COVER PAGE

OFFICIAL PROTOCOL TITLE:	**A Multi-Center Phase III, Randomized, Open-Label Trial of Vigil (bi-shRNAfurin and GMCSF Augmented Autologous Tumor Cell Immunotherapy) in combination with Irinotecan and Temozolomide as a Second-Line Regimen for Ewing's Sarcoma**
PROTOCOL NUMBER:	**CL-PTL-130**
IND NUMBER:	**014205**
NCT NUMBER:	**NCT03495921**
PROTOCOL DATE:	**Amendment 2 dated December 5, 2018**
IRB APPROVAL DATE:	**December 14, 2018**

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STUDY AGENT(S):	**Vigil** **bi-shRNA^{furin} and GMCSF Augmented 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 2 dated December 5, 2018** **Amendment 1 dated June 20, 2018** **Initial Protocol dated September 20, 2017**

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
AST	Aspartate transaminase (also referred to as SGOT)
BUN	Blood urea nitrogen
CBC	Complete blood count
CD	Cluster of differentiation
CO_2	Total carbon dioxide
CR	Complete response
CRF	Case report form
CT	Cancer testis
CT	Computerized Tomography
CTCAE	Common Toxicity Criteria for Adverse Events
ctDNA	Circulating tumor DNA
DFS	Disease-Free Survival
DSMB	Data Safety Monitoring Board
DTIC	dacarbazine
EDC	Electronic Data Capture
EFS	Event Free Survival
ELISPOT	Enzyme-Linked ImmunoSorbent Spot
ERG	ETS-Related Gene
ESFT	Ewing's Sarcoma Family of Tumors
ESPS	Ewing's Sarcoma Prognostic Scores
ETS	E26 transformation-specific or E-twenty-six
FANG	bi-shRNA^{furin} and GMCSF Augmented Autologous Tumor Cell Immunotherapy
FISH	Fluorescent in situ hybridization
FLI-1	Friend Leukemia Virus Integration 1
GMCSF	Granulocyte Macrophage-Colony Stimulating Factor
GVAX	GMCSF Secreting autologous or allogenic tumor vaccine
HLA	Human Leukocyte Antigen
IDMC	Independent Data Monitoring Committee
IE	Ifosfamide and etoposide
IEC	Independent Ethics Committee
IFN	Interferon

Abbreviation	Term
IRB	Institutional Review Board
ITT	Intent to Treat
KPS	Karnofsky Performance Score
LDH	Lactate dehydrogenase
LS	Lansky Performance Score
MRI	Magnetic resonance imaging
NCI	National Cancer Institute
NED	No evidence of disease
NGS	Next Generation Sequencing
NK	Natural Killer
NKT	Natural Killer T cell(s)
ORR	Objective Response Rate
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PD-L1	Programmed death-ligand 1
PFS	Progression Free Survival
PI	Principal Investigator
PR	Partial response
PS	Performance Status
RECIST	Response Evaluation Criteria in Solid Tumors
RFS	Recurrence Free Survival
RT-PCR	Real Time Polymerase chain reaction
TGFβ	Transforming growth factor-β
TIL	Tumor Infiltrating Lymphocytes
TTP	Time to Progression
TTR	Time to Recurrence
VAC	Vincristine, Actinomycin, and Cyclophosphamide

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 in first relapse is dismal (13% 5-year survival). Temozolomide and irinotecan is the consensus standard of care treatment regimen after relapse. Vigil immune response correlates with survival and time to relapse improvement in multiple solid tumors including Ewing's sarcoma. Limited data has demonstrated no adverse effect of the combination therapy with irinotecan, temozolomide and Vigil ($1x10^6$ - $1x10^7$ cells/injection (x4 or more)). Therefore, the combination regimen of Vigil, irinotecan, and temozolomide is hypothesized to increase anti-tumor responses and prolong time to progression in patients with relapsed or refractory Ewing's sarcoma (first relapse).

In an effort to overcome limitations of first-generation immunostimulatory cancer vaccines, we designed a novel autologous whole cell vaccine, Vigil, 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 > 1.0×10^6 cells / injection / month for a minimum of 4 and 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 Vigil induced immunomodulatory effects against Ewing's sarcoma was reflected in positive IFNγ ELISPOT response to autologous tumor cells in 10/12 patients, one RECIST criteria partial response (PR), a second patient achieving complete response (CR) and a total 1-year overall survival (Kaplan Meier method) of 73% in comparison to expected 1-year survival of <25%.

Objective(s):

Primary Objective:

To compare the progression free survival (PFS) of subjects dosed with Vigil immunotherapy in combination with irinotecan and temozolomide vs. irinotecan and temozolomide.

Secondary Objective(s):

- To determine and compare the overall survival (OS) of subjects with relapsed or refractory Ewing's sarcoma dosed with Vigil immunotherapy in combination with irinotecan and temozolomide with patients that are treated with irinotecan and temozolomide.

- To determine the objective response rate (RECIST 1.1) of patients with metastatic Ewing's sarcoma refractory or intolerant to 1 prior line of systemic chemotherapy treated with Vigil immunotherapy dosed with Vigil immunotherapy in combination with irinotecan and temozolomide.
- To determine the Vigil manufacturing success rate by gathering data from tumor tissue collection, Vigil construction, and test procedures.

Methodology:

This is a multicenter, 1:1 randomized Phase III study of intradermal autologous Vigil immunotherapy (1.0 x 10^6 cells/injection; minimum of 4 to a maximum of 12 administrations) in combination with irinotecan and temozolomide in subjects with metastatic Ewing's sarcoma Family of Tumors (ESFT) refractory/intolerant or recurrent to 1 prior line of chemotherapy. Participants undergoing a standard surgical procedure (e.g., tumor biopsy or palliative resection) may have tumor tissue harvested for manufacture of the investigational product, Vigil.

Subjects meeting eligibility criteria including manufacture of a minimum of 4 immunotherapy doses of Vigil will be randomized to receive either:

Group A

(i) oral temozolomide 100 mg/m^2 daily (Days 1 – 5, total dose 500 mg/m^2/cycle),
(ii) oral irinotecan 50 mg/m^2 daily (Days 1 – 5, total dose 250mg/m^2/cycle), and
(iii) Vigil 1.0 x 10^6 cells/injection cells/injection, intradermally on Day 15.

or

Group B

(i) oral temozolomide 100 mg/m^2 daily (Days 1 – 5, total dose 500 mg/m^2/cycle), and
(ii) oral irinotecan 50 mg/m^2 daily (Days 1 – 5, total dose 250mg/m^2/cycle).

One cycle equals 21 days. Screening for the main portion of the study 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. At the time of analysis the subjects will be stratified by initial response to frontline treatment (recurrent versus refractory (progressive)).

Participants 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 activation in response to autologous tumor antigens will be collected at tissue procurement, post-procurement screening and Day 1 (prior to chemotherapy administration) at Cycles 2, 4, and 6, end of treatment (EOT), 3 months after EOT, and every 6 months thereafter. Blood for ctDNA analysis will be collected

at tissue procurement, prior to chemotherapy administration at baseline and on Day 1 prior to chemotherapy administration at Cycles 2, 3, 4, and 6, and EOT.

Number of Patients:

Approximately 140 subjects will have tissue procured so that 114 evaluable subjects will be registered for enrollment into the study.

Tissue Procurement Inclusion Criteria:

Subjects 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). If available, NGS sequencing report should be submitted to Gradalis.
5. Recurrence or refractory to 1 line of systemic chemotherapy including but not limited to doxorubicin, vincristine and ifosfamide.
6. Planned standard of care surgical procedure (e.g., tumor biopsy or palliative resection or thoracentesis) and expected availability of a cumulative soft-tissue mass of ~10-30 grams tissue ("grape" to "golf-ball" size / approximate 2 cm total diameter on imaging) and/or pleural fluid estimated volume ≥ 500mL (from a primary or secondary thoracentesis, yielding in a high volume of tumor cells) for immunotherapy manufacture.
7. Tumor intended for immunotherapy manufacture is not embedded in bone and does not contain luminal tissue (e.g. bowel, ureter, bile duct).
8. Ability to understand and the willingness to sign a written informed protocol specific consent for tissue harvest or a parental/guardian informed consent and pediatric assent when appropriate.

Tissue Procurement Exclusion Criteria:

Subjects 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 HIV or chronic Hepatitis B or C infection.
6. Known hypersensitivity to any temozolomide component or to dacarbazine (DTIC).
7. Known hypersensitivity to irinotecan or its excipients.
8. Known history of allergies or sensitivities to gentamicin.
9. 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.

Study Enrollment Inclusion Criteria:

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

1. Completed manufacture of at least 4 vials of Vigil.
2. Karnofsky performance status (KPS) / Lansky performance status (LS) ≥ 80%.
3. Normal organ and marrow function as defined below:

Absolute granulocyte count	≥1,000/mm^3
Absolute lymphocyte count	≥400/mm^3
Platelets	≥75,000/mm^3
Hemoglobin	≥8.0 g/dL
Total bilirubin	≤ institutional upper limit of normal*
AST(SGOT)/ALT(SGPT)	≤2x institutional upper limit of normal
Creatinine	<1.5 mg/dL

*documented Gilbert's syndrome may be considered after medical monitor review

4. Subject has recovered to CTCAE Grade 1 (except for parameters noted in Item 3, above) or better from all adverse events associated with prior therapy or surgery. Pre-existing motor, sensory neurologic pathology or symptoms, or dermatologic toxicities must be recovered to CTCAE Grade 2 or better.
5. 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.
6. Ability to understand and the willingness to sign a written informed protocol specific consent or a parental/guardian informed consent and pediatric assent when appropriate.

Study Enrollment Exclusion Criteria:

In addition to the procurement exclusion criteria, subjects will NOT be eligible for study registration and randomization if meeting any of the following additional 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 subject'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. Irinotecan will be supplied by Clinigen. Temozolomide will be ordered per standard institutional practice.

Group A

(i) oral temozolomide 100 mg/m^2 daily (Days 1 – 5, total dose 500 mg/m^2/cycle),
(ii) oral irinotecan 50 mg/m^2 daily (Days 1 – 5, total dose 250mg/m^2/cycle), and
(iii) Vigil 1.0 x 10^6 cells/injection or cells/injection, intradermally on Day 15.

or

Group B

(i) oral temozolomide 100 mg/m^2 daily (Days 1 – 5, total dose 500 mg/m^2/cycle), and
(ii) oral irinotecan 50 mg/m^2 daily (Days 1 – 5, total dose 250mg/m^2/cycle).

One cycle equals 21 days.

Duration:

Subjects may receive repeat cycles of treatment until disease progression, unacceptable toxicity, withdrawal of consent or other criterion is met for discontinuation from study. Subjects randomized to Group A, Vigil will receive up to 12 doses depending upon the quantity of Vigil manufactured from the surgical specimen. Within 6 weeks of second relapse or progression, subjects randomized to Group B, will be allowed to cross-over (see Section 10.0) to receive single agent Vigil every 21 days. Subjects who cross-over may receive up to 12 doses of Vigil depending upon the quantity of Vigil manufactured. Cross-over must occur within 2 years of End of Treatment assessments of Group B enrollment. For subjects crossing over, no alternative therapies will be given between End of Treatment and cross-over.

Efficacy Assessments:

- Progression Free Survival (PFS)
- Objective Response Rate (ORR) (RECIST 1.1)
- Overall survival (OS)

Safety Assessments:

- Laboratory assessments
- Physical examination, performance status, height, weight, temperature, blood pressure, and pulse
- Toxicity: CTCAE v 5.0

Exploratory Assessments:

Circulating EWS FLI1 DNA detection 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-L1 expression and tumor infiltrating lymphocyte (TIL) populations.

Statistical Considerations:

The planned sample size of 114 randomized subjects is based on the following assumptions for the PFS endpoint:

- 1:1 randomization;
- Accrual period of 12 months;
- Follow-up period of 6 months;
- Assumed control group median PFS of 6 months from randomization;
- Two-sided test at the alpha=0.0476 level of significance;
- Power 90%;
- Assumed hazard ratio (HR) of 0.45

The planned number of events is 67.

An interim analysis for efficacy, futility, and potential sample size re-estimation will be conducted by an independent data monitoring committee (IDMC) when 40 PFS events have been achieved (60% information fraction). Based on the Lan-DeMets method for group sequential trials using O'Brien-Fleming boundaries (Reboussin, DeMets et al. 2000), the levels of significance for the interim and final analyses are alpha=0.00762 and alpha=0.0476, respectively.

Based on the results of the interim analysis, the IDMC will make one of the following four recommendations to the sponsor:

- Terminate the study for efficacy ($p<0.00762$ at the interim analysis);

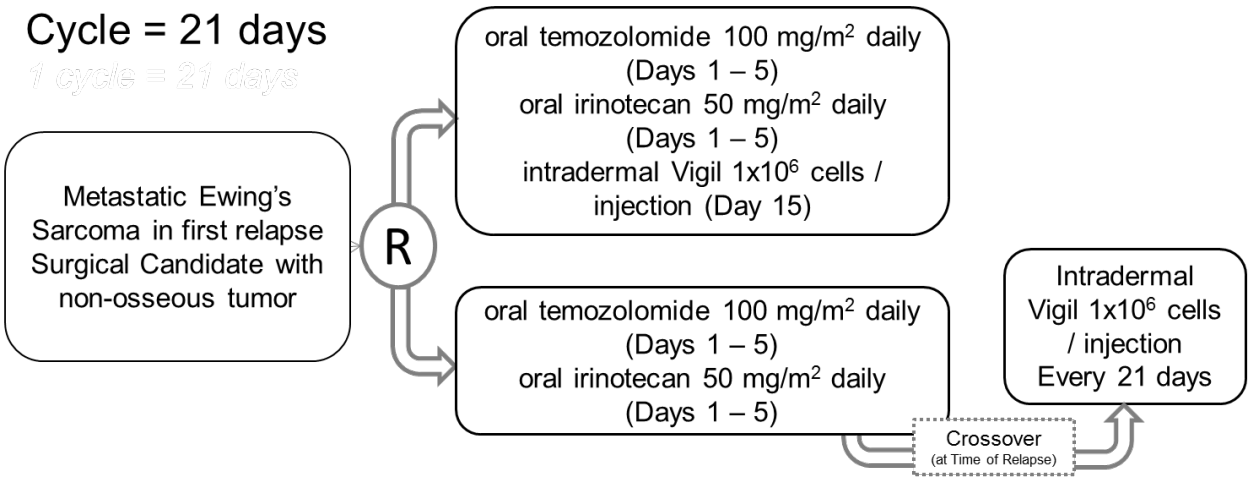
- Continue the study as planned;
- Increase the sample size to 120 events;
- Terminate the study for futility.

If the sample size is increased to 120 events, then the study will have approximately 90% power to detect a true HR of 0.55. Note that if the sample size is increased to 120 events, then the accrual period will be extended to 24 months, with a 6-month follow-up period after randomization of the last patient. Under these assumptions, it is estimated that approximately 164 patients will need to be randomized in order to achieve 120 events.

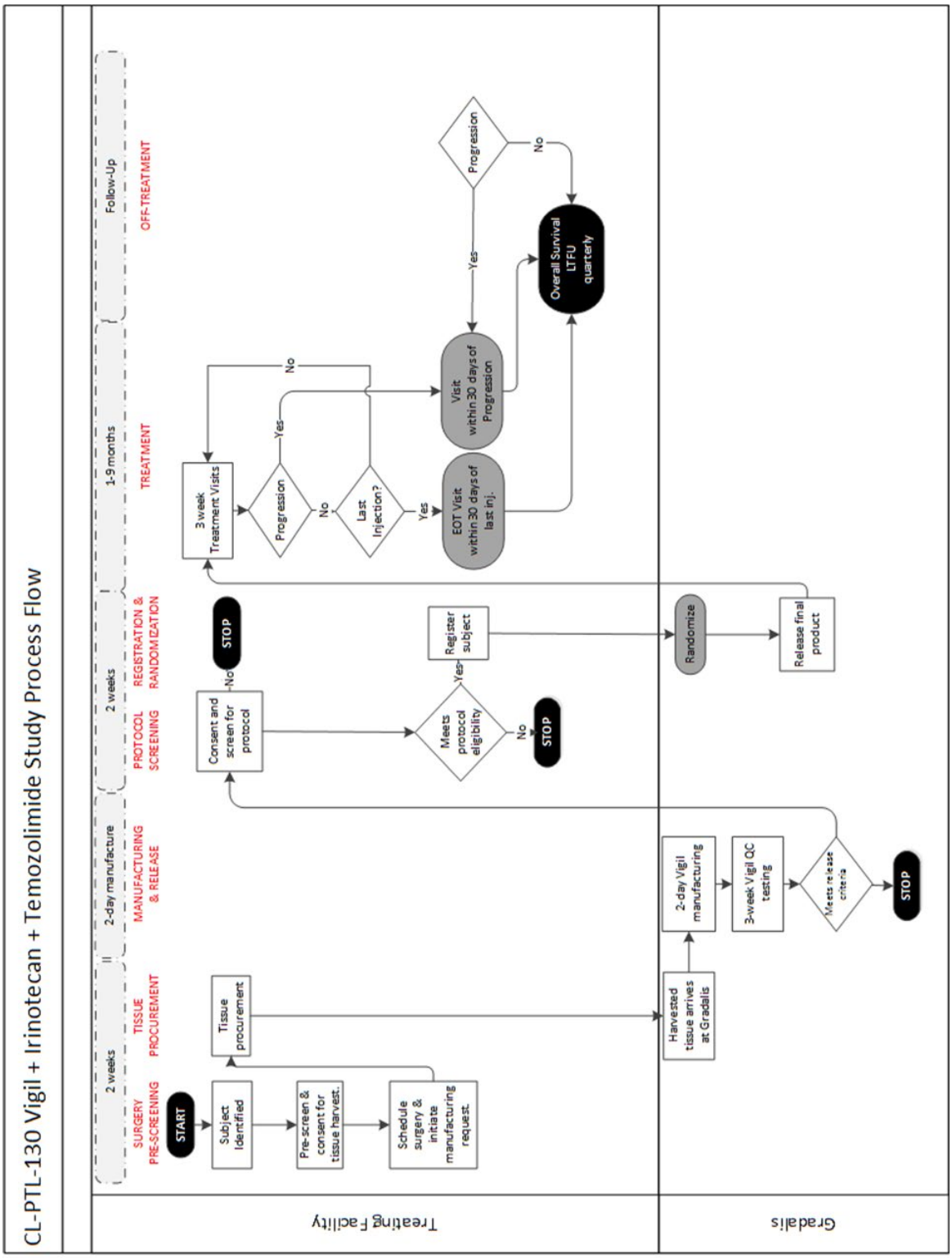
Provided that the IDMC does not recommend terminating the study early for efficacy, the conditional power of the planned final analysis at 67 events will be computed using the methodology of (Jennison and Turnbull 2000) under the assumption that the observed hazard ratio at the interim analysis represents the true effect. In terms of the HR observed at the interim analysis, these rules can be translated (approximately) as follows:

- If the HR is less than 0.43, terminate for efficacy
- If the HR is in the range from 0.43 to 0.51, continue as planned.
- If the HR is in the range from 0.51 to 0.61 (i.e., conditional power in the range from 50% to 80%), increase the sample size to 120 events.
- If the HR is greater than 0.61 (i.e., conditional power less than 50%), terminate for futility.

STUDY SCHEMA



STUDY PROCESS FLOW



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 at diagnosis is 14 – 15 years (Cotterill, Ahrens et al. 2000, Stahl, Ranft et al. 2011). In greater than 90% of patients there is an essentially specific translocation of the EWSR1 gene on chromosome 22q12 with FLI1 on chromosome 11q24 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 (Arvand and Denny 2001).

Patients with initially diagnosed localized disease have a disease-free survival rate (DFS) of 60-70% with standard 5-drug chemotherapy regimen (VAC +IE or VIDE), while patients with metastatic disease have a DFS 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's sarcoma patients enrolled in trials from 1979 to 1993, the 5-year recurrence free survival (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 it is achieved at the cost of an increased toxicity profile (Esiashvili, Goodman et al. 2008). Second or greater line therapy regimens 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 at 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). Presentation with localized disease is associated with a 6-year event free survival (EFS) 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 in this analysis significantly deteriorated to 28% with OS 29% (Guerney, Swensen et al. 1999, Miser, Goldsby et al. 2007, Leavey, Mascarenhas et al. 2008). Other reports have shown worse overall survival in patients who relapse with a marked reduction in overall survival of only 12% at 5 years, and 19% at 2 years (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). In addition, response status to frontline therapy like recurrence (initial response followed by relapse) versus refractory (disease progression on first line regimen) appear to correlate with clinical outcome, while the survival rate is dismal for patients that do not respond to frontline therapy (Casey, Wexler et al. 2009).

Table 1. Relapse from Time of Initial Treatment

PI	Number Patients	Primary Metastatic Disease at Diagnosis	5-year Overall Survival 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.					

The NCCN guidelines do not provide standard of care recommendations for second-line treatment and only 9-13% of patients achieve a second disease free remission (Bacci, Ferrari et al. 2003, Barker, Pendergrass et al. 2005, Rasper, Jabar et al. 2014). 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).

1.3 Salvage chemotherapy in Ewing's sarcoma

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). Although results of progression free survival (PFS) and OS do not significantly vary between dose regimens (Yildiz, Sen et al. 2014), PFS in patients treated with second-line irinotecan/temozolomide, the most commonly used regimen, 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).

Clinical studies have demonstrated the combination of irinotecan and temozolomide to have synergy and significant antitumor activity. Casey et al. showed that the combination of temozolomide and irinotecan is well-tolerated and an active regimen for recurrent or refractory Ewing's sarcoma patients (first relapse) with an overall response of 63% and median TTP of 8.3 months (Casey, Wexler et al. 2009).

A retrospective study of 22 patients with relapsed or refractory Ewing's sarcoma treated with the combination of vincristine, irinotecan, and temozolomide reviewed a shorter treatment course of 5 days for irinotecan instead of the protracted 10-day course. Results showed comparable response rates (68.1 %) with a median TTP of 3 months and suggests the use of the short schedule of administration (Raciborska, Bilska et al. 2013). In a similar study orally administered irinotecan, temozolomide and vincristine showed milder cumulative toxicities employing a 5-day treatment course rather than 10 days in sarcoma patients (Wagner, Perentesis et al. 2010). A pediatric study has directly compared the efficacy of irinotecan with a daily for 5 days versus daily for 5 days for 2 weeks schedule (Mascarenhas, Lyden et al. 2010) in 89 evaluable patients with recurrent rhabdomyosarcoma were randomized to receive vincristine combined with irinotecan. The overall incidence of grade 3–4 adverse events was similar and no significant difference in efficacy was observed. Casey and colleagues showed that irinotecan (iv dx5 x2) and temozolomide (orally dx5) was well tolerated and an active regimen in Ewing's sarcoma in first recurrence (n=11), second recurrence (n=3) or refractory to primary chemotherapy (n=6) (Casey, Wexler et al. 2009).

A more recent review of irinotecan of the past 15 years evaluated oral versus intravenous administration in pediatric sarcoma patients (Wagner, Perentesis et al. 2010, Wagner 2015). Even though the oral dosing requires a higher total dose of irinotecan (bioavailability is less) the metabolism of orally administered irinotecan appears to be more efficient, given that the intestinal tract contains high levels of carboxylesterases, which may pre-systemically metabolize irinotecan to the active moiety, SN-38 and increase the SN-38/irinotecan ratio by threefold or more (Drengler, Kuhn et al. 1999). Although there have been no studies directly comparing the efficacy of the two routes of administration, at similar SN-38 lactone exposures (60 mg/m^2/day oral vs. 20 mg/m^2/day IV), response rates, and toxicity profiles appear to be equivalent. A phase 1 dose escalation (Irinotecan 50mg/m^2/dose escalated to a maximum of 90mg/m^2/dose, and temozolomide 100mg/m^2/dose escalated to a maximum of 150mg/m^2/dose) study in pediatric patients with relapsed or refractory solid tumors of oral irinotecan, temozolomide and temsirolimus demonstrated that lower dose level of irinotecan (50mg/m^2/dose) and temozolomide (100mg/m^2/dose) was well tolerated with less toxicities (Bagatell, Norris et al. 2014). An additional study recommends 50mg/m^2 per day for 5 consecutive days for phase II studies of oral irinotecan (Soepenberg, Dumez et al. 2005). The benefit of oral administration includes not only greater patient convenience, shorter hospitalization and decrease in costs, but most importantly improved quality of life. Thus, summarizing studies of oral irinotecan and temozolomide and in agreement with participating investigators a lower dose regimen of 50mg/m2/d irinotecan and 100mg/m2/d temozolomide administered orally appears to be an acceptable standard of care regimen for second-line Ewing's sarcoma providing clinical activity with low toxicity profiles. In those occasional patients with difficulty taking oral medication (e.g. severe nausea, chronic gastrointestinal complaints, and age-related oral aversion) intravenous

administration may remain a reasonable and acceptable alternative standard of care option (Wagner 2015).

1.4 Immunotherapeutic considerations in Ewing's sarcoma

Ewing's sarcoma demonstrates elements of the immune escape mechanisms determined to be integral to the natural history of cancer. Patients with primary disseminated disease at presentation show an increased frequency of T-regulatory cells in the bone marrow compared to those with localized disease (Brinkrolf, Landmeier et al. 2009). Factors associated with survival are increased number of tumor infiltrating CD8+ T-lymphocytes (p=0.05) which inversely correlates with tumor volume (<200 ml) at diagnosis (Berghuis, Santos et al. 2011). Furthermore, the unique, defining EWS/FLI-1 fusion protein may be an effective immunogenic driver neoantigen less likely to be adaptively downregulated due to its driver status (Liu, Huang et al. 2012, Peng, Huang et al. 2014). Meyer-Wentrup et al. discovered the first immunogenic Ewing's sarcoma specific peptide directly derived from the EWS/FLI1 fusion gene (Meyer-Wentrup, Richter et al. 2005). Ten (10) peptides derived from the 21-mer fusion region were used to prime T cell responses by pulsing single peptides onto mature dendritic cells. Gamma interferon (IFNγ) production was measured in ELISPOT assays as a surrogate marker for T- and NK cell activation. QQSSSYGQQN-pulsed dendritic cells induced significant IFNγ immune response while the other 9 peptide-induced IFNγ production remained unspecific. Interestingly, the response to QQSSSYGQQN was highly peptide specific and could be inhibited by HLA-DR-blocking antibody, known to block HLA class II activation. Fusion-region specific neoantigens, such as described by Meyer-Wentrup and colleagues, could reveal new opportunities to induce and sustain cytotoxic immune responses. Re-activation of immunogenic antitumor response as a treatment strategy for EWS could be exploited either in a monotherapy or in combination with chemotherapy agents such as irinotecan and temozolomide, which produce lower rates of lymphopenia of shorter duration and allow for effective, concurrent immune responsiveness (Kushner, Kramer et al. 2006).

Preliminary results in 83% of Ewing's sarcoma patients treated with Vigil immunotherapy demonstrated CD8+ T-cell activation, as defined by IFNγ ELISPOT assay using unmanipulated autologous tumor cells (verified by Mt. Sinai and plates are assessed for spots by Zellnet, Fort Lee, NJ). Notably, Vigil induced ELISPOT response has been shown to significantly correlate with survival and time to recurrence (TTR) in patients with a variety of solid tumors (Senzer, Barve et al. 2013, Oh, Barve et al. 2016) and specifically with tumor responses (1 CR/NED, 1 PR) in two Ewing's sarcoma patients one of whom is described in a prior publication (Ghisoli, Rutledge et al. 2017). The relationship of the sequential IFNγ ELISPOT level to clinical course in this patient is shown in Figure 1. Evidence of CD4+ immune activation has been identified by analysis of 24 different cancer testis (CT) antigens in Vigil treated Ewing's sarcoma patients. Patient 062 (Figure 1) showed seroconversion of CT47 during Vigil treatment with persistence of antibodies after treatment completion. CT47 expression has been observed to be elevated in sarcoma patients suggesting antigen targeted therapies (Chen, Iseli et al. 2006). Another Ewing's sarcoma patient (PID 095) and long-term survivor (1033 days since procurement) showed similar CT47 CD4 response during Vigil treatment in addition to CD8 T cell activation (ELISPOT response).

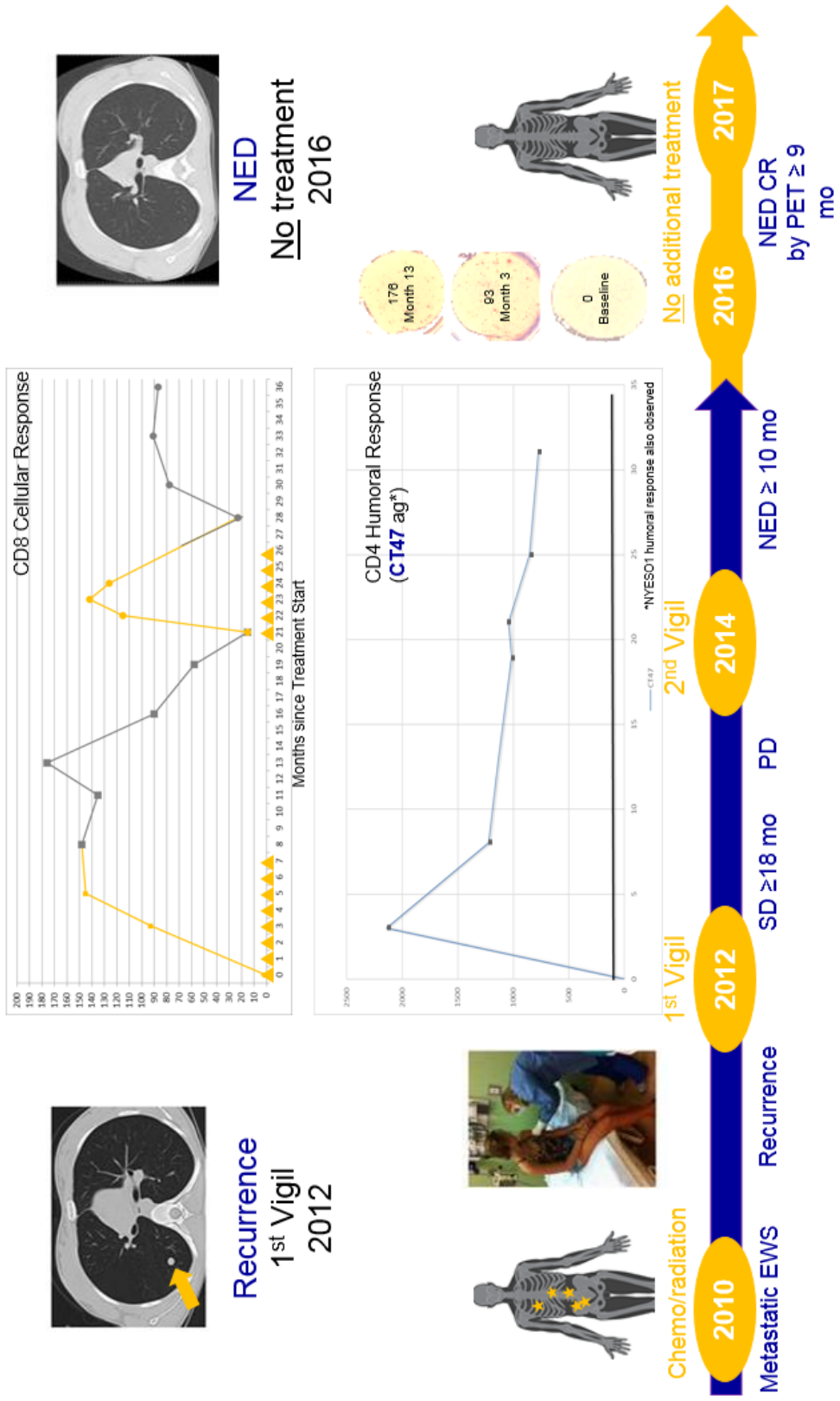


Figure 1. Case Report 1 (F-062). Relapsed Ewing's sarcoma Response to Vigil.

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 x 10^7 cells/injection vs. 2.5 x 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 x 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).

Interestingly, a small sub-cohort (n=15) with a dose level of 1 x 10^6 cells/injection was enrolled and treated on the Phase 1 study with Vigil and showed ELISPOT conversion (12/12 evaluable patients) from baseline to treatment start in a recent analysis (Figure 2 and Figure 3) (Manning 2017). Six (6) of these patients enrolled with advanced, relapsed or refractory Ewing's sarcoma and 5 of the 6 Ewing's patients had prolonged survival of > 2 years (median OS 807.5 days, range 58-1090 days from time of procurement). Four (4) of the low-dose Ewing's patients were also analyzed for ELISPOT response and all 4 showed positive conversion post treatment with Vigil (Figure 4). The median survival from time of procurement of these 4 patients was 1288 days (range 840-1814 days). This data supports a 1x10^6 dose administration of Vigil in Ewing's sarcoma.

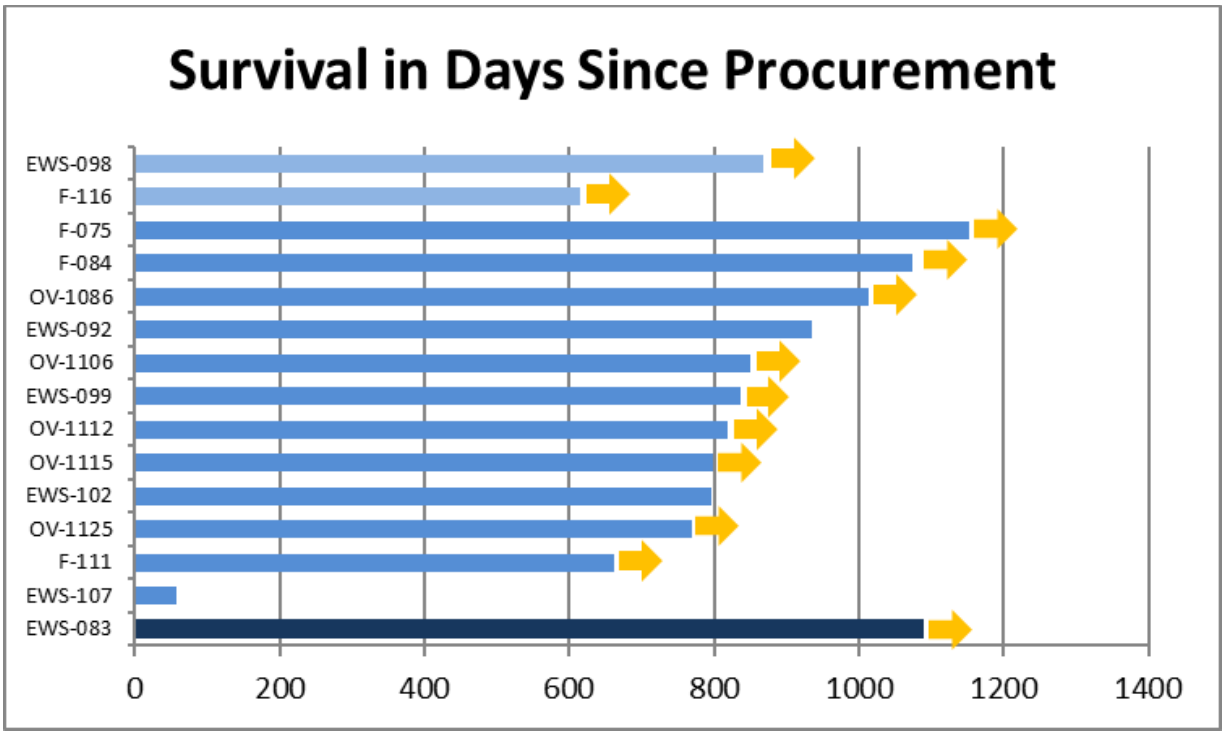


Figure 2. Low Dose Vigil Phase I Survival status. Arrow indicates subjects still alive.

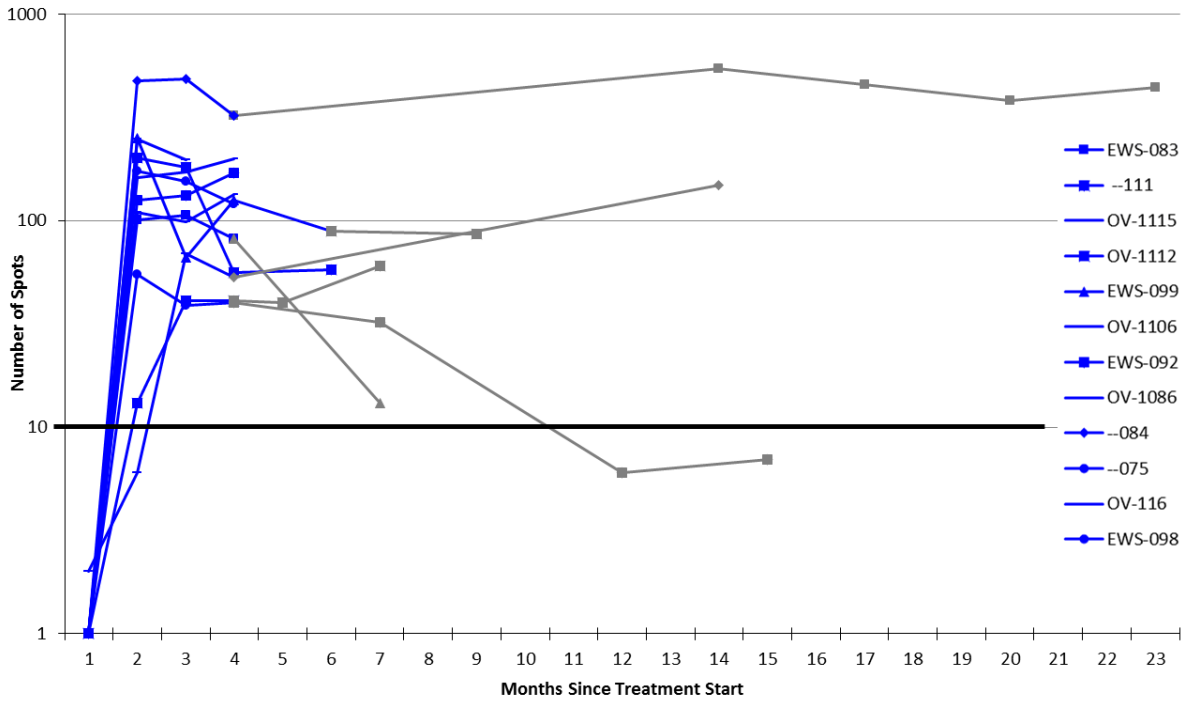


Figure 3. Low Dose Vigil Phase I ELISPOT response status. Gray lines indicates time points off treatment.

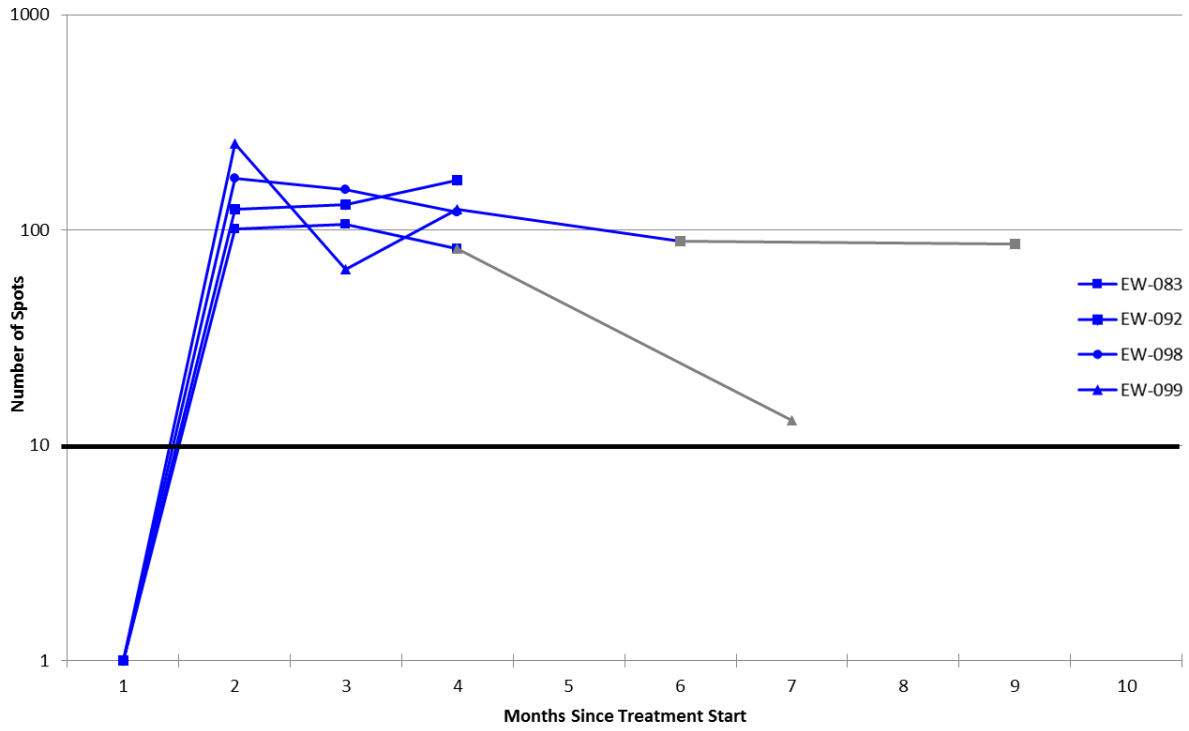


Figure 4. Low Dose Vigil Phase I Ewing's Patients: ELISPOT response status. Gray lines indicates time points off treatment.

Eighty patients in total (54 females, aged 13-84 / 26 males, aged 12-76) entered into Phase I trial involving 19 solid tumor cancers. Four hundred sixty-four (464) Vigil injections were administered and no ≥ Grade 3 toxic events attributed to Vigil have been observed. Dose administered ranged from 1 x 10^6 cells/injection to 2.5 x 10^7 cells/injection via intradermal injection once a month for a minimum of 4 months. The prolonged survival shown in Figure 5 involving predominantly advanced stage, heavily pretreated patients is greater than historical expectation (Senzer, Barve et al. 2013).

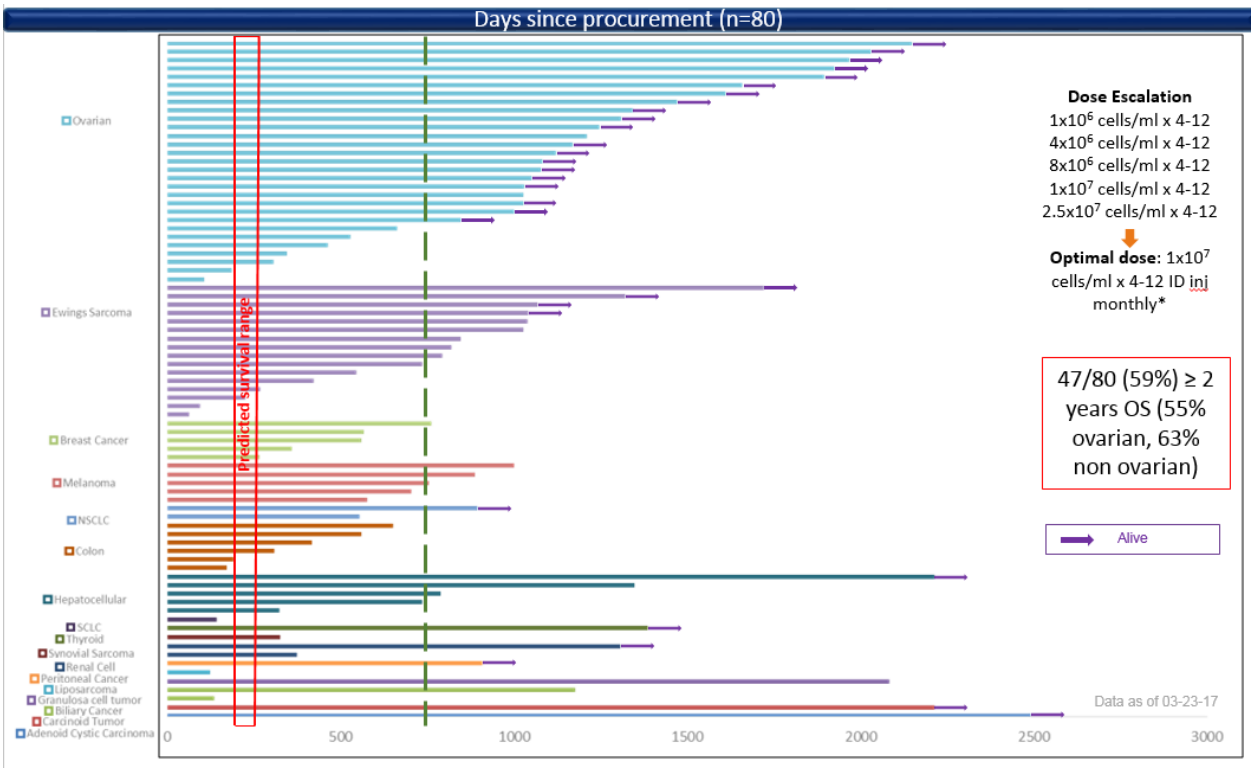
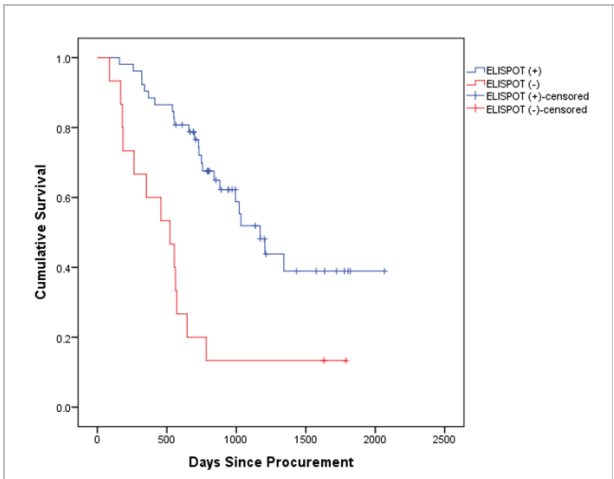
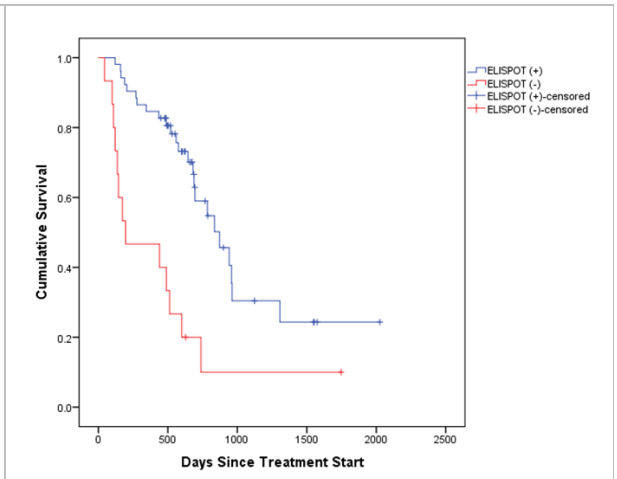


Figure 5. Long term survival by cancer type of patients entered into Vigil Phase I trial.

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 6).





Group	N	Median (mo.)	p-value
ELISPOT (-)	15	17.2	0.00015
ELISPOT (+)	52	38.5	

Group	N	Median (mo.)	p-value
ELISPOT (-)	15	6.4	0.00010
ELISPOT (+)	52	28.7	

Figure 6. Correlated survival and immune response and updated analysis of Vigil Phase 1 patients. 78% ELISPOT conversion rate. 55% ELISPOT (+) patients are still alive vs. 13% ELISPOT (-) are deceased. (Analysis performed on 02/27/17)

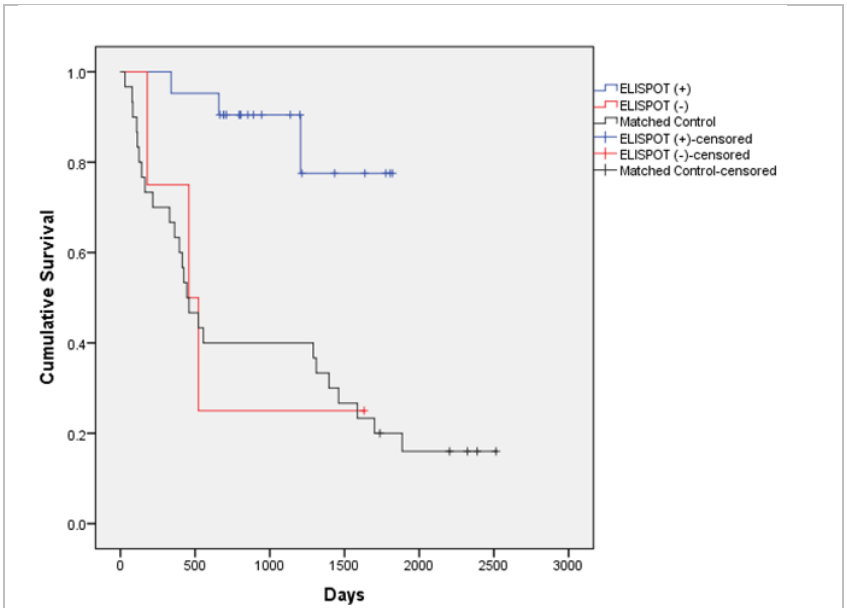
These results have recently been updated (Figure 6). Sixty-seven (67) of these Phase I patients have now undergone IFNγ ELISPOT testing. Fifty-two demonstrated change from negative at baseline (prior to Vigil treatment) to positive IFNγ ELIPSOT reactivity after Vigil (78% conversion rate). However, fifteen 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.

2.1 Vigil Phase II Trial: Ovarian Cancer

Based on positive Phase I results, 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 (cCR) following primary surgical debulking and standard chemotherapy (5-6 cycles, front-line maintenance setting), was initiated (CL-PTL-105). A 1.0×10^7 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. Cross-over was allowed at time of relapse for patients randomized to standard of care therapy. Results have been published by Oh et al (Oh, Barve et al. 2016).

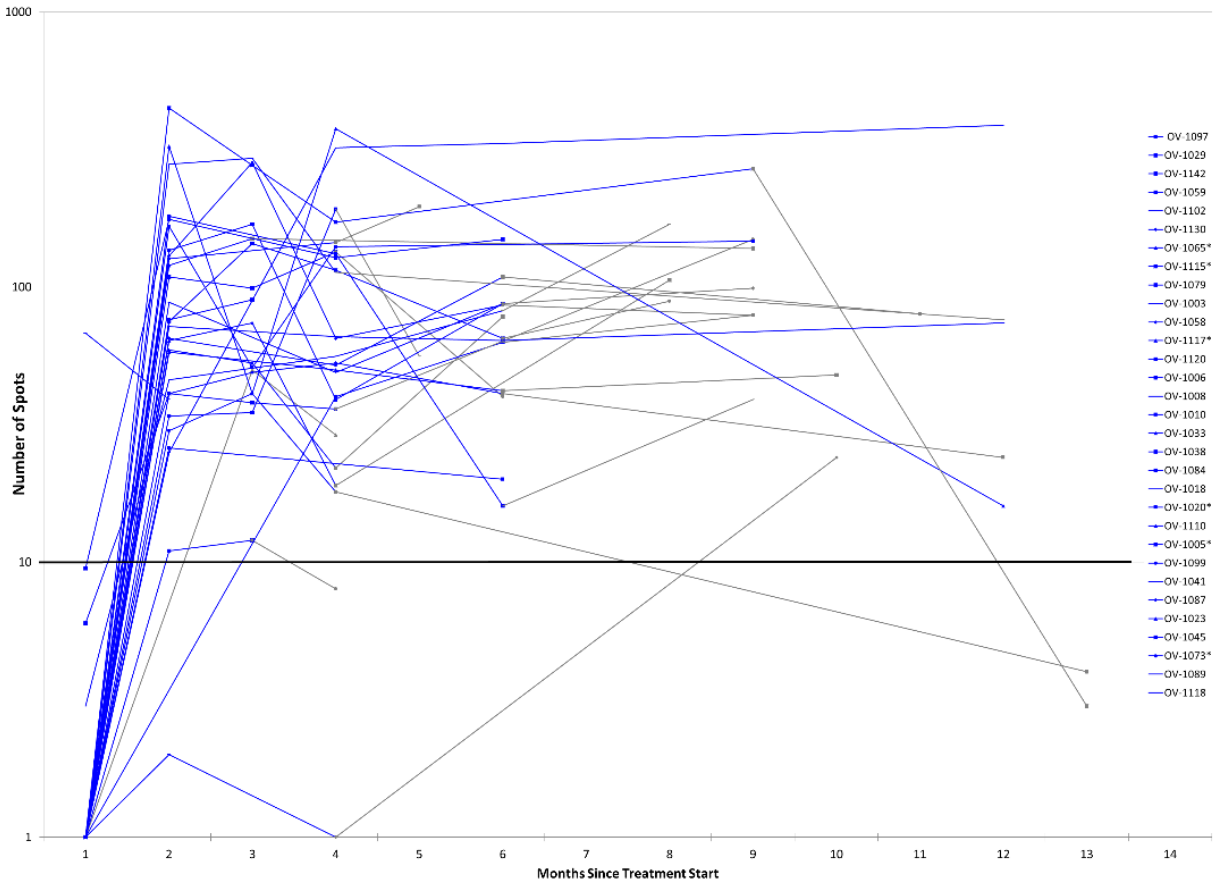
Twenty-one patients were randomized to Vigil, 11 to control standard of care. Discussion of these results lead to a larger (currently ongoing) Phase II/III trial design. Once the Phase II/III trial was initiated study CL-PTL-105 was closed to further accrual and patients not yet randomized in consolidation chemotherapy section entered 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.



Group	N	Median (mo.)	p-value
Control	11	12.4	0.025
Vigil	31	19.8	

Figure 7. Correlated survival of Vigil compared to control in Ovarian Cancer (CL-PTL 105) with immune responses. OV-1049 and OV-1074 were censored at time of cross-over. OV-1049 crossed-over and started Vigil 08/27/14 (5 cycles) and relapsed 4 months after treatment start (12/17/14).



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 (cross-over) Median spots 58 (43-105)

Figure 8. ELISPOT response of Vigil in Ovarian Cancer (CL-PTL-105). Thirty-one (31) patients are ELISPOT (+) post Vigil treatment.

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 IFNγ 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 IFNγ 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) (Figure 8).

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 now 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 of these patients received Vigil. Fourteen concurrent patients who did not receive Vigil after undergoing similar surgery and Vigil manufacture served as control. Nine of the latter were unable to have vaccine released (6 contaminants, 3 insufficient viable tumor cells) and five chose other treatment management. All products constructed fulfilled release criteria of GMCSF production and TGFβ1, β2 knockdown. See demographics in Table 2.

Table 2. 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 Treatment (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. Reported adverse events are shown in Table 3.

Table 3. Phase I (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

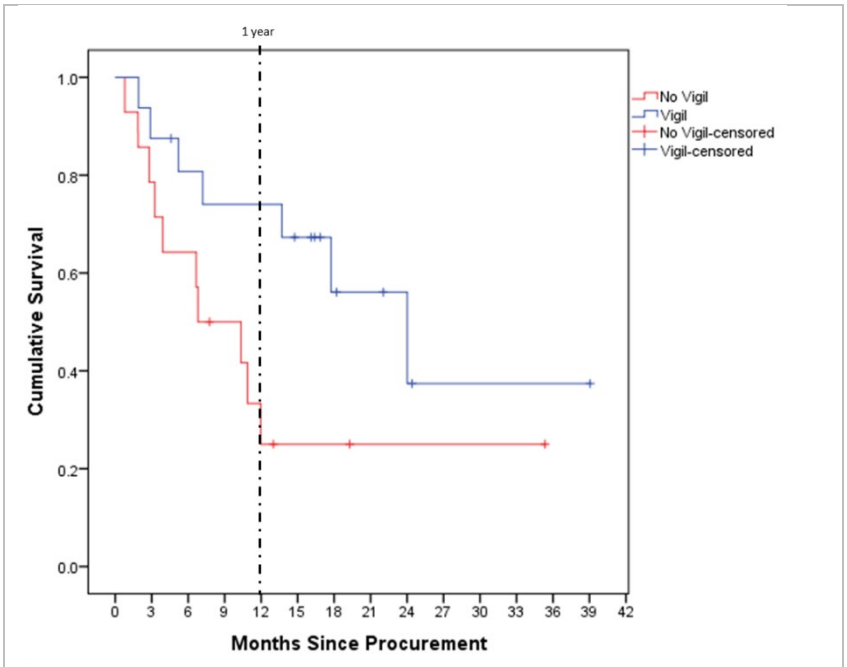
Preferred Term	CTC Grade	Relationship to Study Drug	Number of Subjects	Number of Events
Injection Site Reaction – Induration	1	Definitely Related	1	2
Injection Site Reaction- Erythema	1	Definitely Related	11	31
Injection Site Reaction- Induration	1	Definitely Related	12	55
Injection Site Reaction- Pain	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 adverse events were observed to Vigil. There were 11 serious adverse events (SAEs) reported involving 7 participants. None of the SAEs were related to Vigil.

2.3 Efficacy Evidence of Vigil in Ewing's sarcoma

A survival advantage was seen in the Vigil treated patients compared to control.

Figure 9 shows the results of the Kaplan-Meier analysis of the survival data. Comparison of overall survival from time of procurement (for Vigil manufacture) between the two concurrent groups revealed a 17+ month improvement in survival in the Vigil treated patients. From time of treatment the Vigil treated patients achieved a median survival of 689 days.



Group	N	Median (mo.)	p-value
Matched Control	14	6.8	0.056
Vigil	16	24.0	

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 (ORR 12.5% (1NED, 1PR)) 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 control 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 risk factor scores (the Wheler score for phase I patients (Wheler, Tsimberidou et al. 2012) and the 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 4) thereby supporting evidence of efficacy related to Vigil.

Table 4. Prognostic Indicator Studies of Ewing's sarcoma

Ewing's Sarcoma Prognostic Scores (ESPS)°

	RISK OF LOW SURVIVAL			
Group	**High**	**Intermediate**	**Low**	**Grand Total**
Matched Control	6	8	0	**14**
Vigil	6	10	0	**16**
	High: 16-21 pts	*Intermediate: 11-15 pts*	*Low: 0-10 pts*	

°males higher risk of death

Phase 1 Prognostic Scores*

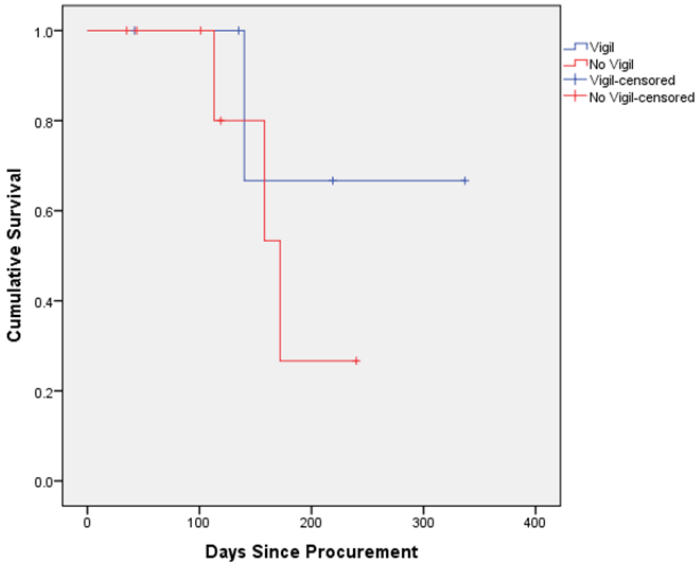
	RISK OF LOW SURVIVAL					
Group	**High**	**High-intermediate**	**Intermediate**	**Low-intermediate**	**Low**	**Grand Total**
Matched Control	0	1	8	5	0	**14**
Vigil	1	6	3	6	0	**16**
	High: 4-5 pts	*High-intermediate: 3 pts*	*Intermediate: 2 pts*	*Low-intermediate: 1 pts*	*Low: 0 pts*	

* Phase 1 Prognostic scores include factors like LDH, number of metastatic sites, albumin, etc.

2.4 Vigil Phase II Trial: Ewing's sarcoma (CL-PTL-121, Part 1)

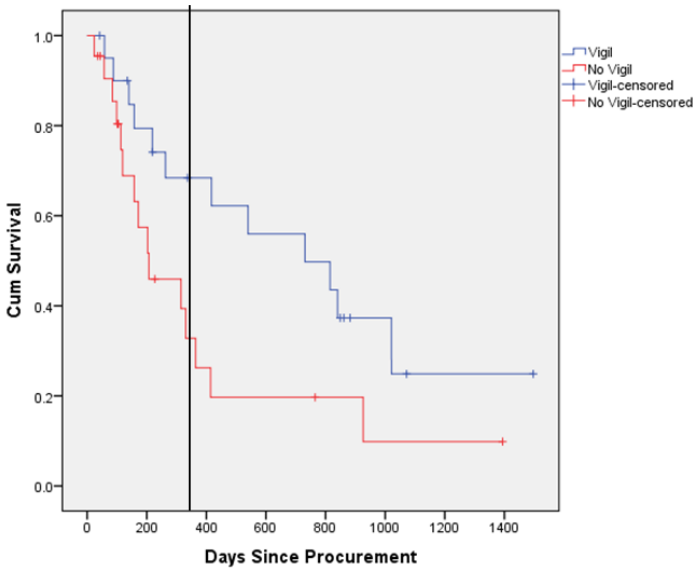
A Phase IIb randomized open label trial (CL-PTL-121) comparing Vigil versus standard of care chemotherapy (gemcitabine/taxol) in metastatic, refractory or recurrent (≥2 prior systemic treatment lines) Ewing's sarcoma patients was initiated and enrolled the first patient 13 months ago. Part 1 of the trial was open to enrollment at 10 sites (5 in development) across the USA but only 13 patients registered into the 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 (5.7 months) 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 (8.9 months). 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 received Vigil in studies CL-PTL-101 and CL-PTL-121 (21 patients) vs. matched (n=14) and randomized controls

(total n=22) provide additional support of potential benefit to Vigil in third-line Ewing's sarcoma (Figure 11). A Kaplan Meier survival of 757 (24.9 months) days was observed with Vigil.



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

Figure 10. Overall Survival Since Procurement on Study CL-PTL-121 Preliminary Results. Two cross-over patients censored. Data updated as of 01/19/17.



Group	N	Mean Survival (mo.)	Median Survival (mo.)	P- value
MC/RC*	22	12.8	6.8	0.041
Vigil	21	24.9	24.0	

Figure 11. Study CL-PTL-101 and -121 Combination Data of Ewing's sarcoma. Overall Survival Since Procurement. Median PFS is 6 months. Expected median PFS is 1.5-2 months (Juergens, Daw et al. 2011; Choy, Butrynski et al. 2014; Yoon, Kwon et al. 2014). Dataset updated 01/23/17. *MC/RC = Matched Control/Randomized Control

2.5 Vigil Phase II Trial: Ewing's sarcoma (CL-PTL-121, Part 2)

Part 2 of CL-PTL-121 was initiated to study the safety of the combination of irinotecan, temozolomide and Vigil in patients with refractory or recurrent (≥1 line of prior treatment line(s)) Ewing's sarcoma.

Ten third-line (received ≥ 2 prior systemic chemotherapy regimens) advanced disease Ewing's Sarcoma patients were entered into Part 2 of CL-PTL-121 study. One patient did not receive any protocol treatment, another patient received only 1 dose of temozolomide/irinotecan and did not receive Vigil before rapid disease progression. These two patients were not evaluable for efficacy. One patient is still being treated on study without progression but is too early for efficacy evaluation (< 6 months SD, stable disease). All other 7 currently evaluable patients for response have received a mean of 8 cycles of Vigil and temozolomide/irinotecan (range 3 to 12). Dose and schedule involved Vigil (1x 10e6 or 1x 10e7 cells/dose on Day 15 every 3 weeks), irinotecan (50 mg/m2/d Days 1-5 orally every 3 weeks) and temozolomide (100 mg/m2/d Days 1-5 orally every 3 weeks). Notably all 7 of these patients had progressed following prior temozolomide/irinotecan before entry into CL-PTL-121.

Demographics are shown in Table 5. No Grade 3 Vigil related toxic effect was observed at dose levels of 1x10e6 (n=5) or 1x10e7 (n=5) cells/injection. No unexpected toxic effect known to be related to irinotecan/temozolomide combination was observed. Table 6 depicts the ≥Grade 3 adverse events reported to date (out of 175 events). Seven (EW-167-3010, EW-167-3012, EW-167-3003, EW-167-3006, EW-167-3011, EW-167-3013, EW-002-3005) of the 10 patients were available to assess durable (≥ 6 months) progression free response. One (EW-167-3014) is alive and doing well but has not yet reached 6-month evaluation. Additionally, the comparison of 1 x 10^6 cell/ml (n=3) vs 1 x 10^7 cells/ml (n=4) did not reveal any differences in safety or efficacy, measured as OS from time of treatment start of mean 169 days (1 x 10^6 cells/ml) vs. 185 days (1 x 10^7 cells/ml).

Table 5. Demographics of subjects enrolled into Part 2 of CL-PTL-121.

	Intent to treat population	**Evaluable patients for response (≥6mo PFS)**
Number of patients	10	7
Age		
Mean	25	24
Range	12 to 46	12 to 46
Gender		
Female	5	4
Male	5	3
Ethnicity		
Caucasian	9	6
Hispanic/Latino	1	1
Other	0	0
Lines of prior systemic Treatment		
Mean	4	5
Range	1 to 13	1 to 13

Table 6. Grade 3 or greater Adverse Events reported, CL-PTL-121, Part 2.

Event Term	CTC Grade	Relationship to VIGIL	Relationship to Temozolomide	Relationship to Irinotecan	Number of Subjects	Number of Events
Platelet Count Decreased	3	Not Related	Probably Related	Probably Related	1	1
Anemia	3	Not Related	Unlikely Related	Unlikely Related	1	2
Chest Wall Pain	3	Not Related	Not Related	Not Related	1	1
Febrile Neutropenia	3	Not Related	Probably Related	Probably Related	1	1
Hypokalemia	3	Not Related	Not Related	Not Related	1	4
Neutrophil count decreased	3	Not Related	Possibly Related	Possibly Related	2	2
	3	Not Related	Probably Related	Probably Related	1	2
	4	Not Related	Probably Related	Probably Related	2	2
Platelet Count Decreased	3	Not Related	Probably Related	Probably Related	2	2
	4	Not Related	Not Related	Not Related	1	1
	4	Not Related	Probably Related	Probably Related	1	1
Pneumonitis	4	Not Related	Not Related	Not Related	1	1
Respiratory Failure	4	Not Related	Not Related	Not Related	1	1
Respiratory, thoracic and mediastinal disorders - Other, Empyema Lt. Thorax Infection	4	Not Related	Not Related	Not Related	1	1
Back Pain	5	Not Related	Not Related	Not Related	1	1
Musculoskeletal and connective tissue disorder - Other, Progressive Ewing's Sarcoma	5	Not Related	Not Related	Not Related	1	1

An objective response by RECIST (partial response, 61% shrinkage of target lesions) was achieved in one patient (EW-167-3006), in addition CT scan abnormalities at baseline cancer sites which demonstrated prior PET avid uptake of FDG of this patient no longer demonstrated PET uptake after Vigil (see Figure 12 and Figure 13). This patient underwent biopsy of pulmonary and pelvic lesions which were still visible by CT scan but no longer PET avid. Results demonstrated no viable tumor presence. Furthermore, entirely necrotic, hyalinize tissue of the pulmonary sample indicates response to on-study therapy. Another patient EW-167-3003 similarly underwent PET scan evaluation and showed change from PET avid at baseline to PET negative 7 months after treatment initiation. This patient later after completion of Vigil developed an infection of the left lung cavity (status post left pneumectomy) and passed away at Month 8 related to progressive infectious pneumonia. Autopsy was performed and the PET negative disease in the posterior chest wall seen on CT scan revealed no viable tumor cells at that site consistent with complete histologic disease response. Additionally, no other sites of microscopic cancer were found at autopsy. Patient EW-167-3010 had durable SD ≥6 months

and remains in good health. Consideration for second resection and assessment of detection of viable tumor at CT detectable disease sites is underway. Previously, we also reported complete histologic response in a prior Vigil monotherapy treatment patient (F-062), who was not RECIST evaluable (Ghisoli, Rutledge et al. 2017). This patient underwent a second harvest and second Vigil treatment with persistent detectable disease following first Vigil treatment and remains alive without recurrence 4 years/months following Vigil start. It was suggested that neoantigen patterns at different sites of cancer growth may have some variation and Vigil activity in part may relate to the neoantigen pattern contained on the autologous tissue harvested. As such, patients with delayed regression or mixed response could be considered for second Vigil harvest and treatment if other more optimal therapies are not available.

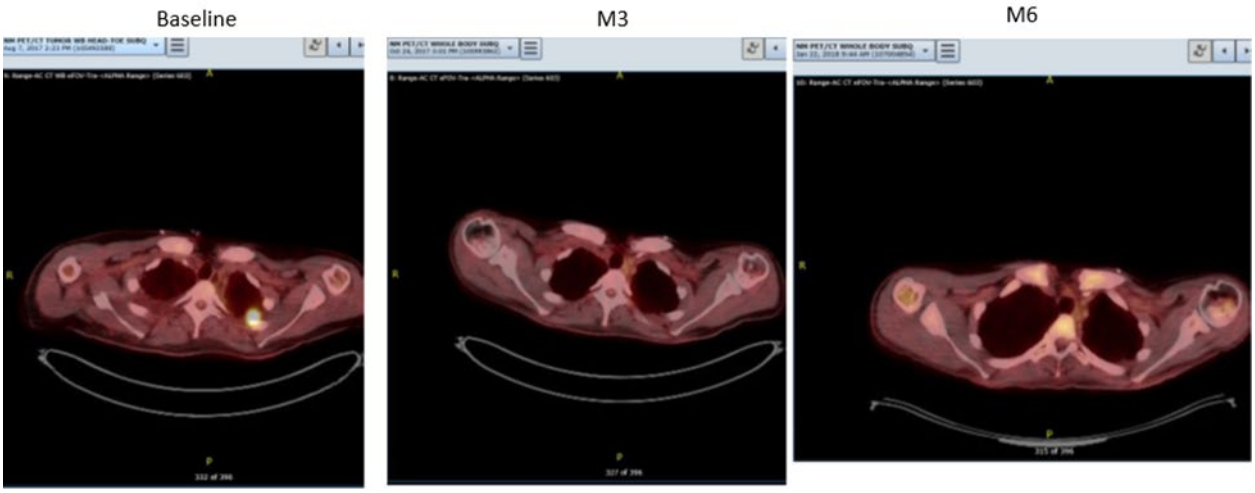


Figure 12. EW-167-3006 Left Upper Lung posterior target lesion PET scan from baseline, Month 3, and Month 6

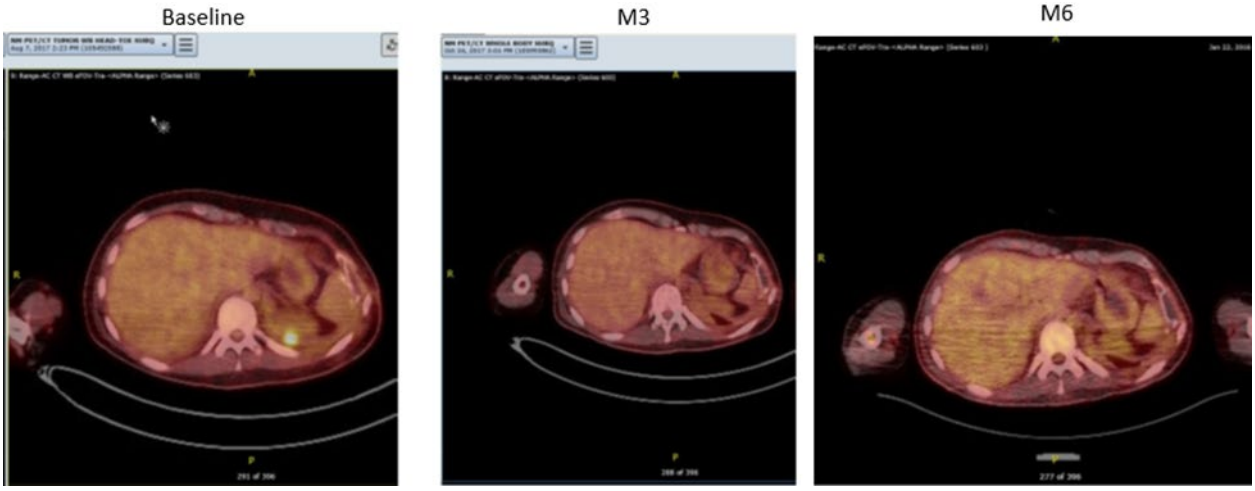


Figure 13. EW-167-3006 Left diaphragm soft tissue target lesion PET scan from baseline, Month 3, and Month 6.

Patient EW-167-3012 had all recurrent disease harvested for Vigil construction and remains without disease recurrence ≥ 6 months. Patients EW-167-3013, EW-167-3011 and EW-002-3005 had progression in < 6 months despite treatment with Vigil, temozolomide and irinotecan. Summary of all participants responses are shown in Figure 14.

Overall, historical disease progression would be expected within 2 months of ≥ third-line disease progression.

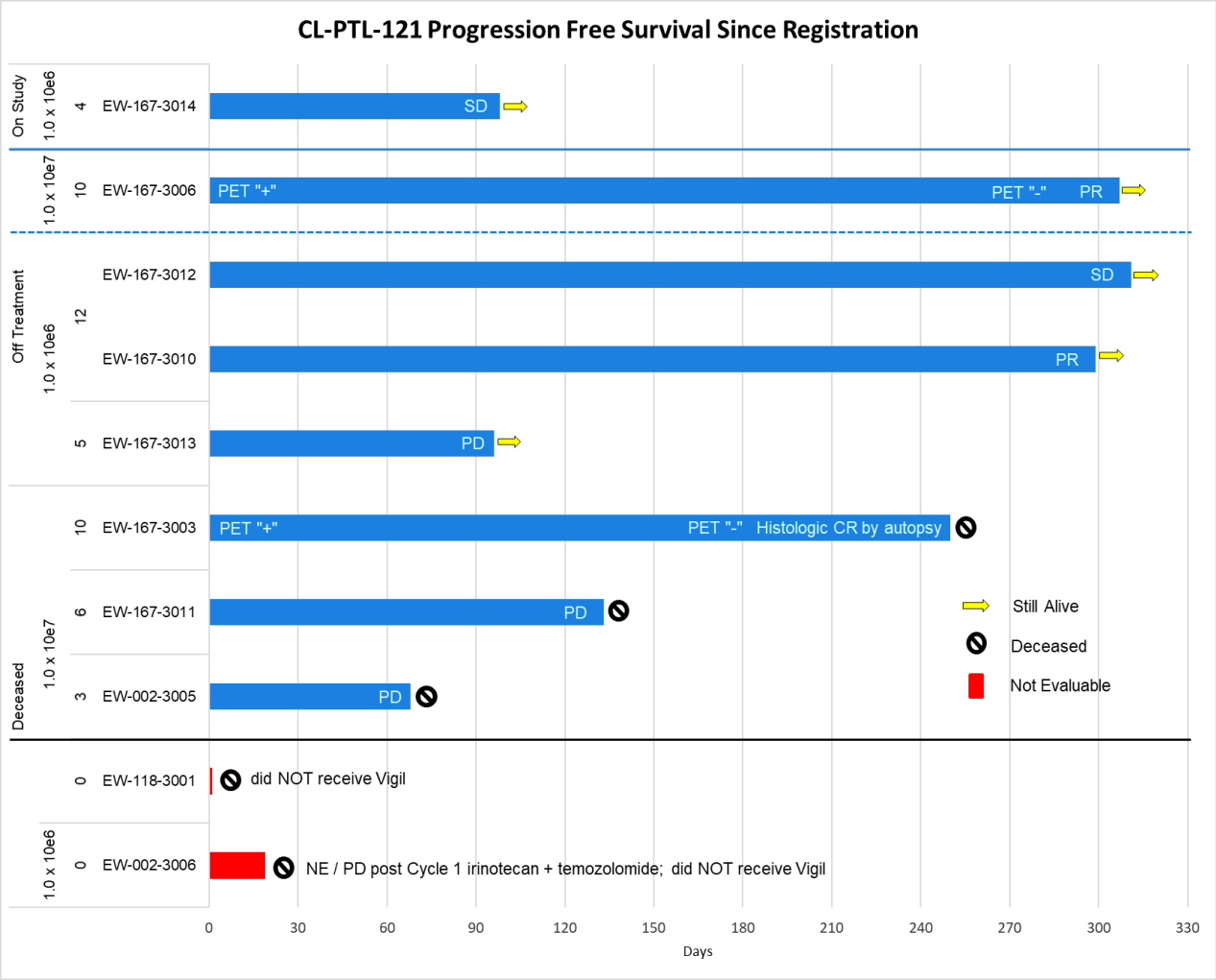


Figure 14. Patient summary of PFS of all 10 intent to treat patients, Part 2 of CL-PTL-121. Fifty-seven percent (57%, 4/7) with a durable clinical response rate (for ≥ 6 months).

3.0 STUDY RATIONALE

The results from the Phase I Vigil trial show product safety (both short-term and long-term through 3+ years), confirm effective transgene expression (GMCSF) and RNAi [furin] silencing, and support the rationale of the immunotherapeutic triad, i.e., autologous tumor cell therapy with

increased GMCSF expression and decreased TGFβ production shown to effectively modulate immunogenicity as evidenced by the correlation of IFNγ-ELISPOT responsiveness with overall survival and the elicitation of the CD4+ mediated circulating antibody responses against CT47 in two of the Phase 1 Ewing's sarcoma patients. The preliminary evidence of safety, immune stimulation and clinical benefit from Vigil in patients with Ewing's sarcoma 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). A lower tumor burden is typically associated with greater delay in progression with other immunotherapies. Mathematical modeling supports the concept of better control of disease progression with the combined use of effective chemotherapy (e.g., temozolomide/irinotecan as second line treatment in Ewing's sarcoma) and immunotherapy (i.e., Vigil) in first relapse patient population (de Pillis, Gu et al. 2006). The utilization of the standard of care temozolomide/irinotecan regimen at investigator consensus defined dose and schedule appears to be minimally immunosuppressive and thus a reasonable option for combination with Vigil. Moreover, the temozolomide and irinotecan regimen at effective dose levels has low myelosuppressive toxicities (irinotecan 50mg/m^2/dose and temozolomide 100mg/m^2/dose) and may increase the tumor antigen release and surface exposure of calreticulin (part of the specific danger-signaling system) as well as reduce the frequency of CD4+ CD25+ regulatory T-cells (Kim, Kim et al. 2010). In addition, given that Ewing's sarcoma expression levels of type-1 associated chemokine ligands CXCL9, CXCL10, and CCL5 correlate with TIL expression chemokine receptors (CXCR3 and CCR5) (Berghuis, Santos et al. 2011) it is notable that temozolomide has been shown to enhance tumor matrix presentation of CXCL9 and CXCL10 in certain tumor models (Tan, Evrard et al. 2015). Temozolomide and irinotecan in combination have shown benign safety profiles without any Grade 3 treatment-related adverse events in seven combination treated Ewing's Sarcoma patients of Part 2 of CL-PTL-121. Furthermore, clinical antitumor activity was demonstrated in 4 of the 7 combination treated patients, all of whom had previously failed temozolomide and irinotecan, as evidenced by 2 partial responses, one histological CR (autopsy) and one prolonged SD currently near 1 year in duration. Thus, the combination of low dose Vigil, irinotecan and temozolomide may not only increase anti-tumor responses but also prolong time to progression and improve the of quality of life in the treatment of Ewing's sarcoma patients.

4.0 OBJECTIVES

4.1 Primary objective

- To compare the progression free survival of subjects dosed with Vigil immunotherapy in combination with irinotecan and temozolomide vs. irinotecan and temozolomide.

4.2 Secondary objective(s)

- To determine and compare the overall survival of subjects with relapsed or refractory Ewing's sarcoma 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 1 prior line of systemic chemotherapy treated

with Vigil immunotherapy dosed with Vigil immunotherapy in combination with irinotecan and temozolomide

- To determine the Vigil manufacturing success rate by gathering data from tumor tissue collection, Vigil construction, and test procedures.

5.0 STUDY DESIGN

This is a multicenter, 1:1 randomized Phase III study of intradermal autologous Vigil immunotherapy (1.0 x 10^6 cells/injection; minimum of 4 to a maximum of 12 administrations) in combination with irinotecan and temozolomide in subjects with metastatic Ewing's Sarcoma Family of Tumors (ESFT) refractory/intolerant or recurrent to 1 prior line of chemotherapy. Participants undergoing a standard surgical procedure (e.g., tumor biopsy or palliative resection) may have tumor tissue harvested for manufacture of investigational product

Subjects meeting eligibility criteria including manufacture of a minimum of 4 immunotherapy doses of Vigil will be randomized to receive either:

Group A

(i) oral temozolomide 100 mg/m^2 daily (Days 1 – 5, total dose 500 mg/m^2/cycle),
(ii) oral irinotecan 50 mg/m^2 daily (Days 1 – 5, total dose 250mg/m^2/cycle), and
(iii) Vigil 1.0 x 10^6 cells/injection, intradermally on Day 15.

OR

Group B

(i) oral temozolomide 100 mg/m^2 daily (Days 1 – 5, total dose 500 mg/m^2/cycle), and
(ii) oral irinotecan 50 mg/m^2 daily (Days 1 – 5, total dose 250mg/m^2/cycle).

If chemotherapy study drugs cannot be administered orally (i.e. size of capsule, nausea, etc.) it is the physician's discretion to request intravenous administration of temozolomide at 100 mg/m^2 daily and/or irinotecan 20 mg/m^2 daily intravenously.

One cycle = 21 days. Screening for the main portion of the study 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. At the time of data analysis the subjects will be stratified by initial response to frontline treatment (recurrent versus refractory (progressive)).

Participants 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 Day 1 (prior to chemotherapy administration) at Cycles 2, 4, and 6, end of treatment (EOT), 3 months after EOT, and every 6

months thereafter. Blood for exploratory ctDNA analysis will be collected at tissue procurement, prior to chemotherapy administration at baseline, prior to chemotherapy administration on Day 1 of Cycles 2, 3, 4, and 6, and EOT.

6.0 STUDY POPULATION

6.1 Sample Size

Approximately 140 subjects will have tissue procured so that 114 evaluable subjects will be registered for enrollment into the study.

6.2 Tissue Procurement Inclusion Criteria

Subjects 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). If available, NGS sequencing report should be submitted to Gradalis.
5. Recurrence or refractory to 1 line of systemic chemotherapy including but not limited to doxorubicin, vincristine and ifosfamide.
6. Planned standard of care surgical procedure (e.g., tumor biopsy or palliative resection or thoracentesis) and expected availability of a cumulative soft-tissue mass of ~10-30 grams tissue ("grape" to "golf-ball" size / approximate 2 cm total diameter on imaging) and/or pleural fluid estimated volume ≥ 500mL (from a primary or secondary thoracentesis, yielding in a high volume of tumor cells) for immunotherapy manufacture.
7. Tumor intended for immunotherapy manufacture is not embedded in bone and does not contain luminal tissue (e.g. bowel, ureter, bile duct).
8. Ability to understand and the willingness to sign a written protocol specific informed consent for tissue harvest or a parental/guardian informed consent and pediatric assent when appropriate.

6.3 Tissue Procurement Exclusion Criteria

Subjects 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 HIV or chronic Hepatitis B or C infection.
6. Known hypersensitivity to any temozolomide component or to dacarbazine (DTIC).
7. Known hypersensitivity to irinotecan or its excipients.
8. Known history of allergies or sensitivities to gentamicin.
9. 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.

6.4 Study Enrollment Inclusion Criteria

Subjects will be eligible for registration if they meet the tissue procurement inclusion criteria and all of the following study enrollment inclusion criteria:

1. Completed manufacture of at least 4 vials of Vigil.
2. Karnofsky performance status (KPS) / Lansky performance status (LS) ≥80%.
3. Normal organ and marrow function as defined below:

Absolute granulocyte count	≥1,000/mm^3
Absolute lymphocyte count	≥400/mm^3
Platelets	≥75,000/mm^3
Hemoglobin	≥8.0 mg/dL
Total bilirubin	≤ institutional upper limit of normal*
AST(SGOT)/ALT(SGPT)	≤2x institutional upper limit of normal
Creatinine	<1.5 mg/dL

*documented Gilbert's syndrome may be considered after medical monitor review
4. Subject has recovered to CTCAE Grade 1 (except for parameters noted in Item 3, above) or better from all adverse events associated with prior therapy or surgery. Pre-existing motor, sensory neurologic pathology or symptoms, or dermatologic must be recovered to CTCAE Grade 2 or better.
5. 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.
6. Ability to understand and the willingness to sign a written informed protocol specific consent or a parental/guardian informed consent and pediatric assent when appropriate.

6.5 Study Enrollment Exclusion Criteria

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

7.0 WITHDRAWAL

7.1 Discontinuation from Study Treatment

Treatment will be continued until progressive disease by RECIST 1.1. However, insofar as some patients in our early vaccine trials had increased survival despite initial, short-term volume increase. There is accumulating evidence that some subjects treated with immune system stimulating agents may appear to demonstrate progression of disease by conventional response criteria before demonstrating clinical objective responses and/or stable disease and ultimately survival benefit. It may be that enhanced inflammation within tumors initially leads to an increase in tumor size or newly visible small lesions. Over time, both the malignant and inflammatory portions of the mass may then decrease leading to overt signs of clinical improvement.

Therefore, selected subjects may be allowed to continue study therapy after investigator-assessed RECIST 1.1 progression and with agreement by the Gradalis medical monitor if the subject is determined to be deriving clinical benefit, tolerating study drug and meeting the following criteria:

1. Absence of symptoms and signs indicating clinically significant PD indicating disease progression.
2. No decline in ECOG performance status.
3. Absence of rapid progression of disease or of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention.

If subsequent imaging shows an objective response or stable disease relative to baseline, treatment with study agents will continue. If subsequent imaging again shows PD, patients will be discontinued from study therapy.

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

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

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

7.2 Treatment Delay

- Treatment may be delayed no more than 3 weeks to allow recovery from toxicity.
- 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 every 3-week schedule thereafter.

If Vigil administration is delayed, then irinotecan and temozolomide should concurrently be delayed.

8.0 INVESTIGATIONAL PLAN

8.1 Subject Screening and Registration

Written documentation of full, non-contingent IRB approval of the protocol and consent document must be on file before a subject can be registered. Study participation begins once written informed consent is obtained. Subjects will be assigned a study identification number upon scheduling of tissue procurement for Vigil manufacture.

No later than the date of surgery, a pre-registration packet will be submitted by the site to Vigil@gradalisinc.com for medical monitor review.

One to eight weeks after tissue procurement has occurred eligibility will be reconfirmed by the study site. The Site will register the subject by sending the registration packet to Vigil@gradalisinc.com for medical monitor review.

Please allow 48 hours for subject 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 and randomization result.

8.2 Tumor Procurement

Refer to the Study Reference Manual for instructions. Surgery for tissue procurement for Vigil manufacture must be scheduled and approved in advance.

The cumulative equivalent of a "grape" to "golf ball size" mass (~10-30 gm of prime tumor tissue / approximate 2 cm total diameter on imaging) is optimal for Vigil manufacturing. If surgeons have the option of collecting more tissue, more doses of Vigil may be prepared (up to 12 doses). Vigil 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 Vigil manufacture.

8.3 Vigil Immunotherapy Manufacturing

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

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

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

8.4 Study Treatment Administration

Treatment will be administered on an outpatient basis.

8.4.1 Schedule, Dose and Administration

	Drug	Dose	Schedule
Group A	Temozolomide	100 mg/m^2 daily, oral	Days 1 – 5, every 21 days
	Irinotecan	50 mg/m^2 daily, oral	Days 1 – 5, every 21 days
	Vigil	1.0 x 10^6 cells/injection, intradermal	Day 15, every 21 days

	Drug	Dose	Schedule
Cross-Over Subjects	Vigil	1.0 x 10^6 cells/injection, intradermal	every 21 days

Subjects will receive Vigil at 1.0 x 10^6 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 may be used.

	Drug	Dose	Schedule
Group B	Temozolomide	100 mg/m^2 daily, oral	Days 1 – 5, every 21 days
	Irinotecan	50 mg/m^2 daily, oral	Days 1 – 5, every 21 days

Please contact the sponsor with any dose adjustment considerations.

Irinotecan Preparation

Order the injectable formulation of irinotecan from central supplier, Clinigen. Draw up 1 cycle of (five doses) into provided oral syringes and dispense to the subject with instructions to refrigerate until administration. Irinotecan may be mixed with cranberry-grape juice before administration to mask the bitter flavor and administered once daily on Days 1 through 5 of each 3-week cycle.

Temozolomide Preparation

Temozolomide capsules for 1 cycle will be obtained by the patient with administration instructions. Temozolomide may be opened and mixed in apple sauce or juice if unable to swallow whole capsules. Temozolomide is administered on Days 1 through 5 of each 3-week course and given at least 1 hour before irinotecan.

Subjects should be instructed to complete a dosing diary for the administration of oral irinotecan and oral temozolomide.

Premedications and post medications

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 immunosuppressants should be avoided due to immune inhibition activity.

Temozolomide / irinotecan: Per package inserts and institutional standards. A home supply of anti-emetic (e.g. ondansetron) is highly recommended to take before temozolomide and as needed. Also, a patient supply of an anti-diarrheal medication (e.g. loperamide) should be available after irinotecan for use as needed.

The patient should be educated with a study calendar indicating days to get chemotherapy and/or Vigil as well as supportive care medications and when Vigil is given.

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 Study 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 Vigil dose will be reduced by 50% and continued.

Dose Modifications for temozolomide and irinotecan

Dose modifications for temozolomide, and irinotecan may be necessary for subject safety and should be carried out in accordance with the Investigator's standard of practice, the package inserts, and institutional standards. Please contact the sponsor regarding any dose modifications.

If irinotecan + temozolomide is administered beyond 12 cycles, it will be administered off study and no longer provided by Gradalis.

If irinotecan + temozolomide is discontinued, Vigil may be administered every 3 weeks until all manufactured product is exhausted or when disease progression is noted.

Study Regimen Delay

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:

- Anti-cancer therapy, including chemotherapy, radiotherapy, or endocrine therapy other than those required per protocol.
- Any investigational drug or device other than Vigil.
- 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 generally prohibited. Contact the Gradalis medical monitor if other steroidal regimens are clinically needed.
- 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 vaccines are allowed on study, if deemed appropriate by the investigator. Live vaccines are prohibited while on study.
- Valproic Acid (due to temozolomide)
- Strong CYP34A inducers and inhibitors (due to irinotecan)

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 both treatment arms, if indicated. Treatment for drug-related adverse events should be administered at the discretion of the investigator.

Peg-filgrastim may be administered after completion of Day 5 temozolomide to facilitate ANC recovery.

8.6 Contraception

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).

8.7 Toxicity

Toxicities will be graded and reported according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE) Version 5. 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.

9.0 SCHEDULE OF ASSESSMENTS

Pre-study Assessments / Surgery Pre-Screening

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 and none of the exclusion 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 and hepatitis 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. Blood collection for immune function analysis will be obtained at tissue harvest. (≤1 week of tissue procurement)
8. Plasma collection for immune function analysis (ctDNA) (≤1 week of tissue procurement)

Screening Assessments / Protocol Screening

The following evaluations will be performed on all subjects within 2 weeks of Cycle 1 (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. A physical examination (including vital signs, height, temperature and body weight) must be obtained.
4. Assessment of concomitant medications
5. Assessment of PS on the Karnofsky or Lansky scale (see Appendix A) must be obtained.
6. Radiological assessment of disease status with computed tomography (CT) chest /abdomen/pelvis (magnetic resonance imaging (MRI) abd/pelvis may be substituted for CT abd/pelvis) within 4 weeks of Cycle 1. 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. Images must be uploaded into WorldPro or submitted to WorldCare.
7. A complete blood count (CBC) with differential and platelet count must be performed. (HIV and hepatitis testing is not required if the subject has no medical history of HIV).
8. 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.
9. Blood collection for immune function analysis (ELISPOT).
10. Plasma collection for immune function analysis (ctDNA).
11. Pregnancy test for those of childbearing potential.
12. Submission to Gradalis, Inc. of preferably 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 on Day 1 of each cycle (1 Cycle = 21 days ±3 days) (unless otherwise specified):

1. *A physical examination, including vital signs and body weight.
2. *A toxicity assessment (CTCAE v 5).
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 6 weeks ± 7 days from Cycle 1 regardless of treatment arm assignment or treatment delay. 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. Blood collection for immune function analysis (ELISPOT) on Day 1 (prior to chemotherapy administration) at Cycles 2, 4, and 6.
8. Plasma collection for immune function analysis (ctDNA) on Day 1 prior to chemotherapy administration at Cycles 2, 3, 4 and 6.
9. *Assessment of Karnofsky Performance Status or LS.
10. Group A and B participants: oral temozolomide 100 mg/m^2 daily (Days 1 – 5, total dose 500 mg/m^2/cycle)
11. Group A and B participants: oral irinotecan 50 mg/m^2 daily (Days 1 – 5, total dose 250mg/m^2/cycle)
12. Group A participants: Vigil 1.0 x 10^6 cells/injection, intradermally on Day 15.
13. Cross-over Subjects: Vigil 1.0 x 10^6 cells/injection, intradermally every 21 days
14. For those randomized to Group A or in cross-over, Vigil administration. Day 16 post Vigil administration assessment of injection site. Instruct the subject on Day 15 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).
15. Submission of Next Generation Sequencing data, if available in medical records.

Subjects who are stable at the end of Cycle 4, with prior approval by the Sponsor may conduct evaluations denoted with an asterisk* on Day 15 of each cycle (1 Cycle = 21 days ±3 days) (unless otherwise specified).

Subjects who become child-bearing while on study will have a pregnancy test performed prior to their next dose. See Section 8.6.

End of Treatment Assessments

The following evaluations will be performed within 30 days after completion of the study 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/Lansky 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. Blood (PBMC) for immune function analysis (ELISPOT).
9. Plasma for immune function analysis (ctDNA).

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 quarterly 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. Blood collection for immune function analysis (ELISPOT) 3 months ±7 days from EOT and every 6 months ±7 days thereafter.
7. Assessment of Performance Status.

Long Term Follow Up

After progression, subjects and their physicians will be contacted quarterly for documentation of post study therapies and 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 and the subject consented to the collection, the clinical site will present the option of the procurement to the participant.

10.0 CROSS-OVER

Subjects randomized to Group B may cross-over to Group A after discussion with, and approval from the Gradalis medical monitor or authorized designee.

Within 6 weeks of second relapse or progression and if alternative therapy has not been given since EOT, subjects randomized to Group B, will be allowed to cross-over to receive single agent Vigil every 21 days following End of Treatment assessment. Subjects who cross-over may receive up to 12 doses of Vigil depending upon the quantity of Vigil manufactured. After the Group B EOT assessment, the subject will start "cross-over" from Assessments During Treatment onward. Vigil and all assessments will occur on Day 1 of each cycle.

Cross-over must occur within 2 years of End of Treatment assessments of Group B enrollment. Subjects who progress ≥ 8 weeks post EOT assessment must re-screen and if they qualify will start "cross-over" from Assessments During Treatment onward. Eligibility criteria for cross-over subjects are listed below.

10.1 Inclusion Criteria for Cross-Over

Subjects will be eligible for cross-over, if they meet all of the following criteria:

1. Evidence of radiologic disease progression by RECIST 1.1 after enrollment into Group B.
2. Successful manufacturing of at least 4 vials of Vigil.
3. Karnofsky performance status (KPS) / Lansky performance status (LS) ≥70%.
4. Normal organ and marrow function as defined below:

Absolute granulocyte count	≥1,000/mm^3
Absolute lymphocyte count	≥400/mm^3
Platelets	≥75,000/mm^3
Total bilirubin	≤ institutional upper limit of normal
AST(SGOT)/ALT(SGPT)	≤2x institutional upper limit of normal
Creatinine	<1.5 mg/dL

*documented Gilbert's syndrome may be considered after medical monitor review

5. Subject has recovered to CTCAE Grade 1 (except for parameters noted in Item 4, above) or better from all adverse events associated with prior therapy or surgery. Pre-existing motor, sensory neurologic pathology or symptoms, or dermatologic toxicities 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 or a parental/guardian informed consent and pediatric assent when appropriate.

10.2 Exclusion Criteria for Cross-Over

In addition to the Procurement and Study Enrollment exclusion criteria, Subjects will NOT be eligible for cross-over if meeting any of the following criteria:

1. With the exception of irinotecan and temozolomide while on study, 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.

11.0 CONDUCT OF THE STUDY

11.1 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.

11.2 IRB

This trial can be undertaken only after review and full approval of the protocol and an 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 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.

11.3 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.

11.4 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.

11.5 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.

11.6 Protocol Questions and Deviations

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

11.7 Data Safety Monitoring Board

An independent data monitoring committee (IDMC) or 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.

12.0 EVALUATION OF TUMORS

12.1 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.

Radiological images should be submitted to WorldCare upon receipt from the imaging facility for third party review.

13.0 DRUG INFORMATION

13.1 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 10^6 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.

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 Study Reference Manual for preparation and handling information.

Vigil concentrate: 1.0×10^6 cells 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.

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 Study Reference Manual.

14.0 ADVERSE EVENTS

14.1 Adverse Event and Serious Adverse Event Definitions

Adverse Event

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

Serious Adverse Event

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

- Initial or prolonged hospitalization (≥ 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 5. All AEs, regardless of severity, will be followed by the Treating Physician until resolution is satisfactory.

14.2 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.

14.3 Vigil 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/arthralgias, 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.

14.4 Recording of an Adverse Event

Adverse events will be recorded for the duration of a subject's time on study: following procurement of the subject's tissue for tissue manufacture and for up to 30 days following the last administration of Vigil. 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.

14.5 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 following procurement of the subject's tissue for tissue manufacture and for up to 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.

15.0 PATIENT COMPLETION AND WITHDRAWAL

15.1 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.

16.0 STATISTICAL CONSIDERATIONS

16.1 Sample Size Justification

This is an open label randomized controlled Phase III 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 log rank test at the alpha=0.0476 level of significance, 67 events will provide 90% power to detect an PFS HR of 0.45. Assuming a Group B (control group) median PFS of 6 months, a one-year accrual period, and a six-month follow-up period after randomization of the last subject, it is estimated that the total sample size (number of subjects) required to achieve 67 events is 114 (57 patients in each arm).

An unblinded interim analysis will be conducted by an independent data monitoring committee (IDMC) when 40 PFS events have occurred. Based on the results of this interim analysis, the planned sample size may be increased to 120 events. If the sample size is increased to 120 events, then the study will have approximately 90% power to detect a true HR of 0.55. Note that if the sample size is increased to 120 events, then the accrual period will be extended to 24 months, with a 6-month follow-up period after randomization of the last patient. Under these assumptions, it is estimated that approximately 164 patients will need to be randomized in order to achieve 120 events. Section 16.6 provides further details.

16.2 Definition of Progression Free Survival (PFS)

- Progression free survival (PFS) is defined as the time from randomization to the event of disease recurrence/progression or death due to any cause.
- Radiographic disease recurrence/progression will be assessed using the RECIST 1.1 criteria. If the disease recurrence/progression assessment involves more than one date, the earliest date will be used as the event date.
- Patients who are alive and recurrence free at the time of analysis data cut-off, or who terminate the study for reasons other than disease progression or death, or who receive non-protocol treatment for Ewing's Sarcoma, will be censored on the last assessment date at which a RECIST 1.1 evaluation of disease status was made.
- Patients missing baseline disease assessment will be censored at date of randomization.
- For equivocal findings of recurrence (e.g., very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If recurrence is confirmed at the next scheduled assessment, the date of recurrence should be the earlier date when recurrence was suspected.

16.3 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.

16.4 Efficacy Analyses

16.4.1 Primary Efficacy Analysis

The primary endpoint of PFS is the time from randomization to progression according to RECIST version 1.1 or death. The distributions of PFS in the two arms will be compared using a two-sided log-rank test at the alpha=0.0476 level of significance

16.4.2 Secondary Efficacy Analyses

A fixed sequence testing procedure will be used to control the overall level of significance for the analysis of the secondary endpoints of OS and ORR. If the primary analysis of PFS is statistically significant ($p<0.0476$), then OS will be analyzed using a two-sided test at the alpha=0.0476 level of significance. In addition, if the analysis of OS is statistically significant ($p<00476$), then ORR will be analyzed using a two-sided test at the alpha=0.0476 level of significance. However, if an earlier analysis is not statistically significant, the remaining analyses will be exploratory rather than confirmatory.

The secondary endpoint of OS is defined as time from randomization to death or to the date of last follow-up. 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 analysis of OS will be conducted using the two-sided log-rank test. Kaplan-Meier OS curves will be displayed by treatment arm. Median OS and percent OS at fixed time points will be estimated.

The secondary endpoint of ORR will be analyzed using Pearson's chi-square test. Overall response rate (ORR) is defined as the proportion of patients who have a partial or complete response to therapy according to RECIST 1.1. ORR will be reviewed 6 months after treatment with Vigil.

The analyses of all other secondary endpoints will be conducted using two-sided tests at the alpha=0.05 level of significance.

16.4.3 Sensitivity Analyses of the Primary Endpoint

Five sensitivity analyses of the primary endpoint will be conducted using each of the following modifications to the definition of PFS:

- If the disease recurrence/progression assessment involves more than one date, the latest date will be used as the event date.
- If disease recurrence or death occurs right after missing data for a scheduled radiographic disease assessment (including missing the assessment or assessment results in an unevaluable status for overall response per RECIST 1.1), the patient will be censored at the date of the last radiographic disease assessment.
- If a patient receives non-protocol treatment for Ewing's Sarcoma, the patient will be treated as having progression at the date of the last radiographic disease assessment.
- Patients missing baseline disease assessment will be treating as had having progression at the date of randomization.
- For equivocal findings of recurrence (e.g., very small and uncertain new lesions; cystic changes or necrosis in existing lesions) for whom treatment continues until the next scheduled assessment, and if recurrence is confirmed at the next scheduled assessment, the date of recurrence should be the date when recurrence was confirmed.

16.4.4 Sensitivity Analysis of Overall Survival

A sensitivity analysis of OS will be conducted in which subjects who are crossed over to Vigil therapy will be censored at the time of crossover.

16.4.5 Subgroup Analyses of the Primary (PFS) and Key Secondary Endpoints (OS, ORR)

Subgroup analyses will be conducted in the following subsets of the ITT population:

- Race / ethnicity
- Study site / region
- Age group (<15 years (low), ≥15 years (high))
- Gender
- Number of doses received (≤median number of doses administered, >median number of doses administered)
- Number of lines of prior treatments including chemotherapy and radiation
- At time of diagnosis: localized vs. metastatic disease
- At time of diagnosis: skeletal vs. extraskeletal
- At time of diagnosis: tumor volume/burden (<100ml or 8cm vs >100ml or 8cm)
- Response to frontline therapy (CR vs. PR vs. SD vs. progressive disease)
- Time to first recurrence: <2 years, > 2 years

16.5 Safety Analyses

Safety endpoints include all adverse events (CTCAE 5), laboratory safety assessments, and physical examination findings.

16.6 Interim Analysis

An interim analysis for efficacy, futility, and potential sample size re-estimation will be conducted by an independent data monitoring committee (IDMC) when 40 PFS events have been achieved (60% information fraction). Based on the Lan-DeMets method for group sequential trials using O'Brien-Fleming boundaries (Reboussin, DeMets et al. 2000), the levels of significance for the interim and final analyses are alpha=0.00762 and alpha=0.0476, respectively.

Based on the results of the interim analysis, the IDMC will make one of the following four recommendations to the sponsor:

- Terminate the study for efficacy ($p<0.00762$ at the interim analysis);
- Continue the study as planned;
- Increase the sample size to 120 events;
- Terminate the study for futility.

Provided that the IDMC does not recommend terminating the study early for efficacy, the conditional power of the planned final analysis at 67 events will be computed using the methodology of (Jennison and Turnbull 2000) under the assumption that the observed hazard ratio at the interim analysis represents the true effect. In terms of the HR observed at the interim analysis, these rules can be translated (approximately) as follows:

- If the HR is less than 0.43, terminate for efficacy
- If the HR is in the range from 0.43 to 0.51, continue as planned.
- If the HR is in the range from 0.51 to 0.61 (i.e., conditional power in the range from 50% to 80%), increase the sample size to 120 events.
- If the HR is greater than 0.61 (i.e., conditional power less than 50%), terminate for futility.

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 AND LANSKY PERFORMANCE SCALES

Karnofsky		Lansky	
Score	Description	Score	Description
100%	No symptoms.	100%	fully active, normal
90%	Able to carry on normal activity; minor signs or symptoms of disease.	90%	minor restrictions in strenuous physical activity
80%	Able to carry on normal activity with effort; some signs or symptoms of disease.	80%	active, but tired more quickly
70%	Cares for self, unable to carry on normal activity or do active work.	70%	greater restriction of play and less time spent in play activity
60%	Requires occasional assistance but is able to care for most of own needs.	60%	up and around, but active play minimal; keeps busy by being involved in quieter activities
50%	Requires considerable assistance and frequent medical care.	50%	lying around much of the day, but gets dressed; no active playing participates in all quiet play and activities
40%	Disabled; requires special care and assistance.	40%	mainly in bed; participates in quiet activities
30%	Severely disabled; hospitalization indicated, although death not imminent.	30%	bed-bound; needing assistance even for quiet play
20%	Very ill; hospitalization necessary; active supportive treatment required.	20%	sleeping often; play entirely limited to very passive activities
10%	Moribund, fatal processes progressing rapidly.	10%	doesn't play; does not get out of bed
0	Patient expired.	0	unresponsive

APPENDIX B

SCHEDULE OF ASSESSMENTS

Procedure	Prestudy / Surgery Pre-Screening	Protocol Screening	Day 1 of each Cycle[1] (unless otherwise noted)	End of TX	Response Follow-Up (q 3mo±7 days) Until Progression
Informed consent	X	X			
Medical History	X				
Physical Examination	X	X	X	X	X
Toxicity (adverse events)		from time of procurement	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 6 weeks ± 7 days from Cycle 1	Within 45 days	X
CBC with differential	X[2]	X	X	X	X
HIV testing, if applicable		X			
Hepatitis testing, if applicable		X			
Serum Chemistry	X[3]	X	X	X	X
PBMC collection for Immune Function Analysis	≤ 1 week of tumor procurement	X	Cycles 2, 4, and 6 Day 1 prior to chemotherapy administration	X	3 months post EOT and q 6 months thereafter
Plasma collection for ctDNA	≤ 1 week of tumor procurement	X	Cycles 2, 3, 4, and 6 Day 1 prior to chemotherapy administration	X	
Pregnancy Test (if applicable)		X			
Tissue Procurement	X				
Vigil administration *depending on randomization*			Day 15 q 21±3 days [4]		
Injection Site Assessment			Day 16 only (may be conducted at home)[5]		
Temozolomide			Days 1 – 5, q21 days		
Irinotecan			Days 1 – 5, q21 days		
Survival Status	X				Long Term Follow Up[6]
Immunohistochemistry		X[7]			

[1] Subjects who are stable at the end of Cycle 4, with prior approval by the Sponsor may conduct evaluations on Day 15 of each cycle (1 Cycle = 21 days ±3 days) (See Section 9.0).

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

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

[4] Cross-over subjects will receive Vigil on Day 1.

[5] Cross-over subjects will have assessment on Day 2.

[6] After progression, subjects will be contacted by phone quarterly for documentation of post study therapies and survival status.

[7] These slides should correlate with tumor procured for vaccine manufacture.

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