A PILOT STUDY OF A POLYVALENT VACCINE-KLH CONJUGATE + OPT-821 GIVEN IN COM BINATION WITH BEVACIZUM AB IN PATIENTS WITH RECURRENT EPITHELIAL OVARIAN, FALLOPIAN TUBE, OR PRIM ARY PERITONEAL CANCER WHO ARE IN SECOND OR GREATER COMPLETE OR PARTIAL CLINICAL REMISSION

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0 PROTOCOL SUMM ARY AND/OR SCHEMA

The majority of patients with advanced epithelial ovarian carcinoma will enter clinical remission following optimal primary surgical cytoreduction and completion of platinum and taxane based chemotherapy. However, almost 70% of these patients will relapse.[1] Many patients will have some degree of response to subsequent chemotherapy. Although some patients can obtain a second or third complete remission with further chemotherapy, subsequent remissions are of progressively shorter duration until chemotherapy resistance uniformly develops.[2] Effective consolidation strategies are needed to prevent relapse or prolong remission.

This is a non-randomized pilot, open label study that will enroll women with recurrent epithelial ovarian, primary peritoneal or fallopian tube cancer who are in clinical remission or have persistent low volume asymptomatic measurable disease following retreatment. We will evaluate the safety and immunogenicity of immunization with a polyvalent antigen – KLH vaccine construct + OPT- 821 in combination with bevacizumab.

Previous studies have demonstrated that the presence of both antibodies and tumor infiltrating T cells are associated with longer survival in patients with epithelial ovarian cancer. We previously completed a phase I safety and immunogenicity pilot study of the polyvalent vaccine-KLH conjugate plus QS-21 in patients with epithelial ovarian, fallopian tube, or peritoneal cancer in a first, second or greater complete clinical remission. The administered vaccine contained GM2, Globo-H, Le^y, Tn-MUC1, Tn(c), STn(c) and TF(c) individually conjugated to KLH and mixed with adjuvant QS-21. In this study, we demonstrated the safe induction of antibody responses against five of the seven antigens contained in the vaccine.[3] Based on cumulative safety data from 299 patients treated at MSK with a variety of similar vaccine constructs (i.e. X antigen-KLH + QS-21), we have a pending phase II multivalent vaccine-KLH + OPT-821 (adjuvant) trial (n = 164 patients) which randomizes patients in second or third remission to the vaccine or adjuvant alone (NCT00693342 with BB-IND 14076) to be conducted with the Gynecology Oncology Group.

Simultaneously, the role of anti-angiogenic agents in the treatment of ovarian cancer is under investigation. Angiogenesis has been shown to play a pivotal role in disease progression. Key ongoing phase III trials (GOG 218, OCEANS) will likely show a

benefit for the addition of bevacizumab to standard platinum and taxane chemotherapy for ovarian cancer. These trials have the potential for changing the standard of care so that the majority of patients will begin bevacizumab therapy in first line therapy, or will have it added to second line therapy if not already receiving it. If our phase II vaccine study also meets its endpoint in the second line setting, illustrating the utility of immune based therapy, the obvious question is whether the vaccine can be safely administered with bevacizumab (as most ovarian cancer patients will likely be receiving it).

The safety profiles of both bevacizumab and our vaccine have been individually established, however, we do not have safety data for the combination of these two new novel approaches.

Table 1 Study Schema (+/- 3 day windows are allowed)

Immunization	Weekly x3			4 Week break				4 Week break				6 Week break						Study end
Immunization Number	#1	#2	#3				#4				#5						#6	
Bevacizumab	Once every 2 weeks starting with vaccine #1 on week 1 until week 11 then q3 weeks until disease progression/study end																	
Bevacizumab	*		*		*		*		*		*			*			*	*
Research Studies	↑						↑							↑			↑	↑
Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	20 or end of study

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objective:

1. To establish the safety of immunization with polyvalent antigen – KLH + OPT-821 vaccine conjugate in combination with bevacizumab in patients with recurrent epithelial ovarian, fallopian tube or primary peritoneal cancer

Secondary Objectives:

1. To assess the immunogenicity of the vaccine when given in the presence of bevacizumab
2. To explore the relationship between a multiplex biomarker panel of angiogenesis markers and progression-free survival

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3.0 BACKGROUND AND RATIONALE

3.1 Dise ase Background

Epithelial Ovarian Cancer (EOC)

Epithelial ovarian cancer is one of the most common gynecologic malignancies and the fifth most frequent cause of cancer death in women in the United States. Over 21,000 cases are diagnosed annually, and there are an estimated 15,000 deaths per year. [4] The majority of patients have widespread disease at presentation.[2] The 5-year survival for advance-stage disease remains less than 30%.[5] Although a complete clinical remission following initial chemotherapy can be anticipated for many patients, a review of second-look laparotomy indicates that less than 50% of patients are actually free of disease.[6] Furthermore, nearly half of patients with a negative second look procedure are destined to relapse and require additional treatment. [7-8] Many patients will achieve a second complete clinical response with additional chemotherapy.[9] However, almost all the patients will relapse after a short remission interval of 9-11 months supporting the concept of persistent minimal residual disease. Effective strategies to prolong remission or to prevent relapse are required, as subsequent remissions are of progressively shorter duration until chemotherapy resistance broadly develops.

Immune directed therapy is theoretically suited for evaluation when disease burden is lowest. Antibodies have been shown to curtail early tissue invasion.[10] Preclinical models have demonstrated the clearance of circulating tumor cells and the elimination of systemic micrometastasis through the use of both passively administered and vaccine induced antibodies. An activated immune response has been shown to be associated with improved clinical outcome in patients with advanced ovarian cancer. This was demonstrated by Zhang et al, who showed that the presence of tumor infiltrating T cells within tumor cell islets was associated with improvement in both progression free and overall survival. [11] Conversely, the infiltration of T-regulatory cells, associated with immunosuppression, appears to confer a worse prognosis.

In recent years, ovarian cancer has been targeted by a variety of novel immune based approaches. Antibody therapy has included oregovomab, a monoclonal antibody therapy targeting the CA125 antigen;[12] abagovomab,[13] an anti-idiotypic antibody targeting CA-125; and trastuzumab,[14] a monoclonal humanized anti-HER2 antibody. Other strategies have included cytokine therapy such as Interferon-γ[15-16] and IL-2.[17] Active immunization with peptides such as Lewis^{y},[18] MUC1,[19] the peptide NY-ESO-1, [20] the HLA restricted peptide NY-ESO-1b and the KH-1-KLH conjugate [20] have also been evaluated.

3.2 Antibody Inducing Vaccines in Ovarian Cancer

3.2.1 Background of Antibody Inducing Vaccines in Ovarian Cancer

We recently performed a pilot trial of a heptavalent vaccine-KLH conjugate plus QS-21 in patients with epithelial ovarian, fallopian tube, or peritoneal cancer in either a first (following persistence and additional treatment), second, or third remission.[3] Vaccinations were administered at weeks 1, 2, 3, 7, 11 and 15. The vaccine was administered in 1 cc total volume using a 25-gauge needle subcutaneously into the buttocks, thighs, abdomen or arms. Dose modification or delay was not permitted. Patients were to be removed from study for a dose limiting toxicity, defined using the NCT toxicity criteria, as 1) >grade 2 allergic reaction, >grade 2 autoimmune reaction,

>grade 3 hematologic or non-hematologic toxicity including fever, or grade 3 injection site reaction. Patients were removed from study for disease progression. Patients had repeat complete blood cell counts, comprehensive biochemistry panel, and serum amylase at regular intervals, and at off-study visit. CT imaging was performed every 3 months while on study, or sooner to restage patients if signs or symptoms, blood tests, or physical examination suggested disease progression.

Serum samples for immune testing were obtained at weeks 1, 5, 7, 9, 13, 15, and 17. ELISA assays were tested against the following target antigens: Tn-MUC1-32mer, GM2, Globo-H ceramide, Le^y ceramide, desialated ovine submaxillary mucin (DOSM) expressing Tn, OSM expressing STn, and desialated porcine submaxillary mucin (DPSM) expressing TF. IgM and IgG antibody titers were measured by ELISA as described previously.[21-23] Fluorescent-activated cell-sorting (FACS) was performed as previously described to demonstrate antibody binding to the cell surface of the cell line MCF-7. MC-7 was chosen as it is known to express each of the 7 antigens.[21-23] The colon cancer cell line, LSC, and the ovarian cancer cell lines OVCAR3 were also used as targets. Complement-dependent cytotoxicity was assayed on MCF-7 cell lines using a 2-hour 51-chromium release assay, as previously described and assessed pre and at the time of peak ELISA reactivity (week 6 or 8).[21-23]

We used the same criteria for immunogenicity as used in the preceding individual pilot trials: Patients had to have a peak IgM titer >1:40, or a peak 8-fold increase in prevailing antibody titer if present at baseline. Eleven patients were included in the safety analysis; nine of the 11 patients remained on study for at least two weeks past fourth vaccination and were included in the immunologic analysis (2 withdrew due to disease progression). The vaccine was well tolerated. Self-limited and mild fatigue (maximum grade 2 in two patients), fever, myalgia, and localized injection site reactions were most frequent. No clinically relevant hematologic abnormalities were noted. No clinical or laboratory evidence of autoimmunity was seen.

Serologic responses by ELISA were largely IgM against each antigen with the exception of Tn-MUC 1 where both IgM and IgG responses were induced. Antibody responses were generally undetectable prior to immunization. After immunization, median IgM titers were as follows: Tn-MUC 1 1:640 (IgG 1:80), Tn 1:160, TF 1:640, Globo-H 1:40, and STn 1:80. Only one response was seen against Le^y and two against GM2. Eight of 9 patients developed responses against at least three antigens. Antibody titers peaked at weeks 4-8 in all patients. FACS and complement-dependent cytotoxicity analysis showed substantially increased reactivity against MCF7 cells in seven of nine patients, with some increase seen in all patients.[24]

Table 2: Summary of Heptavalent Vaccine Responses in Relation to Prior Monovalent Vaccines

Antigen	Median IgM ELISA titers after vaccination (based on previous studies)*			Median IgM ELISA titers after vaccination*	
	Monovalent vaccines			Heptavalent vaccine	
	Pre	Post	Ref	Pre	Post
Tn-M UC1	20	1280	[22]	0	640
Tn (DOSM)	0	640	[25]	0	160
TF (DPSM)	0	1280	[26]	0	640
Globo-H	20	80	[21]	0	40
GM 2	0	160	[27-28]	0	0
Lewis-Y	0	0	[18]	0	0
STn (OSM)	10	640	[29]	0	80

Patients with IgM titer >1:40, or an 8-fold increase in prevailing antibody titer if present at baseline are considered “responders”.

3.2.2 Choice of Antigen and Adjuvant Used in Polyvalent Vaccine

Based on the compilation of prior data, and considering which antigens are best suited for GMP manufacturing and further testing, the polyvalent vaccine to be evaluated here and as part of the planned GOG-0255 study will include 1) GM2-KLH, 2) Globo-H-KLH, 3) Tn-MUC1-32mer-KLH, 4) TF-KLH + OPT-821. OPT-821 will be the immunological adjuvant used. It is derived from the same plant source Quillaja Saponaria Molina as QS-21. (See Section 5.1 for discussion of OPT-821 and its relationship with QS-21, as well as detailed description of the polyvalent-KLH vaccine construct +OPT-821).

3.2.3 Safety Profile of Antibody Inducing Vaccines in Ovarian Cancer

Approximately 90 patients have been enrolled in a series of monovalent vaccine trials at MSK in patients with ovarian cancer in order to characterize the most immunogenic antigens for inclusion in a polyvalent construct. To date, no systemic toxicity related to autoimmunity has been seen. The most common adverse events are localized erythema and induration at the injection site which resolves in 5-7 days, mild to moderate fever for 24 hours in some patients, and occasionally mild fatigue or myalgia

for several days following vaccination. No patients have been removed from study for any of these side effects. Hypothyroidism has been seen in a small number of patients receiving a similar vaccine construct with breast cancer. It is unclear whether this finding was related to vaccination, or reflected the demographics of the population in which autoimmune hypothyroidism is more common. An assessment of thyroid function has been added to the routine screening tests. Specifically, no hypothyroidism has been seen in patients with ovarian cancer receiving previous vaccines at MSK. Detailed side effects are outlined in Section 11.2

3.3 Bevacizumab

3.3.1 Clinical Experience with Bevacizumab in Ovarian Caner

The bevacizumab Investigator Brochure contains descriptions of all completed Phase I, II, and III trials reported to date and details the safety profile.

Angiogenesis is a key process leading to invasion and metastasis of ovarian cancer.[30-31] Elevated VEGF expression is correlated with poor prognosis.[31-33] Inhibitors of VEGF have been shown to both suppress tumor growth and augment the antitumor effects of antineoplastic drugs in both preclinical and human studies.[34-40] The anti-VEGF monoclonal antibody, bevacizumab, is an active agent in patients with recurrent ovarian cancer. Response rates of 15.9% and 21% have been reported when used as single agent, and 24% was seen in conjunction with oral cyclophosphamide.[41-43] Bevacizumab has an acceptable toxicity profile in ovarian cancer.[44-46]

3.3.2 Safety Profile of Bevacizumab

In the initial Phase I and II clinical trials, four potential bevacizumab-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed Phase II and Phase III studies of bevacizumab as well as spontaneous reports have further defined the safety profile. Bevacizumab-associated adverse events identified in phase III trials include congestive heart failure (CHF) primarily in metastatic breast cancer, gastrointestinal perforations, wound healing complications, and arterial thromboembolic events (ATE). These and other safety signals are described in further detail in Section 11 and in the bevacizumab Investigator Brochure.

3.4 Correlative Studies

We will perform 2 separate multiplex assays evaluating markers of angiogenesis and other cytokines. These assays have recently been validated at MSKCC. These novel, automated, immunoassay platforms will simultaneously evaluate multiple biomarkers in a single reaction vessel. Approximately 400µL of whole blood will be collected in an ethylenediaminetetraacetic acid (EDTA) purple top tube. Samples will be taken on day 1 of week 1 (baseline), day 1 of week 17 (postvaccination), end of study visit (post-bevacizumab), or upon progression of disease or significant adverse event.
Patient serum will be removed and stored at -80^{o}C for the multiplex analyses. Please see Appendix A for laboratory procedures.

3.4.1 Angiogenesis Multiplex:

We will perform a 10-plex angiogenesis assay. The angiogenesis panel will include monoclonal antibodies specific for interleukin-8 (Il-8), VEGF, VEGF-R2, intracellular adhesion molecule 1 (ICAM-1), tyrosine kinase with immunoglobulin and

EGF homology domains-2 (TIE-2), placental growth factor (PLGF), tissue inhibitor of metalloproteinase-1 (TIMP-1), hepatocyte growth factor(HGF), fibroblast growth factor-basic (FGF-basic) and E-selectin. Values will be correlated with PFS, and baseline will be compared with progression.

3.4.2 Cytokine Multiplex:

We will perform an 8-plex cytokine assay. The cytokine panel will include tumor necrosis factor (TNF) and the following interleukins (IL): IL1-β, IL -2, IL-5, IL-6, IL-8, IL-10 and IL-12. Cytokines are a diverse group of soluble proteins and peptides which act as regulators of normal and pathological conditions to modulate the functional activities of individual cells and tissues. These proteins mediate interactions between cells directly and regulate processes taking place in the extracellular environment. They function on a variety of cell types, having stimulatory or inhibitory effects on proliferation, differentiation and maturation. Given this pleotrophic effect, measuring the level of only a single cytokine in a biological system provides only limited information on the systemic response. The ability to detect and quantitate multiple cytokines simultaneously will facilitate better understanding of the immune response to the heptavalent-KLH vaccine and adjuvant. Values will be correlated with PFS, and baseline will be compared with progression.

3.5 Rationale for the proposed study

Key ongoing phase III trials (GOG 218, OCEANS) will likely show a benefit for the addition of bevacizumab for patients with ovarian cancer. If these trials are positive patients will begin bevacizumab therapy in first line therapy, or will have it added to second line therapy if not already receiving it. At any rate, the already demonstrated response rate for bevacizumab in ovarian cancer will prompt its use in the recurrent setting and many patients will continue it beyond response. If our phase II polyvalent vaccine study also meets its endpoint in the second line setting, illustrating the importance of immune based therapy in ovarian cancer, the important questions are 1) whether the vaccine can be safely administered with bevacizumab (as most patients will likely be receiving it) and 2) whether immunogenicity to the vaccine and adjuvant is maintained in the presence of bevacizumab. Our correlative studies are aimed at exploring markers that may predict benefit from anti-angiogenic therapies.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a single institution, open label, pilot study of bevacizumab and the polyvalent vaccine-KLH conjugate + OPT-821 in patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer. Eligible patients will have relapsed and completed additional cytotoxic chemotherapy. Patients may be in complete remission or have residual asymptomatic measurable disease.

4.2 Intervention

4.2.1 Polyvalent Vaccine-KLH Conjugate

The intervention includes the subcutaneous injection of polyvalent vaccine-KLH conjugate for 6 vaccinations using the dose and schedule described in section 9.1.

4.2.2 Bevacizumab

The intervention includes the intravenous of bevacizumab q 2 weekly initially and then q 3 weeks using the dose and schedule described in section 9.1

4.2.3 Correlative Studies

Whole blood samples will be collected into EDTA purple top tubes (approximately 4ml) on day 1 of week 1 (baseline), day 1 of week 17 (post vaccination), end of study visit (post-Bevacizumab), or upon POD or significant adverse event. Please see Appendix A for further details on laboratory procedures.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Polyvalent Vaccine-KLH Conjugate + OPT-821

The IND application is held by MSKCC.

5.1.1 Antigens

This trial will be conducted using the following antigens conjugated to KLH (carrier) with OPT-821 as the adjuvant:

- Glycosylated **MUC-1 termed Tn-MUC1 (BB-IND 6013)** was synthesized by Pepceuticals LLD (Leicester, England). The 32 amino acid sequence of the MUC-1 peptide to be used is: CHGVT*S*APDT*RPAPGSTAPPAHGVTSAPDTRPA.

  This 32 amino acid MUC1 sequence contain ~11/2 repeats of the core 20 amino acid sequence and is partially glycosylated with Tn molecules at the * indicated serines or threonines above. An additional terminal cysteine is placed to facilitate attachment of KLH. The Tn-MUC1 is conjugated to KLH using an MBS linker. The dose of Tn-MUC1 is 3µg.

- **Globo H (BB-IND 6861):** The globo H hexasaccharide-KLH was synthesized and conjugated under GMP by Optimer Pharmaceuticals Inc. (San Diego, CA). The dose of Globo H is 30µg.

- **GM 2 (BB-IND pending):** Ganglioside GM2 is extracted from brains obtained from a colony of cats with Tay Sachs Disease (an autosomal recessive GM2 gangliosidosis) by Matreya Inc. (Philadelphia, PA). It was conjugated to KLH by Althea Technologies (San Diego, CA). The dose of GM2 is 30 mcg.

- **TF (c) (BB IND 7862):** TF (c) was synthesized by MSKCC Carbohydrate Synthesis Core. The TF disaccharide is covalently attached to a single threonine and then using Fmoc technology the 3 threonine-TF constructs are linked together with a terminal cysteine for attachment to KLH. The dose of TF(c) is 3 mcg.

5.1.2 Conjugation to KLH

Covalent attachment of KLH to TF(c) and Tn-MUC1 is achieved with m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) which couples the free SH group of cysteine on the antigen to the N terminus and lysine side chains on KLH. Conjugation of KLH to the glycolipid antigens Globo H is by attachment with the MMCCH linker 4-(4- N-maleimidomethyl) cyclohexane-1-carboxyl hydrazide. Conjugation of GM2 to KLH is achieved directly using reductive amination methods.

5.1.3 OPT-821 Adjuvant

OPT-821 is an immunological adjuvant obtained from Optimer Pharmaceuticals Inc. (San Diego, CA). It is mixed with the multiple antigen conjugates at a dose of 100 mcg at the time of vaccine. OPT-821 was derived from the same plant source *Quillaja saponaria* Molina as QS-21. Optimer started with a commercially obtainable QuilA, a partially purified saponin form bark of *Quillaja saponaria* from Brenntag Biosector, Frederikssund, Denmark. The purification consists of a normal phase (silica gel) purification followed by two consecutive reverse phase (C5 and C8) column purifications as described. Solid Quil A was fractionated by silica gel chromatography. Individual fractions containing the desired components were analyzed by an analytical HPLC (Vydac 4 column) and pooled together. Solvents were removed under reduced pressure and the resultant residue was taken up in water before lyophilization. Preparative reverse-phase HPLC (C5 column) under cGMP was used for further purification. After a desalting step through a C8 column, the fractions were pooled and lyophilized to obtain cGMP OPT-821. The product was further analyzed with an analytical HPLC using a Vydac C4 column with a very predominant peak at 27.2 min and a very small shoulder peak at 26.5 min. The MALDI-TOF mass analysis of OPT-821 showed a dominant peak at 2012 (M + Na). Other peaks observed were: 2034 (M + 2Na), 2028 (M + K), 2050 (M + Na + K), and 1990 (M + H).

OPT-821 was evaluated in murine models with the Globo H-KLH conjugate vaccine with various doses purified adjuvant OPT-821 and with saponin adjuvant. Adjuvant activity was measured by production of antibodies by ELISA. Groups of mice (C57BL/6J) were immunized three times at one-week interval with 5 µg of Globo H-KLH (containing 5 µg of Globo H hexasaccharide) plus different dose of OPT-821 (20, 50 and 100µg) subcutaneously in the abdomen or groin. Mice were bled and sera were collected prior to the initial vaccination and at 7 days after third vaccination. Antibody titers were measured by ELISA. Our various preclinical experiments showed that the adjuvant OPT-821 is immunologically active (Dr. Ragupathi, personal communication). Optimer has purified OPT-821 under cGMP for clinical use.

5.1.4 Animal Studies of Polyvalent Vaccine and OPT-821

Repeat dose toxicity studies were performed with OPT-821 with or without the antigenic components (GM2-KLH, Globo H-KLH, MUC1-Tn-KLH and Tf(c)-KLH) administered once weekly for four weeks. Male and female mice (C57BL/6J) were dosed subcutaneously with the vehicle (0.9% normal saline), or OPT-821 at 5 mcg/mouse/dose, or the polyvalent vaccine (GM2-KLH, Globo H-KLH, MUC1-Tn-KLH and Tf(c)-KLH with OPT-821) at two different dose levels. The lower dose level contained 1.5 mcg/mouse/dose of each of the GM2-KLH and Globo H-KLH, and 0.15 mcg/mouse/dose of each of MUC1-Tn-KLH and Tf(c)-KLH, and 5 mcg/mouse/dose of the OPT-821 adjuvant. The doses of each vaccine component including the adjuvant were 4-fold higher in higher dose-level group. The no observable adverse effect level was 1.5 mcg/mouse/dose for each GM2-KLH and Globo H-KLH conjugates and 0.15 mcg/mouse/dose for each Tn-MUC1-KLH and TF(c)-KLH conjugates and 5 mcg/mouse for OPT-821. No treatment related effects were seen in appearance, behavior, body weight or clinical chemistry parameters over the study duration when compared to the control group. A mild to moderate increase in the lymphocyte and monocyte counts was noted at the higher dosing level. Additional information is found in the investigators brochure.

5.1.5 Vaccine Administration

The intervention includes the subcutaneous injection of polyvalent vaccine-KLH conjugate for 6 vaccinations using the dose and schedule described in Table 1. The preparation will be supplied in approximately 1.2 cc total volume and administered subcutaneously in one syringe to a site in the shoulders, buttocks, or thighs. The vaccine will be administered prior to bevacizumab treatment.

5.1.6 Vaccine Storage/Supply

Vials containing vaccine KLH-OPT-821 have 1.2 ml normal saline as the diluent. Vials are to be stored at –30 to –80° Celsius. The antigen doses to be administered are GM2 (30 µg), TF(c) (3 µg), Globo-H (30 µg), Tn-MUC1 (3 µg), KLH (H 600 µg) with adjuvant OPT-821(100µg). Antigen doses were selected based on previous Phase I monovalent vaccine trials as follows: Tn-MUC1-32mer, 3µg [22]; Globo-H, 30µg [21, 23]; GM2, 30µg [47]; Tn(c), 3µg [25]; TF(c), 3µg [26]. OPT-821 is given at 100µg [23, 47]. These conjugates and OPT-821 are vialed together at MSKCC in 1.2 ml normal saline. The vaccines will be distributed by Memorial Sloan Kettering Investigational Pharmacy. All drug should be discarded according to institutional Standard Operating Procedures, and their disposition should be recorded.

5.2 Bevacizumab

Bevacizumab will be given at 7.5 mg/kg via intravenous infusion over 20-30 minutes beginning on day 1 of week 1 once every two weeks initially and then q 3 weeks as outlined, until progression, unacceptable toxicity, or withdrawal of consent. Bevacizumab is a clear to slightly opalescent, colorless to pale brown, sterile liquid concentrate for solution for intravenous (IV) infusion. Bevacizumab will be supplied in 20-cc (400-mg) glass vials containing 16 mL bevacizumab, (at 25 mg/mL). Vials contain bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection (SWFI), USP. Vials contain no preservative and are suitable for single use only. It is not necessary to correct dosing based on ideal weight.

For further details and molecule characterization, see the bevacizumab Investigator Brochure.

5.2.1 Bevacizumab Administration

Bevacizumab will be administered as per Memorial Sloan-Kettering Cancer Center ADULT Chemotherapy/Biologic Therapy Guidelines:

o Add the calculated dose of bevacizumab to 100 ml of 0.9% Sodium Chloride Injection.
o 7.5 mg/kg IV infused over 20 mins once every 14 days until week 11 and then once every 3 weeks. Administration will be as a continuous IV infusion.
o Alternatively at the instruction of the prescriber, it may be infused in as much as 30 mins.

o Anaphylaxis precautions should be observed during study drug administration.
o If an infusion is not tolerated well (ie. fever, chills) then the subsequent infusion(s) should be given over a prolonged infusion time (i.e. 60-90 min).
o If a subject experiences an infusion associated adverse event with the 60-minute infusion, all subsequent doses should be given over 90±15 minutes.

5.2.2 Bevacizumab Storage

Upon receipt of the study drug, vials are to be refrigerated at 2°C–8°C (36°F–46°F) and should remain refrigerated until just prior to use. DO NOT FREEZE. DO NOT SHAKE. Vials should be protected from light.

Opened vials must be used within 8 hours. VIALS ARE FOR SINGLE USE ONLY. Vials used for 1 subject may not be used for any other subject. Once study drug has been added to a bag of sterile saline, the solution must be administered within 8 hours.

5.2.3 Concomitant Medications with Bevacizumab

Low-dose aspirin (≤ 325 mg/d) may be continued in subjects at higher risk for arterial thromboembolic disease. Subjects developing signs of arterial ischemia or bleeding on study should be evaluated for possible bevacizumab discontinuation per Table 4, Bevacizumab Dose Management Due To Adverse Events.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

Eligible patients are those with the following characteristics:

1. Histologically documented epithelial carcinoma arising in the ovary, fallopian tube or peritoneum

2. Patients who have received cytoreductive surgery and chemotherapy with at least one platinum based chemotherapy regimen. Patients who received neoadjuvant chemotherapy are eligible.

3. Patients with relapsed ovarian, fallopian tube or primary peritoneal cancer who have now completed chemotherapy and/or surgery for recurrent disease. Eligible patients are those who would be appropriate to enter a period of observation if standard management were considered

4. Patients who have asymptomatic residual measurable disease on CT scan or who are in complete clinical remission. Patients may have an elevated CA-125. (Complete clinical remission is defined as serum CA-125 ≤ 35 IU/ml, negative physical examination and without objective evidence of disease by computed tomography (CT) of the abdomen and pelvis.)

5. Adequate hematologic, coagulation, renal and hepatic function.
   a. ANC $\geq$ 1,000 cells/mm^3; platelets $\geq$ 100,000 cells/mm^3
   b. PT such that international normalized ratio (INR) is < 1.5 (or an in-range INR, usually between 2 and 3, if a patient is on a stable dose of therapeutic warfarin) Serum creatinine $\leq$ 1.5 mg/dl
   c. Bilirubin, SGOT, Alk Phos < 2.5x upper limit of normal

6. Urine protein: creatinine (UPC) ratio must be < 1. If UPC ratio > 1, collection of 24-hour urine measurement of urine protein is recommended as part of the patient's medical management off-study. See Appendix D for further details.

7. Karnofsky performance status > 70%

8. Expected survival of at least 4 months

9. Age ≥ 18 years. This protocol does **not** include children because the number of children with cancer is limited, and because a nationwide pediatric cancer research network already accesses the majority. Furthermore, the incidence of ovarian, fallopian tube, or peritoneal cancer in children is extremely infrequent.

10. Patients who are ≥ 4 weeks from completion of prior cytotoxic chemotherapy. Prior bevacizumab and/or immunotherapy treatment are permitted

6.2 Subject Exclusion Criteria

Patients are **excluded** from study with the following characteristics:

1. Inability to comply with study and/or follow-up procedures
2. Current or recent (within 4 weeks of the first infusion of this study) participation in another experimental drug study.
3. Active malignancy, other than superficial basal cell and superficial squamous (skin) cell, or carcinoma in situ of the cervix within last five years
4. Patients must have undergone standard cytoreductive surgery as part of primary treatment to be eligible for this study and therefore are not of childbearing potential. Nursing mothers are excluded.
5. Inadequately controlled hypertension (defined as systolic blood pressure >150 mmHg and/or diastolic blood pressure > 90 mmHg)
6. Prior history of hypertensive crisis or hypertensive encephalopathy
7. New York Heart Association (NYHA) Grade II or greater congestive heart failure (see Appendix C)
8. History of myocardial infarction or unstable angina within 6 months prior to Day 1
9. History of stroke or transient ischemic attack within 6 months prior to Day 1
10. Known CNS disease, except for treated brain metastasis
11. Treated brain metastases are defined as having no evidence of progression or hemorrhage after treatment and no ongoing requirement for dexamethasone, as ascertained by clinical examination and brain imaging (MRI or CT) during the screening period. Anticonvulsants (stable dose) are allowed. Treatment for brain metastases may include whole brain radiotherapy (WBRT), radiosurgery (RS; Gamma Knife, LINAC, or equivalent) or a combination as deemed appropriate by the treating physician. Patients with CNS metastases treated by neurosurgical resection or brain biopsy performed within 3 months prior to Day 1 will be excluded
12. Significant vascular disease (e.g., aortic aneurysm, requiring surgical repair or recent peripheral arterial thrombosis) within 6 months prior to Day 1
13. History of hemoptysis (ε 1/2 teaspoon of bright red blood per episode) within 1 month prior to Day 1

14. Evidence of bleeding diathesis or significant coagulopathy (in the absence of therapeutic anticoagulation) or tumor involving major vessels.
15. Major surgical procedure such as laparotomy, open biopsy, or significant traumatic injury within 28 days prior to Day 1 or anticipation of need for major surgical procedure during the course of the study
16. Core biopsy or other minor surgical procedure, excluding placement of a vascular access device, within 7 days prior to Day 1
17. History of abdominal fistula or gastrointestinal perforation within 6 months prior to Day
18. Patients with clinical symptoms or signs of GI obstruction who require parenteral hydration, parenteral nutrition, or tube feeding
19. Patients with evidence of abdominal free air not explained by paracentesis or recent surgical procedure
20. Serious, non-healing wound, active ulcer, or untreated bone fracture
21. Known hypersensitivity to any component of bevacizumab
22. Allergy to seafood
23. Active autoimmune disease (i.e. rheumatoid arthritis, ulcerative colitis etc); or immune deficiency (HIV, hypogammaglobulinemia); or known active infections with Hepatitis B or Hepatitis C; or those receiving immunosuppressive drugs (such as chronic systemic corticosteroids or cyclosporin, etc); or those receiving chronic anti-inflammatory drugs (intermittent use of anti-inflammatory drugs is permitted).

7.0 RECRUITM ENTPLAN

Potential research subjects will be identified by a member of the patient's treatment team, the protocol investigator, or research team at Memorial Sloan-Kettering Cancer Center (MSKCC). If the investigator is a member of the treatment team, s/he will screen their patient's medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study. Potential subjects contacted by their treating physician will be referred to the investigator/ research staff of the study.

The principal investigator may also screen the medical records of patients with whom they do not have a treatment relationship for the limited purpose of identifying patients who would be eligible to enroll in the study and to record appropriate contact information in order to approach these patients regarding the possibility of enrolling in the study.

During the initial conversation between the investigator/research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. The investigator/research staff may also review portions of their medical records at MSKCC in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes.

8.0 PRETREATM ENTEVALUATION

- Clinical: (within 2 weeks before first study treatment)
  - History (demographics, recording of pertinent concurrent medications, adverse event evaluation) and physical examination including vital signs: temperature, heart rate, respiratory rate, and blood pressure assessment.
  - Karnofsky performance status

- Laboratory: (within 2 weeks before first study treatment)
  - Complete blood cell count with differential
  - Comprehensive serum chemistry profile (includes Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.)
  - Serum magnesium
  - Serum CA-125
  - Serum TSH
  - PT/INR
  - Urine protein: creatinine ratio or urine dipstick (and 24 hour collection ifindicated)
  - Amylase

- Imaging and other diagnostic studies: (within 4 weeks before first study treatment)
  - Computed tomography (CT) of abdomen and pelvis.
  - Chest radiograph (or CT chest)
  - Electrocardiogram

9.0 TREATMENT/INTERVENTION PLAN

9.1 Agent Administration

9.1.1 Polyvalent-KLH Vaccine + OPT 821

The administered polyvalent vaccine will contain GM2 (30 µg), Globo-H (30 µg), Tn-MUC1 (3 µg) and TF(c) (3 µg), individually conjugated to KLH (H 600 µg) and mixed with adjuvant OPT-821 (100 µg). Total vial volume is 1.2 ml. 21 patients will be vaccinated.

The vaccine will be administered subcutaneously at weekly intervals for three doses. This will be followed by a four week break, then a fourth vaccination, another four week break, then a fifth vaccination and finally a further six week break and a sixth vaccination.

Table 3: Immunization and Bevacizumab Schedule (+/- 3 day windows are allowed)

Immunization	Weekly x3			4 Week break				4 Week break				6 Week break						Study end
Immunization Number	#1	#2	#3				#4				#5						#6	
Bevacizumab	Starting with vaccine #1 on week 1 once every 2 weeks until week 11 and then q3 weeks until disease progression/study end																	
Bevacizumab	*		*		*		*		*		*			*			*	*
Correlative Studies	↑																↑	↑
Immune Studies	↑						↑							↑				
Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	20 or end of study

9.1.2 Bevacizumab

Bevacizumab will be given via intravenous infusion at 7.5 mg/kg over 20-30 minutes, beginning on day 1 of week 1, once every two weeks initially and then q 3 weeks as outlined.

9.2 Duration of Therapy

A maximum of 6 doses of the polyvalent-KLH vaccine and OPT-821 will be administered to each patient as per the schedule outlined in Table 3. Bevacizumab will be administered once every two weeks until week 11 and then once every three weeks according to the schedule outlined in Table 3. When the 6 vaccinations of the polyvalent-KLH vaccine +OPT821 are completed, patients may still continue to receive bevacizumab on the once every three week schedule.

The protocol treatment will be discontinued for:

- disease progression (as defined by RECIST)

- unacceptable toxicity associated with the polyvalent-KLH vaccine or bevacizumab (outlined in Section 9.3)

- withdrawal of consent

9.3 Dose Delays/ Dose Modifications

9.3.1 Polyvalent-KLH Vaccine + OPT 821

Dose reduction or delay of vaccine are **not** permitted. Patients are removed from study for a vaccine-related Dose Limiting Toxicity (DLT) DLT is defined by:

- ≥ Grade 2 allergic reaction. (Grade 2 is defined as asymptomatic bronchospasm, or urticaria; Grade 3 is defined as symptomatic bronchospasm, requiring parenteral medications, with or without urticaria, allergy related edema or angioedema; Grade 4 is defined as anaphylaxis.)

- ≥ Grade 2 autoimmune reaction (Grade 2 is defined as evidence of autoimmune reaction involving a non-essential organ or function (e.g. hypothyroidism) requiring treatment other than immunosuppressive drugs.)

- ≥ Grade 3 hematologic or non-hematologic toxicity including fever. (Grade 3 fever is > 40° C for ≤ 24 hours).

- ≥ Grade 3 injection site reaction. (Grade 3 is defined as ulceration, or necrosis that is severe or prolonged, or requiring surgery).

Any patient with Grade 2 or greater toxicity is followed with appropriate studies until results return to baseline.

9.3.2 Bevacizumab

There are no reductions in the bevacizumab dose. If adverse events occur that require holding bevacizumab, the dose will remain the same once treatment resumes. Missed doses of bevacizumab will not be made up. Regardless of the reason for holding the bevacizumab, the maximum allowable length of treatment interruption is 2 months. No dose escalation is planned for this study.

Permanent discontinuation of bevacizumab for related adverse events or other criteria:

Any adverse event, laboratory abnormality or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the patient with continued dosing. If bevacizumab is permanently discontinued, the patient may complete the schedule of vaccinations with the polyvalent-KLH vaccine and OPT-821 as per the schedule outlined above in Table 3.

Table 4:
Summary of Bevacizumab Dose Management Due to Adverse Events

Event	Action to be Taken
Hypertension	
No dose modifications for grade 1 events	
Grade 2 or 3	If not controlled to 150/90 mmHg with optimal medical management refer to section 9.3.2.1 below
Grade 4 (including hypertensive encephalopathy)	Discontinue bevacizumab.
Hemorrhage	
No dose modifications for grade 1 or 2 non-pulmonary or non-CNS hemorrhage	
Grade 3 non-pulmonary or non-CNS hemorrhage	Subjects who are also receiving full-dose anticoagulation will be discontinued from receiving bevacizumab. All other subjects will have bevacizumab held until all of the following criteria are met: ⌋ The bleeding has resolved and hemoglobin is stable. ⌋ There is no bleeding diathesis that would increase the risk of therapy. ⌋ There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence. Subjects who experience a repeat grade 3 hemorrhagic event or who experience a delay in resolution according to the above criteria for >3 weeks, will be discontinued from receiving bevacizumab.
Grade 4 non-pulmonary or non-CNS hemorrhage	Discontinue bevacizumab.
Grade 1 pulmonary or CNS hemorrhage	Subjects who are also receiving full-dose anticoagulation will be discontinued from receiving bevacizumab. All other subjects will have bevacizumab held until all of the following criteria are met: ⌋ The bleeding has resolved and hemoglobin is stable. ⌋ There is no bleeding diathesis that would increase the risk of therapy. ⌋ There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence.
Grade 2, 3, or 4 pulmonary or CNS hemorrhage	Discontinue bevacizumab

Venous Thrombosis	
No dose modifications for grade 1 or 2 events	
Grade 3 or 4	Hold bevacizumab treatment. If the planned duration of full-dose anticoagulation is <2 weeks, bevacizumab should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is >2 weeks, bevacizumab may be resumed during the period of full-dose anticoagulation if all of the following criteria are met: ⌋ The subject must have an in-range INR (usually between 2 and 3) if on warfarin ⌋ LMWH, warfarin, or other anticoagulant dosing must be stable prior to restarting bevacizumab treatment. ⌋ The subject must not have had a Grade 3 or 4 hemorrhagic event while on anticoagulation. ⌋ The subject must not have pathological conditions that carry high risk of bleeding (e.g. tumor involving major vessels). Patients with recurrent/worsening venous thromboembolic events after resumption of bevacizumab treatment will be taken off bevacizumab.
Arterial Thromboembolic Event	
New onset or worsening of any arterial thromboembolic event since starting bevacizumab including unstable angina, myocardial infarction, transient ischemic attack, cerebrovascular accident, peripheral arterial ischemia or visceral arterial ischemia or any other arterial thromboembolic event	
≥ Grade 1	Discontinue bevacizumab.
Coagulopathy	
No dose modifications for grade 1 or 2 events	
Grade 3 or 4	Hold bevacizumab, until PT/PTT resolve to grade 1 For patients on therapeutic warfarin with PT/INR higher than the intended therapeutic range, treatment with bevacizumab will be held until PT/INR is within the therapeutic range. Patients experiencing treatment delay >3 weeks because of failure to meet the above criteria will be taken off bevacizumab therapy.
Congestive Heart Failure (Left ventricular systolic dysfunction)	
No dose modifications for grade 1 or 2 events	
Grade 3	Hold bevacizumab until resolution to Grade ≤ 1.
Grade 4	Discontinue bevacizumab.

Proteinuria	
No dose modifications for grade 1 or 2 events	
Grade 3 (UPC> 3.5, urine collection > 3.5 g/24 hr)	Hold bevacizumab treatment until δ Grade 2, as determined by either UPC ratio ≤ 3.5 or 24 hr collection ≤ 3.5 g. Refer to section 9.3.2.2 below.
Grade 4 (nephrotic syndrome)	Discontinue bevacizumab
GI Perforation	
Unexplainable grade 1 (excluding evidence of abdominal free air attributable to other causes such as paracentesis or recent surgical procedure) or ≥ Grade 2 (including intra-abdominal abscess or GI fistul a)	Discontinue bevacizumab.
Fistula	
≥ 1 Grade tracheoesophageal fistula	Discontinue bevacizumab.
≥ 2 Grade other fistula	Discontinue bevacizumab.
Bowel Obstruction Refer to section 9.3.2.3	
Grade 1	Continue patient on study for partial obstruction NOT requiring medical intervention.
Grade 2	Hold bevacizumab for partial obstruction requiring medical intervention. Patient may restart upon complete resolution.
Grade 3 or 4	Hold bevacizumab for complete obstruction. If surgery is necessary, patient may restart bevacizumab after full recovery from surgery, and at investigator's discretion.
Wound dehiscence	
Any grade (requiri ng medical or surgical therapy)	Discontinue bevacizumab. Refer to section 9.3.2.4
Reversible Posterior Leukoencephalopathy	
Any grade (confirmed by MRI)	Discontinue bevacizumab. Refer to section 9.3.2.5
Hypersensitivity Reactions Refer to section 9.3.2.6	

Other Unspecified Bevacizumab-Related Adverse Events Refer to section 9.3.2.7	
Grade 3	Hold bevacizumab until recovery to ≤ Grade 1.
Grade 4	Discontinue bevacizumab.

9.3.2.1 Hypertension:

Hypertension is a known and potentially serious adverse event associated with bevacizumab treatment. Patients receiving bevacizumab should be monitored prior to each dose with measurement of blood pressure. If the patient's blood pressure is elevated (>150/90) at any time, even at outside clinic visits, they should contact their treating physician.

Medications used

Medication classes used for management of patients with grade 2 or 3 hypertension receiving bevacizumab include angiotensin-converting enzyme inhibitors, beta blockers, diuretics, and calcium channel blockers. The use of anxiolytics in conjunction with specific anti-hypertensive agents is not prohibited. The goal for blood pressure control should be consistent with general medical practice guidelines (i.e. < 140/90 mmHg in general and < 130/80 mmHg for patients with diabetes).

Grade 2 and 3 hypertension (CTCAE v4)

Grade 2 hypertension includes systolic BP 140 - 159 mm Hg or diastolic BP 90-99 mmHg) that is recurrent or persistent (>24 hours) where medical intervention with anti-hypertensives is indicated. Grade 2 hypertension also includes a symptomatic increase by >20 mm Hg (diastolic) or to >140/90 mm Hg if previously within normal limits where is monotherapy indicated.

Grade 3 hypertension includes systolic BP ≥160 mmHg or diastolic BP ≥100 mmHg where medical intervention is indicated and more than one drug or more intensive therapy than previously used is indicated.

Dose Delays

- For controlled hypertension, defined as systolic ≤ 150 mm Hg and diastolic ≤ 90 mm Hg, continue bevacizumab therapy.
- For grade 2 or 3 hypertension that is not controlled with optimal medical management or for symptomatic hypertension < grade 4, hold bevacizumab treatment for up to 2 weeks. In the interim, antihypertensive therapy should be initiated or continued.
- If hypertension is controlled (systolic ≤ 150 mm Hg and diastolic ≤ 90 mm Hg) and symptomatic hypertension has resolved by two weeks after holding treatment, continue bevacizumab therapy.
- If hypertension remains uncontrolled or symptomatic hypertension <grade 4 persists two weeks after holding treatment, continue to hold bevacizumab. If symptomatic hypertension has not resolved by 6 weeks after holding treatment

with bevacizumab, treatment with bevacizumab should be discontinued for the remainder of the study.

- Bevacizumab should be discontinued for the remainder of the study in any patient developing grade 4 hypertension.

9.3.2.2 Proteinuria:

Patients receiving bevacizumab should be monitored by urine analysis for urine protein: creatinine (UPC) ratio prior to every other dose of bevacizumab:

- UPC ratio < 3.5 ⇨ Continue bevacizumab.
- UPC ratio ≥ 3.5 and/ or urine collection >3.5g/24hr⇨ Hold bevacizumab until UPC ratio recovers to < 3.5 or urine collection <3.5g/24 hr.
- If therapy is held for > 6 weeks due to proteinuria, discontinue bevacizumab.
- If patient develops grade 4 proteinuria or nephrotic syndrome, discontinue bevacizumab.

9.3.2.3 Intestinal obstruction:

Bevacizumab will be held for occurrence of CTCAE Grade 3 toxicity, until resolution to ≤CTCAE Grade 1 and will be permanently discontinued for occurrence of CTCAE Grade 4 toxicity. Since the development of intestinal obstruction could be a result of cancer progression, the investigator should take steps to evaluate such patients for the possibility of disease progression according, using clinical, laboratory and radiographic information as clinically indicated; in the event of disease progression, all protocol-directed therapy would be discontinued.

9.3.2.4 Wound Disruption/Bowel Perforation, Fistula, or GI Leak:

Treatment with bevacizumab will be discontinued in the event of wound disruption requiring medical or surgical intervention, bowel perforation or fistula (including tracheo-esophageal fistula).

Prior to Initiation of bevacizumab – In the event of superficial wound separations healing by secondary intention with no evidence of fascial dehiscence or infection, therapy with bevacizumab may be initiated with weekly wound examinations until complete closure.

After initiation of bevacizumab – bevacizumab will be discontinued for any new event, regardless of grade.

9.3.2.5 Reversible Posterior Leukoencephalopathy Syndrome (RPLS):

Bevacizumab should be held in patients with symptoms/signs suggestive of RPLS, pending work-up and management, including control of blood pressure. Bevacizumab should be discontinued upon diagnosis of RPLS.

Note: Resumption of bevacizumab may be considered in patients who have documented benefit from the agent, provided that RPLS was mild and has completely resolved clinically and radiographically within 2 to 4 weeks; decision to resume bevacizumab in these patients must be discussed with the study chair and approved by the sponsor.

9.3.2.6 Hypersensitivity Reaction:

In general, the occurrence of a hypersensitivity reaction to bevacizumab is not considered a dose-limiting toxicity. Patients may be retreated at full doses after administration of medication to prevent hypersensitivity reactions, and adjustments in infusion rates should be made (see guidelines for re-treatment with bevacizumab in section 5.2.1). However, if despite these safety measures repeat attempt at infusion of the inciting drug results in a recurrent hypersensitivity reaction, the drug should be discontinued for the remainder of the study. In the event of any grade 3 or 4 allergic or infusion reaction to bevacizumab it will be permanently discontinued.

9.3.2.7 Other:

There will be no dose modifications for alopecia, nausea, constipation, or diarrhea. It is recommended that routine medical measures be employed to manage nausea, constipation, and diarrhea.

Unanticipated Major Surgical Procedures: For any unanticipated (emergent/urgent) major surgical procedure such as laparotomy performed for reasons other than disease progression or CTCAE at least possibly related to bevacizumab treatment should be held > 28 days post-operatively prior to resumption. Treatment delay is not required for minor procedures including a) cystoscopy, b) the removal or insertion of a central venous catheter, nephrostomy tube, or ureteral stent or c) thoracentesis or paracentesis for symptom relief in the absence of disease progression.

Special Modifications Study Treatment: Potential modifications for other non-hematologic toxicities with an impact on organ function of grade 2 (or greater) require discussion with one of the study co-chairs except where noted immediately below:

- For any CTCAE Grade 3 non-hematologic adverse events (except controllable nausea/emesis) considered to be at least possibly related to study treatment, protocol directed treatment should be held until symptoms resolve to ≤ CTCAE Grade 1. If a CTCAE Grade 3 adverse event persists for > three weeks or recurs after resumption of therapy, the patient may be taken off protocol directed treatment after consulting with the Study Chair.
- For any CTCAE Grade 4 non-hematologic adverse event (except controllable nausea/emesis), the patient may be taken off protocol directed treatment therapy after consulting with the Study Chair.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

10.1 Immune Function

Peripheral blood (10-20ml) will be drawn according to the schedule in Table 5 (Section 10.2).

Antibodies against appropriate antigens will be studies by ELISA and FACS where appropriate. One plain red top tube will be collected at weeks 1 and 7.

Collect 10 ml (cc) of whole blood in a plain red top tube. Allow the blood to clot upright at 4˚C at room temperature for a minimum of 30 min but for no longer than 3 hrs. The faster the blood can be processed after the 30 min clotting step the better. The blood must be centrifuged to separate the serum (pale straw-colored liquid) from the blood cells. When the appropriate equipment is available, please centrifuge the blood at

~3,500 x *g* at 4ºC for 10 min. The minimum centrifugation requirements will be ~1000 x *g* at room temperature for 15 min. The longer centrifugation time will help compensate for the slower speed. Avoid centrifugations without refrigeration longer than 15 min as excess heat may build up in the unit and damage the serum.

Immediately freeze serum upright if possible using an appropriate type of freezing/storage space. Ultra-cold conditions such as an ultra cold freezer (δ -70ºC) or liquid nitrogen are ideal. Freezing by direct exposure with dry ice is also acceptable. Serum frozen under ultra-cold conditions represents the highest quality specimen suitable for all types of laboratory testing including proteomic analysis.

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

On week 14, 100ml of heparinized blood will be drawn to evaluate the B-cell response at the clonal level by production of monoclonal antibodies against the immunizing antigens. This will be performed in collaboration with MabVax Therapeutics (San Diego, CA). It will make possible more detailed analysis of the most immunogenic epitopes on these antigens, and more detailed comparison of the avidity of the induced IgG and IgM antibodies than is possible with sera. If the patient goes off study prior to week 14, an additional sample (10-20ml) will be obtain to assess immune status at the off study visit.

Samples should be delivered to Dr Philip Livingston's laboratory (Laboratory of Tumor Vaccinology), MSKCC. The immunology tests will be performed under the direction of the Ludwig Center (as will the immunology tests from the larger randomized GOG trial).

10.2 Assessment

10.2.1 Clinical and Laboratory Assessment

The clinical and laboratory assessment schedule is outlined below and includes parameters to assess the safety of the vaccine, as well as evaluate for signs of disease recurrence and progression. Abnormal findings will be evaluated per standard medical practice, and the abnormality will be classified as related to treatment, disease progression, or neither.

Table 5: Schedule of Asse ssment (+/- 3 days windows allowed)

Week #	0	1	2	3	5	7	9	11	14	17	20 and q3 wks till end of study
Vaccine#		1	2	3		4		5		6	
Bevacizumab		*		*	*	*	*	*	*	*	*
Office visit (toxicity assessment)	*	*	*	*	*	*	*	*	*	*	*
Hx and PE (including BP)	*	*		*	*	*	*	*	*	*	*
Ca- 125	*		*		*		*		*	*	* (alt visits and study end)
CBC, differential	*			*	*	*	*	*	*	*	*
Comprehensive Metabolic Panel	*		*		*		*		*	*	* (alt visits and study end)
Amylase	*										
Urine protein: creatinine ratio	*			*		*		*		*	* (alt visits and study end)
PT	*									*	
TSH	*									*	
EKG	*										
Radiographic Imaging	*							*			* and every three months while on study
Correlative Studies		*								*	*
Immune studies		*				*			*		

10.2.2 Extent of disease evaluation

Extent of disease evaluation: Patients in clinical remission are by definition without clinical or radiographic evidence of disease at protocol entry. Patients with asymptomatic residual disease may have an elevated CA-125 at protocol entry. Criteria for therapeutic response/outcome assessment are determined using RECIST criteria v1.1.[48]

10.2.3 Radiographic imaging

Radiographic imaging (CT abdomen and pelvis) will be obtained q 3 months while on study, or if any clinical symptoms/examination findings warrant further evaluation, or at any time at the discretion of the investigator.

In addition, patients with a normal serum CA-125 level at protocol entry will have radiographic imaging performed if serum CA-125 rises to $\geq$ 70 (per time to treatment failure criteria), confirmed by repeat value.

10.2.4 Length of follow-up

The primary endpoint of the study in this pilot trial is safety. Patients will be followed for the duration of the study. In previous trials, antibodies were generally present by the seventh week. We will proceed with the proposal for additional studies to evaluate efficacy if no systemic toxicity is seen in any patient at the ninth week assessment. Patients will be followed until time to treatment failure.

11.0 TOXICITIES/SIDE EFFECTS

11.1 Toxicity Evaluation

Toxicity will be evaluated according to the National Cancer Institute CTCAE Scale (Version 4.0, published May 28, 2009).[49]

11.2 Vaccination Toxicity

The expected safety of the proposed vaccine is based on accumulating clinical experience with these vaccines in patients with a variety of malignancies including ovarian cancer. Vaccination with the individual components has been well tolerated as previously described.[18, 21, 25-27] In addition, no symptomatic autoimmunity was detected in the pilot heptavalent vaccine trial, with self limited fever, myalgia and injection site reactions as the most common toxicities (all $\leq$ grade 2). No grade 3 or 4 toxicity was seen.[3] The lack of symptomatic autoimmunity has been hypothesized to be related to the preponderance of antigen expression on the secretory borders of cells where they are relatively sequestered from the immune system.

Finally, composite safety data for serious adverse events on single antigen vaccine studies at MSKCC are as follows:

Table 6: Serious Adverse Events in Monovalent Vaccine Trials

Antigen	Total # pts	# SAE's	Protocols	SAE Type
GM2	71	0	94-041	None
GLOBO-H	74	1	96-055,97-086	G2 Angioedema
LEWIS Y	18	1	00-075,02-103	Dermatomyositis
MUC-1	90	4	94-130,99-023	Thyroid (3) Erythema nodosum (1)
TN	25	0	98-002	None
TF	21	0	98-048	None

In the total 299 patients above, 6/299 (2%) of patients had events meeting criteria for SAE and were reported as such. Three of these cases were hypothyroidism. An extensive evaluation of these three patients with hypothyroidism, yielded no evidence for vaccine-induced dysfunction, and hypothyroidism is seen de novo in this patient demographic. These patients received thyroid hormone supplementation without sequelae. However, thyroid studies are now included as part of vaccine safety monitoring. The patient with dermatomyositis carried this diagnosis equivocally prior to enrollment. It is unclear if the erythema nodosum as described was related to vaccination and it has not been seen in other patients. Finally, grade 2 angioedema was seen in 1/299 patients (0.3%) and has not been reported in others. Patients are routinely observed for 30 minutes following vaccination. In reviewing all trials taken together, the most common adverse events are grade 1 or 2 local erythema and induration at the injection site, which resolves in one to three days. Approximately, 25% of patients have mild flu-like symptoms for six to 24 hours after one or more vaccinations.

Side Effects from Vaccine for Patients on Study:

Likely:

1. Mild tiredness

2. Redness and tenderness at vaccination site which resolves in 2-3 days without treatment. The site may become inflamed with significant erythema, and this has been managed conservatively with cool soaks, analgesics for discomfort, acetaminophen most commonly) and it resolves with time. If it occurs, it generally may increase in intensity with the first several vaccinations, and then tends to lessen with subsequent vaccinations.

3. Low grade fever for 24 – 48 hours after vaccination. This may be managed with observation or an anti-pyretic if desired (acetaminophen most commonly used).

Less Likely:

1. Abnormal blood counts

2. High fever

3. Mild elevation in liver function tests (transaminases)

4. Hypothyroidism (This was seen in 3/299 patients previously treated with similar vaccines. While there was no serologic evidence it was related, the protocol requires assessment of thyroid function.)

5. Vasovagal syncope after receiving SC injection (unrelated to vaccine)

6. Nausea

Rare but Serious:

1. Angioedema after vaccination. Grade 2 angioedema was seen in 1/299 previously vaccinated patients (0.3%). Severe allergic reactions have not been reported to date with similar vaccines.

2. Symptoms related to autoimmunity (mastitis, pancreatitis, hepatitis, etc). This entity has not been previously reported with similar vaccines but is theoretically possible.

(See Section 9.1.1 for detailed description of potential side-effects and their management)

11.3 Bevacizumab Toxicity

The most recent safety update is detailed in the current Avastin® Prescribing Information.

Any toxicities associated or possibly associated with bevacizumab treatment should be managed according to standard medical practice. Discontinuation of bevacizumab will have no immediate therapeutic effect. Bevacizumab has a terminal half-life of 21 days; therefore, its discontinuation results in slow elimination over several months. There is no available antidote for bevacizumab.

Subjects should be assessed clinically for toxicity prior to, during, and after each infusion. If unmanageable toxicity occurs because of bevacizumab at any time during the study, treatment with bevacizumab should be discontinued. Any patient whose treatment is delayed must be evaluated on a weekly basis until adequate hematologic and non-hematologic parameters have been met. Missed doses of bevacizumab will not be made up. Regardless of the reason for holding the bevacizumab, the maximum allowable length of treatment interruption is 2 months. No dose escalation is planned for this study.

A number of measures will be taken to ensure the safety of patients participating in this trial. These measures will be addressed through exclusion criteria (see Section 6.2) and routine monitoring as follows.

Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study. Safety evaluations will consist of medical interviews, recording of adverse events, physical examinations, blood pressure, and laboratory measurements. Patients will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of

their participation in the study. Patients discontinued from the treatment phase of the study for any reason will be evaluated ~30 days (28–42 days) after the decision to discontinue treatment.

In the initial Phase I and II clinical trials, four potential bevacizumab-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed Phase II and Phase III studies of bevacizumab as well as spontaneous reports have further defined the safety profile of this agent. Bevacizumab-associated adverse events identified in phase III trials include congestive heart failure (CHF) primarily in metastatic breast cancer, gastrointestinal perforations, wound healing complications, and arterial thromboembolic events (ATE).

These and other safety signals are described in further detail as follows and in the bevacizumab Investigator Brochure.

More likely toxicities are:

Hypertension: An increased incidence of hypertension has been observed in patients treated with bevacizumab. Grade 4 and 5 hypertensive events are rare. Clinical sequelae of hypertension are rare but have included hypertensive crisis, hypertensive encephalopathy, and reversible posterior leukoencephalopathy syndrome (RPLS).(Ozcan et al., 2006; Glusker et al., 2006).

Proteinuria: An increased incidence of proteinuria has been observed in patients treated with bevacizumab compared with control arm patients. In the bevacizumab-containing treatment arms of clinical trials (across all indications), the incidence of proteinuria (reported as an adverse event) was up to 38% (metastatic CRC Study AVF2192g). The severity of proteinuria has ranged from asymptomatic and transient events detected on routine dipstick urinalysis to nephrotic syndrome; the majority of proteinuria events have been grade 1. NCI-CTC Grade 3 proteinuria was reported in up to 3% of bevacizumab-treated patients, and Grade 4 in up to 1.4% of bevacizumab-treated patients. The proteinuria seen in bevacizumab clinical trials was not associated with renal impairment and rarely required permanent discontinuation of bevacizumab therapy. Bevacizumab should be discontinued in patients who develop Grade 4 proteinuria (nephrotic syndrome)

Patients with a history of hypertension may be at increased risk for the development of proteinuria when treated with bevacizumab. There is evidence from the dose-finding, Phase II trials (AVF0780g, AVF0809s, and AVF0757g) suggesting that Grade 1 proteinuria may be related to bevacizumab dose.

Less likely toxicities are:

Thromboembolic Events: Both venous and arterial thromboembolic (TE) events, ranging in severity from catheter-associated phlebitis to fatal, have been reported in patients treated with bevacizumab in the colorectal cancer trials and, to a lesser extent, in patients treated with bevacizumab in NSCLC and breast cancer trials.

Venous Thromboembolism (including deep venous thrombosis, pulmonary embolism, and thrombophlebitis): In the phase III pivotal trial in metastatic CRC, there was a slightly higher rate of venous TE events in patients treated with bevacizumab plus chemotherapy compared with chemotherapy alone (19% vs. 16%). In Study AVF2107g, a Phase III, pivotal trial in metastatic CRC, VTE events, including deep venous thrombosis, pulmonary embolism, and thrombophlebitis, occurred in 15.2% of patients

receiving chemotherapy alone and 16.6% of patients receiving chemotherapy + bevacizumab.

The incidence of NCI-CTC Grade ε 3 venous VTE events in one NSCLC trial (E4599) was higher in the bevacizumab-containing arm compared to the chemotherapy control arm (5.6% vs. 3.2%). One event (0.2%) was fatal in the bevacizumab-containing arm; not fatal events were reported in the carboplatin/paclitaxel arm (see Bevacizumab Investigator Brochure). In metastatic CRC clinical trials, the incidence of VTE events was similar in patients receiving chemotherapy + bevacizumab and those receiving the control chemotherapy alone.

In clinical trials across all indications the overall incidence of VTE events was 2.8%Δ17.3% in the bevacizumab-containing arms compared with 3.2%Δ15.6% in the chemotherapy control arms. The use of bevacizumab with chemotherapy does not substantially increase the risk of VTE event compared with chemotherapy alone. However, patients with metastatic CRC who receive bevacizumab and experienced a VTE event may be at higher risk for recurrence of VTE event.

Arterial Thromboembolic Events: An increased incidence of ATE events was observed in patients treated with bevacizumab compared with those receiving control treatment. ATE events include cerebrovascular accidents, myocardial infarction, transient ischemic attacks (TIAs), and other ATE events. In a pooled analysis of data from five randomized Phase II and III trials (mCRC [AVF2107g, AVF2192g, AVF0780g]; locally advanced or metastatic NSCLC [AVF0757g]; metastatic breast cancer [AVF2119g]), the incidence rate of ATE events was 3.8% (37 of 963) in patients who received chemotherapy + bevacizumab compared with 1.7% (13 of 782) in patients treated with chemotherapy alone. ATE events led to a fatal outcome in 0.8% (8 of 963) of patients treated with chemotherapy + bevacizumab and 0.5% (4 of 782) of patients treated with chemotherapy alone. Cerebrovascular accidents (including TIAs) occurred in 2.3% of patients treated with chemotherapy + bevacizumab and 0.5% of patients treated with chemotherapy alone. Myocardial infarction occurred in 1.4% of patients treated with chemotherapy + bevacizumab compared with 0.7% of patients treated with chemotherapy alone (see the Bevacizumab Investigator Brochure for additional details).

Aspirin is a standard therapy for primary and secondary prophylaxis of arterial thromboembolic events in patients at high risk of such events, and the use of aspirin ≤ 325 mg daily was allowed in the five randomized studies discussed above. Use of aspirin was assessed routinely as a baseline or concomitant medication in these trials, though safety analyses specifically regarding aspirin use were not preplanned. Due to the relatively small numbers of aspirin users and arterial thromboembolic events, retrospective analyses of the ability of aspirin to affect the risk of such events were inconclusive. However, similarly retrospective analyses suggested that the use of up to 325 mg of aspirin daily does not increase the risk of grade 1-2 or grade 3-4 bleeding events, and similar data with respect to metastatic colorectal cancer patients were presented at ASCO 2005 (Hambleton et al., 2005). Further analyses of the effects of concomitant use of bevacizumab and aspirin in colorectal and other tumor types are ongoing.

Rare but serious toxicities are:

Gastrointestinal Perforation: Patients with metastatic carcinoma may be at increased risk for the development of gastrointestinal perforation and fistula when treated with bevacizumab and chemotherapy. Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation. A causal association of intra-abdominal inflammatory processes and gastrointestinal perforation to bevacizumab treatment has not been established. Nevertheless, caution should be exercised when treating patients with intra-abdominal inflammatory processes with bevacizumab. Gastrointestinal perforation has been reported in other trials in non-colorectal cancer populations (e.g., ovarian, renal cell, pancreas, breast, and NSCLC) and may be higher in incidence in some tumor types.

Fistula: Bevacizumab use has been associated with serious cases of fistulae including events resulting in death. Fistulae in the GI tract are common (1%–10% incidence) in patients with metastatic CRC, but uncommon (0.1%Δ1%) or rare (0.01%–0.1%) in other indications. In addition, fistulae that involve areas of the body other than the GI tract (e.g., tracheoesophageal, bronchopleural, urogenital, biliary) have been reported uncommonly (0.1%–1%) in patients receiving bevacizumab in clinical studies and postmarketing reports. Events were reported at various time-points during treatment, ranging from 1 week to > 1 year following initiation of bevacizumab, with most events occurring within the first 6 months of therapy.

Wound Healing Complications: Wound healing complications such as wound dehiscence have been reported in patients receiving bevacizumab. In an analysis of pooled data from two trials in metastatic colorectal cancer, patients undergoing surgery 28-60 days before study treatment with 5-FU/LV plus bevacizumab did not appear to have an increased risk of wound healing complications compared to those treated with chemotherapy alone (Scappaticci et al., 2005). Surgery in patients currently receiving bevacizumab is not recommended. No definitive data are available to define a safe interval after bevacizumab exposure with respect to wound healing risk in patients receiving elective surgery; however, the estimated half life of bevacizumab is 21 days. Bevacizumab should be discontinued in patients with severe wound healing complications.

Hemorrhage : Overall, grade 3 and 4 bleeding events were observed in 4.0% of 1132 patients treated with bevacizumab in a pooled database from eight phase I, II, and III clinical trials in multiple tumor types (bevacizumab Investigator Brochure, October 2005). The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage (see below) and minor mucocutaneous hemorrhage.

Tumor-Associated Hemorrhage: Major or massive pulmonary hemorrhage or hemoptysis has been observed primarily in patients with NSCLC. Life-threatening and fatal hemoptysis was identified as a bevacizumab-related adverse event in NSCLC trials. These events occurred suddenly and presented as major or massive hemoptysis. Among the possible risk factors evaluated (including squamous cell histology, treatment with anti-rheumatic/anti-inflammatory drugs, treatment with anticoagulants, prior radiotherapy, bevacizumab therapy, previous medical history of atherosclerosis, central tumor location, and cavitation of tumors during therapy), the only variables that showed statistically significant correlations with bleeding were bevacizumab therapy and squamous cell histology.

Additional Reported Adverse Events and Potential Risks with Bevacizumab (Avastin®) are:

Infusional reaction: fever, chills, rigor, rash, urticaria, dyspnea

Cardiovascular: hypertension (including hypertensive crisis), hypotension, pericardial effusion*, decrease in cardiac function*, cardiac troponin I elevation, arrhythmias

Hematologic: arterial and venous thrombosis/embolism (including pulmonary embolism, mesenteric vein thrombosis, ischemic bowel, cerebral vascular accident); hemorrhage (including epistaxis, CNS bleeding, GI bleeding, hemoptysis, pulmonary hemorrhage), neutropenia.

Constitutional: headache, infection without neutropenia, asthenia

Skin: rash, urticaria, delay in wound healing or dehiscence of healed wound, pruritus/itching

Gastrointestinal: nausea, vomiting, colitis, stomatitis/pharyngitis, intestinal obstruction, bowel perforation, bowel anastomotic dehiscence, anorexia.

Hepatic: LFT abnormalities

Pulmonary: pulmonary infiltration*, dyspnea

Renal/Genitourinary: proteinuria, nephrotic syndrome

Musculoskeletal: arthralgia, chest pain

Neurologic: dizziness; leukoencephalopathy syndrome, including reversible posterior leukoencephalopathy syndrome (RPLS).

Other: allergic rhinitis

*** indicates reported events with relationship to bevacizumab unclear**
(See Section 9.3.2 for detailed description of the management)

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

- Eligible patients may have asymptomatic residual measurable disease on physical examination and/or CT scan, and/or may have an elevated CA-125; or may be in complete clinical remission.

- Complete clinical remission is defined as a serum CA-125 < 35 IU/ml, CT scan without objective evidence of disease, and normal physical examination. RECIST criteria 1.1 will be used to determine disease response in those patients with measurable disease.[48]

- CA-125 measurements will be followed but will **not** be used to determine progression free survival. A rising CA-125 may, at the discretion of the investigator, prompt an earlier CT scan.

12.1 Base line Assessment

Measurable disease ("Target") is defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded). Each lesion must be ε 10 mm when measured by CT (CT scan slice thickness no greater than 5 mm*); ≥ 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable); and ≥ 20 mm by chest x-ray.

*If CT scan with slice thickness > 5 mm is used, the minimum lesion size must have a longest dimension twice the actual slice thickness.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Clinical lesions will only be considered measureable when they are superficial and ≥ 10 mm diameter as assessed using calipers (e.g. skin nodules). When lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and my also be reviewed at the end of the study.

CT is the best currently available and reproducible method to measure lesions selected for response assessment. MRI may be substituted for contrast enhanced CT for some sites (e.g. for abdomen and/or pelvis), but NOT lung. The minimum size for measurability is the same as for CT (10 mm) as long as the scans are performed with slice thickness of 5 mm and no gap. In the event the MRI is performed with thicker slices, the size of a measurable lesion at baseline should be two times the slice thickness.

Tumors within a previously irradiated field will be designated as "non-target" lesions unless progression is documented or a biopsy is obtained to confirm persistence at least 90 days following completion of radiation therapy.

Bone lesions: Bone scan, PET scan or plain films are NOT considered adequate imaging techniques to measure bone lesions. Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable if the soft tissue component meets the definition of measurability described above. Blastic bone lesions are non-measurable.

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

Non-measurable disease ("Non-Target") is defined as all other lesions, including small lesions (<10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis) as well as truly non-measurable lesions.

Lesions considered truly non-measurable include:

- Leptomeningeal disease
- Ascites
- Pleural or pericardial effusion
- Inflammatory breast disease
- Lymphangitic involvement of skin or lung
- Abdominal masses/abdominal organomegaly indentified by physical exam that is not measureable by reproducible imaging techniques

Baseline documentation of "Target" and "Non-Target" lesions

All measurable lesions up to a maximum of **5 lesions total** (and a **maximum of two lesions per organ**) representative of all involved organs should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the **baseline sum diameters**. The baseline sum diameters will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions (or sites of disease) should be identified as *non-target* lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present" or "absent". All baseline evaluations of disease status should be performed as close as possible to the start of treatment and never more than 4 weeks before the beginning of the treatment.

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

12.2 Response Criteria

Complete Response (CR): Disappearance of all target and non-target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm. Normalization of CA125, if elevated at baseline, is required for ovarian/primary peritoneal/fallopian tube cancer studies.

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

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). **In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm**. (Note: the appearance of one or more **NEW** lesions is also considered progression.) Unequivocal progression of existing non-target lesions is also considered progression (a detailed description and examples of unequivocal progression of existing non-target lesions is provided in the above cited reference).

For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

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

Not evaluable (NE) is when no imaging/measurement is done at all at a particular time point. The patient is not evaluable (NE) at that time point.

Early death is defined as having NO repeat tumor assessments following initiation of study therapy resulting from the death of the patient due to disease or treatment.

Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time will be recorded as '**symptomatic deterioration**'. Every effort should be made to document objective progression even after discontinuation of treatment.

Confirmation of response (for both CR and PR): Complete or partial response may only be claimed if the criteria for each are met at a subsequent time point ($\geq$ 4 weeks later) in studies with a primary endpoint that includes response rate. When response rate is a secondary endpoint (e.g. randomized phase II or III studies with progression-free survival or overall survival as primary endpoint) confirmation is NOT required.

Special note on lymph nodes: Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of < 10 mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

Special note on target lesions that become 'too small to measure': While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2 mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure'. When this occurs it is important that a value be recorded on the D2M form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a **default value of 5 mm** should be assigned (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well).

Evaluation of best overall response is according to Tables 6-7:

Table 6 is used for patients with measurable disease at baseline.

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Table 7 is used for patients with non-measurable disease.

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

Duration of response is defined as the time measurement criteria are first met for CR/PR until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurement recorded on study).

Duration of stable disease is measured from the start of the treatment (in randomized trials, from the date of randomization) until the criteria for progression are met, taking as reference the smallest sum on study (if the baseline sum is the smallest, this is the reference for calculation of PD).

Progression-Free Survival is the period from study entry until recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurement recorded on study), death or date of last contact.

Survival is the observed length of life from entry into the study to death or the date of last contact.

13.0 CRITERIA FOR REMOVAL FROM STUDY

- If at any time the patient develops progressive disease she will be taken off study and referred for alternative therapy.

- If at anytime the patient develops unacceptable toxicity she will be removed from study. Patients will be removed from study for any life-threatening grade 4 toxicity or a vaccine-related Dose Limiting Toxicity (DLT); defined by: ≥ grade 2 allergic reaction, ≥ grade 2 autoimmune reaction, ≥grade 3 hematologic or non-hematologic toxicity including fever, ≥ grade 3 injection site reaction.

- If at anytime the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (i.e. a change in diagnosis), the patient will be removed from the study.

- Subjects who meet the following criteria should be discontinued from the study:

  - Grade 4 hypertension or grade 3 hypertension not controlled with medication
  - Grade 4 congestive heart failure
  - Symptomatic grade 4 venous thromboembolic event
  - Nephrotic syndrome.
  - Bowel Obstruction that has not fully recovered despite medical or surgical intervention
  - Grade 4 hemorrhage or repeat grade 3 hemorrhage (≥ grade 2 pulmonary hemorrhage or CNS hemorrhage)
  - Any grade arterial thromboembolic event
  - Treatment delay in cycles bevacizumab >8 weeks
  - Gastrointestinal perforation
  - Tracheoesophageal fistula (any grade) or grade 4 fistula
  - Wound dehiscence requiring medical or surgical intervention
  - Determination by the investigator that it is no longer safe for the subject to continue therapy
  - Inability to tolerate treatment because of toxicity
  - Inability or unwillingness of subject to comply with study requirements
  - All grade 4 events thought to be related to bevacizumab by the investigator

Any patient with ≥grade 2 vaccine-related DLT will be followed with appropriate studies until results return to baseline. Patients who have an ongoing vaccine-related or bevacizumab-related grade 4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed until resolution of the event or until the event is considered irreversible. (See Section 5.2.3).

- In the absence of a vaccine-related DLT, no further bevacizumab will be given if the patient develops any grade of the following toxicities: wound dehiscence requiring medical or surgical intervention, arterial thromboembolic event, GI perforation, or bowel obstruction or ≥ grade 2 pulmonary hemorrhage or ≥ grade 3 of the following: hypertension, congestive heart failure, venous thrombosis or non-pulmonary hemorrhage. The patient may continue to receive the vaccine as indicated. Any patient with ≥ grade 2 vaccine-related DLT or other ≥ grade 3 toxicity will be followed with appropriate studies until results return to baseline

14.0 BIOSTATISTICS

The endpoints of this pilot trial are safety and confirmation of immunogenicity in the multivalent setting in combination with bevacizumab. Twenty-one evaluable patients will be accrued, with approximately two patients being accrued each month. No systemic toxicity has occurred with the administration of prior vaccines at the center. Toxicity is not expected with this preparation relative to vaccination. However toxicities related to the combination regimen or to bevacizumab alone can be observed since this is the first trial that evaluates safety of the combination regimen. All patients who receive at least one vaccination and one treatment with bevacizumab will be considered evaluable with respect to the safety analysis The trial will be stopped for safety in the event of the following toxicities during the treatment phase of the study (i.e. until week 20 week) (see Table 8 below) .

Bevacizumab-related Toxicities:

1) **Gastrointestinal perforation/ Gastrointestinal Leak**: Unexplainable grade 1 GI perforation (excluding evidence of abdominal free air attributable to other causes such as paracentesis or recent surgical procedure) or ≥ Grade 2 (including intra-abdominal abscess or GI fistula)
2) **GI fistula**: ≥ Grade 1 tracheoesophageal fistula or ≥ Grade 2 other fistula

Vaccine-related Toxicities:

1) **≥ Grade 2 autoimmune reaction**
2) **> Grade 2 allergic reaction**

A repeated significance test that differentiates between acceptable toxicity probabilities of 0.01 and 0.1 will be used to monitor this study. The boundary produced below is based on Type I error of 0.1 and Type II error equal to 0.24. Stop the study if the number of toxicities observed are greater or equal to among the following accrued patients.

Table 8 Stopping rules for toxicities observed

Stop the study if the number of toxicities observed are greater or equal to	Among the following accrued patients
1	10
2	21

The same criteria for immunogenicity will be used as that of the individual pilot trials: patients must have IgM titer > 1:40, or an eightfold increase in prevailing antibody titer if present at baseline. If ≥ 8 of 21 patients should meet these criteria for three or more antigens based on the immune response criteria, the study will be considered positive. This calculation assumes that the probability of immune response under the null hypothesis (i.e. no activity) is 0.2 versus the alternative hypothesis (i.e. target response probability) is 0.5 using a single stage binomial proportion test. Type I error is 4.3% and Type II error is 0.1. In prior trials, antibodies are generally present by completion of the fourth vaccination (week 7) thus immune response will be evaluated by week 7.

The secondary endpoints of this pilot trial are to characterize the nature and duration of the immune response. Antibodies against the individual antigens will be studied by ELISA, and against the appropriate human tumor antigen FACS using human tumor cell lines expressing the respective antigen where appropriate, and 2) a correlation of multiplex biomarker results with PFS, and a comparison of baseline to value at progression will be assessed graphically. The baseline value will be correlated with PFS.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. The PPR fax numbers are (646) 735-0008 and (646) 735-0003. Registrations can be phoned in or faxed. The completed signature page of the written consent/verbal script and a completed Eligibility Checklist must be faxed to PPR.

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

16.1 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb2.mskcc.org/irb/index.htm*

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation.

17.0 PROTECTION OF HUMAN SUBJECTS

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:

The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. The report will be forwarded to the FDA by the Institutional SAE Manager through the IND Office.

All SAE reports must also be forwarded as soon as possible to:

- Genentech Drug Safety
  Fax: (650) 225-4682 or (650) 225-5288

  For questions related to safety reporting, contact:

  Genentech Drug Safety Tel: 1-888-835-2555

  Genentech may contact the reporter for additional information, clarification, or current status of the subject for whom and adverse event was reported.

17.2.1 Genentech Adverse Event Reporting Definitions

A serious treatment emergent adverse event (STEAE) is any sign, symptom or medical condition that emerges during Bevacizumab treatment or during a post-treatment follow-up period that (1) was not present at the start of Bevacizumab treatment and it is not a chronic condition that is part of the patient's medical history, OR (2) was present at the start of Bevacizumab treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy, AND that meets any of the following regulatory serious criteria:

- Results in death
- Is life-threatening
- Requires or prolongs inpatient hospitalization
- Is disabling
- Is a congenital anomaly/birth defect
- Is medically significant or requires medical or surgical intervention to prevent one of the outcomes listed above.

Assessing Causality:

The event should be assessed to decide whether there is a reasonable possibility that bevacizumab caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to bevacizumab administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to bevacizumab administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form."

19.0 REFERENCES

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20.0 APPENDICES

Appendix A: Laboratory Standard Operating Procedures

Appendix B: FIGO Stage Grouping for Primary Carcinoma of the Ovary (1985)

Appendix C: New York Heart Association

Appendix D: Procedure for Obtaining a Urine Protein / Creatinine Ratio

APPENDIX A: Laboratory Standard Operating Procedures

A.1 Correlative Studies

A1A 10-plex Angiogenesis Immunoassay

Summary and Principle

Dec ision Biomar kers, Inc (DBI) has developed a novel, automated immunoassay platform to simultaneously evaluate multiple biomarkers in a single reaction device, the MAX BIOCHIP®. The MA X BIOCHIP® is a polycarbonate cartr idge with a sample chamber, tw o self-contained reagent chambers, and a reaction chamber, all connected by a series of channels interspersed w ith sensors and valves to detect and direct fluid flow . The reaction chamber of the MAXBIOCHIP® cons ists of a microarray of specific antibodies spotted onto a glass slide pre-coated w ith an ultra-thin film of nitrocellulose. To perform an analysis, the MAXBIOCHIP® is inserted into the AVANTRA® Biomarker Workstation. Sample is injected and the instrument automatically directs, in sequence, the flow of sample, solublized biotinylated detection antibodies, labeled streptav idin, and w ash buffer across the microarray. Spent reagents then flow into tw o w aste receptacles. All liquid is self-contained w ithin the BIOCHIP. The resulting fluorescently labeled complexes are then imaged by an internal CCD camera. Instrument softw are correlates spot intensity w ith analyte concentration.

Materials Required: **MAX BIOC HIP® Angio-10 Blocking Reagent (PN 00-0015)** buffer with detergent, preservative and blocking factors, **DBI Pipette Tip (PN 00-0004):** 96 custom loading tips, Optional Components Available: **MAX BIOC HIP®** Angio-10 High control (PN 00-00 19), **MAX BIOC HIP®** Angio-10 Low control (PN 00-0020) *Controls consist of a mixture of all 10 recombinant proteins.*

PRECAUTIONS AND WARNINGS: Reagents are harmful if sw allow ed. Avoid contact w ith skin and eyes. Wear suitable protective clothing. Some reagents contain trace amounts of sodium azide w hich is potentially reactive with copper or lead plumbing. Flush plumbing w ith large amoun ts of w ater during disposal. All samples of a potentially infectious or hazardous origin should be handled in the manner outlined by the Center for Disease Control and the Occupational Health and Safety Administration for blood-borne pathogens and human and animal-source materials.

Reagent storage and stability: Unopened BIOCHIPs are stable until the expiration date show n on the label w hen stored at 2 - 8°C. Keep the MAX BIOCHIP® Angio-10 Blocking Reagent at -20°C prior to first time use. Thaw at room temperature and maintain at 2-8°C for up to one month. **Do not re-freeze.**

Quality Control: Controls are recommended for a complete quality control program. Each laboratory should establish its ow n control means and ranges as w ell as a quality control program to monitor laboratory testing. In addition, each BIOCHIP contains negative and positive control spots w hich monitor instrument and BIOCHIP performance. Please refer to the Lot Specific Control BIOCHIP Values*. Positive and negative control spots on each BIOCHIP may assist the investigator with identifying potential instrument or BIOCHIP problems.*

Suggested Ranges for Lot:20090304 Positive Control Range: 33049-61377 RFU, Upper Lim it Negative Control: 200 RFU

Calibration: Replicate measurements of standards containing all 10 biomarkers are used to generate master curves for all analytes. These are provided on the lot specific CD supplied w ith each shipment of BIOCHIPs.

Results: Refer to the Assay Results tab in the AVANTRA® Express Softw are. An individual sample result may be view ed by single or double c licking on the + next to each BIOCHIP ID. Results below the analytical sensitivity (see Table 8 below) or above the upper limit of the dynamic range w ill not be quantified. Results may be exported to MS Excel⇔ by highlighting the result(s) then selecting the Export Data icon in the AVANTRA® Express toolbar.

Performance Characteristics

Precision: Spot-to-Spot, or intra-assay precision (six spots per analyte from four biochips) and BIOCHIP-to-BIOCHIP, or inter-assay precision (14 BIOCHIPs run over 2 days) w ere calculated for a low-level control for each marker. The data show n in Table 9 below meet the % CV BIOCHIP to BIOCHIP specification of ≤20%.

Analytical Sensitivity: Analytical sensitivity w as ascertained by evaluating replicates of the zero standard, calculating the mean and SD of those values, and determining the concentration equivalent to 2 SD's above the mean. Representative results are show n in Table 10. Results show n are the mean sens itivities of 7 BIOCHIP lots.

Table 9 - Precision

Spot-to-Spot **BIOC HIP to BIOC HIP**

Biom arker	%CV	Biom arker	%CVD
ICA M-1	7	ICA M-1	5
E- Selectin	11	E- Selectin	11
TIMP- 1	4	TIMP- 1	6
IL-8	5	IL-8	3
TIE-2	5	TIE-2	9
HGF	8	HGF	7
FGF- basic	6	FGF- basic	5
PLGF	5	PLGF	5
VEGF-R2	7	VEGF-R2	12
VEGF	12	VEGF	13

BIOC HIP handling: Remove appropriate number of foiled pouches from the refrigerated kit. Allow to equilibrate at room temperature for at least 30 minutes prior to opening pouch. **Do not open individual pouches until ready to run the BIOC HIP.**

Table 10 – Representative Results

Biomarker	Sensitivity	Specification
ICAM-1	7 ng/mL	≤20 ng/mL
E-Selectin	6 ng/mL	≤30 ng/mL
TIMP-1	13 ng/mL	≤55 ng/mL
IL-8	3 pg/mL	≤10 pg/mL
TIE-2	0.3 ng/mL	≤ 1 ng/mL
HGF	93 pg/mL	≤410 pg/mL
FGF-basic	497 pg/mL	≤1500 pg/mL
PLGF	8 pg/mL	≤20 pg/mL
VEGF-R2	150 pg/mL	≤540 pg/mL
VEGF	244 pg/mL	≤350 pg/mL

BIOC HIP Injection Instructions: Please use the Eppendorf pipette provided along w ith the Dec ision Biomar kers pipette tips (w ith w hite collar) for injecting the sample into the BIOCHIP. **The BIOC HIP must be inserted into the AVANT RA® Biom arker Workstation prior to injecting the sam ple.**

Control / Sample Handling and Preparation: Safe laboratory pr actices and personal protective equipment such as gloves, lab coat, and safety glasses should be used w hen handling any samples. EDTA plas ma is the recommended sample type. Avoid multiple freeze-thaw cycles.Warm DBI Angio-10 Blocking Reagent to room temperature. **Ensure that the solution is completely thaw ed.**

a. Thaw controls or patient samples at room temperature and mix gently; avoid prolonged storage at room temperature.
b. Any solid material observed in a sample should be spun dow n by centrifugation. Spin sample at 500xg for five minutes. Transfer supernatant to fresh tube.
c. Gently pulse vortex both the bloc king buffer and the sample.
d. Combine 125 ∝L of control / sample w ith 125 ∝L of DBI Angio-10 Blocking Reagent.
e. Mix thoroughly, either by pipett ing up and dow n at least 5X or vortexing gently.

f. Immediately inject the treated sample into the BIOCHIP following the directions provided.

Directions:

a. Power on the instrument by pressing the green power button on the front of the instrument.
b. On the computer, open the AVANTRA® Express Software
c. The instrument is ready for use when the front panel indicates " Ready for BIOCHIP Insert BIOCHIP".
d. Remove protective red tape, including paper backing, from BIOCHIP and insert BIOCHIP into the AVANTRA® Biomarker Workstation until it snaps into place
e. Enter an optional new Study name, or select a previously used Study name. The Study name will be retained for subsequent samples until changed by the user or the software is restarted. Optional information may be entered into the Operator Comments field.
f. Scan or enter a Control/Sample ID and click "SEND ID" - The sample must be then loaded within 10 minutes of sending an ID
g. Set your DBI provided Eppendorf pipette to 200 ∝L
h. Place a DBI pipette tip (with white collar) onto the pipette and slowly aspirate 200 ∝L of treated control/sample
i. While holding the BIOCHIP in place, insert the DBI pipette tip into the sample septum of the BIOCHIP until the collar touches the septum.
j. Slowly depress the plunger to the first stop to inject the sample.
k. Continue holding the BIOCHIP and **remove the pipette without releasing the pipette plunger**. Dispose of tip appropriately
l. Close and latch the door to the AVANTRA®
m. Push the Start Button.
n. The assay will take approximately one (1) hour to run. Upon completion, the AVANTRA® will signal that the assay is complete. The front panel will indicate "Assay Complete Discard BIOCHIP".

Removing the BIOCHIP: Once the assay is complete, the BIOCHIP can be removed by opening the door and pressing on the two side release tabs. Discard the BIOCHIP in a biohazard waste container. Results may be viewed under the Assay Results tab on the AVANTRA® Express software

A1.b 8-plex Cytokine Immunoassay

Summary and Principle

Decision Biomarkers, Inc (DBI) has developed a novel, automated immunoassay platform to simultaneously evaluate multiple biomarkers in a single reaction device, the MAX BIOCHIP®. The MAX BIOCHIP® is a polycarbonate cartridge with a sample chamber, two self-contained reagent chambers, and a reaction chamber, all connected by a series of channels interspersed with sensors and valves to detect and direct fluid flow. The reaction chamber of the MAX BIOCHIP® consists of a microarray of specific antibodies spotted onto a glass slide pre-coated with an ultra-thin film of nitrocellulose. To perform an analysis, the MAX BIOCHIP® is inserted into the AVANTRA® Biomarker Workstation. Sample is injected and the instrument automatically directs, in sequence, the flow of sample, solublized biotinylated detection antibodies, labeled streptavidin, and wash buffer across the microarray. Spent reagents then flow into two waste receptacles. All liquid is self-contained within the BIOCHIP. The resulting fluorescently labeled complexes are then imaged by an internal CCD camera. Instrument software correlates spot intensity with analyte concentration.

Materials Required : MAX BIOCHIP® Cyto-8 Blocking Reagent (PN 00-0014): Buffer with detergent, preservative and blocking factors, **DBI Pipette Tip (PN 00-0004):** 96 custom loading tips, Optional Components Available: **MAX BIOCHIP®** Cyto-8 High control (PN 00-0016) **MAX BIOCHIP®** Cyto-8 Mid control (PN 00-0017) **MAX BIOCHIP®** Cyto-8 Low control (PN 00-0018) *Controls consist of a mixture of all eight recombinant cytokines.*

PRECAUTIONS AND WARNINGS: Reagents are harmful if swallowed. Avoid contact with skin and eyes. Wear suitable protective clothing. Some reagents contain trace amounts of sodium azide which is potentially reactive with copper or lead plumbing. Flush plumbing with large amounts of water during disposal. All samples of a potentially

infectious or hazardous origin should be handled in the manner outlined by the Center for Disease Control and the Occupational Health and Safety Administration for blood-borne pathogens and human and animal-source materials.

Reagent storage and stability: Unopened BIOCHIPs are stable until the expiration date show n on the label w hen stored at 2 - 8°C. Keep the MAX BIOCHIP® Cyto-8 Blocking Reagent at -20°C prior to first time use. Thaw at room temperature and maintain at 2-8°C for up to one month. **Do not re-freeze.**

Quality Control: Controls are recommended for a complete quality control program. Each laboratory should establish its ow n control means and ranges as w ell as a quality control program to monitor laboratory testi ng. In addition, each BIOCHIP contains negative and positive control spots w hich monitor instrument and BIOCHIP performance. Please refer to the Lot Specific Control BIOCHIP Values. *Positive and negative control spots on each BIOCHIP may assist the investigator with identifying potential instrument or BIOCHIP problems.*

Suggested Ranges for Lot: 20090113 Positive Control Range: 1256-8669 RFU, Upper Lim it Negative Control: 50 RFU

Calibration: Replicate measurements of standards containing all 8 cytokines are used to generate master curves for all analytes. These are provided on the lot specific CD supplied w ith each shipment of BIOCHIPs.

Results: Refer to the Assay Results tab in the AVANTRA® Express Softw are. An individual sample result may be view ed by single or double clic king on the + next to each BIOCHIP ID. Results below the analytical sensitivity (see Table 12) or above 5000 pg/ mL w ill not be quantified. Results may be exported to MS Excel⇔ by highlighting the result(s) then selecting the Ex port Data icon in the AVANTRA® Express toolbar.

Perform ance Characteristics

Precision: Spot-to-Spot, or intra-assay precision (six spots per analyte from 12 BIOCHIPs) and BIOCHIP-to-BIOCHIP, or inter-assay precision (12 BIOCHIPs run over 3 days) w ere calculated for a mid-level control of 150 pg/ mL for each cytokine. The data show n in Table 11 below meet the % CV BIOCHIP to BIOCHIP spec ification of ≤20%.

Table 11– Precision

Spot-to- Spot **BIOCHIP-to-BIOCHIP**

Cytokine	%CV	Cytokine	%CVD
IL-1®	4	IL-1®	4
IL-2	7	IL-2	10
IL-5	4	IL-5	7
IL-6	6	IL-6	6
IL-8	5	IL-8	9
IL- 10	6	IL- 10	7
IL- 12	9	IL- 12	9
TNF⟨	5	TNF⟨	8

Table 12 –Representative Results

Cytokine	Sensitivity (pg/mL)	Specification (pg/mL)
IL-1®	7	≤13
IL-2	9	≤20
IL-5	4	≤13
IL-6	8	≤18
IL-8	3	≤5
IL- 10	4	≤10
IL- 12	9	≤15
TNF⟨	4	≤12

Analytical Sensitivity: Analytical sensitivity w as ascertained by evaluating replicates of the zero standard, calculating the mean and SD of those values, and determining the concentration equivalent to 2 SD's above the mean. Representative results are show n in Table 12. Results show n are the mean sens itivities of 6 BIOCHIP lots.

BIOCHIP handling: **Remove appropriate number of foiled pouches from the refrigerated kit. Allow to equilibrate at room temperature for at least 30 minutes prior to opening pouch.**

Do not open individual pouches until ready to run the BIOC HIP.

BIOC HIP Injection Instructions: Please use the Eppendorf pipette provided along w ith the DBI pipette tips (w ith w hite collar) for injecting the sample into the BIOCHIP. **The BIOC HIP must be inserted into the AVANTRA® Biom arker Workstation prior to injecting the sam ple.**

Control / Sample Handling and Preparation: **Safe laboratory practices and personal protective equipment such as gloves, lab coat, and safety glasses should be used w hen handling any sam ples.** EDTA plas ma is the recommended sample type. Avoid multiple freeze-thaw cycles.

a. War m DBI Cyto-8 Blocking Reagent to room temperature
b. Thaw controls or patient samples at room temperature and mix gently; avoid prolonged storage at room temperature
c. Any solid material observed in a sample should be spun dow n by centrifugation. Spin sample at 500xg for five minutes. Transfer supernatant to fresh tube
d. Combine 225 ∝L of control / sample w ith 25 ∝L of DBI Cyto-8 Bloc king Reagent
e. Mix thoroughly, either by pipett ing up and dow n at least 5X or vortexing gently
f. Immediately inject the treated sample into the BIOCHIP follow ing the directions provided

Directions:

a. Pow er on the instrument by pressing the green pow er button on the front of the instrument
b. On the computer, open the AVANTRA® Express Softw are
c. The instrument is ready for use w hen the front panel indicates " Ready for BIOCHIP Insert BIOCHIP"
d. Remove protective red tape, including paper backing, from BIOCHIP and insert BIOCHIP into the AVANTRA® Biomarker Workstation until it snaps into place
e. Enter an optional new Study name, or select a previously used Study name. The St udy name w ill be retained for subsequent samples until changed by the user or the softw are is restarted. Optional information may be entered into the Operator Comments field
f. Scan or enter a Control/Sample ID and click "SEND ID" - The sample must be then loaded w ithin 10 minutes of sending an ID
g. Set your DBI prov ided Eppendorf pipette to 200 ∝L
h. Place a DBI pipette tip (w ith w hite collar) onto the pipette and slow ly aspirate 200 ∝L of treated control/sample
i. While holding the BIOCHIP in place, insert the DBI pipette tip into the sample septum of the BIOCHIP until the collar touches the septum
j. Slow ly depress the plunger to the first stop to inject the sample
k. Continue holding the BIOCHIP and remove the pipette without releasing the pipette plunger. Dispose of tip appropriately
l. Close and latch the door to the AVANTRA®
m. Push the Start Button
n. The assay w ill take approximately one hour to run. Upon completion, the AVANTRA® w ill signal that the assay is complete. The front panel w ill indicate "Assay Complete Discard BIOCHIP"

Removing the BIOCHIP: **Once the assay is complete, the BIOC HIP can be removed by opening the door and press ing on the two side release tabs. Discard the BIOC HIP in a biohazard w aste container. Results may be view ed under the Assay Results tab on the AVANT RA® Express softw are**

APPENDIX B: FIGO Stage Grouping for Primary Carcinoma of the Ovary (1985)

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

Stage I — Growth limited to the ovaries.

- Stage IA — Growth limited to one ovary; no ascites.
  No tumor on the external surface; capsule intact.
- Stage IB — Growth limited to both ovaries; no ascites.
  No tumor on the external surfaces; capsules intact.
- Stage IC* — Tumor either Stage IA or IB but with tumor on the surface of one or both ovaries; or with capsule ruptured; or with ascites present containing malignant cells or with positive peritoneal washings.

Stage II — Growth involving one or both ovaries with pelvic extension.

- Stage IIA — Extension and/or metastases to the uterus and/or tubes.
- Stage IIB — Extension to other pelvic tissues.
- Stage IIC* — Tumor either Stage IIA or IIB but with tumor on the surface of one or both ovaries; or with capsule(s) ruptured; or with ascites present containing malignant cells or with positive peritoneal washings.

Stage III — Tumor involving one or both ovaries with peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal nodes. Superficial liver metastasis equals Stage III. Tumor is limited to the true pelvis but with histologically verified malignant extensions to small bowel or omentum.

- Stage IIIA — Tumor grossly limited to the true pelvis with negative nodes but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces.
- Stage IIIB — Tumor of one or both ovaries with histologically confirmed implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter. Nodes negative.
- Stage IIIC — Abdominal implants >2 cm in diameter and/or positive retroperitoneal or inguinal nodes.

Stage IV — Growth involving one or both ovaries with distant metastasis. If pleural effusion is present there must be positive cytologic test results to allot a case to Stage IV. Parenchymal liver metastasis equals Stage IV.

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

APPENDIX C: New York Heart Association

Class	Patient Symptoms
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

APPENDIX D: Procedure for Obtaining a Urine Protein / Creatinine Ratio

1) Obtain at least 4 ml of a random urine sample (does not have to be a 24-hour urine)

2) Determine protein concentration (mg/dL)

3) Determine creatinine concentration (mg/dL)

4) Divide #2 by #3 above: urine protein / creatinine ratio = protein concentration (mg /dL) / creatinine concentration (mg /dL)

The UPC directly correlates with the amount of protein excreted in the urine per 24 hrs (i.e. a UPC of 1 should be equivalent to 1g protein in a 24hr urine collection)

Protein and creatinine concentrations should be available on standard reports of urinalyses, not dipsticks. If protein and creatinine concentrations are not routinely reported at an Institution, their measurements and reports may need to be requested.

UPC ratio is calculated using one of the following formulas:

1. [urine protein]/[urine creatinine] – if both protein and creatinine are reported in mg/dL

2. [(urine protein) x0.088]/[urine creatinine] – if urine creatinine is reported in mmol/L

The UPCR has been found to correlate directly with the amount of protein excreted in a 24 hour urine collection. Specifically, a UPCR of 1.0 is equivalent to 1.0 gram of protein in a 24 hour urine collection. The UPCR is derived as follows: protein concentration (mg/dL)/creatinine (mg/dL). Patients must have a UPCR <1.0 to allow participation in the study.