COVER PAGE

OFFICIAL PROTOCOL TITLE:	**A two-part Phase IIb Trial of Vigil (bi-shRNAfurin and GMCSF Augmented Autologous Tumor Cell Immunotherapy) in Ewing's Sarcoma**
PROTOCOL NUMBER:	**CL-PTL-121**
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PROTOCOL DATE:	**Amendment 3 dated March 29, 2017**
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PROTOCOL #:	**CL-PTL-121**
STUDY AGENT:	**Vigil formerly known as FANG (bi-shRNA^{furin} and GMCSF Autologous Tumor Cell Immunotherapy)**
SPONSOR:	**Gradalis, Inc.** **2545 Golden Bear Drive, Suite 110** **Carrollton, TX 75006** **Phone: (214) 442-8124** **Fax: (214) 442-8101**
PROTOCOL DATE:	**Amendment 3 dated March 29, 2017** **Amendment 2 dated September 25, 2015** **Amendment 1 dated June 25, 2015** **Initial Protocol dated March 5, 2015**

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.

_____ _____

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
cCR	Clinically defined Complete Response
CR	Complete response
CRF	Case report form
CTCAE	Common Toxicity Criteria for Adverse Events
CTL	Cytotoxic T lymphocyte
DC	Dendritic cell(s)
ESFT	Ewing's Sarcoma Family of Tumors
ELISA	Enzyme-Linked ImmunoSorbent Assay
ELISPOT	Enzyme-Linked ImmunoSorbent Spot
ER	Endoplasmic reticulum
FANG	bi-shRNA^{furin} and GMCSF Augmented Autologous Tumor Cell Immunotherapy
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
IEC	Independent Ethics Committee
IL	Infiltrating lymphocytes
IRB	Institutional Review Board
ITT	Intent to Treat
KPS	Karnofsky Performance Score
LAK	Lymphokine-activated killer

Abbreviation	Term
LD	Longest diameter
LLC	Large latent complex
LPI	Lead Principal Investigator
MHC	Major histocompatibility complex
MLR	Mixed lymphocyte reaction
MR	Mannose receptor
NCI	National Cancer Institute
NED	No evidence of disease
NGS	Next Generation Sequencing
NK	Natural Killer
NKT	Natural Killer T cell(s)
NSCLC	Non small cell lung cancer
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase chain reaction
PD	Progressive disease
PFS	Progression Free Survival
PI	Principal Investigator
PR	Partial response
PS	Performance Status
RECIST	Response Evaluation Criteria in Solid Tumors
RFS	Recurrence Free Survival
SCLC	Small cell lung cancer
SLC	Small latent complex
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: Ewing's sarcoma is the second most frequently diagnosed primary malignant bone tumor in the US with an annual incidence, from birth to age 20, of 2.9 cases per million population. The survival rate for patients with high-risk recurrent disease (relapse < 2 years) is generally < 10% at 5 years. Moreover, of patients who progress after second line treatment, eighty percent do not achieve a second complete response and of these patients < 10% survive one year. Refractory patients to both frontline and second line therapy have even worse prognosis.

In an effort to overcome limitations of first-generation 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:

1) address the inability to fully identify relevant strong-affinity cancer specific (and associated) antigens,
2) effect antigen recognition by the immune system (i.e. antigen→immunogen),
3) enhance effector potency, and
4) subvert endogenous cancer-induced immune resistance.

A Phase I assessment of Vigil in advanced solid tumor patients including 16 Ewing's sarcoma patients receiving ≥1 cycle (at a dose > 1.0 x 10^6 cells/injection/month for a maximum of 12 vaccinations) demonstrated safety of the Vigil immunotherapy. Furthermore, mechanism of action (i.e., vector effectiveness) was established in the manufactured products with increased mean GMCSF expression and knockdown of TGFβ1 and TGFβ2. The induced immunomodulatory effect in Ewing's sarcoma was reflected in positive IFNγ ELISPOT response to autologous tumor cells in 10/12 assayed patients, one RECIST Criteria partial response (PR), a second patient achieving no evidence of disease (NED) and a total 1-year survival (Kaplan Meier method) of 73%.

Objective(s):

<u>Part 1</u>

Primary Objectives:

- To determine and compare overall survival of patients with metastatic Ewing's sarcoma refractory or intolerant to ≥ 2 prior lines of systemic chemotherapy treated with Vigil immunotherapy vs. gemcitabine/docetaxel.

Secondary Objectives:

- To determine the γIFN ELISPOT conversion rate of subjects treated with Vigil immunotherapy vs gemcitabine/docetaxel.

- To determine and compare the proportion of patients surviving 1 year when treated with Vigil immunotherapy vs gemcitabine/docetaxel.
- To determine and compare the overall survival of patients with relapsed or refractory Ewing's sarcoma who are IFNγ ELISPOT negative at baseline and are treated with Vigil immunotherapy vs. gemcitabine/docetaxel.
- To determine and compare the progression free survival of patients with metastatic Ewing's sarcoma refractory or intolerant to ≥ 2 prior lines of systemic chemotherapy treated with Vigil immunotherapy vs gemcitabine/docetaxel.
- To determine and compare the objective response rate (RECIST 1.1) of patients with metastatic Ewing's sarcoma refractory or intolerant to ≥ 2 prior lines of systemic chemotherapy treated with Vigil immunotherapy vs gemcitabine/docetaxel.
- To determine and compare the safety profile of Vigil immunotherapy vs gemcitabine/docetaxel in patients with relapsed or refractory Ewing's sarcoma.

Part 2

Primary Objective:

- To determine safety profile of Vigil immunotherapy in combination with irinotecan and temozolomide in patients with metastatic Ewing's sarcoma refractory or intolerant to at least 1 prior line of systemic chemotherapy.

Secondary Objectives:

- To determine the γIFN ELISPOT conversion rate of patients dosed with Vigil immunotherapy in combination with irinotecan and temozolomide.
- To determine the progression free survival of patients dosed with Vigil immunotherapy in combination with irinotecan and temozolomide.
- To determine the overall survival of patients with relapsed or refractory Ewing's sarcoma who are IFNγ ELISPOT negative at baseline and are dosed with Vigil immunotherapy in combination with irinotecan and temozolomide.
- To determine the objective response rate (RECIST 1.1) of patients with metastatic Ewing's sarcoma refractory or intolerant to at least 1 prior line of systemic chemotherapy treated with Vigil immunotherapy dosed with Vigil immunotherapy in combination with irinotecan and temozolomide.

Part 1 Methodology:

This is a multicenter, 1:1 randomized Phase IIb study of intradermal autologous Vigil immunotherapy (1.0 x 10^7 cells/injection; minimum of 4 to a maximum of 12 administrations) versus gemcitabine / docetaxel in patients with metastatic Ewing's sarcoma Family of Tumors (ESFT) refractory or intolerant to at least 2 prior lines of chemotherapy. Patients undergoing a standard surgical procedure (e.g., tumor biopsy or palliative resection) may have tumor tissue harvested for manufacture of investigational product. Patients meeting eligibility criteria including manufacture of a minimum of 4 immunotherapy doses will be randomized to receive either (1) intradermal Vigil every 28 days for 4-12

administrations, or (2) gemcitabine 675 mg/m^2 IV at 10 mg/m^2/min D1 and D8 and docetaxel 75 mg/m^2 IV D8 every 21 days. The primary trial objective is to determine the overall survival of patients treated with Vigil versus gemcitabine/docetaxel. Randomization may occur as early as vaccine is released (typically 3 - 4 weeks following tumor procurement) but no later than 8 weeks following tumor procurement. Randomization of patients will be stratified by Karnofsky Performance Status (KPS) ≥ 80% vs < 80%.

Patients will be managed in an outpatient setting. Hematologic function, liver enzymes, renal function and electrolytes will be monitored monthly. Blood for immune function analyses including IFNγ-ELISPOT analysis of cytotoxic T cell response to autologous tumor antigens will be collected at tissue procurement, baseline, and prior to product administration at Cycles 2, 4, end of treatment, and every 6 months thereafter.

Part 2 Methodology:
Based on the limited accrual to Part 1 of this study, Gradalis is opening Part 2 of this clinical protocol to assess the safety of Vigil immunotherapy in combination with irinotecan and temozolomide. Part 2 will be conducted at the same centers as Part 1, studying intradermal autologous Vigil cancer vaccine (1.0 x 10^7 cells/injection; minimum of 4 to a maximum of 12 administrations) in patients with metastatic Ewing's sarcoma Family of Tumors (ESFT) refractory or intolerant to at least 1 prior line of chemotherapy. Patients undergoing a standard surgical procedure (e.g., tumor biopsy or palliative resection) may have tumor tissue harvested for manufacture of investigational product. Patients meeting eligibility criteria including manufacture of a minimum of 4 immunotherapy doses of Vigil will be registered to receive: (i) oral temozolomide 100 mg/m^2 daily (Days 1 – 5, total dose 500 mg/m^2/cycle), (ii) irinotecan 50 mg/m^2 daily (Days 1 – 5, total dose 250mg/m^2/cycle), orally or irinotecan 20mg/m^2 daily (Days 1 – 5, total dose 100mg/m^2/cycle), intravenously (iii) peg-filgrastim 100µg/kg (Day 6) subcutaneously (optional and may be administered at home), and (iv) Vigil 1.0 x 10^7 cells/injection, intradermally on Day 15 and every 3 weeks thereafter. One cycle = 21 days. Registration onto Part 2 may occur as early as one week but no later than 8 weeks following tumor procurement. Vigil is typically released approximately 3 weeks after the completion of the two-day manufacturing process.

Patients will be managed in an outpatient setting. Hematologic function, liver enzymes, renal function and electrolytes will be monitored. Blood for immune function analyses including IFNγ-ELISPOT analysis of cytotoxic T cell response to autologous tumor antigens will be collected at tissue procurement, post-procurement screening and prior to Day 15 Vigil administration at Cycles 2, 4, end of treatment, and every 6 months thereafter. Blood for ctDNA analysis will be collected prior to chemotherapy administration at baseline, Cycle 2 – Week 1 Day 1, Cycle 4 – Week 1 Day 1, and EOT.

Number of Patients:

Part 1: Approximately 62 patients will be enrolled.
Part 2: Approximately 6-9 patients will be enrolled.

Tissue Procurement Inclusion Criteria:

Patients will be eligible for tissue procurement for the Vigil manufacturing process, if they meet all of the following criteria:

1. Histologically confirmed Ewing's Sarcoma Family of Tumors (ESFT).
2. Age ≥2 years.
3. Estimated survival ≥ 6 months.
4. Evidence of EWS translocation by FISH or RT-PCR or Next Generation Sequencing (NGS).
5. Metastatic disease
6. Refractory or intolerant to ≥ 2 lines of systemic chemotherapy (Part 1) or Refractory or intolerant to at least 1 line of systemic chemotherapy (Part 2).
7. Planned standard of care surgical procedure (e.g., tumor biopsy or palliative resection or thoracentesis) and expected availability of a cumulative mass of ~10-30 grams tissue ("golf-ball" size) or pleural fluid estimated volume ≥ 500mL (must be primary tap) for immunotherapy manufacture.
8. Tumor intended for immunotherapy manufacture is not embedded in bone and does not contain luminal tissue (e.g. bowel, ureter, bile duct).
9. Ability to understand and the willingness to sign a written informed consent document for tissue harvest.

Tissue Procurement Exclusion Criteria:

Patients meeting any of the following criteria are not eligible for tissue procurement for the Vigil manufacturing:

1. Medical condition requiring any form of chronic systemic immunosuppressive therapy (steroid or other) except physiologic replacement doses of hydrocortisone or equivalent (no more than 30 mg hydrocortisone or 10 mg prednisone equivalent daily) for < 30 days duration.
2. Known history of other malignancy unless having undergone curative intent therapy without evidence of that disease for ≥ 3 years **except** cutaneous squamous cell and basal cell skin cancer, superficial bladder cancer, in situ cervical cancer or other in situ cancers are allowed if definitively resected.
3. Brain metastases unless treated with curative intent (gamma knife or surgical resection) **and** without evidence of progression for ≥ 2 months.

4. Any documented history of autoimmune disease with exception of Type 1 diabetes on stable insulin regimen, hypothyroidism on stable dose of replacement thyroid medication, vitiligo, or asthma not requiring systemic steroids.
5. Known history of allergies or sensitivities to gentamicin.
6. Known hypersensitivity reactions to docetaxel or to other drugs formulated with polysorbate 80 that would preclude treatment with docetaxel (Part 1 only).
7. History of or current evidence of any condition (including medical, psychiatric or substance abuse disorder), therapy, or laboratory abnormality that might confound the results of the study, interfere with the patient's participation for the full duration of the study, or is not in the best interest of the patient to participate, in the opinion of the treating Investigator.
8. Known HIV or chronic Hepatitis B or C infection.

Study Enrollment Inclusion Criteria:

Patients will be eligible for registration if they meet all of the following inclusion criteria:

1. Successful manufacturing of at least 4 vials of Vigil.
2. Karnofsky performance status (KPS) ≥60% (Part 1) or KPS ≥80% (Part 2).
3. Estimated survival ≥ 4 months (Part 1) or estimated survival of ≥6 months (Part 2).
4. Normal organ and marrow function as defined below:

Absolute granulocyte count	≥1,500/mm^3
Absolute lymphocyte count	≥400/mm^3
Platelets	≥100,000/mm^3
Total bilirubin	≤ institutional upper limit of normal
AST(SGOT)/ALT(SGPT)	≤2x institutional upper limit of normal
Creatinine	<1.5 mg/dL

5. Subject has recovered to CTCAE Grade 1 or better from all adverse events associated with prior therapy or surgery. Pre-existing motor or sensory neurologic pathology or symptoms must be recovered to CTCAE Grade 2 or better.
6. If female of childbearing potential, has a negative urine or serum pregnancy test. If the urine test is positive or cannot be confirmed as negative, a negative serum test will be required for study entry.
7. Ability to understand and the willingness to sign a written informed protocol specific consent.

Study Enrollment Exclusion Criteria:

In addition to the procurement exclusion criteria, patients will NOT be eligible for study registration and randomization if meeting any of the following criteria:

1. Any anti-neoplastic therapy between tissue procurement for Vigil manufacture and start of study therapy.
2. Live vaccine used for the prevention of infectious disease administered < 30 days prior to the start of study therapy.
3. Post-surgery complication that in the opinion of the treating investigator would interfere with the patient's study participation or make it not in the best interest of the patient to participate.

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

Part 1
Patients will receive either 1 x 10^7 cells via intradermal injection every 28 days for a 4 - 12 administrations (depending on the quantity of Vigil manufactured from surgical specimens), or gemcitabine 675 mg/m^2 IV at 10 mg/m^2/min D1 and D8 and docetaxel 75 mg/m^2 IV D8 every 21 days.

Part 2
Patients will receive (i) oral temozolomide 100 mg/m^2 daily (Days 1 – 5, total dose 500 mg/m^2/cycle), (ii) irinotecan 50 mg/m^2 daily (Days 1 – 5, total dose 250mg/m^2/cycle), orally or irinotecan 20mg/m^2 daily (Days 1 – 5, total dose 100mg/m^2/cycle), intravenously (iii) peg-filgrastim 100µg/kg (Day 6) subcutaneously (optional and may be administered at home), and Vigil 1.0 x 10^7 cells/injection, intradermally on Day 15 and every 3 weeks thereafter. One cycle equals 21 days.

Duration: Patients may receive repeat cycles of treatment until disease progression, unacceptable toxicity, withdrawal of consent or other criterion is met for discontinuation from study. Patients randomized to Vigil will receive up to 12 doses depending upon the quantity of Vigil manufactured from the surgical specimen.

Efficacy Assessments:
- Overall survival (OS)
- Progression Free Survival (PFS)
- Response Rate (RECIST 1.1)

Safety Assessments:
- Laboratory assessments
- Physical examination, performance status, height, weight, temperature, blood pressure, and pulse
- Toxicity: CTCAE v 4.03

Exploratory Assessments:
Circulating and intratumoral immune function analysis including, but not limited to IFNγ-ELISPOT analysis.

Evaluation of baseline tumor microenvironment including but not limited to immunohistochemical evaluation of tumor PD-L expression and TIL populations.

1.0 INTRODUCTION

1.1 Summary

Ewing's sarcoma is the second most frequently diagnosed primary malignant bone tumor in the US with an annual age-adapted (birth – 20 years) incidence of 2.9 cases per million population (Esiashvili, Goodman et al. 2008). Approximately 20-30% of cases are diagnosed in the first decade and 10% after age 20. The median age of diagnosis is 14 -15 years old (Cotterill, Ahrens et al. 2000; Stahl, Ranft et al. 2011). Molecularly the disease is distinguished by a translocation of the EWSR1 gene on chromosome 22q12 with in FLI1 or, less commonly, other genes (ERG) of the ETS transcription factor family (Marino-Enriquez and Fletcher 2014). Up to 85% of Ewing's tumors are characterized by the EWS/FLI1 Type 1 (EWS exon 7 to FLI-1 exon 6) or Type 2 (EWS exon 7 to FLI1 exon 5) translocation (11;22)(q24;12) (Arvand and Denny 2001).

Patients with initially diagnosed localized disease have a disease-free survival rate (EFS) of 60-70% with standard 5-drug chemotherapy regimen (VAC +IE or VIDE), while patients with metastatic disease have a EFS of <20%. The 5 year front line treatment survival rate for patients with localized disease approaches 70% with standard of care, but only ~30% for those with metastatic lesions isolated to the lung and <20% for those with bone or bone marrow involvement (Cotterill, Ahrens et al. 2000; Meyers, Krailo et al. 2001; Grier, Krailo et al. 2003). In a large analysis of 975 newly diagnosed Ewing patients enrolled in trials from 1979 to 1993, the 5 year RFS for patients with primary metastatic disease (n=179)was 22%, which was significantly worse than localized disease (p<.0001) (Cotterill, Ahrens et al. 2000). Although the SEER data survival for metastatic disease has improved (5 year survival of 39%) with "current" standard of care, the toxicity profile has also increased during the multi-agent, front line chemotherapy treatment course (Esiashvili, Goodman et al. 2008). Second or greater line therapy regimen only show temporary benefit with dismal survival outcome.

1.2 Relapsed and Refractory Ewing's sarcoma

Thirty to forty percent (30-40%) of Ewing's sarcoma patients who present with localized disease, and 60-80% presenting with primary metastatic disease, will experience relapse or progression with a median time to relapse of 1.3 years (Ozaki, Hillmann et al. 1996; Bacci, Picci et al. 1998; Klingebiel, Pertl et al. 1998; Leavey and Collier 2008; Stahl, Ranft et al. 2011). Patients who relapse have a marked reduction in overall survival with published results in 122 (good prognostic) patients of only 12%, 5 year survival, and 19% 2-year survival (Leavey, Mascarenhas et al. 2008). Factors like site of recurrence (localized, metastatic, combined localized and metastatic) and time to relapse (<2 years or >2 years) play important prognostic factors for survival in recurrent Ewing's sarcoma (Table 1). For relapses that occur within the first 2 years after initial diagnosis, which make up 72% of relapses (Stahl, Ranft et al. 2011), the 2-year OS

was 7% (Shankar, Ashley et al. 2003), 5-year EFS was 5%(Bacci, Ferrari et al. 2003), and 5-year OS was 7% (Stahl, Ranft et al. 2011).

Ewing's Sarcoma can present with localized or metastatic disease at time of diagnosis. Presentation with localized disease is associated with a 6-year event free survival rate of 69% and overall survival (OS) rate of 72% (Leavey, Mascarenhas et al. 2008). If patients present with metastatic disease at diagnosis, the 6-year event free survival significantly deteriorates to 28% with OS 29% (Guerney, Swensen et al. 1999; Miser, Goldsby et al. 2007; Leavey, Mascarenhas et al. 2008). Once first recurrent disease occurs survival is much worse with a 5 year OS of only 13% (Bacci, Ferrari et al. 2003). Recent analysis of 262 first recurrence Ewing's Sarcoma patients treated on INT0091 revealed similar results with 12% 5 year OS, and only 19% 2 year survival. This analysis included 122 (good prognostic) patients who had local recurrence manageable with only surgery and/or irradiation (Leavey, Mascarenhas et al. 2008).

The NCCN guidelines do not provide standard of care recommendations for second-line treatment. Regimens such as topotecan/cyclophosphamide, irinotecan/temozolomide, or docetaxel/gemcitabine have been utilized in second line or later treatment and may prolong life for those who respond (Merchant, Kushner et al. 1999; Saylors, Stine et al. 2001; Wagner, Crews et al. 2004; Wagner, McAllister et al. 2007; Navid, Billups et al. 2008; Navid, Willert et al. 2008; Casey, Wexler et al. 2009; Mora, Cruz et al. 2009; Wagner 2010; Wagner, Perentesis et al. 2010; McGregor, Stewart et al. 2011; Rapkin, Qayed et al. 2012; Raciborska, Bilska et al. 2013; Wagner, Turpin et al. 2013). However, none of these regimens have been determined to have significant advantage in a randomized comparative clinical assessment (Gaspar, Hawkins et al. 2015). The Irinotecan/Temozolomide regimen is the most common second-line regimen utilized. Irinotecan/Temozolomide shows moderate durable responses (Casey, Wexler et al. 2009; Raciborska, Bilska et al. 2013; Palmerini, Jones et al. 2016). Although results of progression free survival (PFS) and OS do not significantly vary between this and other dose intense regimens (Yildiz, Sen et al. 2014), PFS in second-line varies between 3 and 8 months with most commonly reported PFS of 6 months (Casey, Wexler et al. 2009; Raciborska, Bilska et al. 2013; Palmerini, Jones et al. 2016).

Prognostic markers for clinical benefit in second relapse is the response status to prior therapy regimen (i.e. responder 5 year survival 48% vs. non-responder 0% respectively to second-line chemotherapy, p=0.0001), (Barker, Pendergrass et al. 2005). Another study revealed similar results: of 161 patients treated with second-line chemotherapy, had a 5-year survival of 25% in responders (CR/PR) compared to non-responders (SD/PD), (2-year survival 45% vs 10%) (Rasper, Jabar et al. 2014). Few patients respond to second-line achieving a second remission and survival at 5 years is severely limited (Table 1) (Rodriguez-Galindo, Billups et al. 2002; Bacci, Ferrari et al. 2003; Shankar, Ashley et al. 2003; Barker, Pendergrass et al. 2005; McTiernan, Cassoni et al. 2006; Leavey and Collier 2008; Stahl, Ranft et al. 2011), particularly in patients with high risk disease (time to relapse (TTR) within 2 years of front-line treatment). In one large retrospective analysis of 714 patients in first relapse, the 1-

year OS was 43%, 5-year OS was 13%, and 10 year OS was 9% (Stahl, Ranft et al. 2011).

Table 1. Relapse from Time of Initial Treatment

PI	Number Patients	Primary Metastatic Disease at Diagnosis	5-year Overall Suvival After First Relapse	5-year OS: Relapse < 2 years from Initial Diagnosis	5-year OS: Relapse ≥ 2 years from Initial Diagnosis
Stahl 2011	714	35%	13.0%	7.0%	29.0%
Leavey 2008	262	46%	12.0%	7.0%	30.0%
Bacci 2003	195	0%	13.8%	2.5%	14.3%
Rodriguez-Galindo 2002	71	42%	23.7%	5.0%	34.9%
Shankar 2003	64	0%	10.0%	7.0%	45.0%
Barker 2005	55	38%	23.0%	12.0%	48.0%
McTiernan 2006	114	47%	15.2%	6.7%*	31.6%
*Relapse free interval defined as < or ≥ 18 months.					

Second-line chemotherapy for relapsed Ewing's sarcoma generally has had limited efficacy with only 9% to 13% of patients achieving a second disease free remission (Bacci, Ferrari et al. 2003; Barker, Pendergrass et al. 2005; Rasper, Jabar et al. 2014). There is no standard of care for third-line treatment. Multiple groups have evaluated the combination of gemcitabine and docetaxel in the treatment of pediatric sarcomas with particular attention to Ewing's sarcoma (Table 2) (Navid, Billups et al. 2008; Mora, Cruz et al. 2009; Fox, Patel et al. 2012; Rapkin, Qayed et al. 2012). Both Gemcitabine and Docetaxel have been evaluated separately in phase I pediatric solid tumor studies establishing MTD and toxicities in pediatric populations (Seibel, Blaney et al. 1999; Reid, Qu et al. 2004). Gemcitabine with Docetaxel showed a 53% response rate in adult uterine leiomyosarcoma (Hensley, Maki et al. 2002) and showed superior activity in direct comparison to gemcitabine alone (Maki, Wathen et al. 2007). Further studies in adult sarcoma studies showed response rates ranging 16-43% specifically 2 previously treated Ewing's patients responded with 1 PR and 1 SD (Leu, Ostruszka et al. 2004). Gemcitabine in combination with docetaxel began to be used in pediatric settings and an early report evaluated 14 patients with refractory bone sarcomas and showed an ORR of 29% with one SD response out of 2 Ewing's patients (Navid, Willert et al. 2008). A second report of 10 patients included 6 Ewing's patients, of which 4 experienced response (3 CR and 1 PR) and one had stable disease (Mora, Cruz et al. 2009). Of 14 patients with Ewing's sarcoma in another study, two achieved PR and six SD as best response (Fox, Patel et al. 2012). Toxicity primarily involves transient hematopoietic suppression which has been managed with use of Filgrastim and dose reduction.

Table 2. Gemcitabine in Combination with Docetaxel in Refractory Pediatric Sarcomas

Reference	Total Number pts	Efficacy Overall	Gemcitabine Dosing	Docetaxel Dosing	Number Ewings pts	Ewings Respones
(Fox, Patel et al. 2012)	53	9% PR	675 mg/m^2, 90 min, D1,8	75 mg/m^2 D8	14	2 PR, 6 SD
(Mora, Cruz et al. 2009)	10	50% ORR, 30% SD	1000 mg/m^2, 90 min, D1,8	100 mg/m^2 D8 (2-4hr)	6	3 CR, 1 PR, 1 SD
(Rapkin, Qayed et al. 2012)	19	11% ORR, 39% SD	675 mg/m^2, 90 min, D1,8	75 mg/m^2 D8 (1hr)	2	none
(Navid, Willert et al. 2008)	14	29% ORR, 14% SD	675 mg/m^2, 90 min, D1,8	100 (or 75) mg/m^2 D8 (1hr)	2	1 SD

*all received Neupogen starting on Day 9 till recovery

Overall Response Rate (ORR); Stable Disease (SD); Partial Response (PR); Complete Response (CR)

Despite the use of different chemotherapy regimen, the majority of relapsed patients will not achieve a second or third remission, or show only temporary benefit followed by relapse and a grim prognosis.

To date, there is no proven significant benefit of third-line chemotherapy. Prognostic markers for clinical benefit in second relapse is the response status to prior therapy regimen (i.e. responder 5 year survival 48% vs. non-responder 0% respectively to second-line chemotherapy, p=0.0001), (Barker, Pendergrass et al. 2005). Another study revealed similar results: of 161 patients treated with second-line chemotherapy, had a 5-year survival of 25% in responders (CR/PR) compared to non-responders (SD/PD), (2-year survival 45% vs 10%) (Rasper, Jabar et al. 2014). In a relatively large retrospective analysis 86% of 195 patients, did not achieve a second remission and 97% had died with a median of 11.7 mo, while no patient achieved disease control. For the 26 of 195 patients who did go into a second remission, 12 relapsed again (10/12 died and 2 were living with uncontrolled disease at 6 and 13 months)(Bacci, Ferrari et al. 2003).

This reflects the limitations of conventional cytotoxic chemotherapy underscores the desperate need for treatment development in the metastatic Ewing's sarcoma in high-risk first relapse patients(TTR within 2 years of front line treatment) or in second relapse.

Immune escape mechanisms also appear to play a role in the development and metastasis of Ewing's Sarcoma. Patients with primary disseminated disease showed increased frequency of T-regulatory cells in the bone marrow compared to localized disease at presentation (Brinkrolf, Landmeier et al. 2009). Factors associated with survival benefit are increased number of tumor infiltrating CD8+ T-lymphocytes (p=0.05) as well as lower tumor volume (<200 ml) at diagnosis (Berghuis, Santos et al. 2011). Furthermore, the EWS/FLI-1 fusion protein itself presenting uniquely on EWS

tumor cells and absent on normal cells, may be a driver neoantigenic target capable of eliciting an effective cytotoxic immune response (Liu, Huang et al. 2012; Peng, Huang et al. 2014). Re-activation of immunogenic antitumor response as a treatment strategy for EWS could be exploited either in a monotherapy or in combination with lymphocyte-sparing chemotherapy agents like irinotecan and temozolomide, which have been found with lower rates of lymphopenia in treated patients (Kushner, Kramer et al. 2006).

Thus, preliminary results in 83% of Ewing's Sarcoma patients demonstrated actual "turn on" of activated T cells in circulation with marked capacity to release γIFN at exposure to unmanipulated autologous tumor cells as defined by γIFN ELISPOT assay after treatment with Vigil immunotherapy. ELISPOT activity has been verified by Mt. Sinai and plates are third party read, Zellnet, Fort Lee, NJ with a high confidence in this functional immune assessment. ELISPOT results have also demonstrated significant correlation with survival and TTR in previously published work involving other solid tumor patients (Senzer, Barve et al. 2013; Oh, Barve et al. 2016). Moreover, these results have correlated with tumor responses (1 CR/NED, 1 PR) in 2 Ewing's sarcoma patients. One who observed complete regression and is described in prior publication (Ghisoli, Rutledge et al. 2017). Notably γIFN ELISPOT level appeared to correlate lightly to disease response and progression (**Figure 1**) in this patient.

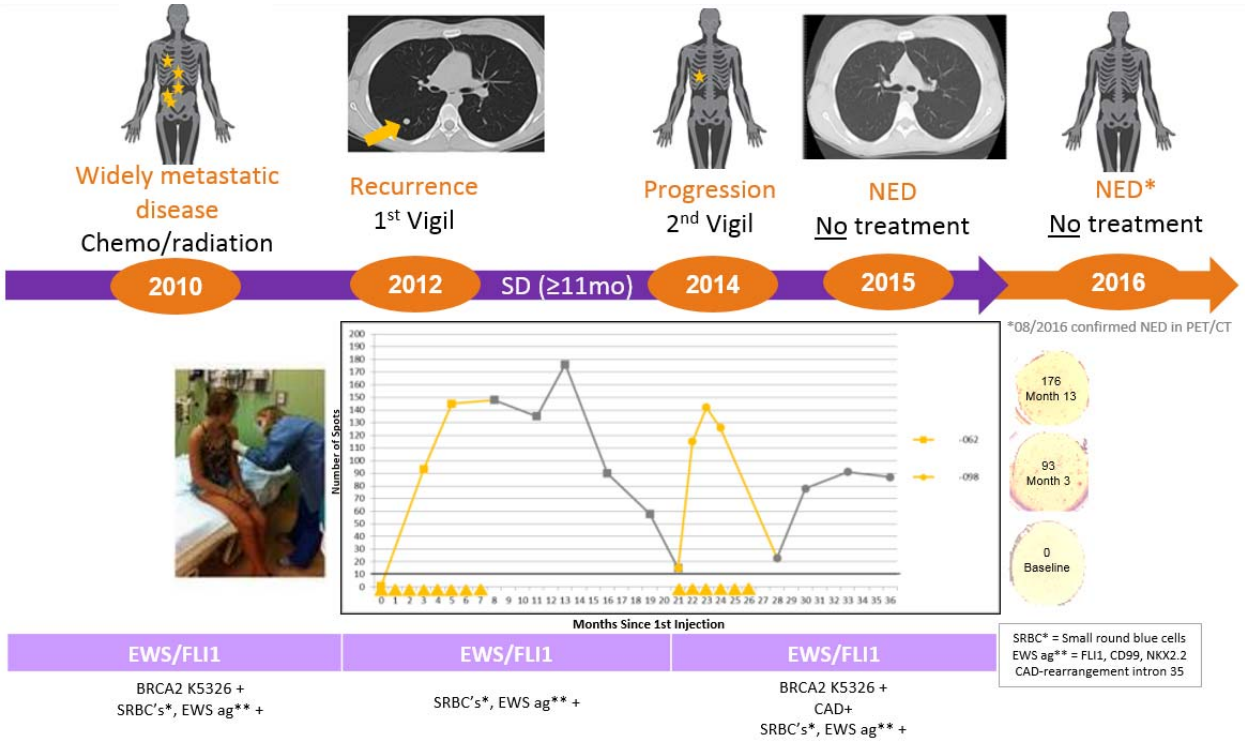


Figure 1. Case Report 1 (F-062). Relapsed Ewing's Sarcoma Response to Vigil

2.0 VIGIL

Vigil immunotherapy comprises 1) autologous tumor cells as a source of the full matrix of a patient's tumor-related antigens and 2) a DNA plasmid with two genetic modifications in order to optimize a "triad" functionality i) patient tumor-specific antigen presentation, ii) dendritic cell activation (GMCSF), and iii) tolerance escape (blocking TGFβ1, β2 activation) (Maples PB 2009; Nemunaitis 2011). To construct Vigil, autologous cancer cells are transfected with a multi-component expression vector encoding GMCSF 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.

Since the start of a Phase I trial of Vigil in advanced cancer patients on 06/08/2009, strong evidence for safety and benefit was initially seen in a sub-analysis of 35 adult and pediatric patients given 176 vaccinations (Senzer, Barve et al. 2013). To determine optimal dosing regimen, analyses were carried out comparing survival, safety and ELISPOT response at 1×10^7 cells/injection vs. 2.5×10^7 cells/injection and at a range of Vigil dosing schedules between 4 and 12 monthly injections. This analysis showed similar results between dose levels and number of doses; thus 1×10^7 cells/injection with a minimum of 4 and a maximum of 12 monthly injections was selected as the treatment regimen for subsequent studies. Vector effectiveness was also confirmed via GMCSF transgene expression and knockdown of Furin, TGFβ1 and TGFβ2 expression (Senzer, Barve et al. 2012; Senzer, Barve et al. 2013).

Eighty patients in total (54 females, aged 13-84 / 26 males, aged 12-76) have been entered into Phase I trial involving 19 solid tumor cancers. Four hundred sixty-four Vigil injections were administered and no ≥ Grade 3 toxic events attributed to Vigil have been observed. Dose variance ranged from 2×10^6 cells/injection to 2.5×10^7 cells/injection via intradermal injection 1x/month for a minimum of 4 months. The prolonged survival shown in **Figure 2** involving predominantly advanced stage, heavily pretreated patients suggests further safety support as it is greater than historical expectation (Senzer, Barve et al. 2013).

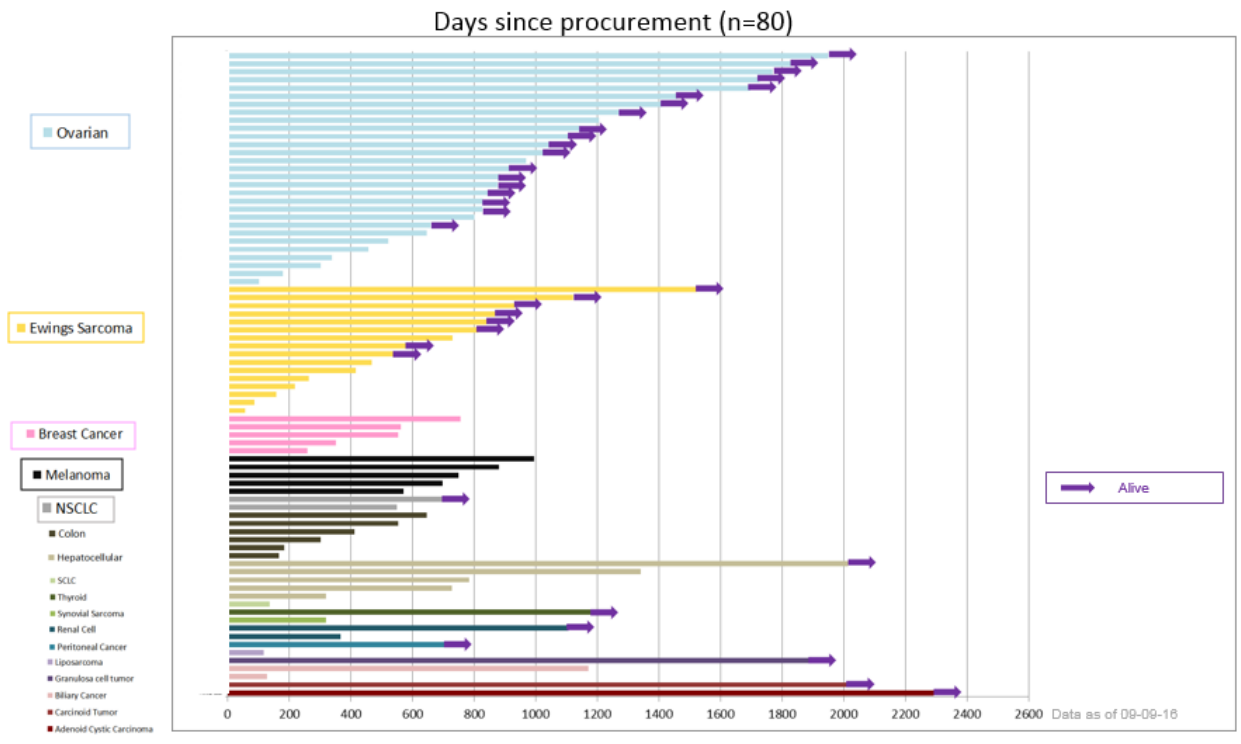
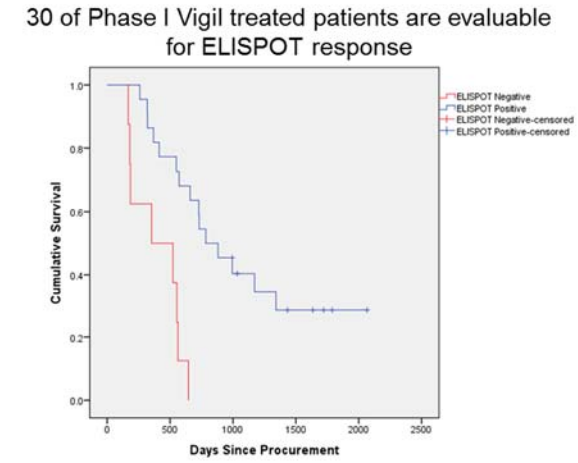


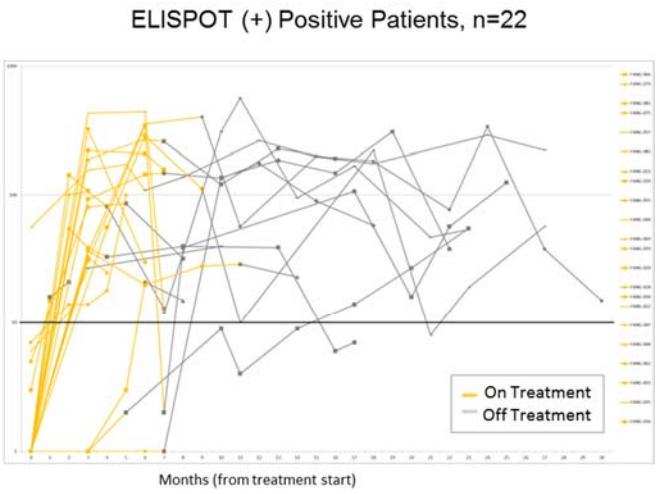
Figure 2. Long term survival by cancer type of patients entered into Vigil Phase I trial

Moreover, correlation of survival benefit with recent follow up involving the first 30 Phase I patients to receive Vigil to γIFN ELISPOT response which measures Vigil turned on circulating immune cells (T effector cells) against autologous tumor suggests efficacy tied to predicted mechanism of Vigil (**Figure 3**).



Group	N	Mean (months)	Median (months)	p-value
ELISPOT (-)	8	13.0	11.6	0.00019
ELISPOT (+)	22	35.8	25.7	

73% conversion rate (22/30 evaluable).

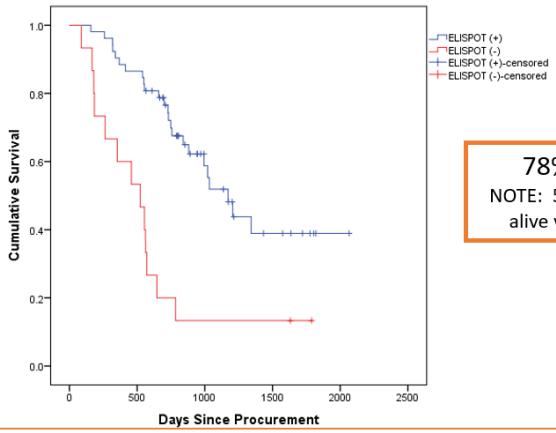


Senzer et al. J Vaccines & Vaccination 2013; 4(8):209

Figure 3. Correlated survival and immune response in first 30 patients entered into Vigil Phase I trial

These results have recently been updated. Sixty-seven of these Phase I patients have now undergone ELISPOT γIFN testing. Fifty-two demonstrated change from negative at baseline (prior to Vigil treatment) to positive γIFN ELIPSOT reactivity after Vigil. However, fifteen failed to show upregulation of circulating active effector T cells against autologous tumor induction by Vigil. Survival correlation continues to be demonstrated in this 4-year updated analysis (**Figure 4**).

67 patients evaluable for ELISPOT assay in Phase 1 of Vigil® in ST



78% ELISPOT conversion
NOTE: 55% ELISPOT (+) patients are still alive vs. 13% of ELISPOT (-) patients

Group	N	Mean (months)	Median (months)	p-value**
ELISPOT (-)	15	19.5	17.2	0.00015
ELISPOT (+)	52	42.2	38.5	

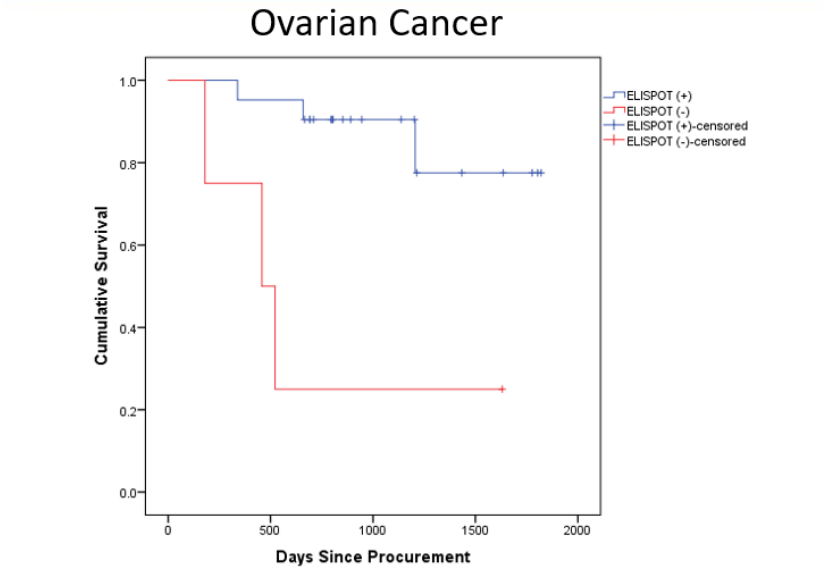
Data as of 02/26/17
Analysis performed on 02/27/17

**Log rank SPSS 22.0

Figure 4. Four-year update of all Phase I patients with γIFN ELISPOT analysis.

2.1 Vigil Phase II Trial: Ovarian Cancer

All ovarian cancer patients who underwent γIFN ELISPOT assay testing underwent a sub analysis from the Phase I population to validate the efficacy findings. Results demonstrated correlation with survival advantage in ovarian cancer patients in which Vigil induced above threshold levels of circulating γIFN expressive T cells to autologous tumor (**Figure 5**).

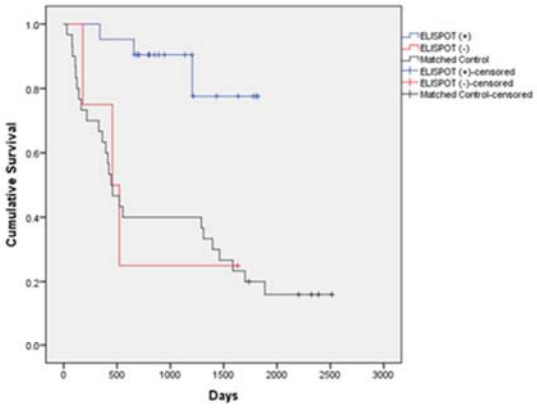


Group	N	Mean (mo)	Median (mo)	p-value*
ELISPOT (-)	4	22.9	15.0	0.003
ELISPOT (+)	21	53.1	Not reached	

Data as of 02/26/17
Analysis performed on 02/26/17
*Log Rank SPSS 22.0

Figure 5. Subset advanced ovarian population analysis of γIFN ELISPOT response to survival

A Mary Crowley Cancer Research Center registry database, randomly selected matched Phase I trial ovarian cancer patients who did not receive Vigil or TAG. This historical sub-analysis revealed survival advantage of Vigil ELISPOT positive patients to the historical control (MCCRC database). Additionally, ELISPOT negative patients had similar survival duration as the matched database (**Figure 6**). Thus, a 2:1 (Vigil:Control) randomized Phase IIa open-label trial of Vigil (tumor harvested at the time of surgical debulking), in patients with Stage III/IV ovarian cancer, who achieved clinical complete response (CR) following primary surgical debulking and standard chemotherapy (5-6 cycles, front-line maintenance setting), was initiated (CL-PTL-105). A 1.0 x 10^7 dose of transfected autologous tumor cells/intradermal injection was administered once a month for up to 12 doses to the treatment group. Standard of care was provided for the control patients. Crossover was allowed at time of relapse for patientsrandomized to standard of care therapy. Results have been published by Oh et al (Oh, Barve et al. 2016).



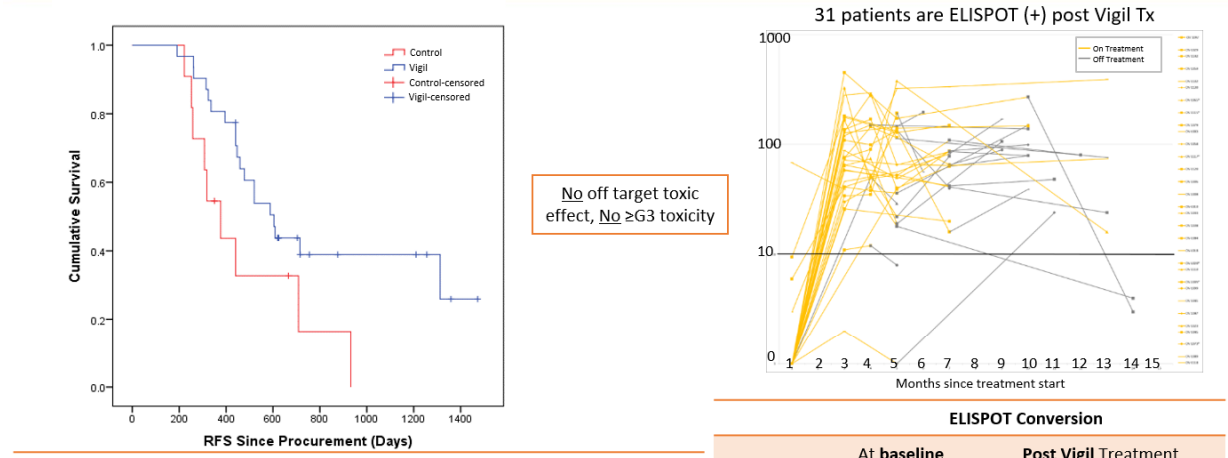
Group	N	No. of Deaths (%)	Mean (days)	Median (days)	p-value
Vigil ELISPOT (-)	4	3 (75%)	697	458	0.001
Vigil ELISPOT (+)	21	3 (14%)	1615	Not reached	
Matched Control	30	25 (83%)	934	445	

Data as of 02/27/17
Analysis performed on 03/03/17

Figure 6. Phase I Ovarian versus MCCRC Match Control Ovarian, Overall Survival

Twenty-one patients were randomized to Vigil, 11 to control standard of care. Discussion of these results lead to a larger (currently ongoing) Phase II/III trial design. Once the Phase II/III trial was initiated study CTL-PTL-105 was closed to further accrual and patients not yet randomized in consolidation chemotherapy section were allowed to enter into the Vigil arm. Thirty-one patients received Vigil as part of the Phase IIa trial (11 were randomized to control standard of care). Demographics revealed no significant differences between the 2 groups.

No ≥ Grade 3 adverse events were observed related to Vigil. Efficacy with respect to time to relapse induced immune response is shown in **Figure 7**. Median time to Vigil relapse by KM analysis (log rank) was 604 days vs. control standard of care, 377 days (p=0.033)



Group	N	Mean RFS (mo)	Median RFS (mo)	p-value
Control	11	15.8	12.4	HR 0.033 Log Rank p=0.039
Vigil	31	27.1	19.8	HR 0.43 95% CI (0.19, 0.96) Cox

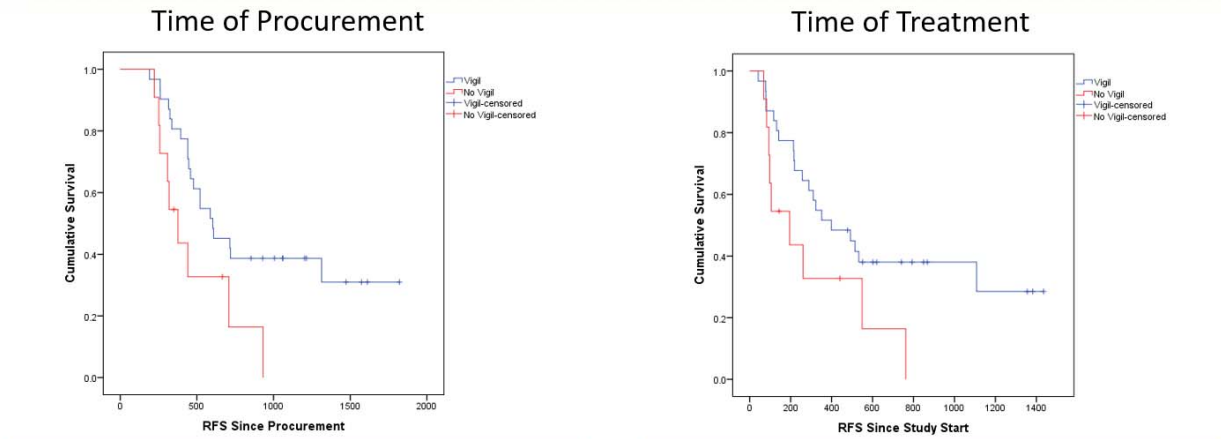
**Recurrence free survival from time of treatment. Mean/Median Control 304/195 days; Vigil 563/352 days; p=0.134

Data as of 04-12-16

ELISPOT Conversion	At **baseline**	**Post Vigil** Treatment
Vigil	1/31 (0.3%)	31/31 (100% conversion) median spots 134 (12-448)
Control	0/8	7/8 (crossover) median spots 58 (43-105)

Figure 7. Correlated survival of Vigil compared to control in Ovarian Cancer (CL-PTL 105) with immune responses

These positive results were associated with robust ELISPOT response (**Figure 7**). Less than 3% of ovarian patients had circulating T cells with capacity to release γIFN and induce autologous tumor necrosis at baseline (prior to Vigil), however following Vigil treatment 31/31 patients demonstrated brisk antitumor immune activity. No control patients demonstrated circulating T cell response against autologous tumor during or after standard of care, or at time of relapse. Ninety percent (90%, 7 of 8) demonstrated autologous tumoricidal activity of circulating T cells by γIFN ELISPOT response, after cross over. Interestingly, ELISPOT reactivity response was only 43% of what was observed with Vigil prior to relapse (134 spots vs. 58 spots). One year follow up analysis of all patients entered into study CL-PTL-105 was recently completed. Results demonstrate continued benefit (**Figure 8**).



Group	N	Mean (mo)	Median (mo)	p-value*
Control	11	15.8	12.4	0.025
Vigil	31	31.0	19.8	HR = 0.41 (95% CI: 0.183, 0.919)

*Log Rank SPSS 22.0, OV-1049 and OV-1074 were censored at the date of crossover.

Data as of 02/16/17
Analysis performed on 02/18/17

Group	N	Mean (mo)	Median (mo)	p-value
Control	11	10.0	6.4	0.058
Vigil	31	22.1	13.1	HR = 0.47 (95% CI: 0.210, 1.046)

*Log Rank SPSS 22.0, OV-1049 and OV-1074 were censored at the date of crossover.

Figure 8. Comparison of Phase II control ovarian cancer patients to Vigil RFS from time of procurement and time of treatment

2.2 Vigil Phase I Trial: Ewing's Sarcoma Subset

Initial results of 12 Ewing's Sarcoma Vigil treated patients entered into CL-PTL-101 were published two years ago (Ghisoli, Barve et al. 2015). A total of 30 Ewing's sarcoma patients have been enrolled into CL-PTL-101 with intent to treat and procurement for EWS Vigil manufacture. All patients had late stage Ewing's sarcoma (majority ≥ third line chemotherapy). Sixteen patients received Vigil. Fourteen concurrent patients (control) did not receive Vigil after undergoing similar surgery and Vigil construction. Nine of the latter were unable to have vaccine released (6 contaminants, 3 insufficient viable tumor cells) and 5 chose other treatment management. All products constructed fulfilled release criteria of GMCSF production and TGFβ1, β2 knockdown. See demographics in **Table 3**.

Table 3. Ewing's Sarcoma Phase I Demographics

	Vigil	**MC***
Tumor Location Harvest (Lung/Soft Tissue/Other)	13/0/3	11/2/1
Sex (M/F)	12/4	7/7
Age median (range)	19 (59-12)	17 (30-12)
Ethnicity (Caucasian/Other)	13/3	12/2
Prior Systemic Tx (Frontline/2nd/≥3rd)	1/5/10	3/4/7
Surgical Candidate (Yes/No)	16/0	14/0
Tissue Harvested (Yes/No)	16/0	14/0
* Matched Control (MC); 3 insufficient viable tumor cells, 6 contaminants, 5 sought other management		

Consistent with Phase I data in adults and Phase II data in ovarian cancer, no significant toxicity was observed in Phase I Vigil treated Ewing's sarcoma patients. Specifically, no product related Grade 3, 4 toxic effects were demonstrated during treatment course and no long term "post treatment" toxicity has been observed. AE's reported are shown in **Table 4**.

Table 4. Phase 1 (CL-PTL-101): Ewing's Sarcoma patients who received Vigil, definitely or probably related adverse events

Preferred Term	CTC Grade	Relationship to Study Drug	Number of Subjects	Number of Events
Bruising	1	Definitely Related	1	1
Erythema @ Injection Site	1	Definitely Related	1	1
Fatigue	1	Probably Related	1	4
Induration / Fibrosis Injection Site Reaction-Induration	1	Definitely Related	2	2
Injection Site Reaction – Induration	1	Definitely Related	1	2
Injection Site Reaction-Erythema	1	Definitely Related	11	31
Injection Site Reaction-Induration	1	Definitely Related	12	55
Injection Site Reaction- Pain	1	Definitely Related	3	3
Injection Site Reaction-Pruritus	1	Definitely Related	1	3
Injection Site Reaction-Swelling	1	Definitely Related	2	3
Injection Site Reaction-Tenderness	1	Definitely Related	1	1
Joint-function	1	Probably Related	1	2
Pain – Back	1	Probably Related	1	1

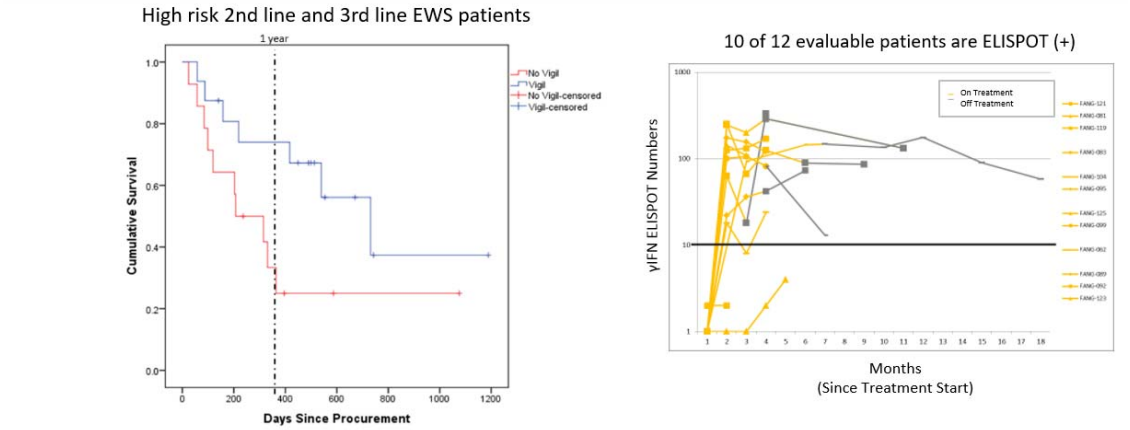
No Grade 3 or 4 related AE's were observed to Vigil. There were 11 SAEs reported involving 7 participants. None of the SAEs were related to Vigil.

2.3 Efficacy Evidence of Vigil in Ewing's Sarcoma

Survival advantage, however, was demonstrated to Vigil compared to the matched comparator.

Figure 9 shows the results of the Kaplan-Meier analysis of the survival data. Overall survival from time of procurement comparing these 2 concurrent groups (Vigil vs. No Vigil) revealed a 17+ month improvement in survival in the Vigil treated patients compared to the No Vigil control group (MC) from a mutually comparable time point,

time of tissue procurement (for Vigil construction). Focus on the Vigil group from time of treatment revealed a median survival of 689 days.



Group	N	Mean Survival (mo)	Median Survival (mo)	P-Value
Matched Control	14	13.4	6.8	0.056
Vigil*	16	23.1	24.0	

Data as of 10/19/15

*ORR 12.5% (1 NED, 1 PR)

83% ELISPOT + conversion

Figure 9. Survival from surgical procurement of advanced Ewing's patients in study CL-PTL-101 successfully harvested for Vigil construction. Comparing those who received Vigil vs. those who did not receive Vigil censored alive as of dates provided on 10/19/15.

The actual 1-year survival of patients who received Vigil (11/15) was 73% vs. those who did not receive Vigil of 23% (3/13). One Vigil and one MC patient remained alive but had not yet achieved actual 1 year survival time point. These results were recently published by Ghisoli et al (Ghisoli, Barve et al. 2016). Retrospective comparison of patient groups to two prognostic indication risk factor scores (Wheler score (Wheler, Tsimberidou et al. 2012) and, Ewing's sarcoma prognostic scores (ESPS) (Cotterill, Ahrens et al. 2000; Bacci, Longhi et al. 2006; Rodriguez-Galindo, Liu et al. 2007; Ladenstein, Pötschger et al. 2009; Jain and Kapoor 2010; Gaspar, Le Teuff et al. 2011; 2016)) suggest similar risk profiles of Vigil and control patients (**Table 5**).

Table 5. Prognostic Indicator Studies of Ewing's Sarcoma

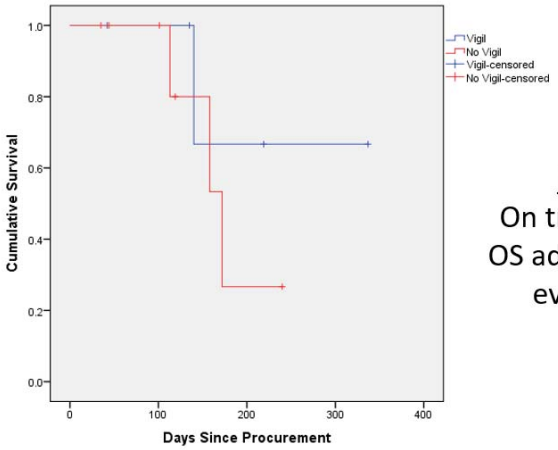
Ewing's Sarcoma Prognostic Scores (ESPS)◦				
Group	**Risk of Low Survival**			
	high	**intermediate**	**low**	**Grand Total**
MC	6	8	0	14
Vigil	6	10	0	16
16-21 points – high, 11-15 point – intermediate, 0-10 points - low				

Phase I Prognostic Scores*					
Group	**Risk of Low Survival**				
	high	**high-intermediate**	**intermediate**	**low-intermediate**	**Grand Total**
MC	0	1	8	5	14
Vigil	1	6	3	6	16
4-5 points – high, 3 points – high-intermediate, 2 points – intermediate, 1 points – low-intermediate, 0 point - low					

◦ (Cotterill, Ahrens et al. 2000; Bacci, Longhi et al. 2006; Rodriguez-Galindo, Liu et al. 2007; Ladenstein, Pötschger et al. 2009; Jain and Kapoor 2010; Gaspar, Le Teuff et al. 2011; 2016)
* (Wheler, Tsimberidou et al. 2012) MC = Matched Control

◦ ESPS incl. factor at diagnosis (tumor site, size, metastatic disease, etc), response to frontline therapy (time to relapse, etc)
* Phase 1 Prognostic Scores incl. factors like LDH, # of metastatic sites, albumin, etc.

A Phase IIb randomized open label trial (CL-PTL-121) comparing Vigil vs. standard of care chemotherapy (Gemcitabine/Taxol) in metastatic Ewing's Sarcoma patients was initiated and enrolled the first patient 13 months ago. Patients with refractory or recurrent disease failing ≥2 prior systemic treatment lines and undergoing surgical disease resection were targeted (for trial CL-PTL-121). The trial is open to enrollment at 10 sites (5 in development) across the USA but only 13 patients have registered into study. Eight were randomized to control and received Gemcitabine/Taxol and 5 received Vigil. No Grade 3 or greater toxicity to Vigil has been observed. Grade 3 or greater toxic events related to chemotherapy were observed in 2 Ewing's patients (hematologic, compromise, edema/facial blisters) and significant chemotherapy related toxicity of Grade 1 and 2 involving fever, fatigue, erythroderma, pruritus, neuropathy, nausea, vomiting was observed as expected. Early assessment of survival is shown in **Figure 10**. Kaplan Meier survival of 172 days was observed in the chemotherapy group while the Vigil (initial treatment) group has not yet reached survival median. Mean survival of the Vigil group is 271 days. This is consistent with the results observed with Phase I/II results in study CL-PTL-101. Futility analysis also revealed that the current trend of positive survival to Vigil is on track to achieve statistical significant survival advantage to Vigil at a 90% power (46 events). Further assessment of all advanced Ewing's patients who have received Vigil in studies CL-PTL-101 and CL-PTL-121 (21 patients) vs. matched controls (n=14) and randomized controls (total n=22) revealed further support of potential benefit to Vigil in third-line Ewing's Sarcoma (**Figure 11**). A Kaplan Meier survival of 524 (17.2 months) days was observed with Vigil.

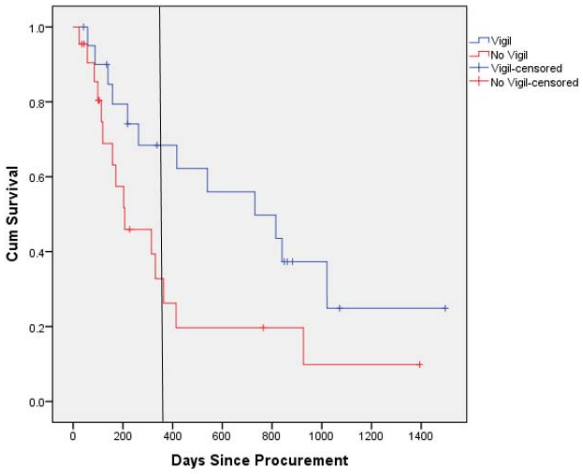


Futility Analysis:
On track to active goal of OS advantage to Vigil at 46 events (90% power)

Group	N	No. of Deaths	Mean OS (mo)	Median OS (mo)	p-value
RC*	8	3	5.7	5.7	0.395
Vigil	5	1	8.9	Not reached	

*RC=Randomized Control
Two crossover patients censored.

Figure 10. Overall Survival Since Procurement on Study CL-PTL-121 Preliminary Results. Two crossover patients censored. Data updated as of 01/19/17. *RC=Randomized Control



Group	N	Mean Survival (mo)	Median Survival (mo)	P- value (Log Rank)
MC/RC*	22	12.8	6.8	.041
Vigil	21	24.9	24.0	

*MC/RC = Matched Control (n=14) /Randomized Control (n=8)

Figure 11. Study CL-PTL-101 and 121 Combination Data of Ewing's Sarcoma. Overall Survival Since Procurement. Dataset updated 01/23/17. *MC/RC = Matched Control/Randomized Control

Additionally, a long-term follow up of one of the two responding Phase 1 Ewing's patients (**Figure 1**) (first patient response characterization is shown in **Figure 12**) revealed long term RECIST qualified response to Vigil. A complete regression related to a second course of Vigil therapy (Ghisoli, Rutledge et al. 2017) was recently confirmed. This patient had extensive disease initially and at rapid progression during frontline chemotherapy (TTR <2 years) underwent Vigil treatment with disease stabilization for > 6 months. Following recurrence in her lungs, she underwent a second Vigil construction and treatment for third-line disease management. Results yielded complete regression by PET scan. ELISPOT response was shown with both Vigil treatments but was shown (in retrospect) to have deteriorated following the first Vigil treatment with recovery in immune response following the second Vigil treatment using the second recurrent disease sample for construction (**Figure 1**). Overall, ten of 12 (83%) of Ewing's Sarcoma patients who received Vigil demonstrated marked elevation of effector T cells in circulation as demonstrated by γIFN ELISPOT assay (**Figure 13**) (Ghisoli, Barve et al. 2015; Ghisoli, Barve et al. 2016). The ELISPOT assessments have been independently validated by Mt. Sinai Human Immune Core Monitoring facility (involving Ewing's Sarcoma samples) (**Figure 14**). Interestingly, very low cell loads 15/5 x 10^3 PBMC's at a 1:3 ratio to the autologous tumor cell stimulant were required as the results were so highly positive that the utilized cell numbers had to be significantly diluted from standard ELISPOT routine in order to distinguish ELISPOTs on the assay.

Treatment History

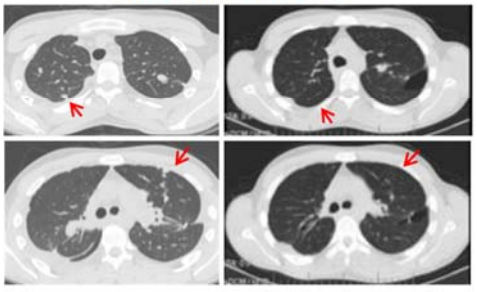
- Intensive chemotherapy from Oct 2008 – May 2009
- Intermittent radiation and experimental agents in 2010, 2011, and 2013

Vigil Ph1: Surgical resection of lung lesion (11/2013)

- 8 doses of Vigil administered Feb-Sept 2014

Patient had radiographically confirmed partial response

Radiology Results



02/19/14 05/19/14

Tumor Measurements

Date	Result
4/08/14	25%↓ target lesions
5/19/14	38%↓ target lesions
7/10/14*	35%↓ target lesions
1/09/2015	Disease Progression

*All non targeted lesions ↓ or completely gone

ELISPOT Conversion

ELISPOT	Positive Spots
Baseline (2/26/2014)	0
Month 2 (3/24/2014)	174
Month 3 (4/21/2014)	155

Figure 12. Case Report 2 (F-089). Relapsed Ewing's Sarcoma Response to Vigil

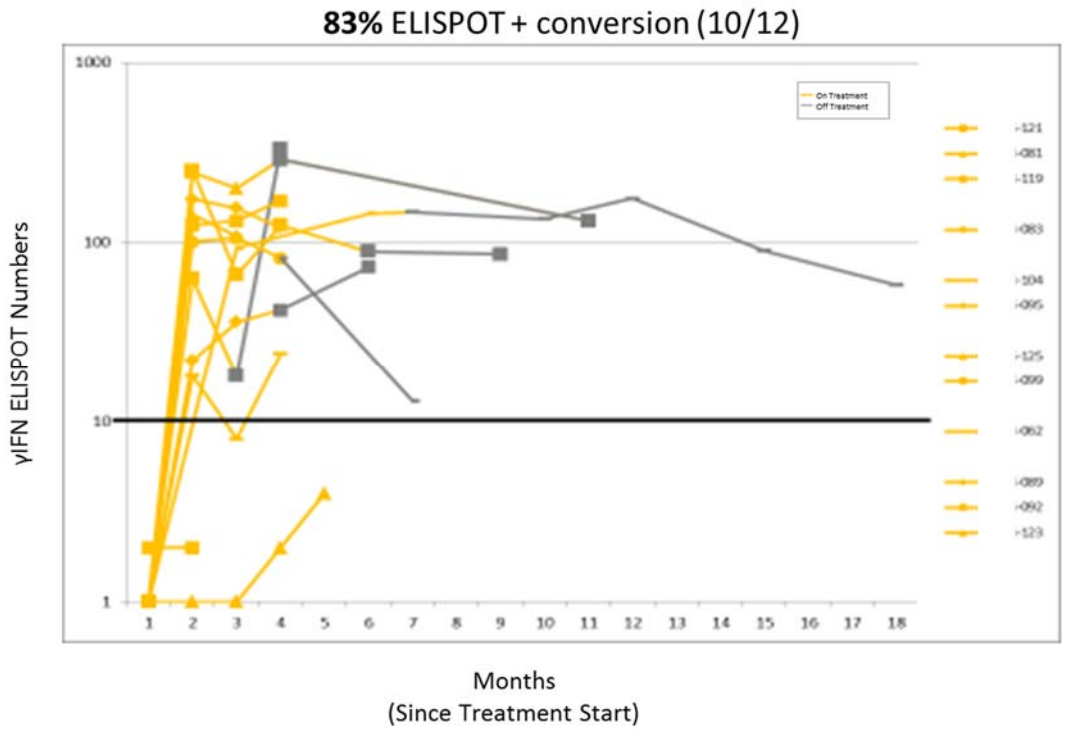


Figure 13. Immune Response by γIFN ELISPOT Assay Related to Vigil.

	Mt Sinai* ZellNet Reader			Gradalis* ZellNet Reader		
Ewing's 123 before Vigil	2	2	2	0	1	0
Ewing's 123 Month 3 after Vigil	54	244	150	247	283	167
Ovarian 1041 before Vigil	0	0	1	0	0	0
Ovarian 1041 Month 2 after Vigil	18	34	15	69	60	91

Figure 14. Mt Sinai – Gradalis Lab: Concurrent Comparison of γIFN ELISPOT Response. Autologous Tumor Cells (pre-transfection) and PBMCs. *ZellNet Consulting, Inc (Fort Lee, NJ) third party spot read out. Results based on 3:1 ratio tumor to PBMC's ($15 \times 10^3/5 \times 10^3$ cells)

3.0 STUDY RATIONALE

The results from the Phase I Vigil trial supported high confidence in safety (both short-term and long-term through 3+ years), confirmed effective transgene expression (GM-CSF) and RNAi [furin] silencing, and supported the triad rationale autologous tumor cell therapy with increased GMCSF expression and decreased TGFβ production, to modulate immunogenicity as shown by the correlation of IFNγ-ELISPOT responsiveness with overall survival (Senzer, Barve et al. 2012; Senzer, Barve et al. 2013). The preliminary evidence of safety, immune stimulation and clinical benefit from Vigil in patients with ESFT treated in the Phase I setting supports evaluation of this therapy in a larger and randomized study and at earlier stage of recurrence (first recurrence). Less tumor burden is typically associated with greater delay in progression with other immunotherapies. Thus, better control of delayed disease progression with increased response to chemotherapy (second line treatment involving Temozolomide/Irinotecan) in concurrence with Vigil immunotherapy in first relapse patient population is suggested. Median time to progression of the Phase I EWS patients (third-line) was approximately 6 months compared to historical experience of similar patients of 2 months (Rodriguez-Galindo, Billups et al. 2002; Bacci, Ferrari et al. 2003; Shankar, Ashley et al. 2003; Barker, Pendergrass et al. 2005; McTiernan, Cassoni et al. 2006; Leavey, Mascarenhas et al. 2008; Juergens, Daw et al. 2011; Stahl, Ranft et al. 2011; Tap, Demetri et al. 2012; Choy, Butrynski et al. 2014; Rasper, Jabar et al. 2014; Ghisoli, Barve et al. 2016). Thus, Part 2 combination of Vigil/Temozolomide/Irinotecan is justified.

4.0 PART 1 OBJECTIVES

4.1 Primary objective(s)

- To determine and compare the overall survival of patients with metastatic Ewing's sarcoma refractory or intolerant to ≥ 2 prior lines of systemic chemotherapy treated with Vigil immunotherapy vs. gemcitabine/docetaxel.

4.2 Secondary objective(s)

- To determine the γIFN ELISPOT conversion rate of subjects treated with Vigil immunotherapy vs gemcitabine/docetaxel.
- To determine and compare the proportion of patients surviving 1 year when treated with Vigil immunotherapy vs gemcitabine/docetaxel.
- To determine and compare the overall survival of patients with metastatic Ewing's sarcoma refractory or intolerant to ≥ 2 prior lines of systemic chemotherapy who are IFNγ ELISPOT negative at baseline and are treated with Vigil immunotherapy vs. gemcitabine/docetaxel.

- To determine and compare the progression free survival of patients with metastatic Ewing's sarcoma refractory or intolerant to ≥ 2 prior lines of systemic chemotherapy treated with Vigil immunotherapy vs gemcitabine/docetaxel.
- To determine and compare the objective response rate (RECIST 1.1) of patients with metastatic Ewing's sarcoma refractory or intolerant to ≥ 2 prior lines of systemic chemotherapy treated with Vigil immunotherapy vs gemcitabine/docetaxel
- To determine and compare the safety profile of Vigil immunotherapy vs gemcitabine/docetaxel in patients with relapsed or refractory Ewing's sarcoma.

5.0 PART 2 OBJECTIVES

5.1 Primary objective

- To determine safety profile of Vigil immunotherapy in combination with irinotecan and temozolomide in patients with metastatic Ewing's sarcoma refractory or intolerant to at least 1 prior line of systemic chemotherapy.

5.2 Secondary Objectives

- To determine the γIFN ELISPOT conversion rate of patients dosed with Vigil immunotherapy in combination with irinotecan and temozolomide.
- To determine the progression free survival of patients dosed with Vigil immunotherapy in combination with irinotecan and temozolomide.
- To determine the overall survival of patients with relapsed or refractory Ewing's sarcoma who are IFNγ ELISPOT negative at baseline and are dosed with Vigil immunotherapy in combination with irinotecan and temozolomide.
- To determine the objective response rate (RECIST 1.1) of patients with metastatic Ewing's sarcoma refractory or intolerant to at least 1 prior line of systemic chemotherapy treated with Vigil immunotherapy dosed with Vigil immunotherapy in combination with irinotecan and temozolomide.

6.0 STUDY DESIGN

Part 1

This is a multicenter, 1:1 randomized Phase IIb study of intradermal autologous Vigil immunotherapy (1.0 x 10^7 cells/injection; minimum of 4 to a maximum of 12

administrations) versus gemcitabine / docetaxel in patients with metastatic Ewing's sarcoma Family of Tumors (ESFT) refractory or intolerant to at least 2 prior lines of chemotherapy. Patients undergoing a standard surgical procedure (e.g., tumor biopsy or palliative resection) may have tumor tissue harvested for manufacture of investigational product. Patients meeting eligibility criteria including manufacture of a minimum of 4 immunotherapy doses will be randomized to receive either (1) intradermal Vigil every 28 days for 4-12 administrations, or (2) gemcitabine 675 mg/m^2 IV at 10 mg/m^2/min D1 and D8 and docetaxel 75 mg/m^2 IV D8 every 21 days. The primary trial objective is to determine the overall survival of patients treated with Vigil versus gemcitabine/docetaxel. Randomization may occur as early as vaccine is released (typically 3 - 4 weeks following tumor procurement) but must occur no later than 8 weeks following tumor procurement. Randomization of patients will be stratified by Karnofsky Performance Status (KPS) ≥ 80% vs < 80%.

Patients will be managed in an outpatient setting. Hematologic function, liver enzymes, renal function and electrolytes will be monitored monthly. Blood for immune function analyses including IFNγ-ELISPOT analysis of cytotoxic T cell response to autologous tumor antigens will be collected at tissue procurement, baseline and and prior product administration at Cycles 2, 4, end of treatment, and every 6 months thereafter.

Part 2

Based on the limited accrual to Part 1 of this study, Gradalis is opening Part 2 of this clinical protocol to assess the safety of Vigil immunotherapy in combination with irinotecan and temozolomide. Part 2 will be conducted at the same centers as Part 1, studying intradermal autologous Vigil cancer vaccine (1.0 x 10^7 cells/injection; minimum of 4 to a maximum of 12 administrations) in patients with metastatic Ewing's sarcoma Family of Tumors (ESFT) refractory or intolerant to at least 1 prior line of chemotherapy. Patients undergoing a standard surgical procedure (e.g., tumor biopsy or palliative resection) may have tumor tissue harvested for manufacture of investigational product. Patients meeting eligibility criteria including manufacture of a minimum of 4 immunotherapy doses of Vigil will be registered to receive: (i) oral temozolomide 100 mg/m^2 daily (Days 1 – 5, total dose 500 mg/m^2/cycle), (ii) irinotecan 50 mg/m^2 daily (Days 1 – 5, total dose 250mg/m^2/cycle), orally or irinotecan 20mg/m^2 daily (Days 1 – 5, total dose 100mg/m^2/cycle), intravenously (iii) peg-filgrastim 100µg/kg (Day 6) subcutaneously (optional and may be administered at home), and Vigil 1.0 x 10^7 cells/injection, intradermally on Day 15 and every 3 weeks thereafter. One cycle = 21 days. Registration onto Part 2 may occur as early as one week but no later than 8 weeks following tumor procurement. Vigil is typically released approximately 3 weeks after the completion of the two-day manufacturing process.

Patients will be managed in an outpatient setting. Hematologic function, liver enzymes, renal function and electrolytes will be monitored. Blood for immune function analyses including IFNγ-ELISPOT analysis of cytotoxic T cell response to autologous tumor antigens will be collected at tissue procurement, and prior to Day 15 Vigil

administration at Cycles 2, 4, end of treatment, and every 6 months thereafter. Blood for ctDNA analysis will be collected prior to chemotherapy administration at baseline, Cycle 2 – Week 1 Day 1, Cycle 4 – Week 1 Day 1, and EOT.

7.0 STUDY POPULATION

7.1 Sample Size

Approximately 62 patients will be enrolled into Part 1. Approximately 6 to 9 patients will be enrolled into Part 2.

7.2 Tissue Procurement Inclusion Criteria

Patients will be eligible for tissue procurement for the Vigil manufacturing process, if they meet all of the following criteria:

1. Histologically confirmed Ewing's Sarcoma Family of Tumors (ESFT).
2. Age ≥2 years.
3. Estimated survival ≥ 6 months.
4. Evidence of EWS translocation by FISH or RT-PCR or Next Generation Sequencing (NGS).
5. Metastatic disease
6. Refractory or intolerant to ≥ 2 prior lines of systemic chemotherapy (Part 1) or Refractory or intolerant to at least 1 line of systemic chemotherapy (Part 2).
7. Planned standard of care surgical procedure (e.g., tumor biopsy or palliative resection or thoracentesis) and expected availability of a cumulative mass of ~10-30 grams tissue ("golf-ball" size) for or pleural fluid estimated volume ≥ 500mL (must be primary tap) immunotherapy manufacture.
8. Tumor intended for immunotherapy manufacture is not embedded in bone and does not contain luminal tissue (e.g. bowel, ureter, bile duct).
9. Ability to understand and the willingness to sign a written informed consent document for tissue harvest.

7.3 Tissue Procurement Exclusion Criteria

Patients meeting any of the following criteria are not eligible for tissue procurement for the Vigil manufacturing:

1. Medical condition requiring any form of chronic systemic immunosuppressive therapy (steroid or other) except physiologic replacement doses of hydrocortisone or equivalent (no more than 30 mg hydrocortisone or 10 mg prednisone equivalent daily) for < 30 days duration

2. Known history of other malignancy unless having undergone curative intent therapy without evidence of that disease for ≥ 3 years **except** cutaneous squamous cell and basal cell skin cancer, superficial bladder cancer, in situ cervical cancer or other in situ cancers are allowed if definitively resected.
3. Brain metastases unless treated with curative intent (gamma knife or surgical resection) **and** without evidence of progression for ≥ 2 months.
4. Any documented history of autoimmune disease with exception of Type 1 diabetes on stable insulin regimen, hypothyroidism on stable dose of replacement thyroid medication, vitiligo, or asthma not requiring systemic steroids.
5. Known history of allergies or sensitivities to gentamicin.
6. Known hypersensitivity reactions to docetaxel or to other drugs formulated with polysorbate 80 that would preclude treatment with docetaxel (Part 1 only).
7. History of or current evidence of any condition (including medical, psychiatric or substance abuse disorder), therapy, or laboratory abnormality that might confound the results of the study, interfere with the patient's participation for the full duration of the study, or is not in the best interest of the patient to participate, in the opinion of the treating Investigator.
8. Known HIV or chronic Hepatitis B or C infection.

7.4 Study Enrollment Inclusion Criteria

Patients will be eligible for registration if they meet all of the following inclusion criteria:

1. Successful manufacturing of at least 4 vials of Vigil.
2. Karnofsky performance status (KPS) ≥60% (Part 1) or KPS ≥80% (Part 2).
3. Estimated survival ≥ 4 months (Part 1) or estimated survival of ≥6 months (Part 2).
4. Normal organ and marrow function as defined below:

Absolute granulocyte count	≥1,500/mm^3
Absolute lymphocyte count	≥400/mm^3
Platelets	≥100,000/mm^3
Total bilirubin	≤ institutional upper limit of normal
AST(SGOT)/ALT(SGPT)	≤2x institutional upper limit of normal
Creatinine	<1.5 mg/dL

5. Subject has recovered to CTCAE Grade 1 or better from all adverse events associated with prior therapy or surgery. Pre-existing motor or sensory neurologic pathology or symptoms must be recovered to CTCAE Grade 2 or better.
6. If female of childbearing potential, has a negative urine or serum pregnancy test. If the urine test is positive or cannot be confirmed as negative, a negative serum test will be required for study entry.
7. Ability to understand and the willingness to sign a written informed protocol specific consent document.

7.5 Study Enrollment Exclusion Criteria

In addition to the procurement exclusion criteria, patients will NOT be eligible for study registration and randomization if meeting any of the following criteria:

1. Any anti-neoplastic therapy between tissue procurement for Vigil manufacture and start of study therapy.
2. Live vaccine used for the prevention of infectious disease administered < 30 days prior to the start of study therapy.
3. Post-surgery complication that in the opinion of the treating investigator would interfere with the patient's study participation or make it not in the best interest of the patient to participate.

7.6 Withdrawal

Discontinuation of Study Treatment

Patients will discontinue **study treatment** if any of the following occur:

1. Disease progression by RECIST 1.1 criteria according to investigator assessment.
2. The patient experiences unacceptable (≥Grade 3) toxicity felt to be related to treatment with the Vigil immunotherapy that persists for >2 weeks. (Part 1 or Part 2)
3. The patient experiences unacceptable (≥Grade 3) toxicity felt to be related to treatment with gemcitabine / docetaxel that persists for >2 weeks. Vigil therapy may be continued in monotherapy. (Part 1)
4. The patient experiences unacceptable (≥Grade 3) toxicity felt to be related to treatment with irinotecan/temozolomide that persists for >2 weeks. Vigil therapy may be continued in monotherapy. (Part 2)
5. Persisting Grade 3 or 4 toxicity unrelated to treatment, defined as failing to normalize within 4 weeks.
6. Any Grade ≥ 3 allergic reactions related to the study agent.
7. Grade 2 autoimmune reactions unless there is evidence of clinical benefit.
8. 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.
9. Cancer therapy other than protocol treatment.
10. Non-compliant with protocol or treatment.
11. Patient refuses to continue treatment.

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

8.0 INVESTIGATIONAL PLAN

8.1 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. Study participation begins once written informed consent is obtained. Patients will be assigned a study identification number upon completion of the screening process.

Two to eight weeks after tissue procurement has occurred eligibility will be reconfirmed by the study site. The Research Nurse or Clinical Research Coordinator will register the patient.

Please allow 48 hours for patient registration as the medical monitor may review the subject's source documents to ensure they meet the eligibility criteria. Once confirmed, the site will be notified of the eligibility (Part 1 and 2) and randomization (Part 1 only) by the Contract Research Organization.

8.2 Tumor Procurement

Refer to the Tissue Procurement Manual for instructions.

The cumulative equivalent of a "golf ball size" mass (~10-30 gm tissue) is optimal for vaccine manufacturing. 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 (<10 gm) tumor masses. **Lesions extending into lumen (e.g., bowel) or tumor embedded in bone cannot be processed**.

Once the procured tissue is received at Gradalis, Inc. samples will be processed for autologous vaccine manufacture.

8.3 Vigil Immunotherapy Manufacturing

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

No tissue or vaccine will be given to the participant or site apart from the outlined clinical protocol.

Any excess tumor tissue, not used for vaccine manufacture will be used towards Vigil vaccine research and process development assays.

8.4 Study Treatment Administration

Treatment will be administered on an outpatient basis.

Schedule, Dose and Administration

Part 1

Subjects will be randomized to receive Vigil or gemcitabine / docetaxel according to the schedule outlined in Appendix B.

Subjects randomized to Vigil will receive 1.0 x 10^7 cells via intradermal injection every 4 weeks for a minimum of 4 administrations to a maximum of 12 administrations depending on quantity of Vigil manufactured from surgical specimens and so long as the patient is clinically stable and without disease progression.

The sites of injection for Vigil 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 will be used. Subjects receiving Vigil will be observed for at least 30 minutes following administration.

OR

Patients randomized to gemcitabine / docetaxel will receive gemcitabine 675 mg/m^2 IV at a rate of 10mg/m^2/min (e.g., 675 mg/m^2 should run over 67.5 minutes) on D1 and Day 8, and docetaxel 75 mg/m^2 IV D8 every 21 days.

Drug	Dose	Schedule
Gemcitabine	675 mg/m^2 IV at a rate of 10mg/m^2/min	Day 1 and Day 8 every 21 days
Docetaxel	75 mg/m^2 IV	D8 every 21 days

Part 2

Subjects will receive Vigil at 1.0 x 10^7 cells via intradermal injection every 3 weeks for a minimum of 4 administrations to a maximum of 12 administrations depending on quantity of Vigil manufactured from surgical specimens and so long as the patient is clinically stable and without disease progression.

The sites of injection for Vigil 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 will be used. Subjects receiving Vigil will be observed for at

least 30 minutes following administration. Vigil will be given in combination with Irinotecan and Temozolimide as outlined below.

Drug	Dose	Schedule
Temozolomide	100 mg/m^2 daily, oral	Days 1 – 5, every 21 days
Irinotecan	50 mg/m^2 daily, oral OR 20 mg/m^2 daily, IV	Days 1 – 5, every 21 days
peg-filgrastim (optional)	100µg/kg, subcutaneous injection	Day 6, every 21 days *may be administered at home*
Vigil	1.0 x 10^7 cells/injection, intradermal	Day 15, every 21 days

Temozolomide, irinotecan and peg-filgrastim should be administered and adjusted per package insert and institutional standards.

Premedications

Vigil: EMLA® may be utilized at the injection site prior to Vigil administration. Analgesics may be employed as necessary. As noted above, systemic steroids or other immunosuppressents should be avoided due to immune inhibition activity.

Gemcitabine / docetaxel: Per package insert and institutional standards. To reduce the frequency and severity of fluid retention, it is recommended to administer dexamethasone 8mg PO bid for 3 days starting one day prior to each docetaxel administration (minimum of 3 doses). (Oral suspensions / modifications are acceptable).

Temozolomide / irinotecan / peg-filgrastim: Per package inserts and institutional standards.

Vigil Immunotherapy Transfer

All manufactured Vigil 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 subject administration.

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

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

Dose Modification for Vigil Toxicity

If > Grade 2 toxicity by NCI Common Toxicity Criteria (excluding Grade 3 injection site reactions) develops related to study treatment the vaccine dose will be reduced by 50% and continued.

Vigil Treatment Delay

- Treatment may be delayed no more than 4 weeks to allow recovery from toxicity.
- Subjects who delay treatment for more than 4 weeks due to toxicities will be considered off study treatment (see Section 5.4 Withdrawal).
- Treatment delay not related to toxic events (including subjects unable to adhere to monthly/3 week cycle injection) may not extend for more than three days unless due to symptoms related to disease or infection in which case up to a 2 week delay is allowed.
- If ≥ one 2 week delay due to disease or infection occurs, subject status must be reviewed by sponsor.
- If subjects miss doses, the doses will be made up the following week and continue on a revised monthly/ every 3 week schedule thereafter.

Dose Modifications for gemcitabine, docetaxel, temozolomide, irinotecan, and peg-filgrastim

Dose modifications for gemcitabine, docetaxel, temozolomide, irinotecan, and peg-filgrastim may be necessary for subject safety and should be carried out in accordance with the Investigator's standard of practice and the package inserts and institutional standards.

If irinotecan + temozolomide is administered beyond 12 months, it will be administered off study. If irinotecan + temozolomide is discontinued, Vigil may be administered every 3 weeks until all manufactured product is exhausted or disease progression is noted.

8.5 Concomitant Medications and Supportive Care

The following medications and interventions, unless otherwise specified, are prohibited from the time of study screening until the End of Treatment visit:

Systemic anti-cancer therapy, including chemotherapy, radiotherapy, or endocrine therapy other than those required per protocol are prohibited from the time of study screening until the End of Treatment visit.

Any investigational drug or device other than Vigil is prohibited from the time of study screening until the End of Treatment visit.

Systemic—oral, IV, injectable—corticosteroids (e.g., dexamethasone) should be avoided in subjects who are administered Vigil. If deemed by the investigator to be necessary, short term (<30 days) systemic steroids ≤ 0.25 mg/kg (max 10mg) prednisone-equivalent per day and inhaled steroids are permitted while on protocol. Other steroid regimens and/or immunosuppressives are prohibited.

Subjects should be provided with full supportive care measures, as clinically indicated, and in accordance with institutional standards. Such care includes medication for pain control and symptom management, antibiotics, bisphosphonates, antiemetics, colony stimulating factors, and transfusions of blood or blood products. Prophylactic GCSF is allowed for the chemotherapy arm. Treatment for drug-related adverse events should be administered at the discretion of the investigator.

Localized radiotherapy is permitted for palliation of painful lesions at the investigator's discretion. However, medical management in place of radiation therapy should be used if clinically appropriate.

Inactivated vaccinations are permitted 2 weeks post administration of investigational product. Nasal flu vaccine is prohibited.

8.6 Toxicity

Toxicities will be graded and reported according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE) Version 4.03 as linked in Appendix C. This document can also be downloaded from the Cancer Therapy Evaluation Program (CTEP) home page <http://ctep.cancer.gov>.

Adverse events will be summarized using the MedDRA coding system or higher. The NCI-CTCAE will be used for AE grading. All AEs, regardless of severity, will be followed by the Treating Physician until resolution is satisfactory.

8.7 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 to explain the reason for this protocol deviation.

Prestudy Assessments

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

1. A signed Informed Consent Form for tissue harvest must be obtained.
2. It has been confirmed that the subject meets all tissue procurement inclusion criteria.
3. A complete medical history must be obtained.
4. A physical examination must be obtained.
5. 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).
6. Routine pre-operative serum chemistries (including but not limited to creatinine, total bilirubin, alkaline phosphatase, and aspartate transaminase (AST) and/or alanine transaminase (ALT).
7. PBMC collection for immune function analysis will be obtained at tissue harvest. (≤24 hours of tissue procurement)

The following evaluations will be performed on all subjects within 2 weeks of randomization (in Part 1) or within 2 weeks of registration (in Part 2) (unless otherwise specified):

1. A signed protocol specific Informed Consent Form must be obtained.
2. It has been confirmed that the subject 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, height, temperature and body weight) must be obtained.
5. Assessment of concomitant medications
6. Assessment of PS on the Karnofsky scale (see Appendix A) must be obtained.
7. Radiological assessment of disease status with CT chest /abd/pelvis (MRI abd/pelvis may be substituted for CT abd/pelvis) 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. Radiographic assessments must be from after the tissue harvest for vaccine manufacture to ensure true baseline disease status is captured.
8. 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).
9. Serum chemistries (creatinine, glucose, total protein, blood urea nitrogen (BUN), total carbon dioxide (CO2), albumin, total bilirubin, alkaline phosphatase, and aspartate transaminase (AST) and/or alanine transaminase (ALT)) and electrolytes (total calcium, chloride, potassium, sodium) must be performed.

10. PBMC collection for immune function analysis.
11. Plasma collection for immune function analysis (ctDNA) (Part 2).
12. Pregnancy test for those of childbearing potential.
13. Female subjects of childbearing potential must consent to use a medically acceptable method of highly effective contraception (oral hormonal contraceptive, condom plus spermicide, or hormone implants) throughout the study period and for 28 days after their final autologous Vigil administration. A method of contraception must be employed by all subjects (male and female).
14. Submission to Gradalis, Inc of 20 unstained tumor slides or tissue block for correlative immunohistochemistry assay. These slides should correlate with tumor procured for vaccine manufacture.

Assessments During Treatment

The following evaluations will be performed once at the beginning of each cycle (1 Cycle = 28 days ±3 days for Vigil treated subjects and 1 Cycle = 21 days ±3 for gemcitabine/docetaxel treated patients in Part 1); (1 Cycle = 21 days ±3 days for Vigil + temozolomide + irinotecan treated subjects in Part 2) (unless otherwise specified):

1. A physical examination, including vital signs and body weight.
2. A toxicity assessment (CTCAE v 4.03).
3. Assessment of concomitant medications.
4. Radiological assessment of disease status with CT chest /abd/pelvis (MRI abd/pelvis may be substituted for CT abd/pelvis) every 12 weeks ± 7 days. 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.
5. A CBC with differential and platelet count.
6. Serum chemistries (creatinine, glucose, total protein, blood urea nitrogen (BUN), total carbon dioxide (CO2), albumin, total bilirubin, alkaline phosphatase, and aspartate transaminase (AST) and/or alanine transaminase (ALT)) and electrolytes (total calcium, chloride, potassium, sodium).
7. PBMC collection for immune function analysis prior to Vigil or chemotherapy administration at Cycles 2, and 4.
8. PBMC collection for immune function analysis prior to Day 15 Vigil administration at Cycles 2, and 4 (Part 2).
9. Plasma collection for immune function analysis (ctDNA) prior to chemotherapy administration at Cycle 2 – Week 1 Day 1 and Cycle 4 – Week 1 Day 1 (Part 2).
10. Assessment of Karnofsky Performance Status.
11. For those randomized to Vigil, Vigil administration. The subject will be observed for at least 30 minutes. Day 2 post Vigil administration assessment of injection site. Instruct the subject on Day 1 to observe the injection site 24 hours after the product administration. (This may be conducted at home). Have the subject follow up with the clinic if an injection site reaction is present. Please note if the

subject reports of any redness, swelling or other response. If the subject identifies any redness, and /or swelling, please ask the subject if it is feasible to take a photograph of the injection site. If photographing the injection site is possible, please instruct the subject to place a measuring tool (bulk supplies provided to the site) next to the injection site reaction in the picture, if feasible. The photograph may be provided to the clinic upon the next visit as a hard copy or electronic jpg image. Please instruct the participant to avoid capturing images that would identify the subject (i.e. face and head). This is applicable to those who receive Vigil in either Part 1 or Part 2 of the protocol.

12. For those randomized to chemo in Part 1: gemcitabine 675 mg/m^2 IV administered at a rate of 10mg/m^2/min on D1 and D8 and docetaxel 75 mg/m^2 IV D8 every 21 days.
13. For those registered into Part 2 of the study: (i) (i) oral temozolomide 100 mg/m^2 daily (Days 1 – 5, total dose 500 mg/m^2/cycle), (ii) irinotecan 50 mg/m^2 daily (Days 1 – 5, total dose 250mg/m^2/cycle), orally OR irinotecan 20mg/m^2 daily (Days 1 – 5, total dose 100mg/m^2/cycle), intravenously (iii) peg-filgrastim 100µg/kg (Day 6) subcutaneously (optional and may be administered at home), and (iv) Vigil 1.0 x 10^7 cells/injection, intradermally on Day 15 and every 3 weeks thereafter.

End of Treatment Assessments

The following evaluations will be performed within 30 days after completion of the study agent or chemotherapy regimen and / or within 30 days of disease progression (whichever event occurs first) (unless otherwise specified):

1. A physical examination, including vital signs and body weight.
2. Toxicity assessment (adverse events).
3. Assessment of concomitant medications taken.
4. Assessment of Karnofsky Performance Status
5. Radiological assessment of disease status with CT chest /abd/pelvis (MRI abd/pelvis may be substituted for CT abd/pelvis) within 45 days of the last injection or disease recurrence or progression
6. A CBC with differential and platelet count.
7. Serum chemistries (creatinine, glucose, total protein, blood urea nitrogen (BUN), total carbon dioxide (CO2), albumin, total bilirubin, alkaline phosphatase, and aspartate transaminase (AST) and/or alanine transaminase (ALT)) and electrolytes (total calcium, chloride, potassium, sodium).
8. PBMC for immune function analysis.
9. Plasma for immune function analysis (ctDNA) (Part 2).

Response Follow Up Assessments

If the study agent 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 subject will be followed every 3 months after the end of study visit until progression.

The following evaluations will be performed quarterly (every 3 months ±7 days) (unless otherwise specified):

1. A physical examination, including vital signs and body weight.
2. Assessment of concomitant medications taken.
3. Radiological assessment of disease status with CT chest /abd/pelvis (MRI abd/pelvis may be substituted for CT abd/pelvis) must be collected. 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.
4. A CBC with differential and platelet count.
5. Serum chemistries (creatinine, glucose, total protein, blood urea nitrogen (BUN), total carbon dioxide (CO2), albumin, total bilirubin, alkaline phosphatase, and aspartate transaminase (AST) and/or alanine transaminase (ALT)) and electrolytes (total calcium, chloride, potassium, sodium).
6. PBMCs for immune function analysis every 6 months from EOT.
7. Assessment of Performance Status.

Long Term Follow Up

After progression, subjects and their physicians will be contacted quarterly for documentation of survival status.

As the intent-to-treat population includes all randomized patients, should a subject be randomized and choose to not receive any treatment on this study, the subject should be asked if he or she is willing to allow for survival follow up through phone call and/or physician contact quarterly. This follow up would include any anti-cancer therapies received and survival status information.

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.

9.0 CONDUCT OF THE STUDY

9.1 Ethics and Regulatory Considerations

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

9.2 IRB

This trial can be undertaken only after review and full approval of the protocol and a 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 Informed Consent Form, and any applicable recruiting materials and subject compensation programs approved.

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

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

- All protocol amendments and Informed Consent Form revisions.
- Reports of all Serious Adverse Events.

The PI provides Gradalis, Inc. with the necessary assurance that an IRB 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 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 that the trial has been completed.

9.3 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 subjects 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 subjects' participation is voluntary, and that they may withdraw consent to participate at any time. They will also be informed that if subjects choose not to participate in the trial alternative treatments are available; such refusal will not prejudice further treatment of their disease. Potential subjects or their legal representatives must be given the opportunity to ask questions about the trial protocol and the procedures involved.

Finally, each subject will be told that his or her records may be accessed by authorized personnel of Gradalis, Inc. and other authorized individuals without violating the subject's confidentiality, to the extent permitted by the applicable laws and/or regulations. By signing the written Informed Consent Form, the subject 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 Informed Consent Form must be obtained from each subject or his or her legal representative; a copy will be given to the person signing the form.

9.4 Confidentiality of Records

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

9.5 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.

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 will be notified of administrative changes according to applicable IRB guidelines.

9.6 Protocol Questions and Deviations

When evaluating a potential patient or while a patient is on study, protocol questions can be directed to the CRO via email or phone using the contact information provided in the Contact Information section of the Study Reference Manual.

9.7 Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) will be established for this trial. Membership will include physicians with appropriate areas of expertise in the therapeutic areas of most interest. A charter will be developed to document their operational methods and timing of safety reviews.

10.0 EVALUATION OF TUMORS

10.1 Tumor Measurements and Response (RECIST 1.1)

Response and progression will also be evaluated in this study using the Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1. The guidelines are available online at: European Organization for Research and Treatment of Cancer (EORTC) RECIST Web page, https://www.eortc.be/Recist/documents/RECISTGuidelines.pdf

11.0 DRUG INFORMATION

11.1 Vigil Investigational Product

Vigil 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 Production

The Vigil manufacturing process is identical to prior Vigil manufacturing (BB-IND 14205) (Maples 2010). Surgically excised tumor is collected in the surgical field and placed in 0.04mg/ml of gentamicin and sterile saline then 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 Vigil plasmid. The cells are incubated overnight to allow transcription of the Vigil 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 x 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.

11.2 Safety Analysis

Vigil plasmid employed in the generation of this product 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 Vigil administrations 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 Pharmacy Reference Manual for preparation and handling information.

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

Route of administration: Intradermal injection

Storage and Shipping

Frozen, unopened vials are stored in the vapor phase of Liquid Nitrogen below -150° C at Gradalis. Each Vigil concentrate will be shipped individually in a portable liquid nitrogen tank. This shipping container will be able to sustain temperature fluctuations for up to 7 days. This will enable sufficient time to reach the different clinical site pharmacies.

12.0 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 1 practices shall be employed with this study medication. Reference the Pharmacy Reference Manual.

13.0 ADVERSE EVENTS

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 (≥ 24 hours). This does not include hospitalizations which are part of routine medical practice.
- 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 4.03 as linked in Appendix C. All AEs, regardless of severity, will be followed by the Treating Physician until resolution is satisfactory.

13.1 Attribution of Causality

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

13.2 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.

13.3 Recording of an Adverse Event

Adverse events will be recorded for the duration of a patient's study treatment (following the first dose of the Investigational Product), and for up to 30 days following the last study treatment. All AEs, regardless of causal relationship are to be recorded in the eCRF 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 eCRF.

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.

13.4 Serious Adverse Event Reporting

All SAEs will be reported to Gradalis, Inc. within 24 hours of notification by the site through email or facsimile. This includes any death from any cause while a patient is receiving the study agent on this protocol, or ≤ 30 days following the last dose of the protocol study agent (Vigil).

The site will supply as much information as is available at the time of the initial notification (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.

14.0 PATIENT COMPLETION AND WITHDRAWAL

14.1 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.

15.0 PART 1 STATISTICAL CONSIDERATIONS

15.1 Sample Size Justification

This is an open label randomized controlled Phase II clinical trial. Information concerning the predicted survival of the control group is limited, based on rarity of disease and few published reports. However expert advisors in the EWS field estimate a conservative one-year survival rate of 25% in the chemotherapy control group. The one-year survival rate of 60% in the Vigil treated group is estimated from EWS patients treated on the Vigil phase 1 protocol. These estimates correspond to a hazard ratio (HR) of 0.383 favoring Vigil over control.

Assuming 1:1 randomization and the use of a two-sided logrank test at the alpha=0.05 level of significance, 46 events will provide 90% power to detect an OS HR of 0.383. Assuming a one-year accrual period, and a one-year follow-up period after randomization of the last subject, it is estimated that the total sample size (number of subjects) required to achieve 46 events is 62 (31 patients in each arm).

15.2 Analysis Populations

The intent-to-treat (ITT) population will include all randomized subjects. All efficacy analyses will be completed in the ITT population.

The Safety population will include all patients who receive study treatment and patients will be analyzed according to actual treatment received. All safety analyses will be completed in the Safety population.

15.3 Efficacy Analyses

15.3.1 Primary Efficacy Analysis

The primary endpoint of OS is time from randomization to death. Kaplan-Meier OS curves will be displayed by treatment arm. Median OS and percent OS at fixed time points will be estimated. The date of last follow-up confirming survival will be used as the censoring date for subjects who are alive and/or do not have a known date of death. The primary analysis of OS will be conducted using a two-sided log rank test at the alpha=0.05 level of significance.

15.3.2 Secondary Efficacy Analyses

The secondary endpoint of PFS is the time from randomization to progression according to RECIST version 1.1 or death. Incomplete data resulting from patients who terminate the study for reasons other than disease progression or death will be censored in time-to-event analyses on the last assessment date at which a RECIST 1.1 evaluation of disease status was made. The distributions of PFS in the two arms will be compared using a two-sided log-rank test.

The secondary endpoint of 1 year survival proportion will be estimated using Greenwood's formula to estimate the standard errors of the Kaplan-Meier survival probability at a specified time point.

The secondary endpoint of ORR will be analyzed using Fisher's exact test.

All secondary analyses will be conducted using two-sided tests at the alpha=0.05 level of significance.

15.4 Safety Analyses

Safety endpoints include all adverse events (CTCAE 4.03), laboratory safety assessments, and physical examination findings.

16.0 PART 2 STATISTICAL CONSIDERATIONS

Since this part of the protocol is an uncontrolled, non-randomized safety cohort with approximately 6-9 subjects studied, there will be no statistical analysis of outcome. Descriptive statistics only will be conducted.

17.0 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 Investigator or their designee 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 eCRFs 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, whichever 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.

17.1 Case Report Form (CRF) Procedures

Data for this study will be captured in the EDC. The investigator or his/her designee is responsible for recording all data relating to the trial on the eCRFs in accordance with the site's contract with Gradalis. The investigator must verify that all data entries on the eCRFs are accurate and correct.

APPENDIX A

KARNOFSKY PERFORMANCE SCALE

STATUS	MEANING
100%	No symptoms.
90%	Able to carry on normal activity; minor signs or symptoms of disease.
80%	Able to carry on normal activity with effort; some signs or symptoms of disease.
70%	Cares for self, unable to carry on normal activity or do active work.
60%	Requires occasional assistance but is able to care for most of own needs.
50%	Requires considerable assistance and frequent medical care.
40%	Disabled; requires special care and assistance.
30%	Severely disabled; hospitalization indicated, although death not imminent.
20%	Very ill; hospitalization necessary; active supportive treatment required.
10%	Moribund, fatal processes progressing rapidly.
0	Patient expired.

APPENDIX B

SCHEDULE OF ASSESSMENTS

Procedure	Prestudy	Screening	At the beginning of each Cycle (unless otherwise noted)	End of TX	Response Follow-Up (q 3mo±7 days) Until Progression
Informed consent	X	X			
Medical History	X	Interval Medical History within 4 weeks			
Physical Examination	X	X	X	X	X
Toxicity (adverse events)			X	X	
Concomitant medications		X	X	X	X
Performance Status		X	X	X	X
Radiological Tumor Assessment (chest/abdomen/pelvis)		within 4 weeks (must be post-procurement)	every 12 weeks ± 7 days	Within 45 days	X
CBC with differential	X[1]	X	X	X	X
HIV testing, if applicable		X			
Hepatitis testing, if applicable		X			
Serum Chemistry	X[2]	X	X	X	X
PBMC collection for Immune Function Analysis	≤ 24 hours prior to tumor procurement	X	(Cycles 2, and 4, prior to Vigil or chemo administration	X	q 6 months
PBMC collection for Immune Function Analysis (Part 2)	≤ 24 hours prior to tumor procurement	X	(Cycles 2, and 4, prior to Day 15 Vigil administration)	X	q 6 months
Plasma collection for ctDNA (Part 2)			(Cy 2, W1D1; Cy 4, W1D1 prior to chemotherapy administration	X	
Pregnancy Test (if applicable)		X			
Vigil administration *depending on randomization of Part 1 and Part 2 participants*			Part 1 – Day 1 q 28±3 days [3] Part 2 – Day 15 q 21±3 days[3]		
Injection Site Assessment			Day 2 or Day 16 only (may be conducted at home)		
Gemcitabine / Docetaxel *depending on randomization*			gemcitabine D1 and 8 docetaxel D8 q 21d		
Temozolomide / Irinotecan *Part 2 participants*			(p.o. temozolomide 100 mg/m^2 daily for 5 days (Days 1 – 5), I.V.		

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

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

[3] If assigned to the Vigil arm, the patient will be observed for at least 30 minutes following Vigil administration.

Procedure	Prestudy	Screening	At the beginning of each Cycle (unless otherwise noted)	End of TX	Response Follow-Up (q 3mo±7 days) Until Progression
			irinotecan 20mg/m^2 daily (Days 1 – 5, total dose 100mg/m^2/cycle), or oral irinotecan 50 mg/m^2 daily for 5 days (Days 1 – 5), q21 days		
Peg-filgrastim *Part 2 participants*			s.c. peg-filgrastim 100ug/kg, Day 6 *optional and may be administered at home*		
Survival Status	X				Long Term Follow Up[4]

[4] After progression, subjects will be contacted by phone quarterly for documentation of survival status.

APPENDIX C

NCI COMMON TOXICITY CRITERIA FOR ADVERSE EVENTS (CTCAE), VERSION 4.03

Publish Date: June 14, 2010

As of June 14, 2010 NCI has introduced version 4.03 of the Common Toxicity Criteria for Adverse Events. These may be obtained at the following web link http://ctep.cancer.gov.

DO NOT USE CTC VERSION 3.0 TO GRADE TOXICITIES IN THIS STUDY!

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