Identifying biologic/molecular profiles
Investigators will not be restricted to utilizing a particular technique for building the classifier. In fact, several classifiers may be identified. However, prior to the validation phase a single classifier corresponding to the primary study objective will be selected and deemed the 'final' classifier. Data from the validation dataset will not be used to select the 'final' classifier.

Reliability
The classifier should provide similar results for the same experimental unit. That is, a biologic specimen with a high prognostic index score should exhibit a high prognostic index score when it is re-evaluated. An index score which cannot be replicated lacks test-retest reliability. This occurs when there are other sources of between-specimen variation that are uncontrolled in the experiment.

In order to assess reliability some specimens will be selected from the training set for repeat assessment. While randomly selecting specimens from the training set for replication is preferable, it may be necessary to randomly select from a subset of the training set due to the availability of adequate biologic material. When possible, the samples will be identified in such a way that the laboratory investigator will be unable to identify which specimens are replicates. These samples will have their biomarkers (i.e. gene expression, serum marker) assessed twice. Since replication can be expensive, depending of the laboratory procedure, the number of samples selected for replication will vary from a dozen to a few dozen, depending on practical considerations like cost and feasibility. The data from the replicated samples will be used to assess reliability of the putative index before proceeding to the validation phase. Reproducibility is a prerequisite for a clinically useful classifier.

Validation
Prior to initiating the validation phase, the 'final' classifier will be completely documented (i.e. computer program or pseudo-code). This documentation will be reviewed by individuals in the GOG Statistical and Data Center (SDC) who are not participating in the analyses. The purpose of this review will be to determine whether the final classifier has been unambiguously defined.

The c-index will be used to measure the classifier's predictive ability. This index assesses the strength of the rank correlation between the predicted outcome and the actual outcome. If the classifier produces a continuous prognostic score and response is dichotomous, then the c-index is comparable to the Wilcoxon two-sample rank score. It can be calculated by taking all possible pairs of individuals in which one individual responded and the other did not respond. In this case, the c-index is the proportion of these pairs in which the responder has a higher predicted probability of responding. A c-index value of 0.5 indicates a useless classifier, and a value of 1.0 indicates perfect prediction. The c-index is Somer's rank correlation index when it is rescaled to vary linearly from 0 to 1. The c-

index can be used when the outcome is partially censored survival time. In this case it measures the proportion of all pairs of individuals in the data set in which the individual with the expected lower risk of failure is known to survive longer.

Other descriptive summaries of predictive ability will also be considered including: Kaplan-Meier curves when the outcome is a time-to-failure or a ROC curve when the outcome is dichotomous.

The publication which describes the results for the primary objective of this study will include a description of the accuracy of the final classifier. While other classifiers may also be described, the final classifier will be clearly distinguished from the other classifiers. The documentation describing the final classifier will be available to other investigators from the SDC upon request.

After the study objectives have been completed, the GOG may elect to make some or all of the validation data set available to other investigators, since the specimens in the training set may become exhausted. Any classifiers developed subsequently will not be permitted to claim that they were independently validated without additional supporting external evidence.

11.6 The anticipated distribution of patients' race and ethnicity for the systemic therapy
portion of this trial is (all are female):

White (not Hispanic)	584
Black (not Hispanic)	39
Hispanic	14
Asian	17
American Indian or Alaskan Native	3
Native Hawaiian or other Pacific Islander	3

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APPENDIX I

FIGO STAGE GROUPING FOR PRIMARY CARCINOMA OF THE OVARY

(1985)

These categories are based on findings at clinical examination and/or surgical exploration. The histologic characteristics are to be considered in the staging, as are results of cytologic testing as far as effusions are concerned. It is desirable that a biopsy be performed on suspicious areas outside the pelvis.

Stage I	Growth limited to the ovaries.
Stage IA	Growth limited to one ovary; no ascites. No tumor on the external surface; capsule intact.
Stage IB	Growth limited to both ovaries; no ascites. No tumor on the external surfaces; capsules intact.
Stage IC*	Tumor either Stage IA or IB but with tumor on the surface of one or both ovaries; or with capsule ruptured; or with ascites present containing malignant cells or with positive peritoneal washings.
Stage II	Growth involving one or both ovaries with pelvic extension.
Stage IIA	Extension and/or metastases to the uterus and/or tubes.
Stage IIB	Extension to other pelvic tissues.
Stage IIC*	Tumor either Stage IIA or IIB but with tumor on the surface of one or both ovaries; or with capsule(s) ruptured; or with ascites present containing malignant cells or with positive peritoneal washings.
Stage III	Tumor involving one or both ovaries with peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal nodes. Superficial liver metastasis equals Stage III. Tumor is limited to the true pelvis but with histologically verified malignant extensions to small bowel or omentum.
Stage IIIA	Tumor grossly limited to the true pelvis with negative nodes but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces.
Stage IIIB	Tumor of one or both ovaries with histologically confirmed implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter. Nodes negative.
Stage IIIC	Abdominal implants >2 cm in diameter and/or positive retroperitoneal or inguinal nodes.
Stage IV	Growth involving one or both ovaries with distant metastasis. If pleural effusion is present there must be positive cytologic test results to allot a case to Stage IV. Parenchymal liver metastasis equals Stage IV.

* In order to evaluate the impact on prognosis of the different criteria for allotting cases to Stage IC or IIC, it would be of value to know if rupture of the capsule was (1) spontaneous or (2) caused by the surgeon and if the source of malignant cells detected was (1) peritoneal washings or (2) ascites.

APPENDIX II

SECONDARY CYTOREDUCTIVE SURGICAL PROCEDURE

Purpose : Maximum resection of recurrent ovarian cancer.

Timing: Surgical exploration should be undertaken within 4 weeks of study entry.

Content of Procedure:

1.0 The abdominal incision must be adequate to explore the entire abdominal cavity and allow safe cytoreductive surgery. A vertical incision is recommended but not required.
2.0 All peritoneal surfaces including the undersurface of both diaphragms and the serosa and mesentery of the entire gastrointestinal tract will be visualized and palpated for evidence of metastatic disease.
3.0 Visible metastatic abdominal and pelvic disease should be resected or ablated completely, if possible.
4.0 Diaphragmatic recurrent disease should be resected. Ablation of disease with electrocautery (e.g. Argon Beam Coagulator) is acceptable.
5.0 Surgical evaluation of the pelvic and paraortic node bearing areas requires resection if not performed on initial staging/debulking procedure. If incomplete nodal resection was previously documented, unresected areas should be excised.
6.0 Solid organ metastases (spleen and liver) should be considered for resection. Treatment by Radio Frequency Ablation (RFA) is acceptable.

Goal: Surgical goal of cytoreduction is to reduce volume of residual disease to smallest quantity possible (no visible residual).

Reporting: The size (two dimensions) and location of residual disease will be recorded.

APPENDIX III

I. Quick Scan Summary of the Specimen Requirements for GOG-0213.

Refer to Section 7.31 of the Protocol for a copy of the Quick Scan Summary Table.

II. Obtaining a GOG Bank ID for Any GOG Protocol (1/3/11)

Only one GOG Bank ID (# # # # - # # - G # # #) is assigned per patient, and all specimens and accompanying paperwork for each patient 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 any GOG protocol with specimen requirements other than GOG-0136 (specimen banking protocol) 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-0213

A. Ordering Specimen Kits for GOG-0213

1. A Dual-Chamber Specimen Kit can be ordered for each GOG-0213 patients who are randomized to the surgery arm of this study from the GOG Tissue Bank using the GOG Tissue Bank's Kit Management application. This application can be accessed via the GOG Web Menu. Plan ahead so that the kits can be shipped by ground transportation whenever possible. **This kit must only be used for the submission of the GOG-0213 pre-op serum and pre-op plasma specimens and the recurrent tumor and normal tissue collected during secondary cytoreductive surgery.** Please submit the archival formalin-fixed and paraffin-embedded primary or metastatic tumor specimen (block or 16 unstained sections) in your own container. For shipping information, please see Section IX.
2. Replacement kits can be ordered as needed based on the number of patients enrolled by your institutions on this protocol and randomized to have secondary cytoreductive surgery. Always try to have replacements available.

B. Materials Provided in the Specimen Kit for GOG-0213

Each Specimen Kit for GOG-0213 will consist of a dual-chamber shipping container for shipping the frozen pre-op serum (SB01), frozen pre-op plasma (PB01), frozen recurrent tumor (RR01) and frozen normal tissue (RN01) on one side and the formalin-fixed recurrent tumor (FR01) and formalin-fixed normal tissue (FN01) on the other side. The following supplies will also be provided within each GOG-0213 kit: foil to wrap the two frozen tissue specimens if snap frozen, two truncated OCT embedding molds if the two types of tissue are OCT-embedded and frozen, two 15-ml screw-cap polypropylene conical tube, two plastic disposable transfer pipette for mixing the serum and plasma specimens, two sets of five 1.8 ml screw-cap cryogenic vials (cryotubes) for the serum aliquots, two sets of five 1.8 ml screw-cap cryogenic vials (cryotubes) for the plasma aliquots, two 15 ml formalin jars for two types of fixed tissue, four plastic zip-lock bags for the frozen specimens, two secondary shipping envelopes with absorbent material, a dry ice label (UN1845), an Exempt Human Specimen Sticker and a pouch for the shipping label.

If there are supplies required to satisfy the specimen requirements for this protocol that are not in provided in the GOG-0213 Specimen Kit or are not available in your clinic, department or institution, please contact the staff at the GOG Tissue Bank by phoning 866-464-2262 (866-GOG-BANC) who will try to help you obtain these additional supplies when possible.

C. Unused Materials or Unused Specimen Kits for GOG-0213

Unused materials or unused Specimen Kits for GOG-0213 will need to be returned to the GOG Tissue Bank. Contact the GOG Tissue Bank if you have any question about the return of unused material.

IV. Submitting Archival Primary or Metastatic Tumor for GOG-0213

A. Requirement

Archival formalin-fixed and paraffin embedded (FFPE) primary or metastatic tumor tissue (FT01) will only be required from women on GOG-0213 who undergo secondary cytoreductive surgery and give permission for their tissue (tumor and/or normal tissue) to be submitted and used for this research study. Patients may participate in this treatment protocol even if they don't give permission for their tissue to be submitted and used for this research study. If tumor cannot be submitted for GOG-0213, please indicate the reason in item 5 on the SP Form such as patient refused, not enough tumor for research, or referring site won't release tumor.

B. Purpose

The GOG Tissue Bank will collaborate with the GOG Statistical and Data Center and the GOG Tissue Utilization Subcommittee to design and create a series of tissue microarrays (TMAs) for GOG-0213 to study markers of recurrence, survival and treatment response or resistance, and prepare sections from conventional blocks and TMAs as needed. Unstained sections from conventional blocks and TMAs will then be distributed to Dr. Michael Birrer at MGH Cancer Center and/or a CEM-approved investigator for biomarker, proteomic and genomic analyses. Laser-capture microdissection will be performed as need to examine cell type-specific expression profiles. The exact choice of the biomarkers and profiles to be evaluated and the assays to be performed in this specimen will be reevaluated based on evolving data in the field.

C. Time Point

The archival formalin-fixed and paraffin-embedded primary or metastatic tumor tissue must have been collected prior to initiating primary chemotherapy. There may be certain patients who receive neoadjuvant chemotherapy prior to surgery, and these details will need to be declared in item 15 of the SP Form for this specimen including agent names with treatment start and stop dates.

D. Format for Labeling the Specimen

Label the archival primary or metastatic tumor specimen (formalin-fixed and paraffin-embedded) with the GOG protocol number (GOG-0213), GOG Bank ID (####-##-G###), specimen code (FT01 for archival formalin-fixed tumor tissue), and collection date (mm/dd/yyyy). This specimen may also be labeled with the pathology accession number and block identifier, but must not be labeled with personal identifiers like patient name or initials.

E. Instructions for Submitting the Archival Primary or Metastatic Tumor Tissue

1. **Identify an Appropriate Tumor Specimen.** Every attempt should be made to provide a tumor block for this research study. Primary tumor is the first choice and metastatic tumor is the second choice. If both can be submitted the primary tumor should be labeled FT01 and the metastatic tumor should be labeled FT02. If it is not possible to provide a block on a permanent or temporary basis, the back-up option will be to provide sixteen unstained sections, 5 micrometer in thickness, on charge glass slides suitable for a standard immunohistochemistry assay. If your institution can not permanently provide a tumor block for this research study, please urge the Pathology Department to allow a tumor block to be submitted to the GOG Tissue Bank on a temporary basis. In this case, please state in field 15 on the SP

Form for FT01 that the tumor block must be returned after the unstained sections and cores for TMA creation are obtained.

2. **Label Tumor Specimen.** Label the primary or metastatic tumor specimen (block or unstained sections) with the GOG protocol number, the GOG Bank ID, the Specimen Code and the collection date.
   * ***Use FT01 for the formalin-fixed primary or metastatic tumor tissue. If both are submitted, use FT01 for the primary tumor and FT02 for the metastatic tumor. The SP Form for FT02 would be considered an optional form for this protocol. In this event, please contact the GOG Statistical and Data Center to have the additional SP Form for FT02 added to the patient form schedule.***
3. **Complete the Form SP**. Complete a GOG Specimen Form (Form SP) as specified in Section VIII. Submit a copy of Form SP with the specimen when it is shipped to the GOG Tissue Bank, submit a copy to the GOG Statistical and Data Center online or by fax, and retain a copy in your files.
   * ***The type of tumor tissue (primary or metastatic) and specimen (block or sections) will need to be specified on the specimen transmittal form (Form SP) submitted for FT01 for GOG-0213. If sections are submitted instead of a tumor block, the reason must be stated in field 15 on the SP Form for this specimen (i.e., the Pathology Department at your institution is prohibited by local or state law from releasing blocks on a permanent or temporary basis for any reason). There may be certain patients who receive neoadjuvant chemotherapy prior to surgery, and these details will need to be declared in item 15 of the SP Form for this specimen including agent names with treatment start and stop dates.***
4. **Ship the Tissue Specimen(s).** Ship the archival tumor specimen(s) (block or unstained sections) to the GOG Tissue Bank **in your own shipping container** as described in Section IX. The archival tumor may also be included in the dual chamber kit if available when the other specimens are ready to ship to the Bank.

V. Fixing and Freezing Recurrent Tumor and Normal Tissue for GOG-0213

A. Requirement

The recurrent tumor will be excised during secondary cytoreductive surgery and a portion will need to be FFPE or fixed in formalin whereas the remainder will need to be frozen (either snap-frozen or OCT-embedded and frozen). Normal tissue is an optional high priority specimen and if collected can either be FFPE or fixed in a jar with formalin whereas the remainder will need to be frozen (either snap-frozen or OCT-embedded and frozen).

Fixed recurrent tumor (FR01) will be required for all patients who give consent for some of their tumor tissue to be used for this research study and are randomized to have secondary cytoreductive surgery. A paraffin block of FFPE recurrent tumor (1st choice) or a piece of recurrent tumor in a jar with formalin (2nd choice) will need to be submitted to satisfy the FR01 requirement.

Frozen recurrent (RR01) will be required for all patients who give consent for some of their tumor tissue to be used for this research study and are randomized to have secondary cytoreductive surgery. A piece of recurrent tumor snap frozen and wrapped in foil or frozen in an OCT mold will need to be submitted to satisfy the RR01 requirement.

Fixed normal tissue (FN01) will be an **optional yet high priority requirement** for all patients who give consent for some of their normal tissue to be used for this research study and are randomized to have secondary cytoreductive surgery. A paraffin block of FFPE normal tissue (1st choice) or a piece of normal tissue in a jar with formalin (2nd choice) will need to be submitted to satisfy the FN01 requirement.

Frozen normal tissue (RN01) will be an **optional yet high priority requirement** for all patients who give consent for some of their normal tissue to be used for this research study and are randomized to have secondary cytoreductive surgery. A piece of normal tissue snap frozen and wrapped in foil or frozen in an OCT mold will need to be submitted to satisfy the RN01 requirement.

B. Purpose

The GOG Tissue Bank will create paraffin blocks from the formalin-fixed recurrent tumor and normal tissue, core appropriate paraffin blocks to create the GOG-0213 tissue microarrays (TMAs), and prepared sections from conventional blocks and TMAs as needed. Unstained sections from conventional blocks and TMAs will then be distributed to Dr. Michael Birrer at MGH Cancer Center and/or a CEM-approved investigator for biomarker, proteomic and genomic analyses. Laser-capture microdissection will be performed as need to examine cell type-specific expression profiles. The exact choice of the biomarkers and profiles to be evaluated and the assays to be performed in these specimens will be reevaluated based on evolving data in the field.

C. Time Point

The fixed and frozen recurrent tumor tissue and normal tissue will be collected during secondary cytoreductive surgery.

D. Format for Labeling the Specimen

Label the tissue specimens from the secondary cytoreductive surgery procedure with the GOG protocol number (GOG-0213), the GOG Bank ID (####-##-G###), the specimen code (see below) and the collection date (mm/dd/yyyy).

- FR01 for the fixed recurrent tumor tissue
- RR01 for the frozen recurrent tumor tissue
- FN01 for the fixed normal tissue
- RN01 for the frozen normal tissue

E. Recommendations for Preparing Fixed or Frozen Tissue Specimens

How quickly should tissue be fixed or frozen? The tissue should be fixed or frozen as quickly as possible. Ideally within 30-60 minutes but certainly within 4 hours of excision from the patient. The faster these specimens can be fixed or frozen, the more valuable the specimens are for research. It may be appropriate to hold occasional meetings of surgical, laboratory, and clinical personnel to emphasize the urgency of processing these specimens rapidly.

What type of freezing method should be used? There are two types of freezing methods provided for your consideration: snap-freezing or OCT-embedding and freezing. When preparing the tissue specimens from the secondary cytoreductive surgical procedure, the choice of freezing method is not mandated for GOG-0213.

How much frozen tissue should be submitted? **Please submit as much frozen tissue as possible for research. Gram quantities with individual pieces ranging from 1 to 5 cm^3 are ideal.** Larger amounts of tissue will allow for replicate laboratory testing and permit validation testing to be performed.

Any suggestions for how to coordinate these efforts? 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 component of GOG-0213 and a protocol like GOG-0136. 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 tissue requirements for this protocol. Sharing operating schedules and providing updates on how the surgery 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 this protocol.

F. Procedures For Excising Tissue For Research

1. Excising recurrent tumor tissue during secondary cytoreductive surgery.
   a. The surgeon should send the excised recurrent tumor tissue from each GOG-0213 patient randomized to undergo surgery to the surgical pathology suite and arrange for immediate tissue sampling within 30-60 minutes of excision when possible.
   b. **Submit as much tumor tissue for research as possible. Gram quantities with individual pieces ranging from 1 to 5 cm^3 are ideal. There is a minimum requirement of 500 mg or 0.5 cm^3 (slightly larger than a pencil eraser).**
   c. The 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 tumor sample, a piece of the recurrent tumor tissue must be formalin-fixed (FR01), and another piece must be snap-frozen or OCT-embedded and frozen (RR01) as described below.
2. Excising normal tissue during surgery.
   a. The surgeon should also try to excise a piece of normal tissue from each GOG-0213 patient randomized to undergo surgery and send it with the tumor tissue when it is sent to the surgical pathology suite so that tissue sampling can be performed within 30-60 minutes of excision when possible. **Normal tissue can be any normal epithelial tissue including non-involved ovary, fallopian tube, uterus, cervix, or skin. When normal epithelium is not available, please submit non-involved peritoneal surface, residual omentum, or retroperitoneal muscle.** Please try to submit normal epithelium whenever possible as this type of tissue will serve as the most appropriate control for the laboratory testing to be preformed for this protocol. **Note for the pathologist,** in the unlikely event that any tumor tissue is subsequently identified within the normal tissue submitted for research, the Pathology Department at the treating institution will be informed and the material will be immediately returned for diagnostic purposes.
   b. Please submit **gram quantities with individual pieces ranging from 1 to 5 cm^3 when possible and a minimum of 500 mg or 0.5 cm^3 of normal tissue (slightly larger than a pencil eraser).**
   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 snap-frozen or OCT-embedded and frozen (RN01) as described below.

G. Procedure For Formalin-Fixing A Tissue Specimen

1. **Label the Formalin-Jar(s).** 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 Bank ID Number, appropriate Specimen Code, and collection date.
   * ***Use FR01 for the formalin-fixed recurrent tumor tissue and FN01 for the formalin-fixed normal tissue.***
2. **Transfer the Tissue into the Formalin-Jar.** Promptly following resection of the tissue, use forceps to transfer the tissue sample to 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 the Tissue in the Fixative.** 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 3 days of the surgery, please have your Pathology Department paraffin-embed this research specimen to preserve the usefulness of this specimen for research purposes. Pathologist review of this embedded tissue is not required, as this material has been designated for research. Alternatively, the formalin-fixed tissue can undergo standard histologic processing and be embedded in a paraffin block.**
4. **Complete Form SP.** Complete a GOG Specimen Form (Form SP) as specified in Section VIII. Include a copy of Form SP with the specimen when it is shipped to the GOG Tissue Bank, and retain a copy in your files.

* ***Indicate if the tissue is recurrent tumor or normal tissue in field 22 on Form SP. If normal tissue, please specify the type of normal tissue that is being submitted in the comment field (item 15 on Form SP) such as normal ovary, Fallopian tube, uterus, cervix, skin, non-involved peritoneal surface, residual omentum, or retroperitoneal muscle.***

5. **Ship the Tissue Specimen(s).** Ship the fixed tissue specimen(s) either in a jar(s) of formalin or embedded in a paraffin block to the GOG Tissue Bank as described in Section IX.

H. Instructions for Preparing the Snap-Frozen Tissue

1. **Label Zip-Lock Bag.** Using a waterproof marker, label a zip-lock bag supplied in the Dual-Chamber Specimen Kit distributed by the GOG Tissue Bank with the GOG protocol number, GOG Bank ID Number, the Specimen Code, and the collection date.
2. **Snap-Freeze Tissue**. Using forceps place the appropriate tissue specimen on a piece of foil supplied in the Single-Chamber Specimen Kit distributed by the GOG Tissue Bank, wrap the foil so that the specimen is completely covered and then immerse the tissue wrapped in foil in liquid nitrogen or a suitable substitute until the tissue is frozen solid.
3. **Transfer Snap-Frozen Tissue to Zip-Lock Bag**. 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. **Immediately Store Snap-Frozen Tissue**. Store the snap-frozen tumor 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. A regular freezer (-20°C) is not adequate. A cryostat is also not appropriate.
5. **Complete the SP Form**. Complete a GOG Specimen Form (Form SP) as specified in Section VIII. Submit a copy of Form SP with the specimen when it is shipped to the GOG Tissue Bank, submit a copy to the GOG Statistical and Data Center online or by fax, and retain a copy in your files.
   * ***Indicate that the item being shipped is a piece of snap frozen tumor in field 9 on Form SP. Alternatively, if a snap-frozen piece and an OCT-mold are both being submitted, select "Other" and enter "OCT-mold and piece" in the specify field. Also indicate if the tissue is recurrent tumor or normal tissue in field 22 on Form SP, and then specify the type of normal tissue that is being submitted in the comment field (item 15 on Form SP) such as normal ovary, Fallopian tube, uterus, cervix, skin, non-involved peritoneal surface, residual omentum, or retroperitoneal muscle.***
6. **Ship the Tissue Specimen(s).** Ship the frozen tissue specimen(s) to the GOG Tissue Bank as described in Section IX.

I. Instructions for Preparing the OCT-Embedding and Freezing Tissue

1. **Label OCT-mold and Zip-Lock Bag.** Using a cryomarker, label a truncated OCT mold and Zip-Lock Bag supplied in the Single-Chamber Specimen Kit distributed by the GOG Tissue Bank with the GOG protocol number, GOG Bank ID Number, the Specimen Code, and the collection date. If more than 0.75 grams or 0.75 cm^3 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. **OCT-Embed and Freeze the Tissue**. 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. Using forceps place the appropriate tissue specimen into the thickened OCT pushing the specimen to the bottom of the mold. Add additional OCT to cover the tissue completely and to fill the mold approximately three-fourths full. 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.
3. **Transfer Frozen OCT-Embedded Tissue to a Zip-Lock Bag**. 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.
4. **Immediately Store Frozen OCT-Embedded Tissue**. Store the frozen OCT-embedded tumor 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. A regular freezer (-20°C) is not adequate. A cryostat is also not appropriate.

5. **Complete the SP Form**. Complete a GOG Specimen Form (Form SP) as specified in Section VIII. Submit a copy of Form SP with the specimen when it is shipped to the GOG Tissue Bank, submit a copy to the GOG Statistical and Data Center online or by fax, and retain a copy in your files.
   * ***Indicate that the item being shipped is an OCT-mold in field 9 on Form SP. Alternatively, if an OCT-mold and a snap-frozen piece are both being submitted, select "Other" and enter "OCT-mold and piece" in the specify field. Also indicate if the tissue is recurrent tumor or normal tissue in field 22 on Form SP, and then specify the type of normal tissue that is being submitted in the comment field (item 15 on Form SP) such as normal ovary, fallopian tube, uterus, cervix, skin, non-involved peritoneal surface, residual omentum, or retroperitoneal muscle.***
6. **Ship the Tissue Specimen(s).** Ship the frozen tissue specimen(s) to the GOG Tissue Bank as described in Section IX.

VI. Preparing Frozen Serum and Plasma for GOG-0213

A. Requirements and Purpose

A pre-op serum specimen and a pre-op plasma specimen will be an optional high-priority requirement for women who are randomized to undergo secondary cytoreductive surgery and consent to allow their serum and plasma to be prepared and used it for this research study.

- The pre-op serum specimen will need to be prepared after obtaining consent for this research study but prior to undergoing secondary cytoreductive surgery from 10 ml of blood drawn into a **plain red-top Vacutainer® tube** as described in Section VI-G, and shipped to the GOG Tissue Bank as described in Section IX.
- The pre-op plasma specimen will need to be prepared after obtaining consent for this research study but prior to undergoing secondary cytoreductive surgery from 10 ml of blood drawn into a **purple-top Vacutainer® tube** with the anti-coagulant EDTA as described in Section VI-H, and shipped to the GOG Tissue Bank as described in Section IX.

Patients may participate in this treatment protocol even if they don't give permission for some of their blood to be used for this research study. **If the serum or plasma specimens cannot be submitted for GOG-0213, please indicate the reason in item 5 on the SP Form, such as patient refused, tried but not able to draw blood, or Non-US site logistically infeasible.**

B. Purpose

Serum and plasma will first be shipped to the GOG Tissue Bank in Columbus Ohio and then aliquots of the pre-op serum specimen and the pre-op plasma specimen will be distributed in batches to Dr. Michael Birrer at MGH Cancer Center and/or a CEM-approved investigator for biomarker and proteomic analyses. The exact choice of the biomarkers and proteomic profiles to be evaluated and the assays to be performed in this specimen will be reevaluated based on evolving data in the field.

C. Time Point

To pre-op serum specimen and the pre-op plasma specimen must be prepared after obtaining consent for this research study but prior to undergoing secondary cytoreductive surgery.

D. Format for Labeling the Specimen

Label the serum specimens with the GOG protocol number (GOG-0213), the GOG Bank ID (####-##-G###), the specimen code (SB01 for the pre-op serum), and the collection date (mm/dd/yyyy).

Label the plasma specimens with the GOG protocol number (GOG-0213), the GOG Bank ID (####-##-G###), the specimen code (PB01 for the pre-op plasma), and the collection date (mm/dd/yyyy).

E. Equipment and Supplies Needed for Preparing Serum Specimens

In addition to the materials provided in each of the Specimen Kits for GOG-0213, you will need gloves, plain red-top Vacutainer® tube(s), tube rack, purple-top Vacutainer® tube with EDTA, a permanent marker, dry ice, a centrifuge, a refrigerator or a bucket with wet ice, and access to appropriate freezing/storage space to collect each serum specimen. ***If you do not have access to a plain red-top Vacutainer® tube and/or a purple-top Vacutainer® tube with EDTA, at your institution, please inform the staff at the GOG Tissue Bank who will try to provide you with these tubes when possible.***

F. Guidelines and Recommendations for Preparing Serum and Plasma Specimens

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

Ideally, the serum and plasma 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; however, the amount of time the serum and plasma is kept in this type of freezer should be kept to a minimum because 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. Serum and plasma kept in a non-cycling -20°C freezer should be surrounded with excess dry ice to allow the serum and plasma to achieve and then maintain a frozen solid state. Storage of serum and plasma in a frost-free -20°C freezer will repeatedly damage the specimen each time the freezer cycles (that is, as the freezer thaws and then refreezes). Serum and plasma frozen under ultra-cold conditions represents the highest quality specimen suitable for all types of laboratory testing. Serum and plasma frozen in a non-cycling -20°C provides a lower -quality specimens suitable for restricted types of laboratory testing. Serum and plasma frozen in a frost-free -20°C freezer provides the lowest-quality specimens which has limited usefulness for research purposes. Tracking the freezing conditions for each serum and plasma specimen is of critical importance to assess specimen quality and suitability for testing.

G. Instructions for Preparing Serum

1. **Label Cryotubes**. Label the screw-cap cryotubes for each time point with the GOG protocol number, the GOG Bank ID, the Specimen Code and the collection date.
   * ***For GOG-0213, label ten 1.8 ml screw-cap with the GOG protocol number (GOG-0213), the GOG Bank ID (##-##-G###), the Specimen Code (SB01) and the collection date (mm/dd/yyyy)..***
2. **Draw Blood**. Draw 10 ml of blood into a **plain red-top** Vacutainer® tube not a serum separator tube.
3. **Allow Blood to Clot**. Allow the blood to **clot upright at room temperature for 30 minutes**.
   * ***If the blood cannot be centrifuged immediately (next step), store the clotted blood at 4°C or in a bucket with excess wet ice for no longer than 3 hrs from the time of the blood draw. The faster the blood can be centrifuged after the 30 min clotting step, the better.***
4. **Centrifuge Blood**. Centrifuge the blood to separate the serum (clear straw-colored liquid) from the fibrin clot and the blood cells.
   * ***The optimal centrifugation conditions are ~3,500 x g at 4°C for 10 min. The minimal centrifugation conditions are ~1000 x g at room temperature for 15 minutes. The longer centrifugation time compensates for the slower speed. Avoid centrifugations without refrigeration longer than 15 min because excess heat may build up in the unit and damage the serum.***

5. **Mix and Aliquot Serum**. Remove the caps from the blood tube, the 15 ml conical tube and the cryotubes. Transfer the serum into the 15 ml conical tube and gently mix the serum. Dispense (aliquot) the serum evenly into as many of the labeled screw-cap cryotubes as possible. Cap the cryogenic vials securely.
   * ***Fill each cryotube with a minimum of 0.25 ml (cc) to a maximum of 1.7 ml (cc) of serum. It is better to separate the serum into more cryotubes with a smaller volume than into fewer cryotubes with a larger volume.***
6. **Freeze Serum**. Freeze the serum in the cryotubes immediately in an upright position, when possible, using an appropriate type of freezing/storage space as described in section with guidelines and recommendations for preparing serum and plasma specimens.
7. **Complete the Form SP.** Complete a GOG Specimen Form (Form SP) as specified in Section VIII. Include a copy of Form SP with the specimen when it is shipped to the GOG Tissue Bank, and retain a copy in your files.
   * ***The type of storage condition prior to shipment (ultra-cold freezer/liquid nitrogen [N_2]/dry ice) and type of blood collection tube (red-top) must be specified on the specimen transmittal form (Form SP) for the serum specimen.***
8. **Ship the Serum**. Ship the frozen serum to the GOG Tissue Bank as described in Section IX.

H. Instructions for Preparing Plasma

1. **Label Cryotubes**. Label the screw-cap cryotubes for each time point with the GOG protocol number, the GOG Bank ID, the Specimen Code and the collection date.
   * ***For GOG-0213, label ten 1.8 ml screw-cap cryotubes with the GOG protocol number (GOG-0213), the GOG Bank ID (##-##-G###), the Specimen Code (PB01) and the collection date (mm/dd/yyyy).***
2. **Draw Blood**. Draw 10 ml of blood into a **purple-top (lavender-top)** Vacutainer® tube with the anticoagulant EDTA until the vacuum is exhausted.
3. **Allow Blood to Clot**. Mix the blood with the anticoagulant by **gently inverting the tube 5-10 times**.
   * ***If the next (centrifugation) step cannot be conducted immediately, store the blood at 4°C in a refrigerator or in a bucket with excess wet ice for no longer than 3 hrs from the time of the blood draw. The faster the blood can be centrifuged after it is mixed with the anticoagulant the better.***
4. **Centrifuge Blood**. Centrifuge the blood to separate the plasma (clear straw-colored liquid) from the blood cells.
   * ***Ideally, centrifuge the blood at ~3,500 x g at 4°C for 10 min. When the ideal equipment is not available, the minimum centrifugation requirements will be ~1000 x g at room temperature for 15 minutes. The longer centrifugation time will compensate for the slower speed. Avoid centrifugations without refrigeration longer than 15 min because excess heat may build up in the unit and damage the plasma.***
5. **Mix and Aliquot Plasma**. Remove the caps from the blood tube, the 15 ml conical tube and the cryotubes. Transfer the plasma into the 15 ml conical tube and gently mix the plasma. Dispense (aliquot) the plasma evenly into as many of the labeled screw-cap cryotubes as possible. Cap the cryogenic vials securely.
   * ***Fill each cryotube with a minimum of 0.25 ml (cc) to a maximum of 1.7 ml (cc) of plasma. It is better to separate the plasma into more cryotubes with a smaller volume than into fewer cryotubes with a larger volume.***
6. **Freeze Plasma**. Freeze the plasma in the cryotubes immediately in an upright position, when possible, using an appropriate type of freezing/storage space as described in section with guidelines and recommendations for preparing serum and plasma specimens.
7. **Complete the Form SP.** Complete a GOG Specimen Form (Form SP) as specified in Section VIII. Include a copy of Form SP with the specimen when it is shipped to the GOG Tissue Bank, and retain a copy in your files.
   * ***The type of storage condition prior to shipment (ultra-cold freezer/liquid nitrogen [N_2]/dry ice) and type of blood collection tube (EDTA) must be specified on the specimen transmittal form (Form SP) for the plasma specimen.***