TO: ALL PRINCIPAL INVESTIGATORS/NURSES/DATA MANAGERS

FROM: PROTOCOL SECTION

DATE: AUGUST 15, 2016

RE: PROTOCOL GOG-0210—Amendments 9 and 10

Protocol Title: “A MOLECULAR STAGING STUDY OF ENDOMETRIAL CARCINOMA”
NCI Version 07/28/2016
Study Chair: William T. Creasman, M.D., (843) 792-4509, creasman@musc.edu

IRB Review Recommendation:

(X) No review required
( ) Expedited review; however, site IRB requirements take precedence
( ) Full board review recommended because there have been changes to the Informed Consent
and/or the risk information

Please direct questions about the recommended level of IRB review to your local IRB. The local IRB is responsible for making this determination. If your local IRB does not agree with the GOG’s recommended level of review, please document the IRB’s decision, and the rationale for the decision, in your study files.

The following changes have been made and become effective August 15, 2016:

SUMMARY OF CHANGES

For Protocol Amendments #9 and 10 to: GOG-0210

NCI Protocol #: GOG-0210
Local Protocol #: GOG-0210

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<td>• Jeffrey Boyd, M.D. has been removed as a Translational Research Chair.</td>
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<td>• Heather Lankes, PH.D. is now the Translational Research Scientist.</td>
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Informed Consent

NCI Version Date is now 07/28/2016.
PROTOCOL GOG-0210
A MOLECULAR STAGING STUDY OF ENDOMETRIAL CARCINOMA

POINTS:
- PER CAPITA – 13 points
- Up to 6 extra points for submission of metastatic or recurrent specimens
- MEMBERSHIP – 3 points

The 13 per capita points will be distributed for the mandatory surgical staging with the collection of detailed surgical-pathologic information (3 points), follow-up data for up to 10 years (3 points), and the epi questionnaire (i.e., 1 point). An additional 6 points will be distributed for the submission of the pre-op serum, the intra-op urine, primary tumor (frozen and formalin-fixed), normal tissue (frozen and formalin-fixed), post-op serum and 3-year follow-up serum specimens. During surgical staging, if gross metastatic tumor tissue is present, an additional point will be distributed for the submission of metastatic tumor (frozen and formalin-fixed) specimens. For patients with persistent or recurrent disease, a recurrent serum specimen will be required for all patients and one point will be distributed for the submission of this specimen. Furthermore, recurrent tumor (frozen and formalin-fixed) specimens will be required for cases where persistent or recurrent tumor tissue can be safely excised, and 4 points will be distributed for the submission of the two recurrent tumor specimens.

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This protocol was designed and developed by the Gynecologic Oncology Group (GOG). It is intended to be used only in conjunction with institution-specific IRB approval for study entry. No other use or reproduction is authorized by GOG nor does GOG assume any responsibility for unauthorized use of this protocol.

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OPEN TO PATIENT ENTRY SEPTEMBER 22, 2003
REVISED JUNE 14, 2004; REVISED NOVEMBER 8, 2004; REVISED SEPTEMBER 18, 2006
REVISED SEPTEMBER 24, 2007; REVISED DECEMBER 27, 2010; REVISED JANUARY 18, 2011; CLOSED TO PATIENT ENTRY DECEMBER 1, 2011, REVISED AUGUST 15, 2016
Patients must meet the eligibility criteria in Section 3.11. Specimen collection is an essential and mandatory component of this molecular staging study. (11/08/2004) (09/18/2006) (09/24/2007)

Patients who are suitable candidates for this study will have to sign an approved informed consent.

A pre-op serum specimen will need to be prepared for all patients from 10 ml of blood drawn on the day of and prior to the surgical staging OR when pre-op bloods are drawn. Collection of the specimen at the time the intravenous line is started for surgery is acceptable provided that the patient has not received pre-operative medications as these medications may alter the profile of proteins in serum.

Patient registration required prior to surgery (12/27/2010)

An abdominal hysterectomy or laparoscopic-assisted vaginal hysterectomy, bilateral salpingo-oophorectomy, bilateral pelvic and para-aortic lymphadenectomy,* and peritoneal cytology will need to be performed with intraperitoneal biopsies when indicated. (06/14/2004) (09/18/2006)

Primary tumor (for all patients) and metastatic tumor (for cases where gross metastatic tumor is present) will need to be dissected from tissue excised during the surgery along with normal adjacent tissue, and both frozen and formalin-fixed specimens will need to be prepared from each type of dissected tissue. In addition, a urine specimen will need to be obtained from pre-op, intra-op or post-op the Foley catheter from all patients. (06/14/2004)

Each patient will need to be asked to complete a Gynecologic Questionnaire within 6 weeks of entry. (06/14/2004)

A post-op serum specimen will need to be obtained from each patient during the post-op clinic visit, which should be within 6 weeks after surgery. (06/14/2004)

All patients will need to be followed every 3 months for the first two years, every 6 months for the next 3 years, and yearly for the next 5 years following the surgery. (1/18/2011)

A follow-up serum specimen will need to be obtained from each patient during the 3-year follow-up clinic visit after the surgery.

For those patients with progressive or recurrent disease, a recurrent serum specimen will need to be obtained for all patients and a recurrent tumor specimen will need to obtained when the tumor tissue can be safely excised.

*Patients with Stage IV intra-abdominal disease NOT debulked to less than 2 cm DO NOT require a lymphadenectomy. (09/18/2006)
Patients must meet the eligibility criteria in Section 3.11, and be a suitable candidate for full surgical staging. Specimen collection is an essential and mandatory component of this molecular staging study.

1. Obtain consent to participate in GOG-0210 from all patients who are suitable candidates for this molecular staging study. Register the patients on study. Satisfy the pre-surgery requirements listed in section 7.1 and enter the patient’s consent choices for the protocol and future research using the online specimen consent application. (09/18/2006)

2. Obtain a pre-op serum specimen from each patient as described in Appendix V, and ask the patient to complete a Gynecologic Questionnaire within 6 weeks of study entry. (06/14/2004)

3. Perform an abdominal hysterectomy or a laparoscopic assisted vaginal hysterectomy, a bilateral salpingo-oophorectomy, a bilateral pelvic (Appendix II) and para-aortic (Appendix III) lymphadenectomy*, and peritoneal cytology on each patient with intraperitoneal biopsies as indicated. Patients will also be enrolled on GOG-136 (specimens will need to be submitted for GOG-0210 and GOG-0136) and/or an appropriate GOG treatment protocol. *Patients with Stage IV intra-abdominal disease NOT debulked to less than 2 cm DO NOT require a lymphadenectomy. (06/14/2004) (09/18/2006)

4. Obtain both frozen & formalin-fixed research specimens as described in Appendix V from primary tumor (for each patient) and metastatic tumor (when gross metastatic tumor is present) excised during the surgical staging procedure along with normal tissue (ideally normal Fallopian tubes, ovaries or cervical tissue but not omental fat). Obtain a urine specimen from the Foley catheter from all patients before, during or after surgery as described in Appendix V. (06/14/2004) (09/18/2006)

5. Within 7 days of surgery, submit a copy of Form SP to the Statistical and Data Center (SDC) electronically via SEDES for each of the following specimens regardless of submission status: the pre-op serum (SB01), urine (UR01), the frozen (RP01) & fixed (FP01) primary tumor, the frozen (RN01) & fixed (FN01) normal tissue, and the frozen (RM01) & fixed (FM01) metastatic tumor specimens, and ship these forms and specimens to the GOG Tissue Bank as described in Appendix V. (09/18/2006)

6. Within 4 weeks of entry, submit Form R (Registration Form), Form OSE (On Study Endometrial Cancer Form) and Form DR (Pre-Treatment Summary Form) to the SDC electronically via SEDES. Treatment for GOG-0210 is full surgical staging so summarize all pre-surgery clinical information including pre-surgery CA125 on Form-DR. (09/18/2006)

7. Within 6 weeks of entry, submit Form C and Form PRE electronically via SEDES and submit the Gynecologic Questionnaire with the coversheet, Form BDR, Form E; copies of the operative report, discharge summary, pathology reports, cytology report, and required pathology slides for GOG Central Pathology Review to the SDC via postal mail. (06/14/2004) (09/18/2006)

8. During the post-op clinic visit (ideally within 6 weeks after surgery and prior to starting any post-operative treatment if possible – if not provide details in item 15 on Form SP), obtain a post-op serum specimen from each patient as described in Appendix V and submit a Q0 form to the SDC. When completing the Q0 form, enter the date of the post-op clinic visit in the field for treatment completion date. (06/14/2004) (09/18/2006)

9. Within 7 days of the post-op clinic visit, submit a copy of Form SP for the post-op serum specimen (SB02) to the SDC electronically via SEDES, and ship this form and specimen to the GOG Tissue Bank as indicated in Appendix V. (09/18/2006)

10. Provide patients with post-operative treatment as needed. Participation in GOG treatment protocols is encouraged. (09/18/2006)

11. Submit follow-up information on Form Q electronically via SEDES for all patients every 3 months for the first two years, every 6 months for the next 3 years, and yearly for the next 5 years following the post-op clinic visit, the treatment completion date. (09/18/2006)(1/18/2011)

12. Obtain a follow-up serum specimen from each patient during the 3-year follow-up clinic visit after the surgery. (09/18/2006)

13. Within 7 days of the 3-year follow-up visit, submit a copy of Form SP for the 3-year follow-up serum (SB03) to the SDC electronically via SEDES, and ship this form and specimen to the GOG Tissue Bank as described in Appendix V. (09/18/2006)

14. For those patients with progressive or recurrent disease, obtain a recurrent serum specimen from all patients and a recurrent tumor specimen when the tumor tissue can be safely excised as described in Appendix V. (09/18/2006)

15. Within 7 days of obtaining the recurrent specimens, submit a copy of Form SP for the recurrent serum (SB04) and the frozen (OR01) & fixed (FR01) recurrent tumor specimens when possible to the SDC electronically via SEDES, and ship these forms and specimen to the GOG Tissue Bank as described in Appendix V. If a pathology report is available for the recurrent tumor, please submit a copy of this report to the SDC via postal mail. (06/14/2004) (09/18/2006)
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6.1 Treatment modifications will not be needed for this protocol since full surgical staging is the only treatment specified. Depending upon the findings of the surgery further treatment may be indicated, but is not specified in this protocol. The physician will discuss the available post-operative treatment options in detail with the patient and further treat the patient as needed. Participation in GOG treatment protocols is strongly encouraged. (9/22/2003) .......................................................... - 35 -

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8.2 Survival will be defined as observed length of life from protocol entry to death, or for living patients, date of last contact regardless of whether or not this contact is on another GOG protocol. .................................................................................................................. - 40 -

8.3 Progression-free survival will be defined as date from protocol entry to date of evidence of disease recurrence or progression, death or date of last contact whichever occurs first. .................................................................................................................. - 40 -

8.4 Cause of death will be carefully documented. ...................................................................................... - 40 -

8.5 Sites of recurrence will be carefully documented. Recurrence will be defined as discovery of disease not previously present by clinical, radiographic, and/or laboratory means. Progression is defined as 50 percent or greater increase in the product from any documented lesion. Histologic confirmation of suspected progressive disease is left to the judgment of the attending physician. .................................................................................................................. - 40 -

8.6 All types of post-operative treatment will be carefully documented in each case including treatments for recurrent or persistent disease. Participation in GOG Treatment Protocols will be encouraged wherever possible. First-line treatments following surgical staging will be reported in detail whereas a brief overview of subsequent treatments will be sufficient. The reporting requirements for the GOG treatment protocol will also satisfy the post-operative treatment reporting requirements for GOG-0210. (9/22/2003) .......................................................................................................................... - 40 -

8.7 The evaluation criteria for the results obtained from each type of laboratory test performed on the GOG-0210 clinical specimens will be described in a project-specific manner in a specific appendix. .................................................................................................................. - 40 -

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9.1 This study will accrue at least 3,500 evaluable patients and these patients will be followed for recurrence and survival for 10 years. It is appreciated that most recurrences will appear in the first three years after entry. (09/24/2003) ........................................................................ - 41 -

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GOG-0210 is a molecular and surgico-pathological staging study of endometrial carcinoma. The overall goal of this pilot protocol is to improve outcome and/or quality of life for patients with endometrial cancer. This fundamental goal will be accomplished through the development of more accurate models of risk, identification of candidate targets for therapeutic intervention and utilization of individualized treatments based on molecular characteristics identified in tumor tissue, normal tissue and/or in readily accessible biological fluids, like serum and urine.

1.0 OBJECTIVES

1.1 Establish a repository of clinical specimens (tissue, urine and serum) with detailed clinical and epidemiologic data from patients with surgically staged endometrial carcinoma.

1.2 Utilize genomic, proteomic and immunoassay results from tissue, urine and serum specimens for the purpose of class prediction and class discovery in endometrial carcinoma to identify and validate molecular characteristics associated with risk of endometrial cancer recurrence, clinical and histological characteristics, and epidemiologic factors.

1.3 Improve the accuracy and resolution of the risk assessment models for predicting endometrial cancer recurrence using informative genomic, proteomic and immunoassay results in combination with clinical, pathological and epidemiologic factors.

1.4 Mine the genomic, proteomic and immunoassay results along with the clinical, histological and epidemiologic data obtained for this research study to identify candidate characteristics to target or exploit that would help prevent and/or treat endometrial carcinoma, and to expand the current understanding of the biology, progression, metastasis and responsiveness of endometrial carcinoma.

2.0 BACKGROUND AND RATIONALE

GOG-0210 addresses a number of the priorities identified in the November 2001 report from the Gynecologic Cancer Progress Review Group (GYN PRG). This protocol will establish a clinical specimen bank for endometrial cancer research--an essential priority identified by the GYN PRG and the first priority of the endometrial cancer roundtable breakout group. The banked tissue, serum and urine specimens with matched clinical and epidemiologic data will serve as an enduring resource for investigators working to define endometrial cancer biology with special attention to unique opportunities and features--the second priority of the endometrial cancer roundtable breakout group. The various types of data collected for this protocol will be used to establish centralized database systems for molecular and clinical correlation--the fifth priority of the endometrial cancer roundtable breakout group. In addition, this protocol has been structured to address two of the overall high-impact priorities identified by the GYN PRG. The first involves the identification of markers of risk, molecular disease classifications, prognostic indicators and new targets for prevention and treatment. The second recognizes the added value of conducting translational research that may improve the quality of life of gynecologic cancer patients. GOG-0210 represents a landmark scientific opportunity to expedite progress in translational research for endometrial cancer and to improve the staging system and clinical management of this disease.

This molecular and surgico-pathological staging study is structured to collect tissue, urine and serum from approximately 3,500 evaluable patients with endometrial cancer along with specific clinical and epidemiologic information for an in-depth research study using different types of...
laboratory tests to learn about the molecular characteristics and biology of this disease. A series of independent and integrated research projects will be undertaken which will utilize high throughput methodologies like genomics and proteomics as well as the more established techniques like immunoassays to perform an in-depth examination of cellular and extracellular factors including chromosomes, DNA, RNA, proteins, lipids and carbohydrates. (09/24/2007)

All of the patients will be surgically-pathologically staged, consistently evaluated, further treated as needed, and followed for up to 10 years after surgery to document further treatment and outcome. It is hoped that the information that is learned from this research study will be used to improve the future treatment of endometrial cancer. The bank of specimens with clinical and epidemiologic information will be available for research only after approval by special committees knowledgeable about the scientific merit of the research and the rights of participants. The results obtained from the comprehensive laboratory testing performed on these clinical specimens with the clinical and epidemiologic data collected for each GOG-0210 patient will represent an enduring resource for endometrial cancer research to study the factors that control the growth and spread of endometrial cancer, and how to predict response to therapy and risk of disease spread, recurrence and overall survival for patients with this disease.

GOG protocol 33 served to define the surgical patterns of disease spread in endometrial carcinoma, and changed practice patterns for the treatment of this disease. This landmark protocol was primarily responsible for providing the evidence that clinical staging had considerable margins of error in determining the extent of disease and, in fact, in 1988 the International Federation of Gynecologists and Obstetricians (FIGO) revised the FIGO classification for carcinoma of the corpus uteri to reflect surgical staging criteria.1

2.1 Hypotheses And Goals (9/22/2003)

The main hypothesis to be tested in this protocol is that a comprehensive molecular classification of endometrial cancer using genomics, proteomics and immunoassays will improve our ability to predict clinical outcome in patients with endometrial carcinoma, specifically with regard to recurrence-free interval following surgical staging. A secondary hypothesis that will be tested is that molecular determinants identified in tissue, urine or serum at the time the patient is surgically staged can be used to individualize post-operative treatment for endometrial carcinoma and improve patient outcome and/or quality of life. Another secondary hypothesis that will be tested is that molecular determinants identified in primary tumor compared with normal tissue, metastatic tumor compared with primary tumor; and recurrent tumor compared with primary and metastatic tumor will represent molecular characteristics to target or exploit to inhibit the progression, metastasis and/or recurrence of endometrial carcinoma. A third secondary hypothesis that will be tested is that molecular determinants identified in serial serum specimens will predict metastatic spread, therapeutic responsiveness and/or recurrence of endometrial carcinoma.

It is hoped that in-depth laboratory testing of patient tumor, urine and serum specimens will not only provide an opportunity to improve our capability to quantify the magnitude of risk for recurrence, but will also allow patients to be screened for resistance to radiation, drugs or hormones, and/or to identify candidate targets or pathways that can be exploited for therapeutic intervention; thus providing the
opportunity to improve future treatment options and quality of life for patients with endometrial carcinoma.

The ultimate goal of GOG-0210 will be to develop a molecular disease classification system that will complement the existing FIGO classification for endometrial carcinoma allowing for more accurate risk assessment and the identification of exploitable characteristics that will be tested in future clinical trials. The main objective of GOG-0210 will be to identify and validate molecular characteristics that predict risk for recurrence and which can be used to direct future trials in endometrial carcinoma. See Appendices XI-XV for a description of some of the specific hypotheses and aims for the independent and integrated translational research projects for this protocol.

The established bank of tissue, serum and urine specimens as well as the centralized database systems with the matched clinical, epidemiologic and laboratory data will serve as enduring resources for GOG investigators and collaborators researching the biology, progression, metastasis and responsiveness of endometrial carcinoma. This type of programmatic strategy for defining the molecular characteristics of endometrial carcinoma will not only revolutionize what is known about this disease during the next five years and beyond, but also provide the intellectual infrastructure required to develop effective treatment strategies to prevent and cure all forms of this disease.

GOG-0210 represents a comprehensive translational research study in endometrial cancer that addresses a number of the priorities identified in the November 2001 Report of the GYN PRG. The GYN PRG report presents the national plan for gynecologic cancer research for the next five years and outlines the top priorities, gaps in knowledge, barriers to progress, and resources for 14 scientific areas and the three types of gynecologic tumors (ovarian, cervix and corpus).

2.2 Overview

Detailed laboratory testing will be carried out on the thirteen clinical specimens collected and banked for this in-depth research study to learn about the molecular characteristics and biology of endometrial cancer. High through put techniques and immunoassays will be used to undertake a comprehensive analysis of chromosomes, DNA, RNA, proteins, lipids and carbohydrates. This molecular staging study for endometrial carcinoma is an ambitious endeavor as endometrial cancer patients who vary in risk of recurrence will need to be recruited, surgically-pathologically staged, consistently evaluated, further treated as needed, and sufficiently followed to provide comprehensive disease overview, treatment and outcome data. Standardized procedures will cover all aspects of specimen procurement and laboratory testing to monitor and control for variability along with an appropriate informatics infrastructure for efficient and confidential data transfer, tracking, storage, reporting and analysis. Facilities with established expertise in data management, banking, genomics, proteomics, immunoassays, statistics and bioinformatics will also be required along with adequate funding to support all components of this protocol from patient accrual to specimen collection, shipping and banking; laboratory testing; specimen and data management; and informatics to enable accurate and timely analysis and reporting of the findings of this molecular staging study. (9/22/2003)
The GOG, through its membership and access to GOG and non-GOG core facilities for data management, statistics, banking clinical specimens, genomics, proteomics, immunoassays and bioinformatics, is uniquely suited and equipped to take on the challenge of this protocol. The specific scientific hypotheses to be tested, and the laboratory methodology to be employed for this molecular staging study will be defined over time so that new advances in research and technology can be appropriately incorporated into this protocol.

Individual and integrated translational research projects for GOG-0210 will be developed in collaboration with the study chairs, the GOG Corpus Committee, the GOG Committee for Experimental Medicine and the GOG Statistical and Data Center using a modification of the GOG Internal Bank Application and Review Process. A Scientific Advisory Board for GOG-0210 will be established as a subcommittee of the Corpus Committee with membership from the Corpus Committee, the Committee for Experimental Medicine and the GOG Statistical and Data Center. The GOG Committee for Experimental Medicine and the Scientific Advisory Board for GOG-0210 will review applications for individual and integrated GOG Internal Bank Projects that plan to target the GOG-0210 resources based on scientific merit, relevance, feasibility and GOG priority. (09/18/2006)

Projects judged to have high scientific merit and to address the objectives and goals of this molecular staging study of endometrial carcinoma from investigators willing to work within the GOG framework and to seek external funding in collaboration with the GOG will be given the highest priority. The GOG-0210 Internal Bank Projects will be cutting-edge, both with respect to the scientific aims and assay platforms used as well as for the statistical analyses and interpretations. (09/18/2006)

Documentation regarding approved GOG-0210 Internal Bank Projects will be presented to the GOG Corpus Committee, the GOG leadership, the Cancer Therapy Evaluation Program (CTEP) and the GOG Operations Committee for review and comments. Additional scientific collaborators including the Endometrial Cancer Specialized Programs of Research Excellence (SPOREs) will be encouraged to participate. (09/22/2003) (09/18/2006)

2.3 Endometrial Carcinoma (9/22/2003)

Adenocarcinoma of the endometrium is the most common female pelvic malignancy. In 2003, the American Cancer Society estimates 40,100 new cancers of the uterine corpus and 6,800 cancer-related deaths (www.cancer.org). About 95% of uterine body cancers are endometrial carcinoma. When compared with other gynecological cancers, endometrial cancer has proportionately fewer deaths. This likely reflects the fact that most endometrial cancer is diagnosed at an early stage (stage I or II when the cancer is confined to the uterus). As with all cancers, early staged disease has a very good prognosis, but rates of recurrence and survival depend upon other prognostic factors identified in GOG protocol 33 and incorporated into the FIGO staging in 1988. According to the Annual Report², patients with stage IA grade 1 disease have a five-year survival of 93% while those with stage I grade 3 cancers have only a 63% five-year survival. Histologic subtype also impacts five-year survival as patients with endometrioid, adenosquamous and mucinous histotypes have about 90% survival
compared with those patients with serous papillary and clear cell carcinomas who have 72% and 80% survival, respectively. A further refinement of the risk factors using results from genomics, proteomics and immunoassays may improve risk assessment, affect post-operative therapy and potentially improve patient management and survival.

In the early 1970s, the GOG carried out a pilot study of surgical evaluation of patients with endometrial cancer. Stage I adenocarcinoma of the endometrium was evaluated prospectively in 140 patients with regard to tumor grade, depth of myometrial invasion, uterine size and pelvic and para-aortic lymph node metastasis. Metastatic spread was observed in 11.4% and 10% of the evaluable pelvic and para-aortic lymph nodes, respectively. At that time, endometrial cancer was clinically staged and the five-year survival rate for stage I disease was only about 75%. This pilot study was pivotal in developing GOG-33 in which over a thousand endometrial cancer patients were surgically staged with criteria established to be adequate staging. This study identified surgical and pathologic factors that correlated with extra-uterine disease and prognosis. Extra-uterine disease was not unusual even when the disease was clinically limited to the corpus. About 25% of all clinical stage I cancer had extra-uterine disease spread which correlated with a less favorable prognosis for these patients. GOG-33 was primarily responsible for providing the evidence that clinical staging had considerable margins of error in determining the extent of disease and in 1988, in Rio de Janeiro, the International Federation of Gynecologists and Obstetricians (FIGO) revised the FIGO classification for carcinoma of the corpus uteri to reflect surgical staging criteria.

There are multiple surgical-pathological risk factors for endometrial cancer. These include histologic subtypes (cell types), tumor grade (the degree of histologic differentiation), depth of myometrial invasion, isthmus-cervix extension (the location of the tumor within the uterus), and extra-uterine disease spread (including adenexal, intraperitoneal as well as pelvic and/or para-aortic lymph node metastasis).

Endometrioid adenocarcinoma is the most frequent type of endometrial carcinoma and the aggressiveness of this histologic subtype is related to the degree of tumor grade. In contrast, serous, clear-cell, undifferentiated and squamous endometrial carcinomas are found relatively infrequently, and these histologic subtypes have an increased likelihood for extra-uterine spread and an unfavorable prognosis.

Hendrickson first reported histologic criteria of papillary serous in 1982. Since that time several investigators have reported their experiences with papillary serous and demonstrated that it behaves differently than the more common endometrioid adenocarcinoma. Subsequent studies classified clear cell tumors into a more aggressive subgroup as well. When compared with endometrioid adenocarcinoma, these two histologic variants are associated with a higher frequency of extra-uterine disease spread, different pattern of failure, and poorer survival. Both early stage and advanced disease states appear to behave aggressively. GOG protocol 94, reporting on one of the largest surgically staged series of Stage I papillary serous/clear-cell patients, found five-year disease-free survival was only 35% compared to Stage I patients in GOG protocol 99 with intermediate risk endometrial adenocarcinoma who had two-year disease-free survival between 88-96% depending on the use of pelvic radiation.
GOG-33 went on to demonstrate that tumor grade is associated with the depth of myometrial invasion and a propensity for pelvic and para-aortic lymph node metastasis. As tumor grade becomes less differentiated (grade 3), the depth of myometrial invasion and the rates of pelvic and para-aortic involvement increase.\(^6\) Invasion to greater than 50% of the myometrium was observed in 50% of the grade 3 lesions; with pelvic lymph node involvement in 30% and para-aortic lymph node involvement in 20% of the grade 3 cases. Morrow reported that grade 3 tumors had a disease-free survival of 70% at 3 years.\(^7\) In 51 patients with a grade 3 tumor, any degree of myometrial invasion, and no risk factors (negative nodes, cytology, lymphovascular space involvement, cervical extension), 8 patients recurred (16%). More recently, data has suggested that grade 3 tumors do as well (or as poorly) as papillary serous/clear cell tumors when matched for stage. Data from the Annual Report from FIGO for Stage I uterine cancers (based on 148 papillary serous, 59 clear cell, and 326 grade 3 tumors) for five-year survival is shown below: (9/22/2003)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Papillary serous</th>
<th>Clear cell</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>74%</td>
<td>83%</td>
<td>62%</td>
</tr>
<tr>
<td>IB</td>
<td>74%</td>
<td>67%</td>
<td>78%</td>
</tr>
<tr>
<td>IC</td>
<td>46%</td>
<td>64%</td>
<td>59%</td>
</tr>
</tbody>
</table>

Deep invasion of the myometrium, greater than 50% of the myometrial thickness, is associated with an increased likelihood of extraterine spread, treatment failure and recurrence.\(^6\)\(^-\)\(^9\) Although grade and depth of invasion are associated with risk of lymph node metastasis, depth is more influential. Only 1% of patients with endometrial involvement only have either pelvic or para-aortic lymph node involvement whereas 25% and 17% of patients with deep muscle invasion have pelvic and para-aortic metastasis, respectively.\(^6\)

GOG protocol 33 also demonstrated that the location of the tumor within the uterus, adnexal involvement, intraperitoneal metastasis, capillary-like space involvement and positive peritoneal cytology are important predictors of pelvic and para-aortic lymph node involvement.\(^6\)

Endometrial carcinoma is a heterogeneous disease both in terms of histology and risk for disease recurrence and death. Scientists, epidemiologists and clinicians often sub-classify this type of cancer as type I or type II disease.\(^20\)\(^,\)\(^21\) This dichotomous classification system reflects differences in histologic subtype, etiology, and tumor behavior. Approximately 85 percent of endometrial cancer cases are (type I) endometrioid histotypes, arise in a background of abnormal endometrial proliferation and are associated with an excellent prognosis certainly in early stage disease. Exposure to estrogen (either endogenous or exogenous) may be associated commonly with endometrioid cancer. Estrogenic stimulation can result in abnormal endometrial proliferation, or endometrial hyperplasia. Patients with atypical endometrial hyperplasia tend to have an increased risk of endometrial carcinoma. Type II disease represent papillary serous tumors of the uterus and other less common histologies including clear cell, undifferentiated and squamous carcinomas that tend to arise from the atrophic (non-proliferative) endometrium. Patients with type II disease tend to have a less favorable prognosis. (9/22/2003)
The basic hypothesis of GOG protocol 33 was that pathological determinants identified at surgery would allow one to individualize postoperative treatment based upon patient risk. The GOG and other investigators have subsequently carried out many protocols evaluating different treatment modalities based on risk assessment determined by surgical-staging. Effective treatments, however, remain to be defined for the endometrial cancer patients with the clinical-pathologic risk factors, and those with recurrent or persistent endometrial carcinoma.

2.4 Report Of The Gynecologic Cancers Progress Review Group (GYN PRG) – November 2001


2.5 Availability Of Clinical Specimens For A Molecular Staging Study Of Endometrial Carcinoma (9/22/2003)

The GOG Tissue Bank was established in 1991 as an NCI-funded repository for collecting and banking tissues and serum for research. From 1991 through 1998, the eligibility for banking GOG clinical specimens for research was restricted to patients with ovarian cancer who either participated (internal bank) or did not participate in a GOG clinical protocol (external bank). Internal bank specimens, therefore, are associated with rather extensive clinical information including demographic and tumor characteristics as well as details regarding treatment and patient outcomes such as toxicity, response, progression/recurrence and survival data when available as a result of the patient’s participation in a GOG clinical protocol and consent to provide clinical specimens for research. The external bank specimens are associated with rather limited clinical information including certain demographic and tumor characteristics available because the patient consented to provide clinical specimens for research, but did not participate in a GOG clinical protocol. In 1998, the GOG Tissue Bank expanded the eligibility for banking GOG clinical specimens for research to include those patients with carcinoma of the cervix or carcinoma of the uterine corpus.

As of March 2003, the GOG Tissue Bank established a repository of tissue and serum specimens from 4494 patients participating in the GOG banking protocol (GOG-0136). This includes specimens from 3342 patients with ovarian cancer (609 cases associated with the GOG internal bank and 2733 cases associated with the GOG external bank), 191 patients with cervical cancer (17 cases associated with the GOG internal bank and 174 cases associated with the GOG external bank), 952 patients with carcinoma of the uterine corpus (68 cases associated with the GOG internal bank and 884 cases associated with the GOG external bank), and nine patients with another type of gynecologic malignancy (two cases associated with the GOG internal bank and seven cases associated with the GOG external bank). The GOG Tissue Bank also banks clinical specimens from patients participating in a GOG clinical protocol with specific specimen collection requirements and translational research objectives, and GOG-0210 will represent this type of study.
The GOG Tissue Bank not only has a proven track record for banking specimens, but also has been extremely successful in distributing high quality internal and external bank specimens to a diverse array of investigators. Before 2000, the GOG Tissue Bank served 124 investigators. Since then, the Bank has distributed GOG specimens to an additional 104 investigators. The results from these various research projects have yielded more than 60 publications and contributed to funded grant applications.

Although tissue and serum specimens have been banked from almost a thousand patients with carcinoma of the uterine corpus, the quality and suitability of these specimens for specific types of laboratory assays is not known, and most specimens are linked with very limited clinical information. The banked specimens were not obtained using the updated specimen collection procedures suitable for the new genomic and proteomic applications or the new specimen form which allows the sites to document critical specimen collection information essential for assessing specimen quality and suitability for specific types of laboratory testing. In addition, 884 of the 952 patients with carcinoma of the uterine corpus who provided clinical specimens for research did not participate in a GOG clinical protocol and therefore, these specimens are not linked with detailed clinical, pathologic, treatment, or outcome data. In addition, the patients were not all surgically staged or consistently managed. Of the remaining 68 cases, 26 are from patients participating in a treatment protocol for advanced and/or recurrent endometrial carcinoma (including five cases from GOG-0122, one case from GOG-0163, three cases from GOG-0177, seven cases from GOG-0184 and 10 cases from other studies including pilots; lymph node sampling was typically an optional component of these trials), 34 are from patients participating in a treatment protocol for early stage endometrial carcinoma (GOG-0137), and eight are from patients participating in a treatment protocol for carcinosarcoma of the uterus. Furthermore, epidemiologic data was not collected for any of these patients.

Currently, the GOG Tissue Bank is the only national repository and distribution center for specimens from patients with a gynecologic malignancy. Aside from the activities of the GOG-0136 banking protocol, the GOG Tissue Bank also banks clinical specimens collected as a direct result of participation in a GOG treatment, prevention or pilot protocol. These specimens are also designated as internal bank specimens and will only be distributed in a protocol-specific manner for approved translational research projects.

GOG-0210 is needed to establish a bank of high-quality tissue, serum and urine specimens matched with clinical, pathologic, treatment, outcome and epidemiologic data from patients with surgically staged endometrial carcinoma for a comprehensive molecular staging study of endometrial carcinoma.

2.6 Endometrial Cancer Research

In spite of unprecedented advances in cancer research and technology, endometrial cancer research has lagged far behind breast, ovarian, and cervical cancer in terms of grant money allocation, and progress. While the GOG has studied patterns of disease spread (GOG-33), and techniques to minimize morbidity of surgery in patients with early staged disease (LAP2), less is known about the molecular characteristics that
predict who will recur, who should receive postoperative treatment and who will respond to radiation, drugs or hormonal therapy. For those patients with advanced or recurrent disease, little is known about the molecular characteristics operating in these tumor cells. The systematic study of genes, transcripts, and proteins in combination with the analysis of individual biomarkers in various types of tissue, serum and urine specimens may shed light on the pathogenesis and therapeutics of endometrial carcinoma.

MOLECULAR BIOLOGY OF ENDOMETRIAL CARCINOMA

As for other human cancers, endometrial carcinoma is believed to result from a series of genetic mutations.\(^22\) A central, unresolved issue in the understanding of endometrial carcinogenesis is the contribution of unopposed estrogen stimulation. It has long been thought that estrogens contribute to the development of endometrial cancer by virtue of their mitogenic effect on the endometrium.\(^23\) A higher rate of proliferation in the endometrium in response to estrogens may lead to an increased frequency of spontaneous mutations. In addition, when genetic damage occurs, regardless of the cause, the presence of estrogens may facilitate clonal expansion. It has also been postulated that estrogens may act as “complete carcinogens,” in the sense that they may function both as initiation factors, causing a direct mutagenic effect on DNA,\(^24\) and as tumor promoters, through their ability to enhance cell proliferation.

CYTOGENETICS, PLOIDY, ALLELIC IMBALANCE, AND CHROMOSOME GAINS AND LOSSES

Cytogenetic studies have described gross chromosomal alterations in endometrial cancers, including changes in the number of copies of specific chromosomes. The extent of cytogenetic abnormalities in a given tumor is generally low, however. Likewise, about 80% of endometrial cancers have a normal diploid DNA content as measured by ploidy analysis.\(^25, 26\) Aneuploidy occurs in 20% of cases and is associated with advanced stage, high grade, non-endometrioid histology, and poor survival.\(^27\) Higher resolution allelotyping studies, in which allelic imbalance is quantitated throughout the genome, are consistent with cytogenetic and ploidy studies in that allelic imbalance (often referred to as “loss of heterozygosity”) occurs at a relatively low frequency in endometrial carcinoma compared to other common solid tumor types. Sites at which allelic imbalance has been observed most frequently include chromosomes 3p, 6p, 8p, 9q, 10q, 14q, 16q, 17p, and 18q.\(^28-32\) Chromosome arms affected by loss of heterozygosity for which the underlying molecular genetic defect has been identified with reasonable certainty are 17p and 10q, which correlate with mutational inactivation of \(TP53\) and \(PTEN\), respectively (see below). Allelic imbalance at chromosome 14q has been associated with a poor clinical outcome.\(^30\) Most recently, the use of comparative genomic hybridization (CGH) to detect gains and losses of specific chromosomal regions at high resolution reinforces the concept that such changes are relatively uncommon in endometrial carcinoma, with copy number changes affecting chromosomes 1, 8, and 10 being the most consistently reported.\(^33-37\) Not unexpectedly, individual tumors with a greater number of gains and losses are associated with a poorer prognosis,\(^36\) and some changes seen in cancers are also present in atypical, but not simple, hyperplasia lesions.\(^35, 37\)
Mutational activation or aberrant expression of a few oncogenes has been described for endometrial cancer, but to a much lesser extent than for tumor suppressor genes (below). Furthermore, no single oncogene has been found to be altered in greater than approximately 30% of the endometrial cancers studied. Activating point mutation of a member of the RAS gene family is the most commonly identified oncogene aberration in human cancers generally, and several studies have confirmed that a RAS mutation, predominantly in codon 12 of the K-RAS gene, is present in 10-30% of endometrial carcinomas. 38-40 In those tumors that contain RAS mutations, this event appears to occur early in the neoplastic process, because the incidence of mutant RAS is the same in endometrial hyperplasia as that found in carcinoma.41-43 Attempts to correlate RAS mutation with clinical outcome have produced conflicting data, possibly reflecting an age dependency of this phenomenon.44

Involvement of the ERBB-2 (also known as HER-2/neu) oncogene in human breast and gynecologic tumorigenesis is through overexpression, with or without gene amplification. It is clear that 10-15% of endometrial cancers display overexpression of ERBB-2 protein compared to normal endometrial epithelium, as quantitated by immunohistochemistry. Some studies have also documented ERBB-2 gene amplification in endometrial cancers, although in only a subset of those tumors showing overexpression. Overexpression of ERBB-2 appears to be confined to a subset of high-grade and/or advanced stage tumors.45-48 Correlation with clinical outcome has been less conclusive, although the trend has been toward a positive correlation between overexpression and worsening of prognosis.

The FMS oncogene encodes a tyrosine kinase that serves as a receptor for macrophage-colony stimulating factor (M-CSF). Expression of FMS in endometrial cancers correlates with advanced stage, high grade, and deep myometrial invasion.49, 50 It has also been shown that FMS and M-CSF are usually co-expressed in endometrial cancers, suggesting that this ligand-receptor pair might mediate an autocrine growth stimulatory pathway.51 In support of this hypothesis, serum levels of M-CSF are increased in patients with endometrial cancer, and M-CSF increases the invasiveness of cancer cell lines that express significant levels of FMS but not those with low levels of FMS.52 Two transcriptional regulators are altered in small fractions of endometrial cancers. The cytoplasmic tail of the E-cadherin tumor suppressor exists as a macromolecular complex with β-catenin and APC proteins, which link it to the cytoskeleton. A critical function of the APC tumor suppressor appears to be negative regulation of β-catenin through phosphorylation, which when accumulated, translocates to the nucleus and induces expression of cyclin D1 and perhaps other genes involved in cell cycle control. Missense mutations in exon 3 of the CTNNB1 gene, which encodes β-catenin, render it resistant to activation by APC; it is therefore categorized as an oncogene. Missense mutations in CTNNB1 are observed in 10-20% of endometrial carcinomas.53, 54

Gene amplification of one or another member of the MYC family has been implicated in many cancer types. Expression of C-MYC has been observed in normal endometrium and endometriosis, with higher expression in the proliferative relative to secretory phase. Several studies suggest that C-MYC is amplified in a fraction of endometrial carcinomas.48, 55
TUMOR SUPPRESSOR GENES

Mutation of the TP53 tumor suppressor gene is the most common genetic abnormality currently recognized in human cancers. Relative overexpression of the p53 protein is frequently observed as a result of many of the common missense mutations that the gene is subject to; thus, immunohistochemical analysis of p53 expression is frequently used as an endpoint in many human tumor studies. Through the analysis of mutation, overexpression, or both, the presence of TP53 mutations in endometrial carcinomas has now been well established, although the incidence is clearly limited to a subset (10-30%) of all tumors studied. Overexpression and/or mutation are associated with poor prognostic features, such as high grade, advanced stage, non-endometrioid histology, and disease recurrence. Several studies have also focused on papillary serous endometrial carcinomas, a majority of which display mutation or overexpression. In a study of over 100 endometrial hyperplasia specimens, TP53 mutations were uniformly absent.

A novel tumor suppressor gene responsible for the hereditary cancer syndrome Cowden’s disease was cloned and characterized in 1997. Named PTEN (or MMAC1), this gene encodes a multifunctional phosphatase that functions to inhibit the phosphatidylinositol-3-kinase pathway and downstream functions. Although endometrial cancer is not recognized as a component of the Cowden’s syndrome, sporadic endometrial carcinomas frequently exhibit loss of heterozygosity in the region of chromosome 10q that contains PTEN. Mutation analyses of PTEN in endometrial carcinomas indicate that this gene is somatically inactivated in 30-50% of all such tumors, representing the most frequent molecular genetic alteration in endometrial cancers yet defined. Interestingly, there is a correlation between the presence of microsatellite instability (see below) and PTEN mutation. In addition, PTEN mutations are observed in 20% of endometrial hyperplasias, suggesting that this is an early event in the development of some type I endometrial carcinomas.

Inherited mutations in genes encoding DNA mismatch repair proteins, primarily MSH2 and MLH1, are responsible for the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome. Endometrial carcinoma is the second most common malignancy (after colorectal) observed in women with mutations in these genes, and is the only known
manifestation of hereditary endometrial cancer. Cancers that arise in these individuals are characterized by frameshift mutations in multiple microsatellite repeat sequences throughout the genome. This microsatellite instability is also observed in 20% of sporadic endometrial carcinomas. In these sporadic cases, acquired mutations in mismatch repair genes are rare; a majority of cases exhibit hypermethylation of the MLH1 promoter, associated with transcriptional silencing and loss of MLH1 function. Additionally, microsatellite instability is present in some cases of complex atypical hyperplasia associated with endometrial carcinoma. Conversely, microsatellite instability is not seen in papillary serous endometrial cancers.

**Molecular Genetic Distinctions between Type I and Type II Cancers.** From this very rudimentary knowledge of molecular genetic alterations in endometrial carcinoma, a categorization scheme in which most known genetic alterations segregate reasonably well into one or the other category begins to emerge (Table 1).

<table>
<thead>
<tr>
<th>Clinical-Pathological Features</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperestrogenism</td>
<td></td>
<td>No estrogen risk factors</td>
</tr>
<tr>
<td>Younger age</td>
<td></td>
<td>Older age</td>
</tr>
<tr>
<td>Endometrioid histology</td>
<td></td>
<td>Non-endometrioid histology</td>
</tr>
<tr>
<td>Associated with hyperplasia</td>
<td></td>
<td>No hyperplasia</td>
</tr>
<tr>
<td>Low grade</td>
<td></td>
<td>High grade</td>
</tr>
<tr>
<td>Good prognosis</td>
<td></td>
<td>Poor prognosis</td>
</tr>
</tbody>
</table>

**Genetic Features**

<table>
<thead>
<tr>
<th>Genetic Features</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diploid</td>
<td>Aneuploid</td>
<td></td>
</tr>
<tr>
<td>Low allelic imbalance</td>
<td>High allelic imbalance</td>
<td></td>
</tr>
<tr>
<td>K-RAS</td>
<td>K-RAS</td>
<td></td>
</tr>
<tr>
<td>MLH1 methylation</td>
<td>TP53</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>ERBB-2</td>
<td></td>
</tr>
</tbody>
</table>

At the present time, several problems preclude the utility of this concept in terms of translational application to clinical problems in endometrial cancer. First and foremost, the extent to which the relevant molecular genetic aberrations involved in endometrial tumorigenesis have been elucidated is very limited. The great majority of mutational changes that drive this process have yet to be defined. Second, it may be argued that characterization of genome-wide changes in gene and protein expression may represent an equally if not more useful endpoint in the molecular classification of cancers for clinical purposes. While the occurrence and selection of genetic mutations drives the
process of tumorigenesis, global changes in gene and protein expression and function are primarily responsible for the tumor phenotype. Third, as previously mentioned, the “type I/type II” dichotomy is an inherently oversimplified generalization that does not reflect the subtle biological continuum (both linear and nonlinear) that undoubtedly underlies the complex etiologic and clinical heterogeneity of endometrial carcinoma. New experimental approaches are clearly needed to overcome these limitations.

**COMPREHENSIVE MOLECULAR CLASSIFICATION OF HUMAN CANCERS**

From the preceding discussion, it is clear that there exist several molecular alterations that correlate with one or another aspect of endometrial cancer histopathology or clinical outcome. Frequently, however, these associations are not reproducible and/or the molecular alterations do not represent independent prognostic variables after accounting for well-established clinical and histopathological prognostic factors. This is perhaps not surprising given the complexity of the cancer phenotype, the number of genes in the human genome, and the inherent limitations in testing one or a small number of molecular genetic markers for predictive value. A combination of circumstances, including the advent of microarray-based technologies and progress in the human genome initiative, provides an ideal opportunity to begin efforts aimed at performing comprehensive molecular profiling that should lead to a greater understanding of the basic biology of endometrial cancers. This understanding will inevitably lead to the development of substantially more effective diagnostic and prognostic tools. In this regard, we are particularly interested in the process of “class prediction” for defining molecular profiles associated with histopathological features and clinical outcome in endometrial cancer. Additionally, the process of systematic and unbiased comprehensive molecular profiling of a large number of cancers linked to clinical data allows for “class discovery,” wherein previously unrecognized tumor subtypes may be defined. We propose to use this approach in discovering important subclasses of endometrial cancers that are otherwise indistinguishable at the clinical and histopathological levels.

**EXPRESSION PROFILING OF ENDOMETRIAL CARCINOMAS.**

Early efforts to apply array-based technologies to the characterization of endometrial carcinoma, described in three published studies, support the promise of this approach. Smid-Koopman et al. used a cDNA expression array to compare RNA samples from two endometrial carcinoma cell lines, two primary endometrial carcinomas, a benign endometrial tissue, and a breast cancer cell line. Three gene products (decorin, TIMP3, and cyclin D1) were differentially expressed between the benign tissue sample and those derived from endometrial carcinoma, suggesting their involvement in cancer progression. Furthermore, a higher degree of similarity in gene expression was observed
between endometrial-derived samples than between endometrial samples and the breast cancer cell line, suggesting the existence of an endometrial-specific gene expression profile. Matsushima-Nishiu et al. also used cDNA microarrays to study the effect of exogenous PTEN expression in endometrial carcinoma cell lines lacking PTEN function. Increased expression of 99 genes and repression of 72 genes, many of which are known to be involved in cell proliferation, differentiation, and apoptosis, was observed, illustrating the potential power of expression profiling in elucidating molecular pathways affected by critical cancer-related genes. In an attempt to discover genes that distinguish malignant from normal endometrial tissues, as well as those differentially regulated during the menstrual cycle, Mutter et al. used oligonucleotide-based microarrays to examine RNA samples from four normal endometrial tissue samples (two proliferative and two secretory) and 10 endometrioid carcinoma tissues. Expression of 100 genes that appear to be expressed in a hormonally related fashion in normal endometrium are expressed in a disordered and heterogeneous fashion in cancers, with tumors resembling proliferative more than secretory endometrium. These studies exemplify the broad range of biological questions that may be addressed using expression profiling of endometrial carcinomas.

EXPRESSION PROFILING OF OTHER HUMAN CANCER TYPES

In the past five years, over 600 papers on expression profiling (also referred to as transcript profiling) of cancers using microarray technology have been published, illustrating the recognized utility of this approach in exploring questions of tumor biology and clinical correlates. Among these are several studies that illustrate particularly well the power of expression profiling in addressing clinical problems in human cancer. The principles of class prediction and class discovery as they apply to the molecular classification of human cancers were exemplified by Golub et al., who used oligonucleotide microarrays to monitor gene expression in acute leukemias as a test case. Class prediction identified and validated a subset of informative genes whose expression was highly correlated with previously-defined classes. The genes that were useful for class prediction for acute myeloid leukemia and acute lymphoblastic leukemia also provided insight into the pathogenesis of acute leukemias and the development of anti-leukemic drugs. The class discovery procedure automatically discovered the distinction between acute myeloid leukemia and acute lymphoblastic leukemia, whose clinical appearance is highly similar.

Subsequent studies have utilized these approaches to provide proof of the “molecular profiling principle” as well as to gain novel insights into clinical cancer problems. Using a specialized, lymphoid cell-specific cDNA microarray, Alizadeh et al. performed expression profiling of diffuse large B-cell lymphomas and identified two molecularly distinct forms of this malignancy that correlated with overall survival. Notably, this molecular classification identified a previously unrecognized subgroup of patients with a poor prognosis who, by clinical standards, were from a low clinical risk group.
The problem of apparent tumor homogeneity was addressed by Bittner et al., who used cDNA array-based expression profiling to distinguish a previously unrecognized subset of cutaneous malignant melanomas. This finding was remarkable in light of the fact that no clinical, histopathological, or molecular factors were previously known to identify subsets of this neoplasm.

In contrast, Perou et al. performed a cDNA array-based analysis of breast tumors, an extremely diverse group of cancers in terms of histopathological phenotype and natural history. Complex, but reproducible patterns of gene expression were observed, pointing toward the probable utility of molecular profiling in distinguishing subtypes of breast cancer.

Dhanasekaran et al. used a 10K-element cDNA microarray to analyze gene expression profiles in benign and malignant human prostate tissues. Signature expression profiles were determined for normal prostate epithelium, benign prostatic hyperplasia, localized prostate cancer, and metastatic, hormone-refractory prostate cancer. Of the many associations between genes and prostate cancer identified, two gene products in particular, hepsin and pim-1, were subsequently studied using high-density prostate tissue microarrays and found to correlate significantly with measures of clinical outcome.

These representative studies provide strong support for the power of microarray-based expression profiling in both class prediction and class discovery approaches to the molecular classification of diverse types of human cancers. Expression profiling studies will serve to identify patterns of gene expression in primary endometrial cancers that portend poor outcome. This molecular profiling portion of the study is designed to identify markers for risk of recurrence of endometrial adenocarcinoma. The rationale is straightforward. New and more effective methods are required to identify those women who are at increased risk for recurrent disease, and who would most benefit from more aggressive treatments at the time of diagnosis. The long-term objective is to use molecular markers that independently predict outcome in combination with conventional clinical-pathologic risk factors in prospective randomized trials of postoperative primary therapies for endometrial cancer. Furthermore, by comparing gene expression in tumor of different histologic subtypes and different stage and grade tumors, we will begin to unravel the difference in gene expression that underlie these important clinical-pathologic variables.

The gene expression array analysis will be complicated by potential differences in epithelial-stromal compositions in the tumor specimens. Three options exist to address the issue of stromal contamination of tumors: 1) dissociate fresh tissue and separate epithelial from stromal elements; 2) use laser capture to microdissect epithelium from stroma in tissue sections; or 3) use whole tumor tissue characterized by epithelium and
stroma. Since epithelial-stromal interactions (paracrine effects) may be important in the hormone-mediated response, it is probably optimal to use intact tissue for the initial analysis. Subsequent analyses will examine cell type specific expression patterns using immunohistochemistry assays, in situ hybridization techniques as well as genomic and proteomic procedures to evaluate purified epithelial compared with stromal cells microdissected from appropriate tissue specimens.

**PROTEOMIC PROFILING OF HUMAN CANCER**

Proteomics is the systematic study of intact and fragmented proteins and their function. This new technology holds great promise for those studying diseases like cancer as it is a high throughput with multiple platforms from protein discovery and profiling protein activities within signaling pathways to the identification of protein fingerprints (patterns) or profiles that predict specific characteristics or outcomes. Biological specimens including isolated cells, tissues, and body fluids can be sources for proteomic analyses. It was hypothesized and shown recently that proteomic fingerprint patterns contain valuable information about the pathologic processes taking place within organs or tissues.\(^94\) One of the proteomic platforms is surface-enhanced laser desorption and ionization with time-of-flight detection (SELDI-TOF). This new technology has been applied to create proteomic fingerprints that reflect in serum what is happening in end organs.\(^95\) Crystallized proteins within biological specimens are bound to selected “bait” surfaces contained in replicates on a biochip. The bait may select for hydrophobic, cationic, or other protein interactions. Selectively bound proteins are then excited off the chip by laser ionization and are registered by the detector as a function of the mass per charge unit. As little as one microliter of serum can be applied to a spot on a biochip. The resultant chromatographic fingerprint is highly reproducible, reliable, accurate and sensitive, with detection down to the attamole (10\(^{-18}\)) range.\(^94,95\) This technology is especially sensitive in the low molecular weight protein regions (< 20,000 Daltons), which have not been easily studied using existing technologies. This is an untapped resource for biomarker discovery.

Advances in artificial intelligence have yielded bioinformatics programs that can apply pattern recognition systems with iterative clustering and survival of the fittest analysis to yield highly discriminative diagnostic algorithms. Recently Petricoin and colleagues used mass spectroscopy to generate proteomic spectra in patients with and without ovarian cancer.\(^96\) They found that a cluster pattern could be identified which could completely segregate cancer cases from non-malignant ones with a sensitivity of 100%, and specificity of 95%. This similar technology will be evaluated by the GOG in patients with advanced ovarian cancer or cervical cancer. In the present protocol, proteomic profiles will be created from patients with endometrial cancer to predict risk of recurrence or extrauterine disease spread for example.
STEROID HORMONES AND THEIR RECEPTORS

The link between estrogen-induced proliferation and progesterone-induced differentiation is well established in normal endometrium. Whereas hormonal regulation is initiated via receptor assembly, ligand binding, and interactions with coregulators and the transcriptional machinery, the functional identifiable hormone response incorporates complex interactions with a multiplicity of growth factors that reside in both the epithelium and the stroma. Our understanding of the etiologic processes that predispose to malignant transformation in the endometrium is very limited. Although the majority of endometrial cancers are hormone dependent, the normal control mechanisms are nonetheless lost, allowing uncontrolled growth, invasion, and metastasis. Evidence from breast carcinoma suggests that alterations in hormone receptors and changes in the hormonal milieu in the microenvironment result in the activation of various growth factor pathways. In contrast, hormone-independent tumors constitutively appear to activate growth factor pathways. These tumors account for a minority of the endometrial cancers but constitute a more aggressive histologic subtype and a decrease response to treatment. Our knowledge of the molecular biology in this subset, particularly the understanding of the “cross-talk” among hormones, specific growth factors, hormone and/or growth factor receptors, and other modulations, is very limited. Furthermore, these molecular processes are influenced by interactions within both the epithelium and the stroma.

The endometrium is one of the most dynamic of human tissues, with cyclic proliferation and shedding, which is regulated by steroid hormones (as reviewed by Clarke and Sutherland, 1990). During the past 20 years, most studies have demonstrated a relationship between the presence and quantity of steroid receptors and the degree of histologic differentiation, the nuclear and architectural grade, FIGO stage, and survival. In the past decade, the availability of monoclonal antibodies to steroid receptors has made it possible to determine the distribution of receptors within the endometrium, an essential technical advance, since non-neoplastic uterine tissues also contain receptors and may yield false-positive biochemical assays. Surprisingly, these conceptual and technical advances have not yet yielded an improved hormonal therapy. This may in part reflect the paucity of data concerning the molecular events and mechanisms of action by which steroid hormones affect cell proliferation in patients with endometrial cancer.

During the past decade, there has been a dramatic shift in the methodology for assessing estrogen receptor (ER) and progesterone receptor (PR), with immunohistochemistry almost universally supplanting biochemical assays of cytosol extracts. Isoforms of ER and PR have been found to have different distributions in normal tissue and in tumor tissue, and different functions. This variability exists, not only within different foci from a single tumor, but also among the neoplastic cells lining individual glands, and when primary tumors are compared with metastatic foci. Benign stromal nuclei, as well as gland cell nuclei express both steroid receptors. Thus, the designation of a tumor simply as positive for steroid receptors is biologically simplistic, and should be recognized as such. Nevertheless, in most studies, the presence and quantity of steroid receptors have been positively correlated with histologic differentiation, FIGO stage, and survival. Various authors have found, by univariate and multivariate analysis, that the presence of one or both steroid receptors was predictive of a decreased
probability of recurrence or improved survival.\textsuperscript{99, 106-110} Ehrlich et al, in a study of 175 patients of all stages, reported that recurrence was related to the absence of ER or PR, and that response to progestin therapy was more common in PR positive tumors.\textsuperscript{106} Although ER and PR are found in about 75\% of primary endometrial adenocarcinomas, in an animal model system, there is a rapid and striking decrease in both receptors in responding tumors to almost unmeasurable levels by biochemical assay following short-term progestin therapy.\textsuperscript{111}

The ligands, estradiol and progesterone, bind to specific intracellular receptors (ER and PR respectively) present in the glands and the stroma of the endometrium. ER and PR are expressed as isoforms, ER-\textalpha, ER-\textbeta, PRA, and PRB, which are functionally distinct and can be detected independently by immunohistochemistry. For PR, it has been documented that each isoform controls the expression of a unique group of genes and proteins that have potential clinical relevance, either as markers of clinical outcome or as participants in critical pathways that control tumor growth and invasiveness,\textsuperscript{112} and similar findings are expected for ER-\textalpha and ER-\textbeta. However, despite years of study, much is still unknown regarding hormone action and receptor function in endometrial cancer, and a number of basic questions have yet to be answered. Also, previous studies on the receptor status of tumors from patients with endometrial cancer have been limited by the lack of data relating to the levels of circulating ligand present for receptor activation. To date, the interactions between the ligand and the receptor in predicting tumor biology or the clinical course of women with endometrial cancer have not been fully evaluated. Therefore, we now propose to provide an integrated analysis of receptor and ligand levels linked to clinical outcome.

Based upon evidence from the breast, it is presumed that tumors with low hormone receptor expression depend upon other pathways for growth, including the members of the epidermal growth factor receptor (EGFR) family.\textsuperscript{113} In breast cancer, estrogen receptor negative tumors often overexpress EGFR, and both EGFR and Her-2 (ErbB2) are associated with poor prognosis.\textsuperscript{114-119} It is not clear whether Type 1 and Type 2 tumors actually represent tumors of different origin, or whether Type 1 tumors can progress to Type 2 tumors over time, resulting in recurrences that differ biologically from the original parent tumor. Further, it is not clear how or if tumors classified as Type 1 or Type 2 differ from a molecular standpoint and what relation this may have to the hormone and growth factor receptors expressed by the tumor cells and the surrounding stromal cells.

\textbf{ASSOCIATION BETWEEN ESTROGEN LEVELS OR MEtabolISM AND CANCER DEVELOPMENT}

Researchers have shown that chronic stimulation by estrogen can lead to the development of neoplasia including cancer. As described in section 2.7, epidemiologic data suggests that postmenopausal women taking unopposed estrogen have a 4- to 8-fold increase in the risk of developing endometrial cancer compared to those not taking estrogen.\textsuperscript{120} As with exogenous estrogen stimulation, endogenous unopposed estrogen stimulation may also lead to neoplasia. Obesity is thought to predispose to endometrial cancer because the peripheral conversion of steroidal precursors leads to the production and circulation of estrogens unopposed by progestins. Not all women, however, who appear to have excess estrogen seem to develop endometrial cancer. The factors that
modulate the promoter effects of estrogen are not well understood. One recent line of investigation examined the expression of estrogen receptor (ER) isoforms, and showed that there was an inverse relationship between ER-α expression and tumor grade.121, 122

Estrogen is metabolized by hydroxylation either at the C-2 or -16α positions. These pathways produce metabolites that vary greatly in their potency and function.123 16α−hydroxyestrogens are active compounds and biologically equivalent to their precursor molecules. For example, 16α−hydroxyestrogens have the mitogenic, promoter activities associated with estrogen. In contrast, 2-hydroxyestrogens do not exert mitogenic activity and, in fact, may serve a protective role. The 2-hydroxyestrogens were shown to exhibit anti-oxidative properties in vitro.124, 125 O-methylation of the 2-hydroxylated estrogen metabolites resulted in compounds that are anti-proliferative, pro-apoptotic and anti-angiogenic.126, 127 Studies in the estrogen receptor positive MCF-7 breast cancer cell line in vitro revealed that 16α−hydroxyestrogens increased cell growth whereas the 2-hydroxyestrogens were inhibitory to cell growth.128 Experiments by Suto and co-workers (1992)129 demonstrated that tumors developed in nude mice implanted with MCF-7 cells after exposure to 16α−hydroxyestrogens but not 2-hydroxyestrogens.

Fishman and colleagues (1984) used a radiometric assay to demonstrated the elevation of 16α-hydroxylated metabolites in women with either breast or endometrial cancer when compared to healthy controls; there was no difference between study and control patients in 2−hydroxylated metabolites detected.130 The authors hypothesize that the altered estrogen metabolism may serve as a marker for the development of estrogen-dependent malignancy. In a pilot study of 14 endometrial cancer patients and 13 controls, a lower estrogen metabolite ratio (the level of 2-hydroxyestrogens to the level of 16α-hydroxyestrogens) was observed among the study patients compared to controls (A. Menzin, unpublished results). An inverse relationship has also been shown between estrogen metabolite ratio and risk of developing breast cancer.131, 132 Among the women in the latter study, those who ultimately developed breast cancer were shown to have a 15% lower ratio of 2:16α−hydroxy metabolites at entry into the study as compared to controls.

Confirmation on an imbalance between 16α− and 2-OH- estrogen metabolites might have diagnostic implications, potentially identifying a subset of women at greater risk for developing endometrial carcinoma. Such a finding would also lend support for an intervention that would shift the metabolism of estrogen away from mitogenic compounds. For instance, indole-3-carbinol (I3C), a substance that is found in cruciferous vegetables (e.g., broccoli, Brussels sprouts, cabbage, and cauliflower), induced a marked increase in 2−hydroxylation with a diminution in 16α−hydroxylation among human subjects.133, 134 Human administration has been associated with minimal toxicity.135, 136 I3C has shown promise as a chemopreventive agent in several model systems.137-140 Kojima and colleagues reported that treatment with I3C increased 2-hydroxylation of estrogen and inhibited the development of spontaneous endometrial cancer in female Donryu rats, which develop endometrial cancer at high rates as they age.141 There are thus far no comparable data available examining the effect of I3C on the development of endometrial carcinoma in humans.
A shortcoming of most clinical investigations of cancer biomarkers is the absence of information to fully characterize the study subjects. By collecting epidemiologic information on patients in this study, it will be possible to assess whether results of this study can be broadly generalized to all patients with similar tumors or must be considered only within the context of restricted patient populations. In addition, this information will enable assessment of predictors of disease recurrence as well as identification of risk profiles associated with certain gene expression arrays and proteomic patterns. Biomarkers can also be used to subdivide heterogeneous groups of tumors into more homogeneous subgroups that exhibit similar expression of markers and perhaps also share unique risk factor relationships. This work should prove helpful in providing insights about biological processes involved in the initiation and progression of this disease.

Previous epidemiologic studies of endometrial cancer have shown it to be a complex disease, with a number of identified risk factors, most of which appear to operate through endogenous hormonal mechanisms. Increased risks have been associated with nulliparity, early ages at menarche, late ages at menopause, obesity and long-term use of estrogen replacement therapy (ERT). In contrast, women with multiple births as well as users of oral contraceptives and cigarette smokers are at decreased risk. Many of these factors can be explained by an unopposed estrogen hypothesis, whereby exposure to estrogens unopposed by progesterone or synthetic progestins leads to increased mitotic activity of endometrial cells, increased DNA replication errors, and somatic mutations resulting in a malignant phenotype.142

Two of the strongest identified risk factors for endometrial cancer are use of exogenous estrogens and obesity. Long-term use of unopposed estrogens has been related to marked increases in endometrial cancer risk (10-20-fold relative risks).143 Epidemiologic studies have generally shown that the excess risk of endometrial cancer associated with estrogens alone can be counteracted by the addition of a progestin; this, however, appears to be dependent on the progestin being given for at least 10 days each month.144, 145 An adverse effect of estrogens on the endometrium is also reflected by studies that show an increased risk of endometrial cancer among tamoxifen-treated breast cancer patients.146 In contrast to menopausal hormones, users of combination oral contraceptives (which contain both an estrogen and a progestin) have been found to have approximately half the risk of non-users, with long-term users in most studies experiencing even further reductions in risk.147

Obesity has also been strongly related to endometrial cancer risk, with studies suggesting a 3-4-fold differential between women in the highest and lowest quartiles of weight or body mass index.148, 149 Among postmenopausal women, this association is believed to be largely due to the conversion of androstenedione to estrone by aromatase in adipose tissue.142 In premenopausal women, obesity-associated anovulation associated with a relative deficit of progesterone may be more central. Some studies suggest that body fat distribution may have an independent effect, with high risks seen for women whose weight distributes abdominally rather than peripherally.148 Whether associations with body fat distribution are reflective of unique hormonal profiles has yet to be resolved.
Given the complex of factors identified for this cancer site, there are many leads that can be pursued through integration of biomarkers with carefully defined risk predictors. Given the recognized role of hormonal factors in the etiology of this disease, it will be of particular interest to focus on a variety of markers, which reflect unique hormonal profiles. It is also noteworthy that some women with endometrioid adenocarcinomas do not have obvious risk factors suggestive of increased estrogen exposure; therefore, the pathology and molecular biology of these tumors could differ from other endometrioid adenocarcinomas. Furthermore, the etiology and biology of pre-menopausal endometrial cancer is poorly understood. In particular, the role of polycystic ovarian disease in its multiple manifestations deserves additional study. We also know very little about the etiology of most aggressive histopathologic types of endometrial carcinomas, including serous and clear cell adenocarcinoma. These tumors generally affect somewhat older women than endometrioid tumors, have a higher incidence among blacks as compared to whites in the U.S., and may differ with regard to risk factors, precursors and biomarkers (especially p53 mutation in serous tumors). Finally, although mismatch repair deficits, loss of PTEN suppressor gene function and ras mutations have been associated with endometrioid adenocarcinomas, the effect that these markers have on prognosis has not been thoroughly examined.

2.8 Inclusion Of Women And Minorities (06/14/2004)

The Gynecologic Oncology Group and GOG participating institutions will not exclude potential subjects from participating in this or any study solely on the basis of ethnic origin or socioeconomic status. Every attempt will be made to enter all eligible patients into this protocol and therefore address the study objectives in a patient population representative of the entire endometrial cancer population treated by participating institutions.
3.0 PATIENT ELIGIBILITY AND EXCLUSIONS

3.1 Eligible Patients

3.1.1 Patients must satisfy one of the following: (9/22/2003) (11/08/2004) (09/24/2007)

- Histologically confirmed non-endometrioid endometrial carcinoma or carcinosarcoma of any stage, grade, histologic subtype as diagnosed from an endometrial biopsy or dilation and curettage

OR

- Histologically confirmed endometrioid endometrial carcinoma as diagnosed from an endometrial biopsy or dilation and curettage AND one or more of the following:
  - Grade 3 disease
  - Positive cervical biopsy
  - Pre-operative imaging (such as ultrasound, CT, CT/PET, MRI, etc.) suggesting deep myometrial invasion, lymph node enlargement or extra-uterine disease
  - Non-Caucasian race
  - Hispanic ethnicity
  - BMI below 25 (normal or underweight)

3.1.2 Patients must be suitable candidates for surgery. Patients may also be entered on GOG-2222 (LAP2). (9/22/2003)

3.1.3 An approved informed consent and authorization permitting release of personal health information must be signed by the patient or guardian. (09/18/2006)

3.1.4 Patients who have met the pre-entry requirements specified in Section 7.1.

3.2 Ineligible Patients

3.2.1 Patients not considered suitable candidates for surgery.

3.2.2 Patients who have had prior retroperitoneal surgery.

3.2.3 Patients who have received prior pelvic or abdominal radiation therapy.

3.2.4 Patients who are pregnant.
4.0 STUDY MODALITIES

4.1 Surgical Procedures (09/18/2006)

See the GOG Surgical Procedures Manual and Appendices II-IV for a description of the surgical procedures to be performed for GOG-0210. Utilization of standardized operative procedures will facilitate evaluability of patients by comparison of the operative report that outlines the procedures performed. Adherence to these minimum standards is essential to provide standard surgical staging information on women participating in a molecular staging study of endometrial carcinoma. (9/22/2003)

Patients will undergo an abdominal hysterectomy (TAH; total abdominal hysterectomy or TVH; total vaginal hysterectomy) or a laparoscopic-assisted vaginal hysterectomy, a bilateral salpingo-oophorectomy (BSO), a bilateral pelvic lymphadenectomy* (Appendix II), and bilateral para-aortic lymphadenectomy* (Appendix III) to at least the level of the inferior mesenteric artery (IMA), and collection of pelvic washings for cytology. Intraperitoneal biopsies will be collected as indicated. High para-aortic lymphadenectomy (Appendix IV) is optional at the discretion of the surgeon. The operative procedure used should be clearly specified in the operative report. (9/22/2003) (06/14/2004)

* Patients with Stage IV intra-abdominal disease NOT debulked to less than 2 cm DO NOT require a lymphadenectomy. (09/18/2006)

4.2 Pathology Procedures, Forms, Reports and Slide Requirements (9/22/2003)

4.21 See the GOG Pathology Manual for general information regarding histologic classification of endometrial carcinoma and for a summary of the specific pathology requirements for GOG-0210.

4.22 Appendix I contains the 1988 FIGO surgical staging classification for carcinoma of the corpus uteri.

4.23 Copies of the pathology report(s) for the diagnostic procedure (an endometrial biopsy or a dilation and curettage) from your institution and a referring site if applicable, and copies of the official pathology report for the surgical staging procedure will need to be submitted to the GOG Statistical and Data Center (SDC) for each patient. (06/14/2004) (09/18/2006)

4.24 Copies of the institution cytology report for the peritoneal washes performed during the surgical staging procedure will also need to be submitted to the SDC along with positive cytology slides as indicated in the Pathology Material Submission Form for GOG-0210. (06/14/2004) (09/18/2006)

4.25 The pathologic findings must be summarized on Form PRE and submitted to the SDC electronically via SEDES. (06/14/2004) (09/18/2006)
4.3 Patient Outcome And Treatment Data Collected During Follow-Up (9/22/2003) (09/18/2006)
Information regarding patient outcome including vital status and disease follow-up status and all post-operative cancer-related treatments will be carefully documented on the standard follow-up forms for the GOG. A set of special instructions are provided on the GOG-0210 Protocol Web page which can be assessed from the Member Access portion of the GOG Website for completion and submission of the standard Form Q0 and Form Q electronically via SEDES.

Form Q0 will be completed at the time of the post-operative clinic visit ideally within 6 weeks after surgery and the date of this clinic visit will represent the treatment completion date and submitted electronically via SEDES. Form Q will be completed every 3 months for the first 2 years, every 6 months for the next 3 years, and then yearly for the next 5 years following the post-operative clinic visit. Form Q will need to be submitted electronically via SEDES. (1/18/2011)

If the patient participates in a GOG treatment protocol such as GOG-0209, the follow-up Q forms submitted to the GOG Statistical and Data Center for the GOG treatment protocol will satisfy the follow-up Form Q reporting requirements for GOG-0210.

When a patient is given post-operative treatment off protocol, document all post-operative cancer-related treatments in the “Non-Protocol Therapy” section of the Q Form. With regard to the level of detail needed for reporting post-operative cancer-related treatment, detailed information will be required for all cancer-related treatment administered prior to progression. After progression, please specify the type of treatment and a list of the agent(s) administered. Please use the “Non-Protocol Therapy” section of the Q Form to report the cancer-related post-operative treatments.

When chemotherapy (cytotoxic and non-cytotoxic) is administered, please provide general information regarding agents utilized with names, doses, cycles, treatment start and stop dates for cancer-related treatments administered prior to progression. After progression, please provide the name of the agents administered.

When radiation is administered prior to progression, please provide a full summary of the radiotherapy. Thereafter, indicate the type of additional radiation therapy provided.

When hormonal treatment is provided prior to progression, please describe the agents administered with dose and schedule as well as treatment start and stop dates. Thereafter, indicate the hormonal agents administered.

See Section 7.2 for a summary of the specimen requirements for GOG-0210 and Appendix V for the specimen procedures for this protocol.

4.5 Gynecologic Questionnaire (9/22/2003) (09/18/2006)
4.51 See Section 5.4.
5.0 TREATMENT PLAN AND ENTRY/RANDOMIZATION PROCEDURE

Sites must submit, all IRB approvals (initial and continuing) on NCI sponsored adult Cooperative Group phase I, II & III prevention and treatment studies to the CTSU Regulatory Office, at the Coalition of Cancer Cooperative Groups in Philadelphia. A CTSU IRB/Regulatory Approval Transmittal Sheet should be submitted along with the CTSU IRB Certification Form or its equivalent. (CTSU forms can be downloaded at https://www.ctsu.org/public/rss2_page.aspx). IRB submissions can be faxed or e mailed (preferred methods) or mailed to

Cancer Trials Support Unit (CTSU)  
ATTN: Coalition of Cancer Cooperative Groups (CCCG)  
Suite1100  
1818 Market Street  
Philadelphia, PA 19103  
FAX: 1-215-569-0206  
CTSURegulatory@ctsu.coccg.org (12/27/2010)  

5.1 Registration

When a suitable candidate has been obtained for protocol entry, the following steps should be taken:

5.11 An approved informed consent form and authorization permitting the release of personal health information must be signed by the patient or guardian. Current FDA, NCI and institutional regulations concerning informed consent will be followed. (9/22/2003) (09/18/2006)

5.12 All eligibility requirements indicated in 3.0 must be satisfied.

5.13 The Fast Fact Sheet data must be gathered. (09/18/2006)

5.14 The institution must register the patient using the web-based registration application or by phone if necessary (800-523-2917). Instructions for web-based registration and randomization can be found by going to the GOG Web Menu page, selecting "Start/finish a patient registration," and then selecting "Directions" found on the left side of the page. Assistance is available from the Statistical and Data Center by phone if necessary (800-523-2917). (09/18/2006)

5.15 The institution must enter the patient’s name, and GOG patient study ID, in the appropriate place in their Log Book to verify the patient’s entry. (9/22/2003)


5.21 SURGICAL STAGING. Patients will be surgically staged by undergoing either an abdominal hysterectomy or a laproscopic-assisted vaginal hysterectomy, a bilateral salpingo-oophorectomy, a bilateral pelvic (Appendix II) and bilateral para-aortic lymphadenectomy* (Appendix III) and peritoneal cytology. Intraperitoneal biopsies will be performed as indicated. High para-aortic
lymphadenectomy (Appendix IV) is optional at the discretion of the surgeon. (06/14/2004)

* Patients with Stage IV intra-abdominal disease NOT debulked to less than 2 cm DO NOT require a lymphadenectomy. (09/18/2006)

5.22 POST-OPERATIVE TREATMENT. Patients will be given post-operative treatment for their endometrial carcinoma as needed. Participation in GOG treatment protocols will be encouraged when appropriate. Details regarding all forms of post-operative treatment for this disease, including treatment for recurrent or persistent endometrial carcinoma, will need to be carefully documented. For patients who receive post-operative treatment off protocol, please document all cancer-related post-operative treatment in the “Non-Protocol Therapy” section of the Q form as described in section 4.3. First-line treatments following surgical staging will need to be reported in detail. It will be sufficient to provide a brief overview for any subsequent treatments. For patients participating in a GOG treatment protocol, the reporting requirements for the treatment protocol will satisfy the treatment reporting requirements for GOG-0210. (09/18/2006)

5.23 FOLLOW-UP. Patients will be followed every 3 months for the first 2 years, every 6 months for the next 3 years, and then yearly for the next 5 years after the post-operative clinic visit approximately 6 weeks after surgery. The Q0 form will be completed during the post-operative clinic visit and the date of this post-op visit will represent the treatment completion date. Form Q will be completed every 3 months for the first 2 years, every 6 months for the next 3 years, and then yearly for the next 5 years after the post-operative clinic visit approximately 6 weeks after surgery. Details regarding the patient’s vital status and disease progression will be carefully documented on the Follow-Up Forms. Q forms submitted for a GOG-0210 who participates in a GOG treatment will satisfy the Q form requirements for GOG-0210. Post-operative treatment information will be documented as indicated in section 4.3 and section 5.22. (1/18/2011)

5.3 Collection And Submission Of Specimens For GOG-0210 (9/22/2003) (06/14/2004) (09/18/2006)
See Section 7.2 for a summary of the specimen requirements for GOG-0210 and Appendix V for the specimen procedures for this protocol.


5.41 This protocol will collect private and potentially sensitive data from participants. This data will be used for research purposes only and will be protected to the full extent permitted by law. Identifying personal information, such as name, address, phone number, or Social Security Number, will not be disclosed to researchers and will not be included in any publication that results from this study. The original questionnaire data will be submitted to the GOG Statistical and Data Center and stripped of all identifiers before submission to Westat for - 33 -
scanning and storage. A unique study identification number will identify all participant data and specimens. (06/14/2004) (09/18/2006)

5.42 All patients will be asked to use a pen not a pencil to complete a clear, centered, single-side and full-sized copy of the Gynecologic Questionnaire which should be printed from SEDES. Although the patient can be asked to complete this questionnaire before or after surgery, special consideration should be given to asking the patient to complete the questionnaire after surgery rather than immediately prior to procedure because of the potential stress on the patient if asked to complete the questionnaire before her diagnostic surgery. The clinical staff should also consider providing the patient with an envelope, marked on the outside with the GOG Study ID number, and asking the patient to seal the questionnaire in the envelope immediately after completion. The patient should be encouraged to only answer questions that she feels comfortable answering. A copy of this questionnaire should be retained in the study chart in a sealed envelope marked with the GOG Study ID, and the original with the coversheet will be submitted to the GOG Statistical and Data Center within 6 weeks of entry. (06/14/2004) (09/18/2006) (09/24/2007)

5.43 Dr. Louise Brinton, in the Division of Cancer Epidemiology and Genetics, will be responsible for providing an electronic copy of the data captured on the Gynecologic Questionnaire for GOG-0210 to the GOG Statistical and Data Center and for working with the GOG to analyze the epidemiologic data provided in the self-administered questionnaire along with appropriate clinical, histopathologic and laboratory data. (9/22/2003) (09/18/2006)
6.0 TREATMENT MODIFICATIONS

6.1 Treatment modifications will not be needed for this protocol since full surgical staging is the only treatment specified. Depending upon the findings of the surgery further treatment may be indicated, but is not specified in this protocol. The physician will discuss the available post-operative treatment options in detail with the patient and further treat the patient as needed. Participation in GOG treatment protocols is strongly encouraged. (9/22/2003)
### 7.0 STUDY PARAMETERS


The following observations and tests are to be performed and recorded on the appropriate form(s). Specimen requirements for translational research are summarized in section 7.2. The pathology slides required for Central Pathology Committee review are summarized in Section 10.2. *(06/14/2004) (09/18/2006) (1/18/2011)*

<table>
<thead>
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<th>Parameter</th>
<th>Prior to Entry</th>
<th>Prior to Surgery</th>
<th>Within 6 Weeks of Entry (06/14/2004)</th>
<th>Post-Op Visit (within 6 weeks after surgery) (06/14/2004)</th>
<th>Follow-up Schedule: every 3 months for the first 2 years, every 6 months for next 3 years, then yearly for the next 5 years</th>
<th>At recurrence or progression</th>
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<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

X - Indicates requirements that are mandatory.

† If the endometrial biopsy or dilation and curettage show endometrioid endometrial cancer, a cervical biopsy and/or pre-operative imaging (such as ultrasound, CT, CT/PET, MRI, etc.) may be needed to determine eligibility. *(09/24/2007)*
7.21 SPECIMEN REQUIREMENTS (09/18/2006)

A total of 13 specimens will be required for this protocol as outlined below.

- The pre-op serum (SB01), urine (UR01), frozen and fixed normal tissue (RN01, FN01), post-op serum (SB02) and 3-year follow-up serum (SB03) will be able to be obtained for all patients.

  The pre-op serum specimen must be prepared from blood drawn prior to surgery. The blood may even be drawn at the time the intravenous line is started for surgery provided that the patient has not received pre-operative medications.

- Frozen and fixed primary tumor (RP01 and FP01, respectively) will be able to be obtained for almost all patients, but a small subset of patients may not have sufficient primary tumor available at the time of the surgical staging procedure for both diagnostic and research purposes. If gross primary tumor is not available at the time of surgery but a primary tumor block or 25 unstained sections on charged slides suitable for a standard immunohistochemistry assay can be submitted to satisfy the fixed tumor tissue requirement for translational research that is ideal.

- Frozen & fixed metastatic tumor (RM01 and FM01) will only be able to be obtained for patients with gross metastatic tumor present during staging.

- Recurrent serum (SB04) will be obtained from the subset of patients who experience disease progression/recurrence.

- Frozen and fixed recurrent tumor (OR01 and FR01, respectively) will be obtained from the subset of patients with disease progression/recurrence who have tumor that can be safely excised.

<table>
<thead>
<tr>
<th>Required Specimen</th>
<th>Prior to Surgery</th>
<th>During Surgical Staging</th>
<th>Post-Op Visit (within 6 weeks after surgery)</th>
<th>Follow-Up Visit (approximately 3 years after surgery)</th>
<th>At recurrence or progression</th>
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<td>SB02</td>
<td>SB03</td>
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A pre-op serum specimen will need to be prepared for all patients from 10 ml of blood drawn on the day of and prior to the surgical staging OR when pre-op bloods are drawn. Collection of the specimen at the time the intravenous line is started for surgery is acceptable provided that the patient has not received pre-operative medications as these medications may alter the profile of proteins in serum.

Extra points will be awarded when the metastatic tumor specimens (1 point for FM01 and RM01), the recurrent serum specimen (1 point for SB04) and the recurrent tumor specimens (4 points for FR01 and OR01) are submitted.

Details regarding specimen collection, shipping, banking and distribution are provided in Appendix V (Specimen Procedures for GOG-0210). A specimen form (Form SP) for each of the thirteen required specimen will need to be submitted to GOG Statistical and Data Center electronically using SEDES regardless of submission status as specified in Section 10.2. A copy of Form SP will also need to accompany each specimen when shipped to the GOG Tissue Bank. Always retain a copy of each SP Form submitted for this protocol in your records.

7.22 The types of laboratory testing performed on the clinical specimens collected and banked from the patients participating in this molecular staging protocol will be described in a project-specific manner in a specific appendix. The essential features for each of the independent and integrated research projects for GOG-0210 will be re-evaluated over time so that new advances in research and technology can be appropriately incorporated into this protocol.


7.23 Additional Details Regarding The Laboratory Testing For Translational Research (06/14/2004)

Various types of laboratory tests including genomic, proteomics and immunoassays will be performed on the tissue, urine and serum specimens collected and banked for the purpose of class prediction and class discovery to improve risk assessment, to identify characteristics that can be targeted or exploited in the prevention or treatment of endometrial carcinoma, and to expand the current understanding of the biology, progression, metastasis and responsiveness of endometrial carcinoma

Light Microscopy will be performed using formalin-fixed and paraffin-embedded tissue specimens to characterize the histopathologic features of the
tissue specimens undergoing molecular profiling, and to satisfy some of the specimen selection criteria for gene expression profiling.

**Affymetrix Gene Expression Microarray Analysis** will be undertaken using RNA isolated from select subsets of frozen primary tumor, normal tissue, metastatic tumor and recurrent tumor to define gene expression patterns.

**Ciphergen SELDI-TOF Mass Spectrometry** will be performed in select subsets of pre-op, post-op, follow-up and recurrent serum specimens to define protein/peptide fragment patterns. The intra-op urine specimen will also be examined in select groups of patients.

**Two-Dimensional Gel Electrophoresis Linked With Mass Spectrometry** (AP Biosystems MALDI-TOF mass spectrometry or with electrospray [ESI] mass spectrometry with a high resolution Micromass Q-TOF mass analyzer) will be performed on select subsets of frozen primary tumor, normal tissue, metastatic tumor and recurrent tumor to define protein/peptide fragment patterns.

**Immunohistochemistry Assays** will be performed on formalin-fixed and paraffin-embedded frozen primary tumor, normal tissue, metastatic tumor and recurrent tumor to characterize the tumor expression of steroid hormone receptor isoforms and members of the EGFR family.

**Enzyme-Linked Immunoassays** will be performed in select subsets of pre-op, post-op, follow-up and recurrent serum specimens to determine the circulating levels of estradiol and progesterone. The level of 2-hydroxyestrogens and 16-hydroxyestrogens will also be quantified in the intra-op urine specimen.

**Bioinformatics and Statistics** will be used for data mining and analyses and for the purpose of examining genomic, proteomic and immunoassay data for class prediction and class discovery.

Additional methodologies will be incorporated as needed, and new technologies that become available will be applied as indicated.
8.0 EVALUATION CRITERIA

8.1 The adequacy of surgery-staging will be assessed according to surgical evaluation and pathology evaluation (guidelines provided in the GOG Surgical Manual, the GOG Pathology Manual and Appendices I-IV). *(9/22/2003)*

8.2 Survival will be defined as observed length of life from protocol entry to death, or for living patients, date of last contact regardless of whether or not this contact is on another GOG protocol.

8.3 Progression-free survival will be defined as date from protocol entry to date of evidence of disease recurrence or progression, death or date of last contact whichever occurs first.

8.4 Cause of death will be carefully documented.

8.5 Sites of recurrence will be carefully documented. Recurrence will be defined as discovery of disease not previously present by clinical, radiographic, and/or laboratory means. Progression is defined as 50 percent or greater increase in the product from any documented lesion. Histologic confirmation of suspected progressive disease is left to the judgment of the attending physician.

8.6 All types of post-operative treatment will be carefully documented in each case including treatments for recurrent or persistent disease. Participation in GOG Treatment Protocols will be encouraged wherever possible. First-line treatments following surgical staging will be reported in detail whereas a brief overview of subsequent treatments will be sufficient. The reporting requirements for the GOG treatment protocol will also satisfy the post-operative treatment reporting requirements for GOG-0210. *(9/22/2003)*

8.7 The evaluation criteria for the results obtained from each type of laboratory test performed on the GOG-0210 clinical specimens will be described in a project-specific manner in a specific appendix.

8.71 Gene Expression Profiling (Appendix VIII).

8.72 Tissue Proteomic Profiling (Appendix IX).

8.73 Serum Proteomic Profiling (Appendix X).

8.74 Immunohistochemistry Assays For Steroid And Growth Factor Receptors (Appendix XI).

8.75 Immunoassays For Steroid Hormones And Estrogen Metabolites (Appendix XII).
9.0 DURATION OF STUDY

9.1 This study will accrue at least 3,500 evaluable patients and these patients will be followed for recurrence and survival for 10 years. It is appreciated that most recurrences will appear in the first three years after entry. (09/24/2007)

9.2 Subsets of certain tumor characteristics; e.g. low grade may be changed from eligibility as the numbers needed for analysis are accrued faster than other characteristics.
10.0 STUDY MONITORING AND REPORTING PROCEDURES

10.1 ADVERSE EVENT REPORTING FOR A TRIAL EVALUATING A SURGICAL PROCEDURE (09/18/2006)

10.11 Definition of Adverse Events (AE)

An adverse event (AE) is any unfavorable and unintended sign, symptom, or disease that occurs in a patient administered a pharmaceutical product or protocol procedure, whether the event is considered related or unrelated to the study treatment.

10.12 Expedited Reporting of Adverse Events occurring within 30 Days of the Study Procedure

The following table summarizes the GOG requirements for expedited reporting of AEs that occur within 30 days of the surgical procedure.

Reporting Requirements for Adverse Events that occur within 30 Days' of the Study Procedure:

From the period of protocol activation through December 31, 2010, Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (CTCAE v3.0) are utilized for defining and grading specific adverse events through the AdEERS system. (12/27/2010)

Beginning January 1, 2011, the NCI Common Terminology Criteria for Adverse Events (CTCAE) v 4.0 will be utilized for AE reporting through the AdEERS system. CTCAE v 4.0 is located on the CTEP website at (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). All appropriate treatment areas should have access to a copy of this Version of CTCAE. CTCAE v 4.0 definition is also available on the GOG member website (https://gogmember.gog.org under MANUALS). (12/27/2010)
Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

- Expedited AE reporting timelines defined:
  “7 calendar days” – A complete AdEERS report on the AE must be submitted within 7 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grades 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities and/or incapacities must be reported via AdEERS if the event occurs following a protocol procedure.

- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

### Additional Instructions or Exceptions to AdEERS Expedited Reporting Requirements for Surgical Trials:

- There are no additional instructions or exceptions to AdEERS expedited reporting requirements for this protocol.
### 10.13 Procedures for Expedited Adverse Event Reporting

**AdEERS Expedited Reports:** Expedited reports are to be submitted using AdEERS available at http://ctep.cancer.gov. The NCI guidelines for expedited event reporting requirements are also available at this site.

### 10.2 GOG DATA MANAGEMENT FORMS (09/18/2006)

The following forms must be completed and submitted to the GOG Statistical and Data Center (SDC) in accordance with the schedule below. All forms except: PMS-form, BDR form, Pathology report, Operative report, Report of Pre-Operative Imaging, Gynecologic Questionnaire including the coversheet must be submitted via the SDC Electronic Data Entry System (SEDES) which is available through the GOG website (www.gogstats.org). Pathology material (Form PMS, pathology reports, pathology slides, cytology report and positive cytology slide) must be submitted together via mail. (9/22/2003) (06/14/2004) (09/24/2007)(12/27/2010)

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* The number of required copies including the original form that must be sent to the GOG Statistical and Data Center.

** Form DR should be completed prior to surgery from the perspective that surgery is the treatment specified for this protocol. It is not necessary to complete Form D2M or Form BDR for GOG-0210. (12/27/2010)
The original Gynecologic Questionnaire including the cover sheet must be submitted for each patient and keep a copy in your files. Ask the patient to complete a clear single-sided print-out of the Gynecologic Questionnaire that is centered on the page and full size. Make sure to add the patient study ID, the date the patient was given the questionnaire on the cover page and the patient study ID to the top corner of each page of the questionnaire prior to submission. Personal health information including patient name, initials, chart #, phone number and date of birth must be removed from the questionnaire prior to submission. In the event that the patient refuses to answer the questionnaire, please write "Patient Refused" on the coversheet and submit the coversheet attached to a blank questionnaire with the patient study ID on each page for scanning. If patient dies before having a chance to answer the questionnaire, please write "Patient Died" on the cover page and submit the coversheet attached to a blank questionnaire with the patient study ID on each page for scanning.

Pathology slides are required for central review by the GOG Pathology Committee. Representative stained slide (or slides) documenting each histologic cell type, grade, maximal myometrial invasion, total myometrial thickness, vascular invasion, cervical invasion (surface and/or stroma), sites of metastatic tumor, positive cytology and one slide to show the most advanced stage of disease must be submitted to the GOG SDC. If tumor is not present in the tissue removed during the surgical staging procedure, make sure to submit slides from the diagnostic procedure to confirm eligibility. When submitting pathology material to the GOG SDC, individual slides must be labeled with GOG Patient ID and patient initials and packed in plastic slide cassettes. Tape plastic slide cassettes shut and wrap in bubble wrap or another type of padded material prior to shipping. Ship pathology slides and two copies of the Pathology Material Submission Form, official pathology report for the diagnostic procedure and the surgical staging procedure as well as the official cytology report and positive cytology slides directly to the Pathology Materials Coordinator at the GOG Statistical and Data Center, Roswell Park Cancer Institute, Research Studies Center, Carlton and Elm Streets, Buffalo, New York, 14263; phone (716) 845-5702. Please include the GOG Patient ID, patient initials, and protocol number on all pages of the pathology reports and cytology report and black out the patient’s name.

Q0 Form should be completed at the time of the post-op clinic visit, which should be within 6 weeks of the surgical staging procedure. Please use the date of the post-op clinic visit as the treatment completion date. The follow-up schedule is quarterly for the first 2 years, semi-annually for 3 additional years and then annually for the next 5 years.

SP Forms for all research specimens must be submitted online to the GOG SDC using SEDES. Research specimens must be shipped to the GOG Tissue Bank in Columbus Ohio (address provided below) with copies of the SP Forms as specified in Appendix V. GOG Tissue Bank / Protocol GOG-0210, Children’s Hospital, 700 Children’s Drive, WA1340, Columbus, OH 43205, Phone: (614) 722-2810, FAX: (614) 722-2897, E-mail: gogbank@pediatrics.ohio-state.edu. Always retain a copy of each SP Form in your files.

This study utilizes the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0) for defining and grading adverse events to be reported on GOG case report forms. A GOG CTCAE v3.0 Manual is available on the GOG member web site (http://www.gog.org under MANUALS) and can be mailed to the institution registering a patient to this study if requested.

This study will be monitored by the Abbreviated Clinical Data Update System (CDUS) Version 1.x. CDUS data will be submitted quarterly to CTEP by electronic means.
11.0 STATISTICAL CONSIDERATIONS

The statistical considerations for this molecular staging study will be developed in collaboration with the GOG Statistical and Data Center as the individual research projects are further defined. It is essential to define the specific hypotheses to be tested, methods to be employed, evaluation criteria, and expected outcomes before the appropriate sample size and power calculations can be performed and the bioinformatic and statistical methods that will need to be employed for data mining and analyses can be defined.

Please see below for some general comments regarding the analysis of genomic and proteomic data for the purpose of identifying informative molecular characteristics associated with risk of endometrial cancer recurrence.

Various types of tissue and serum specimens will undergo in-depth laboratory testing using genomics, proteomics and immunoassays. The primary goal will be to identify and validate informative molecular characteristics which are prognostic of recurrence within three (3) years, within subjects grouped by disease stage, if necessary. Other concomitant clinical and epidemiologic factors will be used in conjunction with the informative genomic, proteomic and immunoassay data for prognosis, including treatment. (Note: post-operative treatment will necessarily not be specified by this protocol, and patients will receive further treatment as needed.)

To identify and validate informative molecular characteristics associated with risk of recurrence, laboratory testing will be performed on primary tumor tissue and the pre-op serum from a specific number of patients who recurred within 3 years and a specified number of patients who did not recur within 3 years. Matching is likely to be 1-1 or possibly 1-2.

The basic approach to be utilized will divide the subject samples into a “training” or model development component, a “testing” component, and then a validation component. Additional approaches such as cross-validation and bootstrapping techniques will also be employed. The general approach for model development for the purpose of class prediction and class discovery using genomic or proteomic array data will involve one or more of the following.

1. **initial dimensionality reduction**, in which the transcripts or proteins/peptide fragments to consider in further analyses is reduced on an individual basis, including analyses of missingness and artifacts;

2. **additional dimensionality reduction**, in which the remaining transcripts or proteins/peptide fragments are used to determine a much smaller dimensional space derived from uncorrelated linear combinations of the variables which suitably represent the data;

3. **profile determination (optional)**, using a cluster analysis methodology, with principal components or factors as input, classify the subjects using the variables remaining after dimensionality reduction; or using self-organizing maps;

4. **outcome or validation analysis procedures** depend upon whether profile analyses were conducted:
a. if a factor analysis was performed, use logistic or Cox proportional hazards analysis with the factors as possible explanatory variables to evaluate the predictive power of the identified factors with respect to 3-year recurrence or progression;

b. if a cluster analysis was performed, determine the proportions of subjects in each cluster who recurred or progressed within 3 years and combine the results into an index of the prognostic power of the clustering;

c. if an ANN analysis was performed, the “testing” and “validation” datasets need to be used to evaluate the model;

NOTE: the cluster and factor analysis methods do not use outcome data in determining the clusters or factors; they therefore do not need “testing” or “validation” datasets.

5. sensitivity analyses should be conducted, assuming that the prognostic model is “successful”; these analyses will perhaps identify the primary transcripts or proteins/peptide fragments responsible for the clustering, factor analytic or ANN results.

There are several different methods for Step 1 and Step 2 from which to choose and no one method has been shown to be superior to the others. Computer programs available for each method in each step:

Missing Values Data for a specific mRNA in a microarray assay or mass/charge point in a proteomic assay may be missing. The two most likely explanations are very low concentrations and “image analysis failure”. In a recent publication on gene expression profiling in small, round blue cell pediatric tumors, 65% of the 6567 genes analyzed had at least one “image analysis failure” among the 88 subjects in the study. Khan et al eliminated these 4259 genes from any analysis. This procedure is clearly problematic, because if they had had twice as many subjects (n=176) the failure rate could have been close to 100%. We will use one or more methods of imputation if we experience a failure rate anywhere near as high as this. In addition, if there are a few subjects who have most of the failures, elimination of those subjects from further analyses would be better than eliminating most of the genes from further analyses.

Initial Dimensionality Reduction The first step in dimensionality reduction in the “training” set is to eliminate those mRNAs or proteins for which there is no statistical indication of a variation between patients who do and who do not have a recurrence. Because the data are quantitative, simple individual t-tests will be performed, possibly with a normalizing transformation (e.g., log or square root transformation) if the distribution is highly skewed. Nonparametric and other approaches, (e.g., Hochberg, Benjamini, 1990; Keselman et al 2002), are also useful. The p-value to exclude will initially be set at 0.10, because in Step 2 even marginally significant mRNAs or proteins may be useful. During Step 4 sensitivity analyses, the p-value will be sequentially lowered.

Additional analyses for genomic microarrays will be conducted using correlational techniques for groups of mRNAs which are considered to be associated in their expression levels. If we
can model the correlation of one or more of these mRNA concentration levels as a function of the others in the group, with correlations above 0.7 say, we can then consider the information to be duplicated and eliminate from further analyses those mRNAs.

Additional Dimensionality Reduction There are numerous procedures which may be used and, in a sense, the decision as to which one to ultimately use depends upon the positive predictive values associated with each. With this as criteria, two validation samples may be necessary.

1. Principal Component Analysis Principal component analysis (PCA) is a common analytic method for reducing dimensionality and accounting for the variance in the original data. The PCA model is as follows:

$$Y = PW,$$  
(1)

where $Y$ is a Mx1 vector random variable from which the data are generated, $W$ is a Mx1 vector random variable composed of M uncorrelated variables, and $P$ is a MxM matrix composed of the eigenvectors of the variance-covariance matrix of $Y$. The principal components (PC) are usually ordered by the amount of variance accounted for by the component. The total number of PCs equals the number of mRNAs and proteins for which data are obtained. Conceptually they are an orthogonal transformation of the original multidimensional variable. Thus, they are uncorrelated by construction. The number of principal components to use for dimensionality reduction should be small and depends upon the cumulative proportion of the variance accounted for by those PCs selected and the incremental increase associated with an additional PC. Determination of the number of PCs is not standardized.153-155

2. Factor Analysis Factor analysis is a related method of dimensionality reduction. Factor analysis postulates the model

$$Y = AF + G,$$  
(2)

where $F$ is a fx1 vector of “common factors”, $A$ is a px$f$ matrix of constants, and $G$ is a px1 vector of uncorrelated “error” terms or specific factors. The common factors are not unique, for there are representations with correlated or uncorrelated common factors. Conditions are placed on the model so that the estimate of $A$ is a non-singular matrix, with inverse $U^c$, say. The we can estimate $F$ for any particular patient as $U^cY$. The estimate for $A$ is not unique and orthogonal rotations within in $f$-dimensional space produce statistically equivalent solutions.

In addition to determining the number of common factors, there is the problem of deciding on whether to use correlated or uncorrelated factors. This latter decision often depends upon whether the uncorrelated factors appear to have a biological interpretation.

Profile Determination There are also numerous analytic competitors for profile determination.
Cluster Analysis

There are several ways in which cluster analysis can be conducted, e.g., hierarachial, K-means, self-organizing networks, reshuffling, multidimensional scaling or PCA. The input variables can be the identified principal components or the factors. The primary problems involve the distance function to be used in a high dimensional Euclidean space, the number of clusters to be used, and the biologic interpretability of the clusters. This is a commonly used statistical methodology.

Outcome or Validation Analyses

1. Clusters Depending upon loss-to-follow-up and competing risks, the rate of recurrence or progression within 3 years can be estimated within each cluster. Rates which are close to 0 or 1 would be associated with profiles in which prognosis was quite effective. Rates which are in the middle range between 0 and 1 indicate profiles in which we do not (yet) have appropriate data for effective prognosis. Clinical, treatment and demographic variables can be included in the analyses.

2. Factors The factors themselves can be used to estimate hazard rates for recurrence or progression within 3 years. Clinical, treatment and demographic variables can also be included in the analyses.

3. Artificial Neural Networks Artificial neural networks (ANN) was developed over the last 40-50 years in an effort to emulate human thinking and perception processes. Despite this history, it can be considered new to medicine, so we provide a more complete, but not very technical, overview. Unlike cluster and factor analyses, ANN does not provide interpretable or apparent profiles which can be used to “explain” the association between the input data and the outcome data. The input data can include clinical, treatment and demographic data.

Terminology emulates that used for real neural networks. ANNs are basically a non-linear, multivariate regression methodologies designed to identify patterns in multivariate data which are associated with different outcomes, e.g., endometrial cancer recurrence. ANNs have a series of “nodes” or “neurons” which carry out non-linear calculations based on weighted input data. The nodes send results to other nodes which use these results as input data. The output is compared to known results. Initial small, randomized weights are provided to the ANN. However, through a series of feedback loops, the weights are adjusted and the ANN “learns” and produces results increasingly in agreement with the known results. This process is performed on a “training” set. We say that the ANN has undergone “training”.

At a particular node or neuron, the incoming signals are combined by a weighted linear function into a single value \( s = \sum w_i a_i \), where the \( a_i \) are the input and the \( w_i \) are the weights. The output comes from applying a possibly nonlinear function to \( s \). A common output function is the logistic function: \( f(s) = 1/(1 + e^{-s}) \). There are “layers” of neurons, often 3. The first layer represents the input neurons, the second layer represents the processing neurons, and the third layer represents the output neurons. Weights are needed for each layer other than the output layer.
Because of a lack of knowledge, usually each neuron in the 1st layer is connected to each neuron in the 2nd layer. There is similar full interconnectedness between layers 2 and 3. During the training process, initial weights are set to small random numbers, again because of a lack of knowledge. Through a very large series of re-runs, the weights are adjusted to increase the agreement between the actual outcome data and the ANN outcome results. The processes by which the weights are altered can be very complicated and there are many choices. The index of agreement with the actual outcome is usually taken to be the mean square error, which is the average squared difference between the actual outcome and the predicted outcome for each subject in the training set. It should be noted that the number of output patterns is adjustable, with more than one output pattern leading to recurrence, say.

Thus, in general, ANNs are “simply” systems of non-linear equations with the unknown parameters or weights within the equations estimated through a series of iterations and a function with which to evaluate the extent of agreement between the ANN output and “reality”. These processes are common to many statistical methods, including those used in genetic segregation and linkage analyses (in which Dr. Sobel received post-doctoral training under Dr. Robert Elston at UNC) and non-linear versions of path analysis. The usefulness of ANN and these other applications is obviously highly dependent upon the modern computer.

In addition to the training dataset, two additional, independent datasets should be used: a “testing” dataset; and a “validation” dataset. The “testing” dataset is used to determine when “overtraining” has occurred. The “training” process results in successively lower mean square error, i.e., presumptively better prediction. However, when each successive set of estimated parameters is applied to the independent “testing” dataset, it is commonly the case that the mean square error decreases at first, but then begins to increase. The training process has become too detailed. The parameter estimates at the minimum value of the mean square error for the “testing” dataset should be applied to the “validation” dataset, whose mean square error is the final indication of the effectiveness of the model.

With cluster and factor analytic approaches, intermediate probabilities (i.e., no close to 0 or 1) of recurrence or progression within 3 years can be the case for some clusters or factor combinations. ANN can accommodate this situation also by allowing the output to be something other than 0 (no) or 1 (yes).

Concerns or Limitations with ANN

Concern #1 The ANN methodology currently requires that the outcome data not be missing, unless we want to use “missing” as an outcome category to be modeled by the input data. Thus, if there is considerable loss-to-follow-up, including death from competing risks, there may be a non-negligible proportion of missing subjects, severely limiting the generalizability of the study.
Concern #2 ANN analyses do not produce a unique model. That is, with
- different randomized initial weights,
- different sample of training cases,
- different order of presentation of cases during training, and
- different number of training cycles,

there are likely to be different solutions. Consequently, it is often suggested that numerous training models be used with an average output calculated from the use of the training models. This can make identification of biologically most important proteins or mRNAs problematic.

Sensitivity Analyses

One of the primary goals is to identify, if they exist, a small number of transcripts and/or proteins/peptide fragments whose presence is either decreased or increased in a manner related to risk of recurrence. This can be done in two steps: (1) by selectively eliminating one or more of the variables used in the cluster, factor or ANN analyses; and (2) by selectively eliminating one or more of the transcripts or proteins/peptide fragments which were used in dimension reduction. With each elimination, the analyses are run again to determine how much, if anything, was lost by the elimination. This process may lead to identification of specific transcripts or proteins/peptide fragments involved in cancer progression, metastasis and recurrence.

Design Considerations

Design considerations will be detailed for each of the individual projects to be developed.
12.0 BIBLIOGRAPHY


Appendix I - SURGICAL STAGING - CARCINOMA OF THE CORPUS UTERI

FIGO CLASSIFICATION (1988)

STAGE 0: Carcinoma in situ. Histologic findings suspicious of malignancy.

(Cases of Stage 0 should not be included in any therapeutic statistics.)

STAGE I: The carcinoma is confined to the corpus.

STAGE IA: The length of the uterine cavity is 8 cm or less.
STAGE IB: The length of the uterine cavity is more than 8 cm.

The Stage I cases should be sub-grouped with regard to the histological type of the adenocarcinoma as follows:

Grade 1 - Highly differentiated adenomatous carcinoma.
Grade 2 - Differentiated adenomatous carcinoma with partly solid areas.
Grade 3 - Predominantly solid or entirely undifferentiated carcinoma.

STAGE II: The carcinoma has involved the corpus and the cervix.

STAGE III: The carcinoma has extended outside the uterus but not outside the true pelvis.

STAGE IV: The carcinoma has extended outside the true pelvis or has obviously involved the mucosa of the bladder or rectum. A bullous edema as such, does not permit a case to be allotted to Stage IV.

STAGE IIA Grade 1, 2, 3: Endocervical glandular involvement only.
STAGE IIB Grade 1, 2, 3: Cervical stromal invasion.

STAGE IIA Grade 1, 2, 3: Tumor invades serosa and/or adnexae and/or positive peritoneal cytology.
STAGE IIB Grade 1, 2, 3: Vaginal metastases.
STAGE IIC Grade 1, 2, 3: Metastases to pelvic and/or para-aortic lymph nodes.

STAGE IVA Grade 1, 2, 3: Tumor invasion bladder and/or bowel mucosa.
STAGE IVB: Distant metastases including intra-abdominal and/oringuinal lymph node.
Histopathology -- Degree of Differentiation

Cases of carcinoma of the corpus should be grouped with regard to the degree of differentiation of the adenocarcinoma as follows:

Grade 1 = 5% or less of a non-squamous or non-morular solid growth pattern.
Grade 2 = 6-50% of a non-squamous or non-morular solid growth pattern.
Grade 3 = More than 50% of a non-squamous or non-morular solid growth pattern.

Notes on Pathological Grading

(1) Notable nuclear atypia, inappropriate for the architectural grade, raises the grade of a grade 1 or grade 2 tumor by 1.

(2) In serous adenocarcinomas, clear cell adenocarcinomas, and squamous cell carcinomas, nuclear grading takes precedence.

(3) Adenocarcinomas with squamous differentiation are graded according to the nuclear grade of the glandular component.

Rules Related to Staging

(1) Since corpus cancer is now surgically staged, procedures previously used for determination of stages are no longer applicable, such as the finding of fractional D&C to differentiate between stage I and stage II.

(2) It is appreciated that there may be a small number of patients with corpus cancer who will be treated primarily with radiation therapy. If that is the case, the clinical staging adopted by FIGO in 1971 would still apply but designation of that staging system would be noted.

(3) Ideally, width of the myometrium should be measured along with the width of tumor invasion.
Appendix II – PELVIC LYMPHADENECTOMY (06/14/2004)

Purpose:  1) Histologic evaluation of the Pelvic nodes.
Indications:  1) Surgical staging of gynecologic malignancy.
Contraindications:  1) Poor surgical risk.

Content of Procedure:
1) Identify the bifurcation of the common iliac, external iliac, hypogastric arteries and veins and the ureters, bilaterally.

2) Any enlarged or suspicious nodes will be excised or biopsied if unresectable.

3) The nodal tissue from the distal one-half of each common iliac artery should be removed and the iliac vessels skeletonized; laterally from the mid portion of the psoas to the ureter medially.

4) The nodal tissue along the external iliac vessels should be removed and the iliac vessels skeletonized; laterally from the mid portion of the psoas medially to the ureter including the hypogastric artery and vein, distally to the circumflex iliac vein.

5) The nodal tissue anterior to the obturator nerve should be removed from the obturator fossa.

6) Ligation of the proximal and distal attachments of the nodal tissue is recommended.

7) An adequate dissection will require nodal tissue from the right and left pelvic areas as demonstrated on pathology, and a minimum of four lymph nodes from each side. (Preferably, there should be pathologically demonstrated lymph nodes from multiple nodal sites from each side of the pelvis). (06/14/2004)

ADVERSE EFFECTS THAT MAY BE ASSOCIATED WITH AN UNEVENTFUL PROCEDURE

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- 68 -
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<td>Fever</td>
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<tr>
<td>Allergic</td>
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Appendix III – PARA-AORTIC LYMPHADENECTOMY (06/14/2004)

**PURPOSE:**
1) Histologic evaluation of para-aortic lymph nodes.

**INDICATION:**
1) Surgical staging of gynecologic malignancy

**CONTRAINDICATION:**
1) Poor surgical risk

**CONTENT OF PROCEDURE:**

1) The skin incision may be of the surgeon’s choosing including midline vertical, transverse, and lateral vertical. Maybe performed via laparoscopy.

2) The bifurcation of the aorta, the inferior vena cava, the ovarian vessels, the inferior mesenteric artery, the ureters and duodenum should be identified.

3) The nodal tissue over the distal vena cava from the level of the inferior mesenteric artery to the mid right common iliac artery is removed.

4) The nodal tissue between the aorta and the left ureter from the inferior mesenteric artery to the left mid common iliac artery is removed.

5) Ligation of the proximal and distal nodal tissue is recommended.

6) An adequate dissection will require nodal tissue from the left and right para-aortic areas as demonstrated on pathology. (06/14/2004)

**ADVERSE EFFECTS THAT MAY BE ASSOCIATED WITH AN UNEVENTFUL PROCEDURE**

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Appendix IV – HIGH PARA-AORTIC LYMPHADENECTOMY (06/14/2004)

PURPOSE: 1) Histologic evaluation of para-aortic nodes.

INDICATION: 1) Surgical staging of gynecologic malignancy

CONTRAINDICATION: 1) Poor surgical risk.

CONTENT OF PROCEDURE

1) The skin incision may be of the surgeon’s choosing including midline vertical, transverse, and lateral vertical. Maybe performed via laparoscopy.

2) The bifurcation of the aorta, the inferior vena cava, the ovarian vessels, the inferior mesenteric artery, the ureters and duodenum should be identified.

3) Right side: removal of the nodal tissue between the aorta medially and the right ureter laterally extending from the level of the origin of the inferior mesenteric artery to the level of the entry of the right ovarian vein into the vena cava superiorly.

4) Left side: removal of the nodal tissue between the aorta medially and the left ureter laterally extending from the level of the origin of the inferior mesenteric artery inferiorly to the level of the entry of left ovarian vein into left renal vein superiorly.

5) An adequate dissection will require nodal tissue from the left and right para-aortic areas as demonstrated on pathology. (06/14/2004)

ADVERSE EFFECTS THAT MAY BE ASSOCIATED WITH AN UNEVENTFUL PROCEDURE

<table>
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</table>
Appendix V - Specimen Procedures For Translational Research (06/14/2004) (09/18/2006)

Questions regarding specimen collection and shipping issues should be directed to:

Translational Research Scientist
GOG Statistical and Data Center
Roswell Park Cancer Institute
Carlton and Elm Streets
Buffalo, New York 14263
Phone: 716-845-7768
Fax: 716-845-8393
E-mail: darcy@gogstats.org

I. Overview Of The Clinical Specimen Requirements For GOG-0210

<table>
<thead>
<tr>
<th>Required Clinical Specimens</th>
<th>Specimen Code</th>
<th>Type Of Patients</th>
<th>Collection Time Point</th>
<th>Shipping Recommendations</th>
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<tbody>
<tr>
<td>Pre-Op Serum</td>
<td>SB01</td>
<td>All patients</td>
<td>On the day of, but prior to surgical staging OR when the pre-op bloods are drawn</td>
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<tr>
<td>Urine</td>
<td>UR01</td>
<td>All patients</td>
<td>Just prior to, during or just after surgical staging</td>
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<tr>
<td>Formalin-Fixed Primary Tumor</td>
<td>FP01</td>
<td>All patients</td>
<td></td>
<td>Ship these eight specimens in a dual-chamber kit to the GOG Tissue Bank within 7 days of the surgical staging procedure</td>
</tr>
<tr>
<td>Frozen Primary Tumor (Snap-frozen or OCT-embedded and frozen)</td>
<td>RP01</td>
<td>All patients</td>
<td>During surgical staging</td>
<td></td>
</tr>
<tr>
<td>Formalin-Fixed Normal Tissue</td>
<td>FN01</td>
<td>All patients</td>
<td></td>
<td></td>
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<tr>
<td>Frozen Normal Tissue (Snap-frozen or OCT-embedded and frozen)</td>
<td>RN01</td>
<td>All patients</td>
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<td>Formalin-Fixed Metastatic Tumor</td>
<td>FM01</td>
<td>Subset of patients with gross metastatic disease</td>
<td>During surgical staging when gross metastatic tumor is available</td>
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<tr>
<td>Frozen Metastatic Tumor (Snap-frozen or OCT-embedded and frozen)</td>
<td>RM01</td>
<td>Subset of patients that recur or progress with gross metastatic disease</td>
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<td>Post-Op Serum</td>
<td>SB02</td>
<td>All patients</td>
<td>During the post-operative visit (within 6 weeks after surgery)</td>
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<td>3-Year Follow-Up Serum</td>
<td>SB03</td>
<td>All patients</td>
<td>During the 3 year follow-up visit</td>
<td>Ship this serum specimen to the GOG Tissue Bank in a single-chamber kit within 7 days of specimen collection</td>
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<td>Recurrent Serum</td>
<td>SB04</td>
<td>Subset of patients that recur or progress</td>
<td>When disease recurrence or progression is documented</td>
<td>Ship these specimen in a dual-chamber kit to the GOG Tissue Bank</td>
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<tr>
<td>Formalin-Fixed Recurrent Tumor</td>
<td>FR01</td>
<td>Subset of patients that recur or progress</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Frozen Recurrent Tumor (OCT-embedded and frozen) OR01 tumor that can be safely excised within 7 days of specimen collection

1 Each specimen will be labeled with a GOG Bank ID number (# # # # - # # - G # # # #), a specimen code and the collection date (m d / y y y y). Specimen codes are assigned as two letters (to signify the specimen type) and two numbers (sequentially assigned for different time points).
2 Please ship the SB01, UR01, RP01, FN01, RN01, FM01 and RM01 specimens to the GOG Tissue Bank along with a completed specimen form for EACH of these eight specimens in the first dual-chamber kit provided by the GOG Tissue Bank for GOG-0210.
3 Please ship the SB02 specimen to the GOG Tissue Bank along with a completed specimen form for this one serum specimen in the first single-chamber kit provided by the GOG Tissue Bank.
4 Please ship the SB03 specimen to the GOG Tissue Bank along with a completed specimen form for this one serum specimen in the second single-chamber kit provided by the GOG Tissue Bank.
5 Please ship the SB04, FR01, and OR01 specimens to the GOG Tissue Bank along with a completed specimen form for EACH of these three specimens in the second dual-chamber kit provided by the GOG Tissue Bank.

II. Obtaining A GOG Bank ID (12/27/2010)

Only one GOG Bank ID Number (# # # # - # # - G # # # #) is assigned per patient. All specimens and accompanying paperwork must be labeled with this coded and confidential tracking number. A GOG Bank ID can be obtained online via the Tissue Bank Portal on the GOG website under Tools on the Web Menu page.

Obtain the GOG patient study ID for all GOG protocols with specimen requirements (except GOG-0136) before requesting a GOG Bank ID from the Tissue Bank Portal.

Please contact the User Support Department at the GOG Statistical and Data Center at support@gogstats.org or by phoning 716-845-7767 or the staff in the GOG Tissue Bank by phoning 866-464-2262 or faxing 614-722-2897 if you need assistance.

III. Requesting Specimen Kits For GOG-0210

A. Specimen Kit Requirements for GOG-0210

1. Up to four GOG-0210 Specimen Kits will be needed to collect and ship the required clinical specimens for this protocol.
2. Three specimen kits (one dual-chamber kits and two single chamber kits) will be needed for each GOG-0210 patient.
   a. The dual-chamber kit will be needed for the pre-op serum specimen, the intra-op urine specimen, the primary tumor specimens, normal tissue specimens and metastatic tumor specimens. The metastatic tumor specimens will only be submitted for the subset of patients with gross metastatic disease at the time of the surgical staging procedure.
   b. One single chamber kit will be needed for the post-op serum specimen.
   c. The second single-chamber kit will be needed for the 3-year follow-up serum specimen.
3. Only a subset of the GOG-0210 patients will recur or progress, and an additional dual-chamber kit will be needed to collect and ship the recurrent serum and recurrent tumor specimens.

B. Ordering The Specimen Kits For GOG-0210

1. The Specimen Kits for GOG-0210 can be ordered from the GOG Tissue Bank during regular business hours (Monday-Friday from 8:30 AM-5:00 PM Eastern Time) by phoning...
C. Materials Provided In The GOG-0210 Specimen Kits

1. The first dual-chamber kit will consist of a dual-chamber shipping container suitable for shipping frozen tissue (primary tumor, normal tissue and metastatic tumor when available), the intra-op urine and the pre-op serum with excess dry ice on one side and formalin-fixed tissue on the other side. In addition, foil to wrap frozen tissue specimens, truncated OCT embedding molds, three 15 ml formalin jars for fixed tissue, a 15 ml screw-cap polypropylene conical tube for mixing the serum specimen, ten 1.8 ml screw-cap cryogenic vials (cryotubes) for the pre-op serum specimen, two 5.0 ml screw-cap cryotubes, plastic zip-lock bags, and eight specimen forms will also be included in the first dual-chamber kit. Finally, a biohazard mailing sticker, dry ice label, two sets of secondary shipping envelopes with absorbent material, and a Federal Express form (pre-billed to the GOG Tissue Bank) will also be included.

2. The two single-chamber kits will each consist of a single-chamber shipping container suitable for shipping the post-op serum specimen or the 3-year follow-up serum specimen with excess dry ice. In addition, a 15 ml screw-cap polypropylene conical tube for mixing the serum specimen, ten 1.8 ml screw-cap cryotubes for dispensing the serum specimen and one specimen form will be included in each of these kits. Finally, a biohazard mailing sticker, dry ice label, a secondary shipping envelope with absorbent material, and a Federal Express form (pre-billed to the GOG Tissue Bank) will also be included.

3. The second dual-chamber kit will consist of a dual-chamber shipping container suitable for shipping the frozen recurrent tumor and serum specimens as well as the formalin-fixed recurrent tumor tissue specimen (when available). This kit will contain a 15 ml screw-cap polypropylene conical tube for mixing the recurrent serum specimen, ten 1.8 ml screw-cap cryotubes for dispensing the recurrent serum specimen, foil to wrap the frozen recurrent tumor specimen, plastic zip-lock bags, truncated OCT embedding molds, one 15 ml formalin jars for fixed tissue, and three specimen forms. Finally, a biohazard mailing sticker, dry ice label, two sets of secondary shipping envelopes with absorbent material, and a Federal Express form (pre-billed to the GOG Tissue Bank) will also be included.
IV. Collecting Fixed and Frozen Tissue Specimens For GOG-0210

A. Requirements and Time Points

Primary tumor and normal tissue specimens will be required for all GOG-0210 patients. Metastatic tumor specimens will only be required from the subset of patients who have gross metastatic disease at the time of the surgical staging procedure. Recurrent tumor specimens will only be required from the subset of patients who recur or progress and have tumor that can be safely excised. Primary tumor, normal tissue and the metastatic tumor (when gross disease is present) will be removed for this research study when the patient is undergoing full surgical staging. The recurrent tumor will be removed for this research study when disease recurrence or progression has been documented and a tumor biopsy can be safely performed.

B. Format For Labeling The Specimen

The formalin-fixed tissue specimens will be labeled with the GOG protocol number (GOG-0210), GOG Bank ID Number, the specimen code (FP01 for the fixed primary tumor, FN01 for fixed normal tissue, FM01 for the fixed metastatic tumor or FR01 for the fixed recurrent tumor), and the collection date.

The frozen tissue specimens will be labeled with the GOG protocol number (GOG-0210), GOG Bank ID Number, the specimen code (RP01 for the frozen primary tumor, RN01 for the frozen normal tissue, RM01 for the frozen metastatic tumor or FT04 for the frozen recurrent tumor), and the collection date. If both OCT-embedded frozen tissue and snap-frozen tissue specimens are submitted for this protocol, both types of frozen specimens can be labeled using the same RP01, RN01 or RM01 specimen code.

C. Guidelines And Recommendations For Preparing The Fixed And Frozen Tissue Specimens

All tumor and normal tissue specimens for GOG-0210 should be formalin-fixed or frozen within 30-60 minutes when possible, and must be formalin-fixed or frozen within 4 hours of excision from the patient. The faster the tissue specimens can be frozen the higher the quality of the RNA extracted. It may be appropriate to hold occasional meetings of surgical, laboratory, and clinical personnel to emphasize the urgency of processing these specimens rapidly.

Please work with one tissue type at a time, remove a piece of tissue sample for formalin-fixation and another piece for freezing using the standard operating procedures provided below. There is only one type or method provided for formalin-fixation. There are two types of freezing methods provided for your consideration.

When preparing the primary tumor, normal tissue and metastatic tumor specimens, the choice of freezing method (OCT-embedding and freezing or snap-freezing) is not mandated for GOG-0210. If both OCT-embedded frozen and snap-frozen primary tumor, normal tissue or metastatic tumor tissue are prepared, both types of frozen specimens should be labeled using the same RP01, RN01 or RM01 specimen code.

When preparing the frozen recurrent tumor tissue specimen, the OCT-embedding and freezing method must be used, not the snap-freezing method. The frozen recurrent tumor specimen should therefore be labeled using the specimen code OR01 for OCT-embedded
and frozen recurrent tumor tissue. Recent experience in collecting frozen tumor biopsy specimens for GOG-0170C demonstrated that when the biopsy tissue was snap-frozen directly in a cryotube the recovery of the specimen for laboratory testing was extremely problematic and inconsistent. Even though a small amount of tumor tissue is often available from a biopsy or similar type of procedure, sufficient high quality tumor tissue was readily and consistently recovered when the tissue was frozen within OCT.

**Quantity Of Tissue Needed For Research:** Please submit as much tissue as possible for research. Gram quantities are ideal. There is a minimum requirement of 500 mg or 0.5 cm³ (slightly larger than a pencil eraser). Larger amounts of tissue will allow for replicate laboratory testing to be performed and will enable multiple assays to be run on the same specimen. Gene expression microarray results can then be directly compared with tissue proteomic results and possibly with immunohistochemical results from the same specimen. The larger tissues will also permit validation testing to be performed following the gene expression profiling studies such as RT-PCR, Northern blotting, and in situ hybridization, for example.

It may be helpful to have meetings among the staff members at your institution such as the GOG surgeons, GOG pathologists, general pathologist, operating room team, nurses, clinical research coordinators and/or tissue procurement specialist that will participate in procuring the tissue specimens for this molecular staging protocol. These types of meetings can help clarify responsibilities and communication methods for keeping the appropriate individuals appraised as to when their services will be need to satisfy the requirements for this molecular and surgico-pathological staging study for endometrial carcinoma. Sharing operating schedules and providing updates on how the full surgical staging procedure is progressing may help ensure that the members of the team are available when needed thus improving the working relationship among the team and the quality of the tissue specimens submitted for GOG-0210.

**D. Procedures For Excising Tissue For Research**

1. **Excising primary tumor tissue during surgical staging.**
   a. The surgeon should send the hysterectomy specimen from all patients to the surgical pathology suite, and arrange for immediate tissue sampling within 30-60 minutes of excision of the uterus when possible.
   b. **Submit as much primary tumor tissue for research as possible.** Gram quantities are ideal. There is a minimum requirement of 500 mg or 0.5 cm³ (slightly larger than a pencil eraser).
   c. The primary tumor tissue for submission to the GOG Tissue Bank will undergoing various types of laboratory testing and should be as clean and as free from necrosis as possible.
   d. Promptly following the dissection of the primary tumor sample, a piece of the tumor tissue must be formalin-fixed (FP01), and another piece must be frozen by OCT-embedding and/or snap-freezing (RP01) as described below.

2. **Excising normal tissue during surgical staging.**
   a. The surgeon should also excise a piece of normal tissue from all patients at the time of the surgery. Normal tissue can be any normal epithelial tissue including ovaries, fallopian tube, uterus, cervix, or skin, although gynecologic tissue is preferred and **fallopian tubes are the ideal choice for the normal tissue** to submit for this molecular staging study. **Avoid the submission of omentum** as the normal tissue specimen when
possible, as fatty omentum does not make a good tissue control for the laboratory
testing proposed.
b. The normal tissue specimen should be sent along with the hysterectomy specimen to the
surgical pathology suite, and arrangements should be made for immediate tissue
sampling within 30-60 minutes of excision of the uterus when possible.
c. Please submit a minimum of 500 mg or 0.5 cm³ of normal tissue, ideally normal
fallopian tubes, (slightly larger than a pencil eraser). This is a minimum
requirement, so please submit as much normal tissue as possible.
d. Promptly following the dissection of the normal tissue specimen, a piece of the normal
tissue must be formalin-fixed (FN01), and another piece must be frozen by OCT-
embedding and/or snap-freezing (RN01) as described below.

3. Excising metastatic tumor tissue when gross metastatic disease is present at the time of the
surgical staging procedure.
a. When gross metastatic tumor is apparent at the time of surgical staging, please submit
as much metastatic tumor tissue as possible for this research study.
b. The metastatic tumor tissue for submission to the GOG Tissue Bank will undergoing
various types of laboratory testing and should be as clean and as free from necrosis as
possible.
c. Promptly following the dissection of the metastatic tumor sample, a piece of the tumor
tissue must be formalin-fixed (FM01), and another piece must be frozen by OCT-
embedding and/or snap-freezing (RM01) as described below.

4. Excise recurrent or persistent tumor when disease recurrence or progression is documented
and a tumor biopsy can be safely performed.
a. When recurrent tumor tissue can be safely obtained, please submit
as much recurrent
tumor tissue as possible.
b. The recurrent tumor tissue for submission to the GOG Tissue Bank will undergoing
various types of laboratory testing and should be as clean and as free from necrosis as
possible.
c. Promptly following the resection of the recurrent tumor tissue, a piece of the tumor
tissue must be formalin-fixed (FR01), and another piece must be frozen by OCT-
embedding and/or snap-freezing (OR01) as described below.

E. Standard Operating Procedure (SOP) For Formalin-Fixing A Tissue Specimen

1. Label the formalin jar(s) provided in the specimen kit distributed by the GOG Tissue Bank
for this protocol. Label each 15 ml formalin jar with the GOG protocol number (GOG-
0210), GOG Bank ID Number, the appropriate Specimen Code specified for this protocol,
and the collection date.
2. Promptly following resection of the tissue, use forceps to transfer the tissue sample in the
pre-labeled jar with 15 ml of 10% buffered formalin, securely fasten the lid, and wrap a
piece of parafilm around the cap and lid several times.
3. Store tissue in the fixative in a 4°C refrigerator until the fixed specimen is shipped to
the GOG Tissue Bank (see below for shipping instructions). Please keep in mind that the
formalin-fixed tissue specimen should undergo standard histologic processing and paraffin-
embedding at the GOG Tissue Bank within 1-3 business days of collecting the tumor
specimen when possible to avoid problems associated with excessive fixation that modify
antigenicity and reduce the usefulness of the tissue specimen. If the formalin-fixed tissue
can’t be shipped to the GOG Tissue Bank within a few days of collection, please
having your Pathology Department paraffin-embed the tissue specimen.
4. Complete a GOG Specimen Form as described below and include a copy of this form with the shipment. When completing a GOG Specimen Form, please take the time to not only provide the header information, but also the general specimen information including the approximate time from tissue excision to fixation, as well as the additional information required specifically for tissue specimens including the type of tissue and the processing method. Form SP can be completed online or by hand. A copy of the completed Form SP must accompany each fixed tissue specimen.

5. Ship the fixed tissue specimen to the GOG Tissue Bank and a completed copy of the GOG Specimen Form in the specimen kit provided by the Bank using the shipping instruction provided below.

F. Standard Operating Procedure (SOP) For Freezing A Tissue Specimen Using The OCT-Embedding and Freezing Method.

1. Label the truncated OCT mold(s) and zip-lock bag(s) provided in the specimen kit distributed by the GOG Tissue Bank for this protocol with the GOG protocol number (GOG-0210), GOG Bank ID number, the appropriate Specimen Code as specified by this protocol and the collection date. Please pre-label the mold with a cryomarker prior to freezing as the ink will not mark on frozen molds. If more than 0.75 grams or 0.75 cm³ of tissue is available for freezing, please split the tissue into two molds, each of which can be labeled with the same specimen code.

2. Cover the bottom of the mold with OCT embedding medium, and holding the mold with forceps place the mold in the vapor phase (not the liquid phase) of liquid nitrogen or a suitable substitute until the OCT becomes opaque and is no longer transparent. Do not allow the gel to become frozen solid.

3. Using forceps place the appropriate tissue specimen into the thickened gel in each mold pushing the specimen to the bottom of the mold.

4. Add additional OCT to each mold to completely cover the tissue and fill the mold until approximately three-fourths full.

5. Holding the mold with forceps, gradually immerse the entire mold into liquid nitrogen or a suitable substitute until the OCT and tissue are completely solid.

6. Using forceps transfer the frozen OCT-embedded tissue specimen to the zip-lock bag labeled with the GOG Bank ID Number, the appropriate Specimen Code and the collection date.

7. Store the OCT-embedded tissue specimen frozen solid in appropriate ultra cold storage space such as an ultra cold freezer (≤ -70°C), in liquid nitrogen (liquid or vapor phase) or in direct contact with excess dry ice until the frozen specimen is shipped to the GOG Tissue Bank (see below for shipping instructions). A regular freezer (-20°C) is not adequate. A cryostat is also not appropriate.

8. Complete a GOG Specimen Form. Please take the time to not only provide the header information, but also the general specimen information including the type of freezing/storage condition utilized for this specimen and the approximate time it took from tissue excision to freezing, as well as the additional information required specifically for tissue specimens including the type of tissue and the processing method. Form SP can be completed online or by hand. A copy of the completed Form SP must accompany each frozen tissue specimen.

9. Ship the frozen tissue specimen to the GOG Tissue Bank with excess dry ice and a completed copy of the GOG Specimen Form in the specimen kit provided by the Bank using the shipping instruction provided below.
GOG-0210
NCI Version Date: 07/28/2016

G. Standard Operating Procedure (SOP) For Freezing A Tissue Specimen Using The Snap-Freezing Method.

1. Using a waterproof marker, label a zip-lock bag supplied in the specimen kit distributed by the GOG Tissue Bank dual-chambered kit with the GOG protocol number (GOG-0210), GOG Bank ID Number, the appropriate Specimen Code, and the collection date.

2. Using forceps place the appropriate tissue specimen on a piece of foil, wrap the foil so that the specimen is completely covered, and immerse the tissue wrapped in foil in liquid nitrogen or a suitable substitute until the tissue is frozen solid.

3. Using forceps transfer the foil-wrapped frozen tissue specimen into the zip-lock baggie labeled with the GOG Bank ID Number, the appropriate Specimen Code and the collection date.

4. Store the snap-frozen primary tumor frozen solid in an appropriate ultra cold storage space such as an ultra cold freezer (≤ -70°C), in liquid nitrogen (liquid or vapor phase) or in direct contact with excess dry ice until the specimens are shipped to the GOG Tissue Bank (see below for shipping instructions). A regular freezer (-20°C) is not adequate. A cryostat is also not appropriate.

5. Complete a GOG Specimen Form. Please take the time to not only provide the header information, but also the general specimen information including the type of freezing/storage condition utilized for this specimen and the approximate time it took from tissue excision to freezing, as well as the additional information required specifically for tissue specimens including the type of tissue and the processing method. Form SP can be completed online or by hand. A copy of the completed Form SP must accompany each frozen tissue specimen.

6. Ship the frozen tissue specimen to the GOG Tissue Bank with excess dry ice and a completed copy of the GOG Specimen Form in the specimen kit provided by the Bank using the shipping instruction provided below.

V. Collecting Serum Specimens For GOG-0210

A. Requirements and Time Points

For GOG-0210, the pre-op serum specimen will need to be prepared for all patients from 10 ml of blood drawn on the day of and prior to the surgical staging OR when pre-op bloods are drawn. Collection of the specimen at the time the intravenous line is started for surgery is acceptable provided that the patient has not received pre-operative medications as these medications may alter the profile of proteins in serum. The post-op serum specimen will be isolated from 10 ml of whole blood drawn on the day of the post-operative clinic visit (within 6 weeks after the surgery). The 3-year follow-up serum specimen will be isolated from 10 ml of whole blood drawn during the 3-year follow-up visit. The recurrent serum specimen will be isolated from 10 ml of whole blood drawn when disease recurrence or progression is documented.

B. Format For Labeling The Specimen

The fixed tissue specimens will be labeled with the GOG protocol number (GOG-0210), GOG Bank ID Number, the specimen code (SB01 for the pre-op serum, SB02 for the post-op serum, SB03 for the 3-year follow-up serum or SB04 for the recurrent serum), and the collection date.
C. Equipment and Supplies Needed For Preparing Each Serum Specimen

In addition to the materials provided in the specimen kit(s) distributed by the GOG Tissue Bank for this protocol, you will need gloves, red top Vacutainer® tube(s), tube rack, a permanent marker, a syringe with a 16-18 gauge needle (or a transfer pipette), dry ice, a centrifuge, a refrigerator or a bucket with wet ice, and access to appropriate freezing/storage space to collect each serum specimen.

D. Guidelines And Recommendations For Preparing The Serum Specimens

Ideally, the serum will be frozen in an ultra cold freezer (≤ -70°C), in liquid nitrogen (liquid or vapor phase) or by direct exposure with excess dry ice. If ultra-cold freezing conditions are not available at your site, a non-cycling -20°C freezer can be used but the amount of time the serum is kept in this type of freezer should be kept to a minimum as this temperature is not cold enough to achieve a frozen solid state (water-based liquids will be frozen solid at ≤ -56°C). A non-cycling freezer is a freezer that will build up frost and requires defrosting by hand. Please surround the serum kept in a non-cycling -20°C freezer with excess dry ice to allow the serum to achieve and then maintain a frozen solid state. Storage of serum in a frost-free -20°C freezer will repeatedly damage the specimen each time the freezer cycles (thaws and then refreezes). Serum frozen under ultra-cold conditions represents the highest quality specimen suitable for all types of laboratory testing including proteomic analysis. Serum frozen in a non-cycling -20°C provides a lower quality specimen suitable for restricted types of laboratory testing. Serum frozen in this manner would not be suitable for proteomic analysis for example. Serum frozen in a frost-free -20°C freezer provides the lowest quality serum with limited usefulness for research purposes. Tracking the freezing conditions for each serum specimen is of critical importance to assess specimen quality and suitability for testing.

Ideally, the serum should be processed from the time the blood is drawn to freezing within 2 hrs when possible and must be frozen within 4 hrs of the blood draw. The faster the serum can be processed from blood draw to freezing the better. Serum processed within 1-2 hrs is the highest quality. Serum processed within 2-4 hrs is a lower quality. Serum processed more than 4 hrs after drawing the blood is the poorest quality serum for testing. Tracking the serum processing time is also critical in assessing specimen quality and suitability for testing.

E. Standard Operating Procedure (SOP) For Collecting Each Serum Specimen.

1. **Label Cryotubes.** Label the 1.8 ml screw-cap cryotubes provided in the specimen kit distributed by the GOG Bank for this protocol with the GOG Bank ID Number, the Specimen Code (S B # #) and the collection date (m m / d d / y y y y). The “S B” portion of the specimen code indicates that the type of specimen is serum. The “# #” portion of the Specimen Code signifies the sequence # associated with the serum time point for a particular GOG protocol.

2. **Draw Blood.** Collect 5-20 ml (cc) of whole blood in an appropriate number of red top tubes. A range is provided for the volume of blood required to provide the sites with the greatest flexibility in participating in GOG studies that require serum collection. Larger volumes are more ideal.

* Please try to draw a minimum of 10 ml of whole blood to prepare the serum for GOG-0210.
3. **Allow Blood To Clot.** Allow the blood to clot upright at 4°C in a refrigerator or in a bucket with excess wet ice for a minimum of 30 min but for no longer than 3 hrs. The faster the blood can be processed after the 30 min clotting step the better.

4. **Centrifuge Blood.** The blood must be centrifuged to separate the serum (clear liquid with yellowish tint) from the fibrin clot and the blood cells. When the appropriate equipment is available, please centrifuge the blood at \( \sim 3,500 \times g \) at 4°C for 10 min. The minimum centrifugation requirements will be \( \sim 1000 \times g \) at room temperature for 15 min. The longer centrifugation time will help compensate for the slower speed. Avoid centrifugations without refrigeration longer than 15 min as excess heat may build up in the unit and damage the serum.

5. **Aliquot Serum.** Remove the caps from the blood tube(s), the 15 ml conical tube for mixing the serum and the 1.8 ml cryotubes. Draw the serum into a sterile syringe with a 16-18 gauge needle (or into a transfer pipette), transfer the serum into the 15 ml conical tube to allow for thoroughly mixing, and then evenly dispense (aliquot) at least 0.5 ml (0.5 cc) of the serum into as many of the pre-labeled 1.8 ml screw-cap cryotubes as possible. Cap the cryogenic vial securely.

6. **Freeze Serum.** Immediately freeze serum upright if possible using an appropriate type of freezing/storage space. Ultra-cold conditions such as an ultra cold freezer (≤ -70°C), liquid nitrogen or direct exposure with excess dry ice are ideal. Utilization of a non-cycling -20°C freezer is acceptable but should be avoided when possible. See the guidelines and rationale for the freezing conditions.

7. **Complete the Specimen Form.** When completing a GOG Specimen Form, please take the time to not only provide the header information, but also the general specimen information including the type of freezing/storage condition utilized for this specimen and the approximate time it took to process the serum from blood draw to freezing, as well as the additional information required specifically for blood products. Form SP can be completed online or by hand. A copy of the completed Form SP must accompany the serum.

8. **Ship Serum To The GOG Tissue Bank.** Ship the frozen serum to the GOG Tissue Bank with excess dry ice and a completed copy of the GOG Specimen Form in the specimen kit provided by Bank for specific GOG protocols. Ship to Attn: Laboratory Technicians, Children’s Hospital, GOG Tissue Bank – Protocol # 0210, 700 Children’s Drive, Room WA1340, Columbus, OH 43205 (Phone: (614) 722-2810) via Federal Express Priority Overnight delivery using Federal Express Account Number 1209-5618-3. (12/27/2010)

**VI. Collecting Urine Specimen For GOG-0210**

A. **Requirements and Time Points**

For GOG-0210, a urine specimen will be collected from the patient’s Foley catheter prior to, during or following the surgical staging procedure and submitted for this research study. Only one urine specimen will be required for each GOG-0210 patient.

B. **Format For Labeling The Specimen**

The intra-op urine specimen will be labeled with the GOG Bank ID Number, the specimen code (UR01), and the collection date.

C. **Equipment and Supplies Needed For Preparing Each Serum Specimen**
In addition to the materials provided in the specimen kit(s) distributed by the GOG Tissue Bank for this protocol, you will need gloves, a permanent marker, and access to appropriate freezing/storage space to collect the one urine specimen.

D. Procedure For Collecting The Urine Specimen

1. Pre-label the two 5.0 ml screw-cap cryotubes provided in the specimen kit distributed by the GOG Bank for this protocol with the GOG protocol number (GOG-0210), GOG Bank ID Number, the Specimen Code (U R # #) and the collection date (m m / d d / y y y y). The “U R” portion of the specimen code indicates that the type of specimen is urine. The “# #” portion of the Specimen Code signifies the sequence # associated with the urine time point and/or collection interval for a particular GOG protocol.

2. After the Foley catheter is placed for the surgical procedure and at least 10 ml of urine have been voided, collect 4 ml of urine from the Foley catheter into each of two 5.0 ml screw-cap cryotubes provided in the specimen kit distributed by the GOG Tissue Bank for this protocol.

3. Immediately freeze urine upright if possible using an appropriate type of freezing/storage space. Ultra-cold conditions such as an ultracold freezer (≤ -70°C), liquid nitrogen or direct exposure with excess dry ice are ideal. Utilization of a non-cycling -20°C freezer is acceptable but should be avoided when possible.

4. Complete a GOG Specimen Form. Please take the time to not only provide the header information, but also the general specimen information including the type of freezing/storage condition utilized for this specimen, as well as the additional information required specifically for fluid products including the utilization of a preservative (i.e., ascorbic acid), and the urine collection method (i.e., removal catheter). Form SP can be completed online or by hand. A copy of the completed Form SP must accompany the urine specimen.

5. Ship the frozen urine specimen to the GOG Tissue Bank with excess dry ice and a completed copy of the GOG Specimen Form in the specimen kit provided by the Bank using the shipping instruction provided below.

VII. Specimen Form Requirements

A. Summary Of The Specimen Form (Form SP) Requirements For GOG-0210

<table>
<thead>
<tr>
<th>Required SP Forms</th>
<th>Specimen</th>
<th>Time Point</th>
<th>Deadline</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP-SB01-0210</td>
<td>For the pre-op serum (SB01)</td>
<td>Collected the day of but prior to initiating the surgical staging procedure OR when the pre-op bloods are drawn.</td>
<td>Form SP must be completed for each of these specimen and submitted to the Statistical and Data Center within 7 days of the surgical staging procedure.</td>
</tr>
<tr>
<td>SP-UR01-0210</td>
<td>For urine (UR01)</td>
<td>Collected just prior to, during or just after the surgical staging procedure.</td>
<td></td>
</tr>
<tr>
<td>SP-FP01-0210</td>
<td>For formalin-fixed primary tumor tissue (FP01)</td>
<td>Collected during the surgical staging procedure.</td>
<td></td>
</tr>
<tr>
<td>SP-RP01-0210</td>
<td>For frozen primary tumor tissue (RP01) – snap frozen or OCT-embedded and frozen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-FN01-0210</td>
<td>For formalin-fixed normal tissue (FN01)</td>
<td></td>
<td></td>
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<td>-------------</td>
<td>----------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-RN01-0210</td>
<td>For frozen normal tissue (RN01) either snap frozen or OCT-embedded and frozen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-FM01-0210</td>
<td>For formalin-fixed metastatic tumor tissue (FM01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-RM01-0210</td>
<td>For frozen metastatic tumor tissue (RM01) either snap frozen or OCT-embedded and frozen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-SB02-0210</td>
<td>For the post-op serum (SB02) Collected during the post-op clinic visit, which should be within 6 weeks of surgery. Form SP must be completed for SB02 and submitted to the Statistical and Data Center within 49 days of the surgical staging procedure.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-SB03-0210</td>
<td>For the 3-year follow-up serum (SB03) Collected during the 3-year follow-up clinic visit. Form SP must be completed for SB03 and submitted to the Statistical and Data Center within 1144 days of the surgical staging procedure.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-SB04-0210 *</td>
<td>For the recurrent serum (SB04) * Collected at the time that disease recurrence or progression is documented * Form SP must be completed for SB04, FR01 and OR01 and submitted to the Statistical and Data Center within 3699 days of the surgical staging procedure.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-FR01-0210 *</td>
<td>For formalin-fixed recurrent/persistent tumor tissue (FR01) *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-OR01-0210 *</td>
<td>For OCT-embedded and frozen recurrent/persistent tumor tissue (OR01) *</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* If the patient does not recur within 10 years of the surgical staging procedure, complete the Specimen Forms for SB04, FR01 and OR01 after the patient is seen during her final follow-up visit for this protocol and indicate that these three specimens could not be collected because the patient did not recur within the 10 years of follow-up.

** Form SP will need to be submitted within 7 days after the post-op clinical visit (post-op visit should be scheduled ~6 weeks after the surgical staging procedure; the form is therefore due within 49 days of the surgical staging procedure) for SB02 to the GOG Statistical and Data Center and to the GOG Tissue Bank.

*** Form SP for SB03 will need to be submitted within 7 days after the 3-year follow-up clinic visit (to be scheduled 3 years ± 6 weeks after the surgical staging procedure; the form is therefore due within 1144 days after surgical staging) to the GOG Statistical and Data Center and to the GOG Tissue Bank.

**** Form SP will need to be submitted within 7 days after the 3-year follow-up clinic visit (to be scheduled 3 years ± 6 weeks after the surgical staging procedure; the 6-week window is to provide the patient and the site with scheduling flexibility; the form is therefore due with 3699 days of the surgical staging procedure) to the GOG Statistical and Data Center and to the GOG Tissue Bank for SB04, FR01 and OR01.

B. Procedure For Completing Form SP (Specimen Form)

One specimen form (Form SP) must be completed and submitted to the GOG Statistical & Data Center within 7 days of the scheduled collection for each specimen required for the protocol regardless of the submission status. Form SP can be completed online or printed and completed by hand. Form SP can be submitted to the GOG Statistical and Data Center online, by fax or by mail. A copy of the completed form must also physically accompany each specimen when shipped to the GOG Tissue Bank.

**Online Submission:** For sites with access to the internet, Form SP can be completed and submitted online for all specimens. The completed form can then be printed for your records and to accompany a specimen being shipped to the GOG Tissue Bank. To access Form SP for online submission, go to the GOG website:

1. Select **GOG Web Menu Login**

2. Enter your username and password. Access to the GOG Web Menu web page can only be obtained via an assigned username and password. If you do not have a GOG Web Menu
username and password, call the GOG Statistical & Data Center User Support Department at (716) 845-7767 Monday - Friday 8:30am - 5:00pm EST or go to the following link https://webreg.gogstats.org/regweb/Help/loginhelp.html, fill out the form, print it, and fax it to (716) 845-8393 Attn. User Support.

3. Select SEDES nonQ from the Registration/Data Entry column. The selected page (SDC Electronic Data Entry System or SEDES) has three different ways you can access Form SP for a patient that your site (parent or affiliate) has enrolled on certain protocols. From the top to the bottom of the screen, these three choices are: (a) Selection of SP forms that are due, (b) Selection of SP forms for amendment, (c) Selection of Teleforms available for a specific protocol.

4. Input the patient study ID number to retrieve Form SP. After accessing Form SP for a specific patient, the form will appear pre-filled with the date, patient Study ID Number, GOG Bank ID Number, and your username, phone number, and e-mail address. If you have selected an SP Form for a specific specimen, the form will also be pre-filled with the Specimen Code and the Time Point description. If the submission field (item 5) checked is “submitted”, certain fields of the form are pre-filled. For example, if SB01 is entered as a Specimen Code, the “serum” (item 8) selection will be automatically filled for you. In addition, once a Specimen Code is entered, other fields of the form become read-only. For example, if SB01 is entered as a Specimen Code, the entire section labeled “Tissue/Cells” becomes read-only since you do not have to enter any information about serum in that area. The bottom section of the form will always be read-only, as the GOG Tissue Bank will complete this section. Certain fields are mandatory and you will not be able to submit the form if they are not completed. Mandatory fields include: “Submitted/Not Submitted” (item 5), “Date Collected” (item 6), “Items Shipped” (item 9), and “Quantity of Items Shipped” (item 10). If any of these are left blank, you will see a prompt when you attempt to submit the form reminding you to fill in the mandatory fields. If you select “Not Submitted” (item 5) the remaining fields on the form become read-only and do not have to be completed except for the field collecting the reason you are not submitting the specimen. “Time Collected” (item 7) does not have to be filled in except for specific studies (pharmacokinetics). When Time Collected is required, the protocol will specifically ask that you fill in this field. “Preservative” (item 20) refers to urine collection and does not have to be filled in for blood products. When submitting a completed Form SP, make sure you also “Print Receipt” so that you have a record of the submission for the patient’s chart.

Paper Submission: Tips for printing and completing Form SP by hand. Staff at the GOG Institution will be responsible for printing out a clear copy of Form SP from the GOG website, and completing the GOG header information and items 1-24. The Specimen Form should be filled out using black ink. Information will need to be entered on the form in a clear and legible manner. To make completion of this form more efficient, the staff at the GOG Institutions should consider filling in certain header and site contact information once, making enough copies of the form so that you will have one form for each specimen required for that patient, and then entering the general specimen information and the specimen specific information on the copies. This will minimize the amount of redundant information that you have to enter on the form. If you select “Not Submitted” (item 5), you are not required to enter any other information except for the field collecting the reason you are not submitting the specimen. A copy of the SP Form will accompany the specimen when it is shipped to the GOG Tissue Bank or the testing laboratory. Always retain a copy of the SP Form for each specimen in your records. Fax or mail a copy of
the completed Form SP for each of the required specimen to the GOG Statistical & Data Center within 7 days of the scheduled collection. Fax the form to: GOG Statistical & Data Center at 716-845-8393. Mail the form to: GOG Statistical & Data Center, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, New York 14263.
VIII. Shipping The GOG-0210 Clinical Specimens

All specimens will be shipped to the GOG Tissue Bank at the following address within 7 days of the scheduled collection:

Attn: Laboratory Technicians
Children’s Hospital
GOG Tissue Bank – Protocol #0210
700 Children’s Drive, WA1340
Columbus, OH 43205
Phone: (614) 722-2810
Fax: (614) 722-2897
E-mail: gogbank@pediatrics.ohio-state.edu

A. Type Of Kits Distributed For Shipping The GOG-0210 Clinical Specimens

Up to four Specimen Kits (two dual-chamber kits and two single-chamber kits) will be distributed by the GOG Tissue Bank for the collection and shipping of the required GOG-0210 clinical specimens. A dual-chamber kit will be provided when multiple specimens need to be shipped at the same time under two different conditions. For example, a dual-chamber kit should be used when shipping frozen specimens with excess dry ice in one chamber and formalin-fixed tissue without dry ice in the other chamber. A single chamber kit will be provided when all the specimens to be shipped at a given time can be transported under the same conditions.

1. The first dual-chamber kit will be used to ship the frozen tissue (RP01, RN01 and RM01), pre-op serum (SB01) and intra-op urine (UR01) specimens packed with excess dry ice in one chamber and the formalin-fixed tissue (FP01, FN01 and FM01) specimens packed without dry ice in the other chamber.

2. The first single-chamber kit will be used to ship the frozen post-op serum specimen (SB02) packed with excess dry ice.

3. The second single-chamber kit will be used to ship the frozen 3-year follow-up serum specimen (SB03) packed with excess dry ice.

4. The second dual-chamber kit will be used to ship the frozen tissue (OR01) and serum (SB04) specimens packed with excess dry ice in one chamber and the formalin-fixed tissue (FR01) specimens packed in the other chamber.
B. Schedule For Shipping The GOG-0210 Clinical Specimens

Please ship the specimens to the GOG Tissue Bank as quickly as possible from the time of collection. Specimens must be shipped within 7 days of the scheduled collection.

1. Ship the first dual-chamber kit with the frozen tissue (RP01, RN01 and RM01), pre-op serum (SB01) and intra-op urine (UR01) specimens (packed with excess dry ice in one chamber) and the formalin-fixed tissue (FP01, FN01 and FM01) specimens (packed in the other chamber) as quickly as possible from the time the last one of these specimens is collected. **Ideally these specimens should be shipped the day the surgical staging is performed and must be shipped within 7 days of the surgical staging procedure.** If these specimens can’t be shipped on the day of the surgical staging procedure, please make sure the frozen specimens are stored using appropriate ultra-cold conditions (a freezer set at ≤–70°C, in liquid nitrogen or surrounded by excess dry ice). If these specimens can’t be shipped within 3 days of the surgical staging procedure, please have your Pathology Department paraffin-embed the formalin-fixed tissue specimens to protect the usefulness of these research specimens. Prolonged tissue fixation can alter antibody reactivity when immunohistochemistry assay are performed on section prepared of these specimens.

2. Ship the first single-chamber kit with the frozen post-op serum specimen (SB02) on the day the patient is in for the post-op clinic visit or within 7 days of that visit (the post-op clinic visit should be within 6 weeks of the surgical staging procedure). If this specimen can’t be shipped the day of the clinic visit, please make sure the frozen SB02 specimen is stored using appropriate ultra-cold conditions (a freezer set at ≤–70°C, in liquid nitrogen or surrounded by excess dry ice).

3. Ship the second single-chamber kit with the frozen 3-year follow-up serum specimen (SB03) on the day the patient is in for the ~3-year follow-up clinic visit or within 7 days of that visit (within 365 days x 3 years plus a 7 week window of the surgical staging procedure). If this specimen can’t be shipped the day of the clinic visit, please make sure the frozen SB03 specimen is stored using appropriate ultra-cold conditions (a freezer set at ≤–70°C, in liquid nitrogen or surrounded by excess dry ice).

4. Ship the second dual-chamber kit with the frozen tissue (OR01) and serum (SB04) specimens (packed with excess dry ice in one chamber) and the formalin-fixed tissue (FR01) specimens (packed in the other chamber) as quickly as possible from the time the last one of these specimens is collected. **Ideally these specimens should be shipped the day the SB04 serum is collected and the biopsy is performed or within 7 days of these events.** If these specimens can’t be shipped on the day they are collected, please make sure the frozen specimens are stored using appropriate ultra-cold conditions (a freezer set at ≤–70°C, in liquid nitrogen or surrounded by excess dry ice). If these specimens can’t be shipped within 3 days of their collection, please have your Pathology Department paraffin-embed the formalin-fixed recurrent tumor tissue to protect the usefulness of this research specimen. Prolonged tissue fixation can alter antibody reactivity when immunohistochemistry assay are performed on section prepared from this specimen.
C. Standard Operating Procedure (SOP) For Shipping Specimens To The GOG Tissue Bank In A Dual-Chamber Kit

The frozen specimens to be included in the shipment will be packed with excess dry ice in one chamber and the non-frozen specimens will be packed (without dry ice) in the second chamber. The first dual-chamber kit will be used for shipping RP01, RN01, RM01, SB01, UR01 in the one chamber and FP01, FN01 and FM01 in the second chamber. The second dual-chamber kit will be used for shipping OR01 and SB04 in one chamber and FR01 in the other chamber.

1. Complete a GOG Specimen Form for each clinical specimen to be submitted to the GOG Tissue Bank.
2. Remove the foam lids from the dual-chamber shipping kit.
3. Layer dry ice into one side of the shipping container until the chamber is about 35% full.
4. Transfer all of the frozen specimens to be included in this shipment into a plastic biohazard secondary envelope with absorbent material and then into the Tyvek envelop.
5. Place the envelop with the frozen specimens on top of the dry ice.
6. Layer additional dry ice on top of the tissue and serum specimens until the chamber is full.
7. Insert the jar(s) with the appropriate tissue specimen in formalin into the other plastic biohazard secondary envelope with absorbent material and then into the Tyvek envelope.
8. Place the Tyvek envelope in the second compartment of the dual-chambered container (without dry ice).
9. Place the Styrofoam covers on top of the dual-chamber container to secure the specimens during shipment.
10. Insert a copy of the completed GOG Specimen Form for each specimen included in the shipment somewhere dry within the kit. The forms should fit in the space between the styrofoam container and the cardboard shipping box.
11. Seal the kit securely with filament or other durable sealing tape.
12. Complete the pre-printed Federal Express air-bill, insert it into the plastic pouch, and attach the pouch to the top of the kit. Send specimens to GOG Tissue Bank at the address provided above.
13. Complete the dry ice label (UN 1845) and stick this label and the Biohazard label to the side of the box.
14. Arrange for Federal Express pick-up through your usual institutional procedure or by calling 1-800-238-5355. When requesting pick-up, be sure to give the account number (1209-5618-3) on the pre-printed air-bill, but stress that pick-up is at your institutional address. (12/27/2010)
15. Ship all of the frozen specimens to be included in this shipment along with a copy of a completed GOG Specimen Form for each specimen to the GOG Tissue Bank at the address provided above via Federal Express Priority Overnight delivery using the GOG Tissue Bank’s Federal Express Account Number (1209-5618-3). Please ship specimens Monday through Thursday to the GOG Tissue Bank for a Tuesday through Friday delivery. (12/27/2010)

D. Standard Operating Procedure (SOP) For Shipping Frozen Specimens To The GOG Tissue Bank In A Single-Chamber Kit

The frozen serum specimen to be included in each shipment will be packed with excess dry ice in the single-chamber kit. The first single-chamber kit will be used to ship SB02. The second single-chamber kit will be used to ship SB03.
1. Complete a GOG Specimen Form for each clinical specimen to be submitted to the GOG Tissue Bank.
2. Remove the foam lid from one of the single-chamber shipping kits.
3. Layer dry ice into the shipping container until the chamber is ~35% full.
4. Transfer all of the frozen specimens to be included in this shipment into a plastic biohazard secondary envelope with absorbent material and then into the Tyvek envelop.
5. Place the envelop with the frozen serum on top of the dry ice.
6. Layer additional dry ice on top of the serum specimen until the chamber is full.
7. Place the Styrofoam cover on top of the container to secure the specimens during shipment.
8. Insert a copy of the completed GOG Specimen Form for each specimen included in the shipment somewhere dry within the kit.
9. Seal the kit securely with filament or other durable sealing tape.
10. Complete the pre-printed Federal Express air-bill, insert it into the plastic pouch, and attach the pouch to the top of the kit. Send specimens to GOG Tissue Bank at the address provided above.
11. Complete the dry ice label (UN 1845) and stick this label and the Biohazard label to the side of the box.
12. Arrange for Federal Express pick-up through your usual institutional procedure or by calling 1-800-238-5355. When requesting pick-up, be sure to give the account number (1209-5618-3) on the pre-printed air-bill, but stress that pick-up is at your institutional address.
13. Ship all of the frozen specimens to be included in this shipment along with a copy of a completed GOG Specimen Form for each specimen to the GOG Tissue Bank at the address provided above via Federal Express Priority Overnight delivery using the GOG Tissue Bank’s Federal Express Account Number (1209-5618-3) to the address provided below.

Please ship specimens Monday through Thursday to the GOG Tissue Bank for a Tuesday through Friday delivery. (12/27/2010)

IX. Banking Clinical Specimens For GOG-0210

The GOG Tissue Bank staff will be responsible for all of the general activities associated with receiving, banking and distributing the clinical specimens submitted for this molecular staging study. The Bank staff will also be responsible for preparing and distributing the Specimen Procurement & Shipping Kits for this protocol. The cost of shipping the GOG-0210 clinical specimens from the GOG participating institutions to the GOG Tissue Bank and from the GOG Tissue Bank to the participating investigators performing the laboratory testing on these specimens will be billed to the GOG Tissue Bank Federal Express Account.

Upon receipt of any shipments containing clinical specimens for GOG-0210, the GOG Tissue Bank staff will immediately assess the type, quantity and condition of the clinical specimens received; complete the appropriate fields in the GOG Specimen Form; inventory the specimens into their database system; and store the specimens under the appropriate conditions. Frozen tissue, serum and urine specimens will be stored at the bank in an ultra-cold freezer (≤ -70°C) or in a liquid nitrogen storage tank. Formalin-fixed tissue will need to be processed as described below. The Bank staff will fax a copy of each of the completed specimen forms included with the shipment to the GOG Statistical and Data Center at 716-845-8393 within 3 business days of receiving any clinical specimens for this protocol.
All formalin-fixed tissue specimens will undergo standard histologic processing and paraffin-embedding at the GOG Tissue Bank with 24 hours of receipt. Each block will be labeled with the GOG protocol number (GOG-0210), GOG Bank ID number, the appropriate specimen code and the collection date. For each formalin-fixed tumor or normal tissue specimen that will undergo laboratory testing for GOG-0210, a 5 µm thick tissue section will be prepared from the appropriate paraffin block and stained with hematoxylin and eosin (H&E). Paraffin blocks will be stored under vacuum protected from light.

The GOG Tissue Bank will provide the GOG Statistical and Data Center with a quarterly electronic report summarizing all the clinical specimens received along with details about specimen type, quantity and condition for GOG-0210.

X. Distributing Clinical Specimens For GOG-0210

The GOG Statistical and Data Center will inform the GOG Tissue Bank when and which clinical specimens to distribute to specific investigators for laboratory testing, and provide the GOG Tissue Bank with appropriate shipping addresses and contact information for investigators who have been approved to use GOG-0210 clinical specimens for research.

The GOG Tissue Bank staff will be responsible for shipping the appropriate clinical specimens to the appropriate investigators. For each shipment, the GOG Tissue Bank staff will also be responsible for e-mailing the investigator and the GOG Statistical and Data Center an electronic file containing the information pertinent to the shipment including an inventory of all specimens included in the shipment with appropriate identifiers for each (GOG protocol number, GOG Bank ID number, specimen code and collection date).

The investigators performing the laboratory testing on the GOG-0210 clinical specimens will not be given access to any personal identifiers like patient name, initials, social security number, date of birth or medical record number. The investigator will be responsible for keeping accurate records of all laboratory testing performed on the GOG-0210 clinical specimens, and for ensuring that the laboratory testing results are linked to the coded, confidential specimen identifiers (GOG protocol number, GOG Bank ID number, specimen code and collection date).

When unstained tissue sections need to be distributed for laboratory testing, the GOG Tissue Bank will be responsible for preparing a specified number of 5 µm thick unstained tissue sections will be prepared on charged slides suitable for standard immunohistochemistry assays. The individual slides will be dipped in paraffin and stored under vacuum protected from light.

The GOG Tissue Bank may be asked to generate tissue microarrays for this protocol, and to distribute unstained serial sections from appropriate GOG-0210 tissue microarrays to approved investigators for laboratory testing.

The GOG Tissue Bank will provide the GOG Statistical and Data Center with a quarterly electronic report summarizing all the clinical specimens distributed for GOG-0210.
Appendix VI - INFORMATION ON THE COLLECTION AND USE OF SPECIMENS FOR RESEARCH

You are being asked to allow samples of your bodily materials (tissue, cells, blood, urine or other material) to be collected and used in research. Such bodily materials are referred to as specimens. Specimens can be used to help doctors and scientists learn more about caring for and treating people with cancer and other diseases. The use of specimens in scientific research can also help doctors and scientists understand why some people develop cancer and others don't, and why some people have cancers that respond or don't respond well to current therapies, for example.

The research that may be done with your specimens is not designed specifically to help you, but it may help others with cancer or other diseases in the future. Reports about research done with your specimens will not be given to you or your doctor, or be put in your health record. The research will not have an effect on your care.

If you agree to participate in one of the studies conducted by the Gynecologic Oncology Group (GOG), your specimens will be collected and used for the research described for that particular study. We will also ask you to decide whether your specimens, if still available after completion of that research study, can be used for future research. You will be asked whether your specimens can be used for cancer research, or for research for health problems other than cancer. You can still participate in a GOG study even if you do not allow your specimens to be used for future research.

When research is performed on specimens connected with clinical information about the person, including the person's disease and how the person responds to treatment for example, doctors and scientists can specifically study how to prevent, detect, treat and cure cancer and other diseases, or how to predict response to therapy, toxicities, recurrence and overall survival. This is why we will ask your permission to use the clinical information that the GOG will collect about you as part of your study participation for future research that will use your specimens. The GOG will utilize all possible methods to protect your privacy and confidentiality. For example, the research investigators that study your specimens will never be given your name, address, phone number, Social Security number or any other personal information. In addition, your specimens will never be labeled with your name or other type of personal identifier. Your clinical specimen will be labeled with a unique series of letters and numbers. The GOG uses the unique series of letters and numbers as confidential codes to keep track of the clinical specimens, and sends research investigators specimens labeled only with these codes.

Your clinical specimens will be used for research purposes and will not be sold. However, the research done with your clinical specimens may help to develop new products and therapies in the future, or may be used to establish a cell line that could be patented and licensed. In any event, there will be no direct financial benefit to you.

If you agree to allow your specimens to be used for future research, there is a chance that your specimens may be used to study genetic changes or differences that are passed on in families and may include such things as DNA analysis to identify a change or difference in your DNA that could contribute to the development of cancer or enable you to respond to a particular therapy for example. Even if your specimens are used for genetic research, they will not be labeled with your name or other personal identifier, and the results will not be put in your health records.

The choice to let us collect your specimens for research is up to you. No matter what you decide to do, it will not affect your care.

If you agree now that your clinical specimens can be collected and used for research, you can change your mind at any time. At that time, please contact the staff at your treating institution, typically your doctor or nurse, and tell them that you have changed your mind about allowing your specimens to be used for research. The staff at your treating institution will update the GOG regarding your wishes about using your specimens for the current research study, for future cancer research and/or for future research for health problems other than cancer. If necessary, the GOG will destroy (incinerate) all of your specimens to make sure that they will no longer be used for research.
When a patient participates in a clinical research study, there is a risk that information from the person’s health records may be released. The staff at your treating institution and at the GOG will protect your records so that your information is kept private and confidential. The chance that your information will be given to someone in error is very small.

There may also be risks associated with the collection of a particular type of specimen. The exact type of specimen to be collected and any associated risks will depend on the study you participate in and this information will be described in the informed consent document for the research study. For example, collection of a blood specimen may result in slight discomfort, bleeding, or bruising at the site of the blood draw. Carefully read the Consent Document that accompanies this pamphlet for a description of the research study, the requirements, the types of medical and laboratory tests to be carried out and the potential benefits and risks associated with the study.

Please see the next section for some frequently asked questions regarding the collection and use of specimens for research. A short glossary is also provided at the end of this pamphlet. An expanded cancer dictionary is provided online at http://cancer.gov/dictionary/.

**Frequently Asked Questions**

1. **Where do specimens come from and how are they used?**
   Specimens such as tissue, cells, blood, urine, saliva, and mucus, come from people (human subjects), like you, who give permission (consent) to have bodily material collected, saved and used for research. These specimens are collected by a trained member of the clinical staff like a doctor or nurse and are used for approved research. People who are trained to handle clinical specimens and also know how to protect the donor’s rights make sure that the highest standards are followed. Your clinical specimens are sent to the GOG Tissue Bank for storage. Staff in the GOG Tissue Bank will then send the clinical specimens to doctors and scientists for research that has received the appropriate approval. Your doctors and nurses do not work for the GOG Tissue Bank, but they have agreed to help collect specimens from their patients who provide consent. Staffs at clinics and hospitals across the country collect clinical specimens from their patients in the same way. In this way, the research is not specific to one person but general to people across the country.

2. **What kinds of laboratory testing will be used on specimens?**
   Researchers are interested in determining how normal cells become cancer cells, how the body reacts to cancer cells, how cancer spreads in the body, and how cancer cells respond to treatment, for example. Clinical specimens play a very important role in this research and your bodily material can be used to study DNA (genetic material), RNA, proteins, and other components of the human body to understand the role they play in cancer and other diseases. There are many laboratory tests that a researcher can use on clinical specimens including an immunohistoassay (to measure the amount of one protein in a tissue, cells or bodily liquid like serum, plasma or urine), in situ hybridization (to measure the amount of one piece of DNA or RNA in a tissue or cells), flow cytometry (to measure the amount of DNA, RNA or a protein in cells), microarrays (to measure the amount of thousands of pieces of RNA in a tissue or cells), proteomics (to measure the amount and size of thousands of proteins or pieces of proteins in tissue, cells or bodily liquid like serum, plasma or urine), or genetic testing (to look for a change called a mutation in a gene like BRCA1 or BRCA2 in your DNA).

3. **Will I find out the results of the research using my specimens?**
   No, you will not receive the results of research done with your clinical specimens. When the research study is completed, the results and conclusions of the study will be published so that the entire scientific community can benefit from the research. The results from research will not affect your treatment or care right now, but they may help people like you in the future.

4. **How could my health records be used in ways that might be harmful to me?**
   Health records could be used against patients and their families to deny insurance or employment to people with certain illnesses like AIDS or cancer. Information found in health records regarding genetic diseases passed down in families may also be used against you or your family members. Research results will not be added to your health record to prevent these types of events.

5. **Who do I contact if I have questions?**
   You may want to contact the staff at your clinic or hospital like your doctor or nurse with any questions. The medical staff will do their best to answer your question(s) or direct you to someone who can. For additional information you can call the National Cancer Institute’s Cancer Information Service at 1-800-4-CANCER (1-800-422-6237) or visit a website that provides accurate and carefully reviewed information like:
Biopsy – removal of a piece of tissue from the body, which is then examined under a microscope to check for cancer cells.

BRCA1 and BRCA2 – two genes that are often changed (mutated) in families with a high risk of developing breast and ovarian cancer. When a person inherits an altered or mutated copy of BRCA1, that individual has an increased chance of developing breast, ovarian or prostate cancer.

Cancer – is a term for diseases in which abnormal cells divide without control. Cancer cells can invade nearby tissues and spread through the blood and lymphatics to other parts of the body.

Cell – the basic unit of any living organism that can reproduce itself exactly. Humans are made from millions of cells that are adapted to carry out particular functions.

Clinical information – includes medical history, diagnosis, treatment, and outcome.

Clinical trial or protocol – Research studies that involve patients. Each study is designed to find better ways to prevent, detect, diagnose, or treat cancer and other diseases, and to answer scientific questions. A clinical trial is a specific type of research activity that involves administration of a test intervention (a drug, surgical procedure, diagnostic test or medical device or example) to humans in order to evaluate the intervention.

DNA (deoxyribonucleic acid) – the substance of heredity; a large molecule that carries the genetic information present in each cell. The other type of nucleic acid found in the body is RNA, which is transcribed from DNA and translated into proteins.

Flow cytometry – a laboratory test to determine the amount of DNA, a piece of DNA or RNA, or a particular protein present in cells.

GOG Tissue Bank – the organization funded by the National Cancer Institute to collect, process, store and distribute clinical specimens from people participating in studies (clinical trials or protocols) conducted by the Gynecologic Oncology Group.

Gynecology – the branch of medicine dealing with diseases and health of the female reproductive organs.

Gynecologic cancer – cancer of the ovaries, cervix, uterine, endometrium, vulva, and vagina for example.

Gynecologic Oncology Group (GOG) – the cooperative clinical trials group focused on the prevention and treatment of gynecologic cancers. The GOG is a national organization dedicated to clinical research in the field of gynecologic cancer. The purpose of the GOG is to improve the treatment of gynecologic cancer through research encompassing surgery, radiation therapy, chemotherapy, pathology, immunology, and/or gynecologic nursing as well as translational or experimental research in clinical specimens.

Human subjects – a person who participates in a research study.

Immunoadsorption – a laboratory test to measure the amount of a specific protein in a thin slice (section) of tissue, cells or liquid like serum, plasma or urine.

In situ hybridization – a laboratory test to measure the amount of a piece of DNA or RNA in thin slice (section) of tissue or cells.

Microarrays – a laboratory test to measure the amount of thousands of pieces of RNA in a tissue or cells to determine an RNA expression pattern or profile in the specimen.

National Cancer Institute (NCI) – a component of the National Institutes of Health (NIH), one of the eight agencies that compose the Public Health Service (PHS) in the Department of Health and Human Services (DHHS). The NCI is the Federal Government's principal agency for cancer research and training. That NCI coordinates the National Cancer Programs which conducts and supports research, training, health information dissemination, and other programs with respect to the cause, diagnosis, prevention and treatment of cancer, rehabilitation from cancer, and the continuing care of cancer patients and the families of cancer patients.

Plasma – the liquid part of blood after adding an anticoagulant and removing the blood cells.

Proteins – substances composed of amino acids that are essential to body structure and proper functioning.

Proteomics – a laboratory test to simultaneously measure thousands of proteins/fragments in a tissue, cells or liquid like serum or urine to determine the protein expression pattern (profile) in the specimen.

Research – a systematic investigation designed to develop or contribute to general knowledge, to discover new information, revise conventional wisdom, and develop new treatments.

RNA (ribonucleic acid) – one of the two nucleic acids found in all cells. In the cell, RNA transfers genetic information from DNA to proteins. RNA is transcribed off DNA and then translated to produce protein.

Saliva – the watery fluid secreted by glands in the mouth.

Serum – the liquid part of blood after coagulation and removal of the fibrin clot and blood cells.

Specimen – a small part or sample of any substance or material obtained for testing. A human specimen specifically represents a bodily material such as tissue, cells, blood, serum, plasma or urine collected for testing.

Tissue – a collection of cells specialized to perform a specific function.

Urine – the liquid waste secreted by the kidney.
Appendix VII - Histologic Assessment For Translational Research

All fixed-tissue specimens submitted for GOG-0210 will undergo standard histologic processing and paraffin-embedding in the GOG Tissue Bank. Each block will be labeled with the GOG Bank ID, the appropriate specimen code and the collection date.

For each formalin-fixed tumor or normal tissue specimen that will undergo laboratory testing for GOG-0210, a 5 µm thick tissue section will be prepared from the appropriate paraffin block and stained with hematoxylin and eosin (H&E).

For each of these cases, the H&E stained tissue section will undergo thorough light microscopic evaluation by the study pathologist, Richard Zaino. A standard report on histologic assessment of the research specimen will be generated. The report will include histologic subtype, tumor grade (when appropriate), and an estimate of neoplastic cellularity and percent necrosis. Neoplastic cellularity will be expressed as the percent of cancer cells within the tissue section, with a range of 0-100%. The percent of necrosis within the tissue section will also be documented with a range of 0-100%.
Appendix VIII - Gene Expression Profiling

The essential features for the gene expression profiling study for GOG-0210 will be re-evaluated over time so that new advances in research and technology can be appropriately incorporated into this protocol. Standardized procedures will be utilized to minimize sources of variability and to maximize consistency, reliability and reproducibility. All RNA extractions, target labeling, prehybridization, hybridization, washing, staining and scanning will be performed in approved core facilities with documented experience in gene expression profiling in human cancer specimens on a fee-for-service basis.

As of March 2003

1. Gene Expression Profiling Project Leaders

Jeff Boyd, Memorial Sloan Kettering Cancer Center; Paul Goodfellow, Washington University School of Medicine; Kathleen Darcy, GOG Statistical and Data Center, Roswell Park Cancer Institute. Drs. Boyd, Goodfellow and Darcy will oversee all components of the independent and integrated gene expression profiling projects, be directly involved in all analyses and provide input for "biological rationale" in interpreting the data; essentially as was done in the creation of molecular portraits of human breast tumors (Perou et al.1999).

2. Core Facilities For Gene Expression Profiling

Genomics Core Laboratory at Rockefeller Research Laboratory/Memorial Sloan Kettering Cancer Center (Agnes Viale, Manager of the Genomics Core Laboratory, add contact information); Multiplexed Gene Analysis Core at the Siteman Cancer Center at Washington University School of Medicine/Barnes-Jewish Hospital (Kate Hamilton at 314.454.8520 or e-mail genechip@pathbox.wustl.edu).

3. Specific Aims and Hypothesis

The first aim will be to perform gene expression profiling in GOG-0210 tissue specimens for the purpose of class prediction and class discovery in endometrial carcinoma to identify and validate molecular characteristics associated with the risk of endometrial cancer recurrence, surgical/pathologic characteristics, and epidemiologic factors.

The second aim will be to utilize the gene expression profiling data collected as part of the first aim to identify candidate characteristics to target or exploit to prevent and/or treat endometrial carcinoma, and to expand the current understanding of the biology, progression, metastasis and responsiveness of endometrial carcinoma.

For these studies, RNA will be extracted from as many as 500 GOG-0210 patients with specific clinical characteristics and outcome, and subjected to gene expression profiling to determine whether distinct expression profiles may be discerned. These profiles will serve as the basis for a new molecular classification of endometrial cancers that may have prognostic and therapeutic importance.
The main hypothesis to be tested is that a comprehensive molecular genetic classification of endometrial cancer, using gene expression profiling technology, will improve our ability to predict clinical outcome, specifically with regard to recurrence-free interval following surgical staging.

4. Gene Expression Array Chip

Chip A of the Affymetrix GeneChip Human Genome U133 (HG-U133) set, which includes various types of controls to assess hybridization success and monitor quality, will be used for the initial phase of the gene expression profiling projects. New advances in array chips and technology will be reviewed for their incorporation into subsequent phases of the gene expression profiling projects.

The Affymetrix U133 A chip contains approximately 21,000 transcripts representing 14,500 known genes and 6,500 ESTs. Each sequence is represented on this microarray by 11 pairs of 25-mer oligonucleotides, with each pair containing an exact sequence match oligonucleotide (positive signal) coupled with an oligonucleotide containing a single base mismatch at the central (13th) position (negative signal). This probe pairing strategy identifies and minimizes the effects of non-specific hybridization and background signal. With this system, RNAs present at a frequency of 1:100,000 can be detected, and detection is quantitative over more than three orders of magnitude. Each array contains oligonucleotide pairs corresponding to three reference genes, allowing standards to be added to the sample prior to hybridization for the normalization and quantitation of data from specimens from different patients evaluate over time. All prehybridization, hybridization, washing, staining and scanning will be performed in the Core facilities on a fee-for-service basis.

5. Equipment

RNA Preparation: Eppendorf Microcentrifuge 5415 D (Fisher Scientific); PowerGen 125 Homogenizer with Saw-Tooth Generator (Fisher Scientific); DU 530 Single Cell Life Science UV/Vis Spectrophotometer (Beckman)

Probe Generation: Eppendorf Microcentrifuge 5415 D (Fisher Scientific); Isotemp Digital Dry Bath Incubator 125D (Fisher Scientific); Peltier Thermal Cycler PTC-100™ (MJ Research); Isotemp Incubator (Fisher Scientific); Agilent 2100 Bioanalyzer (Agilent Technologies)

Array Hybridization: GeneChip® Hybridization Oven 640 (Affymetrix®); Eppendorf Microcentrifuge 5415 D (Fisher Scientific); Isotemp Digital Dry Bath Incubator 125D (Fisher Scientific)

Probe Array Washing and Staining: GeneChip® Fluidics Station 400 (Affymetrix®)

Probe Array Scanning: Agilent GeneArray® Scanner (Agilent Technologies)

Initial Data Analysis: Computer: Compaq Evo, Microsoft Windows 2000 Service Pack 2, Intel Pentium 2 GHz processor, 1.3 GB RAM, 37 GB hard drive; Monitor: NEC MultiSync LCD2010X XtraView; Server: to be named; Software: Affymetrix® Microarray Suite 5.0; Affymetrix® Laboratory Information Management System (LIMS) 3.0
6. Array Controls / Hybridization Controls

GeneChip® Eukaryotic Hybridization Control Kit (Affymetrix®, #900299) for 30 reactions; Control Oligo B2 (Affymetrix®, #900301)

7. Reagents

RNA Preparation: RNeasy Total RNA Isolation Mini Kit (QIAGEN, #74104 for 50 samples, #74106 for 250 samples); RNeasy Total RNA Isolation Midi Kit (QIAGEN, #75142 for 10 samples, #75144 for 50 samples)

Probe Generation: MessageAmp™ aRNA Kit (Ambion, #1750) for 2-5 ug of RNA; SuperScript™ II Double-Stranded cDNA Synthesis Kit (Invitrogen Life Technologies, #11917010) for 10 ug of RNA; BioArray™ HighYield™ RNA Transcript Labeling Kit ,T7 (Enzo Life Sciences, #42655-20) for 10 ug of RNA; RNA 6000 Nano LabChip Kit (Agilent Technologies, # 5065-4476); GeneChip® Sample Cleanup Module (Affymetrix®, #900371)

Hybridization, Washing, and Staining: Human Genome U133 Set (Affymetrix®, #900370); Deionized, Distilled Water, Molecular Biology Grade (AccuGENE™ # 51200); DEPC-treated Water (Fisher Scientific, #BP561-1); Acetylated Bovine Serum Albumin (BSA) solution, 50 mg/ml (Invitrogen Life Technologies, #15561020); Herring Sperm DNA, 10 ug/ul (Promega Corporation, #D1811); 5 M NaCl, RNase-free, DNase-free (Ambion, #9760G); MES Free Acid Monohydrate SigmaUltra (Sigma-Aldrich, #M5287); MES Sodium Salt (Sigma-Aldrich, #M5057); 0.5 M EDTA solution, 100 ml (Sigma-Aldrich, #E7889); R-Phycocerythrin Streptavidin (Molecular Probes, #S-866); PBS, pH 7.2 (Invitrogen Life Technologies, #20012027); 20X SSPE (Fisher Scientific, BP1328-1); Goat IgG, Reagent Grade (Sigma-Aldrich, #I5256); Anti-streptavidin antibody (goat), biotinylated (Vector Laboratories, #BA-0500); 10% Surfact-Amps 20/Tween 20 (Pierce Chemical, #28320); Ethanol Absolute, 200 proof (Sigma-Aldrich, #E7023)

8. Method for RNA Preparation

Ideally, 20 ug of high quality total RNA will be extracted from the tissue specimens under investigation. This minimum requirement will ensure sufficient RNA for duplicate hybridization experiments, should they be required (in the event of a failure or low quality hybridization and to document internal variability and reproducibility). Total RNA will be prepared from the frozen tumor specimen (quarter fractions of the total) using standard organic extraction protocols. At WUSM, Trizol (Invitrogen life technologies, CAT. NO. 15596026 ) will be used. At MSKCC, RNA will be prepared according to the QIAGEN protocol. Following the organic extraction, the RNA will be further purified using a silica-based spin column (Qiagen RNeasy Minikit for RNA cleanup, CAT. NO. 74106). The preparation of RNA specimens will not include DNase treatment. The purified RNA will be suspended in DEPC-treated H2O.
9. Criteria for Assessing RNA Concentration, Purity and Quality

Prior to target preparation, each specimen will be assessed to determine both the quantity, purity and quality of the RNA. RNA purity, with respect to contaminants that absorb in the UV range, such as protein, will be estimated by performing spectrophotometer readings at 260 nm and 280 nm. The \( \frac{A_{260}}{A_{280}} \) ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting \( \frac{A_{260}}{A_{280}} \) ratio can vary greatly. Lower pH results in a lower \( \frac{A_{260}}{A_{280}} \) ratio and reduced sensitivity to protein contamination. For accurate values, absorbance will be measured in 10 mM Tris-HCl, pH 7.5. Pure RNA has an \( \frac{A_{260}}{A_{280}} \) ratio of 1.9-2.1 in 10 mM Tris-HCL, pH 7.5.

The integrity and size distribution of total RNA will be assessed using the Agilent 2100 Bioanalyzer (Agilent Biochips - RNA 600 Pico Labchips, CAT. NO. 5065-4476 run on Agilent 2100 Bioanalyzer, CAT. NO. G2940BA). The 18S and 28S ribosomal bands should appear as sharp bands. The 28S rRNA band should be present with an intensity approximately twice that of the 18S rRNA band. If the rRNA bands are not sharp, but appear as a smear of smaller sized RNAs, the RNA sample is degraded. Only those RNA specimens in which the 28S:18S ribosomal RNA band ratio is approximately 2:1 and for which the 'smear' below the 18S band is minimal will be used for target synthesis. After in vitro transcription labeling, the biotin-labeled transcript will also be analyzed on the Agilent 2100 Bioanalyzer to estimate the yield and size distribution.

10. Method for Probe Generation

Targets (biotinylated cRNA) will be generated using protocols supplied by in the Affymetrix® GeneChip® Expression Analysis Technical Manual. In brief, these methods include reverse transcription to produce double-strand cDNA, linear amplification with T-7 RNA polymerase, and biotin labelling through in vitro transcription. Biotin-labelling is performed using the BioArray\textsuperscript{TM} High Yield\textsuperscript{TM} RNA transcript Labeling Kit (T7) (Enzo, New York, NY).

\textit{B. subtilis} poly-A transcript spikes (\textit{trp}, \textit{thr} \textit{phe} and \textit{lys} at concentrations approximately 10 to 1000 copies per cell starting material) will be included to monitor the efficiency of amplification and labeling reactions and troubleshoot in the event of a poor technical result.

11. Array Hybridization Conditions

Array hybridization will be performed at a temperature of 45°C at 60 rpm for 16 hours.

12. Probe Array Washing and Staining Conditions

Post Hybridization Wash #1: 10 cycles of 2 mixes/cycle with Wash Buffer A (Non-stringent Wash Buffer) at 25°C.

Post Hybridization Wash #2: 4 cycles of 15 mixes/cycle with Wash Buffer B (Stringent Wash Buffer) at 50°C.
13. Probe Array Scanning Conditions and Settings

The scanner is controlled by Affymetrix® Microarray Suite. The probe array is scanned after
the wash protocols are complete. The laser is warmed up prior to scanning by turning the laser
on at least 15 minutes before use. The laser beam setting are as follows: pixel value = 3 um
and wavelength = 570 nm.

14. Initial Data Analysis

After scanning the probe array, the resulting image data created is stored on the hard drive of
the GeneChip® Analysis Suite/Microarray Suite workstation as a .dat file with the name of the
scanned experiment. A grid is automatically placed over the .dat file demarcating each probe
cell. One of the probe array library files, the .cif file, is used by Microarray Suite to determine
the appropriate grid size used. Data is analyzed using the Microarray Suite Expression
Analysis window. The .dat image is analyzed for probe intensities and results are reported in
tabular and graphical formats.

Background subtraction: The first step in the analysis is to correct for background across the
entire array. The calculated background establishes an intensity floor that is subtracted from all
intensity values. The algorithm divides the array into 16 equally spaced zones and assigns an
average background to the center of each zone. It then computes the distance from each cell to
the center of every zone and computes a weighting factor. Finally, the background value is the
results of applying the weighting factor to the zone average. The probe set signal is the
background-adjusted cell intensities.

Scaling-Adjust for labeling intensity: Scaling is a mathematical technique applied to the data
from several different probe arrays (of the same type) to minimize the discrepancies due to
variables such as sample preparation, hybridization conditions or staining. The scale factor is
also applied to the noise value. We use an “all probe sets” type of scaling. This will result in
the adjustment of the trimmed mean signal of a probe array to a user-specified target signal
value (500 in our case).

15. Advanced Data Analysis
We propose to utilize both “supervised” and “unsupervised” statistical methods to identify markers for recurrence and to begin to define the molecular heterogeneity that is a feature of endometrial carcinoma. Supervised methods for distinguishing between two classes of tumors, for example, those who recur and those who do not recur, utilize the class membership of samples in deriving discriminants for distinguishing between the classes based on molecular profiles. Linear and nonlinear regression methods, classification and regression trees, and neural networks are all supervised methods. Unsupervised methods are algorithms for identifying clustering of cases with respect to profiles without using information about class membership. Supervised methods are generally more powerful for deriving predictors of class membership. Unsupervised methods are useful for discovering patterns that may be of biological relevance, although under current treatment regimens, they may not be effective for predicting clinical outcome. Unsupervised methods are sometimes used where supervised methods may be more powerful because of concern that supervised methods may “over-fit” the data and not be predictive of outcome for independent data. For predicting outcome, however, it is often better to utilize supervised methods but to use cross-validation or the “bootstrap” in order to obtain an almost unbiased estimate or predictiveness for independent data. Unsupervised methods may discover clusters that may be useful for classification and relevant to the development of new treatments, but not prognostic with current treatments. We believe that both supervised class prediction and unsupervised pattern discovery are important and will utilize both approaches.

The analysis of data is complex and has been broken into four sections (a-d) for simplicity of presentation.

a. Affymetrix GeneChip software [Microarray Analysis Suite 5.0 (MAS 5.0)] will be used to determine expression levels for each gene. We will also use d-Chip software (add manufacturer information) and will compare its results to those of GeneChip. Any genes with significantly different results will be followed up with both methods. Since the majority of genes are not expected to show changes in expression levels between any of the comparison groups (recurrence vs non-recurrence, grade 1 or 2 vs grade 3 tumor, or other comparisons), we will normalize to constant medians (Gu et al, 2002).

b. The identification of genes that differ between comparison groups will be done in two steps. For all samples from the same group (for the purpose of this section, we will consider stage and grade matched recurrent and non-recurrent endometrioid cancers), the mean and standard deviation of the expression for each gene will be calculated. For example, carcinoma tissues from women who do not recur will be “pooled” at the level of determining their average values from each of the individual measurements. This will allow us to also determine which genes are highly variable, with large standard deviations. After these mean values are determined, the gene expression profiles in all the tissue specimens tested will be compared with each other. Genes that appear to change expression between comparison groups and those that will be followed up with validation studies will be determined by several criteria. A commonly used cut-off for change in expression is a factor of 2. This is a useful, if weak, criterion even though it can lead to false positives, especially for low expression levels. A t-test will also be used to identify those genes with significantly different means.

The set of genes identified for validation will be ranked by a combination of t-test scores, fold increase (taking into account absolute expression values), and “interesting”
annotations. In addition, any genes consistently called "not present" in one comparison group (recurrent or non-recurrent cases) and "present" in another will also be considered for validation studies. The exact rankings will be determined empirically and selected with the intent of minimizing noise that results from false-positives, based in large part on the extensive variation seen in similar studies of breast and colonic tumors (Alon et al., 1999; Perou et al., 1999).

c. Clustering of the expression data will be performed using several complementary methods. In general, genes will be clustered to obtain sets that behave most similarly across the tissue specimens tested. We will focus our interest on genes whose expression changes substantially and consistently. Initial analysis will focus on expression changes with at least a fourfold difference in their mean values in different tissues (Eisen et al., 1998). Subsequent analyses will utilized other thresholds and will determine how much the clusters change and whether new insights emerge.

Genes will be clustered by several methods, including hierarchical (Eisen et al., 1998) and k-means or self-organizing maps (SOMs) (Tamayo et al., 1999, Hughes et al., 2000). The goal of these approaches is to identify sets of genes whose expression varies in correlation with outcome (recurrence, or other known covariates such as stage, grade, patient age, etc.). The normal biological roles of such gene products may highlight specific pathways involved in tumorigenesis. For example, estrogen-responsive genes would be expected to be expressed more abundantly in the so-called type-I endometrial cancers compared with type-II endometrial cancers.

d. Sets of genes that are diagnostic for particular tissue types will also be identified directly. As in Golub et al. (1999), where it was shown that the expression levels of a few genes were sufficient to classify two types of cancers, AML and ALL, the same type of pattern classification approach will be employed to identify genes associated with each tissue type. More recent work has shown that different translocations involving the mixed-lineage leukemia gene are associated with distinct expression profiles (Armstrong et al., 2002). Based on the work from Armstrong et al. (2002), we anticipate being able to define changes in expression associated with histologically defined differences in tumors, and between tumors that recur and do not recur. Pattern classification in our experimental system will be performed both as a method of separation of each type against all others (grades, stage, histology), or to follow the outcome. As in Golub et al (1999), the reliability of these classifications will be evaluated through cross validation studies and in tests on new, independent data sets not included in the initial studies. The genes identified as classifiers will be analyzed for their normal functional roles, as in the first approach, to gain further insight into the molecular changes accompanying the physiological ones.

The principal features of the approach to data analysis are summarized below, beginning with a description of the univariate methodology. To compare the expression levels of a particular gene between two groups of tumors, we will use a Wilcoxon rank sum test. For example, we plan comparisons of gene expression levels between type I and type II tumors. A separate rank sum statistic will be computed for each gene. Because of the large number of genes to be evaluated, it is essential to control the overall false positive error rate utilizing a multiple comparison correction. The usual Bonferroni multiple comparison method would require each rank sum test to be
significant at a level of 0.05 divided by the number of genes considered. Because of the correlation among expression profiles for different genes, however, this is an overly conservative multiple comparison correction. The exact stepwise permutation test approach using the rank sum statistic (Blair et al., 1996) is one method we considered applying. This provides an overall false positive error rate of 5% in a manner that takes advantage of the correlation structure of the gene expression profiles to reduce the conservativeness of the Bonferroni multiple comparison adjustment, and it does not rely on normality assumptions. Korn et al. (2003) more recently showed that this type of approach to control for the expected frequency of false discoveries may not be adequate, and that two stepwise permutation-based procedures should be used to control the actual number and proportion of false discoveries with a specified confidence. To correlate gene expression with survival duration, a univariate Cox proportional hazards regression model will be fit for each gene. The statistical significance of the resulting regression coefficients will be assessed using the two stepwise permutation-based procedures described by Korn and colleagues (2003).

Accurately predicting class membership using multivariate predictors is in general easier than identifying which predictors are statistically significantly differentially expressed among the classes. This is true for two reasons.

First, evaluating the significance of individual genes suffers from the multiple comparisons problem and the need to adjust significance for the number of genes tested. Second, multivariate predictors take advantage of the power of effects of individual predictors to reinforce each other and overcome the noise variation of individual predictors.

Several approaches will be employed for the development of multivariate class predictors. Compound covariates (Tukey, 1993; Hedenfalk et al., 2001) will be the first type of multivariate method utilized to develop a multivariate predictor that is a weighted linear combination of log-average differences for informative genes. The informative genes will be the set of the genes most differentially expressed between the classes being predicted. Genes shown to attain univariate significance levels ≤ 0.001 will be selected. The contribution of an informative gene to the multivariate predictor (its weight) will be proportional to its univariate regression coefficient divided by the standard error of that regression coefficient. For predicting between two classes, for example those that recur versus those that do not recur, informative genes will be identified using the Wilcoxon rank sum test, and weights will be proportional to the value of the test statistic. For classifying a new case, the product of the weight times a normalized log-average difference of the gene will be summed over the set of influential genes. This score will then be compared against a threshold for classification. The classification error rate and optimal threshold will be estimated by cross validation in which the multivariate classifier is re-computed n times, each time with one of the samples excluded. Classification accuracy will be estimated by counting the number of errors in classifying the samples excluded using the classifier derived with that sample excluded. We will include re-selection of the set of informative genes in each step of the cross-validation. Alternative weighting schemes will also be investigated.

Multivariate prediction using logistic regression with principal component predictors based on gene expression profiles from informative genes will also be performed. That
is, starting with the collection of informative genes, a set of uncorrelated linear combinations of the log-average differences whose variances are as large as possible will be computed. These linear combinations are called the principal components. We will use the number of principal components needed to explain 90% of the total variation in log-average differences of gene expression for the informative genes or up to a maximum of 10 principal components. A logistic regression to predict class membership using the principal components as predictor variables will then be performed. Prediction accuracy will be estimated by cross-validation as described above. K-nearest-neighbor classification and linear discriminant analysis will also be examined in terms of classification ability (Dudoit et al., 2002). Similarly, Cox proportional hazards regression with principal component predictors for multivariate prediction of survival outcome will also be used as needed.

The statistical power of multivariate prediction of class membership can be expected to be much greater than for univariate analyses because multiple comparison adjustment to significance levels will not be as extreme as for univariate analyses and because sets of genes will reinforce each other in multivariate predictors.

16. Verification of Changes in Gene Expression and Validation Studies

Two approaches will be taken to verify the changes in message levels seen in the expression profiling studies. Because of the limited tissue resources, we will rely upon quantitative real-time reverse transcriptase PCR (Q-RT-PCR) (Heid et al., 1996). The first step in verification studies will be to perform Q-RT-PCR with cDNA prepared from the same tissue used for the generation of labeled targets. All RT-PCR assays will be biased to the 3’ portions of genes to parallel the distribution of probe sets assessed using the Affymetrix GeneChips. Whenever possible the sample tissues will be studied to confirm up- or down-regulation of genes of interest. The second approach will be to quantitate message levels in additional cases (tumors that recur, those that do not, different stages and histologies) along with normal endometrium. The size of the validation studies (number of specimens investigated) will vary with the size of the effect seen in the initial profiling studies. We anticipate assembling a large collection of tissue and RNAs for use in the expression verification studies.

In situ hybridization and immunohistochemical approaches will also be utilized in the validation component of the gene expression profiling projects to validate the most dramatic differences in RNA and protein expression, respectively, and to determine cell type-specific expression patterns and the intracellular localization. In addition, we propose the coupling of laser capture microdissection (LCM) with expression analysis to ascertain the tissue type from which the signal of interest arose. Finally, we propose to have the GOG Tissue Bank establish tissue arrays from subsets of the GOG-0210 endometrial cancer tissue resource to validate the importance of candidate markers arising from the expression array analyses.

17. Data Transfer

All data files regarding the different gene expression profiling projects for GOG-0210 will be transferred to the GOG Statistical and Data Center and stored in an Ingres II relational database. Appropriate data will then be shared with the project leaders, scientific collaborators and bioinformatics specialists as needed. All data transfers will utilize a secure web-based data
transfer application to be developed by the Information Technology Division of the GOG Statistical and Data Center and made available on the GOG web-site.

18. References


The essential features for the tissue proteomic profiling study for GOG-0210 will be re-evaluated over time so that new advances in research and technology can be appropriately incorporated into this protocol. Standardized procedures will be utilized to minimize sources of variability and to maximize consistency, reliability and reproducibility. Approved core facilities with documented experience in proteomic profiling in human cancer specimens will be utilized on a fee-for-service basis.

As of March 2003

1. Tissue Proteomic Profiling Project Leaders

   Kimberly Leslie, University of New Mexico; Kathleen Darcy, GOG Statistical and Data Center, Roswell Park Cancer Institute. Drs. Leslie and Darcy will oversee all components of the independent and integrated tissue proteomic profiling projects, be directly involved in all analyses and provide input for "biological rationale" in interpreting the data. Elise Kohn from the National Cancer Institute will be asked to provide her expertise as needed for these projects.

2. Core Facilities For Tissue Proteomic Profiling

   Proteomics Core Facility at The University of New Mexico (On-Site Contact To Be Named).

3. Specific Aims and Hypothesis

   The first aim will be to perform proteomic profiling in GOG-0210 tissue specimens for the purpose of class prediction and class discovery in endometrial carcinoma to identify and validate peptide profiles (patterns) associated with the risk of endometrial cancer recurrence, surgical/pathologic characteristics, and epidemiologic factors.

   The second aim will be to utilize the tissue proteomic profiling data collected as part of the first aim to identify candidate characteristics to target or exploit that would prevent and/or treat endometrial carcinoma, and to expand the current understanding of the biology, progression, metastasis and responsiveness of endometrial carcinoma.

   For these studies, tissue lysates will be prepared from as many as 500 GOG-0210 patients with specific clinical characteristics and outcome, and subjected to proteomic profiling to determine whether distinct peptide profiles may be discerned. These profiles will serve as the basis for a new molecular classification of endometrial cancers that may have prognostic and therapeutic importance.

   The main hypothesis to be tested is that peptide profiles of endometrial cancer will predict clinical outcome, specifically with regard to recurrence-free interval following surgical staging. In addition, peptide profiles will be identified that segregate with surgical/pathological characteristics (stage, grade, cell type, extra-uterine disease spread, and type-I versus type-II disease), receptor status (steroid receptor isoform expression or expression of the epidermal growth factor receptor family), and epidemiologic factors.

   The tissue proteomic profiling analyses will determine the protein composition of type-I versus type-II tumors or tumors of varying stages, grades, or cell types for example, and correlate
these findings with disease recurrence or hormone receptor status. These experiments will
encompass first, the identification of unique patterns of peptide expression, and second, the
identification of candidate proteins that discriminate between tumors of different
characteristics. Such studies are designed to (1) identify potential biomarkers that appear to
segregate with clinical outcome, and (2) expand the current understanding of the biology,
progression, metastasis and responsiveness of endometrial carcinoma. The tissue proteomic
profiling data will be compared to the gene expression profiling data and the
immunohistochemical expression data for steroid and growth factor receptors (ER-α, ER-β,
PR-A, PR-B, EGFR, Her-2, ErbB3 and ErbB4) carried out on the same tumor and normal
tissues.

4. Method

Mass spectrometry (MS) linked to two-dimensional gel electrophoresis will be performed on
specific GOG-0210 frozen tumor and normal tissue specimens. Proteins will be extracted using
the ReadyPrep sequential extraction kit (BioRad) where differential solubilization will be
utilized to reduce sample complexity.

Two-dimensional gel electrophoresis will be performed on the individual protein lysates using
an IPGphor (Amersham Pharmacia Biotech) for first dimension separation of proteins on pre-
cast immobilized pH gradient gels (IPG strips). Second dimensional SDS-PAGE
electrophoresis will be performed on 2 different size formats. For preliminary analysis, the
Bio-Rad Criterion electrophoresis Dodeca Cell uses 11cm IPG strips and runs up to 12 gels
simultaneously. Preparatory large format gels will be used to run samples for protein
identification. The protein separation facility uses a Hoefer™ DALT with 18cm IPG gel strips
and can run up to 12 large format (23 x 20cm) gels.

Imaging is accomplished with a Bio-Rad GS-800 calibrated densitometer. Imaged gels will be
analyzed and databased by PDQuest software. Patterns of differential protein expression will
be identified by the software and proteins of interest excised and subjected to in-gel enzymatic
digestion to extract the peptides.

Protein identification will be first attempted by MALDI Time of Flight Mass Spectrometry
(MALDI-TOF) using an AP Biosystems Voyager Elite instrument. Proteins not identified by
MALDI-TOF will be identified with electrospray (ESI) MS-MS with a high resolution Q-Tof™
mass analyzer (Micromass Q-Tof™ 2 ESI mass spectrometer). These two ionization
techniques are complimentary because they are known to produce different MS and tandem
MS/MS spectra from the analysis of the same sample due to the differential ionization of
tryptic peptides. Samples will be introduced into the Q-Tof™ via a Micromass CapLC™ that
is an automated solvent/sample management system specifically for integration with Q-Tof™.
The Micromass ProLyx software will automatically perform database searches with peptide
and sequence data to identify proteins.

Peptide profiles and/or unique peptide loci will be identified that segregate with recurrence,
surgical/pathological characteristics (stage, grade, cell type, extra-uterine disease spread, type I
verses type II disease), epidemiologic factors, steroid receptor isoform expression, and/or
growth factor receptor status.

5. Data Analysis
This section will be developed.

6. Verification and Validation Studies

This section will be developed.

7. Data Transfer

All data files regarding the different tissue proteomic profiling projects for GOG-0210 will be transferred to the GOG Statistical and Data Center and stored in an Ingres II relational database. Appropriate data will then be shared with the project leaders, scientific collaborators and bioinformatics specialists as needed. All data transfers will utilize a secure web-based data transfer application to be developed by the Information Technology Division of the GOG Statistical and Data Center and made available on the GOG web-site.

8. References

References will be added.
Appendix X - Serum Proteomic Profiling

The essential features for the serum proteomic profiling study for GOG-0210 will be re-evaluated over time so that new advances in research and technology can be appropriately incorporated into this protocol. Standardized procedures will be utilized to minimize sources of variability and to maximize consistency, reliability and reproducibility. Approved core facilities with documented experience in serum proteomic profiling will be utilized on a fee-for-service basis.

As of March 2003

1. Serum Proteomic Profiling Project Leaders

Kimberly Leslie, University of New Mexico; Kathleen Darcy, GOG Statistical and Data Center, Roswell Park Cancer Institute. Drs. Leslie and Darcy will oversee all components of the independent and integrated serum proteomic profiling projects, be directly involved in all analyses and provide input for "biological rationale" in interpreting the data. Elise Kohn from the National Cancer Institute will be asked to provide her expertise as needed for these projects.

2. Core Facilities For Serum Proteomic Profiling

Proteomics Core Facility at The University of New Mexico (On-Site Contact To Be Named).

3. Specific Aims and Hypothesis

The first aim will be to perform proteomic profiling in GOG-0210 serum specimens for the purpose of class prediction and class discovery in endometrial carcinoma to identify and validate peptide profiles (patterns) associated with risk of endometrial cancer recurrence, surgical/pathologic characteristics, and epidemiologic factors. The second aim will be to utilize the serum proteomic profiling data collected as part of the first aim to identify candidate characteristics to target or exploit that would prevent and/or treat endometrial carcinoma, and to expand the current understanding of the biology, progression, metastasis and responsiveness of endometrial carcinoma.

For these studies, serum from as many as 500 GOG-0210 patients with specific clinical characteristics and outcome will be subjected to proteomic profiling to determine whether distinct peptide profiles may be discerned. These profiles will serve as the basis for a new molecular classification of endometrial cancers that may have prognostic and therapeutic importance.

The main hypothesis to be tested is that serum peptide profiles will predict clinical outcome, specifically with regard to recurrence-free interval following surgical staging. In addition, peptide profiles will be identified that segregate with surgical/pathological characteristics (stage, grade, cell type, extra-uterine disease spread, and type I verses type II disease), receptor status (steroid receptor isoform expression or expression of the epidermal growth factor receptor family), and epidemiologic factors. These experiments will encompass first, the identification of unique patterns of peptide expression, and second, the identification of candidate proteins that discriminate between different characteristics. Such studies are designed to (1) identify potential biomarkers that appear to segregate with clinical outcome,
and (2) expand the current understanding of the biology, progression, metastasis and responsiveness of endometrial carcinoma.

4. Overview

Surface enhanced laser desorption ionization spectrometry (SELDI) coupled with time-of-flight (TOF) detection and artificial intelligence bioinformatics will be used to perform the separation, detection and analysis of serum proteins from GOG-0210 patients with specific clinical characteristics and outcome. High-quality serum specimens (processed from blood draw to freezing within 4 hours and stored in a frozen-solid state in appropriate ultra-cold storage space) will be tested in batches using a Ciphergen SELDI-TOF instrument and chips that have been validated and optimized. All cases will be batched for analysis and run on the same platforms. Data streams from the GOG serum specimens along with appropriate standards and spike controls will be subjected to artificial intelligence bioinformatics for cluster analysis and appropriate types of data mining and bioinformatics analyses. Quality control and quality assurance assessments will be routinely performed for the SELDI chip and chromatography. Select specimens will be subjected to repetitive testing for quality control purposes; some may be deemed inadequate for subsequent analysis. In addition, positive controls will be run concurrently, intermingled with the GOG serum specimens, and will be used to document between run and within run reproducibility.

The Ciphergen ProteinChip® System, with a Protein Chip® Reader integrated with ProteinChip® Software will be utilized to perform proteomic profiling on the GOG 210 serum proteins captured on Ciphergen’s ProteinChip® Arrays. First, the serum proteins will be directly captured on the chip array without sample preparation and without sample labeling. Second, the signal-to-noise ratio will be enhanced by reducing chemical and biomolecular noise, and in the process target proteins will be selectively retained on the chip and undesired materials washed away. Third, the retained target proteins will be evaluated using the rapid, sensitive laser-induced SELDI process that provides direct information about the mass to charge ratio of the target proteins. Calculations of mass will be based on TOF. TOF refers to the time-of-flight of a protein within the chip reader, and will be used to calculate and display the molecular mass to charge ratio of a protein. The target proteins at one or more locations within the addressable chip array will be directly characterized in situ by engaging in one or more of the on-the-chip binding or modification reactions to characterize protein structure and/or function. Fourth, the data stream will be transferred to the GOG Statistical and Data Center and distributed to approved end-users for the appropriate analysis, data mining and clustering.

Summary of the Proteomic Profile Procedure

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
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<tbody>
<tr>
<td>Step 1.</td>
<td>Serum will be applied directly onto the ProteinChip® Array.</td>
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<tr>
<td>Step 2.</td>
<td>Proteins will be captured, washed, and retained directly on the chip (affinity capture).</td>
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<tr>
<td>Step 3.</td>
<td>A retentate map (data stream) will be generated using surface-enhanced laser desorption/ionization (SELDI).</td>
</tr>
<tr>
<td>Step 4.</td>
<td>The data stream will be transferred to the GOG Statistical and Data Center and distributed to approved end-users for the appropriate analysis, data mining and clustering.</td>
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</tbody>
</table>

The protein chip arrays available from Ciphergen will be used to affinity-capture minute quantities of serum proteins that vary in their surface chemistry. Each aluminum chip contains eight individual chemically treated spots for sample application; this set-up facilitates
simultaneous analysis of multiple serum samples. Typically, a few µl of serum will be applied to one spot on the chip to yield sufficient protein for analysis. Twelve chip arrays aligned side-by-side create a 96-well plate format. A typical experiment using this technology requires up to three hours of work at the bench followed by automated sample analysis within the chip reader for up to three hours. See below for a brief description of some of the hydrophobic, hydrophilic, anion exchange and cation exchange chip arrays that are currently available from Ciphergen.

### Hydrophobic ProteinChip® Arrays
The hydrophobic arrays bind proteins through hydrophobic surface interaction with amino acids such as alanine, valine, leucine, isoleucine, phenylalanine, tryptophan and tyrosine. Binding occurs in aqueous, high salt conditions and binding is reduced by decreasing salt and increasing the concentration of organics. The H4 and the H50 arrays contain a long-chain aliphatic surface that binds proteins by reverse phase interactions.

### Hydrophilic ProteinChip® Arrays
The hydrophilic arrays bind proteins through electrostatic and dipole-dipole interactions as well as hydrogen binding. Proteins with hydrophilic and charged surface amino acids such as serine, threonine and lysine bind well. Binding occurs in aqueous buffers with a water wash prior to analysis. The NP1 and NP2 arrays contain a SiO$_2$ surface for general binding of proteins.

### Anion Exchange ProteinChip® Arrays
Anionic arrays bind proteins through electrostatic interaction of negatively charged amino acids such as aspartic acid and glutamic acid. Binding occurs at high pH with low salt and binding decreases as pH decreases and salt concentration increases. The SAX2 array is a strong anion exchange chip with a higher-capacity quartenary ammonium surface to bind anionic proteins.

### Cation Exchange ProteinChip® Arrays
Cationic arrays bind proteins through electrostatic interaction of positively charged amino acids such as lysine, arginine and histidine. Binding occurs at low pH with low salt and binding decreases as pH and salt concentration increase. The WCX2 array is a weak cation exchange chip with a carboxylate surface to bind cationic proteins. The negatively charged carboxylate groups on the surface of the WCX2 chip interact with the positive charges exposed on target proteins. The binding of target proteins is reduced by increasing the concentration of salt or by increasing the pH of the washing buffers.

### SELDI Processing
On the day the serum undergoes SELDI processing, the appropriate serum specimens will be kept ice-cold upon rapid thawing, a protease inhibitor will be added to the serum (Complete™, Roche), and the serum will be separated into aliquots for immediate testing. Additional 10 µl aliquots of individual serum specimens may be refrozen for replicate testing. Serum that has undergone more than one freeze/thaw cycle will not be utilized for proteomic profiling due to the inconsistent impact of the freeze/thaw on the protein profile (spectra). The chips will be analyzed using the Spot 7 protocol which has the following settings: laser intensity will be set to 170, detector sensitivity will be set to 8, focus lag time will be set to 950 ns, SELDI acquisition parameters will be set to 20, delta will be set to 8, transients per will be set to 10, ending position will be set to 80, and molecular mass range will initially be optimized from 2,000 to 20,000 Daltons using α-cyano-4-hydroxy-cinaminic acid (CHCA) as the matrix. Additional analyses may then be optimized for proteins from 30,000 to 100,000 Daltons using sinnapinic acid as the matrix.

During the initial phase of the serum proteomic profile study, individual serum specimens will be analyzed on the Protein Biology System 2 SELDI-TOF mass spectrometer (Ciphergen Biosystems) using a blast approach. The serum will be evaluated on the SAX2 (anion exchange) chip using eight binding buffers that vary by salt concentration and pH, on the H50 hydrophobic (reversed phase hydrophobic) chip using eight binding buffers that vary in the concentration of salt and organics, and on the IMAC3 (metal affinity) chip. This strategy is designed to provide a broad, but not a comprehensive view of the proteome of interest. Instrument settings will be optimized for the mass range of proteins of interest. The second phase of this study will focus
on the ProteinChip and binding buffer that provide the protein profile(s) with the greatest promise. The main goal of these studies will be to identify and validate peptide profiles in the pre-op serum specimens that segregate cases by recurrence status, surgical/pathological characteristics (stage, grade, cell type, extra-uterine disease spread, type-I versus type-II disease), epidemiologic factors, steroid receptor isoform expression, growth factor receptor status, as well as the level of steroid hormones and metabolites. A secondary goal of these studies will be to identify and validate peptide profiles in the matched pre-op, post-op, 3-year follow-up and recurrent serum specimens that segregate cases by tumor burden, metastatic spread, and recurrence.

6. Mass Determination

Accurate mass determination of protein specimens requires a few basic steps including sample crystallization, sample ionization, flight through a vacuum tube, and detection of ionized proteins. After washing off non-specifically bound proteins and other contaminants from the chip array, a simple energy absorbing molecule solution will be applied and allowed to dry during which time minute crystals form on the chip. These crystals contain the energy absorbing molecule and the retained proteins. After inserting the side-by-side chip arrays into the chip reader, a laser beam will be focused on each spot causing the proteins embedded in the energy absorbing molecule crystals to desorb and ionize. Released ions will then experience an accelerating electrical field that causes them to move through the vacuum tube towards the ion detector. Finally, the ionized proteins will be detected and an accurate mass will be determined based on the time of flight.

The Ciphergen ProteinChip® Reader is a laser desorption/ionization time-of-flight mass spectrometer with state of the art ion optic and laser optic technology. The ion optic technology is derived from a four-stage, time-lag-focusing ion lens assembly that provides precise, accurate molecular weight determination with excellent mass resolving power. The laser optic technology maximizes ion extraction efficiency over the greatest possible sample area, which increases analytical sensitivity and reproducibility. The reader has the capability to switch from positive ion detection to negative ion detection, which broadens the spectrum of proteins amenable to analysis.

Mass accuracy will be assessed daily using Ciphergen's all-in-one molecular weight standard to ensure accurate peptide mass assignments. The peptides in this molecular weight standard include vasopressin (1.08 kDa), somatostatin (1.64 kDa), bovine B-chain (3.50 kDa), human insulin (5.81 kDa) and hirudin (7.03 kDa). Additional molecular standards will be utilized as need.

7. Data Analysis

Ciphergen has developed a specialized software package to control the chip reader and facilitate data collection and analysis. The ProteinChip® Software includes numerous features such as automatic peak detection; multiple spectrum comparison; several alternative data view formats; automated chip-reading protocols; and more. Artificial intelligence bioinformatics will then be used for the various cluster analyses. The most appropriate version of the proteomic cluster algorithm and the bioinformatics data mining tools and software will be employed for the proteomics data analyses.
Data acquisition from a protein chip array experiment will be performed in a standardized fashion and a predetermined set of parameters will be captured for each of the 96 spots (eight spots per array and twelve arrays aligned side-by-side). Mass data will then be presented in a retentate map representing all detected serum proteins. To enhance the appearance and facilitate the interpretation of the protein mass data collected, various presentation formats (data views) will be utilized. A copy of the original data for each experiment will be transferred to the GOG Statistical and Data Center. See below for a brief description of the types of data views commonly employed for SELDI-TOF data including the spectrum view or retentate map, the peak map, difference map view, gel view and 3-D overlay.

### Retentate Map
The retentate map will provide a standard spectral view for mass spectrometry or chromatography by presenting the quantity of protein reaching the ion detector at each particular molecular weight.

### Peak Map
The peak map will provide a limited view of protein data seen in the retentate map. Only the peak height and mass information are displayed in this data view.

### Difference Map View
The difference map view will compare two or more spectra and conveniently highlight unique proteins as well as proteins that are up- or down-regulated between different serum specimens. It will be possible to compare the protein profiles (spectra) from the pre-op serum specimen from different GOG-0210 patients that differ in outcome, clinical characteristics or epidemiologic factors. It will also be possible to compare the pre-op, post-op, 3-year follow-up and recurrent serum specimens within individual GOG-0210 patients. Alternatively, aliquots of the same serum specimen run on two chemically or biologically distinct ProteinChip® Array surfaces will be compared to rapidly determine the surface binding properties of specific proteins of interest.

### Gel View
The gel view will provide an image that resembles the appearance of electrophoretic gels by displaying distinct bands that correspond to proteins of specific mass in terms of a gray scale conversion of the height of each protein peak.

### 3-D Overlay
3-D overlays will be used for comparison of several spectra. The 3-D data view will be particularly useful for studying changes in relative peak heights during endometrial cancer progression in matched pre-op compared with recurrent serum specimens for example.

Advances in artificial intelligence have yielded bioinformatics programs that can apply pattern recognition systems with iterative clustering and survival of the fittest analysis to yield highly discriminative clustering algorithms. Various data mining tools and software have also been developed to facilitate the analysis of proteomics data. The Ciphergen Biomarker Patterns software (Ciphergen Biosystems) will be utilized for these experiments. This software finds hidden correlations to sample phenotypes identified by SELDI protein profiles. The software discovers patterns and presents the results in an easy-to-interpret tree model. Starting with SELDI peak intensity values from a "training set" of serum specimens, Biomarker Patterns Software defines a single splitting rule that best segregates the training set by phenotype. The software repeats the process on each resulting sub-classification of the data to produce a decision tree describing the best set of rules for organizing the samples according to phenotype. The results also include assignment scores of clinical sensitivity and specificity. Once generated, the model can be utilized to classify ‘unknowns’. Patterns consisting of multiple biomarkers may have prognostic and therapeutic importance.

The artificial intelligence bioinformatics higher order cluster algorithm developed by NCI/Correlogics, Inc. and/or that developed by other experienced entities will be integrated into the evaluation and interpretation of the proteomic data from this protocol.

### 8. Data Transfer
All data files regarding the different serum proteomic profiling projects for GOG-0210 will be transferred to the GOG Statistical and Data Center and stored in an Ingres II relational database.
database. Appropriate data will then be shared with the project leaders, scientific collaborators and bioinformatics specialists as needed. All data transfers will utilize a secure web-based data transfer application to be developed by the Information Technology Division of the GOG Statistical and Data Center and made available on the GOG web-site.

8. References


Appendix XI - Immunohistochemistry Assays For Steroid And Growth Factor Receptors

Standardized immunohistochemistry procedures will be utilized to minimize sources of variability and to maximize consistency, reliability and reproducibility in the expression of steroid hormone receptor isoforms and the members of the epidermal growth factor family in GOG-0210 tissue sections. The GOG Receptor Core Laboratory which has documented experience in performing each of these assays will be utilized on a fee-for-service basis.

Although the initial immunohistochemistry projects will utilize traditional unstained serial sections from GOG-0210 formalin-fixed and paraffin-embedded tissue specimens, an ultimate goal for this protocol is to have the GOG Tissue Bank create a series of tissue microarrays using core biopsies from appropriate GOG-0210 tissue specimens from patients that vary in clinical outcome, surgical/pathologic characteristics, epidemiologic factors, or expression of steroid or growth factor receptors.

Immunohistochemical expression will be examined in tumor cells, stroma surrounding tumor cells and normal tissue from the formalin-fixed and paraffin-embedded primary tumor specimens and normal tissue as well as in matched metastatic and/or recurrent tumor specimens when available.

The essential features of the immunohistochemistry projects for steroid and growth factor receptors and the notion of incorporating additional immunohistochemistry projects for other biomarkers will be re-evaluated over time so that new advances in research and technology can be appropriately incorporated into this protocol.

As of March 2003

1. Project Leaders For The Immunohistochemistry Projects

   Kimberly Leslie, University of New Mexico; Meenakshi Singh, University of Colorado; Richard Zaino, Hershey Medical Center; Kathleen Darcy, GOG Statistical and Data Center, Roswell Park Cancer Institute. Drs. Leslie, Singh, Zaino and Darcy will oversee all components of the immunohistochemistry projects, be directly involved in all analyses and provide input for "biological rationale" in interpreting the data. Dr. Richard Lieberman will asked to provide his expertise in digital imaging for these projects as needed.

2. Core Facility For The Immunohistochemistry Projects

   GOG Receptor Core Laboratory, University of Colorado (Meenakshi Singh, site contact).

3. Specific Aims and Hypothesis

   The first aim will be to utilize immunohistochemistry assays for steroid and growth factor receptors in GOG-0210 tumor specimens for the purpose of class prediction and class discovery in endometrial carcinoma to identify and validate tumor and/or stromal expression patterns for one or more of these receptors associated with risk of endometrial cancer recurrence, surgical/pathologic characteristics, and epidemiologic factors. The second aim will be to utilize the immunohistochemistry data collected as part of the first aim to identify candidate characteristics to target or exploit that would prevent and/or treat endometrial
carcinoma, and to expand the current understanding of the biology, progression, metastasis and responsiveness of endometrial carcinoma.

For these studies, tumor tissue from GOG-0210 patients with specific clinical characteristics and outcome will be subjected to immunohistochemical analysis to determine whether distinct expression patterns for steroid and/or growth factor receptors may be discerned. These profiles will serve as the basis for a new molecular classification of endometrial cancers that may have prognostic and therapeutic importance.

The main hypothesis to be tested is that the expression of steroid and growth factor receptors will improve our ability to predict clinical outcome, specifically with regard to recurrence-free interval following surgical staging. We hypothesize that low expression of ERα coupled with high expression and signaling through epidermal growth factor family members (EGFR, Her-2 and ErbB4, but not ErbB3) will be associated with an elevated risk of recurrence. We also hypothesize that distinct patterns of steroid and growth factor receptors will segregate with surgical/pathological characteristics (stage, grade, cell type, extra-uterine disease spread, and type I verses type II disease), epidemiologic factors, and the level of steroid hormones and metabolites. Such studies are designed to (1) identify potential biomarkers that segregate with clinical outcome, and (2) expand the current understanding of the biology, progression, metastasis and responsiveness of endometrial carcinoma.

4. Tissue Sections

The GOG Tissue Bank will initially distribute 20 unstained, 5 µm thick serial sections of appropriate GOG-0210 formalin fixed, paraffin embedded tissue specimens on uncharged glass slides suitable for standard immunohistochemical staining. The individual slides will be labeled with pencil with the GOG Bank ID and the appropriate specimen code (PT01 for primary tumor, PT02 for normal tissue, PT03 for metastatic tumor or PT04 for recurrence tumor). Ultimately the GOG Tissue Bank will generate a series of GOG-0210 tissue microarray and then will distribute an appropriate number of unstained sections from the tissue microarrays for approved immunohistochemistry projects.

5. Primary Antibodies

The primary antibodies that will be employed for these assays are indicated in the table below. The Ventana antibodies will be used to visualize ER-α, PR, and HER-2/neu. The Lab Vision PRB-specific antibody AB6 will be employed to visualize PRB. EGFR will be detected using a Zymed primary antibody and two different primary antibodies will be utilized to visualize phosphorylated EGFR, one from ICN and the other from Santa Cruz Biotechnologies. HER-3 and HER-4 antibodies will also be obtained from Santa Cruz Biotechnologies.

<table>
<thead>
<tr>
<th>Primary Antibody</th>
<th>Manufacturer</th>
<th>Target</th>
<th>Antibody Recognition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-α (6F11)</td>
<td>Ventana</td>
<td>ER alpha isoform</td>
<td>ER-alpha only</td>
</tr>
<tr>
<td>PR (1A6)</td>
<td>Ventana</td>
<td>PR common</td>
<td>Principally PRA</td>
</tr>
<tr>
<td>AB6 (HPRa6)</td>
<td>Lab Vision</td>
<td>PRB</td>
<td>Only PRB</td>
</tr>
<tr>
<td>HER-2 (ErbB-2)</td>
<td>Ventana</td>
<td>c-ErbB-2</td>
<td>HER-2/neu with good specificity</td>
</tr>
<tr>
<td>EGFR</td>
<td>Zymed</td>
<td>c-ErbB-1</td>
<td>Total EGFR, cytoplasm and membrane</td>
</tr>
</tbody>
</table>
6. Immunohistochemistry Procedures

The Ventana NexES automated staining system will be used for all of the immunohistochemistry assays performed. Tissue slides will be deparaffinized through 3 changes of xylene to alcohol to water. Antigen retrieval will be performed by microwaving slides in 1mM EDTA in a pressure cooker (Nordicware, MN) for 30 min. Following a cooling period at room temperature for 20 min, tissue will be incubated with 3% hydrogen peroxide in methanol for 30 min to quench endogenous peroxidases. Slides will be rinsed in Tris buffer pH 7.6 for 10 min. The machine will be programmed to incubate the tissue slides with primary monoclonal antibodies against ERα, PRA+B (to common regions of PRA and PRB), and HER-2/neu manufactured by Ventana. It should be noted that the reliability of the PRB-specific antibody AB6 (Lab Vision), the ICN and Santa Cruz P-EGFR antibodies, the Santa Cruz HER-3 and HER-4 antibodies, and the Zymed EGFR antibody were each confirmed by the GOG Receptor Core Laboratory using the Ventana NexES automated immunohistochemical staining system. The incubation periods will be for 32 minutes at room temperature. Washes, secondary, streptavidin, chromogen incubation will be programmed into the machine. Slides will be rinsed and counterstained with hematoxylin.

7. Antibody And Tissue Controls

The specificity of the primary antibodies will be verified using specific cell lines plated on chamber slides. The well differentiated Ishikawa endometrial cancer cells (that express endogenous ERα, PRA and PRB) and poorly differentiated Hec50co cells (that do not express endogenous ER or PR, but do express HER-2/neu, HER-3, HER-4, and EGFR) will be used for this purpose. These quality assurance slides will be washed once with 1X PBS, air-dried at room temperature for 30 minutes, and then stored at -80°C until needed for staining. Prior to starting the immunohistochemistry procedure, the slides will be thawed at 4°C and fixed in 4% paraformaldehyde at 4°C for 5 minutes. After rinsing slides in 1X PBS, the immunohistochemistry assay will be performed as described above. Positive control tissues (normal and malignant endometrium and breast) will also be available for all receptors.

8. Evaluation Criteria For The Immunohistochemistry Results

Immunohistochemical staining for ERα and PR will be evaluated in a semi-quantitative manner following the initial work of Carcangiu and Chambers (1990) demonstrating excellent correlation in the assessment of ERα and PR level using immunohistochemistry compared with biochemical methods such as ligand binding. Dr. Singh will review each slide in a blinded fashion and indicate the percent of immunopositive tumor cells. The intensity of staining (1+ to 4+) will also be scored, using the positive control tissue sample as 4+. The slides will also be reviewed and scored in a blinded manner by Dr. Zaino and other GOG pathologists as needed, and inter and intra-examiner variability will be recorded. In general, the technique provides reliable semi-quantitation of receptor expression using a review and scoring method now employed by most academic health centers on clinical reports.
Immunohistochemical staining for EGFR, phosphorylated EGFR, HER-2/neu, HER-3, HER-4, ERβ, and PRB will be evaluated in a similar semi-quantitative manner as described above. Dr. Singh will review each slide in a blinded fashion and indicate the percent of immunopositive tumor cells. The intensity of staining (1+ to 4+) will also be scored, using the positive control tissue sample as 4+. These slides will then be reviewed and scored in a blinded manner by Dr. Zaino and other GOG pathologists as needed, and inter and intra-examiner variability will be recorded.

The immunohistochemical results for each receptor will also be categorized into one of three levels based on the percentage of tumor cells that exhibit receptor staining: negative when less than 10% of the tumor cells exhibit receptor staining; positive when 10-50% of the tumor cells exhibit receptor staining; strongly positive when greater than 50% of the tumor cells exhibit receptor staining.

The epithelial as well as the stromal component of each tumor will be evaluated for expression of all receptors. In addition, on slides where non-malignant surrounding endometrium is present, receptor levels will be similarly scored.

The utilization of imaging analysis equipment and software will be evaluated over time to determine when this technology may be suitably applied to the quantification of steroid and growth factor expression patterns in GOG-0210 tissue specimens.

9. Data Transfer

All data files regarding the different immunohistochemistry projects for GOG-0210 will be transferred to the GOG Statistical and Data Center and stored in an Ingres II relational database. Appropriate data will then be shared with the project leaders, scientific collaborators and bioinformatics specialists as needed. All data transfers will utilize a secure web-based data transfer application to be developed by the Information Technology Division of the GOG Statistical and Data Center and made available on the GOG web-site.

10. References


Appendix XII - Immunoassays For Steroid Hormones And Estrogen Metabolites

Standardized quantitative immunoassays will be utilized to minimize sources of variability and to maximize consistency, reliability and reproducibility in the detection of steroid hormones and metabolites in GOG 210 serum and urine specimens. Laboratory testing facilities with documented experience performing each of these assays will be utilized on a fee-for-service basis. The essential features of the steroid hormone and estrogen metabolite projects will be re-evaluated over time so that new advances in research and technology can be appropriately incorporated into this protocol.

As of March 2003

1. Project Leaders For The Steroid Hormone And Estrogen Metabolite Projects

   Kimberly Leslie, University of New Mexico; Andrew Menzin, North Shore University Hospital; Karen Auborn, North Shore-Long Island Jewish Research Institute; Kathleen Darcy, GOG Statistical and Data Center, Roswell Park Cancer Institute. Drs. Leslie, Menzin, Auborn and Darcy will oversee all components of the steroid hormones and metabolites projects, be directly involved in all analyses and provide input for "biological rationale" in interpreting the data.

2. Facility For Quantifying The Levels Of Steroid Hormones and Estrogen Metabolites

   Testing Laboratory at The University Of New Mexico (On-Site Contact To Be Named); Testing Laboratory at North Shore-Long Island Jewish Research Institute (Karen Auborn, site contact).

3. Specific Aims and Hypothesis

   The first aim will be to utilize quantitative immunoassays for steroid hormones and estrogen metabolites in GOG 210 serum and urine specimens for the purpose of class prediction and class discovery in endometrial carcinoma to identify and validate ratios of steroid hormones and estrogen metabolites that are associated with risk of endometrial cancer recurrence, surgical/pathologic characteristics, and epidemiologic factors. The second aim will be to utilize the immunoassay data collected as part of the first aim to identify candidate targets to exploit to prevent and/or treat endometrial carcinoma, and to expand the current understanding of the biology, progression, metastasis and responsiveness of endometrial carcinoma.

   For these studies, serum and urine specimens from GOG 210 patients with specific clinical characteristics and outcome will be tested to determine whether the ratio of steroid hormones and the estrogen metabolite ratio in serum and/or urine have prognostic and therapeutic importance. The main hypothesis to be tested is that the ratio of steroid hormones and the estrogen metabolite ratio will improve our ability to predict clinical outcome, specifically with regard to recurrence-free interval following surgical staging. We hypothesize that a higher ratio of estradiol to progesterone and a lower estrogen metabolite ratio (the level of 2-hydroxyestrogens to the level of 16α-hydroxyestrogens) will be associated with an elevated risk of recurrence. We also hypothesize that the ratio of steroid hormones and the estrogen metabolite ratio will segregate with surgical/pathological characteristics (stage, grade, cell type, extra-uterine disease spread, and type-I versus type-II disease), epidemiologic factors, tumor expression of steroid hormone receptor isoforms, and gene expression of the estrogen metabolizing enzymes as well as estrogen-responsive genes. Such studies are designed to (1) identify potential biomarkers that segregate with clinical outcome, and (2) expand the current...
understanding of the biology, progression, metastasis and responsiveness of endometrial carcinoma.

4. Distribution Of Serum And Urine Specimens

The GOG Tissue Bank will distribute an aliquot of the pre-op, post-op, 3-year follow-up and recurrent (when available) serum specimens from GOG 210 patients with specific clinical characteristics and outcome to the University of New Mexico to quantify the levels of 17β-estradiol and progesterone. The GOG Tissue Bank will distribute an aliquot of the intra-op urine specimen from GOG 210 patients with specific clinical characteristics and outcome to Attn: Dr. Karen Auborn, GOG Protocol 210, HPV Laboratory, North Shore-Long Island Jewish Research Institute, 300 Community Drive, Manhasset, NY 11030 (Phone: 516-562-1184) to quantify the levels of 2-hydroxyestrogens and 16α-hydroxyestrogens.

5. Quantitative Immunoassays For Estradiol and Progesterone

Estradiol and progesterone serum levels will be assayed by enzyme-linked competitive immunoassays and the ratio of estradiol to progesterone will be calculated. An FDA-approved DPC Immunlite automated analyzer platform with reagent cartridges available for estradiol and progesterone will be used to evaluate appropriate GOG 210 serum specimens. These assays are competitive enzyme immunoassays based on enzyme labeled analytes and the use of highly specific antibodies for estradiol and progesterone. The assay calibration range for estradiol is 20-2000 pg/ml and for progesterone it is 0.2-40 ng/ml. On the day the serum is to be tested, the appropriate serum specimens will be kept ice-cold upon rapid thawing and separated into aliquots for immediate testing. Additional 100 µl aliquots of individual serum specimens may be refrozen for replicate testing. Serum that has undergone more than one freeze/thaw cycle will not be utilized for these immunoassays due to the inconsistent impact of the freeze/thaw on the specimen. The assays will be run in triplicate in the presence of positive (serum containing known quantities of estradiol and progesterone) and negative (serum without detectable estradiol or progesterone) controls. In addition, serum from pre-menopausal and post-menopausal women without endometrial cancer will be evaluated for comparison.

5. Quantitative Immunoassays For 2-Hydroxyestrogens And 16α-Hydroxyestrogens

The urine levels of 2-hydroxyestrogens and 16α-hydroxyestrogens will be assayed (Auborn et al., 1998; Klug et al., 1994) using the estramet competitive enzyme-linked immunoassay kit from Immuna Care Corporation (Bethlehem, PA) and the estrogen metabolite ratio (the level of 2-hydroxyestrogens to the level of 16α-hydroxyestrogens) will be calculated. Briefly, 2-hydroxyestrogens and 16α-hydroxyestrogens will be measured in triplicate in intra-op urine specimens preserved with ascorbic acid (1 mg/ml). The estrogen metabolites in the urine specimens, which are excreted as conjugates, will be treated with β-glucuronidase and arylsulphatase (deconjugating enzymes) and neutralized. The estrogen metabolites (antigens) in the urine will be incubated with an antigen-alkaline phosphatase conjugate in appropriate wells of a microtiter plate with monoclonal antibodies captured on the wells of microtiter plates. Enzyme substrate for alkaline phosphatase will then be added to the washed solid phase, and the amount of enzyme product (colored) will be inversely proportional to the concentration of free antigen (estrogen metabolite) – a competitive solid phase enzyme immunoassay. Standard range will be from 0.625 to 10 ng/ml. The assays will be run in triplicate to document intra-assay
variability in the presence of positive (urine containing known quantities of 2-hydroxyestrogens and 16α-hydroxyestrogens) and negative (urine without detectable 2-hydroxyestrogens or 16α-hydroxyestrogens) controls. Replicate testing will be performed in a subset of specimens to monitor the consistency between assay runs. In addition, urine from pre-menopausal and post-menopausal women without endometrial cancer will be evaluated for comparison. Testing will also be performed in aliquots of pre-op serum specimens from a subset of patients to document the level of these estrogen metabolites in serum compared with urine specimens.

8. Evaluation Criteria For The Immunoassay Results

Absorbance levels for the individual specimens will need to be in the linear range in order to be evaluable. The levels of estradiol, progesterone, 2-hydroxyestrogens or 16α-hydroxyestrogens will be interpolated from the estradiol, progesterone, 2-hydroxyestrogens or 16α-hydroxyestrogens standard curve, respectively. The ratio of estradiol to progesterone and the ratio of 2-hydroxyestrogens and 16α-hydroxyestrogens will then be calculated.

9. Data Transfer

All data files regarding the different steroid hormone and estrogen metabolite projects for GOG 210 will be transferred to the GOG Statistical and Data Center and stored in an Ingres II relational database. Appropriate data will then be shared with the project leaders, scientific collaborators and bioinformatics specialists as needed. All data transfers will utilize a secure web-based data transfer application to be developed by the Information Technology Division of the GOG Statistical and Data Center and made available on the GOG web-site.

10. References


Appendix XIII - FACILITIES AND TESTING LABORATORIES

STATISTICS AND DATA MANAGEMENT
- GOG Statistical and Data Center at Roswell Park Cancer Institute in Buffalo, New York (John Blessing, Director; On-Site Data Manager Contact To Be Named)

CLINICAL SPECIMEN BANKING
- GOG Tissue Bank at The Biopathology Center in Children’s Research Institute in Children’s Hospital in Columbus, Ohio (Stephen Qualman, Director; Yvonne Moyer, Project Manager; Lisa Beaverson, Research Assistant)

GENE EXPRESSION PROFILING
- Genomics Core Laboratory at Rockefeller Research Laboratory/Memorial Sloan Kettering Cancer Center (Jeff Boyd and Agnes Viale, Manager of the Genomics Core Laboratory)
- Multiplexed Gene Analysis Core at the Siteman Cancer Center at Washington University School of Medicine/Barnes-Jewish Hospital (Paul Goodfellow and Kate Hamilton at 314.454.8520 or e-mail genechip@pathbox.wustl.edu)

PROTEOMIC PROFILING
- Proteomics Core Facility at The University of New Mexico (Kimberly Leslie and On-Site Contact To Be Named)

IMMUNOASSAYS
- GOG Receptor Core Laboratory at The University of Colorado (Kimberly Leslie and Meenakshi Singh)
- Testing Laboratory at The University Of New Mexico (Kimberly Leslie and On-Site Contact To Be Named)
- Testing Laboratory at North Shore-Long Island Jewish Research Institute (Andrew Menzin and Karen Auborn)

BIOINFORMATICS
- Bioinformatic Core at Rockefeller Research Laboratory/Memorial Sloan Kettering Cancer Center (Adam Olshen, Department of Epidemiology and Biostatistics)
- Bioinformatic Core at the Siteman Cancer Center at Washington University School of Medicine/Barnes-Jewish Hospital (Sunita Koul at 314.362.4662 or e-mail sunita@pathbox.wustl.edu)
- Bioinformatic Core at The University Of New Mexico (Contacts To Be Named)

EPIDEMIOLOGY
Environmental Epidemiology Branch in the Division of Cancer Epidemiology and Genetics at the National Cancer Institute (Louise Brinton and On-Site Contact To Be Named)