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# CONFIDENTIAL

PROTOCOL GOG-3003

# A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE II STUDY OF VTX-2337 (IND #78,416) IN COMBINATION WITH PEGYLATED LIPOSOMAL DOXORUBICIN (PLD) IN PATIENTS WITH RECURRENT OR PERSISTENT EPITHELIAL OVARIAN, FALLOPIAN TUBE OR PRIMARY PERITONEAL CANCER

***This protocol is open to participation by U.S. Sites***

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OPEN TO PATIENT ENTRY AUGUST 13, 2012

## PROTOCOL SCHEMA

### Eligible Patients:

Patients with confirmed epithelial ovarian, fallopian tube or primary peritoneal cancer that is persistent or recurrent despite primary therapy, and who have measurable disease. Recurrence must occur < 12 months after completing platinum-based first- or second-line therapy. Up to two (1–2) prior cytotoxic regimens are allowed. Prior PLD is NOT allowed.

### Treatment Arms:

Treatment assignment will be blinded and assigned in a randomized manner.

Arm 1: PLD 40 mg/$m^2$ plus VTX-2337 3.0 mg/$m^2$

Arm 2: PLD 40 mg/$m^2$ plus placebo

The dosing schedule will be the same for both treatment arms, and will be based on a 28-day cycle. The starting dose schedule is PLD on Day 1 plus VTX-2337 or placebo on Day 3, Day 10, and Day 17 for the first 4 cycles (induction schedule). Starting with cycle 5, the dose regimen will be PLD on Day 1 plus VTX-2337 or placebo on Day 3 **only**, without additional doses of VTX-2337 or placebo on Days 10 and Day 17.

### Study Schedule:

The blinded treatment cycle is repeated every 28 days until disease progression (based on Immune-Related RECIST) or until adverse effects prohibit further therapy.

NOTE: The recommended total cumulative dosage of PLD is 550 mg/$m^2$. However, once this cumulative PLD dose is reached, patients may—at the Investigator's discretion—continue study participation.

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# 1.0 OBJECTIVES

## 1.1 Primary Objectives:

1.11 To compare the overall survival (OS) of patients treated with VTX-2337 + PLD versus those treated with PLD alone in women with recurrent or persistent, epithelial ovarian, fallopian tube or primary peritoneal cancer.

1.12 To compare the progression-free survival (PFS) between the two treatment groups using Immune-Related Response Evaluation Criteria In Solid Tumors (irRECIST).

## 1.2 Secondary Objectives:

1.21 To compare the progression-free survival (PFS) between the two treatment groups using Response Evaluation Criteria In Solid Tumors (RECIST 1.1).

1.22 To compare the nature, frequency and severity of drug-related adverse events (AEs) between the two treatment groups.

## 1.3 Exploratory Objectives:

1.31 To compare the best overall response rate (ORR) and duration of response (based on the probability of being in response function [PBRF]) between the two treatment groups using irRECIST and RECIST 1.1.

1.32 To compare the disease control rate (DCR) between the two treatment groups using irRECIST and RECIST 1.1.

1.33 To assess the impact of immune status and response on the clinical effects (OS, PFS, DCR, ORR, PBRF, AEs) of study treatment.

1.34 To assess the effect of TLR8 polymorphisms and BRCA1/BRCA2 mutations on the clinical effects (OS, PFS, DCR, ORR, PBRF, AEs) of study treatment.

1.35 To assess the effect of immune cell subsets, as measured by immunohistochemistry and micro RNA in primary tumor tissue (e.g. immune score), on the clinical effects (OS, PFS, DCR, ORR, PBRF, AEs) of study treatment.

1.36 To assess whether the presence of autoantibodies to tumor-derived proteins are predictive of the clinical effects (OS, PFS, DCR, ORR, PBRF, AEs) of study treatment.

## 2.0 BACKGROUND AND RATIONALE

Epithelial ovarian, fallopian tube and primary peritoneal cancers carry the highest fatality to case ratio for all gynecologic malignancies diagnosed in the United States; it was estimated that in 2010, 21,880 new cases would be diagnosed and 13,850 women would die of the disease. 1 Over the past two decades, there have been only modest improvements in overall 5-year survival, and while 5-year survival has increased steadily from 30% to 50% overall, it has improved by only 5%, from 20% to only 25% for women with advanced-stage tumors. This plateau in mortality has been attributed to limitations in standard cytotoxic therapy, including intrinsic and acquired drug resistance and the lack of specificity of these agents for mechanisms of disease progression. Furthermore, such treatment, in the management of recurrent disease, has been associated with high impact of toxicity on quality of life.

There is abundant evidence that host anti-tumor cell mediated immune mechanisms play a role in controlling the natural history of ovarian carcinoma. Ovarian carcinoma may be recognized and attacked by the immune system. The tumor may contain lymphocytic infiltrates [2–4], and these tumor-associated lymphocytes exhibit oligoclonal expansion [5, 6] recognize tumor antigens [2,7–9] and display tumor-specific cytolytic activity in vitro. [10] Coukos and colleagues have demonstrated that among patients with advanced epithelial ovarian cancer treated with platinum-based primary chemotherapy, the absence of tumor infiltrating T lymphocytes (TIL) in fresh primary tumors was associated with poor prognosis with respect to PFS and overall survival. [11] The five-year overall survival rate was 38.0% among patients whose tumors contained TIL and 4.5% among patients whose tumors contained no TIL within tumor islets.

Significant progress has been made recently in our understanding of immune evasion mechanisms operating in some patients with ovarian cancer. In human cancer, $CD4^+$ $CD25^+$ $FoxP3^+$ T regulatory (Treg) cells were first demonstrated in ovarian cancer patients [12, 13], where increased Treg frequency predicts poor patient survival. [13, 14] The association of both the antitumor immune response (intraepithelial T cells) with prolonged survival and immune escape mechanisms with poor survival suggests that the immune system may modulate the biology of ovarian cancer. Indeed, pilot clinical data indicate that ovarian cancer patients respond to immunotherapy including interleukin-2 (IL-2), [15, 16] CTLA-4 antibody [17, 18] and adoptive transfer of ex vivo expanded TIL.[19]

It is increasingly clear that tumor antigen directed T cell responses, while important, are only one component of anti-tumor immune responses. Specifically, NK cells, dendritic cells, and even neutrophils may contribute to the eradication of established tumors. [20, 21] Clearly, an integrated, pro-inflammatory immune response is required to overcome the anatomical barriers and the complex counter-regulatory immune mechanisms that suppress anti-tumor immune effector populations. This integration is likely to occur

through dendritic cells and monocytes—cells capable of secreting a wide variety of inflammatory mediators (e.g. interleukin-12, interleukin-1b; tumor necrosis factor-alpha), integrating innate and adaptive immune responses, and regulating the priming and amplification of $CD4^+$ and $CD8^+$ T cell responses.

Interestingly, certain chemotherapeutic agents may dramatically augment the anti-tumor immune response, in part through their killing of tumor cells in a manner that promotes uptake of tumor antigens by dendritic cells (DCs). One notable example is doxorubicin, which promotes killing of tumor cells via an 'immunogenic' pathway that is caspase dependent and results in the surface exposure of calreticulin and internalization of antigens by phagocytic DCs [22, 23]. In addition, non-myelotoxic administration of doxorubicin can cause activation and maturation of DCs, resulting in increased IL-12 secretion, a critical factor required for T cell priming. [24]

PLD is a formulation of doxorubicin encapsulated in polyethylene-glycol (PEG)-coated liposomes associated with a dramatic alteration in pharmacokinetics characterized by a prolonged circulation time, reduced clearance, and a small volume of distribution.[2, 3] PLD liposomes become extravasated through abnormally permeable tumor vessels. Once concentrated in tumors, the liposomes of PLD can deliver high levels of doxorubicin to destroy malignant cells without affecting normal tissue. A randomized phase III study was conducted to compare PLD 50 mg/m$^2$ every 4 weeks ($n$=239) versus topotecan 1.5 mg/m$^2$/d for 5 consecutive days every 3 weeks ($n$=235) (which is indicated for the treatment of metastatic carcinoma of the ovary after failure of initial or subsequent chemotherapy) in patients with advanced epithelial ovarian carcinoma following failure of first-line platinum-based chemotherapy. The overall objective response rates for PLD and topotecan were 20% and 17%, respectively (p=0.390). Toxicities were mainly mucosal and skin. [25] Based on the improved tolerability profile and perceived clinical equivalence, 40 mg/m$^2$ of PLD has become the community standard dose. [26]

One of the most basic mechanisms for activation of the immune system is through Toll-like receptors (TLRs). TLR engagement alerts the immune system and leads to the activation of innate immune cells. Additionally, triggering of TLR induces DC maturation. [20, 21] Accordingly, we hypothesized that the combination of a specific Toll-like receptor agonist with the capacity to activate DCs and monocytes might provide synergy with doxorubicin and would stimulate a variety of immune pathways relevant to the generation of anti-tumor immunity. Targeting Toll-like receptor 8 (TLR8) would be expected to provide a unique opportunity given the fact that, in humans, TLR8 is expressed on both myeloid-lineage (CD11c+) DCs and monocytes (CD14+). [27]

Dr. George Coukos tested this hypothesis in his laboratory at the University of Pennsylvania, combining PLD with a novel, small molecule TLR8 agonist (VTX-2337) to treat established OVCAR tumor xenografts in a novel model using immune-deficient

mice that have been reconstituted with human hematopoietic system, the human immune system (HIS) Nod/SCID/ILRγc knock out (NSG) mice. These mice are repopulated with all cells derived from human myeloid and lymphoid lineages and provide a unique model to study interactions of drugs with the human immune system in vivo. A dramatic anti-tumor effect was noted when PLD was combined with VTX-2337 in tumor-bearing NSG mice. The TLR8 agonist significantly activated the human immune system as evidenced by up-regulation of human cytokines and activation of human monocytes and dendritic cells, followed by potent activation of human effector immune cells against HLA-matched ovarian cancer xenografts. The positive interaction between PLD and VTX-2337 could be in part explained by sensitization of tumor cells to innate and adaptive effector mechanisms.

**Study VRXP-A101**

VTX-2337 was evaluated in a Phase I clinical study conducted at the Mayo Clinic (Scottsdale, AZ) and the Translational Genomics Research Institute (TD2, Scottsdale, AZ) to assess the safety, tolerability, and biological activity of the compound in late-stage cancer patients (IND #78,416). Thirty-three subjects with various late-stage, refractory solid malignancies were evaluated in 8 successive cohorts of a standard, dose-ascending protocol with doses ranging from 0.1 mg/m$^2$ to 3.9 mg/m$^2$ of VTX-2337 as a stand-alone agent administered weekly via subcutaneous (SC) injection. VTX-2337 was overall clinically well tolerated, with the predominant adverse events being transient Grade 1 or 2 fever, chills, flu-like symptoms and injection site reactions. No significant drug-related hematologic, gastrointestinal, neurologic or cardiac toxicities were observed. A single subject in the study (Cohort 8; 3.9 mg/m$^2$) experienced a dose-limiting toxicity of Grade 3 hypotension. The patient recovered completely with no sequelae. Accordingly, the maximum dose evaluated in this study (3.9 mg/m$^2$) was tolerated.

The pharmacokinetics (PK) of VTX-2337 were assessed in all subjects and demonstrated dose-dependent exposure. Biological activity was assessed in all subjects using a multiplex immunoassay (Rules Based Medicine humanMAP v1.6) that measures serum level of 98 biomarkers, including cytokines, chemokines, and other inflammatory markers. A subset of these serum proteins had previously been established as biomarkers of TLR8 activation in cell culture and in animal studies using cynomolgus monkeys. Biological activity of VTX-2337 in the VRXP-A101 participants was shown to be dose-dependent, with the robust induction of multiple inflammatory markers at various time points. Clinical responses were assessed by RECIST at 8 weeks. No CRs or PRs were seen in the study, but approximately 25% of subjects had evidence of stable disease (SD) at 8 weeks. It should be noted that VTX-2337 was intended to have stand-alone biological activity, but was not anticipated to induce a CR or PR as a single agent, particularly in late-stage patients. The goal of the Phase I study was to identify a suitable, active, well-tolerated dose of VTX-2337 for combination studies.

**Study GOG-9925**

As previously noted, VTX-2337 provides a clear additive effect to PLD in a humanized mouse model of ovarian cancer. In these studies, repeat cycles of PLD and VTX-2337 were administered, and VTX-2337 was dosed several days after starting PLD. This schedule was based on the observations that 1) PLD requires approximately 48 hours to initiate apoptosis of ovarian cancer cells in vitro, and 2) antigens would first be released from dying tumor cells starting at approximately 36–48 hours in vivo. Based on the proposed mechanism of action which suggests that VTX-2337 both enhances the pro-apoptotic effects of PLD and enhances the internalization, processing and 'cross-priming' of tumor antigens by myeloid-dendritic cells and monocytes, it is believed that this dosing regimen—in which PLD is administered approximately 48 hours prior to VTX-2337—will provide optimal biological synergy for these agents. These data, collectively with the clinical data from study VRXP-A101, lead to the first clinical evaluation of PLD plus VTX-2337 in women with advanced ovarian cancer (GOG-9925).

This multi-center phase 1b protocol was initiated in March 2011 and has enrolled 13 women with recurrent or persistent epithelial ovarian or fallopian tube cancer. GOG-9925 assesses the combination of PLD administered on Day 1 plus VTX-2337 administered on Days 3, 10, and 17 of a 28-day cycle. The dose level of PLD is held constant at 40 $mg/m^2$, and 3 dose levels of VTX-2337 have been evaluated in a standard dose-escalation schema: 2.5 $mg/m^2$ (n=3), 3.0 $mg/m^2$ (n=3), and 3.5 $mg/m^2$ (n=7). Study data indicate that the combination therapy is adequately tolerated. There have been no dose-limiting toxicities; no serious, unexpected drug-related adverse events; and no evidence of synergistic toxicities between PLD and VTX-2337 (i.e., the toxicities associated with the combination therapy is consistent with the known toxicities of PLD and VTX-2337 when administered alone). The PK data verifies there is no metabolic interaction between the two drugs. Refer to section 4.3 for a list of the most common adverse events associated with the administration of PLD + VTX-2337 (all dose levels).

Pharmacodynamic data confirms the biological activity of VTX-2337 at all 3 dose levels, with induction of multiple inflammatory markers (cytokines and chemokines) indicative of TLR8 stimulation and immune activation. These results are consistent with previous pre-clinical and clinical studies of VTX-2337 as described above, and demonstrate that concomitant administration of PLD with VTX-2337 does not abrogate the inflammatory immune response elicited by this agent. Of the 13 patients enrolled on this regimen, 10 were evaluated for tumor response by RECIST 1.1, with 1 patient achieving a complete response, 7 patients with disease stabilization, and 2 patients with progressive disease. One patient did not complete the first cycle of combination therapy and was not evaluable for efficacy. Two patients were enrolled based on biochemical evidence of recurrent disease; one achieved a complete biochemical response and one had biochemical progression.

In selecting the dose for the current protocol, the clinical tolerability of the combined regimen, along with pharmacodynamic (PD) data were carefully considered. At the 3.5 mg/m$^2$ dose level of VTX-2337, one patient withdrew from treatment after receiving 3 cycles of therapy, in part due to toxicities associated with VTX-2337 (i.e., Grade 2 chills, fatigue, injection site reaction, nausea, and vomiting). A second patient enrolled at this level had a dose reduction to 3.0 mg/m$^2$ after 2 cycles due to Grade 3 drug-related events (nausea, vomiting, chills, and fever). These events suggest that long-term dosing of VTX-2337 at 3.5 mg/m$^2$ may not be clinically optimal. When considered in the context of PD data which clearly illustrate the biological activity of VTX-2337 at 2.5 and 3.0 mg/m$^2$, the selection of 3.0 mg/m$^2$ of VTX-2337 as the recommended phase 2 dose is reasonable and clinically appropriate.

2.1 Translational Research

2.11 Immune effects of VTX-2337 in a humanized mouse model, in the primate and in humans:

The pharmacokinetic and pharmacodynamic responses to VTX-2337 have been extensively characterized in cynomolgus monkeys. Plasma levels of a large multiplexed panel of immune biomarkers were quantified using the human multiple analyte panel (humanMAP) inflammation panel from Myriad/Rules Based Medicine (Austin, TX). Subcutaneous administration of VTX-2337 to cynomolgus monkeys was evaluated at doses ranging from 0.1 to 30 mg/kg and produced transient changes in the plasma levels of multiple cytokines, chemokines, acute phase proteins, and shed cell surface antigens all consistent with activation of the innate immune system. All doses evaluated were considered pharmacologically active, although the lowest dose of 0.1 mg/kg induced changes in only a subset of the analytes that were responsive to the higher doses. Analytes showing the greatest dynamic range in response to increasing doses of VTX-2337 included C-reactive protein, G-CSF, IL-6, MCP-1, and MIP-1β. Elevations in plasma IL-1β, TNFα, and IL-12p70 were seen at the higher doses.

This entire panel of immune biomarkers was also measured in a Phase I clinical trial in late stage oncology patients (VRXP-A101). Plasma samples were collected from patients at 0, 4, 8, and 24 hr following subcutaneous administration of VTX-2337. Increases in multiple cytokines and chemokines including G-CSF, IL-6, MCP-1, MIP-1β, and TNF-α were observed across a wide dose range. As in cynomolgus monkeys, the response in humans showed a clear dose dependence. When elevated, increased plasma levels of biomarkers were trending back to

baseline at 24 hours. The concentration of VTX-2337 that elicited a response in man was similar to that characterized in cynomolgus monkeys.

In addition, when VTX-2337 was used in HIS-NSG mice at similar doses as in the human phase I study or the cynomolgus monkey, it produced similar responses from the human hematopoietic system. We observed similar elevation of inflammatory cytokines in peripheral blood with similar PD profiles as in the human or the non-human primate, and we documented rapid and dose dependent activation of dendritic cells and macrophages. Finally, when biologically effective doses of VTX-2337 were used in combination with PLD in HIS-NSG mice bearing OVVCAR5 s.c. tumors, we observed a potent positive interaction between the drugs, with more potent activation of the human immune system, activation of DCs, down regulation of T regulatory cells, Th1 polarization of T cells, increase in tumor infiltrating T cells and more dramatic tumor growth suppression by the combination of the two drugs.

2.12 Considerable variability in immune reactivity can exist between individuals. Despite the fact that VTX-2337 activates a specific receptor (TLR8) on a restricted class of dendritic cells and monocytes, marked differences in responsiveness to treatment with this agent may relate to pre-existing difference in immune activation at baseline. We hypothesize that measureable differences in response to VTX-2337 at baseline (prior to dosing) may predict the pharmacodynamic response, and potentially clinical responses, to this agent in combination with chemotherapy.

We have employed a validated assay (TruCulture$^{TM}$) to measure the baseline response to VTX-2337. In preliminary studies using healthy volunteers and cancer patients and the TruCulture system, we have demonstrated variability between individuals following immune activation with lipopolysaccharide or VTX-2337, particularly with regards to IFN-gamma and interleukin-12 production. Data from TruCulture at baseline will be correlated with TLR8 SNP data (below), immune responses after treatment, and clinical responses (where applicable).

2.13 TLR8 polymorphisms:

Genes encoding TLRs, particularly those encoding the intracellular TLR (TLR3, TLR7, TLR8 and TLR9) are evolutionarily conserved. Still, various single nucleotide polymorphisms (SNPs) in the TLR8 gene have been identified from large scale sequencing efforts in multiple ethnic populations. Recently, a common TLR8 SNP [denoted TLR8 A1G (rs3764880)] has been found to be associated with various infectious

diseases, notably progression of HIV and TB. [28, 29] This allele is present in all populations tested, and is found at a frequency of approximately 30% in most ethnic groups. While one report suggests that the TLR8 A1G encodes a variant of TLR8 which decreases activity relative to the wild type allele, there are scant data, at present, to support this conclusion.

Given the relatively high allele frequency of this common TLR8 genetic variant, the potential functional differences in this allele, and the human genetic association between this variant and several infectious diseases, it is reasonable to genotype individuals from clinical trials investigating VTX-2337. We have developed a reliable method to analyze DNA from blood or saliva to detect the TLR A1G SNP. We propose genotyping all subjects in the proposed trial for the A1G SNP to assess the potential relevance of this allele to clinical responses (related to efficacy and safety) following treatment with VTX-2337.

## 2.2 Inclusion of Women and Minorities

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 ovarian, fallopian tube and peritoneal primary cancer population treated by participating institutions.

# 3.0 PATIENT ELIGIBILITY AND EXCLUSIONS

## 3.1 Eligible Patients

3.11 Patients must have recurrent or persistent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma. Histologic documentation of the original primary tumor is required via pathology report.

3.12 Patients with the following histologic cell types are eligible: serous adenocarcinoma, endometrioid adenocarcinoma, mucinous adenocarcinoma, undifferentiated carcinoma, clear cell adenocarcinoma, mixed epithelial adenocarcinoma, transitional cell carcinoma, malignant Brenner's tumor or adenocarcinoma not otherwise specified (N.O.S.).

3.13 Patient must have measurable disease as defined by RECIST 1.1 (section 8.11). Measurable disease is at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded). Each lesion must be ≥ 10 mm when measured by CT, MRI, or caliper measurement by clinical exam; or ≥ 20 mm when measured by chest x-ray. Lymph nodes must be ≥ 15 mm in short axis when measured by CT or MRI.

Patients must have at least one “target lesion” as defined by RECIST 1.1. Tumors within a previously irradiated field will be designated as “non-target” lesions unless progression is documented or a biopsy is obtained to confirm persistence at least 90 days following completion of radiation therapy.

3.14 Patients must have received treatment with a platinum-based chemotherapeutic regimen for management of primary disease containing carboplatin, cisplatin or another organoplatinum compound. This initial treatment may have included intraperitoneal therapy, consolidation, non-cytotoxic agents (biologic/targeted therapy) or extended therapy administered after surgical or non-surgical assessment.

Patients are allowed to receive, but are not required to receive, one additional cytotoxic regimen for management of recurrent or persistent disease.

Patients are allowed to have received, but are not required to have received, biologic/targeted therapy (e.g., bevacizumab and/or PARP inhibitor) as part of their primary treatment regimen or for management of recurrent or persistent disease.

3.15 Patients must have platinum-resistant disease, defined as progression < 12 months after completion of first- or second-line platinum-based chemotherapy. The date (platinum-free interval) should be calculated from the last administered dose of platinum therapy.

Patients with platinum-refractory primary disease, defined as having disease progression while receiving first-line platinum-based chemotherapy, are NOT eligible. Disease progression while receiving second-line platinum therapy is allowed.

3.16 Patients must have adequate bone marrow, renal, hepatic, and neurologic functions as defined by the following:

3.161 Bone marrow function:

- Absolute neutrophil count (ANC) $\geq$ 1,500/mm$^3$
- Platelets $\geq$ 100,000/mm$^3$
- Hemoglobin $\geq$ 9 g/dL

3.162 Renal function: creatinine $\leq$ 1.5 x institutional upper limit normal (ULN).

3.163 Hepatic function:

- Bilirubin < 1.2 mg/dL
- SGOT (AST) and SGPT (ALT) $\leq$ 3.0 x ULN
- Alkaline phosphatase $\leq$ 2.5 x ULN

3.17 Patients must have recovered from effects of recent surgery, radiotherapy or chemotherapy:

3.171 Patients should be free of active infection requiring parenteral antibiotics.

3.172 Any hormonal therapy directed at the malignant tumor must be discontinued at least one week prior to the first date of treatment on this study. Continuation of hormone replacement therapy is permitted.

3.173 Any other prior therapy directed at the malignant tumor, including chemotherapy, biologic/targeted agents and immunologic agents, must be discontinued at least three weeks prior to the first date of treatment on this study.

3.174 Any prior radiation therapy must be completed at least four weeks prior to the first date of treatment on this study.

3.18 Patients must have a GOG performance status of 0 or 1.

3.19 Patients of childbearing potential must have a negative pregnancy test prior to the study entry and be practicing an effective form of contraception. If applicable, patients must discontinue breastfeeding prior to the first date of treatment on this study.

3.110 Patients must meet the entry requirements and undergo the baseline procedures specified in section 7.0.

3.111 Patients must have signed an IRB-approved informed consent form and authorization permitting release of personal health information.

3.2 Ineligible Patients

3.21 Patients who have had treatment with VTX-2337, doxorubicin, PLD, or any other anthracycline.

3.22 Patients who have received an investigational agent < 30 days prior to the first date of treatment on this study.

3.23 Patients who have received oral or parenteral corticosteroids < 2 weeks prior to the first date of treatment on this study or who require ongoing systemic immunosuppressive therapy for any reason. The use of topical, inhaled, and intranasal corticosteroids is allowed.

3.24 Patients with active autoimmune disease.

"Active" refers to any condition currently requiring therapy. Examples of autoimmune disease include systemic lupus erythematosus, multiple sclerosis, inflammatory bowel disease and rheumatoid arthritis.

3.25 Patients with a history of other invasive malignancies, with the exception of non-melanoma skin cancer, are excluded if there is any evidence of the other malignancy being present within the last three years. Patients are also excluded if their previous cancer treatment contraindicates this protocol therapy.

3.26 Patients who have received prior radiotherapy OTHER THAN for the treatment of ovarian, fallopian tube or primary peritoneal cancer within the last three years are excluded.

3.27 Patients who have received prior chemotherapy OTHER THAN for the treatment of ovarian, fallopian tube or primary peritoneal cancer within the last three years are excluded.

3.28 Patients with history or evidence upon physical examination of CNS disease, including primary brain tumor, seizures not controlled with standard medical therapy, any brain metastases, or history of cerebrovascular accident (CVA, stroke), transient ischemic attack (TIA) or subarachnoid hemorrhage within six months of the first date of treatment on this study.

3.29 Patients with clinically significant cardiovascular disease. This includes:

3.291 Uncontrolled hypertension, defined as systolic > 150 mm Hg or diastolic > 90 mm Hg.

3.292 Myocardial infarction or unstable angina within 6 months of the first date of treatment on this study.

3.293 History of serious ventricular arrhythmia (i.e., ventricular tachycardia or ventricular fibrillation) or cardiac arrhythmias requiring anti-arrhythmic medications, except for atrial fibrillation that is well controlled with anti-arrhythmic medication.

3.294 Baseline ejection fraction ≤ 50% as assessed by echocardiogram or MUGA.

3.295 New York Heart Association (NYHA) Class II or higher congestive heart failure (Appendix II).

3.296 Grade 2 or higher peripheral ischemia [brief (< 24 hrs) episode of ischemia managed non-surgically and without permanent deficit].

3.210 Patients who are pregnant or nursing.

3.211 Patients under the age of 18.

3.212 Patients with clinical symptoms or signs of gastrointestinal obstruction and/or who require drainage gastrostomy tube and/or parenteral hydration or nutrition.

## 4.0 STUDY MODALITIES

### 4.1 Pegylated Liposomal Doxorubicin (PLD)

Refer to the PLD package insert to confirm the most current information on the following:

4.11 Formulation: PLD (doxorubicin HCl liposome injection) is supplied as a sterile, translucent, red liposomal dispersion in 5 mL, 10 mL, or 30 mL glass, single use vials. Each vial contains doxorubicin HCl at a concentration of 2 mg/mL.

4.12 Storage: Refrigerate unopened vials of PLD at 2°–8°C (36°–46°F). Avoid freezing. Prolonged freezing may adversely affect liposomal drug products; however, short-term freezing (less than 1 month) does not appear to have a deleterious effect on PLD.

4.13 Preparation: PLD doses must be diluted in 5% Dextrose Injection, USP prior to administration in accordance with the package insert and/or institutional procedures. . Aseptic technique must be strictly observed since no preservative or bacteriostatic agent is present in PLD. Diluted PLD should be refrigerated at 2°C–8°C (36°F–46°F) and administered within 24 hours.

Do not mix with other drugs.

Do not use with any diluent other than 5% Dextrose Injection.

Do not use any bacteriostatic agent, such as benzyl alcohol.

PLD is not a clear solution but a translucent, red liposomal dispersion.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if a precipitate or foreign matter is present.

4.14 Procedure for Proper Handling and Disposal: Caution should be exercised in the handling and preparation of PLD.

The use of gloves is required.

If PLD comes into contact with skin or mucosa, immediately wash thoroughly with soap and water.

PLD should be considered an irritant and precautions should be taken to avoid extravasation. With intravenous administration of PLD, extravasation may occur with or without an accompanying stinging or

burning sensation, even if blood returns well on aspiration of the infusion needle. If any signs or symptoms of extravasation have occurred, the infusion should be immediately terminated and restarted in another vein. PLD must not be given by the intramuscular or subcutaneous route.

PLD should be handled and disposed of in a manner consistent with other anticancer drugs.

4.15 Adverse Effects: Consult the PLD package insert for the most current and complete information.

4.16 Clinical Supply: PLD is commercially available from various national drug wholesalers and distributors. All commercially available sources are allowed, including Doxil®, Lipodox®, and generic PLD.

Every reasonable attempt should be made to obtain PLD through usual commercial channels. In the event commercially-supplied PLD is unavailable or cannot be obtained, **contact VentiRx Pharmaceuticals immediately** (see below). A limited supply of PLD may be available from VentiRx Pharmaceuticals for use by eligible patients participating in GOG-3003.

4.17 Drug Ordering: Consult the American Hospital Formulary Service Drug Information guide, Facts and Comparisons, or the package insert for additional information.

In the event that PLD cannot be obtained commercially, contact VentiRx Pharmaceuticals immediately—and prior to patient registration if applicable—via email at PLD@ventirx.com. Be sure to include your institution name, GOG number, and telephone contact information.

4.2 VTX-2337 (IND #78,416) or Placebo ("Investigational Drug")

4.21 IND Sponsor: VentiRx:

If there are questions regarding the IND, please contact:

VentiRx Pharmaceuticals
Seattle, WA
Email: ClinicalTrials@ventirx.com
Phone: (206) 689-2259

4.22 Investigator Brochure: All investigators who receive a copy of the protocol must obtain a copy of the Investigator's Brochure (IB). The IB can be downloaded from the GOG-3003 web site by clicking on the "Investigator's Brochure" link.

If there are any questions related to the IB, please contact:

VentiRx Pharmaceuticals
Seattle, WA
Email: ClinicalTrials@ventirx.com
Phone: (206) 689-2259

4.23 Formulation: VTX-378 is a novel, small, organic molecule TLR8 agonist with a molecular weight of 458.6. This active pharmaceutical ingredient (API) is formulated at neutral pH in Captisol®, a cyclodextrin-based solubilizing agent, to produce the investigational drug VTX-2337. Placebo is the identical formulation of VTX-2337, except it does not contain the API VTX-378.

4.24 How Supplied: Upon registration, patients will be assigned a unique subject number and randomly assigned to receive VTX-2337 or placebo. Approximately 4 business days following registration (section 4.29), the Investigator will receive a patient-specific drug kit containing blinded vials of VTX-2337 or placebo ("investigational drug"). The drug kit will contain an external label that includes the clinical protocol number ("GOG-3003"); unique subject number (XXX-3003-YYY, where XXX is the institution number and YYY is the sequential subject number); a unique, random 5-digit drug kit number, and a place for the subject's initials to be recorded.

Within each patient-specific drug kit, investigational drug is provided as a lyophilized cake in a 3 mL clear glass, single-use vial with a secured rubber cap. Each vial contains VTX-2337 or an equivalent volume of placebo, and is affixed with a label similar to the following:

| | |
|---|---|
| XX mg VTX-2337 or Placebo for subcutaneous injection.<br>XXXXX<br>Prepare according to clinical protocol GOG-3003<br>Store 2–8ºC<br>Caution: New drug—limited by federal law to investigational use.<br>*VentiRx Pharmaceuticals, Inc* | XX mg VTX-2337 or Placebo for subcutaneous injection.<br>XXXXX for protocol GOG-3003<br>Patient: _____<br>Date: _____ |
| ↑ This portion attached to vial | ↑ Detach this portion for patient record |

Prior to administration, vials of VTX-2337 or placebo must be reconstituted and prepared according to the Storage and Preparation Instructions provided with each shipment of investigational drug. Blinded

vials of investigational drug will be shipped in a qualified shipper at 2–8ºC via traceable courier.

4.25 Storage: Upon receipt, investigational drug supplies should be removed from the shipping package, inventoried, and placed in refrigerated storage at 2ºC–8ºC (36ºF–46ºF) and protected from light.

4.26 Stability: The formulation of investigational drug contains no preservatives and is intended for single use only. Doses of VTX-2337 or placebo should be made for each patient and for each dose in the study. Prepared vials of investigational drug are stable for 8 hours at room temperature and for 24 hours when refrigerated at 2°C–8°C.

Preparations of the investigational drug should be drawn from the vial into the syringe within approximately 60 minutes prior to dosing.

4.27 Preparation: Prior to administration, investigational drug must be prepared according to the Storage and Preparation Instructions supplied with each shipment of study drug. Investigational drug should be prepared and transferred using aseptic technique in a biological safety cabinet. Investigational drug supplies are intended for single use only.

4.28 Clinical Supply: Investigational drug will be provided free of charge by VentiRx Pharmaceuticals and distributed by Fisher Clinical Services.

4.29 Drug Ordering: No drug will be shipped to any site until regulatory approval has been obtained (see section 5.0). There will NOT be an initial drug supply forwarded to all investigational sites upon initial regulatory approval. Upon registration of an eligible patient, the investigational site will be supplied with a patient-specific, blinded, investigational drug kit. The drug kit will arrive 4 business days after the date of randomization.

To order additional drug for a specific patient, the site will complete the drug order/re-order (DORA) form and submit it to the GOG statistical center via EDS (SEDES).

Drug re-order requests received in SEDES by 5:00 PM EST will be processed for shipment two business days later. Investigational drug is shipped via traceable courier for overnight delivery.

| Patient Registration | Investigational Drug Received |
|---|---|
| Monday | Thursday |
| Tuesday | Friday |
| Wednesday | Tuesday |

| Patient Registration | Investigational Drug Received |
|---|---|
| Thursday | Tuesday |
| Friday | Wednesday |

Investigational Drug will be shipped from Fisher Clinical Services directly to the institution where the patient is to be treated. The transfer of investigational drug between institutions is not permitted.

4.210 Drug Accountability: The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all investigational drug (blinded VTX-2337 or placebo) using the GOG-3003 Investigational Drug Accountability Form (can be downloaded from the regulatory forms link on the GOG-3003 web page).

4.211 Drug Transfers: Drug transfers are not permitted.

4.212 Drug Destruction: Upon completion of the study, all investigational drug should be destroyed according to the site's local standard operating procedures and the destruction must be recorded on the GOG-3003 Investigational Drug Accountability Form. A copy of this form must be provided to VentiRx at ClinicalTrials@ventirx.com; please reference "GOG-3003 Study Drug Accountability" in the email subject line.

4.213 Adverse Effects:

**Adverse Events Associated with the Administration of Single-Agent VTX-2337 (By Decreasing Frequency)**

| Adverse Event | ≤ Grade 2 | Grade 3* |
|---|---|---|
| **Events Occurring in > 20% of Patients** | | |
| Injection-site reaction | X | |
| Chills | X | |
| Pyrexia | X | |
| Influenza-like illness | X | |
| **Events Occurring in 10–20% of Patients** | | |
| Fatigue | X | |
| Pain (NOS) | X | |
| **Events Occurring in < 5% of Patients** | | |
| Cytokine release syndrome | X | |
| Hypotension | | X |

* There have been no Grade 4 or Grade 5 events associated with the use of VTX-2337 to date.

4.3 PLD + VTX-2337 Adverse Effects

**Adverse Events Associated with the Administration of PLD + VTX-2337 (By Decreasing Frequency)**

| Adverse Event | Severity | | |
|---|---|---|---|
| | ≤ Grade 2 | Grade 3 | Grade 4 |
| **Events Occurring in > 20% of Subjects** | | | |
| Injection site reaction | X | | |
| Fatigue | X | | |
| Fever | X | X[1] | |
| Anemia | X | X[2] | |
| Chills | X | X[1] | |
| Headache | X | | |
| Neutrophil count decreased | X | X[2] | X[1] |
| White blood cell decreased | X | X[1] | |
| Mucositis oral | X | X[2] | |
| Nausea | X | | |
| Vomiting | X | X[1] | |
| Arthralgia | X | | |
| Palmar-plantar erythrodysesthesia syndrome | X | X[1] | |
| Anorexia | X | | |
| Constipation | X | | |
| Diarrhea | X | | |
| Dysphagia | X | | |
| Platelet count decreased | X | | |
| Rash maculo-papular | X | | |
| **Events Occurring in 10–20% of Subjects** | | | |
| Abdominal pain | X | | |
| Bloating | X | | |
| Flu like symptoms | X | | |
| Gastroesophageal reflux disease | X | | |
| Infusion related reaction | X | | |

[1] Occurs in < 10% of subjects
[2] Occurs in < 20% of subjects

## 5.0 PATIENT ENTRY/RANDOMIZATION PROCEDURE AND TREATMENT PLAN

Before patient entries will be accepted, submit the following regulatory documents to the GOG Administrative Office via mail **(Attn: Regulatory Department, Protocol GOG-3003):**

- IRB approval*
- IRB-approved informed consent
- IRB Membership list or FWA assurance letter
- Study-specific signed original FDA Form 1572 for institution PI******
- Current CV (signed and dated within one year) for institution PI and sub-investigators listed on FDA Form 1572
- Medical license for institution PI and sub-investigators listed on the FDA Form 1572
- Lab license, certificates, and required Normal Lab Values (NLV) for labs listed on FDA Form 1572
- Signed original GOG Investigator Signature Page for PI******
- Signed original GOG Financial Disclosure Form for all investigators listed on FDA Form 1572******
- Shipping Information Form******

**Please allow 7−10 days for processing of regulatory documents before screening the first patient.** All copies of the above should be filed into a study-specific regulatory binder at your institution.

* All initial, continuing and amendment reviews must be sent to the GOG Administrative Office.

** Please see GOG-3003 protocol documentation page to download forms by clicking on the “Regulatory Forms” link.

The GOG Administrative Office will forward these documents to VentiRx for review and approval. VentiRx will notify the GOG Administrative Office when the documents have been approved (typically within 24 hours) and patients can be accepted onto the study.

### 5.1 Patient Entry and Registration

This trial is open to U.S. GOG Member Institutions. The GOG Statistical and Data Center (SDC) utilizes a web-based patient registration system (available at the GOG web menu page). When a suitable candidate has been identified for protocol entry, the following steps should be taken:

5.11 An IRB-approved informed consent form and authorization permitting the release of personal health information must be signed by the patient or guardian. Current FDA, and institutional regulations concerning informed consent will be followed.

5.12 All eligibility requirements indicated in section 3.0 must be satisfied.

5.13 The Fast Fact Sheet data must be gathered.

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.

5.15 The institution will enter the patient's name, GOG patient identification number, and date of registration in the appropriate place in their Log Book to document the patient's entry.

5.2 Treatment Plan: Assignment to treatment arm will be randomized and blinded.

Arm 1: PLD 40 mg/m$^2$ on Day 1 plus VTX-2337 3.0 mg/m$^2$ on Day 3, Day 10, and Day 17 of a 28-day cycle for the first four cycles. Thereafter, PLD 40 mg/m$^2$ on Day 1 plus VTX-2337 3.0 mg/m$^2$ on Day 3.

Arm 2: PLD 40 mg/m$^2$ on Day 1 plus placebo on Day 3, Day 10, and Day 17 of a 28-day cycle for the first four cycles. Thereafter, PLD 40 mg/m$^2$ on Day 1 plus placebo on Day 3.

5.21 Administration of PLD

PLD will be administered in a manner consistent with the current labeled dosing regimen.

PLD is administered as an intravenous infusion on Day 1 of a 28-day cycle at an initial rate of 1 mg/min to minimize the risk of infusion reactions. If no infusion-related adverse reactions are observed, the rate of infusion can be increased to complete administration of the drug over one hour (60 min).

As per section 9.1, the recommended total cumulative dosage of PLD is 550 mg/m$^2$. Therefore, once this cumulative dose of PLD is reached (with no dose reductions, this would be approximately 13 cycles of therapy), patients will—at the Investigator’s discretion—either continue with PLD + VTX-2337/placebo or discontinue both treatments and enter the long-

term assessment portion of the trial. Note that patients who continue to receive PLD beyond a cumulative dose of 550 mg/m$^2$ must undergo echocardiogram or MUGA scan every 2 cycles or in accordance with institutional standards to monitor for cardiac toxicity. Patients will discontinue treatment should their resting left ventricular ejection fraction (LVEF) demonstrate an absolute decrease of > 10% below the institutional lower limit of normal or should they develop Grade 3 (or greater) left ventricular systolic dysfunction (symptomatic due to drop in ejection fraction responsive to intervention).

5.22 Administration of VTX-2337 or Placebo

VTX-2337 or placebo will be administered as subcutaneous injection on Day 3 (~48 hours after the completion of the infusion of PLD), Day 10, and Day 17 of a 28-day cycle for the first 4 cycles. The 3 doses of investigational drug should be spaced approximately 7 days (1 week) apart from each other.

For subsequent cycles (Cycles 5 and later), VTX-2337 or placebo will be administered as subcutaneous injection on Day 3 only of a 28-day cycle.

5.3 Sequence and timing of drug administration

PLD will be administered on Day 1. VTX-2337 or placebo will be administered on Day 3, approximately 48 hours after completing the 60-minute PLD infusion. Therefore, PLD should be administered on a Monday, Tuesday, or Wednesday only. During the induction dose schedule (cycle 1 through cycle 4), additional doses of VTX-2337 or placebo will be on Day 10 and Day 17 of the 28-day combination cycle.

5.4 Administration

5.41 PLD: Do not use with in-line filters. Rapid flushing of the infusion line should be avoided. Do not administer as a bolus injection or undiluted solution.

Acute infusion-related reactions have occurred in up to 10% of patients treated with PLD. Serious and sometimes life-threatening or fatal allergic/anaphylaxis-like infusion reactions have been reported. Medications to treat such reactions, as well as emergency equipment, should be available for immediate use.

PLD should be administered intravenously at an initial rate of 1 mg/min to minimize the risk of infusion reactions. If no infusion-related adverse

reactions are observed, the rate of infusion can be increased to complete administration of the drug over one hour.

**Due to their immunosuppressive effect, administration of systemic steroids (e.g., dexamethasone) as an antiemetic and/or preparative regimen for hypersensitivity reactions should be avoided if other means of treatment are available and medically appropriate.**

Preparative antihistamine use is allowed (e.g., diphenhydramine 25 mg IV) prior to FIRST dose (and subsequent doses if hypersensitivity reaction is seen with a previous dose).

5.42 VTX-2337 or Placebo: Doses are administered via subcutaneous injection. Investigational drug should be drawn from the dilution vial into the syringe within 60 minutes prior to dosing.

To help mitigate potential adverse events commonly associated with the administration of VTX-2337, such as fever and myalgia, patients should be administered 650–1000 mg acetaminophen by mouth within 30 minutes prior to each dose of VTX-2337 or placebo. Patients should be given an additional dose of 650–1000 mg of acetaminophen by mouth approximately 4–6 hours after administration of the investigational drug. Due to their suppressive effects, the use of NSAIDS within 24 hours of dosing should be avoided if clinically feasible.

To reduce the effects of injection site reaction that may be associated with administration of investigational drug, an ice pack may be applied to the site of injection for approximately 30 minutes prior to injection and every 1–2 hours following injection on the day of administration. Additionally, at the discretion of the investigator, single doses of investigational drug may be divided and administered at two different anatomical sites.

Standard medications to treat possible hypersensitivity reactions and/or symptoms of cytokine release syndrome should be readily available at the time of treatment, including epinephrine, H1 antihistamines (e.g., diphenhydramine), H2 antihistamines (e.g., ranitidine), narcotics, IV fluids for volume expansion, and supplemental oxygen. (See also section 6.2.) **Due to their immunosuppressive effect, administration of systemic steroids (e.g., dexamethasone) should be avoided in this setting if other means of treatment are available and appropriate.**

The dose of investigational drug should be administered with a 1 mL syringe suitable for subcutaneous injection. To administer the injection, 1–

2 inches of fatty tissue should be pinched up to avoid injection into the muscle layer. The needle can be inserted at either 45 or 90 degrees; a 45-degree angle is recommended when less than 2 inches of tissue can be pinched. Appropriate anatomic areas for subcutaneous injection include the fatty tissue over the triceps, thighs, or lower back.

The abdomen is not a preferred anatomic area for injection of VTX-2337 in this patient population. This is due to a potential increase in the severity of injection site reaction when proximal to an abdominal incision and/or scarring. If abdominal injection cannot be avoided, it should be administered ≥ 8 cm away from any incision.

# 6.0 TREATMENT MODIFICATIONS AND EMERGENCY UNBLINDING

In order to maintain dose-intensity and cumulative dose-delivery on this study, reasonable efforts will be made to minimize dose reduction and treatment delays as specified. Any patient whose treatment is delayed due to study-drug related toxicity must be evaluated on a weekly basis until adequate hematologic and non-hematologic parameters have been met. No intra-patient dose escalation is planned for this study.

Patients should be monitored for study-drug related toxicity as outlined in section 7.1. The investigator should carefully assess all treatment-associated toxicities and, whenever possible, determine if they can reasonably be attributed to PLD alone, VTX-2337 alone, or the combination regimen. If appropriate, dose delays and/or adjustments should be restricted to the suspected causative agent.

## 6.1 PLD Dose Modification Guidelines

Refer to the PLD label for current commendations regarding dose delays and reductions.

Once the dose of PLD has been reduced due to drug-related toxicity, it should not be increased at a later time. If more than 2 dose reductions are required due to PLD-related toxicity, consult the study chair to determine if PLD should be discontinued and the patient removed from study.

In the event of a delay in PLD dosing due to drug-related toxicity, doses of VTX-2337 or placebo should also be delayed.

Upon restarting the dose of PLD, the standard dosing regimen for the combination therapy should be resumed (e.g., PLD on Day 1 and investigational drug on Days 3, 10, and 17 during Cycles 1–4, or PLD on Day 1 and investigational drug on Day 3 during Cycles 5 and later).

### 6.11 Modifications for Hematologic Toxicity

The use of growth factors is not restricted in this study. Investigators may follow their institutional guidelines/standard practices for use of growth factors for supportive care.

**PLD Dose Modification Guidelines for Hematological Toxicity**

| Toxicity Grade | ANC | Platelets | Modification |
|---|---|---|---|
| **1** | 1,500–1,900 | 75,000–150,000 | Resume treatment with no dose reduction |
| **2** | 1,000–< 1,500 | 50,000–< 75,000 | Wait until ANC ≥ 1,500 and platelets ≥ 75,000; redose with no dose reduction |
| **3** | 500 –999 | 25,000–< 50,000 | Wait until ANC ≥ 1,500 and platelets ≥ 75,000; redose with no dose reduction |
| **4** | < 500 | < 25,000 | Wait until ANC ≥ 1,500 and platelets ≥ 75,000; redose at 25% dose reduction or continue full dose with cytokine support |

## 6.12 Modifications for Hand-Foot Syndrome

**PLD Dose Modification Guidelines for Hand-Foot Syndrome (HFS)**

| Toxicity Grade | Dose Adjustment |
|---|---|
| **1:** mild erythema, swelling, or desquamation not interfering with daily activities | **Redose unless patient has experienced previous Grade 3 or 4 HFS.** If so, delay up to 2 weeks and decrease dose by 25%. Return to original dose interval. |
| **2:** erythema, desquamation, or swelling interfering with, but not precluding normal physical activities; small blisters or ulcerations <2 cm in diameter | **Delay dosing up to 2 weeks or until resolved to Grade 0-1.** If after 2 weeks there is no resolution, PLD should be discontinued. If resolved to Grade 0–1 within 2 weeks, and there are no prior Grade 3–4 HFS, continue treatment at previous dose and return to original dose interval. If patient experienced previous Grade 3–4 toxicity, continue treatment with a 25% dose reduction and return to original dose interval. |
| **3:** blistering, ulceration, or swelling interfering with walking or normal daily activities; cannot wear regular clothing | **Delay dosing up to 2 weeks or until resolved to Grade 0–1.** Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, PLD should be discontinued. |
| **4:** diffuse or local process causing infectious complications, or a bed ridden state or hospitalization | **Delay dosing up to 2 weeks or until resolved to Grade 0–1.** Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, PLD should be discontinued. |

### 6.13 Modifications for Stomatitis

**PLD Dose Modification Guidelines for Stomatitis**

| Toxicity Grade | Dose Adjustment |
|---|---|
| **1:** painless ulcers, erythema, or mild soreness | **Redose unless patient has experienced previous Grade 3 or 4 toxicity.** If so, delay up to 2 weeks and decrease dose by 25%. Return to original dose interval. |
| **2:** painful erythema, edema, or ulcers, but can eat | **Delay dosing up to 2 weeks or until resolved to Grade 0-1.** If after 2 weeks there is no resolution, PLD should be discontinued. –If resolved to Grade 0–1 within 2 weeks and there was no prior Grade 3–4 stomatitis, continue treatment at previous dose and return to original dose interval. If patient experienced previous Grade 3–4 toxicity, continue treatment with a 25% dose reduction and return to original dose interval. |
| **3:** painful erythema, edema, or ulcers, and cannot eat | **Delay dosing up to 2 weeks or until resolved to Grade 0–1.** Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, PLD should be discontinued. |
| **4:** requires parenteral or enteral support | **Delay dosing up to 2 weeks or until resolved to Grade 0–1.** Decrease dose by 25% and return to PLD original dose interval. If after 2 weeks there is no resolution, PLD should be discontinued. |

### 6.14 Modifications for Hepatic Toxicity

**PLD Dose Modification Guidelines for Impaired Hepatic Function**

Limited clinical experience exists in treating patients with hepatic impairment with PLD. Based on experience with doxorubicin HCl, it is recommended that the PLD dosage be reduced if the bilirubin is elevated as follows:

serum bilirubin 1.2 to 3.0 mg/dL: give ½ normal dose
serum bilirubin > 3 mg/dL: give ¼ normal dose

## 6.2 VTX-2337 Dose Modification Guidelines

VTX-2337 is a non-cytotoxic therapy. In previous clinical and non-clinical studies of this agent, no drug-related laboratory toxicities have been observed (i.e., no hematological toxicity).

The most common AE associated with VTX-2337 administration is Grade 1 or Grade 2 injection site reaction. Acute symptoms typically resolve within 48 hours of injection, but more persistent reactions are not uncommon. Grade 2 reactions may include a painful, raised, fluid-filled blister that mimics a sterile abscess. Antibiotic intervention is not typically warranted, but a culture of the fluid should be obtained—using sterile technique—if infection is suspected (e.g., associated

with fever persisting > 24 hours after injection). Symptomatic treatment with ice, acetaminophen, and narcotics is acceptable. Due to their suppressive effects, the use of NSAIDS within 24 hours of dosing should be avoided if clinically feasible.

The most common systemic drug-related adverse events—such as Grade 1/Grade 2 chills, flu-like symptoms, and fever—typically resolve in < 48 hours and do not require dose reductions or a delay in the dosing regimen. Less commonly, dosing with VTX-2337 may result in cytokine release syndrome (CRS). CRS is most likely to be Grade 1, but more severe reactions are possible (see below).

Treatment of systemic drug-related events should be consistent with the severity of the reaction as well as institutional standards, and may include: acetaminophen, $H_1$- and $H_2$-receptor antagonists, narcotics, IV fluids for volume expansion, and supplemental oxygen. Due to their immunosuppressive effect, administration of NSAIDs and systemic steroids (e.g., dexamethasone) should be avoided if other means of treatment are available and medically appropriate.

CRS is a symptom complex, and is characterized by systemic symptoms which may include fever, nausea, chills, tachycardia, hypotension, dyspnea, asthenia, headache, and rash. VTX-2337 directly targets TLR8, which is expressed on immune cells. CRS may result from the release of cytokines from the activated immune cells, or from 'downstream' events that can be associated with cytokine production. Symptoms of CRS may be acute or delayed by several hours after dosing with VTX-2337.

In most cases the CRS will be Grade 1. However, a severe, life-threatening reaction resulting from a substantial release of cytokines is possible. Severe CRS is a medical emergency, and urgent intervention must be taken to prevent life-threatening complications. This may include administration of sympathomimetic amines for pressor support and/or hospitalization for acute monitoring and intervention.

**VTX-2337 Dose Modification Guidelines**

| Grade | Action |
|---|---|
| Cytokine Release Syndrome | |
| 1 | Dose reduction not required<br>Consider prophylactic precautions with next dose |
| 2 | Consider prophylactic precautions—including volume expansion and treatment with oral or IV antihistamines—with next dose<br>Reduce dose by 1 level at Investigator's discretion |

| Grade | Action |
|---|---|
| Cytokine Release Syndrome | |
| 3 | Use prophylactic precautions with next dose<br>Reduce dose by 1 level |
| Injection Site Reaction | |
| 1 | Dose reduction not required<br>Consider administering subsequent injections at distal anatomic site(s) or dividing the dose and administering at 2 different anatomical sites |
| 2 | Administer subsequent injections at distal anatomic site(s) and/or divide the dose and administer at 2 different anatomical sites until resolved to ≤ Grade 1 |
| | Delay dosing for up to 2 weeks if needed |
| | If > 2 week delay or > 2 delays are required with consecutive injections, reduce dose by 1 level |
| 3 | Delay dosing until resolved to ≤ Grade 1<br>Reduce dose by 1 level |
| All Other Drug-Related AEs | |
| ≥ 3 | Reduce dose by 1 level |

If study-drug related toxicity occurs which does not meet the guidelines for dose reduction noted above, but is nevertheless considered by the investigator to be unacceptable or otherwise warrants a reduction in dose, a decrease in VTX-2337 or placebo by 1 dose level is acceptable following consultation with the study chair.

Delays in investigational drug dosing should not delay or shift the timing of the PLD dosing (doses of VTX-2337 are skipped and not made up). In the event of Grade 3 or Grade 4 toxicities attributed to investigational drug, subsequent doses should be delayed until recovery to ≤ Grade 1.

Once the dose of investigational drug has been reduced due to drug-related toxicities, it should not be increased at a later time. Patients who require > 2 dose reductions of investigational drug due to drug-related toxicity should discontinue treatment with VTX-2337 (or placebo). Patients should remain on study (i.e. continue receiving treatment with PLD and undergo all protocol-related assessments) until confirmed disease progression. Thereafter, patients should be followed long-term for survival per protocol.

**Investigational Drug Dose Reduction Schedule**

| Dose Level (DL) | Investigational Drug Dose ($mg/m^2$) |
|---|---|
| Starting Dose | 3.0 |
| Dose Reduction 1 | 2.5 |
| Dose Reduction 2 | 2.0 |
| Dose Reduction 3 | Discontinue Investigational Drug* |

*Patients should remain ON study (i.e. continue receiving treatment with PLD and undergo all protocol-related assessments) until confirmed disease progression. Thereafter, patients should be followed long-term for survival per protocol.

6.3 Emergency Unblinding

The treatment assignment of any individual subject may be accessed only in case of an emergency, such as a life-threatening serious adverse event (SAE) where knowledge of the treatment code is required for medical management of the event. Whenever feasible, the investigator should obtain approval from the study chair prior to unblinding, and should document the unblinding (including notification of study chair and reason for unblinding) in the subject's medical record.

To unblind a subject's treatment assignment, contact the GOG Statistical and Data Management Center at:

(800) 523-2917: Monday through Friday, 9:00 am to 5:00 pm Eastern Time

(716) 901-2853: After hours

If there is no answer, leave a message including a telephone number for a return call. A staff member from the GOG Statistical and Data Management Center will return your call.

For quality control, the GOG Statistical and Data Management Center will require the protocol number (i.e.,'GOG-3003'), the patient ID number (e.g., '999-3003-001'), and the patient initials (e.g., 'TDR') to unblind the subject.

**Remember, this is only in the event of an emergency.** This procedure is to be used by the investigator when the investigator needs to know whether the subject is taking VTX-2337 or placebo to manage the acute illness.

The treatment assignment should be maintained as confidential (i.e., only personnel essential for subject treatment should be informed of the treatment assigned) and placed in a sealed envelope.

The treatment code for an individual subject may also be broken at the discretion of the study sponsor to facilitate regulatory reporting of suspected unexpected serious adverse reactions (SUSAR).

# 7.0 STUDY PARAMETERS

## 7.1 Procedures and Tests

The following observations and tests are to be performed and recorded on the appropriate form(s). Unless otherwise specified, all required assessments on the day of PLD or VTX-2337 dosing should be performed prior to dosing.

**Table 7-1: Schedule of Protocol Activities and Assessments**

| | Pre-Treatment | Cycle 1 | | | | Cycles 2–4 | | | | Wk 12 (±7 days) | Cycles 5+ | | | Q8 wks (±7 days) | End of Treatment | Q3–12 Months |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cycle Week:** | | 1 | | 2 | 3 | 1 | | 2 | 3 | | 1 | | 4 | | | |
| **Cycle Day:** | | 1 | 3 | 10 | 17 | 1 | 3 | 10 | 17 | | 1 | 3 | 21–28 | | | |
| PLD | | X | | | | X | | | | | X | | | | | |
| VTX-2337 or Placebo (1) | | | X | X | X | | X | X | X | | | X | | | | |
| History & Physical | 2 | 5 | | | | 5 | | | | | 5 | | | | X | |
| Vital Signs | 2 | X | X | X | X | X | X | X | X | | X | X | | | X | |
| ECG | 2 | | | | | | | | | | | | | | X | |
| Echocardiogram or MUGA | 2 | | | | | | | | | | | | 8 | | 8 | |
| CBC/Differential/ Platelets | 3 | | | | | 6 | | | | | 6 | | | | X | |
| Serum creatinine/ BUN/electrolytes | 3 | | | | | 7 | | | | | 7 | | | | X | |
| Bilirubin/SGOT/ SGPT/Alkaline phosphatase/Ca/ $PO_4$/Mg | 3 | | | | | 7 | | | | | 7 | | | | X | |
| Serum Pregnancy | 4 | | | | | 4 | | | | | 4 | | | | X | |
| CT or MRI | 2, 9 | | | | | | | | | 9 | | | | 9 | 9 | |
| Chest Imaging | 2 | | | | | | | | | 10 | | | | 10 | 10 | |
| CA-125 | 3 | | | | | | | | | 11 | | | | 11 | X | |
| Adverse Event Assessment | | | X | X | X | X | X | X | X | | X | X | | | X | |
| Long Term Assessment | | | | | | | | | | | | | | | | 12 |

***See next page for footnotes.***

1. All cycles: Day 10 and Day 17 doses may be ± 1 day. Cycles 5+, Day 3 dose may be +1 day.
2. Must be performed within 28 days prior to initiating protocol therapy.
3. Must be performed within 14 days prior to initiating protocol therapy.
4. For women with child-bearing potential only. Obtain within 3 days prior to dosing for pretreatment test. Obtain every other cycle (odd numbered cycles).
5. Complete physical examination required on Cycle 1 Day 1 only if screening PE was performed > 2 weeks previously. On subsequent cycles, a problem-oriented PE should be performed ≤ 4 days prior to the Day 1 dose of PLD.
6. Perform ≤ 4 days prior to each dose of PLD or pre-dose on the day of dosing. Results must be known prior to administration of PLD.
7. Perform ≤ 4 days prior to each dose of PLD or pre-dose on the day of dosing.
8. If the cumulative dose of PLD exceeds 550 mg/m$^2$, then repeat echocardiogram or MUGA scan every other cycle or according to institutional standards. Repeat at the End of Treatment visit. The same method should be used for each patient throughout the study.
9. CT or MRI of abdomen and pelvis. The same imaging modality—encompassing the same field(s)—must be used for the same patient throughout the study.

   Use the CT/MRI Scheduling Tool on the GOG-3003 website (see example Appendix V) to generate scan dates for patients. Enter the Cycle 1 Day 1 date; the protocol-required imaging dates will be generated, including the ± 7-day window for each date. Maintain the imaging schedule until progression of disease is documented (or the patient initiates subsequent non-protocol therapy). If the patient stops VTX-2337/placebo and continues PLD, they should do so ON study, and the CT/MRI schedule should be maintained until progression of disease (or initiation of subsequent non-protocol therapy). If subsequent non-protocol therapy is initiated prior to progression of disease being documented and prior to the next scheduled scan date, obtain CT/MRI prior to initiating subsequent therapy (End of Treatment scan).

   **The first on-study radiographic tumor assessment is performed at Week 12 (± 7 days)**. Thereafter, tumor imaging is performed every 8 weeks (56 ± 7 days) until disease progression is confirmed or patient is put on subsequent non-protocol therapy. Also repeat tumor imaging at any time if clinically indicated based on symptoms or physical signs suggestive of progressive disease.

   **Patients with progressive disease (irPD) detected before or at the 12 week imaging assessment, but without rapid clinical deterioration, require confirmation of irPD with a second, consecutive scan obtained ≥ 4 weeks from the initiation documentation. Patients will continue to receive study treatment until irPD is confirmed at this later time point. (See section 8.2.)**

   All tumor assessment time points should be calculated from the Cycle 1 Day 1 dose of PLD. That is, if dose delays are required due to drug-related toxicities or other reasons, the radiographic tumor assessments should not be delayed but rather should be performed according to the original schedule (see Appendix V).

10. Repeat chest imaging at Week 12 and then every 8 weeks (56 ± 7 days) if initially abnormal or if required to monitor tumor response. See Section 8.12.
11. Obtain at Week 12, and then every 8 weeks (56 ± 7 days).
12. After End of Treatment visit, follow the patient long term to assess vital status and for Q form completion. Long-term follow-up visits should occur every 3 months for 2 years, then every 6 months for 3 years, and annually thereafter.

7.2 Pathology Requirements

7.21 Pathology report for histologic confirmation of primary tumor.

7.22 Pathology report for histologic confirmation of recurrent or persistent disease is not required for this protocol.

7.23 Stained slides to confirm eligibility by Central Pathology Committee Review are not required for this protocol.

7.3 Translational Research Requirements

7.31 Specimen Requirements

**The patient must give permission for her specimens to be used for this mandatory translational research component.** Participating institutions are required to submit the patient's specimens as outlined below (unless otherwise specified).

A detailed description of the Specimen Procedures can be found in Appendix III.

| **Required Specimen (Specimen Code)** | **Collection Time Point** | **Ship To** |
|---|---|---|
| FFPE Primary Ovarian Tumor (FP01)*<br>1st Choice: block<br>2nd Choice: 20 unstained slides (charged, 5µm) | Prior to all treatment | GOG Tissue Bank within 8 weeks of registration[1] |
| Pre-Treatment Whole Blood (WB01)<br>7–10mL drawn into a purple top (EDTA) tube | Collect at any time prior to administering PLD on Cycle 1, Day 1 | GOG Tissue Bank the day the blood is collected[1] |
| Pre-Treatment TruCulture™ (TC01)<br>1mL drawn into each of 4 TruCulture tubes and processed[2] | Collect at any time prior to administering PLD on Cycle 1, Day 1 | GOG Tissue Bank within 2 weeks of registration[1] |
| C1D3 Pre-VTX-2337/Placebo Plasma (PB01)<br>Prepare from 7-10mL of blood drawn into a purple top (EDTA) tube | Collect prior to administering VTX-2337 or Placebo on Cycle 1, Day 3 | |
| C1D3 8 Hour post-VTX-2337/Placebo Plasma (PB02)<br>Prepare from 7-10mL of blood drawn into a purple top (EDTA) tube | Collect 8 hours (± 1 hour) after administering VTX-2337 or Placebo on Cycle 1, Day 3 | |

* A copy of the corresponding pathology report must be shipped with all tissue specimens sent to the GOG Tissue Bank

1 GOG Tissue Bank / Protocol GOG-3003, Nationwide Children's Hospital, 700 Children's Drive, WA1350, Columbus, OH 53205, Phone: (615) 722-2865, FAX: (615) 722-2897, E-mail: GOGBank@nationwidechildrens.org

2 Please refer to Appendices III and IV for TruCulture™ specimen processing

7.32 Laboratory Testing

7.321 Pharmacogenomics

DNA will be isolated from whole blood by the GOG Tissue Bank. DNA will be shipped to the University of Washington, Tumor Vaccine Group (Seattle, WA) and used to examine single nucleotide polymorphisms (SNPs) in the TLR8 gene and to examine mutations in the BRCA1 and BRCA2 genes by conventional methods.

7.322 Baseline Immune Responsiveness (TruCulture™)

Processed TruCulture™ tubes will be shipped to Myriad/Rules Based Medicine (Austin, TX) to assess baseline immune responsiveness.

7.323 Immune Monitoring (HumanMAP®)

Plasma will be shipped to Myriad/Rules Based Medicine (Austin, TX) and used to assess specific analytes including chemokines, cytokines, and other inflammatory mediators by protein array technology.

7.324 Tumor Infiltrating Lymphocytes

Unstained sections of tumor will be shipped to Dr. George Coukos (University Hospital of Lausanne) for immunohistochemical and micro RNA analysis of T cell and other immune cell phenotype markers.

7.325 Proteomics

Plasma will be shipped to Dr. Karen Anderson (Biodesign Institute at Arizona State University) for assessment of autoantibodies to tumor-derived proteins by high-density programmable protein microarray.

# 8.0 EVALUATION CRITERIA

## 8.1 Assessment of Solid Tumors

In this study, disease parameters (section 8.11) and methods for tumor evaluation (Section 8.12) will be in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) guideline Version 1.1. [30]

RECIST 1.1 response criteria are primarily designed to evaluate the early effects of cytotoxic agents, and depend on tumor shrinkage to demonstrate biologic activity. However, clinical evidence of tumor responses seen with immunotherapeutic agents such as VTX-2337 can take longer to achieve, and may occur after a period of disease stabilization or following an initial increase in tumor burden. In light of the limitations of utilizing RECIST1.1 to evaluate immunomodulatory agents, immune-related response criteria (irRC) have been proposed to systematically detect the novel response patterns observed with immunologic agents. [31] Therefore, in this study, tumor response will be evaluated with an irRC modification of RECIST v1.1 for the study's co-primary endpoint (See also Section 8.2). In addition, tumor response will be evaluated by RECIST 1.1 as a secondary assessment of efficacy.

Patients will receive study treatment until disease progression per irRECIST.

### 8.11 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 10 mm with CT scan, as ≥ 20 mm by chest x-ray, or ≥ 10 mm with calipers by clinical exam. All tumor measurements must be recorded in decimal fractions of centimeters.

Note: Tumor lesions that are situated in a previously irradiated area will not be considered measurable unless progression is documented or a biopsy is obtained to confirm persistence at least 90 days following completion of radiation therapy.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness should be ≤ 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), are considered non-measurable disease. Leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and

abdominal/pelvic masses (identified by physical exam and not CT or MRI), are considered as non-measurable.

Notes:

Bone lesions: Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above. Blastic bone lesions are non-measurable.

Cystic lesions: that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. "Cystic lesions" thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions: All measurable lesions, up to a maximum of 2 lesions per organ and 5 lesions in total—representative of all involved organs—should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, and in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement, in which circumstance the next largest lesion which can be reproducibly measured should be selected.

The sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) of all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline SD will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease), including any measurable lesions over and above the 5 target lesions, should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

8.12 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is required.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is ≤ 5 mm. MRI is also acceptable in certain situations (e.g., for body scans), but NOT lung.

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline, and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, subsequent image acquisitions should use the same type of scanner and follow the baseline imaging protocol as closely as possible. If possible, body scans should be performed with breath-hold scanning techniques.

PET-CT: At present, the low-dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for

use with RECIST measurements. PET-CT scans are not always done with oral and IV contrast. In addition, the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed. **For these reasons, the GOG will not allow PET-CT use for response criteria**.

FDG-PET: FDG-PET will not be used to assess tumor response in this study.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not allowed.

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

It is mandatory to obtain cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when measurable disease has met criteria for response or stable disease. This confirmation is necessary to differentiate response or stable disease versus progressive disease, as an effusion may be a side effect of the treatment.

## 8.13 Response Criteria (RECIST 1.1)

**THIS SECTION IS FOR REFERENCE FOR THE SECONDARY ENDPOINT ONLY.** All subjects will receive study treatment until disease progression per **irRECIST.** Refer to Section 8.2.

Determination of response should take into consideration all target (see 8.131) and non-target lesions (see 8.132) and if appropriate, biomarkers (see 8.133).

8.131 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression by RECIST 1.1 [but not irRECIST; see section 8.2 below].)

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Not evaluable (NE): When at least one target lesion is not evaluated at a particular time point.

8.132 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Note: If CA-125 is initially above the upper normal limit, it must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of CA-125 level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Not evaluable (NE): When at least one non-target lesion is not evaluated at a particular time point.

Although a clear progression of only "non-target" lesions is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

8.133 Evaluation of Biomarkers

If serum CA-125 is initially above the upper normal limit, it must normalize for a patient to be considered in complete clinical response.

8.134 Evaluation of Best Overall (unconfirmed) Response

The best overall response is the best time point response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest sum recorded since baseline). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria in some circumstances.

**Time Point Response for Patients with Measurable Disease at Baseline (i.e., Target Disease)**

| Target Lesions | Non-Target Lesions | Biomarker CA-125 | New Lesions* | Time Point Response |
|---|---|---|---|---|
| CR | CR | Within normal limits | No | CR |
| CR | Non-CR/Non-PD | Any value | No | PR |
| CR | NE | Any value | No | PR |
| PR | Non-PD or NE | Any value | No | PR |
| SD | Non-PD or NE | Any value | No | SD |
| NE | Non-PD | Any value | No | NE |
| PD | Any | Any value | Yes or No | PD |
| Any | PD** | Any value | Yes or No | PD |
| Any | Any | Any value | Yes | PD |

*See RECIST 1.1 manuscript for further details on what is evidence of a new lesion

** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

**Time Point Response for Patients with Only Non-Measurable Disease at Baseline (i.e., Non-Target Disease)**

| Non-Target Lesions | Biomarker CA-125 | New Lesions* | Time Point Response |
|---|---|---|---|
| CR | Within normal limits | No | CR |
| CR | Above normal limits | No | Non-CR/non-PD* |
| Non-CR/non-PD | Any value | No | Non-CR/non-PD* |
| NE | Any value | No | NE |
| Unequivocal PD | Any value | Yes or No | PD |
| Any | Any value | Yes | PD |

*See RECIST 1.1 manuscript for further details on what is evidence of a new lesion

** 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

8.135 Best Overall Confirmed Response

Confirmation of CR and PR for determination of best overall response is required for studies with a primary endpoint that includes response.

**Confirmed CR and PR for Best Overall Confirmed Response**

| Time Point Response First time point | Time Point Response Subsequent time point | BEST overall confirmed response |
|---|---|---|
| CR | CR | CR |
| CR | PR | SD, PD or PR* |
| CR | SD | SD provided minimum criteria for SD duration met, otherwise, PD |
| CR | PD | SD provided minimum criteria for SD duration met, otherwise, PD |
| CR | NE | SD provided minimum criteria for SD duration met, otherwise, NE |
| PR | CR | PR |
| PR | PR | PR |
| PR | SD | SD |
| PR | PD | SD provided minimum criteria for SD duration met, otherwise, PD |
| PR | NE | SD provided minimum criteria for SD duration met, otherwise, NE |
| NE | NE | NE |

*If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have

reappeared after CR). However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR or SD, not CR at the first time point. Under these circumstances, the original CR should be changed to PR or SD and the best response is PR or SD.

**In non-randomized trials where response is part of the primary endpoint, confirmation of CR or PR is needed to deem either one the "best overall response". Responses (CR and PR) require confirmation ≥ 4 weeks from initial documentation.**

The minimum criterion for SD duration is 6 weeks.

Patients with a global deterioration of health status requiring discontinuation of treatment or die without objective evidence of disease progression at that time should be reported to be off study treatment due to "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

## 8.2 Response Assessment: Immune-Related RECIST

As previously noted, this study will assess tumor response with an immune-related modification of RECIST 1.1 for the co-primary endpoint (see Table 8.2-1 below).

Determination of response via irRECIST should take into consideration all target and non-target lesions.

A key distinction between standard RECIST 1.1 criteria and immune-related response criteria is that irRECIST requires early evidence of progressive disease (i.e., a determination of irPD ≤ 12 weeks after starting study treatment) be confirmed by repeat, consecutive imaging ≥ 4 weeks after the initial documentation in the absence of rapid clinical deterioration. During this interim ≥ 4 week period, patients should continue to be followed per protocol, including continued dosing of the study drug(s).

Additionally, the immune-mediated responses expected from VTX-2337 require activation of the immune system prior to the observation of clinical responses, and such immune activation may take weeks to months to be clinically evident. Some patients with advanced cancer may have objective volume increase of tumor lesions within 12 weeks following the start of dosing on study. Such patients may not have had sufficient time to develop the required immune activation or, in some patients, tumor volume increases may represent infiltration of lymphocytes into the original tumor. In traditional oncology studies, such increases in tumor volume during the first 12 weeks of the study would constitute

progressive disease and lead to discontinuation of study treatment and of imaging to detect response, thus disregarding the potential for subsequent immune-mediated clinical response. Therefore, in this study, the first imaging assessment will be performed at Week 12, followed by assessment every 8 weeks thereafter.

**Table 8.2-1 Tumor Response Evaluation: Comparison Between RECIST 1.1 and irRECIST**

| Criteria | RECIST1.1 | irRECIST |
|---|---|---|
| New measurable lesions (≥10 mm) | Always represents PD | Incorporated into tumor burden |
| New non-measurable lesions (< 10 mm) | Always represents PD | Does not define progression but precludes irCR |
| Non-Target lesions | Changes contribute to defining BOR of CR, PR, SD, and PD | Contribute to defining irCR (complete disappearance required) |
| CR | Disappearance of all lesions | Disappearance of all lesions |
| PR | ≥ 30% decrease in the sum of the longest diameter of all target lesions compared with baseline, in absence of new lesions or unequivocal progression of non-target lesions | ≥ 30% decrease in tumor burden compared with baseline |
| SD | Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study | Neither a 30% decrease in tumor burden compared with baseline nor a 20% increase compared with nadir can be established |
| PD | At least 20% increase in sum of diameters of target lesions, taking as reference the smallest sum on study. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5mm. The appearance of one or more new lesions is also considered progression. | At least 20% increase in tumor burden compared with nadir (at any single time point)* |

*** Patients with an initial finding of progressive disease (irPD) before or at the 12 week imaging assessment, but without rapid clinical deterioration, require confirmation of irPD with a second, consecutive scan obtained ≥ 4 weeks from the initiation documentation. Patients will continue to receive study treatment until irPD is confirmed at this later time point. Best overall response (BOR) will therefore include responses occurring at any time before disease progression and after early progression (i.e., within the first 12 weeks of the study).**

8.21 Response in Measurable Lesions

At baseline, the sum of the longest diameters (SumD) of all target lesions (up to 2 lesions per organ, up to total 5 lesions) is measured. At each subsequent tumor assessment (TA), the SumD of the target lesions and of new, measurable lesions (≥ 10 mm [lymph nodes ≥ 15 mm in shortest diameter]; up to 2 new lesions per organ, total 5 new lesions) are added together to provide the total measurable tumor burden (TMTB):

TMTB = SumD target lesions + SumD new, measurable lesions

Percentage changes in TMTB per assessment time-point describe the size and growth kinetics of both old and new, measurable lesions as they appear. At each TA, the response in target and new, measurable lesions is defined based on the change in TMTB (after ruling out irPD) as follows:

Complete Response (irCR): Complete disappearance of all target and new, measurable lesions, with the exceptions of lymph nodes which must decrease to < 10 mm in short axis

Partial Response (irPR): Decrease in TMTB ≥ 30% relative to baseline (see below).

Stable Disease (irSD): Not meeting criteria for irCR or irPR, in absence of irPD.

Progressive Disease (irPD): Increase in TMTB ≥ 20% relative to nadir.

8.22 Response in Non-measurable Lesions

At each TA, the presence of any new, non-measurable lesions is assessed. The presence of such lesions will rule out an overall response of irCR. An increase in the size or number of new, non measurable lesions does not necessarily imply an overall response of irPD; if these lesions become measurable (≥ 10 mm) at a subsequent TA, their measurement will at that point start to contribute to the TMTB.

In addition, the response in non-target lesions is defined as follows:

Complete Response (irCR): Complete disappearance of all non-target lesions

Stable Disease (irSD): Non-target lesions are stable

Progressive Disease (irPD): Unequivocal increases in number or size of non-target lesions. To achieve unequivocal progression of non-target lesions, there must be an overall level of substantial worsening of non-target disease that is of a magnitude that the treating physician would feel it is important to change therapy.

NOTE: Equivocal findings of progression of non-target lesions (e.g., small and uncertain new lesions; cystic changes or necrosis in existing lesions) should be considered irSD, and treatment may continue until the next scheduled assessment.

8.23 Evaluation of Biomarkers

Serum CA-125 must be within normal limits for a patient to be considered in complete clinical response

8.24 Overall Response

The OR according to the irRC is derived from the responses in measurable lesions (based on TMTB) as well as the presence of any non-measurable lesions as follows:

Complete Response (irCR): Complete disappearance of *all lesions* (whether measurable or not); lymph nodes must decrease to < 10 mm in shortest dimension. Serum CA-125 within normal limits.

Partial Response (irPR): Decrease in TMTB ≥ to 30% relative to baseline.

Stable Disease (irSD): Not meeting criteria for irCR or irPR, in absence of irPD.

Progressive Disease (irPD): Increase in TMTB ≥ 20% relative to nadir.

The immune-related best overall response (irBOR) is the best irRC OR over the study as a whole, recorded between the date of first dose until the last TA prior to subsequent therapy (including tumor resection surgery) for the individual patient in the study. As with the primary definitions of tumor response, early progression (i.e., irPD occurring prior to Week 12) will not preclude an irBOR of irCR, irPR or irSD resulting from the Week 12 assessment. An assessment of irPD at or after Week 12 will preclude a subsequent irBOR of irCR, irPR or irSD. However, any post-progression clinical activity in subjects with irBOR of irPD may be summarized for exploratory purposes.

**Table 8.2-2 Best Overall Response (irBOR)**

| **Target Lesions** Baseline (Index) and New Measurable Lesions | **Non-Target Lesions*** | | **irRC Overall Response** |
|---|---|---|---|
| **Total Measurable Tumor Burden (TMTB)** | **Baseline Lesions** | **Unequivocal New Lesions** | |
| irCR | irCR | No | irCR |
| irCR | irSD | No | irPR |
| irPR | irCR or irSD | No | irPR |
| irSD | irCR or irSD | No | irSD |
| irPD | Any | Yes or No | irPD |
| Any | Unequivocal Progression | Yes or No | irPD |
| Any | Any | Yes | irPD |

***NOTE: Any increase in the size or number of non-measurable lesions does not necessarily imply an overall response of irPD. If these lesions become measurable (≥ 10 mm) at a subsequent TA, their measurement will at that point start to contribute to the TMTB. To achieve unequivocal progression of non-target lesions, there must be an overall level of substantial worsening in non-target disease that is of a magnitude that the treating physician would feel it is important to change therapy. Equivocal findings of progression of non-target lesions (e.g., small and uncertain new lesions; cystic changes or necrosis in existing lesions) should be considered irSD, and treatment may continue until the next schedule assessment.**

8.3 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for irCR or irPR (whichever is first recorded) until the first date that progressive disease (irPD) is objectively documented (taking as reference for progressive disease the smallest measurements recorded [nadir] since the treatment started).

The duration of overall irCR is measured from the time measurement criteria are first met for irCR until the first date that irPD is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for irPD are met.

8.4 Progression-Free Survival

Progression-Free Survival (PFS) is defined as the duration of time from randomization to time of progression (irPD) or death, whichever occurs first.

8.5 Survival

Survival is defined as the duration of time from randomization to time of death due to any cause, or the date the patient was last confirmed to be alive.

## 9.0 DURATION OF STUDY

9.1 Patients will receive therapy until disease progression per irRECIST, or intolerable toxicity intervenes. Patients may refuse study treatment at any time.

Note: The recommended total cumulative dosage of PLD is 550 mg/m$^2$. Once this cumulative dose of PLD is reached (with no dose reductions, this would be 13 cycles of therapy), patients may—at the Investigator's discretion—continue on study and receive additional doses of PLD and investigational drug (VTX-2337 or placebo). Note that patients who continue to receive PLD beyond a cumulative dose of 550 mg/m$^2$ must undergo echocardiogram or MUGA scan every 2 cycles or in accordance with institutional standards to monitor for cardiac toxicity. Patients will discontinue treatment and be removed from protocol should their resting LVEF by echocardiogram or MUGA demonstrate an absolute decrease of > 10% below the institutional lower limit of normal or should they develop Grade 3 cardiac toxicity (symptomatic congestive heart failure).

9.2 A patient is considered off study therapy when the patient has progressed (irPD) or died, a subsequent drug or therapy (directed at the disease) is initiated, or all study therapy is discontinued. Report all treatment received on Form D2R and adverse events on Form T until the patient qualifies as being off study therapy.

9.3 Following treatment completion, all patients will be followed with physical exams and histories every three months for the first two years, and then every six months for the next three years, and then annually thereafter. Patients will be monitored for delayed toxicity and survival with Q forms submitted at these time points to the GOG Statistical and Data Center, unless consent is withdrawn.

# 10.0 STUDY MONITORING AND REPORTING PROCEDURES

Sponsor: VentiRx (IND # 78,416)

## 10.1 ADVERSE EVENTS AND REPORTING

The following subsections describe the minimum requirements for assessing and reporting adverse events, including serious adverse events and serious suspected adverse reactions, for subjects participating in trial GOG-3003. The Investigator is additionally responsible for complying with all local institution and institutional review board requirements for reporting adverse events.

### 10.11 Definitions

10.111 Adverse Event (AE): An adverse event is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related. An AE can therefore be any unfavorable and unintended sign, symptom, or disease which is temporally associated with the use of the investigational drug, whether or not considered related to (i.e. to have a causal relationship with) the investigational drug.

AEs include exacerbation of a pre-existing illness, an increase in frequency or intensity of a pre-existing episodic event or condition, a condition detected or diagnosed after investigational drug administration even though it may have been present prior to the start of the study, or a continuous persistent disease or symptom present at baseline that worsens following the start of the study.

Medical conditions and diseases that are present before starting PLD or VTX-2337 are only considered AEs if they worsen after starting treatment with the investigational drug. Any medical condition with an onset or diagnosis before the first administration of PLD on Cycle 1 Day 1 will be included in the subject's medical history. Abnormal laboratory values or test results constitute adverse events if they induce clinical signs or symptoms or if they require intervention or therapy.

10.112 Serious Adverse Event (SAE): An adverse event or suspected adverse reaction (see Section 10.15) is considered "serious" if, in the view of either the Investigator or sponsor, it results in any of the following outcomes:

- death,

- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

10.113 Suspected Adverse Reaction (SAR): An adverse event for which there is a reasonable possibility that the investigational drug caused the adverse event (i.e., that administration of VTX-2337 "possibly", "probably", or "definitely" caused or contributed to causing the adverse event). See section 10.15.

10.12 Reporting Requirements

10.121 Adverse Events: Information regarding all AEs, whether volunteered by the subject, discovered via questioning by the Investigator or clinical personnel, or identified through physical examination, laboratory test or other means will be collected from the time of the first dose of PLD until the End of Treatment visit. Suspected adverse reactions (i.e., AEs that are assessed as "possibly", "probably", or "definitely" related to VTX-2337) which are ongoing at the End of Treatment visit will be followed until resolution or until Grade $\leq 2$ and stabilized. Suspected adverse reactions that begin after the End of Treatment visit may be reported at any time.

Whenever possible, adverse events should be described in terms of a change in the baseline status (e.g., "increased frequency of migraines") or with a diagnosis or summary term in place of individual clinical signs or symptoms (e.g., "upper respiratory infection" vs. "runny nose", "sneezing", and "cough").

10.122 Serious Adverse Events: Serious Adverse Events (SAEs) will be collected from the time of the first dose of PLD or VTX-2337 until the End of Treatment visit . Serious suspected adverse reactions (i.e., SAEs that are assessed as “possibly”, “probably”, or “definitely” related to VTX-2337) which are ongoing at the End of Treatment visit will be followed until resolution or until ≤ Grade 2 and stabilized. Serious suspected adverse reactions that begin after the final dose of VTX-2337 must be reported at the time they occur, and as described in section 10.17.

The Investigator is responsible for notifying the Institutional Review Board (IRB) or other appropriate committees of all SAEs in accordance with institutional and IRB policies.

10.13 Criteria for Determining Adverse Event Severity

Adverse events will be categorized and severity will be graded according to the National Cancer Institute’s (NCI) Cancer Therapy Evaluation Program (CTEP) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Note that “seriousness” and “severity” are distinct concepts. “Serious” is a term applied to an AE that meets specific regulatory requirements; it is typically associated with events that pose a threat to the subject’s life or functioning. “Severity” refers to the AE intensity classification. A severe AE may be of minor medical significance (e.g. Grade 3 headache), while an AE that is graded as mild in severity may be serious (e.g. Grade 1 stroke requiring overnight hospitalization).

10.14 Criteria for Determining Adverse Event Expectedness

An adverse event (Section 10.111) or suspected adverse reaction (Section 10.15) is considered “unexpected” if it is not listed in the Investigator Brochure or is not listed at the specificity or severity that is observed.

For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the Investigator Brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the Investigator Brochure listed only cerebral vascular accidents. “Unexpected”—as used in this definition—also refers

to adverse events or suspected adverse reactions that are mentioned in the Investigator Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Therefore, "unexpected" also refers to an adverse event or suspected adverse reaction that has not been previously observed (i.e. included in the Investigator Brochure) rather than from the perspective of such an experience not being unanticipated from the subject's disease state.

10.15 Criteria for Determining Adverse Event Causality

The following categories for determining the relationship of an AE to the investigational drug are as follows:

| Relationship | Attribution | Description |
|---|---|---|
| Unrelated to investigational agent/intervention | Unrelated | The AE *is clearly **NOT** related* to the intervention |
| | Unlikely | The AE *is **doubtfully related*** to the intervention |
| Related to investigational agent/intervention | Possible | The AE ***may be related*** to the intervention |
| | Probable | The AE ***is likely related*** to the intervention |
| | Definite | The AE ***is clearly related*** to the intervention |

10.16 Serious Unexpected Suspected Adverse Reactions

Federal regulations require that VentiRx Pharmaceuticals report any suspected adverse reactions that are both serious (Section 10.112) and unexpected (Section 10.14). Such events must be reported to the Food and Drug Administration (FDA) and all participating investigators (i.e., all investigators to whom VentiRx is providing drug under its INDs or under any Investigator's IND) within 15 calendar days after VentiRx receives the information and determines that it qualifies for reporting. Such notification must occur within 7 calendar days if the serious unexpected suspected adverse reaction (SUSAR) was fatal or life-threatening.

Therefore, once the Investigator has determined that an SAE has occurred, it is important to adhere to the reporting timeframes outlined in Section 10.17. After receiving the Investigator's SAE report from GOG, including the Investigator's assessment of causality, VentiRx will also assess the event for seriousness and the relationship to VTX 2337, and will determine if the event was unexpected.

Investigational New Drug Safety Reports (INDSRs) will be reported to the FDA by VentiRx Pharmaceuticals; copies of the report will be distributed to GOG who will distribute them to all participating clinical investigators. The Investigator is responsible for notifying the relevant IRB of all INDSRs in accordance with institutional and IRB policies.

10.17 Procedures for Serious Adverse Event Reporting:

The Investigator must immediately (≤ 24 hours of clinical site personnel becoming aware of the event) report to GOG any serious adverse event—whether or not considered drug related, including those listed in the Investigator Brochure—and must include an assessment of whether there is a reasonable possibility that the investigational drug caused the event. Follow-up information or new information regarding an ongoing SAE must be provided promptly to GOG.

Initial and follow-up serious adverse reports are to be submitted using SEDES.

Reporting to VentiRx: The GOG Regulatory Department will forward all SAE reports to VentiRx within 24 hours of first awareness to VentiRx Pharmaceuticals.

## 10.2 GOG DATA MANAGEMENT FORMS

The following forms must be completed and submitted to the GOG Statistical and Data Center (SDC) in accordance with the schedule indicated below. Protocol forms and instructions can be submitted through or printed from the SDC Electronic Data Entry System (SEDES) online application found at the GOG Web Menu page. All amendments to forms submitted through SEDES must also be submitted through SEDES. The original form and required copies for forms NOT submitted online must be mailed to the GOG SDC. Pathology material (Form F, pathology reports) should be submitted together via postal mail. The GOG Uploader Application in SEDES is an alternate method for submitting Form BDR, Operative reports, discharge summaries, Form F, pathology reports.

| Form | Due within | | Copies* | Comments |
|---|---|---|---|---|
| | Weeks | Event | | |
| Specimen Consent Application | 1 | Registration | N/A | Online |
| Form R (Registration Form) | 2 | Registration | 1 | Mandatory Submission via SEDES |
| Form OHR (Recurrent Ovarian Cancer On-Study Form) | 2 | Registration | 1 | Mandatory Submission via SEDES |

| Form | Due within | | Copies* | Comments |
|---|---|---|---|---|
| | Weeks | Event | | |
| Form DR (Pre-Treatment Summary Form) | 2 | Registration | 1 | Mandatory Submission via SEDES |
| Primary disease:**<br>Pathology Report | 6 | Registration | 1 | Submit to SDC via postal mail or via report uploader |
| Recurrent or Persistent Disease:**<br>Pathology Report (only if histologically documented) | 6 | Registration | 1 | |
| Form BMR (Biomarker Reporting Form) *** | 2 | Registration, beginning of cycle 4 and every other cycle thereafter | 1 | Mandatory Submission via SEDES |
| Form D2R (Cycle Dose Drug Form) | 2 | Beginning of each subsequent cycle and after the last cycle | 1 | Mandatory Submission via SEDES |
| Form irRECIST (Immune Related RECIST Form) | 2 | Registration and subsequent Clinical Response Assessments | 1 | Mandatory Submission via SEDES |
| Form T (Common Toxicity Reporting Form) | 2 | Beginning of each subsequent cycle and after last cycle | 1 | Mandatory Submission via SEDES |
| Form SP-FP01-3003 FFPE pre-treatment primary ovarian tumor**** | 8 | Registration | N/A | Mandatory Submission via SEDES† |
| Form SP-WB01-3003 pre-treatment whole blood | 1 | Registration | N/A | Mandatory Submission via SEDES† |
| Form SP-TC01-3003 pre-treatment TruCulture | 2 | Registration | N/A | Mandatory Submission via SEDES† |
| Form SP-PB01-3003 C1D3 pre-VTX-2337/placebo plasma | 2 | Registration | N/A | Mandatory Submission via SEDES† |
| Form SP-PB02-3003 C1D3 8 hour post-VTX-2337/placebo plasma | 2 | Registration | N/A | Mandatory Submission via SEDES† |
| Form Q0 (Treatment Completion Form) | 2 | Completion of study treatment | 1 | Mandatory Submission via SEDES |
| Form Q (Follow up) | 2 | Disease progression; death; follow up; change in treatment | 1 | Mandatory submission via SEDES, Submit quarterly for 2 years, semi-annually for 3 more years, annually thereafter |

* The number of required copies including the original form which must be sent to the Statistical and Data Center. No copies are required for forms submitted through SEDES. Forms submitted through SEDES should not be sent through post or fax.

** Pathology slides for Central Pathology Committee Review are not required on this study.

*** Serial CA-125 values should be reported on Form BMR.
**** A copy of the corresponding pathology report must be shipped with all tissue specimens sent to the GOG Tissue Bank.
† Form SP must be submitted via SEDES regardless of whether the specimen is submitted for research.

## 11.0 STATISTICAL CONSIDERATIONS

The primary objectives of this study are to assess the efficacy of the combination of PLD plus VTX-2337 relative to that of PLD alone by comparing the overall survival and progression-free survival based on irRECIST between the two treatment groups.

Patients will be stratified by platinum-free interval (≤ 6 months and > 6 to 12 months) and GOG performance status (0 versus 1). The randomization will be stratified using blocked randomization, and will be done in a 1:1 ratio to one of the following arms:

Arm 1: PLD 40 mg/m$^2$ plus VTX-2337 3.0 mg/m$^2$

Arm 2: PLD 40 mg/m$^2$ plus placebo

11.1 The parameters to be employed to assess toxicity and efficacy are:

11.11 Primary endpoints

11.111 Overall survival

11.112 Progression free survival based on irRECIST

11.12 Secondary endpoints

11.121 Progression free survival based on RECIST 1.1

11.122 Toxicities assessed using CTCAE v 4.

11.13 Exploratory endpoints

11.131 Best overall response rate based on irRECIST and RECIST 1.1

11.132 Duration of best overall response rate based on irRECIST and RECIST 1.1 based on the probability of being in response function (PBRF)

11.133 TLR8 polymorphisms and BRCA1/BRCA2 mutations and their relationships to clinical effects (PFS, ORR, and AEs) of treatment

11.134 Baseline immune status and its relationship to clinical effects (PFS, ORR, and AEs) of treatment

11.2 The study accrued 297 subjects over a period of 18 months (October 2012–April 2014).

11.3 Study Design

This is a randomized, blinded, placebo-controlled, two-arm phase II design.

Commensurate with protocol Amendment 3, overall survival (OS) and progression-free survival as assessed by irRECIST (PFS by irRECIST; irPFS) will be co-primary endpoints. A parallel-testing approach will be utilized, whereby the final analysis of both endpoints will occur when the requisite number of events has been met for the OS endpoint, as specified below. To control the overall type I error at 0.10 (10%) while testing both co-primary endpoints, the OS endpoint will be tested at the one-sided 0.08 (8%) alpha level, and the irPFS endpoint will be tested at the one-sided 0.02 (2%) alpha level.

The primary analyses will be of overall survival and irPFS using a log rank test stratified by the randomization strata: platinum-free interval (≤ 6 months versus > 6 to 12 months) and GOG performance status (0 versus 1). OS and irPFS will be summarized and displayed by treatment arm using Kaplan-Meier methods. The hazard ratio between the two treatment arms, as well as the associated one-sided 90% confidence interval, will be presented using a Cox proportional hazards regression model. The null and alternative hypotheses to be tested are:

$$H_0: \theta \geq 1 \quad \text{vs.} \quad H_1: \theta < 1,$$

where $\theta = \lambda_E / \lambda_C$ is the hazard ratio for death for the OS endpoint and the hazard ratio of first disease progression (irPD) or death for the irPFS endpoint, where $\lambda_E$ and $\lambda_C$ are the hazard rates in the experimental (PLD + VTX-2337) and control (PLD + placebo) arms, respectively.

Overall survival: In order to have 88.2% power to detect a 30% reduction in the hazard of death ($\theta = 0.70$) in the combination therapy arm versus the monotherapy arm based on a one-sided significance test with alpha controlled at 0.08 for the overall survival endpoint, 211 events are required.[32, 35] Therefore, the final analysis will be done when at least 211 deaths have been reported among all of the patients enrolled.

In a previous randomized trial of Doxil 40 mg/m$^2$ versus gemcitabine 1000 mg/m$^2$ in ovarian cancer patients who had received only one platinum/paclitaxel regimen and who recurred or progressed within 12 months after completion of primary treatment, the median OS was 12.9 months in the 76 patients who received Doxil.[33] Based on this, we anticipate median OS to be approximately 12 months in the PLD + placebo arm of this study. The hazard ratio of 0.70, for which the study is powered, corresponds to an increase in median OS to 17.1 months. Based on this assumption, in order to observe 211 events, we expect to enroll 290 patients over a period of 26 months or less, and a 14 month post-accrual follow-up period will be necessary.

Progression-free survival: As noted above, analysis of PFS by irRECIST will occur in parallel with OS, and will take place when at least 211 overall survival events have occurred. Assuming that 90% of enrolled patients will have died or had a disease progression event (irPD) at the time of the final analysis, there will be approximately 265 PFS events at that time. In this case, the study will have 83.3% power to detect a 31% decrease in the hazard of first progression or death ($\theta_{PFS}$=0.69). This effect size is comparable to increasing the reference group's expected median PFS duration from 5.5 months to 8.0 months under the exponential failure time model assumption.

The primary efficacy analyses of overall survival and progression-free survival by irRECIST, the secondary efficacy analyses of progression-free survival by RECIST 1.1, and best response based on irRECIST and RECIST 1.1 will use the intent-to-treat (ITT) principle; all randomized patients will be included in these analyses and all patients will be grouped according to their randomized arm.

11.4 Secondary and Exploratory Analyses

Statistical inference regarding the treatment effect on secondary efficacy endpoints (PFS by RECIST 1.1 and tumor response) will be made only if the primary analysis of overall survival or irPFS is statistically significant (as detailed above in Section 11.3); no other adjustments for multiple testing will be made. One-sided tests with alpha = 0.10 will be used.

PFS by RECIST 1.1 will be compared between the two treatment arms using the previously described stratified log rank test.

Objective tumor response will be compared between the two treatment arms using a stratified exact test. Duration of response will be compared between the treatment arms based on the probability of being in response function (PBRF). [34]

NCI CTCAE version 4.0 will be used to classify toxicities observed during treatment. Patients will be tabulated according to the maximum severity for each organ system or preferred term. Analyses of safety and toxicity parameters will include all patients who receive any study therapy.

Baseline immune status and changes from baseline in immune status will be examined with respect to overall survival, PFS, tumor response, and adverse events, and treatment effects on these clinical endpoints will examined relative to immune status. TLR8 SNPs will be examined in a similar manner.

11.5 Data from this protocol will be reviewed before each semi-annual meeting by the Study Chair in conjunction with the Statistical and Data Center. In some instances (e.g., because of unexpectedly severe toxicity), the Statistical and Data Center may elect—after consultation with the Study Chair, the study Sponsor, and the Medical Oncology Committee—to recommend early closure or suspension of this study.

The frequency and severity of all toxicities are tabulated from submitted case report forms and summarized for review by the Study Chair, Developmental Therapeutics Committee, and GOG Data Safety and Monitoring Board (DSMB) in conjunction with each semi-annual GOG meeting.
All serious and/or unexpected events are communicated to the Study Chair, sponsor, and regulatory agencies on an ongoing basis as mandated in the protocol. These reports are reviewed by the Study Chair (or designated co-chair) for consideration of investigator notification, amendment, or immediate study suspension. In this case, all participating institutions will then receive notification of the toxicities and reason for study suspension. Under these circumstances, accrual cannot be re-activated until the study is reviewed by the GOG Data and Safety Monitoring Board. However, patients currently receiving treatment may continue to receive treatment in accordance with protocol guidelines at the discretion of their physicians, unless directed otherwise.

11.6 Interim Analysis

No interim analyses of PFS or OS are planned.

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## GOG General Chemotherapy Guidelines

- For 21 or 28 day cycles, a patient will be permitted to have a new cycle of chemotherapy delayed up to 7 days without this being considered to be a protocol violation for major life events (e.g., serious illness in a family member, major holiday, vacation which is unable to be re-scheduled). Documentation to justify this decision should be provided.
- It will be acceptable for individual chemotherapy doses to be delivered within a "24-hour window before and after the protocol-defined date" for "Day 1" treatment of 21 or 28 day cycles. If the treatment due date is a Friday, and the patient cannot be treated on that Friday, then the window for treatment would include the Thursday (1 day earlier than due) through the Monday (day 3 past due).
- For weekly regimens, it will be acceptable for individual chemotherapy doses to be delivered within a "24-hour window," for example; "Day 8 chemotherapy" can be delivered on Day 7, Day 8, or Day 9 and "Day 15 chemotherapy" can be given on Day 14, Day 15, or Day 16.
- Chemotherapy doses can be "rounded" according to institutional standards without being considered a protocol violation (most institutions use a rule of approximately ± 5% of the calculated dose).
- Chemotherapy doses will be based on the subject's weight at baseline and will remain the same throughout the study. However, the doses will be recalculated if the patient has a weight change of greater than or equal to 10% from baseline.
- Maximum body surface area used for chemotherapy dose calculations will be 2.0 $m^2$. For chemotherapy dose calculations that use mg/kg, there will be no maximum kilogram amount used (doses will be calculated on actual weight in kilograms).

Congestive Heart Failure
New York Heart Association Criteria

| Class | Definition |
|---|---|
| **I** | No limitation: Ordinary physical activity does not cause undue fatigue, dyspnea, or palpitation |
| **II** | Slight limitation of physical activity: Such patients are comfortable at rest. Ordinary physical activity results in fatigue, palpitations, dyspnea, or angina. |
| **III** | Marked limitation of physical activity: Although patients are comfortable at rest, less than ordinary physical activity will lead to symptoms. |
| **IV** | Inability to carry on physical activity without discomfort: Symptoms of congestive heart failure are present even with rest. With any physical activity, increased discomfort is experienced. |

Source: Criteria Committee, New York Heart Association, Inc. Diseases of the heart and blood vessels. Nomenclature and criteria for diagnosis. 6th ed. Boston, Little, Brown and Co, 1964: 114.

# Specimen Procedures

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## I. Summary of Specimen Requirements

| Required Specimen (Specimen Code) | Collection Time Point | Ship To |
|---|---|---|
| FFPE Primary Ovarian Tumor (FP01)*<br>1st Choice: block<br>2nd Choice: 20 unstained slides (charged, 5µm) | Prior to all treatment | GOG Tissue Bank within 8 weeks of registration[1] |
| Pre-Treatment Whole Blood (WB01)<br>7-10mL drawn into a purple top (EDTA) tube | Collect at any time prior to administering PLD on Cycle 1, Day 1 | GOG Tissue Bank the day the specimen is collected[1] |
| Pre-Treatment TruCulture™ (TC01)<br>1mL drawn into each of 4 TruCulture tubes and processed[2] | Collect at any time prior to administering PLD on Cycle 1, Day 1 | GOG Tissue Bank within 2 weeks of registration[1] |
| C1D3 Pre-VTX-2337/Placebo Plasma (PB01)<br>Prepare from 7-10mL of blood drawn into a purple top (EDTA) tube | Collect prior to administering VTX-2337 or Placebo on Cycle 1, Day 3 | |
| C1D3 8 Hour post-VTX-2337/Placebo Plasma (PB02)<br>Prepare from 7-10mL of blood drawn into a purple top (EDTA) tube | Collect 8 hours (± 1 hour) after administering VTX-2337 or Placebo on Cycle 1, Day 3 | |

* A copy of the corresponding pathology report must be shipped with all tissue specimens sent to the GOG Tissue Bank

1 GOG Tissue Bank / Protocol GOG-3003, Nationwide Children's Hospital, 700 Children's Drive, WA1340, Columbus, OH 43205, Phone: (614) 722-2865, FAX: (614) 722-2897, Email: GOGBank@nationwidechildrens.org

2 Please refer to Section V and Appendix IV for TruCulture™ specimen processing

## II. Obtaining a GOG Bank ID

Only one GOG Bank ID (# # # # - # # - G # # #) is assigned per patient. All specimens and accompanying paperwork must be labeled with this coded patient 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 patient's study ID (GOG #) for all protocols with specimen requirements before requesting a Bank ID from the Tissue Bank Portal. **Be sure to indicate if the patient has a previous GOG # when registering.** This will ensure that the patient is only assigned one Bank ID.

The GOG ID – Bank ID Lookup on the Tissue Bank Portal can be used to search for an existing Bank ID. To lookup an existing Bank ID, enter the patient's GOG # and click Lookup Bank ID. To lookup GOG #(s) associated with a given Bank ID, enter the Bank ID (without dashes) and click Lookup GOG #.

Please contact User Support at the GOG Statistical and Data Center if you need assistance or have assigned more than one Bank ID to a patient (Email: support@gogstats.org; Phone: 716-845-7767).

## III. Requesting Specimen Kits

The GOG Tissue Bank will supply a kit for the collection and shipment of plasma samples. Myriad Rules Based Medicine (RBM) and VentiRx Pharmaceutical will provide supplies for the collection and processing of the TruCulture™ specimen.

Please note: Once processed, the TruCulture specimen should be shipped to the GOG Tissue Bank in the specimen kit with the plasma samples.

### A. Ordering Specimen Kits

1. One GOG Tissue Bank specimen kit per patient can be ordered online via the Kit Management link on the GOG website (under Data Entry on the Web Menu page). Each site may order two kits per protocol per day (daily max = 6 kits).

   Please contact the GOG Tissue Bank if you need assistance (Email: GOGBank@nationwidechildrens.org; Phone: 866-GOG-BANC/866-464-2262).

   Please plan ahead to allow time for kits to be shipped by ground transportation.

2. **TruCulture collection and processing supplies** will be shipped automatically from Myriad RBM and VentiRx at the time an eligible patient is registered for the study. Please allow 3–4 business days from patient registration for receipt. To ensure automatic shipment of TruCulture supplies, download the "Shipping Information Form" from the GOG Regulatory link, complete the form, and submit it to the GOG Administrative Office via mail (ATTN: Regulatory Department, Protocol GOG-3003) at the time of IRB approval.

TruCulture kits contain frozen materials and will be shipped Monday through Thursday for Tuesday through Friday delivery. TruCulture kits will not be shipped on Friday. If TruCulture supplies do not arrive within 4 business days of patient registration, please contact VentiRx at ClinicalTrials@ventirx.com.

## B. Materials Provided in the Specimen Kits

1. One GOG Tissue Bank specimen kit will be provided per patient. Each kit will consist of:

   - single-chamber shipping container
   - twenty cryovials
   - two transfer pipettes
   - two 15mL conical tubes
   - one secondary shipping envelope with absorbent material
   - one Tyvek envelope
   - Dry ice label (UN1845)
   - Exempt Human Specimen sticker

2. For each enrolled patient, RBM will supply:

   - four TruCulture tubes: three stimulant tubes, one null tube
   - one Monovette® syringe priming tube
   - one butterfly needle
   - four Seraplas valve separators with plungers

3. At the time the first patient is registered, VentiRx will provide the following supplies to be used for the duration of the study:

   - Digital Dry Block Heater with temperature probe
   - Heat block with 12-13mm diameter and depth of 40–55mm

## C. Unused Materials and Specimen Kits

Unused GOG Tissue Bank materials and specimen kits should be returned to the GOG Tissue Bank. Contact the bank if you have any questions about the return of unused materials.

Unused TruCulture collection and processing supplies should be discarded. Unused heat blocks do not need to be returned.

## IV. Submitting Formalin-Fixed, Paraffin-Embedded Tissue

### A. Requirement

**The patient must give permission for her specimens to be used for this mandatory translational research component.** The participating institution is required to submit specimens as outlined in Section I.

Formalin-fixed, paraffin embedded (FFPE) tissue should be the most representative of the primary tumor. Primary tumor should be collected prior to all treatment. Only one block may be submitted per tissue type.

Every attempt should be made to provide a FFPE block; however, if a block cannot be provided on a permanent basis, then 20 unstained slides (charged, 5µm) should be submitted. All tissue sections should be cut sequentially from the same block.

The type of specimen (block or slides) and should be specified on Form SP.

All FFPE tissue should be submitted with the corresponding pathology report.

### B. Purpose

FFPE will be used for immunohistochemical analysis of T cell markers.

### C. Time Points

FFPE primary tumor should be collected prior to receiving any treatment.

### D. Format for Labeling the Specimen

A waterproof permanent marker or printed label should be used to label the specimen with:

GOG Bank ID (# # # # - # # - G # # #)
GOG protocol number (GOG-3003)
specimen code (FP01)
collection date (mm/dd/yyyy)
surgical pathology accession number
block number

When labeling slides, only label on the top, front portion of the slide. Do not place a label on the back of the slide or over the tissue. The label must fit on the slide and should not be wrapped around the slide or hang over the edge.

## V. Submitting Whole Blood

### A. Requirement

**The patient must give permission for her specimens to be used for this mandatory translational research component.** The participating institution is required to submit specimens as outlined in Section I.

A lavender/purple top (EDTA) tube should be used for whole blood collection.

The type of blood collection tube (EDTA) should be specified on Form SP.

### B. Purpose

Whole blood specimens will be used for pharmacogenomics.

### C. Time Points

One whole blood specimen should be collected any time prior to administering PLD on Cycle 1, Day 1.

### D. Format for Labeling the Specimen

A waterproof permanent marker or printed label should be used to label the specimen with:

GOG Bank ID (# # # # - # # - G # # #)
GOG protocol number (GOG-3003)
specimen code (WB01)
collection date (mm/dd/yyyy)

### E. Instructions for Preparing the Specimen

1. Label the lavender/purple top (EDTA) collection tube(s) as described above. Multiple tubes may be used to collect the required amount.

2. Draw 7–10mL of blood into the labeled lavender/purple top tube(s). A minimum of 3mL is needed for processing.

3. Immediately after collection, gently invert the tube 5–10 times to mix the blood and EDTA.

4. Whole blood specimens should be refrigerated (4°C) until the specimens can be shipped. Ship whole blood to the GOG Tissue Bank the day the specimen is collected. If the whole blood absolutely cannot be shipped the day it is collected, the tube(s) should be refrigerated (4°C) until the specimen can be shipped.

## VI. Submitting TruCulture Specimens

### A. Requirement

**The patient must give permission for her specimens to be used for this mandatory translational research component.** The participating institution is required to submit specimens as outlined in Section I.

Four TruCulture tubes should be used for blood collection and processing.

The specimen type (whole blood) and the type of blood collection tube (Other, specify TruCulture) should be specified on Form SP.

### B. Purpose

TruCulture specimens will be used to determine baseline immune responsiveness.

### C. Time Points

TruCulture specimens should be collected any time prior to administering PLD on Cycle 1, Day 1.

### D. Format for Labeling the Specimen

A waterproof permanent marker or printed label should be used to label the specimen with:

GOG Bank ID (# # # # - # # - G # # #)
GOG protocol number (GOG-3003)
specimen code (TC01)
collection date (mm/dd/yyyy)

### E. Instructions for Preparing the Specimen

1. Prior to use, thaw the tubes for 1 hour at room temperature in a non-styrofoam or insulating rack. Never thaw the tubes at > 37˚C.

   *Note: Tubes may be thawed in the refrigerator overnight, as long as tubes have reached room temperature prior to blood draw.*

2. Draw 1mL of blood into each of the four TruCulture tubes using the TruCulture kits provided.

   *Note: Draw the stimulated tubes first and the null tube last.*

3. Place the culture tubes immediately in a dry heat block at 37°C for 24–26 hours.

4. After incubation, manually insert the plunger into the culture tube and separate the supernatant from the cells. Instructions for this step are provided in Appendix IV (or view the TruCulture instructional video at http://www.rulesbasedmedicine.com/products-services/truculture/truculture-video).

5. Immediately **freeze the TruCulture specimens in an upright position** in a -20°C freezer until ready to ship.

Please contact Ashley Garrett at Myriad RBM if you have questions about collecting and processing TruCulture specimens (Email: ashley.garrett@rulesbasedmedicine.com; Phone: 512-835-8026, x336).

## VII. Submitting Plasma

### A. Requirement

**The patient must give permission for her specimens to be used for this mandatory translational research component.** The participating institution is required to submit specimens as outlined in Section I.

A lavender/purple top (EDTA) tube should be used for blood collection to prepare plasma.

The type of blood collection tube (EDTA) should be specified on Form SP.

### B. Purpose

Plasma specimens will be used for immune monitoring.

### C. Time Points

Plasma should be collected:

1. Cycle 1, Day 3, prior to administering VTX-2337/placebo (PB01) and
2. Cycle 1, Day 3, 8 hours (±1 hour) after administering VTX-2337/placebo (PB02).

### D. Format for Labeling the Specimen

A waterproof permanent marker or printed label should be used to label the specimen with:

GOG Bank ID (# # # # - # # - G # # #)
GOG protocol number (GOG-3003)

specimen code (PB##, see above)
collection date (mm/dd/yyyy)

### E. Instructions for Preparing the Specimen

1. Label cryovials and a 15mL conical tube as described above. For plasma specimens going to the GOG Tissue Bank, use 2mL cryovials.

2. Draw 7–10mL of blood into lavender/purple top (EDTA) tube(s).

3. Immediately after collection, gently invert the blood collection tube 5–10 times to mix the blood and EDTA.

4. Centrifuge the blood at 1000g for 15 minutes at 4ºC (preferred) or room temperature to separate the plasma (top, straw-colored layer) from the red blood cells (bottom, red layer).

5. Transfer the plasma into a pre-labeled 15mL conical tube and gently mix.

6. Quickly, evenly dispense (aliquot) the plasma into the pre-labeled cryovials and cap the tubes securely. Place a minimum of 0.25mL into each cryovial.

7. Immediately **freeze the plasma in an upright position** in a -70$^{o}$C to -80$^{o}$C freezer or by direct exposure with dry ice until ready to ship. If a -70$^{o}$C to -80$^{o}$C freezer is not available for storage, store and ship on dry ice within 24 hours of collection.

## VIII. Submitting Form SP

### A. Form SP Requirements

Form SP must be completed and submitted online to the GOG Statistical and Data Center (SDC) using the SDC Electronic Data Entry System (SEDES). Form SP must be submitted for each specimen required for the protocol regardless of the specimen submission status. Specific instructions for completing Form SP are available via SEDES by scrolling down to the SP Forms for the specific protocol.

### B. Instructions for Submitting Form SP Online

Form SP must be submitted online using SEDES which is available on the GOG Web Menu under *Registration/Data Entry*. A copy of the completed form must also accompany each specimen shipped to the GOG Tissue Bank. Retain a printout of the completed form for your records. Form SP does not need to be sent to the GOG Tissue Bank when specimens are not collected.

To access Form SP for online submission, log onto the GOG Web Menu and use SEDES to electronically enter Form SP data. Any questions about access or problems should be directed to the User Support at the GOG Statistical and Data Center (Email: support@gogstats.org; Phone: 716-845-7767).

## IX. Shipping Specimens

### A. FFPE

FFPE tissue and a copy of the corresponding pathology report should be shipped using your own container at your own expense to:

GOG Tissue Bank / Protocol GOG-3003
Nationwide Children's Hospital
700 Children's Dr, WA1340
Columbus, OH 43205
Phone: (614) 722-2865
FAX: (614) 722-2897
Email: GOGBank@nationwidechildrens.org

**Do not ship FFPE tissue for Saturday delivery.**

### B. Whole Blood Specimens

All whole blood specimens should be shipped to the GOG Tissue Bank (address above).

Whole blood specimens can be shipped to the GOG Tissue Bank **Monday through Friday for Tuesday through Saturday delivery**. Please do not ship whole blood the day before a holiday. Use your own shipping container to ship specimens via **FedEx priority overnight**.

When shipping whole blood specimens, **please be aware that your Institution must comply with IATA standards** (www.iata.org). If you have questions regarding your shipment, contact the GOG Tissue Bank at GOGBank@nationwidechildrens.org or by phoning 866-GOG-BANC (866-464-2262).

To ship whole blood specimens you will need (1) a sturdy shipping container (e.g., a cardboard or styrofoam box), (2) a leak proof biohazard envelope with absorbent material*, (3) a puncture and pressure resistant envelope (e.g. Tyvek envelope), (4) an Exempt Human Specimen Sticker, and (5) a pre-paid FedEx air bill.

**If you will be shipping whole blood specimens from more than one patient, please put each specimen in a separate plastic zip-lock bag before placing the specimens in the shipping bag. You may include up to four different blood specimens in one biohazard envelope.*

If you do not have these materials available at your Institution, you may order them from any supplier (e.g., Saf-T-Pak; Phone: 800-814-7484; Website: www.saftpak.com).

**Instructions for Shipping Whole Blood Using Your Own Shipping Container**

1. Place the whole blood specimen in a biohazard envelope containing absorbent material. Expel as much air as possible before sealing the bag.

2. Wrap the biohazard envelope in bubble wrap or another padded material.

3. Place the padded tube(s) into a Tyvek envelope. Expel as much air as possible before sealing the envelope.

4. Place the Tyvek envelope in a sturdy shipping contained (e.g., cardboard FedEx box).

5. Insert a copy of the SP Form(s) into the box.

6. Attach an Exempt Human Specimen Sticker to the outside of the shipping container.

7. Print a pre-paid FedEx air bill using the Kit Management application (found under Data Entry on the Web Menu page). Attach the air bill.

8. Make arrangements for FedEx pick-up through your usual institutional procedure or by calling 800-238-5355.

## C. Frozen Specimens

All frozen specimens should be shipped to the GOG Tissue Bank (address above) using the specimen kit(s) provided.

Frozen specimens should be shipped **Monday through Thursday for Tuesday through Friday delivery**. Do not ship frozen specimens the day before a holiday.

Frozen specimens should be stored in an ultra-cold freezing/storage space (i.e., ultra cold $\leq$-70$^{o}$C freezer, liquid nitrogen, or direct exposure with dry ice) until the specimens can be shipped.

**Instructions for Shipping Frozen Specimens in a Specimen Kit**

1. Pre-fill the chamber of the specimen kit about 1/3 full with dry ice.

2. Place each set of frozen specimens in a separate zip-lock bag.

3. Place the zip-lock bags in the biohazard envelope containing absorbent material. Put the secondary envelope into a Tyvek envelope. Expel as much air as possible before sealing all envelopes.

4. Place the Tyvek envelope containing the frozen specimens into the kit and fill the chamber to the top with dry ice.

5. Insert the SP Forms.

6. Place the foam cover on top of the kit. Tape the outer box of the kit closed with filament or other durable sealing tape. Please do not tape the inner (foam) chamber.

7. Print a pre-paid FedEx air bill using the Kit Management application (found under Data Entry on the Web Menu page). Attach the air bill.

8. Attach the dry ice label (UN1845) and the Exempt Human Specimen sticker.

9. Arrange for FedEx pick-up through your usual Institutional procedure or by calling 800-238-5355.

## X. Distributing Specimens for Translational Research

The GOG Statistical and Data Center and Tissue Bank (or alternate laboratory) will coordinate the distribution of specimens to approved investigators for translational research. Specimen selection will be based on information regarding specimen procurement and condition as well as patient eligibility and consent, statistical considerations, and relevant clinical information and evaluation criteria.

For each shipment, an inventory of all specimens sent will be provided to the investigator and the Statistical and Data Center.

Investigators will not be given access to any personal identifiers.

Investigators will be responsible for the direct supervision and oversight of translational research performed and for keeping accurate records.

Investigators will ensure the results are linked to the appropriate specimen-specific identifiers and are responsible for transferring relevant laboratory data to the Statistical and Data Center.

At the discretion of the Chair of the Committee on Experimental Medicine and the Director of the GOG Tissue Bank, investigators may be required to ship any specimens (or by-products) remaining after the completion of the translational research to the GOG Tissue Bank.

### A. FFPE

Unstained sections of FFPE primary tumor will be distributed to Dr. George Coukos:

Dr. George Coukos
University of Pennsylvania
421 Curie Blvd., BRB2/3, Rm. 1209
Philadelphia, PA 19104
Phone: 215-662-3316
Fax: 215-615-0555
Email: gcks@mail.med.upenn.edu

### B. Whole Blood

The GOG Tissue Bank will isolate DNA from whole blood. Aliquots of DNA will be distributed to the University of Washington, Tumor Vaccine Group:

University of Washington, Tumor Vaccine Group
ATTN: Yi Yang
815 Mercer St, Rm 219
Seattle, Washington 98109
Phone: 206-685-8893
Fax: 206-685-3128
Email: yyangti@u.washington.edu

### C. TruCulture Specimens

Frozen TruCulture specimens will be distributed to Myriad RBM:

Bobby Gonzalez, Project Manager
Myriad RBM
3300 Duval St
Austin, TX 78759
Phone: 512-835-8026, x301
Fax: 512-835-4687
Email: bobby.gonzalez@rulesbasedmedicine.com

### D. Frozen Plasma

Frozen plasma will be distributed to Myriad RBM (address above).

## XI. Banking Specimens for Future Research

Specimens will remain banked in the GOG Tissue Bank and made available for approved research projects if the patient has provided permission for the use of her specimens for future cancer and/or non-cancer research. The patient's choices will be recorded on the

signed informed consent document and electronically via the online Specimen Consent Application. At the time of specimen selection for project distribution, the most recent consent information will be used.

**GOG Institutions can amend a patient's choices regarding the future use of her specimens at any time if the patient changes her mind.**

If the patient revokes permission to use her specimens, the GOG Tissue Bank will destroy or return any remaining specimens to insure the patient's wishes are honored. The patient's specimens will not be used for any <u>further</u> research; however, any specimens distributed for research prior to the revoking of consent cannot be returned or destroyed. In addition, the patient cannot be removed from any research that has been done with her specimens prior to revoking consent.

Please note, when return of specimens is requested, shipping will be at the expense of the submitting institution.


MYRIAD RBM™

**TruCulture is For Research Use Only. Not Intended for Use in Diagnostic Procedures.**

TruCulture is covered by the following patents:
US6410334B1, AU199954193A, EP1102988A2, AT308045T, DE59912716D1

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### Intended Use

TruCulture® is a whole blood culture system incorporating proprietary media and a mechanical separation system. Blood cells are cultured and then separated from media for analysis of soluble or cellular components.

This product is for research use only. It is not intended for use in diagnostic procedures or patient management.

### Background

The immune system consists of a complex, multi-layered signaling network that provides specific and sensitive responses to stimuli. Responses can vary greatly between individuals yet patterns for any given individual are remarkably consistent if standardization of sample collection and minimization of sample manipulation are employed. For accurate immunomonitoring data, it is essential that one conserve as close to *in vivo* conditions in an *in vitro* system.

### Product Description

TruCulture® is a simple, self-contained whole blood culture tube supplied with or without an immune system stimulant. After blood collection and incubation, a valve is manually inserted to separate the cells from the supernatant. The specimen can then be prepared for analysis according to the desired downstream applications, i.e. use the supernatant for protein biomarker analysis or use the cellular fraction for gene expression analysis. The TruCulture tubes have been developed and optimized for protein biomarker analysis using Myriad RBM's InflammationMAP® testing service that measures 46 protein biomarkers, chemokines, and acute phase reactants. Gene expression analysis results will vary depending on the methods used.

## BLOOD COLLECTION AND HANDLING

### Equipment Provided for Specimen Collection

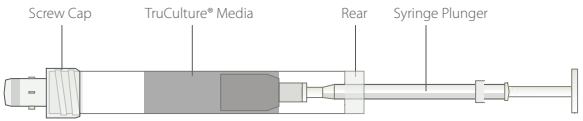
1. TruCulture® System
   a. TruCulture syringe-tube with stimulated or null media
   b. Sarstedt Monovette® priming tube
   c. Sarstedt sterile Multifly® needle set, 19G – 21G
   d. Seraplas valve separator with plunger

### Equipment Not Provided

1. Dry Block heater capable of maintaining consistent 37˚C temperature, external calibration device recommended
2. Heat blocks with 12 MM to 13 MM diameter and a hole depth of 40-55 mm (e.g. VWR Catalog # 13259-130)
3. Tube rack to freeze specimens upright. No Styrofoam racks should be used.
4. If sample analysis will not be completed by Myriad RBM, the following supplies may be necessary depending on your application:
   a. Protein biomarker analysis
      i. Transfer pipettes and tubes for supernatant
      ii. Vortex
      iii. Centrifuge capable of spinning down cells (400 – 500 g, brake turned off)
      iv. Reagents and equipment required for analysis
   b. Gene Expression
      i. Transfer pipettes
      ii. Transfer tubes
      iii. Reagents and equipment required for analysis
5. Eye protection, gloves and other personal protective equipment as necessary for prevention from exposure to blood borne pathogens using standard precautions.

### Prevention of Backflow

TruCulture® tubes contain serum-free cell culture media with or without immune stimulants. It is important to avoid possible backflow from the tube to prevent the possibility of the media entering the blood circulation. To protect against backflow, pull the syringe plunger back until it clicks and locks safely, and after venipuncture do not push the syringe plunger in for any reason.



## Recommended Draw Order

1. Use the Monovette® priming tube provided to fill the Multifly tubing system completely with blood.
2. TruCulture® tubes must be collected immediately thereafter to avoid activation of platelets.
3. If stimulated and null TruCulture tubes will both be collected, the stimulated tubes must be drawn first.
4. Any other tubes included in the study can be drawn from the same needle after filling the TruCulture tubes.

## Warnings and Precautions

1. Practice Standard Precautions. Use gloves, gowns, eye protection, other personal protective equipment, and engineering controls to protect from blood splatter, blood leakage, and potential exposure to blood borne pathogens.
2. Handle all biologic samples and blood collection "sharps" (lancets, needles, luer adapters, and blood collection sets) according to standard guidelines and the policies and procedures of your facility. Obtain appropriate medical attention in the event any exposure to biologic samples (for example, through a puncture injury), since they may transmit viral hepatitis, HIV (AIDS), or other infectious diseases.
3. Discard all blood collection "sharps" in biohazard containers approved for their disposal.



## Storage

TruCulture® tubes should be stored at -20˚C until ready for use.

All other TruCulture System components should be stored at room temperature.

## Specimen Collection and Culture Instructions

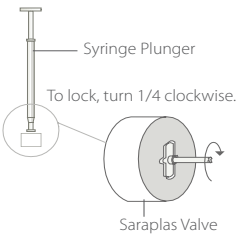
1. Follow universal precautions during blood collection to minimize exposure hazard.
2. Thaw the required number of TruCulture tubes for 1 hour at room temperature, in a non-styrofoam or insulating rack. Never thaw the tubes at >37˚C.
3. Label the tubes as appropriate.
4. Prior to drawing blood, press the plunger into the TruCulture tube until it stops.
5. Using the Multifly needle system provided, connect its adaptor to the front end of an empty Monovette syringe (priming tube) and lock it by turning clockwise.
6. Puncture the vein, ensure the cannula position is safe and the blood flows easily. Draw just enough blood to fill the tubing system of the multifly needle set completely.
7. Remove the priming tube and replace with the first TruCulture tube.

**Note: Do NOT, for any reason, depress the plunger after the TruCulture® tube has been attached to the inserted Multifly set.**

8. Fill the TruCulture tube slowly with blood by pulling the syringe plunger gradually until it snaps into its final position with a gentle click.
9. Wait for 5 seconds until the blood volume shows no further increase
10. Disconnect the TruCulture tube from the Multifly adaptor and gently mix the tube contents by inverting 3 times end over end, being careful to avoid foaming.
11. Break away the plunger close to the rear of the TruCulture tube.
12. Remove any blood remaining in the tube cap by gently tapping the bottom of the TruCulture tube on the bench top.
13. Place in 37˚C block thermostat with the tube-cap end point up.
14. Repeat steps 8 through 13 to fill additional TruCulture tubes if required.
15. Remove cannula when the desired number of tubes have been filled.
16. Incubate all TruCulture tubes at 37˚C in the block thermostat (or equivalent) for a study-defined period of time, preferably not to exceed 48 hours. The study-defined time should be strictly adhered to for all specimens of the same cohort. Any deviations should be noted, and it is recommended that the exact start and stop time of the cultures are recorded for each tube.
17. If the study-defined incubation time is less than 24 hours, centrifugation may be necessary to sediment layers. If clear layers are not visible after incubation, spin the tubes at 400 – 500 g for 10 min using a centrifuge with the brake off.

**Specimen Separation and Preparation Instructions**

18. Within 10 minutes prior to ending the incubation in step 16, assemble the Seraplas valve separators by inserting the plungers into the slot of the separator and lock them with a clockwise turn.
19. Carefully remove the TruCulture tubes from the incubator. Avoid shaking.
20. Remove the screw cap from each tube and slowly insert the assembled Seraplas valve until it is about 5 mm (1/4 inch) above the cell sediment level.




**Note: It is important to keep the TruCulture® tubes in an upright position during this procedure.**

21. Disconnect and remove the sticks from the separators with a counter-clockwise turn. The valve will stay in the TruCulture tube.

22. Close the TruCulture tubes with the screw caps (hand-tight).
23. Freeze the TruCulture tubes at -20˚C immediately, in an upright position.

**Note: If specimens are intended for gene expression analysis, remove supernatant and collect cell layer in the appropriate lysis buffer prior to freezing in order to preserve nucleic acid integrity. Please contact Myriad RBM at info@MyriadRBM.com for our recommended procedure.**

24. If tubes are being shipped to an external lab for protein biomarker analysis, ship them on dry ice, in an upright position. Do not use Styrofoam racks.
25. If tubes are being shipped to Myriad RBM for MAP analysis, ship frozen tubes upright on dry ice following the instructions outlined in the Human Sample Submission form located at www.myriadrbm.com/order/sample-submission-forms.aspx.

**In-house or Alternative Lab Protein Biomarker Analysis**

26. If tubes will be analyzed for protein biomarker analysis in house or at a facility other than Myriad RBM, thaw the tubes in an upright position prior to beginning the analysis.
27. Pour off the supernatant into a separate tube, removing as much of it as possible without disturbing the valve.
28. Vortex the tube with the supernatant.
29. Spin the supernatant at 400 g for 10 min to remove any particulates.
30. Begin applicable protein biomarker analysis protocol.

**Ordering Information:**

*List of Standard TruCulture® Stimulants*

Other stimulants are available, but require a custom manufacturing lot and a commitment to purchase a minimum number of tubes.

| Stimulant | Major Target Cells |
|---|---|
| Lipopolysacharride (LPS) | Monocytes, (T cells) |
| Zymosan | Granulocytes, monocytes |
| Anti-CD3 antibody | Th1>Th2 |
| Anti-CD3 antibody + Anti-CD28 antibody | Th1, Th2, regulatory T cells |
| Staphylococcal Enterotoxin type B (SE-B) | Th1>>Th2 |
| LPS + SE-B | Monocytes, T cells and Th1>>Th2 |

Example of CT/MRI Scheduling Tool
Located on the GOG-3003 website

**GOG-3003**

**CT/MRI Scheduler**

**Subject ID:**

**Initials:**

| C1D1 | Week 12 | | | Week 20 | | | Week 28 | | | Week 36 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Earliest | Target | Latest | Earliest | Target | Latest | Earliest | Target | Latest | Earliest | Target | Latest |
| 4-Nov-13 | 20-Jan-14 | 27-Jan-14 | 3-Feb-14 | 17-Mar-14 | 24-Mar-14 | 31-Mar-14 | 12-May-14 | 19-May-14 | 26-May-14 | 7-Jul-14 | 14-Jul-14 | 21-Jul-14 |

Enter the date for Cycle 1, Day 1. The remaining cells will populate automatically.