STATISTICAL ANALYSIS PLAN

Vitamin D, Leptin, Vitamin D Receptor Polymorphism, and Treatment-Related Morbidity in Ovarian, Primary Peritoneal and Fallopian Tube Cancer

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Abbreviations

PFS	Progression free survival
SAP	Statistical analysis plan

Contents

1. **Objectives**
    a. Primary Objectives
        1. To assess the treatment-related morbidity (including 30-day postoperative surgical morbidity and chemotherapy-related morbidity) and 1-year disease-free survival in patients with deficient serum 25(OH)D levels at the time of ovarian, primary peritoneal, or fallopian tube cancer diagnosis who did and did not receive vitamin D repletion.
    b. Major Secondary Objectives
        1. To describe serum 25(OH)D levels, serum leptin levels, and VDR FokI SNP status among newly diagnosed ovarian, primary peritoneal, or fallopian tube cancer patients.
        2. To determine the proportion of patients with ovarian, primary peritoneal, or fallopian tube cancer that have deficient vitamin D at diagnosis and have normal serum 25(OH)D after oral vitamin D supplementation during primary therapy.
        3. To identify potential relationships between serum 25(OH)D and serum leptin levels when controlling for BMI.
        4. To evaluate change of serum leptin levels with repletion of 25(OH)D in patients with low levels at the time of ovarian primary peritoneal, or fallopian tube cancer diagnosis.
    c. Translational Research Objectives
        1. To evaluate VDR FokI SNP status relationship with serum 25(OH)D levels and treatment-related morbidity and cancer outcomes.

2. **Design information**
    a. General statistical considerations

This study will contain 2 cohorts, those with normal serum 25(OH)D and those with low serum 25(OH)D at enrollment. The study will take place in women with ovarian, primary peritoneal, and fallopian tube cancer, subsequently referred to as ovarian cancer in this document due to the overlap in disease and treatment.

Patients with low serum vitamin D levels, defined as <20 ng/mL, will be provided a supply of cholecalciferol (vitamin D3), 50,000 IU weekly for 8 weeks that will be dispensed in the Stephenson Cancer Center outpatient pharmacy to standardize therapy. They will then be provided with a prescription for 2,000 IU of cholecalciferol (vitamin D3) daily following completion of 8 weeks of therapy. Treatment with cholecalciferol will not impact initiation of treatment for ovarian cancer. Compliance with cholecalciferol repletion will be assessed with medication reconciliation at every gynecologic oncology visit.

    b. Sample Size and Power

The anticipated enrollment is 100 patients based on estimated new diagnoses of ovarian cancer treated in the Division of Gynecologic Oncology annually at the Stephenson Cancer Center. Based on the previously mentioned unpublished data, we presume that at least 25 of the prospectively enrolled patients will be vitamin D deficient. All of the vitamin D deficient women will be repleted with oral vitamin D. We will compare outcomes between these patients

and 75 vitamin D deficient ovarian cancer patients from previous years (these will include patients from the above-mentioned previous retrospective review as well as other patients with banked blood samples) using logistic regression. We will control for age, stage, and histology. With 25 prospective patients and 75 retrospective patients we will have 80% power to detect differences of 32% in chemotherapy delay between ovarian patients who were not vitamin D deficient (33%) and who were vitamin D deficient (65%).

3. Study Populations

Inclusion and exclusion criteria are sometimes modified during the duration of the protocol. Before beginning analysis, the statistical team will review the criteria in the SAP to confirm it is up to date with the current criteria.

a. Eligible Patients
    1. The study population will consist of women with newly diagnosed ovarian, fallopian tube, or primary peritoneal cancer and no previous treatment.
    2. A retrospective cohort of women with ovarian, fallopian tube, or primary peritoneal cancer and low serum 25(OH)D that did not receive repletion identified from available tumor bank serum samples.

b. Inclusion Criteria
    1. Patients must have a histologic diagnosis of ovarian, fallopian tube, or primary peritoneal carcinoma per pre-treatment biopsies by laparoscopy, or interventional radiology, paracentesis, or CT guided core biopsy. Histologic documentation of the original primary tumor is required via the pathology report.
    2. Patients must have adequate:
        1. Renal function: Glomerular Filtration Rate (GFR) ≥ 30.
        2. Gastrointestinal absorption: No underlying malabsorption syndrome (i.e. inflammatory bowel disease, celiac disease)
        3. Ability to tolerate oral medication.
    3. Patients taking vitamin D at the time of enrollment without a diagnosis of vitamin D deficiency.
    4. Patients with childbearing potential must have a negative pregnancy test prior to the study entry and be practicing and effective form of contraception. If applicable, patients must discontinue breastfeeding prior to study entry.
    5. Patients must have signed an IRB-approved informed consent and authorization permitting release of personal health information.
    6. Patients must be at least 18 years old.

c. Exclusion Criteria
    1. Patients with a known pre-existing diagnosis of a vitamin D deficiency.
    2. Patients with renal disease and a GFR<30.
    3. Patients with primary hyperparathyroidism.
    4. Patients with other invasive malignancies, with the exception of nonmelanoma skin cancer. Patients with concomitant endometrial cancer diagnosed at the time of their ovarian cancer are allowed to participate if the endometrial cancer is FIGO stage IB or less.
    5. Patients of childbearing potential, not practicing adequate contraception, patients who are pregnant, or patients who are breastfeeding are not eligible for this trial.

4. Analysis Populations

The analysis populations are defined as below.

• **Safety population:** All patients who receive at least one dose of vitamin D will be included in the analyses of compliance and safety.

• **Evaluable population:** For tumor response analysis, the evaluable population will comprise all patients who have sufficient baseline and on-study measurements of tumor response parameters.

5. Summary of Study Population

a. Patient Disposition

1. Demographic, clinical, and disease characteristics and outcomes will be collected from all patients prospectively. Descriptive statistics will be used to summarize the demographic and clinical characteristics of patients. Chi-square and t-tests will be used to make comparisons between groups.
2. The association between serum leptin and 25(OH)D levels will be compared among groups, including any change in leptin levels observed in the vitamin D supplementation group. Because there is a positive linear association between BMI and serum leptin levels, BMI will be corrected for during analysis for -potential relationships between serum leptin and 25(OH)D.

b. Study Drug Administration and Compliance

1. Pill counts at each patient visit will be utilized to document compliance with oral medication.

6. Antitumor Evaluation

1. Treatment related morbidity (including 30-day postoperative surgical morbidity and chemotherapy-related morbidity) will be summarized by descriptive statistics as appropriate and compared across groups (chi-square for categorical variable).
2. Progression-Free Survival (PFS) at end of study (EOS) is defined as the time in months from first dose of the drug to the date of death or the first documented date of progression, whichever comes first. For patients who did not die and did not have progression, the PFS is censored at the last documented tumor assessment.

   Survival curve for PFS will be generated using the Kaplan-Meier method. Median PFS with 95% CI will be computed.

7. Translation Analyses

1. The proportion of patients with VDR FokI SNP will be assessed. We will also compare levels of vitamin D at diagnosis, differences in treatment-related morbidity or mortality including completion of treatment, treatment delays, survival and response to vitamin D supplementation across 3 SNP allele groups (homozygous mutated, heterozygous mutated, homozygous wild type).

8. Safety Analyses

a. Adverse Events

1. The descriptions and grading scale of the AE will be graded by the Investigator using the NCI CTCAE, version 4. For AEs that are not included in the CTCAE, the grading categories (mild, moderate, severe, life-threatening, and fatal) described in Table 1 will be used. The frequency of patients experiencing a specific AE will be tabulated by dose level, cycle, system organ class, preferred term, seriousness, worst severity, timing of occurrence, outcome, and relationship to study drug. In addition, the number and percentage of patients experiencing a specific AE will be tabulated similarly.

Table 1: The grading categories for AEs not included in the CTCAE

Severity	Grade	Description
Mild	1	The AE is noticeable to the patient but does not interfere with routine activity.
Moderate	2	The AE interferes with routine activity but responds to symptomatic therapy or rest.
Severe	3	The AE significantly limits the patient's ability to perform routine activities despite symptomatic therapy.
Life Threatening	4	The AE places the patient at risk of death at the time of the event.
Death	5	The AE results in the death of the patient.

9. Handling of Missing Data

Every effort will be made to collect information at all defined visits including at early withdrawal or dropout. Reasons for missing data will be summarized. However, there will be no imputation of missing data.