Protocol #: CL-PTL 105

PROTOCOL TITLE: Open-label Phase II Trial of Adjuvant bishRNAfurin and GMCSF Augmented Autologous Tumor Cell Vaccine (FANG™) for High-Risk Stage III/IV Ovarian Cancer

NCT 01309230

PROTOCOL DATE: September 25, 2015

PROTOCOL TITLE:	**Open-label Phase II Trial of Adjuvant bi-shRNA^{furin} and GMCSF Augmented Autologous Tumor Cell Vaccine (FANG™) for High Risk Stage III/IV Ovarian Cancer**
Protocol #:	**CL-PTL 105**
STUDY AGENT:	**Vigil™ formerly known as FANG™ (bi-shRNA^{furin} and GMCSF Autologous Tumor Cell Vaccine)**
SPONSOR:	**Gradalis, Inc.** **2545 Golden Bear Drive, Suite 110** **Carrollton, TX 75006** **Phone: (214) 442-8124** **Fax: (214) 442-8101**
PROTOCOL DATE:	**Amendment #9 dated September 25, 2015** **Amendment #8 dated June 19, 2014** **Amendment #7 dated January 9, 2014** **Amendment #6 dated April 15, 2013** **Amendment #5 dated January 31, 2013** **Amendment #4 dated May 1, 2012** **Amendment #3 dated October 5, 2011** **Amendment #2 dated June 23, 2011** **Amendment #1 dated February 18, 2011** **Initial Protocol dated November 16, 2010**

INVESTIGATOR PROTOCOL SIGNATURE PAGE

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I have read and understand the contents of the indicated clinical protocol and will adhere to the trial requirements as presented, including all statements regarding confidentiality. In addition, should I choose to participate as an investigator, I and my sub-investigator(s) agree to conduct the study as outlined herein, in accordance with Good Clinical Practices (GCPs), the Declaration of Helsinki, in compliance with the obligations and requirements of clinical investigators and all other requirements listed in Title 21 Code of Federal Regulations (CFR) Part 312.

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Name of Investigator (please print) Signature of Investigator

_____

Date

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ABBREVIATIONS

Abbreviation	Term
AE	Adverse event
ALT	Alanine transaminase (also referred to as SGPT)
ANC	Absolute neutrophil count
APC	Antigen Presenting Cells
AST	Aspartate transaminase (also referred to as SGOT)
BUN	Blood urea nitrogen
CBC	Complete blood count
CD	Cluster of differentiation
CMV	Cytomegalovirus
CO_2	Total carbon dioxide
CR	Complete response
CRF	Case report form
CTCAE	Common Toxicity Criteria for Adverse Events
CTL	Cytotoxic T lymphocyte
DC	Dendritic cell(s)
DTH	Delayed-type hypersensitivity
ECOG PS	Eastern Cooperative Oncology Group Performance Score
ELISA	Enzyme-Linked ImmunoSorbent Assay
ELISPOT	Enzyme-Linked ImmunoSorbent Spot
ER	Endoplasmic reticulum
FANG™	bi-shRNA^{furin} and GMCSF Augmented Autologous Tumor Cell Vaccine
FL	Flt-3-Ligand
GMCSF	Granulocyte Macrophage-Colony Stimulating Factor
GMP	Good Manufacturing Practice
GVAX	GMCSF Secreting autologous or allogenic tumor vaccine
HLA	Human Leukocyte Antigen
IBC	Institutional Biosafety Committee
IEC	Independent Ethics Committee
IL	Infiltrating lymphocytes
IRB	Institutional Review Board
LAK	Lymphokine-activated killer

Abbreviation	Term
LD	Longest diameter
LLC	Large latent complex
LPI	Lead Principal Investigator
MARY CROWLEY	Mary Crowley Cancer Research Centers
MHC	Major histocompatability complex
MLR	Mixed lymphocyte reaction
MR	Mannose receptor
NCI	National Cancer Institute
NED	No evidence of disease
NK	Natural Killer
NKT	Natural Killer T cell(s)
NSCLC	Non small cell lung cancer
PCR	Polymerase chain reaction
PD	Progressive disease
PI	Principal Investigator
PR	Partial response
PS	Performance Status
RECIST	Response Evaluation Criteria in Solid Tumors
SCLC	Small cell lung cancer
SD	Stable disease
SLC	Small latent complex
STMN1	Stathmin 1
TAA	Tumor Associated Antigens
TAP	transporter associated with Ag processing
TGFβ	Transforming growth factor-β
TIL	Tumor infiltrating lymphocytes
TNF	Tumor necrosis factor
Treg	Regulatory T cell
TTR	Time to recurrence
ULN	Upper limits of normal
WNL	Within normal limits

SYNOPSIS

Summary: Despite a gradual improvement in their overall survival over the past decade, approximately 75% of women with Stage IIIC ovarian cancer who achieve a complete clinical response will relapse as will 50% of those achieving pathologic complete response at a median time of 18-24 months. Phase III studies of both maintenance and consolidation therapeutic interventions have not translated into an overall survival advantage. Preliminary studies of immunotherapy in patients with ovarian cancer suggest target accessibility (potential immunogenicity) to immune mediated approaches. In an effort to overcome limitations of immunostimulatory cancer vaccines, we designed a novel autologous whole cell vaccine, Vigil™ formerly known as FANG™, incorporating the rhGMCSF transgene and the bifunctional shRNA^{furin} (to block proprotein conversion to active TGFβ1 and β2) to 1) address the inability to fully identify cancer associated antigens, 2) effect antigen recognition by the immune system (i.e. antigen$\rightarrow$immunogen), 3) enhance effector potency, and 4) subvert endogenous cancer-induced immune resistance.

A Phase I assessment of Vigil™ vaccine in 33 advanced solid tumor patients (1 of them being a pediatric patient 15 years of age) receiving ≥1 vaccination (at a dose of 1.0 x 10^7 or 2.5 x 10^7 cells/injection/month for a maximum of 12 vaccinations) demonstrated safety of the Vigil™ vaccine. Furthermore, proof of principle was established in the manufactured vaccines with increased mean GMCSF expression post-transfection to 1135 pg/10^6 cells/ml and knockdown of furin, TGFβ1 and TGFβ2 at 78%, 93%, and 95%, respectively). In addition, although a Phase I study, the data suggested an overall survival benefit.

Given the preliminary Phase II evidence of prolongation of time to relapse in randomized Vigil™ vs. No Vigil™ treated patients (p=0.005), as of this amendment all patients screening for enrollment into the main portion of the trial (including those who previously had tumor tissue harvested), will receive Vigil™ vaccine and will no longer be randomized. An extensive Phase II/III protocol is designed to more definitively assess benefit. Additional safety data in ovarian cancer patients receiving 1x10^7 cells/ID injection on a monthly basis (maximum 12) is required.

This is a Phase II open-label study of maintenance intradermal autologous Vigil™ cancer vaccine administered to women with Stage III/IV epithelial ovarian cancer who attain a clinical complete response (including a post-treatment, prevaccination baseline serum CA-125 level of ≤ 20 units/ml) after surgical cytoreduction and a total of six courses of front-line (pre- and post- or post-surgical) doublet chemotherapy. Patients meeting eligibility criteria will receive at least 5 but no more than 6 cycles of NCCN Category 1 chemotherapy. Thereafter, patients will receive Vigil™ vaccine at a dose of 1.0 x 10^7 cells/injection for minimum of 4 to a maximum of 12 vaccinations (based on number of vaccine doses manufactured and patient eligibility. Trial endpoints

include time to recurrence (TTR), documentation of immune responses, and correlation of immune response and clinical effect.

Objective(s):
Primary Objectives:

- To assess time to recurrence (TTR) following the administration of bi-shRNA^{furin} and GMCSF autologous tumor cell (Vigil™) vaccine.

Secondary Objectives:

- To identify and determine the effect of Vigil™ autologous tumor cell vaccine on immune surrogate markers.
- To assess the predictive potential of initial tumor infiltrating lymphocyte (TIL) and tumor associated macrophage (TAM) phenotypes.
- To enlarge the safety database of Vigil™ autologous tumor cell vaccine in patients with minimal disease.

Methodology:
This is a Phase II open-label trial of Vigil™ autologous tumor cell vaccine. Tumor will be harvested at the time of surgical debulking (standard of medical care).

Patients achieving clinical complete response (CR) following primary surgical debulking and doublet chemotherapy will be stratified for i) surgical stage (Stage IV or suboptimal debulking (>1 cm residual) Stage III disease versus Stage III patients with optimal debulking (<1 cm residual)) and ii) post-operative chemotherapy, pre-vaccine CA-125 of greater than 10 but less than or equal to 20 U/mL versus 0≤10 U/ml then randomized 2:1 (Note: patients with Stage IIIc ovarian cancer will be additionally evaluated as a subset using descriptive statistical endpoint only). Patients will receive 1.0 x 10^7 cells / intradermal injection of gene transfected autologous tumor cells, Vigil™, once a month for up to 12 doses as long as sufficient material is available. A minimum harvest aliquot to produce 4 monthly injections will be required for entry into the study. These patients will be managed in an outpatient setting. Hematologic function, liver enzymes, renal function and electrolytes will be monitored monthly. Immune function analysis including ELISPOT analysis of cytotoxic T cell function to autologous tumor antigens will be monitored at (≤24 hours before) tissue harvest, ≤24 hours before the first cycle chemotherapy (post debulking), ≤24 hours before the third cycle chemotherapy (post debulking), baseline (screening); prior to Vigil™ injection at Months 2, 4, 6 and EOT. CA-125 will be monitored at baseline, every month for the first year, every 3 months ± 2 weeks for the second and third year.

Number of Patients: Approximately fifty (50) patients with high risk Stage III/IV ovarian cancer.

Inclusion Criteria:

1. Histologically confirmed Stage III/IV papillary serous or endometrioid ovarian cancer.
2. Per Amendment #8, treatment naïve, high risk ovarian cancer will no longer be stratified, but the following information will be collected:
    a. Stage IV or suboptimal (>1 cm residual) Stage III disease versus Stage III patients with optimal (≤1 cm residual) disease,
    b. CA-125 ≤10 U/ml versus CA-125 greater than 10 to 20 U/ml
    c. IP chemotherapy versus IV chemotherapy
3. Clinically defined CR (no cancer related symptoms, normal physical examination and CT scan abdomen/pelvis and chest x-ray, and CA-125 ≤ 20 U/ml) following completion of surgical debulking. Patients enrolled must complete at least 5 but no more than 6 cycles platinum/taxane adjuvant or interval debulking + chemotherapy (or chemotherapy as per recommendations of NCCN guidelines, category 1 (IP chemotherapy included)). (Patients who complete surgery/chemotherapy with a CA-125 >20 U/mL pre-randomization have the option of being followed up to 2 months if serial CA-125 values continue to decrease at a rate of ≥50% per month.
4. Availability of "golf-ball" size ~10-30 grams tissue at time of primary surgical debulking.
5. Successful manufacturing of 4 vials of Vigil™ vaccine.
6. Recovered from all clinically relevant toxicities related to prior protocol specific therapy (including neuropathy ≤Grade 2).
7. ECOG performance status (PS) 0-2 prior to tumor debulking laparotomy.
8. ECOG PS 0-1 prior to Vigil™ vaccine administration.
9. Normal organ and marrow function as defined below:

Absolute granulocyte count	≥ 1,500/mm^3
Absolute lymphocyte count	≥ 200/mm^3
Platelets	≥ 75,000/mm^3
Total bilirubin	≤ 2 mg/dL
AST(SGOT)/ALT(SGPT)	≤ 2x institutional upper limit of normal
Creatinine	< 1.5 mg/dL

10. Patients must be off all "statin" drugs for ≥ 2 weeks prior to initiation of therapy. Ability to understand and the willingness to sign a written informed consent document for tissue harvesting.
11. Ability to understand and the willingness to sign a written informed protocol specific consent.

Exclusion Criteria:

1. Surgery involving general anesthesia, radiotherapy, or immunotherapy within 4 weeks prior to randomization. Chemotherapy within 3 weeks prior to Vigil™ vaccine administration. Steroid therapy within 1 week prior to vaccine administration.
2. Patients must not have received any other investigational agents within 4 weeks of Vigil™ vaccine administration.

3. Patients with history of brain metastases.
4. Patients with compromised pulmonary disease.
5. Short term (<30 days) concurrent systemic steroids ≤ 0.25 mg/kg prednisone per day (maximum 7.5 mg/day) and bronchodilators (inhaled steroids) are permitted; other steroid regimens and/or immunosuppressives are excluded.
6. Prior splenectomy.
7. Prior malignancy (excluding nonmelanoma carcinomas of the skin and carcinoma *in situ* cervix) unless in remission for ≥ 2 years.
8. Kaposi's Sarcoma.
9. Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
10. Patients with known HIV.
11. Patients with chronic Hepatitis B and C infection.
12. Patients with uncontrolled autoimmune diseases.

Medication and Doses: Autologous Vigil™ vaccine will be supplied by Gradalis, Inc.

Patients will receive 1.0 x 10^7 cells via intradermal injection one day each month, ≥3 weeks following completion of doublet chemotherapy (no longer than 2.5 months post chemotherapy), for a maximum of 12 doses as long as sufficient material is available and subject is clinically stable.

Duration: If sufficient material is available, the patient may continue therapy up to 12 doses, unless disease progression is documented (see Section 4.0, Study Design). If the patient experiences ≥ Grade 2 toxicity (excluding Grade 3 injection site reaction), they may continue on treatment with doses delayed or reduced if, in the opinion of the PI, this is considered to be in the best interest of the patient.

Efficacy Assessments:

- Time to disease recurrence
- Immune surrogate markers
- Quality of Life (FACT-O, Version 4)

Safety Assessments:

- Physical examination, performance status, height, weight, temperature, blood pressure, and pulse
- Toxicity: CTCAE v 3.0

1.0 INTRODUCTION

1.1 Immune Therapy in Cancer

For decades immune-based therapies have been investigated in many types of cancer, including melanoma, prostate, renal cell, non-Hodgkin's lymphoma, bladder cancer, and renal cell carcinoma (Quan, Dean et al. 1997; Alexandroff, Jackson et al. 1999; Eton, Legha et al. 2002; Kwak 2003; Kaufman, Wang et al. 2004; Coppin, Porzsolt et al. 2005). In a majority of these trials, induction of tumor specific cellular immune responses have been documented. However, in only a minority has there been translation into clinical effectiveness (Walden 2007). There have been several hypotheses to explain this demonstrable lack of anticancer immune activity in solid tumors. These include ineffective priming of tumor-specific T cells, lack of high-avidity of primed tumor-specific T cells, and physical or functional disabling of primed tumor-specific T cells by the primary host and or tumor-related mechanism. For example, in NSCLC a high proportion of the tumor-infiltrating lymphocytes are immunosuppressive T regulatory cells (CD4+ CD25+) that secrete transforming growth factor-β (TGFβ) and express a high level of cytotoxic T lymphocyte (CTL) antigen-4 (Woo, Yeh et al. 2002). These cells have been shown to impede immune activation by facilitating T cell tolerance to tumor associated antigens rather than cross-priming CD8+ T cells, resulting in the inhibition of killer T cells that recognize the tumor without attacking it (Dohadwala, Luo et al. 2001; Neuner, Schindel et al. 2002; Woo, Yeh et al. 2002). Elevated levels of immune inhibitors such as IL-10 and TGFβ are found in the circulation in patients with advanced cancer, and animal models have shown immune suppression is mediated by these cytokines serving both as a barrier to antigen recognition and T-cell activation, as well as a defense against the body's immune effector system (Rook, Kehrl et al. 1986; Tsunawaki, Sporn et al. 1988; Fontana, Frei et al. 1989).

Recently, however, several advances have been made with respect to mechanisms by which the immune system can actually be manipulated to facilitate tumor antigen recognition and enhance anticancer immune effector activity in cancer. Two independent approaches involving gene-based vaccines, specifically, Belagenpumatucel-L and GVAX®, have demonstrated, in Mary Crowley Cancer Research Centers and other institutional studies, remarkable safety and significant benefit with respect to response and survival benefits in advanced cancer patients (Nemunaitis ; Eager, Harle et al. 2007; Nemunaitis and Nemunaitis 2007; Murray N 2010). Moreover, enhancement of tumor antigen recognition following treatment was correlated with patient benefit.

1.2 Immune Function Capacity in Cancer Patients

Tumor cells often continue to express normal cellular antigens in addition to neo-antigens that are recognized by the immune system of the tumor-bearing host. The production of tumor specific antibodies by B cells can be elicited by the immunization of animals with tumor cells, and tumor-associated determinants have been defined by their ability to stimulate an antibody response in association with their presence on tumor cells. Detectable titers of antibodies that bind to tumor-associated antigens have been demonstrated in cancer patients, but there is no compelling evidence that administration of exogenous antibodies to tumor-associated determinants cause solid tumors to regress in humans. However, there is an accumulating body of evidence to suggest that many tumors in experimental model systems and from cancer patients express molecules that are targeted by the T cell arm of the immune system.

Rejection of antigen-expressing tumor cells has been shown to be mediated by specific host cytolytic T cells (Prien RT 1957; Kripke 1974). Tumor infiltrating lymphocytes, or TIL, have been shown to mediate durable regression of established tumors in mice with advanced tumor burdens (Yang, Perry-Lalley et al. 1990; Barth RJ 1991). In patients bearing metastatic tumors, a number of groups have demonstrated the existence of anti-tumor CTL responses. Peripheral blood mononuclear cells as well as TIL contain populations of cells and individual clones that demonstrate tumor specificity; they lyse autologous tumor cells, but not natural killer targets, allogeneic tumors cells, or autologous fibroblasts (Anichini, Mazzocchi et al. 1989; Darrow, Slingluff et al. 1989; Knuth, Wolfel et al. 1989; Topalian, Solomon et al. 1989; Van den Eynde B 1989). Therefore, tumor specific antigens exist on metastatic human tumors, and are capable of stimulating a specific T cell response that can be expanded *ex vivo* to achieve clinical objective responses in tumor bearing patients.

Endogenously synthesized antigens of virtually all mammalian cells are processed in an endoplasmic reticulum compartment and converted to small epitope peptides that are subsequently displayed on the cell surface in association with Class I MHC molecules to stimulate cytotoxic CD8+ cells (Townsend, Gotch et al. 1985; Maryanski, Pala et al. 1986; Townsend, Rothbard et al. 1986). However, although exogenous (extracellular) antigens are processed by MHC II molecules to activate CD4+ cells, a subgroup of specialized antigen processing cells (dendritic cells) are capable of presenting very small numbers of exogenous peptides in association with Class I MHC molecules to stimulate T cell recognition (Rock, Gamble et al. 1990; Christinck, Luscher et al. 1991) in a process designated “cross-presentation” (Hirschowitz, Foody et al. 2004).

The role of dendritic cells (DCs) in cell-mediated immunity has been extensively investigated (Gilboa, Nair et al. 1998; Timmerman and Levy 1999; Keilholz,

Weber et al. 2002; Conrad and Nestle 2003; Cranmer, Trevor et al. 2004). DCs have been found to play a central role in the induction of anti-tumor immunity in tumor-bearing hosts via the process of antigenic cross-presentation (Hirschowitz, Foody et al. 2004). Recent data suggest a distinct mannose receptor (MR) in DCs through which endocytosed antigen can be diverted from the classical MHC II restricted presentation pathway to the MHC I pathway (Burgdorf, Kautz et al. 2007). As a result, by way of differential receptor-dependent uptake, DCs either present antigens through the MHC II pathway activating of CD4+ cells or cross-present through the MHC I pathway activating CD8+ T cells. CD4+ cells further augment the activity of natural killer cells and macrophages, in addition to amplifying antigen-specific immunity by local secretion of cytokines (Germain 1994; McAdam, Schweitzer et al. 1998; Pulendran, Smith et al. 1999; Banchereau, Briere et al. 2000; Akbari, DeKruyff et al. 2001). Recent studies in animal models have shown that immunization with DCs loaded with defined tumor antigens in the form of peptides, proteins, or RNA are capable of priming CTL responses and inducing tumor immunity (Flamand, Sornasse et al. 1994; Mayordomo, Zorina et al. 1995; Porgador and Gilboa 1995; Boczkowski, Nair et al. 1996). DCs mixed *in vivo* with irradiated tumor cells have been shown to produce a protective immune response against a challenge with autologous tumor cells (Coveney, Wheatley et al. 1997). Tumor-specific CTL response was also detectable. Clinically, infusion of DCs has shown evidence of antitumor activity in patients with solid tumors (Hirschowitz, Foody et al. 2004; Ribas 2005; Small, Sacks et al. 2007). These attributes make DCs a pivotal component in therapeutic strategies of many current immune-based therapies in cancer.

1.3 Immune Function Capacity in Ovarian Cancer Patients

The majority of women diagnosed with cancer of the ovary present in advanced stage (Jemal, Siegel et al. 2009) with 5-year survival rates of 46.7% for Stage IIIa, 41.5% for Stage IIIb, and 32.5% for Stage IIIc (Heintz, Odicino et al. 2006). Despite a gradual improvement in overall survival over the past decade, approximately 75% of the women with Stage III/IV ovarian cancer who achieve a complete clinical response will relapse, as will 50% of those achieving pathologic complete response at a median time of 16-24 months depending on risk factors (Gadducci, Sartori et al. 1998; Markman, Liu et al. 2003; Gadducci, Cosio et al. 2005). Phase III studies of both maintenance and consolidation therapeutic interventions have not had an impact on overall survival (Mei, Chen et al. ; Foster, Brown et al. 2009; McGuire 2009; Pecorelli, Favalli et al. 2009). It is therefore pertinent that expression of all three immunosuppressive TGFβ isoforms is increased in ovarian tumor as compared with normal ovarian tissue (Henriksen, Gobl et al. 1995; Bristow, Baldwin et al. 1999) with significant increases in TGFβ1 in both primary (2.9 fold; $p \leq 0.002$) and recurrent (4.4 fold; $p \leq 0.002$) ovarian cancer (Bristow, Baldwin et al. 1999). Secreted TGFβ from ovarian cancer cells generate immunosuppressive Treg cells (CD4+CD25+) from peripheral CD4+CD25- cells (Li, Ye et al. 2007). Both Treg tumor infiltration as

well as the granzyme B+/FOX3p+ ratio are associated with outcome in those patients treated with neoadjuvant chemotherapy (Polcher, Braun et al.). The contribution of optimal surgical cytoreductive surgery to prognosis may be due, in part, to the associated reduction in circulating Tregs (Napoletano, Bellati et al. 2009). In a recently described transgenic LSL-K-ras$^{G12D/+}$p53$^{loxP/loxP}$ murine model of induced metastatic ovarian cancer in the setting of a mature immune system (Scarlett, Rutkowski et al.), a shift in dendritic cell phenotype from immunostimulatory to immunosuppressive was shown to be due to tumor-cell derived TGFβ1 and PGE2 both of which were found to be secreted at high levels in advanced tumors (Scarlett, Rutkowski et al.). Further, TGFβ has been linked to the emergence of cisplatin resistance (Li, Balch et al. 2009).

Furin, an upstream regulator of TGFβ activity, is a member of the subtilisin-like proprotein convertase family. Proteolytic cleavage by furin is required for TGFβ convertase activation (i.e. pro-TGFβ→ TGFβ). All three of the TGFβ isoforms contain the RXXR motif at their cleavage site albeit with different amino acid sequences (Kusakabe, Cheong et al. 2008). High levels of furin mRNA and furin are widely expressed in human tumors and, specifically, in ovarian tumors (Page, Klein-Szanto et al. 2007). The presence of furin in tumor cells likely contributes significantly to the maintenance of tumor directed, TGFβ-1 peripheral immune tolerance (Pesu, Watford et al. 2008).

According to the serial immune function analyses by Coleman (Coleman, Clayton et al. 2005), five of nine patients with Stages III-IV ovarian adenocarcinoma who achieved post-chemotherapy remission retained the capacity of $CD8^+$ T-cell responses to a panel of 11 viral peptides restricted by at least six common HLA class I alleles, whereas four patients with disease progression displayed low or reduced responses at different stages of treatment. Chemotherapy produced no apparent effect on naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), or effector memory ($CD45^-CCR7^-$) T-cells.

1.4 Background on Transforming Growth Factors beta (TGFβ)

Transforming growth factors beta (TGFβ) are a family of multi-functional proteins that regulate the growth and function of many normal and neoplastic cell types (Sporn, Roberts et al. 1986; Massague 1987; Border and Ruoslahti 1992; Jachimczak, Bogdahn et al. 1993). TGFβ is synthesized as a prepro-TGFβ precursor, processed in the Golgi apparatus with removal of the propeptide and secreted as either the small latent complex (SLC) or with latent TGFβ-binding protein (LTBP) as the large latent complex (LLC) (Li, Wan et al. 2006). Following dissociation from the matrix associated latent complexes, the dimeric TGFβ activates a tetrameric TGFβ receptor complex comprised of TGFβRII and TGFβRI (ALK5) resulting in the phosphorylation of Smad2 and Smad 3 which translocates to the nucleus complexed with Smad4 where a number of

transcription factors are engaged. TGFβ2 signal transduction has been found to affect the expression of more than 20 different genes (Baker and Harland 1997; Heldin, Miyazono et al. 1997; Stiles, Ostrow et al. 1997; Yingling, Datto et al. 1997). TGFβ exerts a wide range of effects on a variety of cell types and has been shown to stimulate or inhibit cell growth, induce apoptosis and increase angiogenesis (Jennings, Kaariainen et al. 1994; Merzak, McCrea et al. 1994; Ashley, Kong et al. 1998; Ashley, Sampson et al. 1998; Jennings and Pietenpol 1998). Although TGFβ has been shown to be an effective tumor suppressor in epithelial cells and in the early phases of tumorigenesis, once the tumor escapes its growth regulatory effects, likely as the result of genetic instability, TGFβ appears to function as a tumor promoter (Bierie and Moses 2006; Pardali and Moustakas 2007) by virtue of its involvement in all six of the essential hallmark cancer-related processes as defined by Hanahan and Weinberg (Hanahan and Weinberg 2000). The three known TGFβ ligands (TGFβ1-3) are ubiquitous and expressed in the majority of tumors (Arteaga 2006). These isoforms are highly homogenous in their mature regions with greater heterogeneity between their propeptide forms (Graycar, Miller et al. 1989). Therefore, isoform specificity is likely controlled through propeptide sequences resulting in highly dynamic and spatially regulated expression. In addition to misregulation of the ligand, there are a variety of reported mutations and downregulation by hypermethylation of CpG islands in the receptors and Smads (Kim, Im et al. 2000; Levy and Hill 2006). Many tumors, including breast, colon, esophageal, gastric, hepatocellular, pancreatic, SCLC and NSCLC produce high levels of active TGFβ isoforms (Constam, Philipp et al. 1992; Eastham, Truong et al. 1995; Friedman, Gold et al. 1995; Jakowlew, Mathias et al. 1995; Kong, Anscher et al. 1995; Yamada, Kato et al. 1995; Eder, Stenzl et al. 1996; Bierie and Moses 2006; Levy and Hill 2006). Furthermore, overexpression of TGFβ has been correlated with tumor progression and poor prognosis (Bierie and Moses 2006; Levy and Hill 2006). Elevated TGFβ2 levels have also been linked with immunosuppression in both afferent and efferent limbs (Sporn, Roberts et al. 1986; Massague 1987; Bodmer, Strommer et al. 1989; Border and Ruoslahti 1992; Chen, Hinton et al. 1997; Bierie and Moses 2006; Li, Wan et al. 2006). Tumor-derived TGFβ1 and PGE2 induce the upregulation of PD-L1 in immunocompetent splenic dendritic cells and are causally related to the shift in dendritic cell phenotype from immunostimulatory to immunosuppressive transgenic in the LSL-K-ras$^{G12D/+}$p53$^{loxP/loxP}$ murine model of induced metastatic ovarian cancer(Scarlett, Rutkowski et al.). TGFβ2 inhibits T cell activation in response to antigen stimulation as well as targeting cytotoxic T cell cytolytic pathways (Thomas and Massague 2005). Additionally, TGFβ2 has antagonistic effects on the Natural Killer (NK) cells as well as the induction and proliferation of the lymphokine-activated killer (LAK) cells (Rook, Kehrl et al. 1986; Kasid, Bell et al. 1988; Tsunawaki, Sporn et al. 1988; Hirte and Clark 1991; Ruffini, Rivoltini et al. 1993; Naganuma, Sasaki et al. 1996).

The immune suppressor functions of TGFβ are likely to play a major role in modulating the effectiveness of cancer cell vaccines. TGFβ inhibits GMCSF

induced maturation of bone marrow derived dendritic cells (DCs) (Yamaguchi, Tsumura et al. 1997) as well as expression of MHC Class II and co-stimulatory molecules (Geissmann, Revy et al. 1999). It has been shown that antigen presentation by immature DCs result in T cell unresponsiveness (Steinman, Hawiger et al. 2003). TGFβ also inhibits activated macrophages (Ashcroft 1999) including their antigen presenting function (Du and Sriram 1998; Takeuchi, Alard et al. 1998). Therefore, both the ubiquitous expression of the TGFβ isoforms as well as the inhibitory effects of these isoforms on GMCSF immune modulatory function (see below), support a broad based tumor target range for the application of a TGFβ suppressed / GMCSF expressing immune enhancing therapeutic.

1.5 Background on GMCSF

One factor that may limit the immunogenicity of tumor cells is the influence of cytokines in the tumor microenvironment (Mach and Dranoff 2000). There is evidence that cytokines have a role in tumor formation and it has been demonstrated that manipulation of the cytokine balance can be exploited for cancer therapy. Forni et al. (Forni, Giovarelli et al. 1985) showed that modifying intracellular cytokine levels could alter the outcome of the host response. Protective immunity against later tumor challenge was also reported in some instances (Forni, Giovarelli et al. 1985).

More specifically, tumor cells genetically modified to secrete GMCSF have consistently demonstrated the most potent induction of anti-tumor immunity compared to other cytokines (Dranoff, Jaffee et al. 1993). Results suggest that treatment with GMCSF protein may translate into clinical advantage, perhaps through immune stimulation (Anderson, Markovic et al. 1999; Spitler, Grossbard et al. 2000; Rini, Weinberg et al. 2003). Interestingly, systemic cytokine administration has not induced direct anticancer response in randomized controlled trials, possibly due to a failure of the approach to recreate accurately the paracrine function of cytokines in tissue microenvironments (Dranoff 2004).

Increasing evidence suggests that GMCSF is involved in the augmentation of tumor antigen presentation (Dranoff, Jaffee et al. 1993; Huang, Golumbek et al. 1994). DCs mediate a crucial role in priming antigen-specific immune responses (Zeng, Wang et al. 2001). DCs express diverse receptors that allow for recognition and capture of antigens in peripheral tissues, process this material efficiently albeit by different routes into the MHC Class I and II presentation pathways, upregulate costimulatory molecules upon maturation, and migrate to secondary lymphoid tissues (Banchereau, Briere et al. 2000). It was observed following injection of GVAX® in patients that an intense local reaction consisting of dendritic cells, macrophages, and granulocytes occurs (Dranoff, Jaffee et al. 1993; Mach and Dranoff 2000).

Dendritic cells have proven to be incredibly versatile with regards to their response to inflammatory conditions and pathogens, which has made it difficult for researchers to determine the appropriate functional characteristics DCs must attain for cancer vaccination (Banchereau, Briere et al. 2000). In one study B16 melanoma cells engineered to secrete either GMCSF or Flt-3-Ligand (FL) and their immunologic effects were reported (Mach and Dranoff 2000). Although both cytokines provoke a marked expansion of DCs locally and systemically, GMCSF stimulated greater levels of protective immunity. Three profound differences have been described, which may account for the disparity in response. First, GMCSF induces a subset of DCs that are superior for the phagocytosis of apoptotic tumor cells (Young and Inaba 1996; Pulendran, Lingappa et al. 1997; Shen, Reznikoff et al. 1997). Second, compared to FL, GMCSF evoked higher levels of costimulatory molecules, which is characteristic of greater functional maturation. This enhanced activity results in more efficient T cell stimulation, thereby broadening the arsenal of induced lymphocyte effector mechanisms (Murtaza, Kuchroo et al. 1999). Third, GMCSF promoted uniformly high levels of CD1d on DCs, in contrast to FL, which triggered a more heterogeneous expression (Kawano, Cui et al. 1997). CD1d is a nonclassical MHC Class I molecule that presents lipid antigens (Yamaguchi, Furukawa et al. 1990). The CD1d lipid complex activates NKT cells, a population of lymphocytes that display a restricted Class I MHC-like receptor (Bendelac, Rivera et al. 1997). Importantly, natural killer T cells (NKT) cells may play pivotal roles in both endogenous and therapeutic responses to tumors (Smyth, Crowe et al. 2002).

1.6 Background on Gene Vaccines in Cancer

Gene therapy has received considerable attention in recent years. Vaccination with tumor cells designed to augment tumor antigen presentation and induce specific anti-tumor immunity has yielded promising but limited results (Holladay, Heitz et al. 1992). Advances in our understanding of cancer biology and developments in vector technologies are advancing the therapeutic potential of tumor vaccine approaches. It is now possible to genetically modify tumor cells for vaccination to express specific tumor suppressor genes, immune modulators, drug sensitive genes and antisense gene fragments (Huber, Richards et al. 1991; Culver, Ram et al. 1992; Trojan, Blossey et al. 1992; Dranoff, Jaffee et al. 1993; Ram, Culver et al. 1993; Trojan, Johnson et al. 1993; Fakhrai, Dorigo et al. 1996; Swisher, Roth et al. 1999). Extensive experiences by others, as well, demonstrate safety with the use of GMCSF gene based vaccines as a potential vaccine therapeutic in cancer patients (Table 1).

Table 1: Summary of clinical trials testing GMCSF gene-transduced vaccines

Trial	Cancer (stage)	Vaccine	Vector	n	Response	Dose (number of irradiated tumor cells)	GM-CSF production (ng/10^6 cells every 24 h)	Concurrent treatment
Simons (Simons, Jaffee et al. 1997)	Renal cell (IV)	Autologous	Retrovirus	16	1 PR (7 mo)	4 x 10^6 – 4 x 10^8	17-149	None
Chang (Chang, Li et al. 2000)	Melanoma (IV)	Autologous	Retrovirus	5	1 CR (≥36 mo)	1 x 10^7	56-100	RhIL-2 3.6 x 10 μ/kg x 15 doses*
Soiffer (Soiffer, Lynch et al. 1998)	Melanoma	Autologous	Retrovirus	29	1 PR, 3 MR, 1 mixR, 3 pts DFS s/p surg/XRT, ≥36, ≥20 mo	ND	84-965	None
Jaffee (Jaffee, Hruban et al. 2001)	Pancreatic cancer (I, II, III)	Allogeneic	Plasmid	14	3 pts DFS 4-5 years	1 x 10^7 – 5 x 10^8	ND	Surgery to disease-free adjuvant XRT/chemo
Simons (Simons, Mikhak et al. 1999)	Prostate	Autologous	Adenovirus	8	No responses immune activation observed	1 x 10^7 or 5 x 10^7	143-1403	None
Salgia (Salgia, Lynch et al. 2003)	NSCL (IV)	Autologous	Adenovirus	35	2 pts DFS >3 years s/p surgery	1 x 10^6 – 1 x 10^7	Mean 233	Surgery
Kusumoto (Kusumoto, Umeda et al. 2001)	Melanoma (IV)	Autologous	Adenovirus	9	No clinical response	2 x 10^6 – 1 x 10^7	80-424	None
Mastrangelo (Mastrangelo, Maguire et al. 1999)	Melanoma (IV)	Intratumoral injection	Vaccinia virus	7	1 PR, 1 CR (injected lesion)	NA	NA	None
Hu (Hu, McNeish et al. 2003)	Solid tumor	Intratumoral injection	Herpes simplex type 1	15	1 PR (injected lesion)	NA	NA	None
Soiffer (Soiffer, Hodi et al. 2003)	Melanoma (IV)	Autologous	Adenovirus	35	1 CR, 1 PR, 3-year follow-up 10 patients alive, 4 DFS	1 x 10^6 – 1 x 10^7	745	None
Simons (Simons, Nelson et al. 2002)	Prostate	Allogeneic	Adenovirus	34	Survival 31 mo with 3 x 10^8 cells vs. 22 mo with 1 x 10^8 cells	1 x 10^8 – 3 x 10^8		None
Simons (Simons, Higaro et al. 2003)	Prostate	Allogeneic	Adenovirus	65	33 evaluable (1 PSA PR, 2 PSA MR)	1 x 10^8 – 3 x 10^8		None
Nemunaitis (Nemunaitis, Sterman et al. 2004)	NSCLC (IIIB/IV)	Autologous	Adenovirus	63	3/33 CR Dose-related survival advantage ($p < 0.05$)	5-100 x 10^6	44-236	None
Nemunaitis (Nemunaitis, Sterman et al. 2004)	NSCLC (IB/II)	Autologous	Adenovirus	20	7/10 DFS at 16 mo	5-100 x 10^6		None
Tani (Tani, Azuma et al. 2004)	Renal (IV)	Autologous	Retrovirus	4	2 patients alive ≥40 and ≥58 mo	1.4-3.7 x 10^8		None
Simons (Simons, Carducci et al. 2006)	Prostate (hormone naive)	Allogeneic	Adenovirus	21	76% decrease PSA velocity	1.2x10^8	ND	None
Small (Small, Sacks et al. 2007)	Prostate (hormone refractory)	Allogeneic	Adenovirus	55	Median survival metastatic group n=(34) Low dose-24 m High dose-34.9 m	Prime: 5x10^8 Boost: 1x10^8 or 3x10^8	ND	None
Urba (Urba, Nemunaitis	Prostate (hormone	Allogeneic	Adenovirus	19	PSA doubling ↑ 29 → 57 wks	1-5 x 10^8	ND	None

Trial	Cancer (stage)	Vaccine	Vector	n	Response	Dose (number of irradiated tumor cells)	GM-CSF production (ng/10^6 cells every 24 h)	Concurrent treatment
et al. 2008)	naïve)							
Nemunaitis (Nemunaitis, Jahan et al. 2006)	NSCL IIIB/IV	Autologous and transfected K562	Adenovirus	49	None	5 x 10^6 auto 5 x 10^8 K562	2624	None
Laheru (Laheru, Lutz et al. 2008)	Pancreatic	Allogeneic	Adenovirus	50	Survival 4.3 mo vs. 2.3 mo when combined with cytoxan	5 x 10^8	ND	Cohort 13 and cyclophosphomide
Senzer (Senzer 2009)	Melanoma IIIC/IV	Intratumoral injection	Herpes simplex 1 virus	50	61% 1-year survival	1 x 10^6→1 x 10^8 pfu/ml	ND	None
Hofbauer (Hofbauer, Baur et al. 2008)	Melanoma, leiomyosarcoma	Intratumoral injection	Canarypox (ALVAC)	—	SD	—	ND	None
Cornelio (G. H. Cornelio 2008)	Miscellaneous	Reximmune-C	Retrovirus	7	Median OS 5 mo	2.5x10^9 or 5.0x10^{10} pfu	ND	None
Higano (Higano, Corman et al. 2008)	Prostate (hormone refractory)	Allogeneic	Adenovirus	80	Median survival Hi 35 mo. Int 20 mo. Lo 23.1 mo.	Hi 100x10^6 or 200x10^6 (q28dx6) Int 200x10^6 (q14dx12) Lo 500x10^6 x1→300x10^6 (q14dx11)	Serum levels only	None
Ho (Ho, Vanneman et al. 2009)	AML	Autologous		10	9/10 durable CR			Allogeneic, nonmyeloablative HSCT
Emens (Emens, Asquith et al. 2009)	Breast	Allogeneic	Plasmid	28	Not reported	5.0x10^7 or 5.0x10^8 cells	16.8±29.4 pg/mL (5x10^7) 153.6±36.3 pg/mL (5x$10^{*)}$	Cyclophosphamide, doxorubicin

Additional studies involving the use of adenoviral vectors to transfer the GMCSF® gene have been approved by the National Institutes of Health in patient with prostate, lung, breast, colon and head and nec k cancer, as well as in melanoma patients.
*Following vaccine injection, four patients received *ex vivo* expanded lymphocytes (CD3 negative) from harvested vaccine-primed lymph nodes.
chemo: Chemotherapy; CR: Complete response; DFS: Disease-free survival; GMCSF®: Granulocyte-macrophage colony-stimulating factor; NA: Not available; mixR: Mixed response; mo: Months; MR: Minor response; ND: Not described; NSCLC: Non-small cell lung cancer; PR: Partial response; PSA: Prostate-specific antigen; pts: Patients; RhIL: Recombinant human interleukin; s/p: Status post; surg: Surgery; XRT: Radiation therapy.

1.7 Blockade of multiple TFGβ isoforms is needed to reverse immunosuppression

Overexpression of two or more of the TGFβ isoforms has been demonstrated in melanoma, gliomas, prostate, gastric, colorectal, ovarian and gastric cancers (Tsamandas, Kardamakis et al. 2004; Dallas, Zhao et al. 2005; Polak, Borthwick et al. 2007; Vagenas, Spyropoulos et al. 2007). It was previously noted that expression of all three immunosuppressive TGFβ isoforms is increased in ovarian tumor as compared with normal ovarian tissue (Henriksen et al, 1995; Bristow et al, 1999) with significant increases in TGFβ1 in both primary (2.9 fold; $p \leq 0.002$) and recurrent (4.4 fold; $p \leq 0.002$) ovarian cancer (Bristow, Baldwin et al. 1999). TGFβ1 and -β2 both bind to TGFβ receptor 1 and suppress DC and helper T cell function through Smad and MAP kinases (Larmonier, Marron et al. 2007; Park, Letterio et al. 2007). The recent correlative study by Polak showed that tumor infiltrating tolerogenic DCs and suppressor T cell lymphocytes in malignant melanoma can be correlated with immunosuppressive TGF-β1, -β2 and IL-10 expression (Polak, Borthwick et al. 2007). This mechanism of tumor-

associated immunosuppression is likely to contribute to tumor escape to immune response.

The genetic modification with a TGFβ2 antisense-encoding plasmid represents one of many currently examined approaches to inhibit local TGFβ activity. Others include the use of neutralizing antibodies, soluble receptors, receptor kinase antagonist drugs, antisense reagents, and a number of less specific drugs such as angiotensin II antagonist and tranilast (Prud'homme 2007). Systemic TGFβ blockade may result in severe inflammatory disease, although this has not been observed, presumably because the neutralization is only partial. To attain localized TGFβ blockade as is applicable to achieve optimal immunostimulation with a GM-CSF-modified cancer cell vaccine, the desired outcome may be accomplished expediently through the incorporation of a *de novo* gene modifying moiety into the vaccinating cancer cells *ex vivo*, thereby bypassing pharmacokinetic and toxicity concerns from systemic introduction of anti-TGFβ neutralizing antibodies, soluble receptors, or receptor kinase antagonists. In view of the overlapping immunosuppressive activities of the various TGFβ isoforms, it would be particular advantageous if such a gene modifying moiety can globally attenuate the activity of TGFβ1, -β2, and -β3.

1.8 Background on TAG (TGFβ2 antisense and GMCSF vaccine)

Based on the hypothesis that the combination of GMCSF and TGFβ2 antisense genes will enhance potency of immune stimulation against cancer specific antigens, a combined plasmid, TAG was constructed (Gradalis, Inc. Carrollton, TX) (Kumar 2009). Components of this plasmid are the clinically utilized pUMVC3 vector backbone (University of Michigan), a bacterial origin of replication, a kanamycin resistance gene, a CMV IE promoter / enhancer and intron A driving the hGMCSF cDNA followed by a 2A linker sequence and a 930 base pair fragment of the hTGFβ2 cDNA molecule in antisense orientation followed by a rabbit hemoglobin poly-A tail (Kumar 2009; Maples PB 2009).

The TAG expression vector was electroporated into the autologous cells *ex vivo*. Therefore, only the cells present at the time of electroporation would incorporate the transfected DNA.

The vector utilized is expected to remain extra-chromosomal. Amplification of the insert by PCR suggested that the vector is non-rearranged in the cells. Our previous human and animal vaccination studies with TGFβ2 antisense vaccine and GVAX® vaccine (GMCSF) had not demonstrated deleterious effects due to unwanted gene expression. It is theoretically possible that the structure or expression of a gene near an integration site (if the vector was incorporated into a chromosome) may be affected by insertion of the added DNA. However, all

cells used for vaccinations were irradiated with 10,000 cGy to block their growth potential.

GMCSF expression in the TAG product despite being significant is lower with the electroporation processing (1ng per 10^6 tumor cells per 24 hours). Thus, although survival in one GVAX® study (Nemunaitis, Sterman et al. 2004) correlated with GMCSF expression ≥40ng/10^6 cells/24 hours, in a second study there was no correlation (see below). Further, in addition to documented variability in level of GMCSF expression between manufacturing processes, the levels of expression achieved with the TAG vaccine are deemed clinically relevant as 1) use of a plasmid rather than a viral vector obviates the obfuscating effects of elicited anti-viral neutralizing antibodies, 2) use of a plasmid likewise prevents the development of elicited antibodies interfering with long-term gene expression, and 3) concurrent suppression of TGFβ2 would minimize tumor associated inhibition of GMCSF induced dendritic cell maturation (Yamaguchi, Tsumura et al. 1997), which we hypothesize would improve product activity and theoretically lower minimal toxic effect related to GMCSF and or adenoviral presence. In addition, our experience with the bystander GVAX® vaccine with a 25-fold higher GMCSF secretion than the autologous vaccine in which no objective tumor responses were seen suggests that levels of GMCSF protein expression from the manufactured vaccine is not an exclusive or possibly even necessary factor in vaccine activity.

Thirty-eight patients (BB-IND 13650) underwent harvest for TAG vaccine. Thirty-two were successfully manufactured under GMP conditions (Maples PB 2009). GMCSF expression and TGFβ2 knockdown met product release criteria. Three (all gastrointestinal tumors with luminal access) had bacterial contaminants and could not be released. Three had insufficient cells. Twenty-two advanced refractory cancer patients have been treated (Maples, Kumar et al. 2009; Maples PB 2009; Nemunaitis 2009). No significant toxic effect was observed. No Grade 3/4 toxicity was demonstrated to TAG (Tables 2 and 3). Significant increase in GMCSF expression and knockdown of TGFβ2 but not TGF-β1 were demonstrated as components of product release criteria. In preliminary results we have observed 17 of 22 (77%) evaluable patients with stable disease or better of at least 3 months. Two withdrew early for personal reasons with stable disease after 1 cycle; two had progressive disease at the 2 month assessment; and one withdrew consent at month 3 with progressive disease (Tables 4 and 5). One patient achieved CR, confirmed by imaging studies (Figure 1) (melanoma). Three patients remain alive under observation following successful administration of all manufactured vaccines (008, 013, and 023). Two additional patients are continuing on trial (037 and 041). Eight of 18 evaluable patients survived > 1 year following initiation of treatment. Thus the TAG vaccine appears to be safe and has evidence of clinical efficacy (Olivares J 2011).

Table 2: Frequency of Adverse Events Possibly, Probably, or Definitely Related to the TAG vaccine

Preferred Term	Grade	Relation to TAG	Number of Events	Number of Subjects
Edema	1	Definitely	1	1
Fatigue	1	Possibly	1	1
Fatigue	2	Possibly	1	1
General Pain (NOS)	1	Definitely	4	1
Injection Site Reaction	1	Definitely	3	3
Injection Site Reaction	1	Probably	2	2
Injection Site Reaction	2	Probably	1	1
Rash	1	Possibly	1	1
Rash	2	Possibly	1	1
Fatigue	2	Possibly	1	1
Left Arm Soreness	1	Probably	1	1
Left Arm Weakness	1	Probably	1	1

Table 3: Serious Adverse Experiences reported on TAG vaccine

ID#	Reported Term	Grade	Drug Related Assessment	Unexpected
007	Small Bowel Obstruction	3	Not Related	Yes
007	Dehydration	3	Not Related	No
010	Seizure	3	Not Related	Yes
010	GI Bleed	3	Not Related	Yes
010	Brain Metastasis	3	Not Related	Yes
017	Pneumonia (resulting in death)	5	Not Related	Yes
031	Pericardial effusion	3	Not Related	Yes
032	Weight Loss	2	Not Related	No
032	Vomiting	3	Not Related	No
032	Aspiration Pneumonia	3	Not Related	No

Table 4: Demographics of Subjects on TAG

Age (years)	16-64	24
	≥65	14
Gender	Male	17
	Female	21
Cancer	Adrenocortical	2
	Bladder Cancer	2
	Breast Cancer	4
	Colon Cancer	4
	Colorectal	1
	Duodenal Cancer	1
	Gastric	1
	Leiomyosarcoma	1
	Melanoma	5
	Neuroendocrine	2
	NSCLC	7
	Ovarian	1
	Rectal Cancer	1
	Renal Cancer	1
	Urachal Adenocarcinoma	1
	Prostate	1
	Hemangiopericytoma	1
	Esophageal	1
	Cervical Cancer	1

Table 5: Response status of subjects on TAG

	2.5×10^7 cells / injection	**1.0×10^7 cells / injection**
Stable disease ≥ 3 months after vaccine received	10	6
Unevaluable	2	0
Progressive disease	3	0
Complete response	0	1
Active on treatment < 3 months	4	0

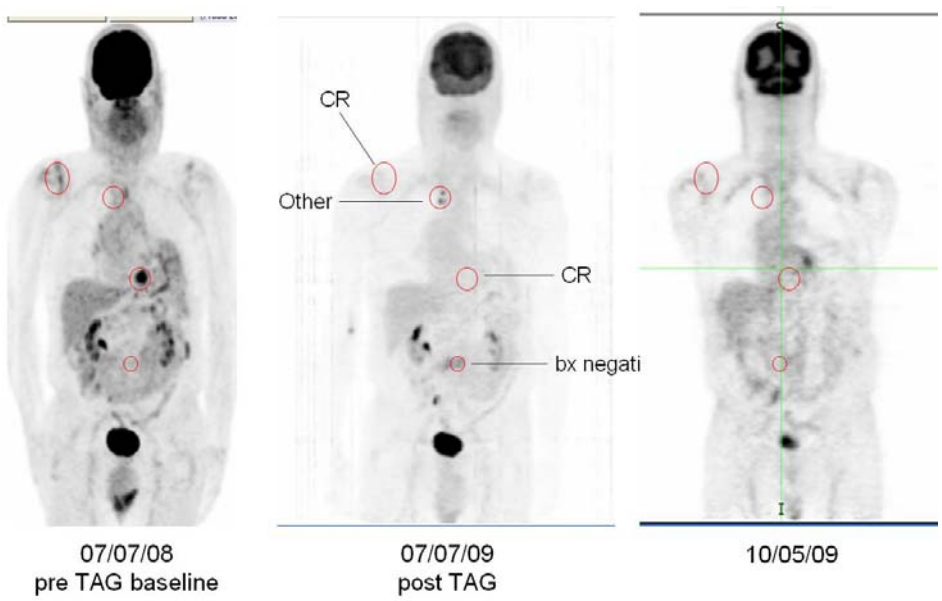


Figure 1: Patient 013 had diffusely metastatic melanoma and had previously failed multiple standard therapies. PET CT 11 cycles after TAG demonstrated significant response on 7/7/09 in comparison to baseline on 7/7/08. New 2,3 FDG uptake however in upper paratracheal nodes in association with a viral syndrome confused demonstration of CR. Re-scan 3 months later with viral syndrome resolved revealed CR. Residual uptake at L 2 was followed up with a MRI scan and biopsy which revealed no malignancy.

Harvested malignant tissue was processed to a single cell suspension, and cells were transfected with the TAG expression vector via electroporation. All processed TAG vaccines demonstrated GMCSF secretion and low to undetectable levels of TGFβ2, as shown in Figures 2A and 2B.

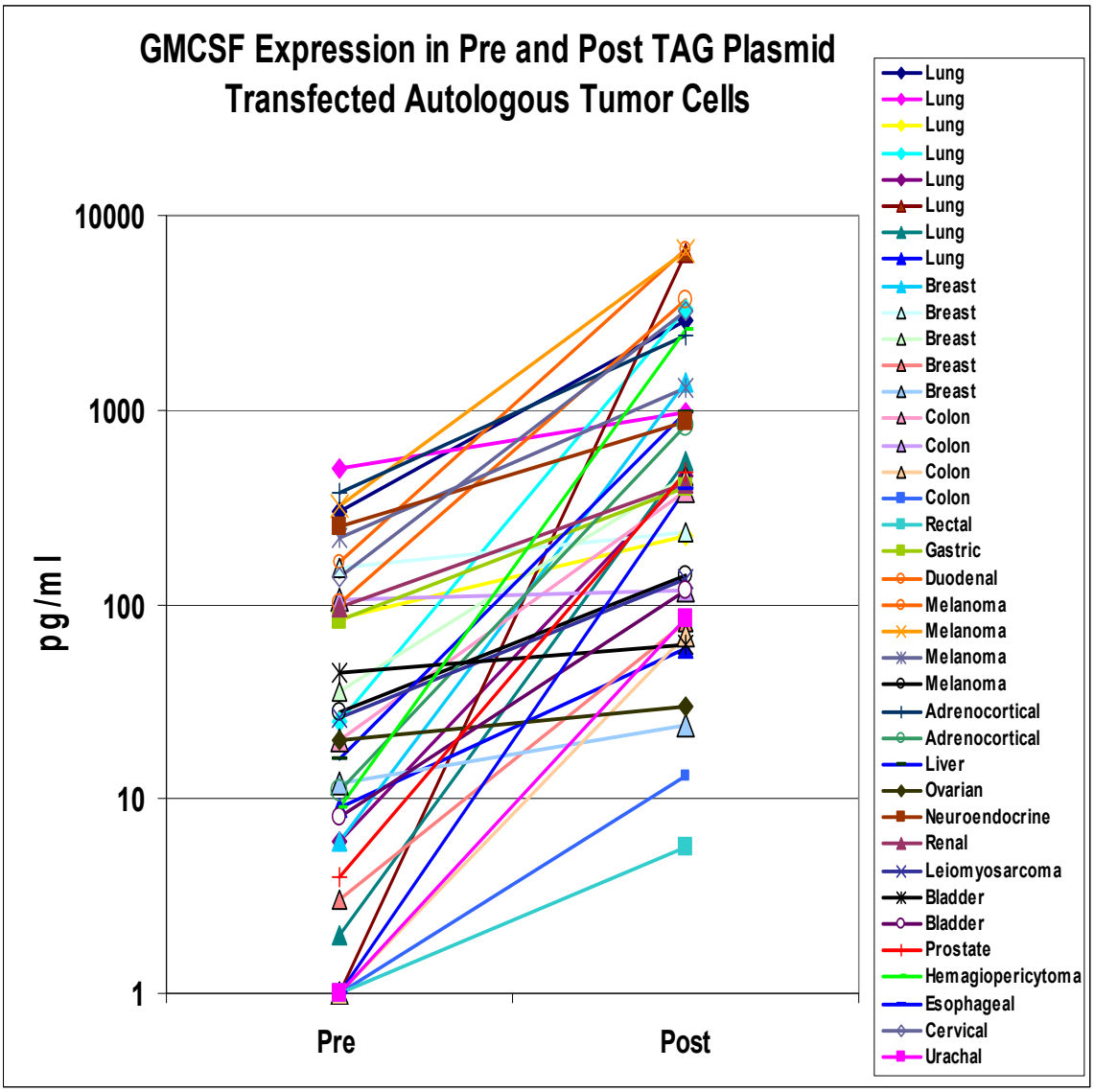


Figure 2A: GM-CSF expression in TAG vaccines.

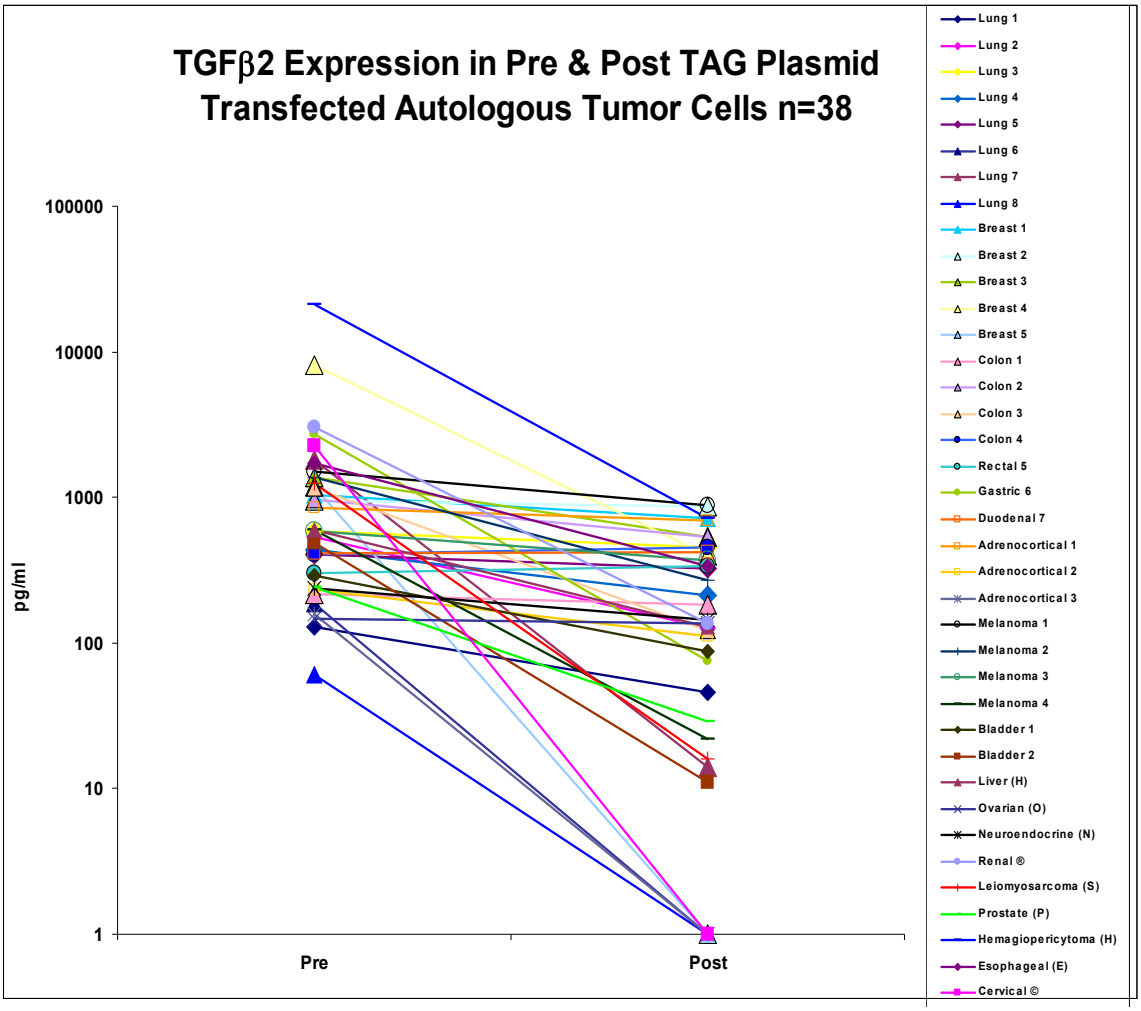


Figure 2B: TGFβ-2 expression in TAG vaccines.

1.9 Background on Furin

Furin, an upstream regulator of TGFβ activity, is a member of the subtilisin-like proprotein convertase family. The members of this family are proprotein convertases that process latent precursor proteins into their biologically active products. This encoded protein is a calcium-dependent serine endoprotease that can efficiently cleave precursor proteins at their paired basic amino acid processing sites by the consensus sequence –Arg-X-K/Arg-Arg (RXK/RR), with -RXXR- constituting the minimal cleavage site (Thomas 2002). Furin is synthesized as an inactive zymogen and translocated into the endoplasmic reticulum (ER), where the propeptide is cleaved autocatalytically. Furin is activated when it exits the ER and transits to the mildly acidic trans Golgi apparatus, where it is further cleaved by the resident, activated furin or by an

associated molecule in trans. Its dissociation from the catalytic domain leads to its activation. Following release and intracellular trafficking, furin can be tethered at the cell membrane by the cytoskeletal protein filamin. Proteolytic cleavage by furin is required for TGFβ convertase activation (i.e. pro-TGFβ→ TGFβ). All three of the TGFβ isoforms contain the RXXR motif at their cleavage site albeit with different amino acid sequences (Kusakabe, Cheong et al. 2008). Data suggest that the unique structure of the TGFβ2 latency associated peptide (LAP) region might attenuate furin mediated cleavage but do not exclude a role for furin in its processing.

Furin is the primary proconvertase in TGFβ processing events. It is worth noting that there appears to be a disparity between the complete cleavage of TGFβ *in vivo* versus the partial cleavage *in vitro* (Dubois, Laprise et al. 1995). TGFβ in turn appears to amplify furin gene transcription through an amplification loop (Blanchette, Day et al. 1997; McMahon, Laprise et al. 2003).

High levels of furin mRNA and furin protein are widely expressed in human tumors and, specifically, in ovarian cancer (Schalken, Roebroek et al. 1987; Cheng, Watson et al. 1997; Mbikay, Sirois et al. 1997; Bassi, Mahloogi et al. 2000; Bassi, Mahloogi et al. 2001; Bassi, Fu et al. 2005). There is evidence that furin may be an important target in the treatment of prostate cancer (Uchida, Chaudhary et al. 2003), colorectal cancer (Scamuffa, Basak et al. 2008; Scamuffa, Siegfried et al. 2008), and breast cancer (Lapierre, Siegfried et al. 2007). In addition, furin plays an important role in immune regulation (Pesu, Muul et al. 2006; Pesu, Watford et al. 2008). In APCs, cytotoxic T lymphocyte-sensitive epitopes in the trans-Golgi compartment were processed by furin and the less frequented TAP independent pathway (Lu, Wettstein et al. 2001). The conditional deletion of endogenous-expressing furin in T lymphocytes allowed for normal T-cell development, but impaired the function of regulatory and effector T cells, which produced less TGFβ1. Furin-deficient Tregs were less protective in a T-cell transfer colitis model and failed to induce Foxp3 in normal T cells. Additionally, furin-deficient effector cells were inherently over-active and were resistant to suppressive activity of wild-type Treg cells. Thus, furin expression by T cells appears to be indispensable in maintaining peripheral tolerance, which is due, at least in part, to its non-redundant, essential function in regulating TGFβ1 production (Pesu, Watford et al. 2008).

We and others have found that up to a 10-fold higher level of TGFβ1 may be produced by human colorectal, lung cancer, melanoma, and ovarian cancer cells, and likely impact the immune tolerance state by a higher magnitude (Bommireddy and Doetschman 2007; Fogel-Petrovic, Long et al. 2007; Polak, Borthwick et al. 2007). The presence of furin in tumor cells likely contributes significantly to the maintenance of tumor directed, TGFβ1 peripheral immune tolerance (Pesu, Watford et al. 2008). Hence, furin knockdown represents a novel and attractive approach for optimizing immunosensitization. Initial studies

to validate the effect of furin blockade on TGFβ expression was carried out with the furin inhibitor decanoyl-Arg-Val-Lys-Arg-CMK (Dec-RVKR-CMK), a peptidyl chloromethylketone that binds irreversible to the catalytic site of furin and blocks its activity ((Henrich, Cameron et al. 2003)). Treatment with Dec-RVKR-CMK either completely or partially reduces the activity of furin substrates BASE (β-site APP-cleaving enzyme), MT5-MMP, and Boc-RVRR-AMC (Pearton, Nirunsuksiri et al. 2001).

TGFβ1 and TGFβ2 specific immunoassays (R&D Systems Quantikine ELISA) were used to quantify the effect of furin blockade on secreted cytokine levels of the human colorectal line CCL-247. Dec-RVKR-CMK (30 nM) reduced TGFβ1 level from 250 pg/ml to below detectable levels, and inhibited TGFβ2 expression by approximately 50% (from 35 to 18 pg/ml). Dec-RVKR-CMK was similarly effective in reducing TGFβ1 and TGFβ2 production in the human melanoma line CRL-1585 (reductions of 100% and 82%, respectively) and the Human NSCLC line H460 (reductions of 100% and 93%, respectively). These findings indicate that TGFβ isoform expression can be effectively reduced through furin blockade.

To consider the applicability of furin knockdown for inhibiting TGFβ isoform expression, similar assessments were performed following transfection with furin-specific siRNAs. Prospective siRNA targeting sites in the furin mRNA were determined by the published recommendations of Tuschl and colleagues and the additional selection parameters that integrates BLAST searches of the human and mouse genome databases (http:jura.wi.mit.edu/bioc/siRNAext). siRNAs targeting eligible translated and 3'UTRs were purchased from Ambion (A-1, -2,-3) and Integrated DNA Design Technologies/IDT (I-1,-2,-3; Figure 3). Proprietary siRNAs from Ambion were designed with an additional algorithm that utilized a proprietary classification approach support vector machine (SVM) approach for enhanced specificity and activity. siRNAs from IDT utilizes a proprietary rational design algorithm that integrates both traditional 21-mer siRNA design rules as well as new 27-mer Dicer-substrate specific design criteria.

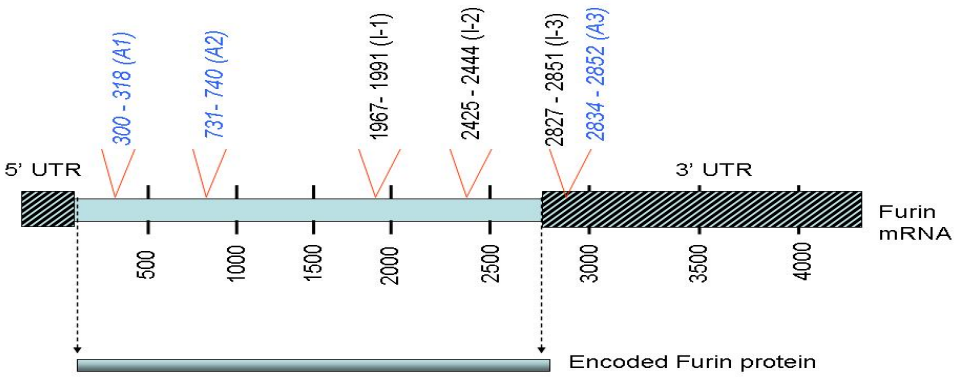


Figure 3: siRNA targeted regions of furin mRNA.
Prospective siRNA targeting regions in 3'-UTR and encoding regions of furin mRNA and the targeted sequence by each siRNA construct is shown.

The transfection of CCL-247 cells was carried out with Lipofectamine 2000 with 10nM -60nM siRNA and $1x10^5$ cells/ml. Treatment with each of the 6 siRNA constructs resulted in marked reduced levels of TGFβ1 and TGFβ2 in the culture supernatants. Depletion ranged from 60-72% for TGFβ1 and comparable levels (57-77%) for TGFβ2 (Figure 4). By comparison, the inhibitory effects of scrambled siRNA treatment were less profound (<20%). Further, siRNAs that targeted the encoding (A-1, A-2, I-1, I-2) or the 3'-UTR (A-3, I-3) of furin miRNA appeared to be equally effective for TGFβ1 and β2 depletion. Similar levels of cytokine reduction were observed over the dose range tested (10-60 nM) for each siRNA.

The viability of untreated CCL-247 cells was 84% at 24 hours post-culture, based on trypan blue dye exclusion viability analysis. By comparison, 70% of cells were viable after treatment with the fuin inhibitor Dec-RVKR-CMK. $siRNA^{furin}$ treated cultures displayed a viability of $\geq$ 70% for all siRNA tested at the same time point, as compared to 80% following control siRNA treatment. There was no remarkable survival advantage following treatment with any of the 6 siRNA tested. These findings indicate that the observed TGFβ reductions cannot be explained by the marginal cytotoxicity of Dec-RVKR-CMK or $siRNA^{furin}$ transfection. The relatively robust viability of transfected cultures implies that immunosensitization through transgene expression is likely to remain active over an extended time period, (i.e. $\geq$ 48 hrs).

$siRNA^{furin}$ treatment similarly reduced TGF levels the human melanoma line CRL-1585 (37-59% reduction of TGF-β1, 25-51% reduction of TGF-β2) and the NSCLC line H460 (78-94% reduction of TGF-β1, 72-79% reduction of TGF-β2)

(Figure 4). Our findings indicate that siRNA-mediated furin knockdown is effective for the depletion of TGF-β isoforms.

a B

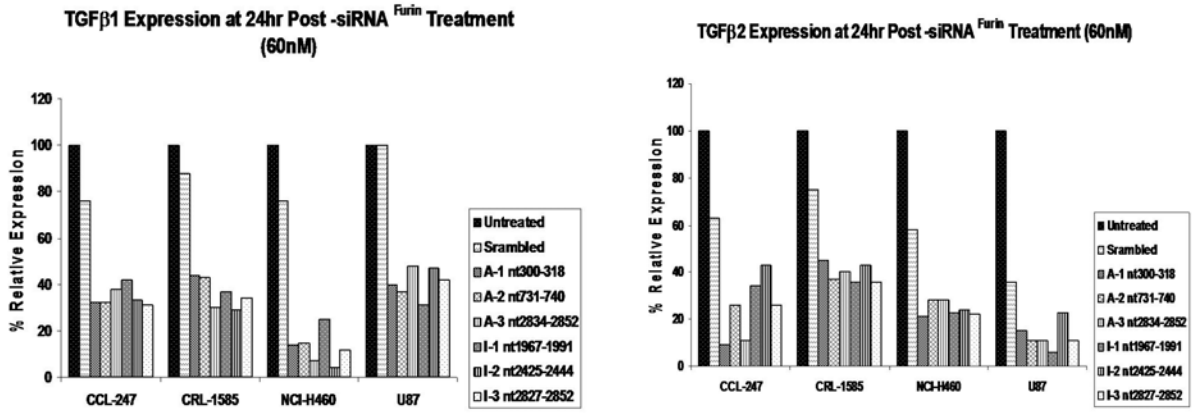


Figure 4: TGF-β 1 (Figure 4a) and TGF-β2 (Figure 4b) expression in human cancer lines following siRNA^{furin} knockdown.

Currently, it is unclear why TGFβ reduction in CRL-1585 was comparatively modest. All three lines displayed similar transfection efficiencies (75-85%) by assessment with a GFP expression plasmid. Followup studies are underway to quantify furin reduction at the mRNA and protein levels, in order to correlate knockdown outcome with TGFβ reduction outcomes.

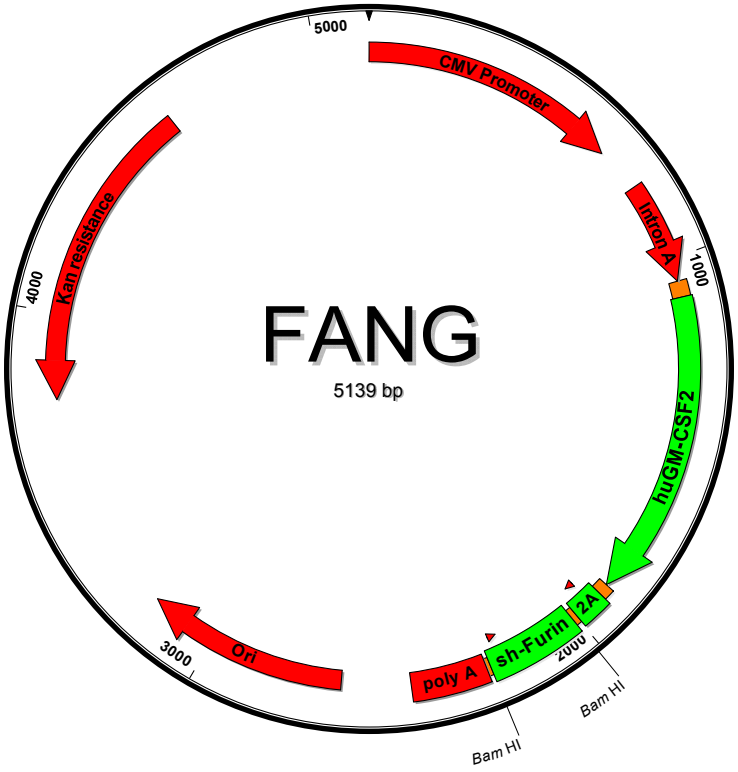
Gradalis, Inc., is at the forefront in the design of "bifunctional" vectors that embed both siRNA and miRNA functional components in a miR 30 scaffold for optimized gene knockdown (Rao 2009; Rao D 2010). The siRNA component is encoded as a hairpin composed of complete matching sequences of the passenger and guide strands. Following cleavage of the passenger strand by Ago 2, an endonuclease with RNase H like activity, the guide strand is incorporated into the RNA-induced silencing complex (RISC), which binds to and cleaves complementary target mRNA (cleavage dependent process). In distinction, the miRNA component of the "bifunctional" vector incorporates mismatches between the passenger and guide strands within the encoding shRNA hairpin in order to achieve lower thermodynamic stability. This configuration allows the passenger strand to dissociate from RISC without cleavage (cleavage independent process) (Matranga, Tomari et al. 2005; Leuschner, Ameres et al. 2006), and the RISC incorporated guide strand acts both through translational repression and sequestration of the target mRNA in the cytoplasmic processing bodies (P-body).

We have previously demonstrated the markedly enhanced effectiveness of bi-functional shRNA^{STMN1} in knockdown of stathmin (oncoprotein 18) which encodes a protein that regulates microtubule remodeling of the cytoskeleton and shown to be upregulated in a high proportion of patients with solid cancers (Rana, Maples

et al. 2008). The bifunctional shRNA construct achieved effective knockdown against stathmin 1, with a 5 log dose enhancement in potency of tumor cell killing compared with the identically targeted siRNA (Rao 2009; Rao D 2010).

A similarly designed bifunctional shRNA was designed to effect furin knockdown. The bi-shRNA^{furin} consists of two stem-loop structures with miR-30a backbone; the first stem-loop structure has complete complementary guiding strand and passenger strand, while the second stem-loop structure has two bp mismatches at positions 11 and 12 of the passenger strand. Our strategy is to use a single targeted site for both cleavage and sequestration. By the use of a proprietary algorithm, the encoding shRNAs are proposed to accommodate mature shRNA to be loaded onto more than one types of RISC (Azuma-Mukai, Oguri et al. 2008). Our reason for focusing on a single site is that multi-site targeting may double the chance for "seed sequence" induced off-target effect (Jackson, Koduvayur et al. 2006).

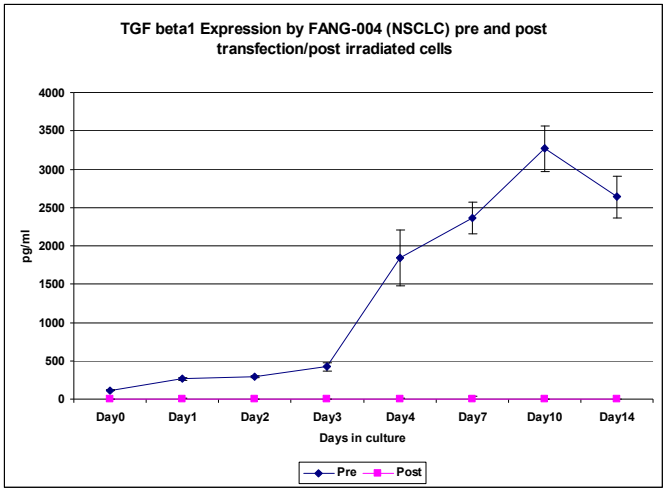
Figure 5: Plasmid construct of FANG™.

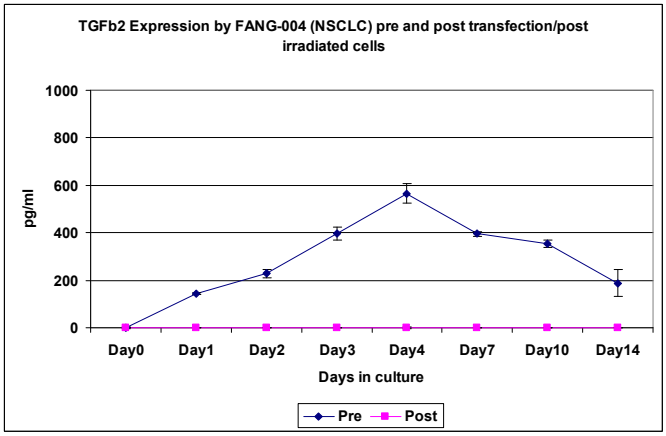


Construction of the FANG™ expression vector is based on the TAG plasmid (Kumar 2009) with the only difference being the replacement of the TGFβ2 antisense DNA sequence with the bi-shRNA^{furin} DNA sequence (Figure 5). The two stem-loop double stranded DNA sequence was assembled with 10 pieces of synthetic complementing and interconnecting oligonucleotides through DNA

ligation. The completed 241 base pairs DNA with Bam HI sites at both ends was inserted into the Bam HI site of the TAG expression vector in place of the TGFβ2 antisense sequence. Otherwise the two expression vectors (TAG and FANG™) are identical (confirmed by sequencing). Orientation of the inserted DNA was screened by PCR primer pairs designed to screen for the shRNA insert and orientation. The FANG™ construct has a single mammalian promoter (CMV) that drives the entire cassette, with an intervening 2A ribosomal skip peptide between the GMCSF and the furin bifunctional shRNA transcript, followed by a rabbit polyA tail. There is a stop codon at the end of the GMCSF transcript. Insertion of picornaviral 2A sequences into mRNAs causes ribosomes to skip formation of a peptide bond at the junction of the 2A and downstream sequences, leading to the production of two proteins from a single open reading frame (Funston, Kallioinen et al. 2008). We have found that the 2A linker to be effective for generating approximately equal levels of GMCSF and anti-TGFβ transcripts with the TAG vaccine, and elected to use the same design for Vigil™.

Electroporation of GMP FANG™ plasmid into patient tumor cells (the cGMP vaccine manufacturing process) demonstrated GMCSF protein production and concomitantly TGFβ1 and –β2 knockdown as predicted. Figure 6 depicts a FANG™-transfected NSCLC tumor's expression profile over 14 days (FANG-004). A summary of the 14 day expression (after manufacturing) profiles of the 23 tumors processed to date is depicted in Figure 7. Of note is the comparison of the TAG-038 and FANG-004 vaccines produced from the same patient's NSCLC tumor. The TAG vaccine has similar GMCSF expression and TGF-β2 knockdown but TAG did not effect the TGF-β1 expression (which is about 10X that of pre-transfection TGF-β2) (Figure 8).





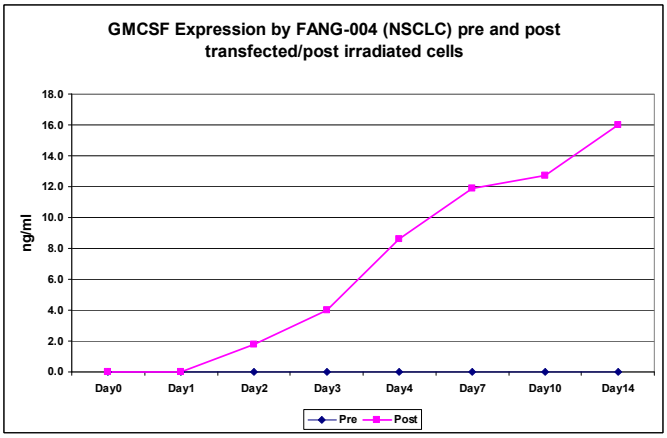


Figure 6: Summary of TGFβ1, -β2, and GMCSF expression in FANG-004 tumor cells pre and post FANG™ cGMP plasmid transfection. Cells were incubated for 14 days and media was periodically sampled for cytokine analysis.

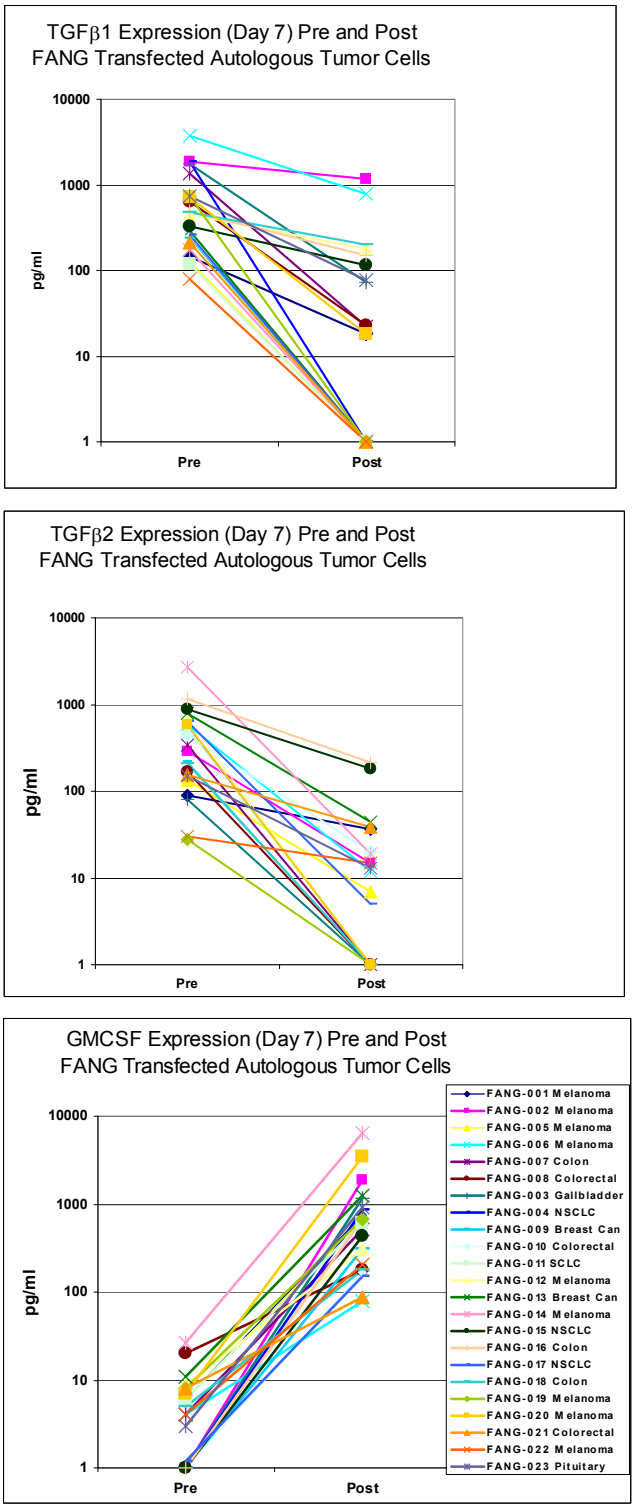


Figure 7: Summary of TGFβ1, -β2, and GMCSF protein production pre and post FANG™ plasmid transfection. ELISA values from Day 4 of the 14-day determinations of cytokine production in manufactured autologous cancer cells.

Data represents autologous vaccines independently generated from 23 patients who underwent Vigil™ processing (FANG 001 – 023).

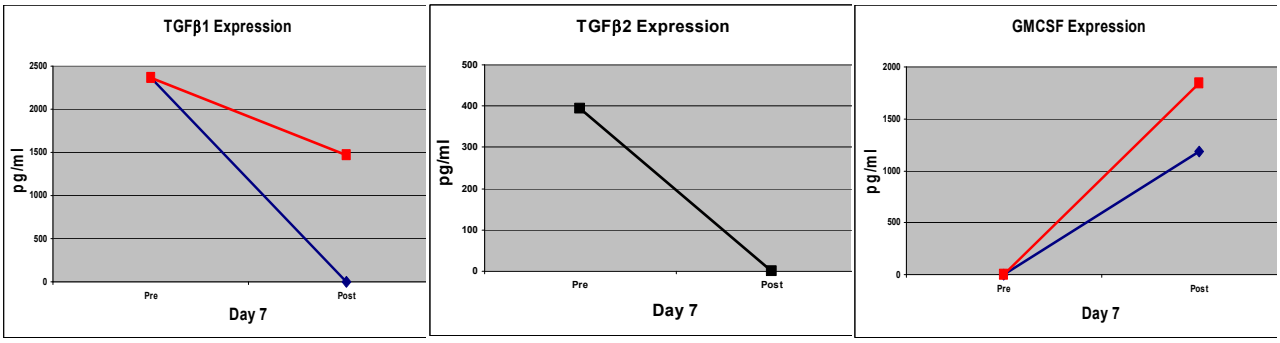


Figure 8: Manufactured consecutively from the same patient tumor. Values represent ELISA determinations of cytokine production at Day 7 post transfection in harvested autologous cancer cells transfected with either Vigil™ (blue) or TAG plasmid (red). The TGFβ2 post transfection values are the same for TAG (ACTV-038) and Vigil™ (FANG-004), hence the black line.

1.10 Phase I Results

The Vigil™ vaccine is comprised of autologous tumor cells as a source of TAA's (tumor associated antigens) and two genetic modifications in order to optimize a "triad" functionality--patient tumor-specific antigen presentation, dendritic cell activation (GMCSF), and tolerance escape (blocking TGFβ1, β2 activation) (Maples PB 2009; Nemunaitis 2011). To construct Vigil™, autologous cancer cells are transfected with a multiple component expression vector encoding GMCSF for recruitment and differentiation of antigen presenting dendritic cells (DCs) and a downstream bi-functional small hairpin RNA for specific knockdown of furin, a proprotein convertase critical for maturational proteolytic processing of immune relevant TGFβ isoforms.

We recently completed a Phase I trial with Vigil™ vaccine in which 56 cancer patients underwent successful vaccine construction and 33 patients (1 of them being a pediatric patient 15 years of age) received ≥1 vaccination. No significant toxic effect was observed. A marked elevation of interferon gamma producing T cells was observed in a subset of patients which correlated with survival.

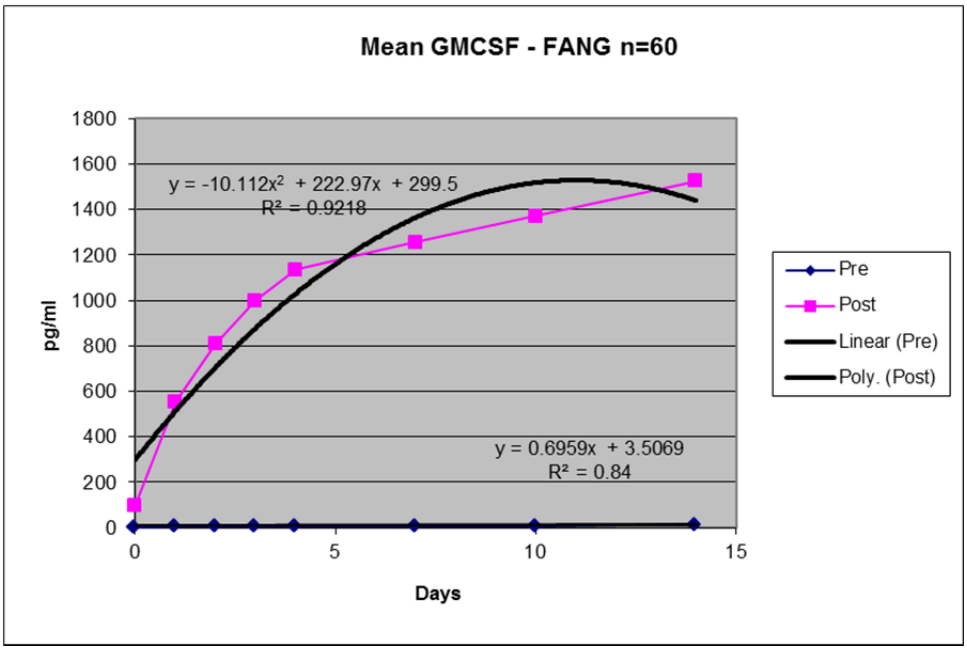
The capacity of Vigil™ to deplete TGFβ1 and TGFβ2 isoforms is hypothesized to 1) effect silencing of a well documented, primarily endogenous family of immunosuppressive cytokines and 2) specifically inhibit TGFβ-suppression of GMCSF induced maturation of DCs, expression of MHC Class II and co-stimulatory molecules.

Product transgene expression of GMCSF and downregulation of expression of TGFβ1, -β2, and Furin are shown in Figure 9. Mean post-transfection GMCSF expression value was 1135 pg/10^6 cells/ml (Day 4 of QC assay), the GMCSF release specification of ≥30pg GMCSF/10^6 cells/ml was met for each vaccine manufactured). Mean post-transfection TGFβ1 and β2 knockdown were 93% and 95%, respectively (Day 4 of QC assay). Furin knockdown was 78%.

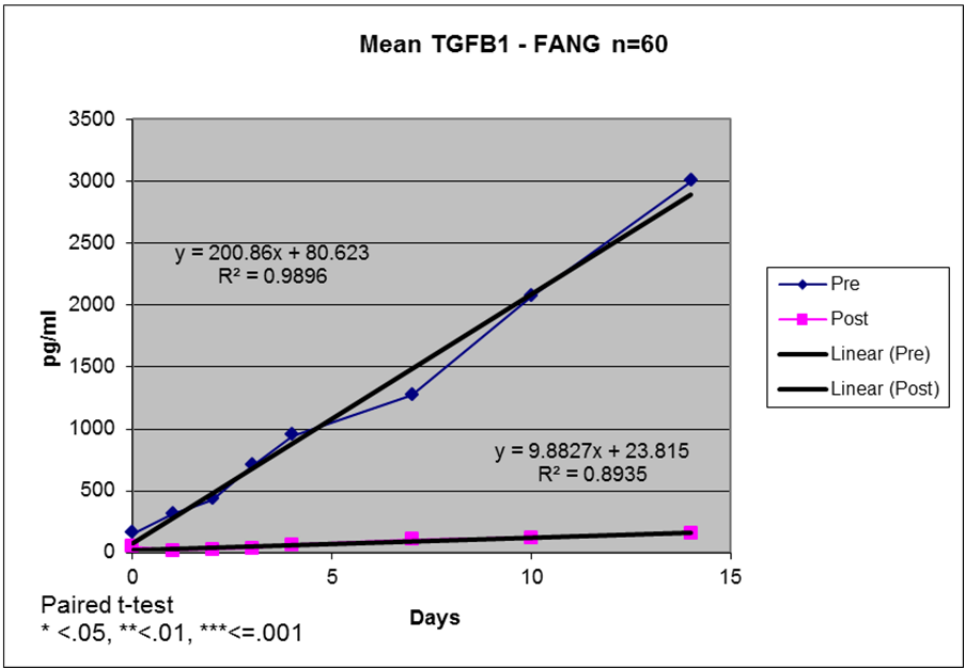
There was no difference in the rate of adverse events across the 2 dose cohorts. Two possibly related Grade 3 treatment-related events were observed: abdominal pain and neutropenia. There were no treatment-related serious adverse events. The most common Grade 1, 2 adverse event occurring at ≥5% frequency related to study medication was injection site erythema. No relationship between dose and adverse event frequency or severity was observed.

Of the 33 patients who have received ≥1 vaccine dose, 31 were evaluable for tumor response (1 adult and 1 pediatric patient progressed following first injection). Mean and median survival of all Vigil™ treated adult patients (n=32) from time of procurement was 584 days and 562 days, respectively. Their mean and median survival from time of treatment was 477 days and 490 days, respectively. Factors such as age, sex, dose level, pre-treatment expression levels of TGFβ1 and β2 and Furin and vaccine transgene expression or knockdown did not correlate with survival. However, breakdown of survival comparing Vigil™ treated adult patients receiving ≥4 vaccines (n=24) vs. <4 vaccines (n=8) was significantly different ($p<0.001$) as shown in Figure 10. Although not randomized, comparison to patients fulfilling the same inclusion criteria who did not receive Vigil™ formerly known as FANG™ from time of procurement (no FANG™) is shown in Figure 11. Difference in survival (median 122 days for no FANG™, n=29 and 562 days for FANG™, n=32) achieved statistical significance ($p<0.00001$). Conservative assessment of only patients who survived 4 or more months since procurement revealed a median survival of 251 days for the no FANG™ patients (n=11) and 572 days for FANG™ patients (n=30) ($p=0.005$). Interestingly, all four patients with advanced metastatic melanoma achieved ≥1 year of survival following treatment with Vigil™ (specifically, 967, 835, 560, and 490 days each).

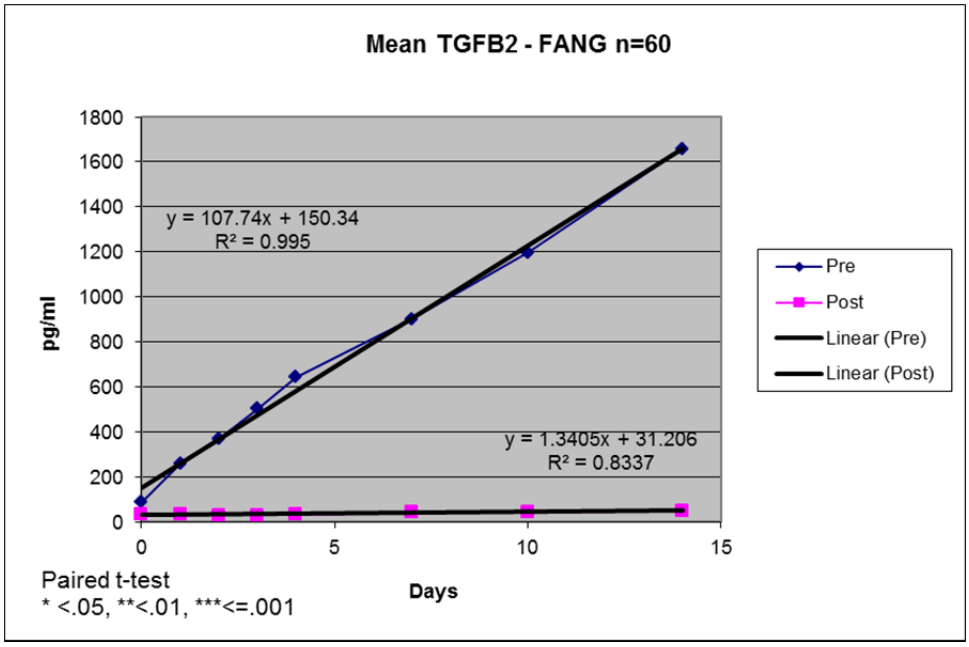
a


Mean GMCSF - FANG n=60
y = -10.112x² + 222.97x + 299.5
R² = 0.9218
y = 0.6959x + 3.5069
R² = 0.84
pg/ml
Days
Pre
Post
Linear (Pre)
Poly. (Post)

b


Mean TGFB1 - FANG n=60
y = 200.86x + 80.623
R² = 0.9896
y = 9.8827x + 23.815
R² = 0.8935
pg/ml
Days
Pre
Post
Linear (Pre)
Linear (Post)
Paired t-test
* <.05, **<.01, ***<=.001

c



d

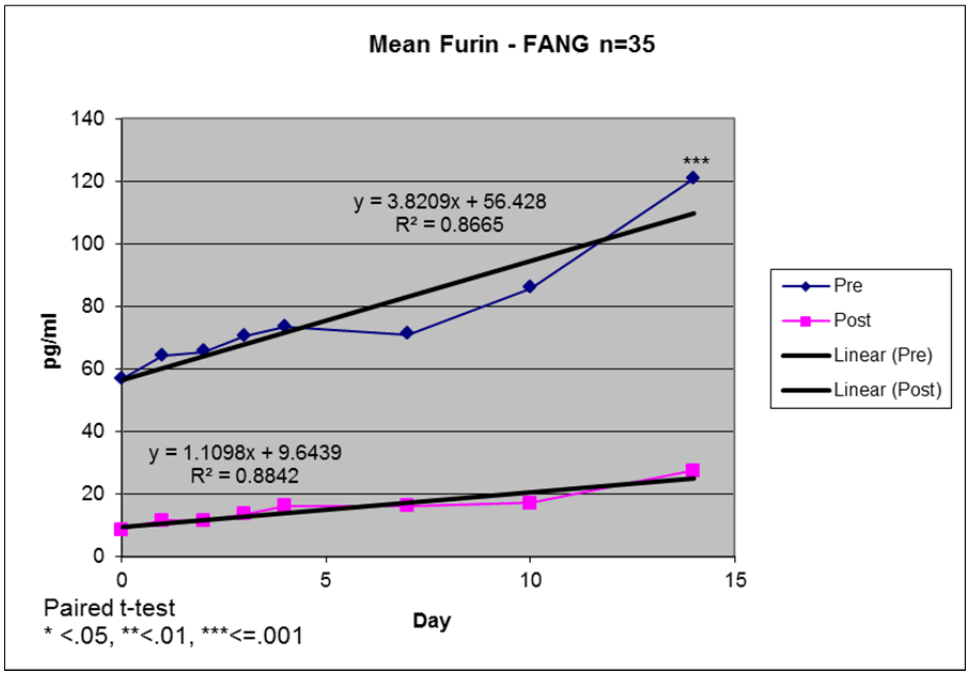


Figure 9. Protein data results for GMCSF gene expression (a), TGFβ1 knockdown (b), TGFβ2 knockdown (c), and Furin knockdown (d) from 56 successful vaccines and 4 vaccines not meeting the minimum dose requirement but for which protein data were collected. Failed vaccines (n=5) did not have protein data collected.

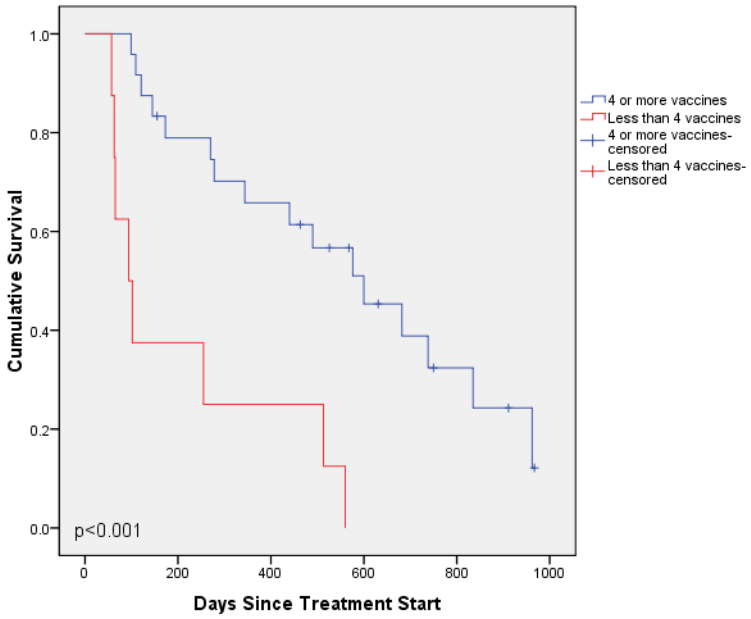


Figure 10. Survival comparison of treated adult patients who received <4 FANG™ vaccinations (red) vs. ≥4 vaccinations (blue), n=32, p<0.001. (Data as of 01/08/13)

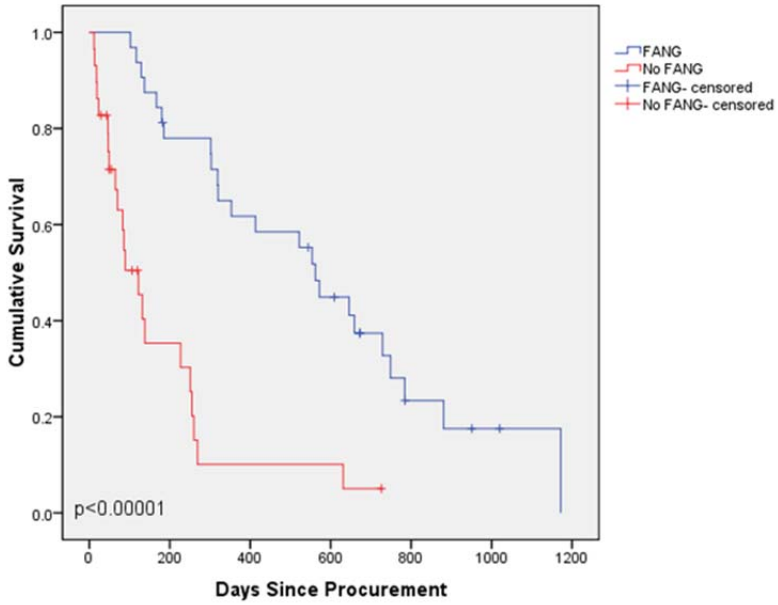


Figure 11. Survival comparison of adult patients receiving FANG™ (blue) to those not receiving FANG™ (red), n=61, p<0.00001. (Data as of 01/08/13)

Sequential ELISPOT analysis at baseline and prior to the 4th Vigil™ vaccine was performed in 24 patients. Results are shown in Figure 12. Twenty-two had no

detectable autologous tumor specific cytotoxic CD8+ T-cell activity at baseline. Two patients showed ELISPOT reaction at baseline (≥10 spots), but while one showed reactivity ≥2 times that of baseline at the Month 3 assessment, the other did not have a ≥2 times increase in ELISPOT spots, and thus was considered a negative responder. Twelve patients had an increase in immune response from a baseline mean of 6 IFNγ producing T-cell spots to an end of Cycle 3 mean of 106 IFNγ producing T-cell spots (p = 0.011). The other 12 patient showed neither reactivity nor enhancement of immune response from a baseline mean of 3 spots through Cycle 3. These 2 populations were statistically different from each other at the end of Cycle 3 in ELISPOT response (106 spots vs. 3 spot, p=0.007). Comparison of survival between the 12 patients demonstrating positive ELISPOT response and those 12 not demonstrating response at completion of Cycle 3 demonstrated a statistically significant increase in survival from time of procurement (p=0.040) and time of treatment start (p=0.023, Figure 13a, b) in the former group. Additionally, patients who received ≥3 doses of Vigil™ and had a positive ELISPOT had better survival than patients who did not receive Vigil™ (p<0.001).

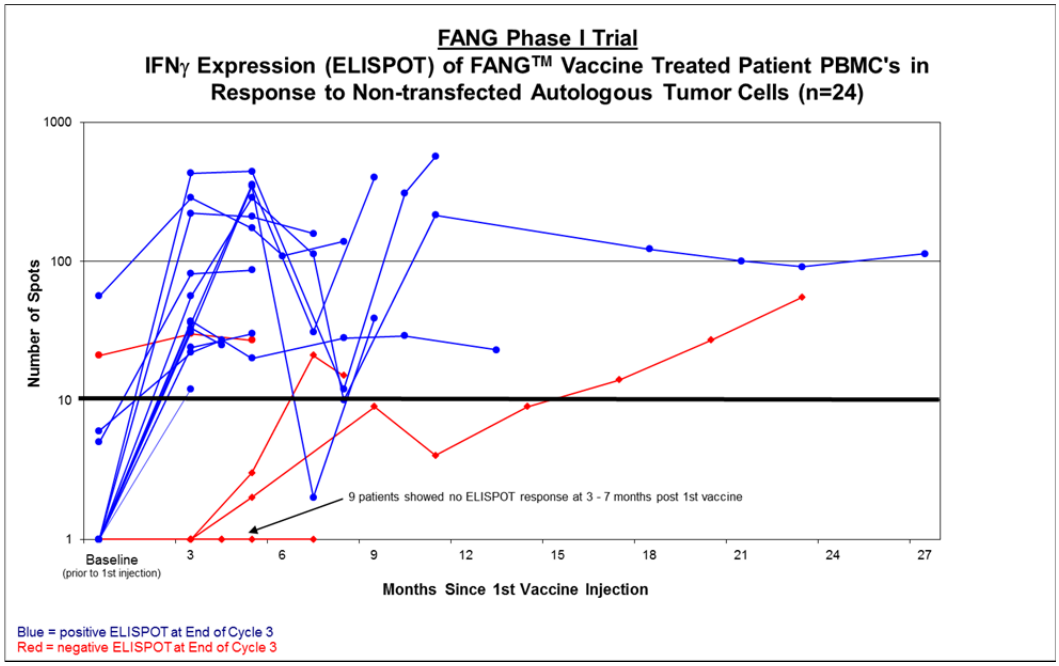
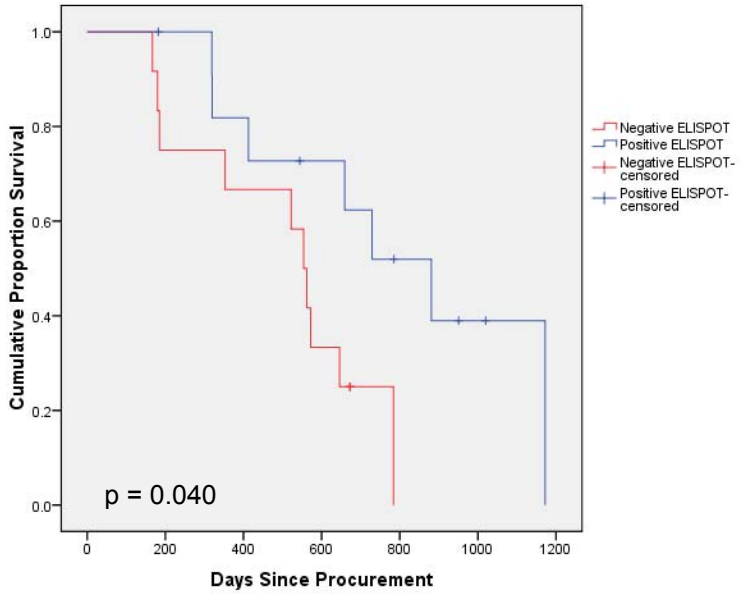


Figure 12. IFN-γ (ELISPOT) in FANG™ vaccine treated patients peripheral blood mononuclear cells in response to non-transfected autologous tumor cells. Blue lines indicate patients achieving ≥10 IFN-γ producing lymphocytes (positive response) after 3 cycles of FANG™ vaccine. Red lines indicate patients not achieving positive ELISPOT response after 3 cycles of the FANG™ vaccine. (Data as of 01/08/13)

All patients demonstrating positive response after 3 complete cycles of Vigil™ vaccine who had long term assessment continued to show positive ELISPOT response during vaccine treatment and after discontinuation of vaccine therapy (Figure 12). Two patients who were in the non-responsive group at the end of Cycle 3 demonstrated a late positive response after 5 cycles of Vigil™ vaccine. One of these patients had advanced metastatic melanoma with survival of 490 days since treatment start, and the other had hepatocellular cancer with a survival of 738 days since treatment start.

a



b

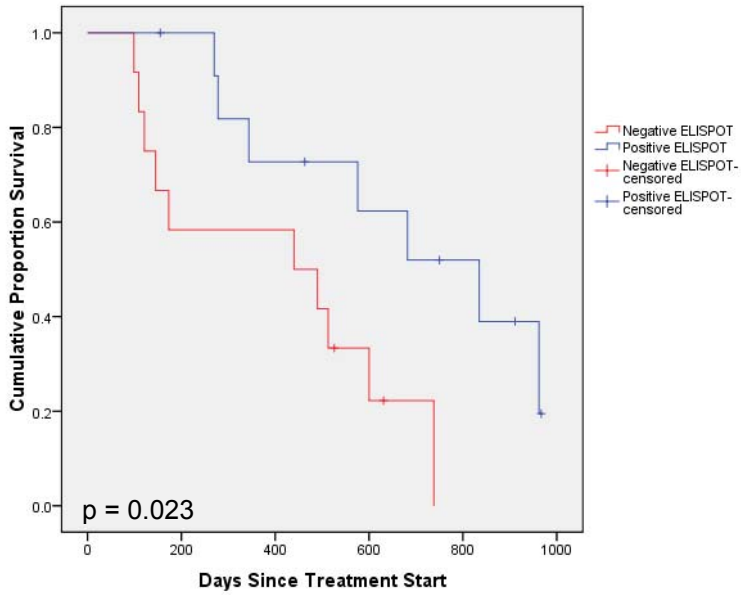


Figure 13: Survival comparison of patients achieving positive ELISPOT response prior to the 4th injection (blue) vs. those not achieving positive ELISPOT response prior to the 4th injection (red) from time of procurement, p=0.40 (a) and time of first vaccination, p=0.023 (b) (n=24).

This Phase I study was designed to assess the safety of a RNAi mediated, GMCSF expressing autologous tumor cell vaccine, Vigil™, and to evaluate the

triad immunotherapeutic concept (Nemunaitis 2011) of concurrent autologous tumor cell antigen provision, immunostimulation and inhibition of autologous whole cell component endogenous immunosuppression.

Enhanced effectiveness of the bi-shRNA vis-à-vis downregulation of expression as compared with siRNA and shRNA has been previously demonstrated (Liu, Rao et al. ; Rao, Maples et al. 2010). In the context of comparative Vigil™ plasmid functionality with the prior generation TAG vaccine, the median GMCSF expression was similar but TGFβ2 knockdown was more effective, i.e., 92.5% as compared to slightly more than 54% knockdown with TAG. However, most significantly, TGFβ1 knockdown, which is not affected in TAG, was 93% with Vigil™. These results validate the rationale and confirm the effectiveness of inhibition of expression of immunosuppressive TGFβ isoforms via a bi-shRNA mediated knockdown of the proprotein convertase Furin as well as the feasibility of an integrated GMCSF + RNAi moiety. The high degree of TGFβ1 and β2 knockdown herein achieved combined with the clinical and immunological data presented justify advancement of the current manufacturing process. A 3+ year follow up of 74 Phase I patients with successful vaccine manufacture, of whom 35 received Vigil™, confirmed tolerability and safety with no evidence of long-term toxicity. In addition, survival continues to correlate with ELISPOT response (median 836 days vs. 440 days with positive and negative ELISPOT respectively) (Senzer N 2013)

Results support the hypothesis that the Vigil™ vaccine is safe and, in addition, provide a Phase I database justifying continued clinical evaluation and expansion of immune assessment assays.

2.0 STUDY RATIONALE

This study adopts an alternative approach to immune therapy assessment by eschewing the treatment of late stage disease and evaluating adjuvant-based disease prevention in patients with minimal disease but at high risk for recurrence. According to the serial immune function analyses by Coleman (Coleman, Clayton et al. 2005), five of nine patients with Stages III-IV ovarian adenocarcinoma who achieved post-chemotherapy remission retained the capacity of $CD8^+$ T-cell responses to a panel of 11 viral peptides restricted by at least six common HLA class I alleles. Chemotherapy produced no apparent effect on naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), or effector memory ($CD45^-CCR7^-$) T-cells. These findings affirm the strategy of vaccine deployment following chemotherapy, and justify selection of Stage III/IV epithelial ovarian cancer patients who have achieved complete clinical or pathological remission following cytoreductive surgery and front-line chemotherapy for this Vigil™ vaccine study. Exploratory analysis of two Phase III studies (D9901 and D9902) with the recently FDA approved Provenge® has identified prolonged

survival benefits in prostate cancer patients initially treated with Provenge® and subsequently received docetaxel (median survival of 34.5 months, compared to 25.4 months in patients treated with placebo followed by docetaxel) (Petrylak 2006). Thus the potential for vaccine mediated immune modulation both posterior and anterior to chemotherapy is a clinically attractive strategy worthy of exploration.

With regard to the appropriateness of a placebo arm in this group of patients, to date, in conjunction with platinum/taxol base regimens, maintenance therapies have shown neither a DFS endpoint advantage (Sabbatini, Harter et al. 2013) nor an OS (overall survival) endpoint advantage low dose taxol (Markman, Liu et al. 2009), Abagovomab (Sabbatini, Harter et al. 2013), and bevacizumab (Burger, Brady et al. 2011; Perren, Swart et al. 2011) while giving rise to a spectrum of extended treatment related side effects and questions of results-related economic justification (Lesnock, Farris et al. 2011). In the two recent randomized Phase III studies of bevacizumab, GOG-0218 (15 mg/kg x 16 cycles) and ICON7 (7.5 mg/kg x 12 cycles), in which there was a RFS endpoint advantage, the bevacizumab and control curves converged after discontinuation of bevacizumab prior to progression at 24 months and ~22 months, respectively, implying that continuation of bevacizumab until progression be considered, consistent with the results of the OCEANS trial in women with platinum-sensitive recurrent disease (for a significant RFS endpoint albeit there was still no significant difference in OS at first interim analysis) (Aghajanian, Blank et al. 2012). This must be weighed against the added exposure to bevacizumab associated adverse effects. As subsequently pointed out, the 3.8 month gain in PFS came at the patient cost of “23% risk for developing Grade 2 hypertension, 10% risk for Grades 3 to 4 hypertension, and 2.3% risk for Grade 3 or worse GI perforation, hemorrhage, or fistula formation”(Hensley 2011). In addition, based on Quality Adjusted Life Year (QALY), the cost of chemotherapy plus just 16 cycles of bevacizumab is $326,530/QALY versus $13,402/QALY for the same chemotherapy and maintenance low-dose paclitaxel (Lesnock, Farris et al. 2011). Likewise in a cost-effectiveness analysis of GOG-0218, the cost effectiveness ratio (i.e. the cost per year of PFS) was $247,616, $1,800,000 and $5,500,000 for the paclitaxel (P) and carboplatin (C); P+C and bevacizumab (B); and P+C+B and maintenance B, respectively (Cohn, Kim et al. 2011). In the absence of a confirmed OS advantage, at least for front-line therapy for ovarian cancer, the additional cost is hard to justify (Hensley 2011; Tomao, Tomao et al. 2013); particularly so without FDA approval. Similarly Grade ≥2 toxic effects including peripheral neuropathy (15.5), infection/fever (19.9%), and dermatologic events (70.8%), are observed with maintenance therapy involving taxol (Mannel, Brady et al.). Despite a recurrence-free-interval delay in the SWOGS9761/GOG 178 study of 12 vs. 3 cycles of maintenance Taxol (22 vs. 14 months), long-term follow up revealed no survival advantage (53 vs. 48 months; p=0.34) (Markman, Liu et al. 2009). In addition, a Phase III study of maintenance Taxol x 6 cycles vs. observation revealed neither a progression-free survival nor overall survival advantage (Conte 2007). The attendant significant morbidity and lack of survival

benefit qualified the National Comprehensive Cancer Network (NCCN) commentary on use of Taxol maintenance as 2B with the further proviso recommending use in a clinical trial.

3.0 OBJECTIVES

3.1 Primary objective(s)

- To assess time to recurrence (TTR) following the administration of bi-shRNA^{furin} and GMCSF autologous tumor cell (Vigil™) vaccine.

3.2 Secondary objective(s)

- To identify and determine the effect of Vigil™ autologous tumor cell vaccine on immune surrogate markers in this group of patients.
- To assess the predictive potential of initial tumor infiltrating lymphocyte (TIL) and tumor associated macrophage (TAM) phenotypes.
- To enlarge the safety database of Vigil™ autologous tumor cell vaccine in patients with miminal disease.

4.0 STUDY DESIGN

4.1 Design

This is a Phase II open-label trial of maintenance Vigil™ autologous tumor cell vaccine. Tumor will be harvested at the time of surgical debulking (standard of medical care). Subsequently, patients achieving clinical CR following primary surgical debulking and doublet chemotherapy will be stratified for i) surgical stage (Stage IV or suboptimal debulking (>1 cm residual) Stage III disease versus Stage III patients with optimal debulking (<1 cm residual)) and ii) post-op chemotherapy, pre-vaccine CA-125 >10 ≤ 20 U/mL versus 0≤10 U/ml, then randomized 2:1 (Note: patients with Stage IIIc ovarian cancer will be additionally evaluated as a subset using descriptive statistics only). Patients will receive 1.0 x 10^7 cells / intradermal injection of gene transfected autologous tumor cells, Vigil™, once a month for up to 12 doses as long as sufficient material is available. Enough harvested tissue to provide a minimum of 4 monthly injections will be required for entry into the study. These patients will be managed in an outpatient setting. Hematologic function, liver enzymes, renal function and electrolytes will be monitored monthly. Immune function analysis including

ELISPOT analysis of cytotoxic T cell function to autologous tumor antigens will be monitored at (≤24 hours before) tissue harvest, ≤24 hours before the first cycle of chemotherapy (post debulking), ≤24 hours before the third cycle of chemotherapy (post debulking), baseline (screening), prior to Vigil™ injection Months 2, 4, 6 and at EOT. The dates of the last dose of chemotherapy and the administration of Vigil™ vaccine #1 will be recorded.

Treatment will be continued until disease recurrence or exhaustion of the patient's vaccine supply. If ≥ Grade 2 toxicity by NCI Common Toxicity Criteria (excluding Grade 2 fever ≤ 24 hours and Grade 2 and 3 injection site reactions) develops related to study treatment the vaccine dose will be reduced by 50% and continued on a monthly basis. If a single patient develops ≥ Grade 3 toxicity (other than injection site reaction) related to study treatment the trial will be placed on hold for reevaluation of design in discussion with FDA. During this hold, no new subjects will initiate dosing, but subjects already being dosed may continue dosing as scheduled if deemed clinically appropriate by the PI.

5.0 STUDY POPULATION

5.1 Sample Size

Approximately fifty (50) treatment naïve patients with high risk Stage III/IV ovarian cancer who achieve clinical CR following surgical debulking and chemotherapy will be randomized in this trial.

5.2 Inclusion Criteria

Patients will be eligible for tissue procurement for the Vigil™ vaccine manufacturing process if they meet all of the following critieria:

1. Presumptive Stage III/IV papillary serous or endometrioid ovarian cancer.
2. Per Amendment #8, treatment naïve, high risk ovarian cancer will no longer be stratified, but the following information will be collected:
   a. Stage IV or suboptimal (>1 cm residual) Stage III disease versus Stage III patients with optimal (≤1 cm residual) disease,
   b. CA-125 ≤10 U/ml versus CA-125 greater than 10 but less than or equal to 20 U/ml
   c. IP chemotherapy versus IV chemotherapy
3. Availability of "golf-ball" size 10-30 grams tissue at time of primary surgical debulking.

4. ECOG performance status (PS) 0-2 prior to tumor debulking laparotomy.
5. Ability to understand and the willingness to sign a written informed consent document for tissue harvest.

Patients will be registered for inclusion in this study if they meet all of the following criteria:

1. Histologically confirmed Stage III/IV papillary serous or endometrioid ovarian cancer.
2. Clinically defined CR (no cancer related symptoms, normal physical examination and CT scan abdomen/pelvis and CXR, and CA-125 ≤20 U/ml) following completion of primary surgical debulking. Patients enrolled must complete at least 5 but no more than 6 cycles platinum/taxane adjuvant or interval debulking + chemotherapy (or chemotherapy as per recommendations of NCCN guidelines, category 1 (IP chemotherapy included)). (Patients who complete surgery/chemotherapy with a CA-125 >20 U/mL pre-registration have the option of being followed up to 2 months if serial CA-125 values continue to decrease at a rate of CA-125 decrease of ≥50% per month.)
3. Successful manufacturing of 4 vials of Vigil™ vaccine.
4. Recovered from all clinically relevant toxicities related to prior protocol specific therapies (including neuropathy to ≤Grade 2).
5. ECOG performance status (PS) 0-1.
6. Normal organ and marrow function as defined below:

Absolute granulocyte count	≥ 1,500/mm^3
Absolute lymphocyte count	≥ 200/mm^3
Platelets	≥ 75,000/mm^3
Total bilirubin	≤ 2 mg/dL
AST(SGOT)/ALT(SGPT)	≤ 2x institutional upper limit of normal
Creatinine	< 1.5 mg/dL

7. Patients must be off all “statin” drugs for ≥ 2 weeks prior to initiation of therapy.
8. Ability to understand and the willingness to sign a written informed protocol specific consent document.

5.3 Exclusion Criteria

Patients will be excluded from this study if they meet any of the following criteria:

1. Surgery involving general anesthesia, radiotherapy, or immunotherapy within 4 weeks prior to randomization. Chemotherapy within 3 weeks prior to Vigil™ vaccine administration. Steroid therapy within 1 week prior to vaccine administration.
2. Patients must not have received any other investigational agents within 4 weeks prior to Vigil™ vaccine administration.
3. Patients with history of brain metastases.
4. Patients with compromised pulmonary disease.
5. Short term (<30 days) concurrent systemic steroids ≤ 0.25 mg/kg prednisone per day (maximum 7.5 mg/day) and bronchodilators (inhaled steroids) are permitted; patients requiring other steroid regimens and/or immunosuppressives at randomization are excluded.
6. Prior splenectomy.
7. Prior malignancy (excluding nonmelanoma carcinomas of the skin and carcinoma *in situ* cervix) unless in remission for ≥ 2 years.
8. Kaposi's Sarcoma.
9. Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
10. Patients with known HIV.
11. Patients with chronic Hepatitis B and C infection.
12. Patients with uncontrolled autoimmune diseases.

5.4 Withdrawal

Off Study Treatment

Patients will be taken **off study treatment** if any of the following occur:

1. The patient experiences unacceptable (≥Grade 3) toxicity felt to be related to treatment with the tumor cell vaccination that persists for >1 week.
2. Persisting Grade 3 or 4 toxicity unrelated to treatment, defined as failing to normalize within 4 weeks.
3. ≥ Grade 2 allergic reactions related to the study agent.
4. Grade 2 autoimmune reactions unless there is evidence of clinical benefit.
5. ≥ Grade 3 autoimmune reactions.
6. An intercurrent illness, which would in the judgment of the investigator, affects assessments of clinical status to a significant degree or requires discontinuation of study treatment.

7. Non-protocol therapy (including, but not limited to, chemotherapy not listed as Level 1 Category of Evidence per NCCN Guidelines Version 3.2012, radiotherapy, hormonal therapy, immunotherapy, or surgery) is administered during study treatment.
8. Non-compliant with protocol or treatment.
9. Patient refuses to continue treatment.

The date of and reason for discontinuation must be noted in the Case Report Form (CRF). Every effort should be made to complete the appropriate assessments.

Stopping Rules

Patients with disease recurrence will not receive further vaccine treatment. Withdrawal of patients from the study treatment will be in the event of unexpected and unacceptable vaccine-related toxicity, ≥ Grade 2 allergic reactions, Grade 2 autoimmune reactions unless there is evidence of clinical benefit, ≥ Grade 3 autoimmune reactions related to the study agent, or clinically significant disease progression.

6.0 INVESTIGATIONAL PLAN

6.1 Patient Registration and Enrollment

Written documentation of full, non-contingent IRB approval of the protocol and consent document must be on file before a patient can be registered. Patients will be assigned a tissue procurement number upon harvest and a vaccine number / patient number will be assigned upon completion of the manufacturing process.

Once eligibility has been confirmed, the site Research Nurse or Clinical Research Coordinator will email or fax the Registration Form and applicable de-identified records to MCCRO@marycrowley.org or 214-658-1990. If sent by fax, and email should be sent to the above email address to alert the CRO of the incoming fax.

The received documents will be reviewed for completeness and sent to the Medical Monitor for review and randomization (if applicable). If records are not complete or if question arise, the site Research Nurse or Clinical Research Coordinator will be contacted for more information.

Please allow 48 hours for patient registration / randomization. Once confirmed, the completed Registration Form will be returned to the site indicating the assigned patient cohort.

6.2 Tumor Procurement

The investigative sites will ensure patients are scheduled for surgery for tumor procurement. Gradalis, Inc. will manufacture vaccine from the procured tissue, and thus will need to be notified at least 24 hours before the scheduled procurement in order to prepare for vaccine manufacturing (when possible). Additionally, tumors are to be procured on Mondays, Tuesdays, Wednesdays and Thursdays only due to the manufacturing being a 2-day process. Please contact the Sponsor if scheduling adjustments need to be made. Refer to the Tissue Procurement Study Reference Manual for instructions.

The equivalent of a “golf ball size” mass (10-30 gm tissue) and/or ascites fluid (preferably first paracentesis) is necessary for vaccine manufacturing. Radiological scans should confirm the presence of a lesion >3cm. If surgeons have the option of collecting more tissue, more doses of vaccine may be prepared (up to 12 doses). Vaccine manufacturing is rarely successful with small tumor masses. Lesions extending into bowel lumen cannot be processed.

Once the procured tissue is received at Gradalis, Inc. samples will be processed for autologous vaccine manufacture. No tissue or vaccine will be given to the participant or site apart from the outlined clinical protocol.

If available without compromising vaccine manufacture or pathology requirements, an aliquot of tumor will be collected for TIL and TAM immunophenotyping. Any excess tumor tissue, not used for vaccine manufacture will be used towards Vigil™ vaccine research and process development assays.

6.3 Vaccine Manufacturing

Gradalis, Inc. will manufacture vaccine from the procured patient tumors. Gradalis, Inc. will release vaccine once all release criteria have been met and eligibility has been confirmed.

6.4 Study Treatment Administration

Treatment will be administered on an outpatient basis. The site must contact the Mary Crowley CRO to obtain participant randomization assignment.

Recommended Chemotherapy

Patients will receive 6 cycles of chemotherapy as either adjuvant or interval debulking + chemotherapyschedule. Recommended regimens are those classified as Level 1 Category of Evidence per NCCN Guidelines Version 3.2012. Regimens other than these should be discussed with the sponsor. Patients will not receive maintenance therapy other than that specified by protocol.

Vaccination Schedule and Dose

Patients will be vaccinated according to the schedule outlined in Appendix B. Patients will receive Vigil™ vaccine at 1 x 10^7 cells via intradermal injection for a minimum of 4 doses and a maximum of 12 doses starting ≥3 weeks following completion of chemotherapy (no longer than 2.5 months post chemotherapy).

Patients with viable cells in sufficient numbers (i.e. at least 4 doses of 1.0 x 10^7 cells / injection) will receive monthly intradermal injections of the tumor cell vaccine for up to 12 months as long as sufficient material is available. Patients whose vaccine manufacturing fails to achieve a minimum of 4 doses will not undergo treatment.

Administration of Vigil™ vaccine

The sites of injection will be rotated between the right and left upper arms. If the ipsilateral axillary lymph nodes were radiated or surgically removed during prior therapy, alternative sites (e.g., anterior thigh) will be used. The patient will be observed for at least 30 minutes (with a 10% window) following vaccination. During this observation period, vital signs will be taken every 10 minutes (with a 10% window). If clinically stable, vaccine administration may continue for up to 12 doses given on a monthly basis as long as sufficient material is available.

Vigil™ Vaccine Transfer

All manufactured vaccine will be stored in the vapor phase of liquid nitrogen until ready for use. The site will contact Gradalis, Inc. when the study agent is needed for patient vaccine administration.

Gradalis will complete a Drug Transfer and Administration Form to release the patient vaccine. The clinic will sign off on the form upon receipt of the vaccination.

Please reference the Pharmacy Reference Manual for vaccine preparation and handling information.

Treatment Delay

1. Treatment may be delayed no more than 4 weeks to allow recovery from toxicity.
2. Patients who delay treatment for more than 4 weeks due to toxicities will be considered off study treatment (see Section 4.4 Off Study Treatment).
3. Treatment delay not related to toxic events (including subjects unable to adhere to monthly injection) will not be delayed for more than three days. Unless a delay due to symptoms related to disease or infection in which case up to a 2 week delay is allowed.
4. If ≥ one 2 week delay due to disease or infection occurs, patient status must be reviewed by sponsor.
5. If patients miss doses due to toxicities, the doses will be made up the following week and continue on a revised monthly schedule thereafter.

Dose Modification for Toxicity

If ≥ Grade 2 toxicity by NCI Common Toxicity Criteria (excluding Grades 2 and 3 injection site reactions) develops related to study treatment the vaccine dose will be reduced by 50% and continued on a monthly basis. If a single patient develops ≥ Grade 3 toxicity (other than site reaction) related to study treatment the trial will be placed on hold for re-evaluation of design in discussion with FDA.

6.5 Toxicity

Toxicities will be graded and reported according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE) Version 3.0 as linked in Appendix C. This document can also be downloaded from the Cancer Therapy Evaluation Program (CTEP) home page <http://ctep.info.nih.gov>.

Should a report of an infection (any grade) arise, please identify the bacteria or fungus related and report the event via Serious Adverse Event form. In particular, if any organisms are identified as anaerobic, infectious disease should be consulted for management using standard of care relevant to anaerobic bacterium.

6.6 Schedule of Assessments

The schedule of assessments for the trial is shown in Appendix B. If a required observation or procedure is missed, documentation is required on the Protocol Deviation Form, to explain the reason for this protocol deviation.

Prestudy Assessments

The following evaluations will be performed within 4 weeks of the tumor debulking laparotomy (unless otherwise specified):

1. A signed Patient Informed Consent Form for tissue harvest must be obtained.
2. It has been confirmed that the patient meets all tissue procurement inclusion criteria and none of the exclusion criteria.
3. A complete medical history must be obtained.
4. A physical examination (including vital signs, height, and body weight) must be obtained.
5. Assessment of PS on the ECOG scale (see Appendix A) must be obtained.
6. Radiological assessment of tumors (i.e., chest X-ray, (chest CT or MRI only if indicated), pelvic/abdominal CT or MRI) used to stage the extent of disease must be performed within 6 weeks. The methods used for prestudy assessments (e.g., CT or MRI) should be used throughout the study. If possible, the same equipment should be used each time.

7. A complete blood count (CBC) with differential and platelet count must be performed. (HIV testing is not required if the subject has no medical history of HIV).
8. CA-125 tumor assessment (at initial diagnosis per medical history).
9. Routine pre-operative serum chemistries (including but not limited to creatinine, total bilirubin, alkaline phosphatase, and aspartate transaminase (AST) and/or alanine transaminase (ALT).
10. Immune function analysis consisting of ELISPOT analysis of cytotoxic T cell reaction to autologous tumor antigens will be obtained at tissue harvest. (within 24 hours before tissue procurement or on the day of harvest)

Following tissue procurement, patients will be contacted every 6 weeks ±2 weeks to assess interest / eligibility of the main randomized portion of the trial and survival status. Blood for immune function analysis will be collected prior to the first and third cycles of (≤24 hours before) chemotherapy post debulking. Medical records should be provided to support continued eligibility.

The following evaluations will be performed on all subjects within 2 weeks of registration / randomization (unless otherwise specified):

1. A signed protocol specific Patient Informed Consent Form must be obtained.
2. It has been confirmed that the patient meets all inclusion criteria and none of the exclusion criteria.
3. An interval medical history must be obtained within 4 weeks.
4. A physical examination (including vital signs, oxygen saturation, height, temperature and body weight) must be obtained.
5. Assessment of concomitant medications
6. Assessment of PS on the ECOG scale (see Appendix A) must be obtained.
7. A tumor clinical assessment of the patient's disease (i.e., by physical examination) must be performed within 4 weeks.
8. Radiological assessment of disease status (i.e., chest X-ray, (chest CT or MRI only if indicated), pelvic/abdominal CT or MRI) used to stage the extent of disease must be performed within 4 weeks. The methods used for prestudy assessments (e.g., CT or MRI) should be used throughout the study. If possible, the same equipment should be used each time.
9. A complete blood count (CBC) with differential and platelet count must be performed. (HIV testing is not required if the subject has no medical history of HIV).
10. CA-125 tumor assessment within 4 weeks following completion of surgery chemotherapy. If the CA-125 is >20 U/mL, the patient can continue with serial CA-125 evaluations up to 2 months if the levels

continue to decrease and there is no objective evidence of disease recurrence.
11. Serum chemistries (creatinine, glucose, total protein, blood urea nitrogen (BUN), total carbon dioxide (CO_2), albumin, total bilirubin, alkaline phosphatase, and aspartate transaminase (AST) and/or alanine transaminase (ALT)) and electrolytes (total calcium, chloride, potassium, sodium) must be performed.
12. Immune function analysis consisting of ELISPOT analysis of cytotoxic T cell reaction to autologous tumor antigens.
13. Urinalysis must be performed.
14. EKG must be performed.
15. FACT-O, Version 4 assessment

Assessments During Treatment

The following evaluations will be performed monthly (every 28 days ±3 days) during the therapy (unless otherwise specified):

1. A physical examination, including vital signs and body weight.
2. A toxicity assessment (adverse events).
3. Assessment of concomitant medications taken.
4. Tumor response by clinical assessment of the patient's disease (i.e., by physical examination).
5. Radiological assessment of disease status (i.e., chest X-ray, (chest CT or MRI only if indicated), pelvic/abdominal CT or MRI) must be performed ≤ 1 week prior to Cycle 4) and then at standard of care intervals. The methods used for prestudy assessments (e.g., CT or MRI) should be used throughout the study. If possible, the same equipment should be used each time.
6. A CBC with differential and platelet count.
7. CA-125 tumor assessment until recurrence. (monthly for the first year, then every 3 months ± 2 weeks for the second and third year.)
8. Serum chemistry and electrolytes.
9. Immune function analysis consisting of effector T cell response (ELISPOT assay will be monitored - Months 2, 4, 6 (prior to Vigil™ administration).
10. Assessment of PS on the ECOG scale (see Appendix A).
11. Vaccine administration. The patient will be observed for at least 30 minutes (with a 10% window) following vaccination. During this observation period, vital signs will be taken every 10 minutes (with a 10% window).
12. Day 2 assessment of injection site.
13. FACT-O, Version 4 assessment at baseline, Month 3, Month 6, Month 12 and Month 18 (or until recurrence).

Radiological assessment of disease status (i.e., chest X-ray, (chest CT or MRI only if indicated), pelvic/abdominal CT or MRI) must be collected if CA-125 >35 U/mL after two consecutive measurements taken one month apart.

Off Treatment Assessments

The following evaluations will be performed within 30 days following the last Vigil™ administration and within 30 days of disease recurrence (unless otherwise specified):

1. A physical examination, including vital signs and body weight.
2. Toxicity assessment (adverse events) (Vigil™ administered patients only).
3. Assessment of concomitant medications taken.
4. Assessment of PS on the ECOG scale (see Appendix A).
5. A tumor clinical assessment of the patient's disease (i.e., by physical examination).
6. Radiological assessment of disease status (i.e., chest X-ray, (chest CT or MRI only if indicated), pelvic/abdominal CT or MRI) used to stage the extent of disease. (within 45 days of the last injection or disease recurrence)
7. A CBC with differential and platelet count.
8. CA-125 tumor assessment.
9. Serum chemistry and electrolytes.
10. Serum for immune function analysis consisting of ELISPOT analysis of cytotoxic T cell function to autologous tumor antigens.

Follow Up Assessments

If the Vigil™ vaccine is discontinued (for reasons such as completion of all available doses of vaccine, intolerable toxicity, treatment interruption of more than 4 weeks, intercurrent illness, protocol deviation at PI's discretion), the patient will be contacted by phone every 3 months after the end of study visit for:

- disease status
- request of medical records, when applicable (laboratory findings, radiological scans, and progress report)
- documentation of 2nd line therapy
- recording of response and duration of response to that therapy
- survival
- assessment of any additional cancer treatments)

Note: Patients who die or withdraw consent are considered **off study** and no further information except survival data will be collected.

Based on findings during the study or during the follow up portion of the trial, Gradalis may request for additional blood and / or tissue samples from the research participant. Collection of whole blood (40ml) and / or tissue samples (via biopsy or clinically indicated surgical removal) will be **optional** and used to study the effects of the study agent (included, but not limited to testing of biomarkers, predictors or biological responses, toxicity, relationship between genotype and study agent responses).

Should Gradalis request for additional blood or tissue, the clinical site will present the option of the procurement to the participant and obtain written informed consent.

7.0 CONDUCT OF THE STUDY

7.1 Ethics and Regulatory Considerations

This study must have the approval of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC), and the Institutional Biosafety Committee (IBC). Before the investigational drug is shipped to the investigator, the investigator will provide Gradalis, Inc. with a copy of the IRB or IEC and IBC approval letter stating that the study protocol and informed consent form have been reviewed and approved.

7.2 IRB and IBC

This trial can be undertaken only after review and full approval of the protocol and a Patient Informed Consent Form has been obtained from a properly constituted IRB. This written approval must be dated and it must clearly identify the protocol, any amendments, the Patient Informed Consent Form, and any applicable recruiting materials and subject compensation programs approved.

Since this trial will also be reviewed by a properly constituted IBC, this trial will not be undertaken until the protocol has been reviewed and received full approval from a properly constituted IBC. This written approval must be dated and it must clearly identify the protocol and any amendments.

The decision concerning the conduct of the study will be made in writing to the sponsor. Copies of this decision and of all IRB and IBC correspondence will be kept on file at the study site; copies will be provided to the Mary Crowley Research Office.

During the trial, the PI is required to send various documents to the IRB and IBC for review:

1. All protocol amendments and Patient Informed Consent Form revisions.
2. Reports of all Serious Adverse Events.

The PI provides Gradalis, Inc. with the necessary assurance that an IRB and IBC is responsible for the initial and continuing review and approval of the proposed clinical study in accordance with 21 CFR 312.60. At least once a year, the IRB and IBC will be asked to review and re-approve the clinical trial protocol; the request must be documented in writing. At the end of the trial, the PI will notify the IRB and IBC that the trial has been completed.

7.3 Written Informed Consent

The informed consent document should meet the requirements of the latest version of the Declaration of Helsinki and any applicable regulations and guidelines. It must be approved by an IRB or IEC.

Prior to entry into the trial and before any protocol-required procedures are performed, the Investigator must explain the nature of the trial, its intended purpose, and the implications of participation to potential patients or to their legal representatives. They will be told about the possible risks and benefits, and the possible adverse experiences. They will be informed that patients' participation is voluntary, and that they may withdraw consent to participate at any time. They will also be informed that if patients choose not to participate in the trial alternative treatments are available; such refusal will not prejudice further treatment of their disease. Potential patients or their legal representatives must be given the opportunity to ask questions about the trial protocol and the procedures involved.

Finally, each patient will be told that his or her records may be accessed by authorized personnel of Mary Crowley, Gradalis, Inc. and other authorized individuals without violating the patient's confidentiality, to the extent permitted by the applicable laws and/or regulations. By signing the written Patient Informed Consent Form, the patient or his or her legal representative is authorizing such access. Following this explanation and prior to entry into the trial, the written,

dated, and signed Patient Informed Consent Form must be obtained from each patient or his or her legal representative; a copy will be given to the person signing the form.

7.4 Confidentiality of Records

The Investigator is required to retain, in a confidential manner, sufficient information on each patient (i.e., full name, current address, and social security number) so that the patient may be contacted by the FDA, Gradalis, Inc., or by Mary Crowley should the need arise.

7.5 Modification of Protocol

Any changes to this protocol that affect study objectives, study design, study procedures, patient population, or significant administrative procedures will require a formal amendment to the protocol. Any proposed protocol amendments must be sent in writing to the applicable IRB. Prior to implementation, an amendment must be approved by the Gradalis, Inc., and approved by the applicable IRB or IEC and IBC.

General administrative changes to the protocol are minor corrections and/or clarifications that do not affect the manner in which the study is to be conducted. Such administrative changes will be agreed upon by the Gradalis, Inc., and will be documented in a memorandum. The applicable IRB or IEC and IBC will be notified of administrative changes according to applicable IRB guidelines.

7.6 Protocol Questions and Deviations

When evaluating a potential patient or while a patient is on study, protocol questions can be sent to MCCRO@marycrowley.org. Mary Crowley CRO will work with Gradalis in obtaining an answer / clarification. Please allow 24 hours for a response. For urgent questions affecting patient safety, please contact the Gradalis personnel by the phone provided in the Gradalis Contact Information section of the Study Reference Manual.

For Planned Protocol Deviation requests, the site will complete the Eligibility Inquiry/Planned Protocol Deviation Form and email or fax it to MCCRO@marycrowley.org or 214-658-1990. If sent by fax, an email should be sent to the above email address to alert Mary Crowley of the fax. Mary Crowley CRO will work with Gradalis and the Medical Monitor to review the

request. Please allow 48 – 72 hours for review. Once complete, the form will be returned to the site.

8.0 EVALUATION OF TUMORS

8.1 Disease Evaluation

Disease recurrence will be evaluated in this study using the Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST) (unidimensional measurement) of the tumor lesions are used in the RECIST criteria (Eisenhauer, Therasse et al. 2009).

Disease recurrence is defined as the appearance of any measurable or evaluable lesion or as asymptomatic CA-125 levels greater than 35 U/mL at two consecutive measurements, at least one month apart. The time to recurrence is measured from date of first treatment until the first date that recurrence is objectively documented whether local, regional, or distant.

9.0 DRUG INFORMATION

9.1 Investigational Product

The Vigil™ vaccine is made up of irradiated autologous tumor cells which have been electroporated *ex vivo* with the Vigil™ plasmid designed to suppress expression of both the TGFβ1 and TGFβ2 proteins while simultaneously expressing rhGMCSF protein.

Vigil™ Vaccine Production

The Vigil™ Phase II ovarian vaccine cGMP manufacturing process is identical to prior Vigil™ vaccine cGMP manufacturing (BB-IND 14205) (Maples 2010). Surgically excised tumor is collected in the surgical field and placed in sterile saline and packaged for transport to the manufacturing facility. The tumor is mechanically and enzymatically dissociated into a single cell suspension. The cells are counted and then transfected with the FANG™ plasmid. The cells are incubated overnight to allow transcription of the FANG™ plasmid. The following morning the cells are harvested, washed, and then irradiated at 10,000cGy in a standard Blood Bank irradiator. The irradiated cell suspension is then enumerated, aliquoted and frozen (1×10^7 cells). The freeze media consists of

10% DMSO (dimethyl sulfoxide; Cryoserv USP; Bionichepharma US), 1% Human Serum Albumin (ABO Pharmaceuticals) in Plasma-Lyte A at pH 7.4 (Baxter). After freezing the cells are stored in the vapor phase of liquid nitrogen until all release testing is completed, all necessary approvals are obtained and the patient is ready for treatment.

9.2 Safety Analysis

The FANG™ plasmid employed in the generation of this autologous cell vaccine has been tested for identity, sterility, purity and strength.

Irradiated Gene Modified Tumor Cells

To ensure safety, all gene-modified tumor cells to be used in patient vaccinations must be irradiated 10,000 cGy prior to freezing. This is the same irradiation process as for the TAG vaccine, BB-IND 13650 and prior vaccines (Belagenpumatucel-L and GVAX® published trial results and BB-IND 13401 and BB-IND 12118) (Kumar 2009; Maples PB 2009; Maples PB 2009). The selection of this radiation dose is based on the desire to utilize the lowest possible radiation dose for the transfected cells to optimize the level and duration of bifunctional shRNA^{furin} transcription and GMCSF protein production and maximize the safety of vaccine cell injections at the same time. In addition, investigators have demonstrated that irradiating cultured tumor cells of different histologic origins at 10,000 cGy completely arrests tumor colony formation.

Preparation

Reference the Study Reference Manual for vaccine preparation and handling information.

Drug concentrate: 1.0×10^7 cells per injection in a volume of 1mL.

Route of administration: Intradermal injection

Storage

Frozen, unopened vials are stored in the vapor phase of Liquid Nitrogen.

Administration of vaccine

Patients with viable cells in sufficient numbers for at least 1.0 x 10^7 cells / injection for 4 doses will receive monthly intradermal injections of the tumor cell vaccine (as assigned cohort and available cell yield following tumor harvest and processing) as long as sufficient material is available. Patients who fail to achieve successful manufacturing of a minimum of 4 doses will not undergo treatment. The sites of injection will be rotated between the right and left upper arms. If the ipsilateral axillary lymph nodes were radiated or surgically resected during prior therapy, alternative sites (e.g., anterior thigh) will be used. The patient will be observed for at least 30 minutes (with a 10% window) following vaccination. During this observation period, vital signs will be taken every 10 minutes (with a 10% window). If clinically stable vaccine administration may continue for up to 12 doses given on a monthly basis as long as sufficient material is available.

9.3 Concomitant Therapy

All concomitant treatments, including blood and blood products, must be reported on the source documentation. The following concomitant medications must be documented on the Con Meds page of the CRF:

- Prophylactic antibiotics
- Premedications
- Antifungals
- Antinauseants, antipyretics

Short term (<30 days) concurrent systemic steroids ≤ 0.25 mg/kg prednisone per day (maximum 7.5 mg/day) and bronchodilators (inhaled steroids) are permitted; other steroid regimens and/or immunosuppressives are excluded.

10.0 OCCUPATIONAL SAFETY

Study medications are not expected to pose significant occupational safety risks to investigational staff under normal conditions of use and administration. However, precautions should be taken to avoid direct contact with study medication. Biosafety Level 2 practices shall be employed with this study medication. Reference the Study Reference Manual.

11.0 ADVERSE EVENTS

11.1 Adverse Event and Serious Adverse Event Definitions

Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Serious Adverse Event

An AE (experience) or reaction occurring at any dose should be classified as a serious adverse event (SAE) if any of the following occur:

- Initial or prolonged hospitalization
- A life-threatening condition (i.e. an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe)
- Significant disability/incapacity (i.e. the AE resulted in a substantial disruption of the subject's ability to carry out normal life functions)
- Congenital anomaly/birth defect
- It does not meet any of the above serious criteria, but may jeopardize the subject or may require surgical or medical intervention to prevent one of the outcomes listed above.
- Death

Unexpected Adverse Event

An unexpected event is any AE that is not identified in nature, severity or frequency in the Clinical Investigator's brochure or the drug package insert.

Grading Adverse Events

Adverse events (AEs) will be recorded throughout the trial. Toxicities and AEs will be graded and reported using the Common Toxicity Criteria for Adverse Events (CTCAE) Version 3.0 as linked in Appendix C. All AEs, regardless of severity, will be followed by the Treating Physician until resolution is satisfactory.

11.2 Attribution of Causality

The relationship of each event to treatment will be assessed by the Treating Physician and recorded on the CRF.

11.3 Expected Side Effects

Tumor cell vaccines have been previously administered to patients with cancer. Side effects were minimal, the most frequent of which included local reactions at the site of injection. Potential adverse events are listed below.

Local skin reactions at the site of injection:

Erythema, tenderness, induration, urticaria/rash, pruritus.

Other expected adverse events:

Fever, myalgias/arthralagias, chills/rigors, nausea, fatigue, headache, thrombocytopenia and other cytopenias, hyperglycemia, vomiting, hypotension, infection at the immunization site.

In addition there may also be a risk of autoimmune disease development, although to date no evidence of this has been seen in any vaccination study. There may also be worsening of tumor related symptoms secondary to immune mediated attack on patient's tumor.

11.4 Recording of an Adverse Event

Adverse events will be recorded for the duration of a patient's study treatment, and for up to 30 days following the last study treatment. All AEs, regardless of causal relationship are to be recorded in the CRF and source documentation. Additional information about each event, such as treatment required, eventual outcome, and whether or not therapy had to be interrupted or dosages reduced, will also be recorded on the CRF.

Pre-existing conditions will be recorded at baseline on the Medical History Form. If a pre-existing condition does not change, it does not have to be reported as an AE on subsequent cycles.

11.5 Serious Adverse Event Reporting

All SAEs will be reported by email or facsimile upon becoming aware of the event within 24 hours to Gradalis, Inc. This includes any death from any cause while a patient is receiving treatment (Vigil™) on this protocol, or ≤ 30 days following the last dose of protocol treatment (Vigil™).

The site will supply as much information as is available at the time of the initial facsimile (study number, patient initials, patient study number, onset date, relationship, patient demographics, event, dosing regimen of study agent) to:

Gradalis, Inc. 2545 Golden Bear Drive, Suite 110 Carrollton, TX 75006 Vigil@gradalisinc.com	
Direct: (214) 442-8124	Fax: (214) 442-8101

Gradalis, Inc. will report adverse events to the FDA in compliance with 21 CFR 312.32.

12.0 PATIENT COMPLETION AND WITHDRAWAL

12.1 Indication for Taking Patients Off Study

The Investigator must notify the sponsor at any time following discontinuation of a patient on study for the occurrence of a serious or unexpected AE associated with the use of the study medication.

13.0 DATA EVALUATION

13.1 Statistics and Estimated Sample Size

The primary objective of this study is to investigate whether maintenance Vigil™ vaccine after a clinically defined complete response to induction platinum/paclitaxel chemotherapy results in prolonged time to recurrence (TTR) compared with standard of care observation. TTR was used for the purpose of sample-size estimation. Based on prior published experience (Markman, Liu et al. 2003), the median RFS after a clinical complete response to induction therapy for the control arm was estimated to be approximately 16 months for those with stage IV or suboptimal (> 1 cm residual) stage III disease and 24 months for stage III patients with optimal (< 1 cm residual) disease. Given an average RFS of 24 and 16 months, respectively, a 10% increase in RFS (i.e. TTR) would provide adequate statistical power to support expanding the study (Table 6). This analysis was performed using a statistical power calculator (http://www.dssresearch.com/KnowledgeCenter/toolkitcalculators/statisticalpowercalculators.aspx) to conduct a 1-tail test of a sample average in comparison to the historical value, a standard deviation of 1.0, and an alpha error level of 5%. In order to expand the protocol treatment base and increase the rate of accrual while maintaining the objectives of the protocol, the study, which was initially limited to patients with stage IIIc ovarian cancer, is being expanded to patients with Stage III/IV disease. In the SEER database: 1988-2001(Kosary 1988-2001), women with Stage IIIc disease comprised 19.4% of the ovarian cancer population; those with Stage III inclusive, 35.3%; and those with Stage IV, 31.7%. Thus, the eligibility expansion increases the base catchment population from 19.4% of women with ovarian cancer to 67% of which only a subset will meet protocol criteria.

In the completed Phase I study of Vigil™ vaccine in patients with solid tumors experiencing progressive disease following prior therapy, 50% of patients with serial assessments (n=18) demonstrated conversion from γIFN ELISPOT

negative to positive by Month 4 (Senzer N 2012). The percent γIFN ELISPOT conversions and the timing thereof will be recorded in the current patient population of patients with minimal or no residual disease using descriptive statistics to assess the hypothesis that there is an inverse relationship between tumor volume and immune responsiveness based in part on the quantitative relationship between tumor volume and the production of immunosuppressive factors.

Although all patients will be evaluatated for RFS, subgroup analyses will be separately perfomed on patients with negative ELISPOT at baseline and those with positive ELISPOT at baseline.

Table 6: Statistical Power of 2:1 Randomization Trial per Surgical Stage Stratification Group (1 tailed t test)

Surgical Stage Stratification	Vigil™ Vaccine (TTR in months)	Observation Control (TTR in months)	Power	n
Stage III patients with optimal (< 1 cm residual)	26.4	24	1.0	30
Stage IV or suboptimal (> 1 cm residual) stage III disease	17.6	16	1.0	30

Exploratory analysis of two Phase III studies (D9901 and D9902) with the recently FDA approved Provenge documentated prolonged survival benefits in Provenge-treated prostate cancer patients (25.9 months vs. 21.4 months and 25.8 vs. 21.7 months, respectively). Supporting our clinical objective, patients who subsequently received docetaxel experienced a median survival of 34.5 months compared to 25.4 months in patients treated with placebo followed by docetaxel. Descriptive statistics will be employed to format the response rates and response durations to 2nd line therapy in patients that relapse in both FANG™ and observational groups.

13.2 Definition of Evaluable Patients

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations will be performed as closely as possible to the beginning of treatment and never more than 2 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of treatment.

Clinical Lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules, palpable lymph nodes). In the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended.

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

Conventional CT and MRI: These techniques should be performed with cuts of 10mm or less in slice thickness contiguously.

Spiral CT: Spiral CT should be performed using a 5mm contiguous reconstruction algorithm.

Ultrasound: When the primary endpoint of the study is objective response evaluation, ultrasound should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. Ultrasound may also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

13.3 Time to Recurrence of Disease (TTR)

Time to recurrence will be measured from the treatment start date (date of first dose) to either the date the patient is first recorded as having disease recurrence (even if the patient went off treatment because of toxicity), or the date of death if the patient dies due to any causes before recurrence.

13.4 Time to Treatment Failure

Time to treatment failure will be measured as the date from when the patient started treatment (first dose) to the date the patient is withdrawn due to: AE(s), progressive disease/insufficient therapeutic response, death, failure to return, refused treatment/did not cooperate/withdrew consent, or started a new

treatment. Patients who do not fail treatment will be censored at the date of last follow-up.

13.5 Survival

Survival will be measured, as the time from start of treatment to the date of death or the last date the patient was known to be alive.

Long-term survival information will be collected.

14.0 STUDY RECORDS

14.1 Documentation

A log of all patients evaluated for this protocol must be maintained at each site. Patients excluded from admission will be provided with a clear explanation of the specific reasons why they have been excluded from the study. Patients who are included will be assigned a patient identification number.

For each patient treated with the study drug(s), the Research Coordinator is required to prepare and maintain case histories that include all observations and other data pertinent to the investigation. This will include all source documents needed to verify the accuracy of all observations and other data contained in the CRFs on each study patient.

The Investigator or his/her designee is required to retain the records related to the trial for a period of 2 years following the date a marketing application is approved for the indication being investigated. If no application is to be filed or if the application is not approved for such indication, the records must be retained until 2 years after the investigation is discontinued and the regulatory agencies are notified.

The Investigator shall retain study drug disposition records and source documents for the maximum period required by the country and institution in which the study will be conducted, or for the period specified by Gradalis, which ever is longer. The Investigator must contact Gradalis, Inc. prior to destroying any records associated with the study.

If the Investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another investigator, IRB). Notice of such transfer will be given in writing to Gradalis, Inc.

14.2 Case Report Form (CRF) Procedures

CRFs will be supplied by Gradalis, Inc. for recording all data from each patient. CRFs must be typewritten or printed legibly using black ballpoint pen or completed electronically. The investigator or his/her designee is responsible for recording all data relating to the trial on the CRFs. The investigator must verify that all data entries on the CRFs are accurate and correct.

If an item is not available or is not applicable, it should be documented as such; no blank spaces should be left on a CRF.

APPENDIX A

ECOG Performance Status Scale

ECOG Performance Status Scale	
Grade	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of light or sedentary nature—(e.g., light housework or office work).
2	In bed <50% of the time. Ambulatory and capable of all self care, but unable to carry out any work activities; up and about > 50% of waking hours.
3	In bed >50% of the time. Capable only of limited self-care, confined to bed or chair >50% of waking hours.
4	100% bedridden. Completely disabled; cannot carry out any self care; totally confined to bed chair.
5	Dead

APPENDIX B

Schedule Of Assessments

Procedure	Prestudy	Screening	Monthly (q 28±3 days)	End of TX	Follow-Up
Informed consent	X	X			
Phone Contact	X[1]				
Medical History	X	Interval Medical History within 4 weeks			
Physical Examination	X	X	X	X	
Toxicity (adverse events)			X	X	
Concomitant medications		X	X	X	
Performance Status	X	X	X	X	
Clinical Tumor Assessment		X	X	X	
Radiological Tumor Assessment (abdomen/pelvis)[2]	within 6 weeks	within 4 weeks		X	
CBC with differential	X	X	X	X	
HIV testing, if applicable		X			
CA-125 tumor assessment[3]	X	X	X	X	
Serum Chemistry	X[4]	X	X	X	
Immune Function Analysis	At tissue procurement and prior to the 1st and 3rd cycles of chemotherapy post debulking	X	Months 2, 4, 6 prior to vaccine administration	X	
Urinalysis		X			
EKG		X			
Vaccine administration[5]			X		
Injection Site Assessment			Day 2 only		
FACT-O[6]		X	X		
Survival Status	X			X	X[7]

[1] Following tissue procurement, patients will be contacted every 6 weeks ±2 weeks to assess interest / eligibility of the main randomized portion of the trial and survival status.

[2] Radiographic assessments taken at baseline, ≤ 1 week prior to Cycle 4 , at SOC intervals, and when CA-125 >35U/mL.

[3] CA-125 taken at baseline, monthly for the first year, every 3 months ± 2 weeks for the second and third year.

[4] Obtain from medical records from standard preoperative hematology and chemistry panels.

[5] The patient will be observed for at least 30 minutes (with a 10% window) following vaccination. During this observation period, vital signs will be taken every 10 minutes (with a 10% window).

[6] FACT-O assessment will be collected at baseline, Month 3, Month 6, Month 12, and Month 18.

[7] After EOT, subjects will be contacted by phone quarterly for disease status, medical records (when applicable), documentation of 2nd line therapy and recording of response and duration of response to that therapy, additional cancer treatments as well as survival.

APPENDIX C

NCI Common Toxicity Criteria For Adverse Events (CTCAE), Version 3.0

Publish Date: MARCH 31, 2003

As of April 02, 2003 NCI has introduced version 3.0 of the Common Toxicity Criteria for Adverse Events. These may be obtained at the following web link http://ctep.cancer.gov/reporting/ctc.html or on the Mary Crowley CTMS.

DO NOT USE CTC VERSION 2.0 TO GRADE TOXICITIES IN THIS STUDY!

APPENDIX D

FACT-O, VERSION 4

FACT-O (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FACT-O (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-O (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
O1	I have swelling in my stomach area	0	1	2	3	4
C2	I am losing weight	0	1	2	3	4
C3	I have control of my bowels	0	1	2	3	4
O2	I have been vomiting	0	1	2	3	4
B5	I am bothered by hair loss	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
C7	I like the appearance of my body	0	1	2	3	4
BMT5	I am able to get around by myself	0	1	2	3	4
B9	I am able to feel like a woman	0	1	2	3	4
O3	I have cramps in my stomach area	0	1	2	3	4
BL4	I am interested in sex	0	1	2	3	4
BMT7	I have concerns about my ability to have children	0	1	2	3	4

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