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A Phase II Study of Concurrent IGFBP-2 Vaccination and Neoadjuvant Chemotherapy to Increase the Rate of Pathologic Complete Response at the Time of Cytoreductive Surgery

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STUDY SYNOPSIS

Title

A Phase II Study of Concurrent IGFBP-2 Vaccination and Neoadjuvant Chemotherapy to Increase the Rate of Pathologic Complete Response at the Time of Cytoreductive Surgery

Study Population

Newly diagnosed patients with ovarian/fallopian tube/peritoneal cancer recommended for neoadjuvant chemotherapy with carboplatin and paclitaxel with subsequent cytoreductive surgery due to extent of metastases.

Rationale

The importance of immunity in ovarian cancer has been well established [1]. Most ovarian cancer patients have low levels or no evidence of Type I tumor infiltrating lymphocytes (TIL). Integration of vaccine strategies designed to elicit tumor specific Type I T-cells early in treatment may result in improved clinical outcomes in women with ovarian cancer. Patients particularly at risk for poor outcomes are those that are not surgically resectable. An approach to patients with advanced disease not thought to be amenable to surgical resection is neoadjuvant chemotherapy followed by interval cytoreductive surgery [2]. After neoadjuvant chemotherapy and surgery, patients with no residual disease have the best chance of survival [3]. The presence of a Type I adaptive immune response in the tumor is known to have prognostic significance. This effect is most likely due to CD8 cytotoxic T-cells which directly kill cancer as well as T-helper 1 (Th1) cells which secrete cytokines to activate local antigen presenting cells to expand destructive immunity.

Our group has been developing vaccines specifically aimed at stimulating Type I immunity for several years. We have recently made several advances that could facilitate successful application of selective Th1 vaccines to the clinic for the treatment of ovarian cancer. First, we have identified epitopes within the natural sequence of self-tumor antigens that selectively elicit either Th1 or are regulatory and induce antigen specific Th2 immune responses [4]. The Th1 selective epitopes, when used in a vaccine, can elicit unopposed Type I immunity and are effective in inhibiting cancer growth in pre-clinical models. If Th2 inducing epitopes are included in a vaccine, Th2 cells elicited via immunization will abrogate the anti-tumor response of Th1 cells. The second advance has been the identification of biologically relevant antigens associated with the development of epithelial to mesenchymal transition (EMT) and drug resistance such as IGFBP-2. Type I mediated elimination of cancer cells expressing IGFBP-2 may significantly enhance the effect of chemotherapy in ovarian cancer while immune surveillance stimulated by vaccination eliminates micro-metastatic disease. A final advance is the strategic use of chemotherapy concurrent with vaccination to augment the immune response. Platinum and taxanes have been shown to activate cytotoxic T-cells, augment Th1 responses, and inhibit T-regulatory (Treg) cells and myeloid derived suppressor cells (MDSC) which can limit Type I immunity. In addition, we have demonstrated the Type I cytokines released by T-cells induced by vaccination can have direct anti-proliferative effects on cancer cell growth and will sensitize the

tumor to chemotherapy resulting in greater cell kill. Neoadjuvant chemotherapy is known to enhance host immune responses and sequential chemoimmunotherapy has been suggested to improve disease control in advanced ovarian cancer. [5] The combined use of chemotherapy and an IGFBP-2 vaccine could be effective in enhancing the cytotoxic effects of chemotherapy. The purpose of this study is to increase the rate of complete response (CR) at the time of cytoreductive surgery in patients with advanced stage ovarian cancer

Objectives

Primary:

1. Determine whether the addition of an IGFBP-2 vaccine to neoadjuvant chemotherapy increases the rate of pathologic complete response (CR) induction.

Secondary:

1. Determine whether the addition of an IGFBP-2 vaccine to neoadjuvant chemotherapy increases progression free survival at 12 months.
2. Determine whether the addition of an IGFBP-2 vaccine to neoadjuvant chemotherapy improves overall survival.
3. To determine whether IGFBP-2 vaccination in combination with chemotherapy increases the level of TIL in the tumor.
4. To assess the level of IGFBP-2 Th1 elicited with vaccination concurrent with chemotherapy.

Exploratory:

1. To explore whether there is a predictive genomic signature for CR induction when IGFBP-2 vaccination is used in combination with chemotherapy.

Study Design This will be a Phase II single arm, non-randomized study in patients with advanced stage (III/IV) ovarian/fallopian tube/peritoneal cancer that are about to start neoadjuvant carboplatin / paclitaxel chemotherapy with subsequent cytoreductive surgery.

Number of Patients The study will accrue a maximum of 38 patients.

Outcome Measures

Primary Endpoints:

1. Rate of pathologic complete response after neoadjuvant chemotherapy.

Secondary Endpoints:

1. Progression free survival at 12 months.
2. Overall survival.
3. Immunohistochemical staining for tumor infiltrating T cells correlated with complete cytoreduction.
4. Th1 antigen specific T cells in peripheral blood correlated with surgical outcome.

Exploratory Endpoint:

1. Whole exome sequencing of tumor correlated with surgical outcome.

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Appendix A ECOG Performance Scale
Appendix B Calendar of Events
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Appendix D Common Chemotherapy Side Effects
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Abbreviations

APC	Antigen presenting cells
CR	Complete remission
CRC	Clinical Research Center
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTL	Cytotoxic T lymphocytes
DFS	Disease-free survival
DLT	Dose limiting toxicity
DSMP	Data and Safety Monitoring Plan
ECD	Extracellular domain of HER2
EMT	Epithelial to mesenchymal transition
ES	Epitope spreading
FHCRC	Fred Hutchinson Cancer Research Center
GMP	Good manufacturing practice
HER2	HER-2/neu
HLA	Human leukocyte antigen
i.d.	Intradermal
ICD	Intracellular domain of HER2
IDS	Investigational Drug Services
IFN-g	Interferon-gamma
IGFBP	Insulin-like growth factor binding protein
MDSC	Myeloid derived suppressor cells
MHC	Major histocompatibility complex
NED	No evidence of disease
OS	Overall survival
PBMC	Peripheral blood mononuclear cells

PFS	Progression-free survival
RFS	Relapse-free survival
rhuGM-CSF	Recombinant human granulocyte-macrophage colony stimulating factor; GM-CSF; LEUKINE® (sargramostim)
SI	Stimulation index
TGF-beta	Transforming growth factor beta
TIL	Tumor infiltrating lymphocytes
Th	T helper cells
Treg	T regulatory cell
TVG	Tumor Vaccine Group
ULN	Upper limit of normal
UPN	Unique patient number
UW	University of Washington

1. INTRODUCTION

Ovarian cancer is immunogenic, and immunity may confer a better prognosis.[6, 7] If immunity could be generated in the majority of advanced stage ovarian cancer patients during treatment induced remissions, perhaps clinical outcomes could be improved. A vaccine targeting immunogenic biologically relevant proteins in ovarian cancer could offer such a possibility. Unfortunately, there are several obstacles to vaccine development such as the lack of identified commonly expressed ovarian cancer antigens and the inability of the immune response elicited with vaccination to overcome the immunosuppressive ovarian cancer tumor microenvironment.[8]

IGFBP-2 is a tumor antigen in ovarian cancer patients and an excellent target for active immunization. The protein is overexpressed in most invasive ovarian cancers, is associated with invasive potential and the development of metastasis, and has shown to be a predictor of poor prognosis. Moreover, studies in animal models demonstrate that vaccination against IGFBP-2 significantly inhibits tumor growth without causing any toxicity, as does the infusion of IGFBP-2 specific T cells.[9]

We have extensive experience in developing vaccine strategies designed to elicit Type I inflammatory $CD4^+$ T helper immunity (Th1).[10] A focus on eliciting $CD4^+$ tumor specific Th1 cells with vaccination has several distinct advantages over immunization strategies designed to elicit predominantly $CD8^+$ T cells. First, vaccine stimulated tumor antigen-specific $CD4^+$ Th1 cells may home to the tumor and the inflammatory cytokines they secrete, such as IFN-gamma (g), may modulate the tumor microenvironment. Th1 cytokines enhance the function of local antigen presenting cells (APCs) and augment endogenous antigen presentation.[11] Increased processing of endogenous tumor cells results in epitope spreading, the development of an immune response to the multiple immunogenic proteins expressed in the tumor.[12] In addition, by providing a robust $CD4^+$ Th1 T cell response, tumor-specific $CD8^+$ T cells will be elicited and proliferate endogenously.[13] Finally, antigen specific $CD4^+$ T cells would provide the environment needed to enhance and sustain tumor specific T cell immune responses over time. We have identified multiple Th epitopes derived from IGFBP-2 which can be exploited in a polyepitope vaccine.

The trial described here is a Phase II, single arm study of a plasmid based DNA vaccine targeting IGFBP-2 amino acid (aa) sequence spanning from aa1-163. Studies from our group, described below, have identified this region of the protein as the most immunogenic encoding several native MHC class II peptides. Our primary objective is to assess the clinical efficacy of IGFBP-2 vaccination given concurrently with platinum-based neoadjuvant chemotherapy in patients with newly diagnosed advanced stage ovarian cancer. Our secondary objectives are to determine the immunogenicity of the vaccine, whether the vaccine elicit an IGFBP-2 Th1 immune response, increase the level of TILs, and whether administering vaccine with neoadjuvant chemotherapy improves progression free survival at 12 months and overall survival in this population. We will also explore whether there is a predictive genomic signature for complete response (CR) with IGFBP-2 vaccination given concurrently with neoadjuvant chemotherapy.

2. BACKGROUND

2.A. Rationale for immune therapy in ovarian cancer. Ovarian cancer, the most lethal of gynecologic cancers, is well suited to immunologic approaches to therapy. The majority of patients initially respond well to standard therapies, cytoreductive surgery and chemotherapy, obtaining a complete remission or minimal residual disease state. The time to relapse after definitive treatment in advanced stage patients can often be measured in months and years, providing a sufficient window of opportunity for the development of a therapeutic immune response against this low microscopic tumor burden. Moreover, ovarian cancer is immunogenic, and patients have been found to possess pre-existent immunity to tumor-associated proteins and

T cells that recognize autologous tumors.[6, 14-17] The presence of tumor infiltrating T cells in ovarian cancers has also been shown to confer a favorable prognosis while regulatory T cells and immunosuppressive B7-H4 expressing macrophages have an inverse association with survival.[1, 7, 18-20] Microarray expression analysis of advanced serous ovarian cancers has further demonstrated that the upregulation of immune function genes, particularly those associated with Type I immunity (Th1), is strongly correlated with improved survival.[21] The development of an effective therapeutic immune response via active immunization resulting in the eradication of minimal residual disease in ovarian cancer patients requires both an immune target that is expressed on the majority of ovarian cancers (we propose IGFBP-2) and a method of vaccination that can actively modulate the immunosuppressive factors in the ovarian cancer microenvironment (we propose plasmid DNA encoding multiple IGFBP-2 Th1 epitopes).

2.B. IGFBP-2 as a target for ovarian cancer immune therapy. The insulin like growth factor (IGF) pathway has become recognized for its role in the growth regulation of ovarian cancer. IGF signaling stimulates proliferation and inhibits apoptosis in cancer cells.[22] Insulin like growth factor binding proteins, such as IGFBP-2, transport IGFs from the circulation into tissues and form an important regulatory network controlling cell proliferation, migration, and apoptosis.[23] IGFBP-2 is one of six IGFBPs and is found at elevated levels in the sera of ovarian cancer patients.[24, 25] While IGFBP-2 can directly promote tumor growth, the protein is also a regulator of PI3K/Akt activation and may facilitate malignant transformation.[26, 27] Other studies have suggested that IGFBP-2 promotes invasion as well as metastasis.[28] IGFBP-2 upregulation measured by immunohistochemical staining with IGFBP-2 specific antibodies is specific for high grade invasive tumors as compared to low malignant potential tumors or cysts ($p<0.0001$).[28] IGFBP-2 is widely overexpressed in ovarian cancers. Quantitative real-time PCR performed on 113 epithelial ovarian cancers measured a 38-fold higher mean IGFBP-2 expression in epithelial ovarian cancers compared to normal ovarian epithelium.[24] In a large study of over 200 ovarian cancers, high levels of IGFBP-2 expression, as assessed by immunohistochemical staining, were detected in greater than 80% of serous tumors (n=173) and greater than 70% of endometrioid tumors (n=26).[29] Furthermore, overexpression of IGFBP-2 was preserved across all stages of invasive disease (stage I-IV).[29] In a study of nearly 100 patients with ovarian cancer, persistently elevated IGFBP-2 serum levels after cytoreductive surgery were associated with a greater rate of disease relapse, regardless of IGFBP-2 level in the tumor.[25] Taken together, these studies establish that IGFBP-2 is both significantly expressed and detected in the vast majority of epithelial ovarian cancers and suggest that stimulating immune eradication of IGFBP-2 expressing ovarian cancer cells may prolong remission or prevent relapse.

While IGFBP-2 is upregulated in cancer cells, basal levels of IGFBP-2 are found in many normal tissues. In particular, colon, lung, and thyroid tissue weakly express IGFBP-2 in a majority of samples tested. Studies from our group and others have indicated that overexpression or abundance of a protein within a cell makes that protein more amenable to immune recognition. Indeed, the immunogenicity of the HER-2/neu (HER2) protein is directly related to the level of its overexpression in primary breast tumors.[30] Theoretically, marked abundance of a protein in the cell may alter the peptide repertoire display as compared to peptides presented when the protein is at basal levels. Due to the potential risk of immune reaction directed against normal tissues after IGFBP-2 vaccination, however, patients will be monitored frequently by both blood analyses and physical exam during the course of this study.

2.C. IGFBP-2 is a human tumor antigen. We have shown that IGFBP-2 is a tumor antigen in patients with ovarian cancer.[9] Sera from patients with ovarian cancers (n=120) were more likely to have antibody immunity to IGFBP-2 than samples from volunteer donors (n=200), $p=0.03$. Moreover, we could identify peptide epitopes derived from IGFBP-2 that had high likelihood of binding to human class II MHC molecules. Previous studies from our group had demonstrated that high affinity binding peptides that were responsive across multiple DR alleles were likely to be native epitopes of tumor antigens, such as HER2.[30] We developed a combined scoring

system using widely available algorithms for predicting potential human class II binding in an effort to rank individual peptides that had a high likelihood of being encompassed in a sequence that bound with predicted high affinity across multiple HLA DR alleles, e.g. promiscuous epitopes. 14 peptides were constructed. Ten (71%) of the 14 evaluated peptides stimulated significant IFN-g ELISPOT responses in volunteer donors and ovarian and breast cancer patients. In addition, IGFBP-2 protein specific T cells could be generated after in vitro priming with the identified peptides.[9]

Human IGFBP-2 has a high degree of homology with murine IGFBP-2 (82%) and for this reason we questioned whether immunity to IGFBP-2 would impact tumor growth in mice. IGFBP-2 peptides p8-22, p251-265, and p291-305 were studied because they were shown to be native epitopes of human IGFBP-2, and all have significant homology with murine IGFBP-2 protein. We chose to evaluate therapeutic efficacy in the neu-transgenic (neu-tg) mouse model. Although the neu-tg mouse develops breast cancer, we hypothesized it would be a reasonable model to adapt to the study of ovarian cancer for several reasons; (1) there are few transgenic mouse models of ovarian cancer, (2) the immune system of the neu-tg mouse has been extensively characterized by our group. The animals do not mount significant immunity to their tumors via the same mechanisms that are in effect in patients with ovarian cancer, e.g. Tregs, (3) the tumors naturally up-regulate IGFBP-2, and (4) the mice express murine IGFBP-2 in normal tissues, thus, would provide an adequate animal model for toxicity testing prior to a clinical trial. Animals were immunized with a vaccine composed of all three peptides. Immunized mice developed murine IGFBP-2-specific IgG antibody immunity, the level of IGFBP-2 specific IgG was significantly higher post- than pre-vaccine in vaccinated mice ($p<0.05$). The vaccine generated both peptide and murine IGFBP-2 protein-specific IFN-g secreting T cells. IFN-g ELISPOT responses were significantly higher to the peptide mix ($p=0.0022$) and murine recombinant protein ($p=0.002$) as compared to no antigen wells. Mice demonstrated no toxicities associated with vaccination.

Mice were immunized twice, two weeks apart and then challenged with $1x10^6$ tumor cells. IGFBP-2 peptide vaccination inhibited tumor growth by approximately 50% compared with control groups. To assess the therapeutic efficacy of IGFBP-2-specific T cells, $1x10^7$ of in vitro cultured IGFBP-2 peptide specific T cells were adoptively transferred to 10-day tumor bearing mice. A single infusion of IGFBP-2 specific T cells inhibited tumor growth by 60%.[9] To further determine which cell subset was the mediator of the anti-tumor effect, mice were selectively depleted of $CD4^+$ and $CD8^+$ T cells just prior to and during IGFBP-2 immunization. The anti-tumor effect of vaccination was impacted by $CD8^+$ T cell depletion but not by $CD4^+$ T cell depletion.[9] Data suggests tumor antigen specific $CD4^+$ Th1 cells home to the tumor and secrete inflammatory cytokines, which modulate the tumor microenvironment and enhance the function of local APC. The actions of tumor specific Th1 in the tumor microenvironment facilitates cross priming allowing tumor antigen specific $CD8^+$ T cells to be elicited endogenously. This broadening of the immune response, or epitope spreading, has also been elicited in our clinical vaccine trials and is associated with improved survival.

In subsequent studies by our group, we screened PBMC derived from 40 donors for IGFBP-2 epitope specific T cells that preferentially secreted IFN-g or IL-10. In this population based study we identified 6 peptides that were associated with preferential IFN-g secretion and 8 peptides that preferentially induced IL-10 secretion in response to antigen. Peptides that elicited IFN-g secretion were clustered in the N terminus of the protein sequence (aa 1-163) whereas peptides that elicited IL-10 preferentially were clustered in the C terminus (aa 164-328). We constructed plasmid DNA vaccines using pUMVC3 including sequences 1-328 (whole protein) and the N terminus (1-163) and immunized mice. The vaccine derived from the N terminus elicited both IGFBP-2 peptide ($p=0.003$) and protein ($p=0.001$) specific immunity as assessed by ELISPOT as compared to vector alone controls. The full length sequence was non immunogenic. Moreover, the N terminus construct significantly inhibited tumor growth in the neu-tg mouse ($p<0.0001$) as compared to vector control or the full length construct which were not significantly different from

each other. Mice demonstrated no untoward effects to vaccination. These data demonstrate that IGFBP-2 is immunogenic in cancer patients, including ovarian cancer, and can mediate anti-tumor immunity in a biologically relevant murine model. Moreover, an extended sequence derived from the N terminus of IGFBP-2 contains multiple class II epitopes and elicits Th1 immunity that may be therapeutic.

2.D. Plasmid DNA is an optimal vaccine platform for eliciting long lasting tumor antigen specific Th1 immunity. We propose to deliver IGFBP-2 antigen in a plasmid based DNA vaccine. We have developed an extended Th epitope plasmid DNA based vaccine for HER2 (pNGVL3-hICD) and determined the optimal dose and route of administration of such a construct. The clinical application of plasmid based vaccines has several advantages over the use of synthetic peptides. Plasmid DNA can produce antigen inside a cell, enhancing the generation of a CTL response via antigen uptake in the class I processing pathway. Unlike peptides which are quickly degraded in the skin, plasmid DNA can stably transfect skin cells and result in a depot of antigen which may generate persistent immunity.[31] We have recently completed a 66 patient dose escalation study of pNGVL3-hICD which targets the HER2 ICD (an extended sequence similar to the IGFBP-2 vaccine). Vaccines were administered intradermally (i.d.) at a 10mcg, 100mcg or 500mcg/dose (22 patients/arm). The magnitude of pNGVL3-hICD induced IFN-g antigen specific ELISPOT response was significantly higher in the 100mcg vs. 10mcg dose; HER2 ICD (p=0.001), and ECD (p=0.0005). As the pNGVL3-hICD vaccine targets the HER2 ICD, the ECD responses reflect the development of epitope spreading and occurred in 77% of patients. The HER2 specific Th1 responses induced with high-dose pNGVL3-hICD (500mcg dose) were statistically identical to those induced with 100mcg pNGVL3-hICD. Thus, 100mcg was selected as the maximum biologic dose for antigen specific plasmid based DNA vaccines using this construct. Simultaneously, and in a similar patient population (optimally treated HER2+ Stage III/IV breast cancer), we had an ongoing Phase II study of a HER2 ICD peptide based vaccine. The median post-vaccination ICD IFN-g secreting Th1 response after synthetic peptide immunization (in the first 25 patients) was shown to be statistically identical to that generated with the intermediate and high dose of pNGVL3-hICD vaccine (p=0.400). Additionally, peptide-induced epitope spreading immune responses to the ECD were not significantly higher when compared to DNA induced responses (p=0.353).

Studies of plasmid based vaccines in infectious disease have demonstrated that the plasmid can stably transfect cells and persist at the site of vaccination for extended periods of time (>1yr). Data has suggested that this depot effect can enhance the generation of immunologic memory.[32] We biopsied the vaccine site at 1 and 6 months after plasmid immunization in 52 of 66 patients and the majority of subjects (75%) demonstrate persistent plasmid expression in skin at 6 months. Moreover, patients immunized with pNGVL3-hICD continue to show persistent HER2 ICD immunity of high magnitude post-vaccination. At 1 year after the end of active immunization 80% of subjects still have elevated levels of HER2 ICD immunity. In contrast, our recently published HER2 synthetic peptide vaccine trial reported approximately half of the patients had persistent HER2 ICD immunity at 1 year.[12]

2.E. The plasmid backbone, pNGVL3 (pUMVC3), is associated with minimal side effects when used in vaccination. Two key concerns associated with DNA vaccination are (1) potential integration of plasmid DNA into the host chromosomal DNA following direct injection, and (2) the potential risk of foreign DNA inducing an autoimmune state. Both of these theoretical safety concerns were addressed as part of the pNGVL3-hICD Phase I study. First, lymphocytes from study patients were evaluated for presence of plasmid vector pre- and 1 month post-vaccination. To date, there has been no detection of DNA plasmid in lymphocytes from patients immunized with any dose of pNGVL3-hICD. Additionally, DNA plasmid persistence in the skin (positive skin biopsies) as described above has not been associated with any adverse clinical sequelae to date.

Secondly, ANA, anti-dsDNA and complement levels (C3) were evaluated as markers of autoimmune disease as part of eligibility screening and then at each vaccine visit starting at

baseline and up to 12 months post vaccine. While a few patients developed positive ANA titers, the titers were low level and had resolved by the end of vaccination. Moreover, no patients developed anti-dsDNA antibodies, abnormal C3 levels and/or any clinical signs/symptoms suggestive of autoimmune disease. Thus, similar to published pre-clinical animal data our phase I findings have demonstrated only non-significant increases in autoimmune markers and no evidence of autoimmune disease induction or acceleration after plasmid DNA administration.[29]

Based on this phase I pNGVL3-hICD data, we expect minimal if any autoimmune toxicity. Yet, given that the administration of pUMVC3-hIGFBP-2 is the first-in-man, autoimmune parameters including ANA, C3, thyroid function, and anti-dsDNA will be evaluated prior to the first vaccine (baseline), prior to the third vaccine, and one, six and twelve months after the last vaccine. However, eligibility pre-screening for autoimmunity with ANA, anti-dsDNA, and C3 will not be done. Rationale for this is based on our phase I pNGVL3-hICD findings in which extensive screening of these autoimmune markers was conducted as part of eligibility. Specifically, 83 patients were screened for ANA, anti-dsDNA, and C3. All patients screened with this autoimmune panel had to pass the first criteria of not having an “active autoimmune process”. Of the 83 patients who were screened based on a negative history of an “active autoimmune process” only 5 patients (6%) had positive ANAs which were non-specific and very low titer. None of these patients had a positive anti-dsDNA or abnormal complement levels on screening. These findings corroborate current recommendations for the use of autoimmune markers including ANA and anti-dsDNA as initial screens of autoimmune disorders in that these markers are most useful only in patients with known or suspected autoimmune disease. Thus, in the absence of a known or suspected autoimmune disorder, these laboratory markers are non-specific and do not signify the presence of autoimmune illness.

These data suggest that plasmid based DNA vaccination, using sequences derived from tumor associated proteins such as IGFBP-2, which contain numerous Th1 epitopes, can be safely administered and result in long lived immunity.

2.F. Neoadjuvant chemotherapy and modulation of the tumor immune microenvironment

The approach to patients with advanced disease not thought to be amenable to optimal surgical resection is often neoadjuvant chemotherapy followed by interval cytoreductive surgery.[2] While this approach is considered not inferior to primary surgery, its use has important implications for surgical milestones and the immune response to ovarian cancer. With the neoadjuvant approach, only complete cytoreductive surgery to no residual disease improves survival; the common definition of optimal cytoreduction to largest residual tumor diameter of less than 1 cm, which offers less precision in assessing total tumor burden, no longer does.[3] Furthermore, when neoadjuvant chemotherapy is used, tumor infiltrating T cells, the immune measure that has been associated with improved prognosis, is also no longer significant.[3]

Although cytotoxic chemotherapy has traditionally been viewed as being immunosuppressive, recent studies have shown that specific agents may possess significant immune stimulatory effects.[33] Platinum-based chemotherapies, the most widely used in the primary treatment of ovarian cancer, are now also being appreciated for their immune potentiating effects.[34] The use of therapeutic cancer vaccines in conjunction with platinum-based chemotherapy has also been shown to successfully augment antitumor responses in phase 2 clinical trials for lung cancer.[35] There is further evidence of T-cell activation in omental biopsies after neoadjuvant chemotherapy, specifically CD4+ T cells with enhanced interferon gamma production and antitumor Th1 gene signatures.[36]

We hypothesize that vaccination against IGFBP-2 can synergize with neoadjuvant chemotherapy to improve the rate of complete pathologic complete response at time of surgery. We further hypothesize that this will be accomplished through induction of a Th1 immune response, antigen specific T cells, and tumor infiltrating lymphocytes.

3. OBJECTIVES

3.A. Primary objectives

1. Determine whether the addition of an IGFBP-2 vaccine to neoadjuvant chemotherapy increases the rate of pathologic complete response (CR).

3.B. Secondary objectives

1. Determine whether the addition of an IGFBP-2 vaccine to neoadjuvant chemotherapy increases progression free survival at 12 months.
2. Determine whether the addition of an IGFBP-2 vaccine to neoadjuvant chemotherapy improves overall survival.
3. To determine whether IGFBP-2 vaccination in combination with chemotherapy increases the level of TIL in the tumor.
4. To assess the level of IGFBP-2 Th1 elicited with vaccination concurrent with chemotherapy.

3.C. Exploratory

To explore whether there is a predictive genomic signature for CR induction when IGFBP-2 vaccination is used in combination with chemotherapy.

4. DRUG INFORMATION

4.A. Chemotherapy (this will be performed per standard of care)
The accepted concurrent chemotherapy regimen under this clinical trial is paclitaxel (175 mg per square meter of body surface) administered as a 3-hour infusion immediately followed by an intravenous infusion of carboplatin (area under the curve of 6) [37] over 1 hour per Vergote et al [2]. However, carboplatin at an area under the curve of at least 5 are allowed [2].

4.B. IGFBP-2 Vaccine

The IGFBP-2 plasmid-based DNA vaccine, which contains the IGFBP-2 N terminus sequence (1-163), can be used clinically (BB-IND 14906, Sponsor Mary L. Disis).

We recently completed a Phase I study with the IGFBP-2 vaccine in 25 patients with ovarian cancer. Vaccination was safe and 90% of patients developed IGFBP-2 immunity.

4.C. Synthesis and characterization

Plasmid DNA (pUMVC3-hIGFBP-2) for clinical use will be amplified, quantified and vialed by the Biologics Production Facility at the Fred Hutchinson Cancer Research Center under GMP laboratory conditions.

4.D. Formulation and stability

The vaccine will be vialed as single doses containing DNA suspended in tromethamine/EDTA (TE) as a stabilizing buffer. The vaccine formulation is labeled to accurately reflect the product identity, concentration, lot number and fill date. These labels are attached to vials immediately post-fill. Vials will be subjected to microbial, sterility and stability testing to ensure safety and stored at -20ºC ± 5ºC until use.

4.E. GM-CSF

Recombinant human GM-CSF (rhuGM-CSF; Sargramostatin, LEUKINE) will be used as adjuvant admixed with IGFBP-2 DNA plasmid based vaccine. GM-CSF is a growth factor that supports the survival, clonal expansion, and differentiation of hematopoietic progenitor cells including dendritic APCs. In general, the use of GM-CSF is associated with little toxicity and rhuGM-CSF, when administered IV or SC, is generally well tolerated at doses ranging from 50-500 mcg/m^2/day. Severe toxicity is extremely rare in patients treated with rhuGM-CSF. For the current study, rhuGM-CSF will be used at a total injection dose of 100 mcg.

Administration of rhuGM-CSF may aggravate fluid retention in patients with pre-existent edema, capillary leak syndrome, or pleural or pericardial effusions. In some patients with pre-existing renal or hepatic dysfunction, elevation of the serum creatinine or bilirubin and hepatic enzymes has occurred during rhuGM-CSF administration. Dose reduction or interruption of rhuGM-CSF administration has resulted in a decrease to pretreatment values. Occasional transient and reversible supraventricular arrhythmia has been reported in uncontrolled studies, particularly in patients with a previous history of cardiac arrhythmia. Stimulation of marrow precursors with rhuGM-CSF may result in a rapid rise in white blood cell count. If rhuGM-CSF is being used for the purpose of hematopoietic reconstitution, dosing should be stopped if the ANC exceeds 20,000/cm^3. The dose being used in this study should not have an effect on ANC levels. RhuGM-CSF may stimulate the growth of myeloid malignancies; therefore caution must be exercised in its use in these malignancies or myelodysplastic syndromes. Our group has given over 1100 vaccine injections mixed with 100 mcg GM-CSF to patients on previous studies. During those studies patients sometimes complained of mild to moderate flu like symptoms (fever, chills, achy, fatigue) for 1 – 2 days following vaccination which may be related to the use of GM-CSF.

Patients with contraindications to receiving rhuGM-CSF will not be eligible for study as noted in Section 5.B.4. Contraindications will include: (1) previous allergic reactions to GM-CSF, (2) laboratory values outside of an adequate range as described in Section 5.A.4 and (3) cardiac conditions as described in Section 5.B.1.

RhuGM-CSF is a sterile, white, preservative-free lyophilized powder in 250 mcg vials. Reconstituted rhuGM-CSF will be admixed and administered with IGFBP-2 DNA plasmid based vaccine.

4.F. Final vaccine preparation

The pUMVC3-hIGFBP-2 vaccine is supplied in single-use vials as a sterile, frozen solution. Each single-use vial has a volume of 1.2 mls. The pUMVC3-hIGFBP-2 vaccine will be supplied as frozen vials and each vial will carry a label bearing the drug identification and conditions for storage. Investigational Drug Services (IDS) pharmacy at the University of Washington (UW) will prepare and dispense the drug for administration per their standard operating procedure as follows:

- 170mcg DNA / 1 ml TE. Total DNA per vial is 200mcg. 0.6 ml (100mcg) of vaccine will be delivered to patients enrolled at this dose-level.

4.G. Vaccine product accountability

The IGFBP-2 DNA plasmid based vaccine and rhuGM-CSF will be managed by the UW IDS. UW IDS will be responsible for the study drug disposition (drug receipt, dispensing, transfer or return) and shall be documented on the Investigational Drug Accountability Record.

5. PATIENT SELECTION

5.A. Inclusion criteria

1. Patients with newly diagnosed advanced stage (III/IV) ovarian cancer (ovarian/fallopian tube/peritoneal cancer) who have been recommended to receive neoadjuvant carboplatin / paclitaxel chemotherapy with subsequent cytoreductive surgery.
2. Patients must have ECOG Performance Status Score of ≤ 2 (Appendix A).
3. Patients must have recovered from major infections and/or surgical procedures, and in the opinion of the investigator, not have any significant active concurrent medical illnesses precluding protocol treatment.
4. Estimated life expectancy of more than 6 months.
5. Adequate laboratory values within 30 days of enrollment to study defined as follows:
    a. WBC ≥ 3000/mm^3
    b. Hgb ≥ 10 g/dl
    c. Hct ≥ 28%
    d. Serum creatinine ≤ 2.0 mg/dl or creatinine clearance > 60 ml/min
    e. Total bilirubin ≤ 2.5 mg/dl
    f. AST/SGOT ≤ 3 times ULN
    g. Blood glucose <1.5 ULN
6. All patients who are having sex that can lead to pregnancy must agree to contraception for the duration of the study.
7. Patients must be at least 18 years of age.

5.B. Exclusion criteria

1. Patients with any of the following cardiac conditions:
    1. Symptomatic restrictive cardiomyopathy
    2. Unstable angina within 4 months prior to enrollment
    3. New York Heart Association functional class III-IV heart failure on active treatment
    4. Symptomatic pericardial effusion
2. Uncontrolled diabetes
3. History of (non-infectious) pneumonitis that required steroids or current pneumonitis
4. Patients with any contraindication to receiving rhuGM-CSF based products
5. Patients with any clinically significant autoimmune disease uncontrolled with treatment
6. Patients who are currently receiving an anti-IGF-IR monoclonal antibody as part of their treatment regimen
7. Patients who are simultaneously enrolled in any other treatment study
8. Patients who are pregnant of breastfeeding

6. EXPERIMENTAL DESIGN

6.A. Study design

This will be a phase II, single arm, non-randomized study. A total of 38 patients will be enrolled.

6.B. Sample size

To test the primary hypothesis on the pathological complete response induction rate, we will enroll 38 patients to treat with IGFBP-2 vaccine with neoadjuvant chemotherapy. This target sample size will provide 80% power to detect significance increase in pathological complete response with the one-sided type I error rate 0.05, when compared to the average historical pathological response rate at 0.075 with neoadjuvant chemotherapy alone.[38, 39] 7.5% is a fixed benchmark and this is a single arm trial.

6.C. Outcome measures

A. Primary endpoints:

1. The increased rate of pathologic complete response (CR) will be assessed.

B. Secondary and exploratory endpoints:

1. Evaluation of PFS will be followed through 12 months. Though not statistically powered to this endpoint, large differences in PFS if observed between the treatment groups will be noted and described.

2. Evaluation of OS will be followed and compared between the treatment arms. Though not statistically powered to this endpoint, large differences in OS if observed between the treatment groups will be noted and described.

3. Relate the response to the level of IGFBP-2 Type I T-cells elicited with vaccine by IFN-g ELISPOT assay to evaluate for the presence of Th1 and Th2 T cells responsive to IGFBP-2 epitopes.

   The level of IGFBP-2 Type 1 T-cells elicited will also be correlated to tumor burden at definitive surgery.

4. Increase level of TIL in the tumor will be evaluated by Immunohistochemical staining from tumor blocks obtained from cytoreductive surgery post neoadjuvant chemotherapy and vaccination. Immunohistochemical staining for CD3, CD4, CD8, and CD27 will be performed and quantitated using published methods and will be correlated with surgical CR [3, 40].

C. Exploratory endpoints:

1. Predictive signature of CR induction when vaccinated with an IGFBP-2 vaccine in combination with neoadjuvant chemotherapy.

7. PLAN OF TREATMENT (SEE APPENDIX B)

7.A. Active Treatment – Period (7A-7C): Initial Evaluation (may be performed within 2 weeks prior to initial vaccine visit)

1. Sign consent form before initiation of study treatment.
2. Medical history and complete physical examination which includes weight, vital signs, baseline symptom assessment and ECOG scoring (Appendix A).
3. Clinical labs used for determination of eligibility as defined above in (Section 5.A.4) may be used as baseline labs prior to vaccine 1 if < 30 days from enrollment. These include:
    a. Complete blood count with differential and platelet count.
    b. Comprehensive metabolic panel which includes electrolytes, glucose, creatinine, blood urea nitrogen, AST, ALT, alkaline phosphatase, and total bilirubin.
    c. CA-125.
4. Baseline ANA, C3, thyroid function tests, and anti-dsDNA.
5. Research blood:
    a. Approximately 200 mls for immunologic monitoring as described in Section 7.F. below.
6. Collect archived tissue from the primary diagnosis, if available. We plan to compare pre and post treatment samples to understand how the addition of IGFBP-2 vaccine alters the study responses.

7.B. Immunizations (approximately 2 weeks after each chemotherapy visit)

1. A physical examination will be done prior to each vaccination which will include weight, vital signs, medication review, symptom/toxicity assessment.
2. Patients who are having sex that can lead to pregnancy must have a negative urine pregnancy test. This excludes patients who have undergone permanent sterilization (i.e., tubal ligation, hysterectomy or menopause as defined per the NCCN Guidelines. See Appendix E).
3. Collection of lab results from their oncologist prior to their chemotherapy.
4. Tetanus diphtheria (Td) immunization if one has not been administered within six months (prior to first vaccine only).
5. Vaccinations will be given during neoadjuvant chemotherapy of combination carboplatin and paclitaxel and prior to interval cytoreductive surgery.
    a. Carboplatin and paclitaxel will be given IV every three weeks per standard of care by the patient's primary Gynecology Oncology or Medical Oncology physician.
        i. 3 chemotherapy cycles will be completed prior to surgery
            1. Additional chemotherapy cycles may be given but no additional vaccines
        ii. Clinical labs obtained at the beginning of each chemotherapy cycle will be reviewed prior to each vaccination.
    b. Vaccinations will be given intradermally approximately two weeks after each combination chemotherapy for 3 doses.

i. A total of three vaccines will be given on the arm or leg

ii. As much as possible, each vaccine will be administered within the same draining lymph node site

iii. All vaccines will be administered in the outpatient setting at the UW Clinical Research Center.

6. Post-immunization monitoring: Patients will be observed for a minimum of 60 minutes post immunization and post-vaccine vital signs will be documented (See Section 7.F. below).

7.C. Evaluation after the final vaccine

1. Approximately one to two weeks after the final vaccination:
   a. A physical examination will be performed which will include weight, vital signs, symptom/toxicity assessment.
      - Complete blood count with differential and platelet count.
      - Comprehensive metabolic panel which includes electrolytes, glucose, creatinine, blood urea nitrogen, AST, ALT, alkaline phosphatase, and total bilirubin.
      - CA-125.
      - ANA, C3, thyroid function tests, and anti-dsDNA.
   b. Research blood.
      - Up to 200 mls for immunologic monitoring as described in Section 7.F. below.
2. Collect pathology report, operative report, and tissue from surgery after neoadjuvant chemotherapy and vaccination. Immunohistochemical staining for CD3, CD4, CD8, and CD27 will be performed and quantitated and will be correlated with surgical CR. In addition, whole exome sequencing on vaccinated patients' tumors and we will correlate expression profiles with primary platinum sensitive, resistance and refractory outcomes to determine differences induced by vaccination.

7.D. Follow-Up (approximately 6 months after the last vaccine)

1. A physical examination will be performed which will include weight, vital signs, symptom/toxicity assessment as well as:
   a. Complete blood count with differential and platelet count.
   b. Comprehensive metabolic panel which includes electrolytes, glucose, creatinine, blood urea nitrogen, AST, ALT, alkaline phosphatase, and total bilirubin.
   c. CA-125.
   d. ANA, C3, thyroid function tests, and anti-dsDNA.
2. Research blood
   a. Up to 200 mls of blood will for immunologic monitoring as described in Section 7.F. below.
      -

7.E. Long-Term Follow-Up

Once a year for five years the patient's primary oncologist will be contacted for recent laboratory evaluation of toxicity, CA-125, and the patient's disease free and overall survival status. Clinical notes and/or laboratory values will be requested directly from the primary care physician.

7.F. Management of potential study risks

Although patients have a small chance of experiencing an allergic-type reaction to the vaccine, if a reaction were to happen, it would usually occur within one hour of the vaccination. For this reason, all patients will undergo observation and monitoring of vital signs for a minimum of 60 minutes post-vaccination.

Large volume blood draws (up to 200 mls) are required at designated study visits for immunologic monitoring. The amount of blood obtained for immunologic monitoring assays at designated time points is in strict adherence with guidelines set by the Bloodworks Northwest and are associated with minimal risks. However, as with any large volume blood draw patients should be well hydrated prior to the blood draw. Thus, during the consent process, and at subsequent visits, patients will be instructed to hydrate sufficiently prior to visits requiring large volume blood draws. Study physician will review recent hematocrit and hemoglobin values to determine a safe amount of blood to be drawn (not to exceed 200 mls for immunologic monitoring).

7.G. Storing samples for future testing

If any research blood or tumor tissue is left over after the study's immunologic monitoring, it may be stored for future research related to the development of other immunotherapies. The choice to store any leftover samples is up to the patient and whatever their decision, it will not affect their participation in this study.

8. Subject Withdrawal/Study Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator.

If a subject withdraws from the study, we will discuss with the subject participating in LTFU and post vaccine blood as outlined in 7.C and 7.D. If the subject agrees to participate in the LTFU and post-vaccine blood draws we will proceed with these as described above in 7.C., 7.D., and If the subject declines any further involvement in the follow-up of the study then they will not be contacted for LTFU and post-vaccine blood draws.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Unacceptable adverse experiences as described in Section 9
- Concurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- Noncompliance with trial treatment or procedure requirements
- Administrative reasons

We will continue to monitor clinically significant toxicity until the toxicity resolves or returns to an individual's baseline.

If patients are willing, we will continue to follow them for disease status and/or research blood to follow immunity as described in Section 7.D. above. We will report the study termination to the Fred Hutchinson Cancer Research Center Cancer Consortium Institutional Review Board at the time we become aware of the study termination (with 10 days).

9. EVALUATION AND MANAGEMENT OF TOXICITY

Patients will be asked to report local injection site reactions; and systemic reactions post vaccine visit. Toxicity will be evaluated after each vaccine visit. Toxicity grading will be evaluated according to the CTEP CTCAE v. 4.0 and the Data and Safety Monitoring Plan (DSMP) described in Appendix C.

9.A. IGFBP-2 Vaccine

For grade 1 or 2 vaccine-related reactions, patients may be treated with acetaminophen or Benadryl as clinically indicated at the discretion of the study clinician.

Table 1. Expected and Allowable Grade 3 and 4 toxicities (CTCAE v4.0) that are not DLT and felt to be related to vaccination

Category	Toxicity/Adverse Event	Allowable Duration
General disorders and administration	Flu-like symptoms	2 weeks
	Fever	2 weeks
	Malaise	2 weeks
Musculoskeletal and connective tissue disorders	Arthralgia	2 weeks
	Myalgia	2 weeks
Investigations	Lymphocyte count decreased	2 weeks
	Hemoglobin decrease	2 weeks
	White blood cells decreased	2 weeks
Gastrointestinal Disorders	Constipation	2 weeks
	Diarrhea	2 weeks
	Ascites	2 weeks
	Nausea/vomiting	2 weeks
Metabolism and Nutritional Disorders	Dehydration	2 weeks

If grade 3 or 4 toxicity at least possibly related to the vaccination (except as described above in Table 1) is observed, treatment if indicated, will be initiated per standard of care and that patient will be permanently removed from the study treatment. Additionally, if grade 3 or 4 toxicity at least possibly related to vaccine-induced immune responses is observed, a regimen of corticosteroids will be administered. The following dose schedule will be used:

Day 1: Intravenous Solu-Medrol at 1 mg/kg
Day 2: Intravenous Solu-Medrol at 1 mg/kg

Day 3-4: Prednisone at 30 mg BID PO q day
Day 4-5: Prednisone at 15 mg BID PO q day
Day 5-6: Prednisone at 10 mg BID PO q day
Day 6-7: Prednisone at 10 mg PO q day
Day 8-9: Prednisone at 5 mg PO q day

9.B. Chemotherapy – Carboplatin and Paclitaxel

Table 2. Expected and Allowable Grade 3 and 4 toxicities (CTCAE v4.0) that are not DLT and felt to be related to chemotherapy [2].

Category	Toxicity/Adverse Event	Allowable Duration
Blood and Lymphatic System Disorders	Anemia	2 weeks
	Neutropenia	2 weeks
Gastrointestinal Disorder	Abdominal Ascites	2 weeks
	Abdominal Pain	2 weeks
	Constipation	2 weeks
	Diarrhea	2 weeks
	Nausea/Vomiting	2 weeks
	Fatigue/General Weakness	2 weeks
General Disorders and Administration Site Conditions	Fever	2 weeks
	Dehydration	2 weeks

Appendix D provides a description of the common side effects of carboplatin/ paclitaxel that can serve as a reference when trying to determine attribution of vaccine reactions.

9.C. Dose Limiting Toxicity

DLT is defined as any incidence of Grade 3 or greater toxicities (other than those listed above in Tables 1 and 2) using the CTCAE v 4.0. Specifically, we will consider any Grade 3 or greater non-hematologic toxicity events a DLT with the only exceptions listed in Tables 1 and 2. Furthermore, any of the following adverse events that is at least possibly related to vaccines will be regarded as a DLT.

1 Any Grade 3 or higher non-hematologic adverse events with the exceptions of transient nausea, vomiting and diarrhea, responding to supportive care.
2 Any Grade 3 or higher allergic reaction
3 Any Grade 2 or higher autoimmune reaction
4 All Grade 2 or higher hypersensitivity reactions
5 Grade 3 or higher thrombocytopenia
6. Any Grade 4 or higher hematologic adverse events lasting more than 7 days

Any patient who develops a DLT will be permanently removed from the study and will not be replaced.

When recording adverse events we will be making every attempt to distinguish which treatment contributes to the adverse event: (1) IGFBP-2 vaccine, (2) chemotherapy, (3) both IGFBP-2 vaccine and chemotherapy or (4) neither of the treatments.

10. DOSE AND SCHEDULE MODIFICATIONS

There will not be any modifications of vaccine dose. There may be allowances for the timing of the administration of vaccine to accommodate for special circumstances and must be approved by the Principal Investigator and documented.

Neoadjuvant chemotherapy dose reduction or dose delay will be allowed under this study if dose reduction or delay are managed in accordance with published neoadjuvant chemotherapy reports and guidelines [2, 41]. Neoadjuvant dose reductions or delays will be managed by the patient's primary treating Gyn. Oncologist or Medical Oncologist. If a dose of neoadjuvant chemotherapy is delayed then subsequent vaccination will be delayed for 2 weeks +/- 2 days after neoadjuvant chemotherapy is reinitiated, in order to maintain the two week window between chemotherapy and vaccination.

11. STATISTICAL CONSIDERATIONS

11.A. Study hypotheses

We hypothesize that the addition of an IGFBP-2 vaccine to neoadjuvant chemotherapy will

- Increase the rate of pathological complete response induction (primary)
- Increase progression free survival at 12 months (secondary)
- Improve overall survival (secondary)
- Increase the level of TIL in the tumor (secondary)
- Elicit IGFBP-2 Th1 (secondary)

11.B. Power and sample size calculation

To test the primary hypothesis on the pathological complete response induction rate, we will enroll 38 patients to treat with IGFBP-2 vaccine with neoadjuvant chemotherapy. This target sample size will provide 80% power to detect significance increase in pathological complete response to 20% with the one-sided type I error rate 0.05, when compared to the average historical pathological response rate of 7.5% with neoadjuvant chemotherapy alone.[38, 39] 7.5% is a fixed benchmark and this is a single arm trial.

11.C. Projected gender and ethnic distribution

The ethnic and gender distribution chart below (Table 3) reflects estimates of race and gender of the population to be included in this study. Men do not develop ovarian cancer, thus, the study population will be female. These estimates are based on the following general statistics of the population in Washington State based on the recent 2010 US Census data. The population pool from which patients will be drawn is 83.8% Caucasian, 7.0% Asian American, 3.9% African American, 1.8% American Indian or Alaska Native and 0.5% Native Hawaiian or Other Pacific Islander.

Table 3. Distribution of ethnicity and gender.

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	3	0	3
Not Hispanic or Latino	35	0	35
Ethnic Category: Total of All Subjects *	38	0	38
Racial Categories			
American Indian/Alaska Native	1	0	1
Asian	3	0	3
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	2	0	2
White	32	0	32
Racial Categories: Total of All Subjects *	38	0	38

11.D. Patient accrual and study monitoring

We expect to recruit 2-3 patients per month, therefore accrual will take approximately 13 months for the first stage of the study, additional 6 months for follow-up to study termination.

Sequential boundaries will be used to monitor the grade 3 dose-limiting toxicity rate and the grade 4 DLT rate. The accrual will be halted if excessive numbers of dose-limiting toxicities are seen, that is, if the number of dose-limiting toxicities is equal to or exceeds b_n out of n patients with full follow-up (see Table 4 and 5 below). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 5% when the rate of dose-limiting toxicity is equal to the acceptable rate of grade 3 DLT rate at 15%, and the grade 4 DLT rate at 5%.[42] In addition, the study will stop if there is any fatality not attributed to disease progression, surgery and complications from surgery, or chemotherapy.

Table 4. Stopping boundary for grade 3 DLT rate 15%

Number of Patients, *n*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundry, b_n	-	-	3	3	4	4	4	5	5	5	6	6	6	6	7	7	7	7	8	8
Number of Patients, *n*	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	8	8	8	9	9	9	9	10	10	10	10	10	11	11	11	11	12	12	-	-

Table 5. Stopping boundary for grade 4 DLT rate 5%

Number of Patients, *n*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundry,b_n	-	2	2	2	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4
Number of Patients, *n*	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary,b_n	4	4	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	-	-

11.E. Data analysis plan

The clinical and pathologic characteristics, including age, stage, surgical outcome, progression free survival and overall survival will be tabulated for the first and if recruited, the second set of patients, as well as the combined set. The rate of pathological complete response (CR) induction will be compared to the historical control rate by a binomial test; the progression free survival and overall survival will be plotted by Kaplan-Meier curve, and compared to the survival data reported by Vergote et al which reported median PFS of 12 months and median OS of 30 months for patients treated with neoadjuvant chemotherapy by a log-rank test.[43] Immunohistochemical staining for CD3, CD4, CD8, and CD27 will be performed and quantitated using published methods and will be correlated with surgical CR by the Man–Whitney U or one-way ANOVA test were used, depending on the distribution of TIL outcomes. The level of IGFBP-2 Type 1 T-cells elicited will be evaluated by IFN-g ELISPOT assay and correlated to tumor burden at definitive surgery.

For the exploratory signature study endpoint, we hypothesize that we can selectively eliminate cancer cells destined to become chemoresistant by vaccinating against EMT associated proteins. We will perform whole exome sequencing on vaccinated patients' tumors. We will interrogate the data set for genes that predict an ordinal chemo-response variable (sensitive, resistant, refractory). We will use the LASSO regularized regression method to generate preliminary data for a predictive signature. We will correlate mutational profiles with primary platinum sensitive, resistance and refractory outcomes, leveraging the TCGA data publicly available, to determine differences induced by vaccination.

12. ADMINISTRATIVE CONSIDERATIONS

12.A. Institutional review board

In accordance with federal regulations, an Institutional Review Board that complies with the regulations in 21 CFR 56 must review and approve this protocol and the informed consent form prior to initiation of the study.

12.B. Consent

The P.I., study physician or their designated physician extender must explain verbally and in writing the nature and duration of the study and possible consequences of the treatment. Patients must also be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment. At the initiation of eligibility screening and prior to enrollment, the investigators or their associates will obtain permission from the patient to access medical records for research purposes.

12.C. Reporting adverse events

Adverse events will be reported using the NCI's CTEP CTCAE v 4.0. A copy of the CTCAE v 4.0 can be downloaded from the CTEP homepage (http://ctep.info.nih.gov). Guidelines for adverse event reporting to the FDA and the NCI are described in the DSMP, Appendix C. The Monitoring

Plan (as mandated by the NIH/NCI) Policy and Procedure for this phase I study is described in Appendix C.

All serious adverse events are communicated to the Principal Investigator, FDA, NIH Office of Science Policy (OSP) formally NIH OBA, Medical Monitor, FHCRC/UW CC IRB, UW IBC, Clinical Research Center (CRC).. In addition, the study will be monitored by Clinical Research Services (CRS) according to the FHCRC – Cancer Consortium monitoring plan.

12.D. Confidentiality of patient records

All eligible patients will be assigned a unique study ID number that will not contain any personally identifying information, such as name, initials, medical record number, social security number, etc. The study ID number will be used on all specimens, research study records and associated documents collected in the research record. To maintain confidentiality, we protect the link between the patients' personal identifying information and study ID number by limiting who has access to the patients' chart documentation. Only specified clinical research staff has access to the data, which remain locked at all times when not in use.

All hard copy research records collected on potential and enrolled patients are stored in a locked filing cabinet that can only be accessed by approved clinical research staff when not in use. This staff includes: Principal Investigator, study physician(s), and designated clinical research staff. These are also the only people that have access to the link between the patients' personal identifying information and their assigned UPN codes.

In terms of the protections and security of electronic clinical data it is being performed by the University of Washington Medicine Information Technology (IT) Services. The UW Medicine IT information security policy is to protect UW Medicine information and information systems. It also ensures compliance with University of Washington policy and state and federal regulations.

12.E. Study team roles and responsibilities

Dr. Liao, the Principal Investigator, will be responsible for the oversight of the research. Dr. Liao is responsible for ensuring that all information and documentation related to the conduct and safety of the study is disseminated to the proper agencies in the proper timeframe.

Research physicians/physician extenders will be responsible for conducting consent conferences at the initial visit as well as performing monthly assessments prior to vaccination.

Research Nurse(s)/Coordinator(s) will be responsible for initial screening and scheduling patient visits. The Research Nurse(s)/Coordinator(s) will ensure that the clinical data is entered into the database in a timely manner, so as to have real time data to review and report any safety concerns trends. They will also be responsible for maintaining regulatory documentation to the various agencies involved with this research and ensure that all research team members are following the protocol and all regulations.

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Appendix A. ECOG Performance Scale

Performance status: Patients will be assessed according to the current ECOG performance scale.

ECOG performance scale

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

As published in Am. J. Clin. Oncol: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.*

The ECOG/Zubrod Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

Appendix B. Calendar of Events

Visit Time Point	Procedures
Baseline Visit Some procedures may be done up to two weeks prior to initial vaccine visit	• Consent • Medical history and complete physical examination • Vitals signs-including weight • ECOG scoring • Baseline symptom assessment • Clinical labs if greater than 30 days old: complete blood counts (with differential and platelet count), serum chemistries (electrolytes, glucose, AST, ALT, alkaline phosphatase, bilirubin, creatinine, blood urea nitrogen), ANA, C3 and anti-ds-DNA, thyroid function tests • CA125 • Research blood: approximately 200 mls* • Archived tissue collection, if available
First Vaccine: 2 weeks after chemotherapy	• Medical history and complete physical examination • Vitals signs-including weight • ECOG scoring • Baseline symptom/toxicity assessment (if not collected previously) • Urine pregnancy test (if applicable) • Tetanus diphtheria immunization • pUMVC3-hIGFBP-2 vaccine • Post vaccine monitoring
Second Vaccine: 2 weeks after chemotherapy	• Medical history and complete physical examination • Review of pre chemotherapy lab results • Vitals signs-including weight • Symptom/toxicity assessment • Urine pregnancy test (if applicable) • pUMVC3-hIGFBP-2 vaccine • Post vaccine monitoring
Third Vaccine: 2 weeks after chemotherapy	• Medical history and complete physical examination • Review of pre chemotherapy lab results • Vitals signs-including weight • Symptom/toxicity assessment • Urine pregnancy test if applicable) • pUMVC3-hIGFBP-2 vaccine • Post vaccine monitoring
Evaluation after the final vaccine (Approx. 1 week after final vaccine Prior to surgery	• Medical history and complete physical examination • Vitals signs-including weight • Symptom/toxicity assessment • Clinical labs: complete blood counts, serum chemistries, ANA, C3 and anti_ds-DNA, CA125, thyroid function tests • Research blood: approximately 200 mls*
Month 6	• Medical history and complete physical examination • Vitals signs-including weight • Symptom/toxicity assessment

Visit Time Point	Procedures
Post the last vaccine	• Clinical labs: complete blood counts, serum chemistries, ANA, C3 and anti-ds-DNA, CA125, thyroid function tests • Research blood: approximately 200 mls*
Once yearly follow-up for 5 years	• Collection of physician notes to review toxicity and clinical status
* During the consent process, and at subsequent visits, patients will be instructed to hydrate sufficiently prior to visits requiring large volume blood draws.	

Appendix C. Data and Safety Monitoring Plan

A. Purpose

To ensure that the Tumor Vaccine Group (TVG) follows NIH/NCI/CTEP/FDA guidelines with respect to: (1) accurate assessment and timely reporting of adverse drug reactions associated with investigational drugs, (2) adherence to protocol, and (3) accurate reporting of data. The DSMP will be approved by Fred Hutchinson Cancer Research Center (FHCRC) /University of Washington (UW) – Cancer Consortium IRB (FHCRC/UW – CC IRB) in Seattle, Washington.

The Principal Investigator (PI), for this project, is responsible for the conduct of the study. The PI will be involved in the study design, delegating responsibility/authority and will be involved with final analysis. The Sponsor of the study is responsible for study design, , monitoring of the study conduct and final analysis of the study data. Regulations defining the responsibilities for assessment and reporting of all adverse events (AE), serious AE (SAE), and unexpected AEs are defined by the Code of Federal Regulations: 21 CFR 312.32 and Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 published by the Cancer Therapy Evaluation Program (CTEP), a division of the NCI/NIH.

This clinical study will rely upon the monitoring of the trial by the IND Sponsor, Principal Investigator (P.I.) in conjunction with a Research Nurse, research staff, Statistician, an Independent Medical Monitor and an Independent Study Monitor assigned by the Clinical Research Support (CRS) of the FHCRC/UW–CC IRB.

The Independent Medical Monitor for this study is Ron Swensen, M.D., Gynecologic Oncology Physician at the Valley Medical Center. Dr. Swensen has conducted research in gynecologic oncology and immune therapy. The Medical Monitor will be notified that the study is open prior to patients being recruited in order to orient committee participants to the study. The Medical Monitor will then discuss the study every 6 months throughout the study treatment period with the P.I., Research MD(s), Research Nurse(s), Research Coordinator(s), and/or other related clinical research staff. The Medical Monitor will review patient recruitment and retention, adherence to protocol, follow-up, data quality, and participant risk versus benefit.

If a SAE occurs the PI will be notified along with the IND Sponsor, FDA, NIH OSP, Medical Monitor, FHCRC/UW CC-IRB, UW IBC, and UW CRC.

B. Objectives

1. To ensure that the PI and Clinical Research Staff follow federal and institutional regulatory guidelines with respect to timely reporting of adverse reactions associated with investigational drugs.
2. To define classification of adverse drug reactions as expected or unexpected.
3. To define classification of adverse drug reactions as serious or non-serious.
4. To ensure compliance and accuracy of documentation of SAE reactions reportable to: (1) FDA (2) NIH OSP, (3) Medical Monitor, (4) FHCRC/UW CC-IRB,, (5) UW IBC, (6) UW CRC and (7) IND Sponsor.

C. Adverse event reporting policy and procedures

1. Evaluation of adverse events: Patients are monitored for the development of toxicities by assessing adverse events lab values throughout the study. The development of laboratory autoantibody responses will also be clinically assessed at baseline visit, and approximately 1 week and 6 months after the last vaccine for the development of anti-DNA antibodies (ANA, C3, thyroid function tests, and anti-dsDNA). All adverse events for all systems are graded on a scale of 1-5 and attribution is assigned, using the Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.0).

2. Definitions of adverse events (AEs):

   a. *AE* - any unfavorable and unintended sign (including abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure.

      An adverse event may include

      - an exacerbation of a pre-existing illness that would stop treatment of a standard chemotherapy treatment.
      - an increase in frequency or intensity of a pre-existing episodic event or condition.
      - a condition detected or diagnosed after study drug administration.
      - continuously symptoms that were present at baseline and worsen following the start of the study.

      An adverse event does not include:

      - medical or surgical procedures unrelated to the treatment of the disease (e.g., surgery, endoscopy, tooth extraction, or transfusion); however, the condition that leads to the procedure may be an adverse event.
      - pre-existing diseases, conditions, or laboratory abnormalities present or detected at the start of the study that do not worsen.
      - hospitalizations or procedures that are done for elective purposes not related to an untoward medical occurrence (e.g., hospitalizations for cosmetic or elective surgery or social/convenience admissions).
      - the disease being studied or signs/symptoms associated with the disease unless more severe than expected for the patient's condition.
      - overdose of study drug without any clinical signs or symptoms.

   b. *Expected AE* - an event that may be reasonably anticipated to occur as a result of the study procedure and is described in the IND 14906 and/or consent form.

   c. *Unexpected Adverse Event* - an AE that is not described in the IND 14906 and/or consent form and is unanticipated. An event that might have been anticipated but is more serious than expected or occurs more frequently than expected, would be considered an unexpected adverse event.

   d. *Life-threatening AE* - the patient was at substantial risk of dying at the time of the adverse event or it is suspected that the use or continued use of the product would have resulted in the patient's death.

   e. *Serious AE* - grade 4 or 5 toxicity or any AE occurring at any dose that results in any of the following outcomes: death, a life threatening AE, inpatient hospitalization or

prolongation of existing hospitalization due to the AE, a persistent or significant disability/incapacity or a congenital anomaly/birth defect that is expected and related.

f. Hospitalizations that do not meet these criteria are:

- reasons described in the protocol, e.g., drug administration, protocol-required testing.
- social reason in the absence of an AE.

Note: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations; for example, important medical events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Any adverse event is considered a serious adverse event if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

3. Scale of scoring adverse events

a. Grade 1 = mild adverse event

b. Grade 2 = moderate adverse event

c. Grade 3 = severe adverse event

d. Grade 4 = life-threatening or disabling adverse event

e. Grade 5 = death related to adverse event.

4. Attribution of adverse event

a. 5 (definite): the adverse event is clearly related to the investigational agent.

b. 4 (probable): the adverse event is likely related to the investigational agent.

c. 3 (possible): the adverse event may be related to the investigational agent.

d. 2 (unlikely): the adverse event is doubtfully related to the investigational agent.

e. 1 (unrelated): the adverse event is clearly NOT related to the investigational agent.

5. SAE event reporting

This procedure is outlined below by regulatory agency (Table 3).

FDA (for trials using an Investigational New Drug (IND) – Serious adverse events will be reported in accordance with the requirements under 21 CFR 312.32.

NIH Office of Biotechnology Activities (NIH OSP) - any serious adverse event that is fatal or life-threatening that is unexpected and associated with the use of a gene transfer product must be reported as soon as possible but no later than 7 days after the sponsor's initial receipt of the information. Unexpected serious adverse events that are associated with the gene transfer product, but are not fatal or life-threatening must be reported as soon as possible but no later than 15 calendar days from the sponsor's initial receipt of the information. An adverse event that is initially considered not associated with the use of the gene transfer product but is subsequently determined to be associated must be reported within 15 calendar days of the determination. All other adverse events will be reported to NIH OSP in the annual report. The NIH Office of Biotechnology Activities will accept the report in either a narrative format. We will send a copy of the MedWatch Form 3500A. .

Independent Medical Monitor - At a minimum, the medical monitor should comment on the outcomes of serious adverse event(s) to the vaccine or prepare an unbiased written report of the event. They should also indicate whether they agree with the details of the report provided by the study investigator.

Fred Hutchinson Cancer Research Center/UW Cancer Consortium IRB - We will follow the current AE reporting policy of the FHCRC – CC IRB.

University of Washington IBC - We will follow the current AE reporting policy of the UW IBC.

University of Washington Clinical Research Center (UW CRC) - The CRC will receive copies of the documents submitted to the FHCRC/UW CC-IRB within 7-10 calendar days.

IND Sponsor – Will be notified within 24 hours of the event occurring in order to report to the FDA if needed.

Table 3. Adverse event reporting

	Expected	Expected	Unexpected	Unexpected
	Non-Serious	Serious	Non-Serious	Serious (including grade 4 & 5 toxicity)
	Attribution * (Possible, Probable, Definite)	Attribution * (Possible, Probable, Definite)	Attribution * (Possible, Probable, Definite)	Attribution * (Possible, Probable, Definite)
FDA	Annual Progress Report	Annual Progress Report	Annual Progress Report	FDA Form 3500A or Narrative Format – ASAP but within 7-15 days)
NIH OSP	Annual Report	Annual Report	Annual Report	FDA Form 3500A or Narrative Format – ASAP but within 7-15 days)
Medical Monitor	Bi-annual Meeting (twice a year)	Bi-annual Meeting (twice a year)	Bi-annual Meeting (twice a year)	FHCRC Adverse Event Expedited Reporting Form – Following the FHCRC/UW CC-IRB policy
FHCRC/UW – Consortium IRB	Continuation Review Report	Continuation Review Report	Continuation Review Report	FHCRC Adverse Event Expedited Reporting Form – Following the FHCRC/UW CC-IRB policy
UW IBC	Routine Report, as appropriate	Routine Report, as appropriate	Routine Report, as appropriate	Follow UW IBC reporting policy
UW CRC	Copy of Continuation Review Report	Copy of Continuation Review Report	Copy of Continuation Review Report	Copy of FHCRC/UW CC-IRB Adverse Event Expedited Reporting Form

6. Procedure for reporting SAEs:

   a. Identify the classification/attribution of the adverse event as defined above using the Modified Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.0).

   b. After appropriate medical intervention has been instituted, the Principal Investigator or his/her designee will be notified within 24 hours.

c. File appropriate reports immediately by phone/fax with appropriate agencies, as described in Table 3.

d. Notify the patient's primary physician or referring physician within a medically appropriate timeframe, depending on the classification of the adverse event.

e. Submit written reports to appropriate agencies.

f. Document the adverse event in the patient's research file, using a progress note to describe the event and treatment, if appropriate.

g. File copies of all forms/correspondence relating to the adverse event in the patient's research file.

D. Clinical trials monitoring operational procedures

1. Clinical data documentation

a. *Internal study monitoring*:

All patients actively enrolled in the study are seen every 3 weeks during the treatment period. Clinical labs are evaluated (1) during eligibility, within 2 weeks prior to vaccines 1, 2, and 3 as well as 1 week and 6 months after the last vaccine, for the development of toxicity (adverse events) related to the vaccine. Any clinically significant abnormal lab values may be faxed to the patient's physician.

A Research Physician, physician extender or P.I. sees each study participant at each visit with the following evaluations being completed: physical assessment and adverse events summary (these evaluations are part of the source documentation that is filled out at each visit). Grade 1 and 2 non-serious and expected adverse events will be reviewed with the P.I. or designee regularly. All other adverse events will be reported to the P.I. or designee at the time they become known and reported as outlined above in Section C. Clinical research staff member(s) will review patient research files for patient recruitment and retention, protocol adherence, follow-up, data quality, and participant risk versus benefit.

b. *Biannual review by the independent Medical Monitor*:

The Medical Monitor will review data with the P.I., designee, and/or other members of the clinical team, approximately every 6 months. All patients are reviewed for adverse events. Conduct of the study is reviewed and changes/clarifications can be requested/discussed with the Medical Monitor

c. *Biannual study audit*:

Institutional support of trial monitoring will be in accordance with the FHCRC/UW Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, FHCRC Clinical Research Support (CRS) coordinates data and compliance monitoring conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), FHCRC Scientific Review Committee (SRC) and the FHCRC/UW Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating patients. The IRB reviews the study progress and safety information to assess continued

acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

2. Data validity/integrity

Systems to insure data integrity have been put into place to provide multiple checks to data entry. Patient eligibility is initially reviewed by the Research Coordinator/Research Nurse and the PI or designee during the screening portion of the study. The chart is then reviewed by a study physician after the consent conference and before study procedures start. It is then ultimately signed off on by the PI or designee.

Clinical laboratory monitoring data is reviewed by a qualified clinician (Research MD, physician extender, Research Nurse and/or P.I.) and any abnormalities are assessed. Patient records are kept in the patient's research file and reviewed at biannual monitoring visits. Data is taken from the patient's source documentation, the patient's research file, and entered into a database which links data to patient by a Unique Patient Identifier and is accessible by password code only. Data entry is made by the Research Clinical Research Staff members. A percentage of all data entry is reviewed by a member of the clinical research team and/or the Independent Study Monitor.

Appendix D. Common Side Effects of Carboplatin/ Paclitaxel

COMMON, SOME MAY BE SERIOUS In 100 people receiving Carboplatin and Paclitaxel, more than 20 and up to 100 may have:
• Hair loss • Infection, especially when white blood cell count is low • Anemia which may cause tiredness, or may require blood transfusions • Bruising, bleeding • Diarrhea, nausea, vomiting, constipation • Sores in mouth which may cause difficulty swallowing • Changes in taste • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Pain • Muscle weakness • Numbness and tingling in fingers and toes

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Carboplatin and Paclitaxel, from 4 to 20 may have:
• Heart attack or heart failure which may cause shortness of breath, swelling of ankles, and tiredness • Abnormal heartbeat • A tear or a hole in the stomach which may cause belly pain or that may require surgery • Damage to the lungs which may cause shortness of breath • Blood clot which may cause swelling, pain, shortness of breath

RARE, AND SERIOUS In 100 people receiving Carboplatin and Paclitaxel, 3 or fewer may have:
• Changes in vision • Damage to organs which may cause hearing and balance problems

Appendix E. NCCN Guidelines for Menopause Status

The National Comprehensive Cancer Network (NCCN) defines menopause as "generally the permanent cessation of menses, includes a profound and permanent decrease in ovarian estrogen synthesis." According to their guideline, the criteria for determining menopause are:

- Prior bilateral oophorectomy
- 60 or older
- Age less than 60 years; amenorrheic for 12 or more months in the absence of chemotherapy, tamoxifen, toremifene, or ovarian suppression; and follicle-stimulating hormone (FSH) and plasma estradiol in the postmenopausal range.
- If taking tamoxifen or toremifene, and age is under 60 years, then FSH and plasma estradiol level should be in the postmenopausal range.
- It is not possible to assign menopausal status to women who are receiving a leuteinizing hormone-releasing hormone agonist or antagonist. In women premenopausal at the time of adjuvant chemotherapy, amenorrhea is not a reliable indicator of menopausal status.