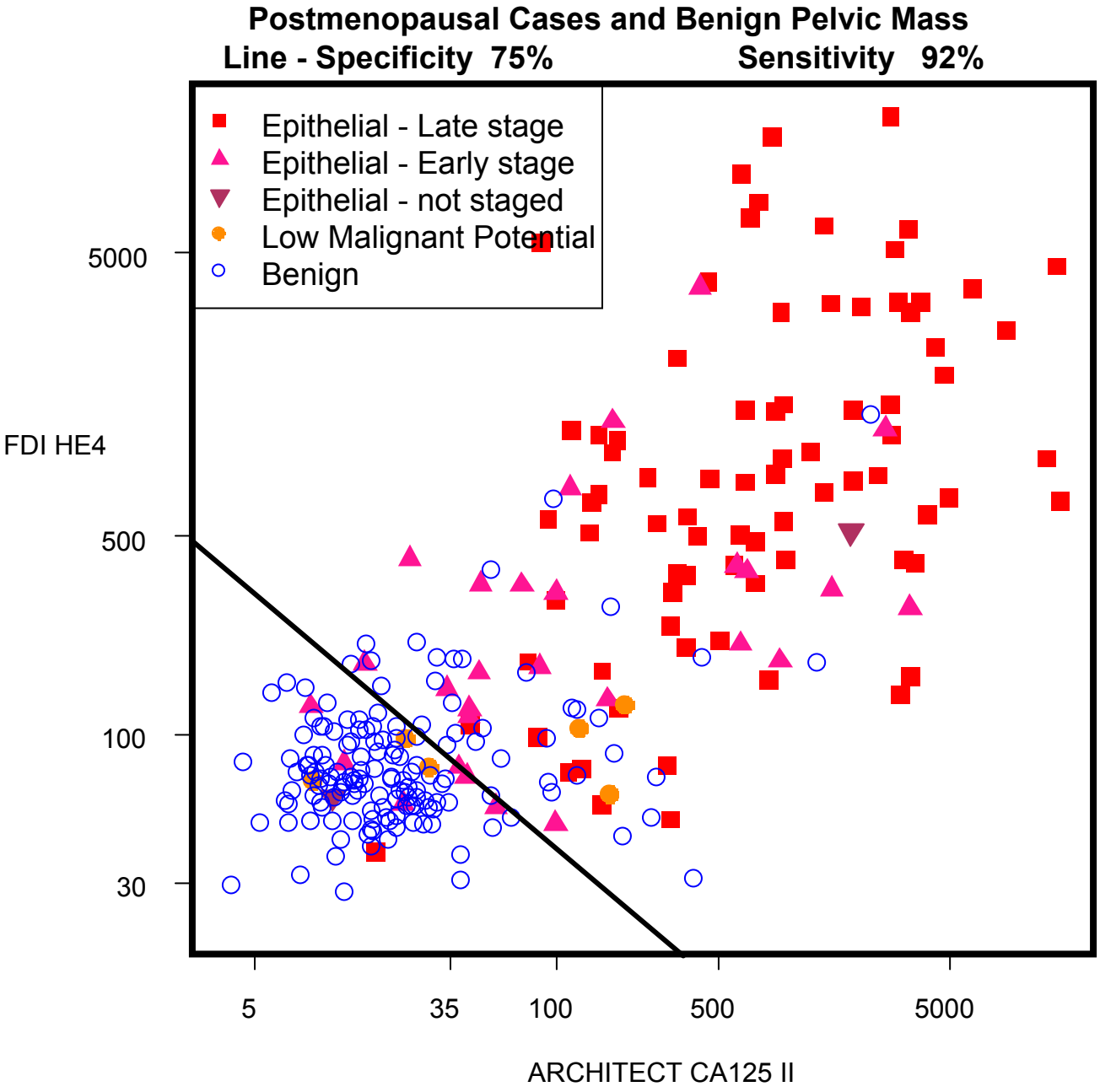


Figure 5 X-Y Scatterplot of CA 125II concentrations (X axis, log scale) and HE4 concentrations (Y axis, log scale) for postmenopausal women only



Several conclusions are evident from this data. First, when specificity is held at 75%, the sensitivity for epithelial ovarian cancer + low malignant potential (borderline) tumors is 89% (Table 24). More importantly, 121 of 129 of patients with an invasive epithelial ovarian cancer were classified into the High Risk subgroup giving a sensitivity of 94% for detecting invasive epithelial ovarian cancer alone. This level of sensitivity and specificity provides excellent stratification of patients into low and high risk groups. Although this test should not be used alone, but in conjunction with all available diagnostic information, several generalizations can be made relative to the performance of the test itself. When applied in clinical practice, the CA 125/HE4 algorithm would triage 56% of all patients to the obstetrician/gynecologist, and 44% of all patients to the gynecologic oncologist. Of the patients managed by the gynecologist, only 6% (17/279) would be expected to harbor ovarian cancer (Table 24), and of that 6%, only 6% of those (1/17) would have late stage cancer (Stages III/IV) (Table 25). Thus, if a gynecologist performed 1000 surgeries, based on the results of this test, 60 patients would have cancer, and of those 60 patients, 3-4 would have late stage cancer. Conversely, if the gynecologic oncologist treated only the patients at high risk as defined by this test, 60% (134/223) would have cancer (Table 24), with 66% of those (89/134) having late stage cancer (Table 25).

When the results are evaluated separately for premenopausal women, the stratification is less accurate than for postmenopausal women, as expected. However, the premenopausal population of women with pelvic mass presents a more challenging clinical dilemma. The PP using CA 125II and HE4 provides 76% (26/34) sensitivity in premenopausal women (Table 26). Thus the population triaged to the gynecologist includes 67% of all premenopausal patients, which includes 5% invasive epithelial ovarian cancers or low malignant potential tumors (8/157) (Table 26). More importantly, 16 of the 18 premenopausal patients with an invasive epithelial ovarian cancer were classified giving a sensitivity of 89% for detecting invasive epithelial ovarian cancer in the premenopausal group. None of these cancers would be of late stage (0/8) based on results of this study (Table 27). The gynecologic oncologist would treat only 33% of all premenopausal patients, however 34% (26/77) of these patients would be found to have cancer (Table 26), of which 35% would be late stage (9/26).

For postmenopausal patients, the stratification of patients is excellent, showing 92% (108/117) sensitivity for detecting invasive epithelial ovarian cancer and low malignant

potential tumors at 75% specificity (Table 28). When considering invasive epithelial cancers alone, 105 of 111 patients with an invasive epithelial ovarian cancer were classified in the High Risk subgroup resulting in a sensitivity of 95% in the postmenopausal group. A total of 46% of all postmenopausal patients would be triaged to the gynecologist with 7% (9/122) of those harboring cancer. A total of 54% of postmenopausal patients would be treated by the gynecologic oncologist with 74% (108/146) of those patients found to have cancer (Table 28).

In all cases, the cancers with predictive probabilities below the cut-points, tend to be low malignant potential (borderline) tumors or early stage cancers. In fact, only one Stage III/IV and two unstaged patients had predictive probabilities below the cut-points.

Overall, this test provides excellent stratification of patients with pelvic mass into subgroups with low and high risk. This stratification provides an accurate basis for triage of patients into the appropriate subspecialty of clinicians. This could be of benefit to patients and to providers to provide the most efficient use of limited resources.

13.1.1. COEFFICIENT OF VARIATION FOR ADDITIONAL ANALYSES

An analysis was completed to derive the estimates for the variation of the PI and the predictive probabilities associated with the thresholds set in the Additional Analyses for premenopausal and postmenopausal women. The CVs change according to the predictive probabilities and as expected, the CV of the PP is largest when the PP is smallest in both premenopausal and postmenopausal groups. The maximum CV of the PP is 13.5% in premenopausal women and 6.27% in postmenopausal women and in both cases, the accuracy of the PI is less than 15%. The CV of premenopausal and postmenopausal PP is represented in Figures 6 and 7, and details of the derivation of these data are presented in Attachment 16.12.

Figure 6 CV of Premenopausal PP given HE4 and CA 125 values

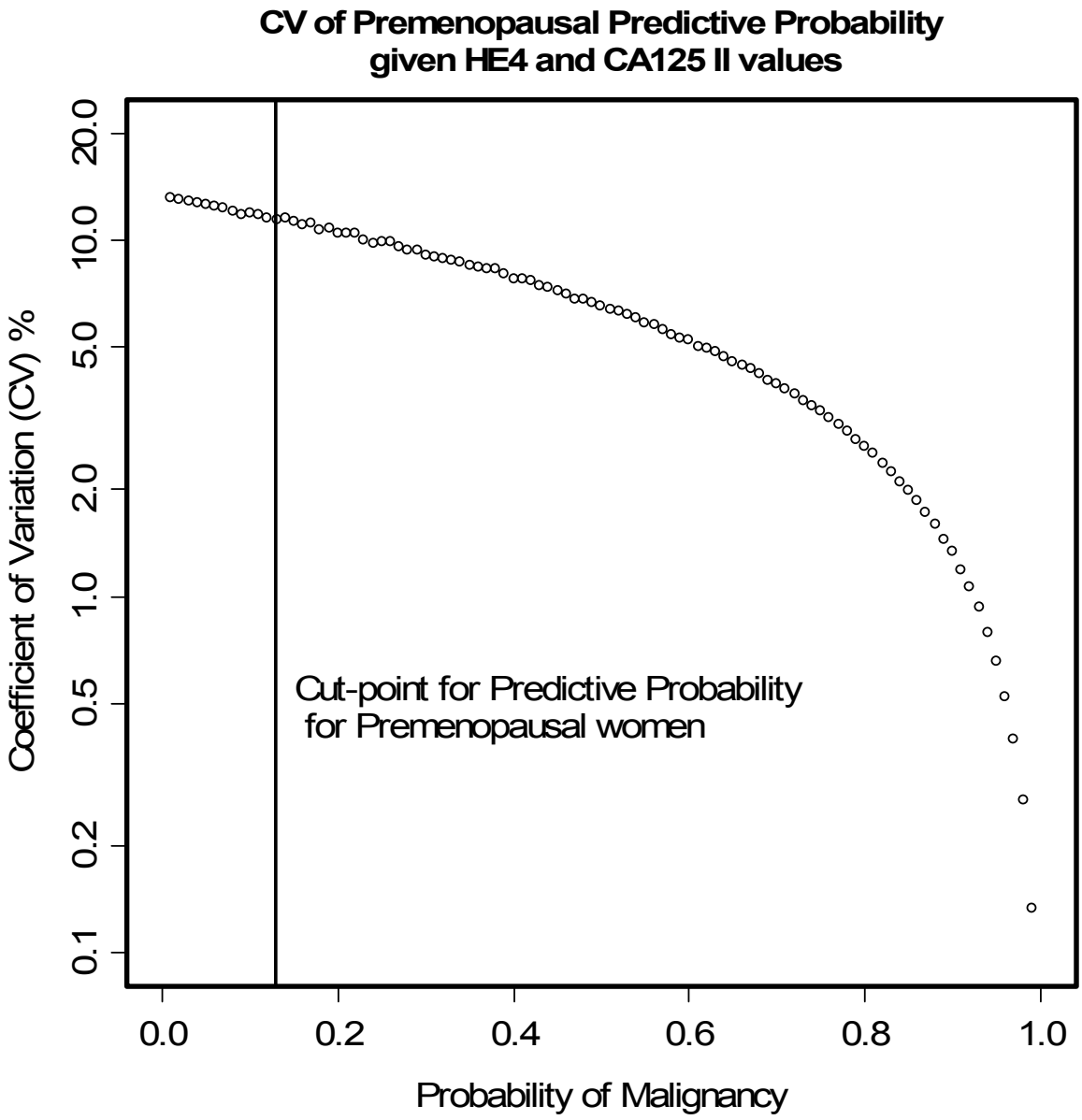
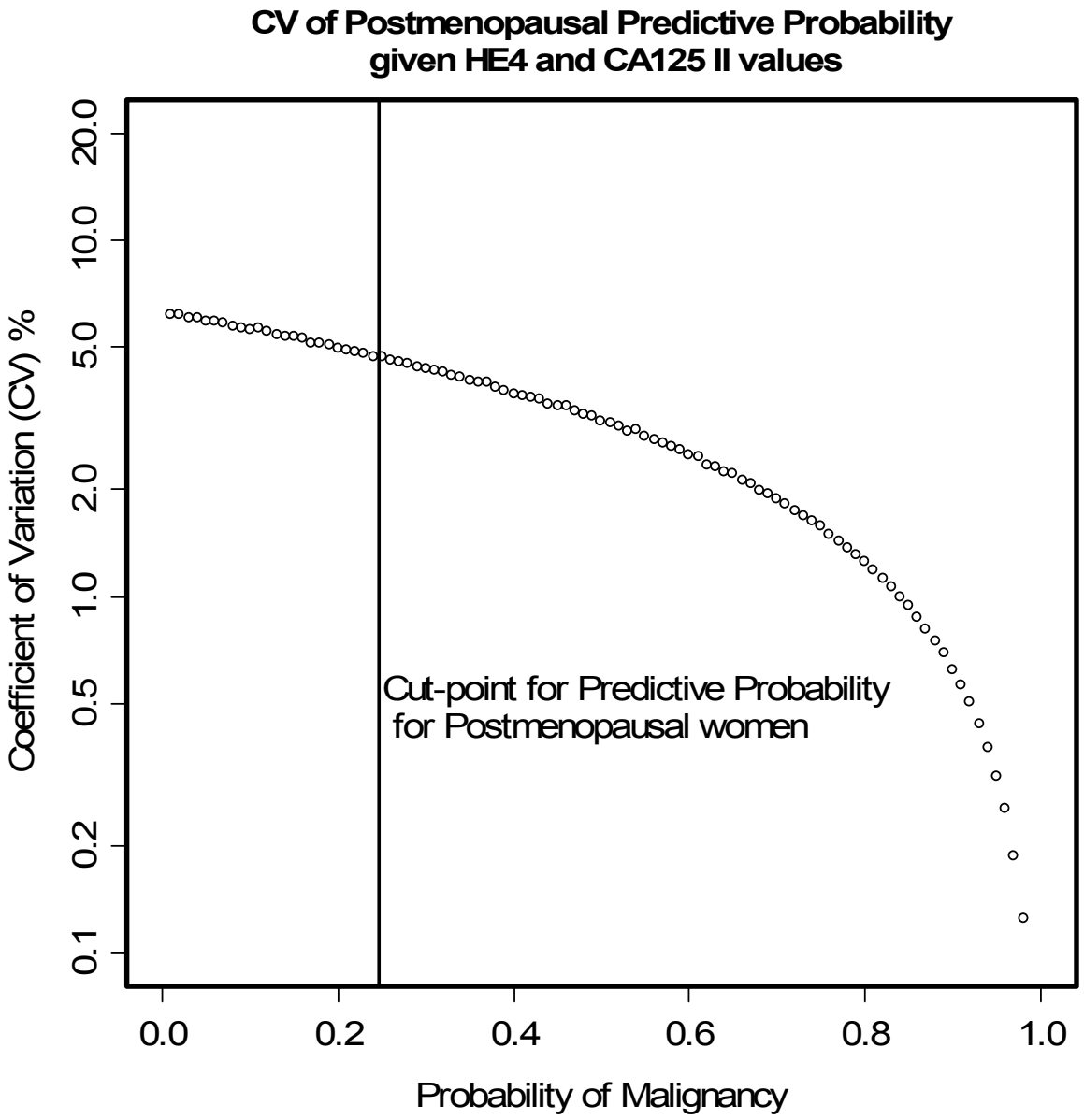


Figure 7 CV of Postmenopausal PP given HE4 and CA 125 values



13.3 ANCILLARY ANALYSES

13.3.1 DOT PLOTS

Shown below are three dot plots of HE4 assay values plotted as a log function and subdivided by the type of disease and by the stage of ovarian cancer (Figure 8). Similarly, Figure 9 is a plot of CA 125 assay concentrations on a log scale, and lastly,

the Predictive Probabilities are presented in Figure 10 on a linear scale for the same subgroups.

Figure 8 Dot plot of HE4 values on a log scale subdivided by disease subtype and stage of ovarian cancer.

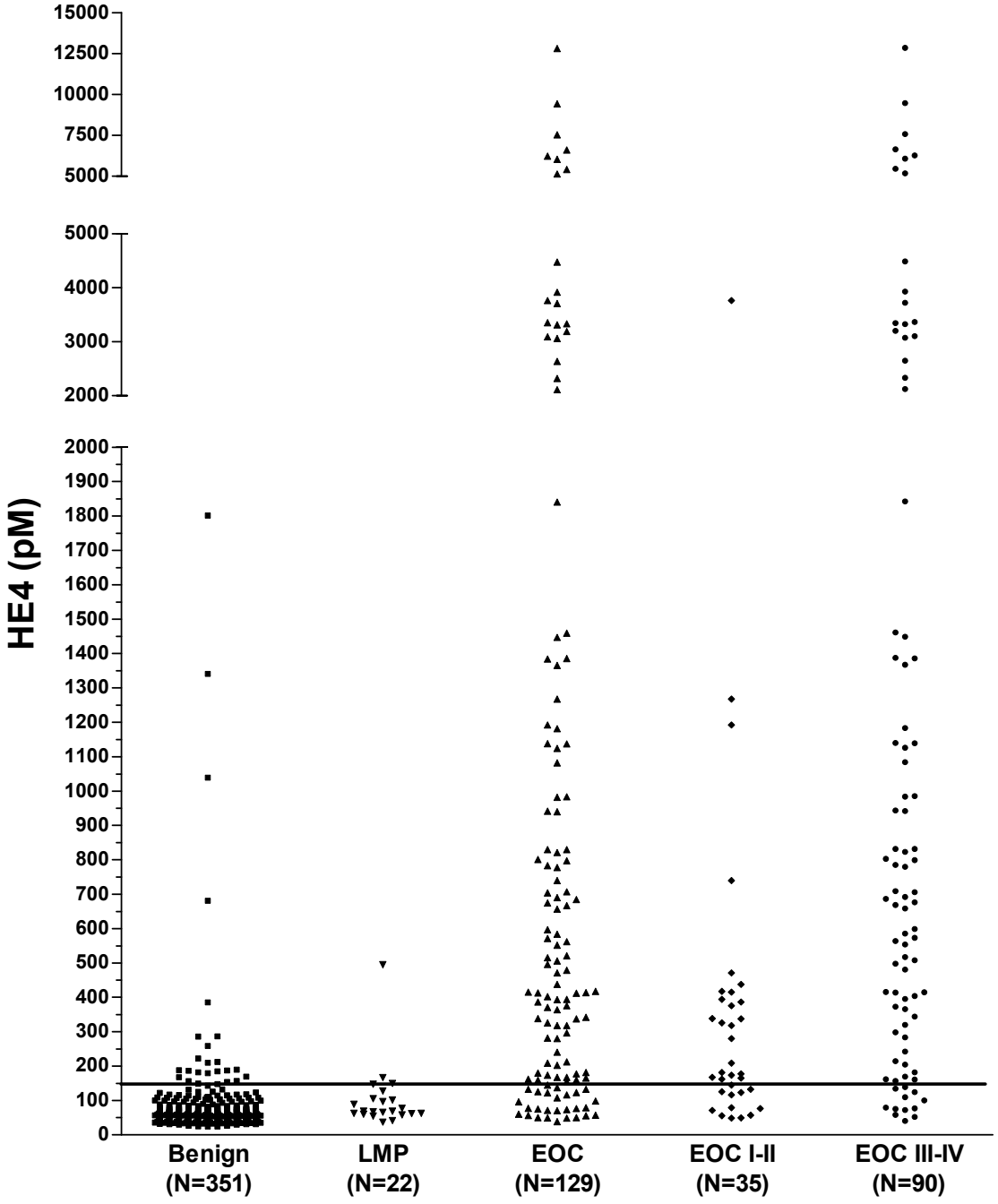


Figure 9 Dot plot of CA 125II values on a log scale subdivided by disease subtype and stage of ovarian cancer.

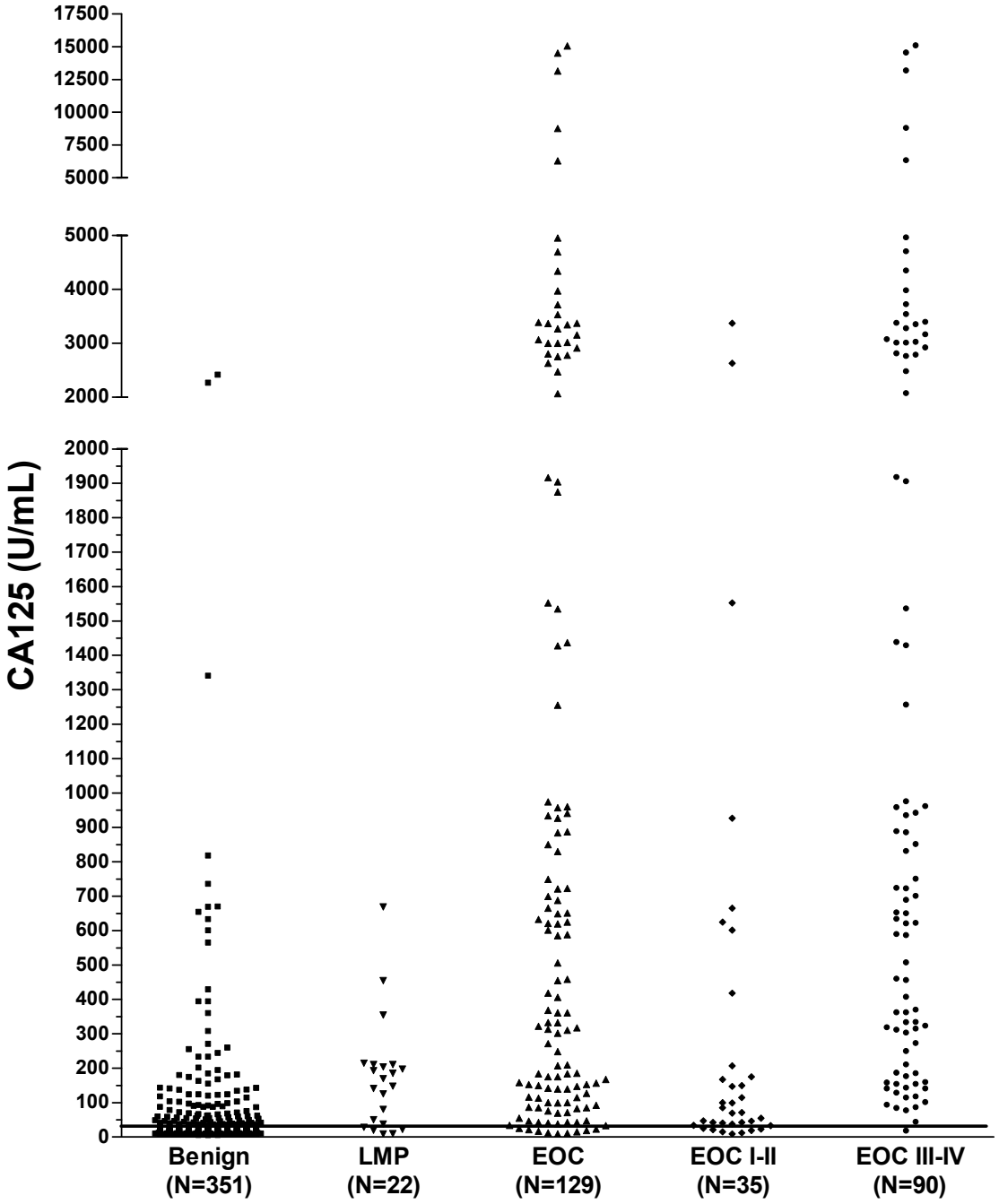
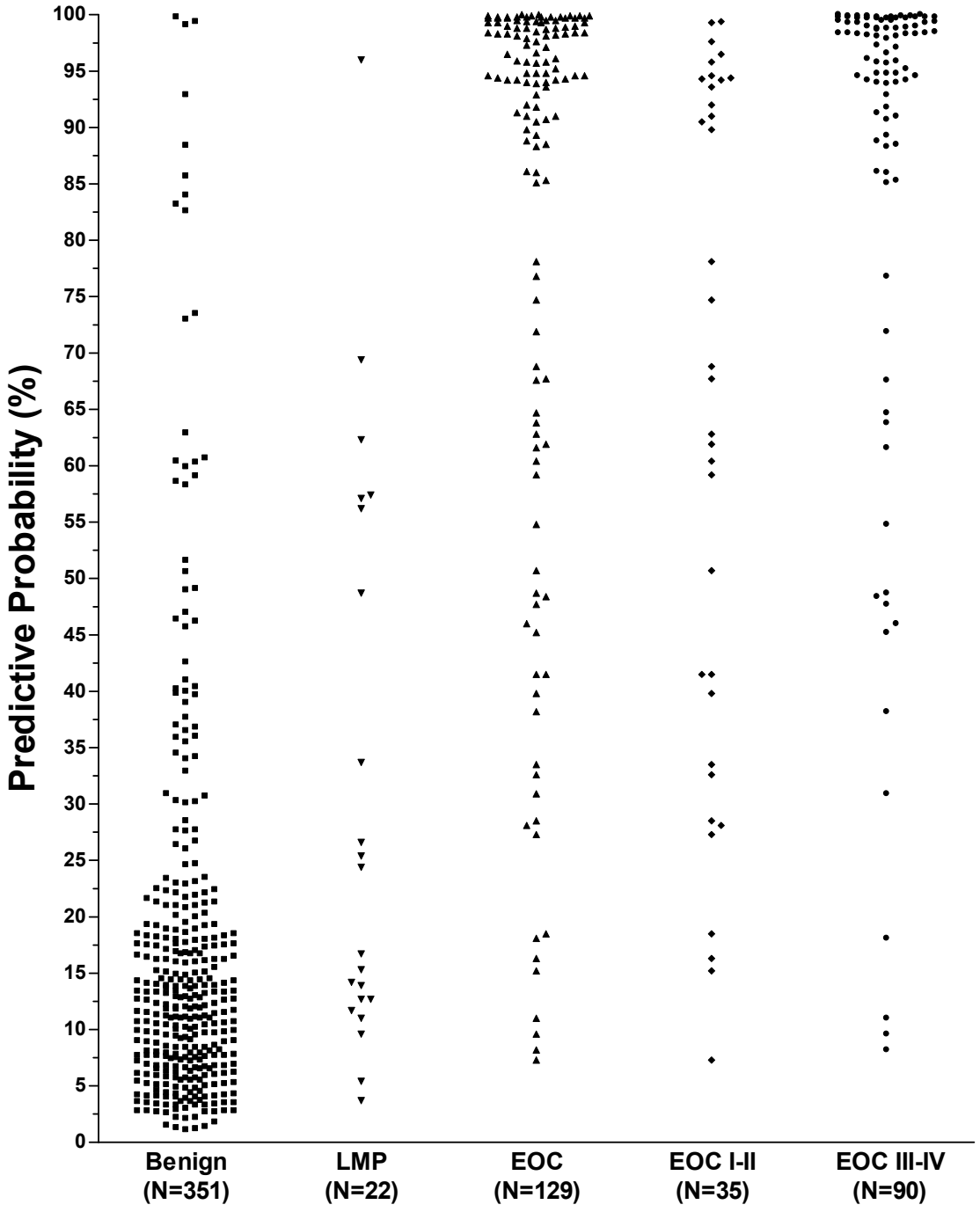


Figure 10 Dot plot of Predictive Probabilities on a linear scale subdivided by disease subtype and stage of ovarian cancer.



The dot plots above support the observation that the combination of CA 125 II and HE4 as a PP provide better stratification than either biomarker alone. The Upper Limit for HE4 was determined to be 150 pM, compared to that for CA 125 of 35 U/mL. When these limits are applied, it is apparent from the graphs that HE4 is more specific, but CA 125 appears to be more sensitive. Neither assay provides an optimum ability to stratify patients into low and high risk subgroups. However, the combination of markers with a logistic regression algorithm, provides improved separation of patients with benign diseases from those with cancer, as shown by the comparison of Figure 10 (PP) with Figures 8 and 9 (HE4 and CA 125 alone).

13.3.2 RECEIVER OPERATOR CHARACTERISTIC CURVES

We also evaluated the biomarker data using traditional ROC analysis. We compared the AUC separately for HE4 alone, CA 125 alone, and the PP. Results are shown in Figure 11 for all benign disease patients and patients with all stages of epithelial ovarian cancers and low malignant (borderline) tumors. Figure 12 shows the ROC curves for all benign disease patients and patients with all stages of epithelial ovarian cancers only. Figure 13 shows ROC curves for all benign disease patients and patients with Stages I/II epithelial ovarian cancers and low malignant potential (borderline) tumors. Figure 14 shows the ROC curves for all benign disease patients and patients with Stages I/II epithelial ovarian cancers only. Figure 15 shows the ROC curves for all premenopausal benign disease patients and patients with Stages I/II epithelial ovarian and low malignant potential (borderline) tumors. Figure 16 shows the ROC curves for all postmenopausal benign disease patients and patients with Stages I/II epithelial ovarian and low malignant potential (borderline) tumors.

Figure 11 **ROC curves for all benign disease patients and patients with all stages of epithelial ovarian cancers and low malignant potential tumors. LN(CA125) + LN(HE4) in the figure refers to the ROMA.**

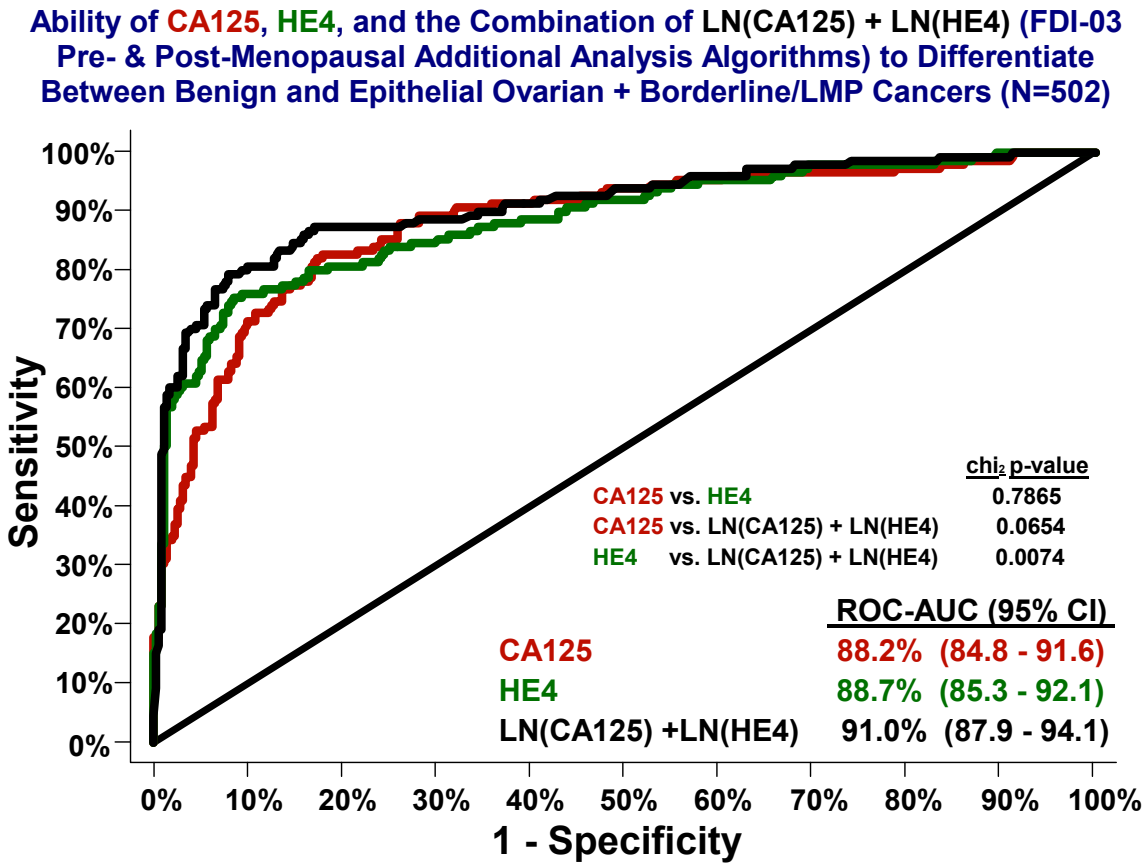


Figure 12 **ROC curves for all benign disease patients and patients with all stages of epithelial ovarian cancers only. LN(CA125) + LN(HE4) in the figure refers to the ROMA.**

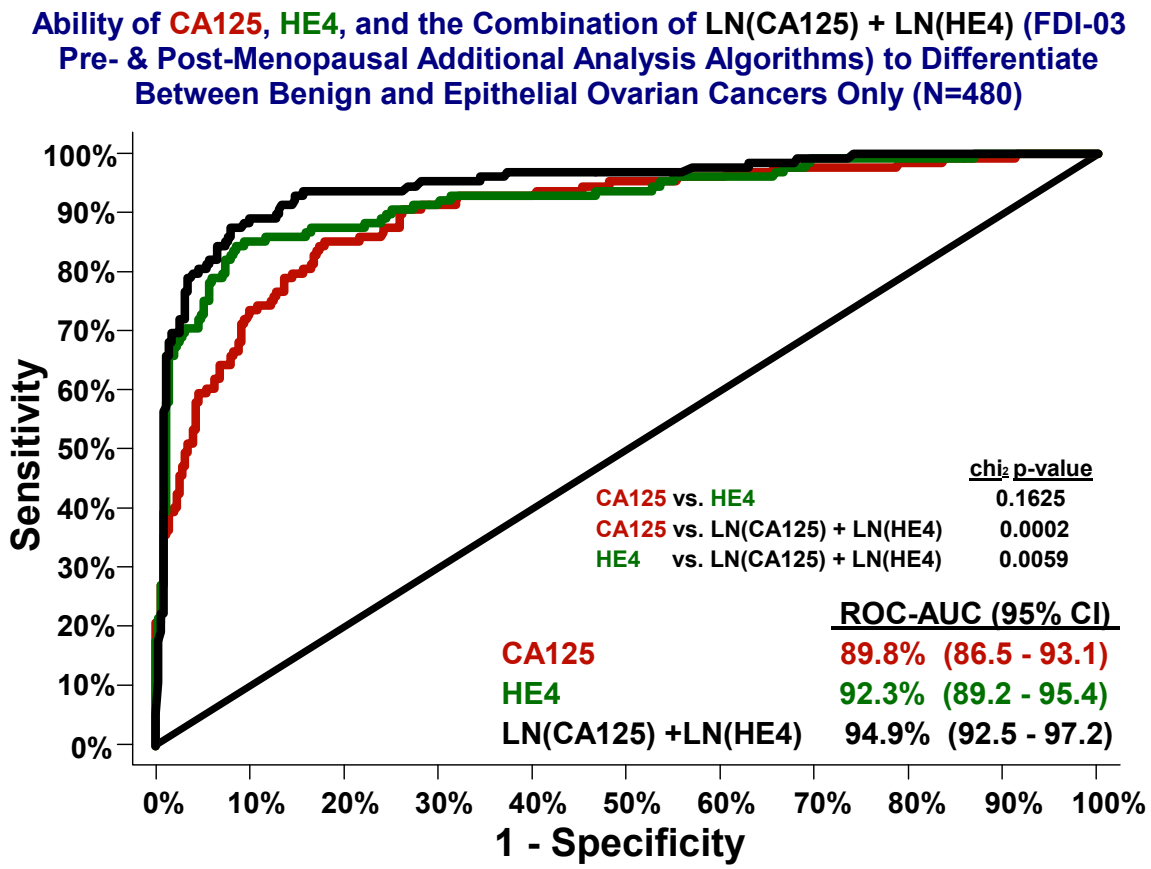


Figure 13 **ROC curves for all benign disease patients and patients with Stages I/II epithelial ovarian cancers and low malignant potential tumors. LN(CA125) + LN(HE4) in the figure refers to the ROMA.**

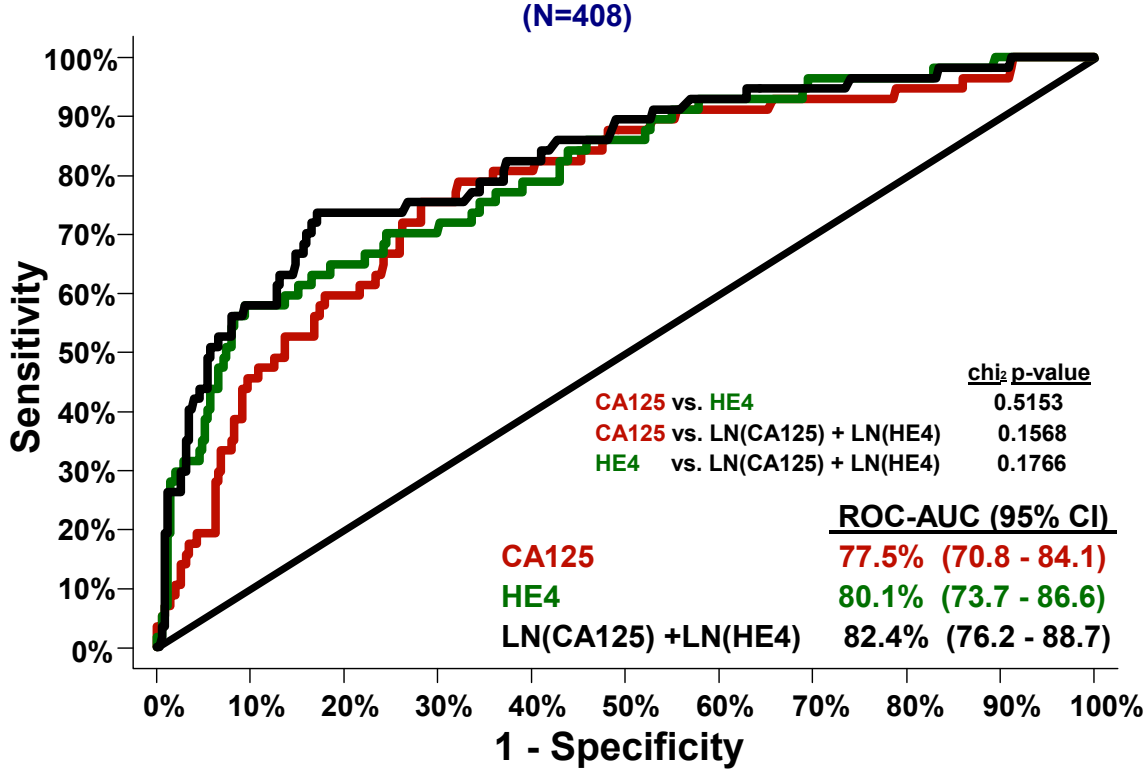


Figure 14 **ROC curves for all benign disease patients and patients with Stages I/II epithelial ovarian cancers only. LN(CA125) + LN(HE4) in the figure refers to the ROMA.**

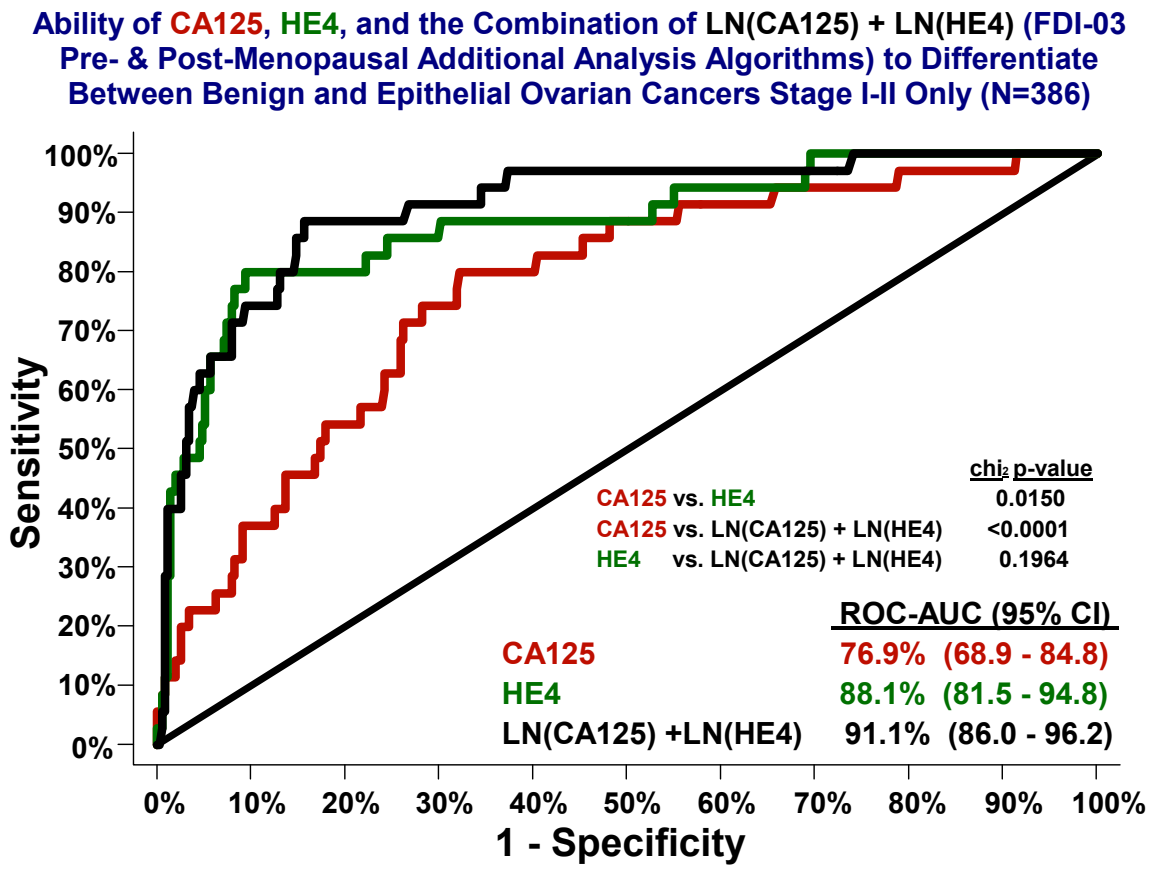


Figure 15 **ROC curves for all premenopausal benign disease patients and patients with epithelial ovarian cancers and low malignant potential tumors. LN(CA125) + LN(HE4) in the figure refers to the ROMA.**

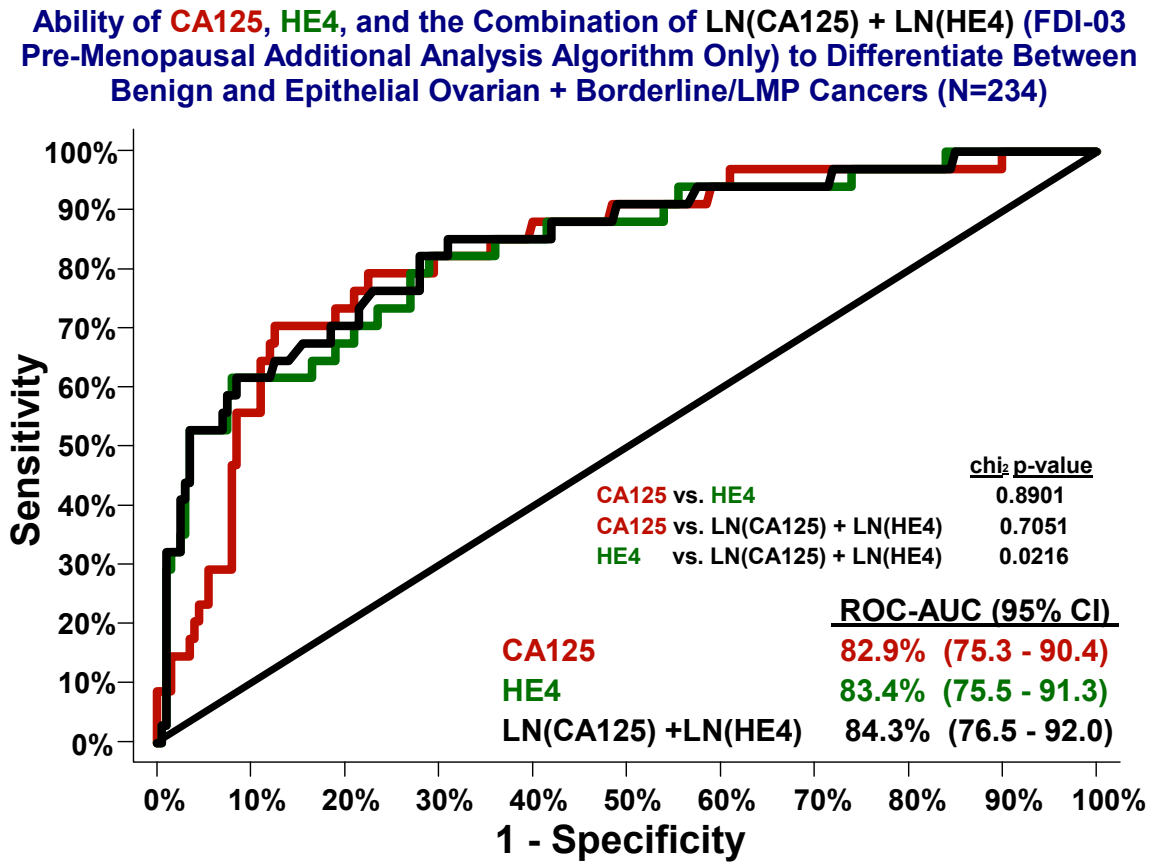
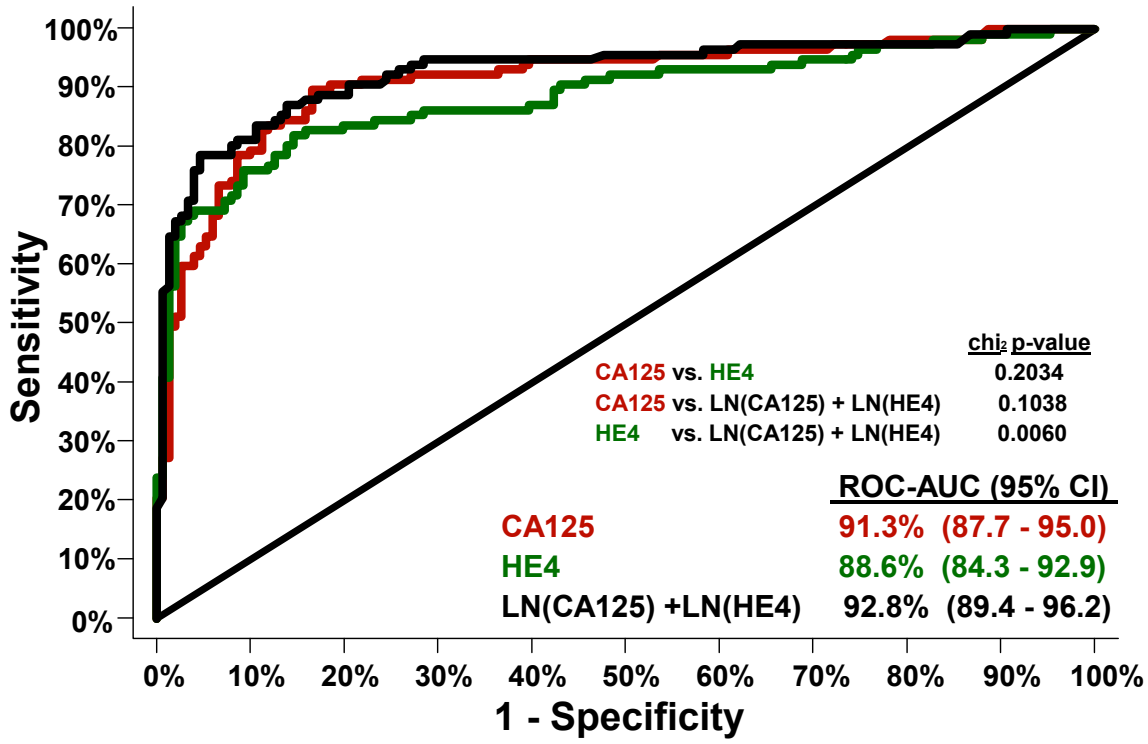


Figure 16 **ROC curves for all postmenopausal benign disease patients and patients with epithelial ovarian cancers and low malignant potential tumors. LN(CA125) + LN(HE4) in the figure refers to the ROMA.**



These data demonstrate that for all ROC curves shown above in Figures 11-16, the AUC for the PP was higher than for either CA 125 or HE4 alone.

In addition, when only early stage epithelial ovarian cancers (Stage I/II) were evaluated, CA 125 showed inferior performance to PP in early stage disease ($p \leq 0.0001$, Figure 14), and the AUC for CA 125 was statistically significantly lower when compared with the AUC for HE4 in Stage I/II cancers ($p=0.0150$, Figure 14), again using only epithelial ovarian cancers. The AUCs for CA 125, HE4 and the PP were 76.9%, 88.1% and 91.1% respectively indicating a significant improvement through the use of the PP as compared to the use of CA 125 in the early stage epithelial cancers.

While the two biomarkers showed similar AUCs for epithelial and low malignant potential ovarian cancers combined, they showed different profiles for individual patients. This is evidenced by the data below in Table 34 that lists representative patients with PP above the cutoffs, but that had low levels of one biomarker and elevated levels of the other. These data demonstrate that the combination of two biomarkers in a logistic model to predict risk of ovarian cancer is clearly better than the use of either biomarker alone.

Table 34 Representative Patients with Predictive Probabilities above the respective cut-offs for premenopausal (13.1%) and postmenopausal (27.7%) women, where the PP is driven by only one biomarker.

Biomarker Driving PP	Menopausal Status	Histological Subtype	PP*	[CA 125] (U/mL)	[HE4] (pM)
CA 125	premenopausal	Low Malignant Potential	13.9	203.6	62.6
	premenopausal	Low Malignant Potential	16.7	214.8	18.5
	postmenopausal	Low Malignant Potential	54.4	210.5	65.6
	premenopausal	Epithelial Ovarian Cancer	18.1	322.1	75.6
	postmenopausal	Epithelial Ovarian Cancer	45.2	156.6	56.6
	postmenopausal	Epithelial Ovarian Cancer	54.8	313	50.3
HE4	premenopausal	Epithelial Ovarian Cancer	94.6	33.2	470.9
	postmenopausal	Epithelial Ovarian Cancer	41.5	33.7	144.5
	postmenopausal	Epithelial Ovarian Cancer	35.1	18.8	167.5
	postmenopausal	Epithelial Ovarian Cancer	61.9	23.2	417.1
	postmenopausal	Epithelial Ovarian Cancer	32.6	14.9	177.8

*Derived from Additional Analysis using different equations for premenopausal and postmenopausal women

14 OVERALL CONCLUSIONS FROM THE STUDY

1. The Intended Use for the HE4 EIA is "The HE4 EIA is an enzyme immunometric assay for the quantitative determination of HE4 in human serum. The HE4 EIA used in conjunction with the ARCHITECT CA 125 II assay creates a predictive probability of epithelial ovarian cancer using a mathematical function referred to as the Risk of Ovarian Malignancy Algorithm (ROMA™), for use in premenopausal and post-menopausal women presenting with an adnexal mass who are candidates for surgical intervention. Subjects categorized as low risk for epithelial ovarian cancer using the ROMA value may have surgical intervention performed by a non-oncology specialist. The results must be interpreted in conjunction with other clinical findings in accordance with standard clinical management guidelines. The assay is not indicated as an aid in a decision to proceed to surgery."

   NOTE: In the labeling, the mathematical function determining Predictive Probability (PP) will be referred to as the ROMA.

The population of patients that support the Intended Use from this study includes the "Intent-to-Evaluate" population of women with known menopausal status and invasive epithelial ovarian cancers and low malignant potential tumors.

Based on results found with this population, the triage of women with pelvic mass into populations with Low Risk and High Risk determined by PP represents a significant improvement in the management of these patients. By defining a Low Risk population, half of women referred to gynecologic oncologists with pelvic masses using the current clinical standard of care could remain in the community with their gynecologist for surgery. This Low Risk population includes 75% of all benign gynecologic cases. The proportions of women with cancer in the two groups are appropriate for improved management of these patient groups.

2. At a set specificity of 75%, the combined use of CA 125II and HE4 in a logistic algorithm provides 89% sensitivity for premenopausal and postmenopausal patients combined for detecting invasive epithelial ovarian cancer and low malignant potential tumors and a 94% sensitivity for detecting invasive epithelial ovarian cancer alone. The algorithm also provides a 95%, 93%, and 94% NPV for premenopausal women, postmenopausal women, and all women combined, respectively when considering both epithelial ovarian cancers and low malignant potential tumors.

3. If this algorithm were applied in clinical practice, over 50% (56% in this study) of patients referred to gynecologic oncologists using the current standard of care could have their surgery performed safely by gynecologists in community health care settings (Table 24). Only 6% (17/279, Table 24) of patients that remain under the gynecologist's care would be expected to harbor ovarian cancer. Of the 6% of patients found to have cancer, only 6% (1/17, Table 25) would be expected to harbor late stage invasive epithelial ovarian cancer.

4. Of the 44% of patients in the high risk subgroup triaged to the gynecologic oncologist, only 40% (89/223) would be found to have benign diseases. A total of 89% (134/151) of all invasive epithelial ovarian cancers and low malignant potential tumors would be included in this High Risk subgroup, and 66% (89/134) would have late stage (stages III/IV) invasive epithelial ovarian cancer. In addition, 94% (121/129) of all invasive epithelial ovarian cancer would be classified to the High Risk subgroup.

5. The CA 125II and HE4 biomarkers behave differently in premenopausal and postmenopausal women with pelvic mass (Figures 4, 5). For this reason, the PP should be determined using the separate equations derived as shown in Section 13.3. This provides for cut-points for PP of 13% for premenopausal women and 28% for postmenopausal women, rounded for convenience in implementation.

6. The factor for HE4 is higher in the logistic regression equations described in Section 13.3. This is particularly true for premenopausal women where the factor for HE4 is 38-fold higher than that for CA 125II (2.38 vs 0.0626). This difference in the factors in the logistic regression equations demonstrates that the model relies more heavily on the HE4 concentrations than the CA 125II values.

7. While the logistic regression factors for HE4 are greater than the factors for CA 125II for both premenopausal and postmenopausal women, both biomarkers contribute to the PP for individual patients (Table 34).

8. HE4 as an individual biomarker showed a higher AUC by ROC analysis than for CA 125II (Figures 11-16). This is particularly true for early stage patients, and especially for early stage patients with invasive epithelial ovarian cancer (Figures 13, 14).

9. The stratification of pelvic mass patients into groups with low and high risk of ovarian cancer is maximized by the use of a PP compared with individual concentrations of CA 125II or HE4 alone. This is demonstrated visually in Figures 8-10. The PP values show a visually distinct separation near the cutoffs selected from this study (13.1% premenopausal and 27.7% postmenopausal), and cluster as the PP nears 100% (Figure 8).

10. The data presented in this report supports the intended use for the HE4 EIA because the low and high risk subgroups for premenopausal and postmenopausal women provide excellent stratification of women with benign pelvic masses from those with cancerous pelvic masses. This improves the current standard of care by decreasing the number of women referred unnecessarily to a gynecologic oncologist for treatment of their pelvic mass.

15 REFERENCES

1. National Institutes of Health Consensus Development Conference Statement. Ovarian Cancer: screening, treatment, and follow-up. *Gynecol Oncol*. 1994;55:S4-S14.

2. Finkler NJ, Benacerraf B, Lavin PT, Wojciechowski C, Knapp RC: Comparison of serum CA 125, clinical impression and ultrasound in the preoperative evaluation of ovarian masses. Obstet Gynecol 1988;72:659-64.

3. ACOG Practice Bulletin. Clinical Management Guideline for Obstetrician-Gynecologists. Management of Adnexal Masses. *Obstet Gynecol.* 2007;110:201-213.

4. Gostout BS, Brewer MA. Guidelines for referral of the patient with an adnexal mass. *Clin Obstet Gynecol.* 2006;49(3):448-458.

5. Paulsen T, Kjaerheim K, Kaern J, Tretli S, Trope C. Improved short-term survival for advanced ovarian, tubal, and peritoneal cancer patients operated at teaching hospitals. *Int J Gynecol Cancer.* 2006;16(suppl 1):11-17.

6. Earle CC, Schrag D, Neville BA, Yabroff KR, Topor M, Fahey A et al. Effect of surgeon specialty on processes of care and outcomes for ovarian cancer patients. *J Natl Cancer Inst.* 2006;98(3):172-180.

7. Engelen MJ, Kos HE, Willemse PH, et al. Surgery by consultant gynecologic oncologists improves survival in patients with ovarian carcinoma. *Cancer*. 2006;106(3):589-598.

8. American Cancer Society. Cancer facts and figures 2000. Atlanta, GA:American Cancer Society; 2000.

9. American Cancer Society. Cancer facts and figures 2007. Atlanta, GA:American Cancer Society; 2007.

10. Reis LA, Harkins D, Krapchow M, et al. Seer cancer statistics review, 1975-2003. Bethesda (MD):National Cancer Institute;2006

11. Maggino T, Gadducci A, D'Addario V, et al: Prospective Multicenter Study on CA 125 in postmenopausal pelvic masses. *Gynecol Oncol.* 1994:54;117-123.

12. Roman LD, Muderspach LI, Stein SM, Laifer-Narin S, Groshen S, Morrow CP Pelvic examination, tumor marker level, and Gray-Scale and Doppler sonography in the prediction of pelvic cancer. *Obstet Gynecol.* 1997:89;493-500.

13. DePriest PD, Shenson D, Fried A, et al. A morphology index based on sonographic findings in ovarian cancer. *Gynecol Oncol.* 1993:51;7-11.

14. Jacobs I, Oram D, Fairbanks J Turner J, Frost C, Grudzinskas JG. A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer. *Br J Obstet Gynaecol.* 1990:97;922-929.

15. Tingulstad S, Hagen B, Skjeldestad FE, et al. Evaluation of a risk of malignancy index based on serum CA 125, ultrasound findings, and menopausal status in the pre-operative diagnosis of pelvic masses. *Br J Obstet Gynaecol*. 1996:103;826-831.

16. Torres JC, Derchain SF, Faundes A, Gontijo RC, Martinez EZ, Andrade LA.. Risk-of-malignancy index in preoperative evaluation of clinically restricted ovarian cancer. *Sao Paulo Med J.* 2002120(3); 72-76.

17. Manjunath AP, Pratapkumar, Sujatha K, Vani R. Comparison of three risk of malignancy indices in evaluation of pelvic masses. *Gynecol Oncol.* 2001:81;225-229.

18. Bailey J, Tailor A, Naik R, Lopes A, Godfrey K, Hatem HM, Monaghan J. Risk of malignancy index for referral of ovarian cancer cases to a tertiary center: does it identify the correct cases? *Int J Gynecol Cancer.* 2006;16(suppl 1):30-34.

19. Sohaib SA, Sahdev A, Van Trappen P, Jacobs IJ, Reznek RH. Characterization of adnexal mass lesions on MR imaging. *AJR Am J Roentgenol.* 2003:180;1297-1304.

20. Kupesic S, Kurjak A. Contrast-enhanced, three-dimensional power Doppler sonography for differentiation of adnexal masses. *Obstet Gynecol.* 2000:96;452-458

21. Schutter EMJ, Sohn C, Kristen P, et al. Estimation of probability of malignancy using a logistic model combining physical examination, ultrasound, serum CA 125, and serum CA 72-4 in postmenopausal women with a pelvic mass: An international multicenter study. *Gynecol Oncol.* 1998:69;56-63.

22. Nardo LC, Kroon ND, Reginald PW. Persistent unilocular ovarian cysts in a general population of postmenopausal women: Is there a place for expectant management? *Obstet Gynecol.* 2003:102;589-593.

23. Giede KC, Kieser K, Dodge J, Rosen B. Who should operate on patients with ovarian cancer? An evidence-based review. *Gynecol Oncol.* 2005;99:447-461.

24. Bristow RE, Zahurak ML, del Carmen MG, et al. Ovarian cancer surgery in Maryland: volume-based access to care. *Gynecol Oncol.* 2004:93;353-360.

25. Kumpulainen S, Kuoppala T, Leminen A, et al. Surgical treatment of ovarian cancer in different hospital categories - A prospective nation-wide study in Finland. *Eur J of Cancer.* 2006:42;388-395.

26. Board RE, Bruijns CTPH, Pronk AE, et al. Stage- and CA 125-related survival in patients with epithelial ovarian cancer treated at a cancer center. Int *J Gynecol Cancer.* 2006;16 (suppl 1):18-24.

27. McGowan L, Lesher LP, Norris HJ, Barnett M. Misstaging of ovarian cancer. *Obstet Gynecol.* 1985;65(4):568-572.

28. Olaitan A, Weeks J, Mocroft A, Smith J, Howe K, Murdoch J. The surgical management of women with ovarian cancer in the south west of England. *Br J Cancer.* 2001;85(12):1824-1830.

29. Carney ME, Lancaster JM, Ford C, Tsodikov A, Wiggins CL. A population-based study of patterns of care for ovarian cancer: who is seen by a gynecologic oncologist and who is not? *Gynecol Oncol.* 2002;84:36-42.

30. Malkasian GD, Jr, Knapp RC, Lavin PT, et al. Preoperative evaluation of serum CA 125 levels in premenopausal and postmenopausal patients with pelvic masses: discrimination of benign from malignant disease. *Am J Obstet Gynecol.* 1988;159(2):341-346.

31. Lu KH, Patterson AP, Wang L, et al. Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. *Clin Cancer Res.* 2004;10:3291-3300.

32. Moore RG, Brown AK, Miller MC, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. *Gynecol Oncol.* 2008;108(2):402-408.

33. Lachin JM. Sample size determinations for rxc comparative trials. *Biometrics.* 1977;33:315-324.

34. World Health Organization. Research on the Menopause in the 1990s. Technical Report Series, 866, Geneva Switzerland.

35. Messinis IE. Ovarian feedback, mechanism of action and possible clinical implications. *Hum Reprod Update*. 2006;12: 557 – 571.

36. Burger HG, Dudley EC, Robertson DM, Dennerstein L. Hormonal changes in the menopause transition. *Recent Prog Horm Res*.2002;57:257 - 275.

37. Gracia CR, Sammel MD, Freeman EW, et al . Defining menopause status: creation of a new definition to identify the early changes of the menopausal transition. *Menopause*: 2005;12(2): 128-135.

38. Hale GE, Zhao X, Hughes CL, Burger HG, Robertson DM, Fraser IS. Endocrine Features of Menstrual Cycles in Middle and Late Reproductive Age and the Menopausal Transition Classified According to the Staging of Reproductive Aging Workshop (STRAW) Staging System. *J Clin Endocrinol Metab.* 2007; 92(8):3060-3067.

39. Cooper GS, Thorp JM. FSH levels in relation to hysterectomy and to unilateral oophorectomy. *Obstet Gynecol*.1999;94:969-972.

16 ATTACHMENTS

ATTACHMENT 16.1 HE4 EIA PACKAGE INSERT

ATTACHMENT 16.2 ARCHITECT CA 125II PACKAGE INSERT

ATTACHMENT 16.3 ARCHITECT FSH PACKAGE INSERT

ATTACHMENT 16.4 PROTOCOL AND PROTOCOL AMENDMENTS

ATTACHMENT 16.5 CASE REPORT FORMS

ATTACHMENT 16.6 TEMPLATE INFORMED CONSENT FORM

ATTACHMENT 16.7 TEMPLATE IRB APPROVAL

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039	Walter Gajewski, MD New Hanover Regional Medical Center Wilmington, NC	**New Hanover Regional Medical Center,** Wilmington, NC
040	Laura Havrilesky, MD Duke University Medical Center Durham, NC	**Duke University Health System,** Durham, NC
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063	Alexander Burnett, MD University of Arkansas for Medical Sciences Little Rock, AR	**Arkansas Cancer Research Center** **University of Arkansas for Medical Sciences,** Little Rock, AR

ATTACHMENT 16.8 BIO/CURRICULUM VITAES: INVESTIGATORS, CENTRAL PATHOLOGISTS, AND STATISTICIANS

ATTACHMENT 16.9 FINANCIAL DISCLOSURE FORMS: INVESTIGATORS

List of Final Financial Disclosure Forms

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ATTACHMENT 16.10 DATASETS

ATTACHMENT 16.11 PROTOCOL DEVIATION LIST

ATTACHMENT 16.12 STATISTICAL REPORT: ADDITIONAL ANALYSIS

ATTACHMENT 16.13 STATISTICAL REPORT: ANCILLARY STUDIES

ATTACHMENT 16.14 STATISTICAL REPORT: UPDATED ANCILLARY STUDIES

ATTACHMENT 16.15 STATISTICAL REPORT: JUSTIFICATION FOR FSH CUTPOINT