Phase II study of Vigil® DNA engineered immunotherapy as maintenance in advanced stage ovarian cancer

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HIGHLIGHTS

- Vigil (DNA engineered immunotherapy), well tolerated in frontline ovarian cancer.
- Significant induction of delay in relapse (p = 0.033) shown between Vigil/control.
- Correlation of T cell activation (ELISPOT response) and clinical benefit

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ABSTRACT

Objectives. The majority of women with Stage III/IV ovarian cancer who achieve clinical complete response with frontline standard of care will relapse within 2 years. Vigil immunotherapy, a GMCSF/bi-shRNA furin DNA engineered autologous tumor cell (EATC) product, demonstrated safety and induction of circulating activated T-cells against autologous tumor in Phase I trial Senzer et al. (2012, 2013). Our objectives for this study include evaluation of safety, immune response and recurrence free survival (RFS).

Methods. This is a Phase II crossover trial of Vigil (1.0×10^7 cells/intradermal injection/month for 4 to 12 doses) in Stage III/IV ovarian cancer patients achieving cCR (normal imaging, CA-125 ≤ 35 units/ml, physical exam, and no symptoms suggestive of the presence of active disease) following primary surgical debulking and carboplatin/paclitaxel adjuvant or neoadjuvant chemotherapy. Patients received Vigil or standard of care during the maintenance period.

Results. Forty-two patients were entered into trial, 31 received Vigil and 11 received standard of care. No ≥ Grade 3 toxicity related to product was observed. A marked induction of circulating activated T-cell population was observed against individual, pre-processed autologous tumor in the Vigil arm as compared to pre-Vigil baseline using IFNγ ELISPOT response (30/31 negative ELISPOT pre Vigil to 31/31 positive ELISPOT post Vigil, median 134 spots). Moreover, in correlation with ELISPOT response, RFS from time of procurement was improved (mean 826 days/median 604 days in the Vigil arm from mean 481 days/median 377 days in the control arm, p = 0.033).

Conclusion. In conjunction with the demonstrated safety, the high rate of induction of T-cell activation and correlation with improvement in RFS justify further Phase II/III assessment of Vigil.

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1. Introduction

The current standard of care in Stage III/IV ovarian cancer is bulk reductive surgery either preceded by neoadjuvant or followed by consolidation with paclitaxel and carboplatin. Following such frontline management, the majority of advanced ovarian patients will be in complete clinical response (cCR; i.e., normal CT scan or MRI of the abdomen/pelvis, normal chest X-ray, normal physical examination, CA-125 antigen level ≤35 units/ml, no symptoms suggestive of the presence of active disease). However, approximately 75% of these women who achieve a cCR will relapse at 13–18 months after surgery [3–5]. Maintenance therapy has not proven beneficial. In conjunction with carboplatin/paclitaxel based regimens, maintenance therapies have shown neither a recurrence free survival (RFS) advantage [6] nor an overall survival (OS) endpoint advantage [6–9]. A recent meta-analysis of maintenance chemotherapy in 1644 women with ovarian cancer in 8 randomized, controlled trials showed no significant difference in three-, five- and 10-year OS or progression free survival (PFS) [10]. A subsequent, independent trial-sequential analysis of these same data reached the same conclusion [11]. In addition to lack of survival benefit, it is also noteworthy that the toxicity profile during clinical investigation of various maintenance therapies did give rise to a spectrum of extended treatment related side effects thereby further raising questions of results-related economic justification to the plethora of maintenance approaches tested [12].

Four large trials of bevacizumab as consolidation and/or maintenance therapy have been performed in advanced ovarian cancer. Two of those were in the frontline setting [9,13], one in platinum-sensitive recurrent disease [14,15], and one in platinum-resistant recurrent disease, involving over 4300 patients. PFS was improved by 3–4 months but no OS improvement was demonstrated. Importantly, toxicity (Grade 3 hypertension, bowel perforation) limited therapy continuation in some [16] reflecting a narrow therapeutic window.

Preliminary studies of immunotherapy in patients with ovarian cancer present compelling evidence that these cancers are immunogenic and are appropriate targets for immune mediated approaches [17]. It is our premise that immune mechanisms induced by Vigil can impact the clinical outcome of ovarian cancer. Expression of furin and consequent immunosuppressive TGFβ isoforms are increased in ovarian tumor as compared with normal ovarian tissue [18]. Moreover significant increases in TGFβ1 have been observed in both primary (2.9 fold; $p \leq 0.002$) and recurrent (4.4 fold; $p \leq 0.002$) ovarian cancer tissue [18]. In an effort to overcome one of the limitations of whole cell (whether autologous or allogeneic) cancer vaccines, i.e., the presence of intrinsic immunosuppressive proteins such as TGFβ, we designed a novel DNA engineered autologous whole cell therapy, Vigil® Ovarian (gemogenovatucel-T) (formerly known as FANG®), incorporating the rhGMCSF (recombinant human granulocyte-macrophage colony stimulating factor) cDNA and the bifunctional shRNA (short hairpin ribonucleic acid; a novel RNA interference moiety) targeting furin (to block furin mediated conversion of TGFβ proproteins into active, immunosuppressive TGFβ1 and β2). Further, by using autologous tumor tissue, Vigil provides the complete tumor-specific antigen matrix within the patient's own cells, thus an individualized, HLA-identical tumor specific immunotherapeutic approach. Others have shown tumor response advantage of multi-antigen over single antigen vaccines and the combination of GMCSF expression/ with TGFβ knockdown has proven effective in preclinical tumor models [19,20].

A Phase I assessment of Vigil in solid tumor patients, including patients with ovarian cancer, who received ≥1 injection (at a dose of 1.0×10^7 or 2.5×10^7 cells/injection/month for up to 12 doses) demonstrated marked safety of Vigil immunotherapy [1]. In addition, Phase I Vigil treated patients appeared to have an OS benefit when compared to similar concurrent non randomized patients entered into trial who also underwent successful tumor harvest and Vigil construction but did not receive Vigil treatment (562 days versus 122 days,

$p < 0.00001$). Recent long term follow up beyond 3 years continues to demonstrate long term safety of Vigil treated advanced cancer patients as well as a survival advantage over those with Vigil constructed but not administered [2]. A survival advantage is also by the results in patients with advanced Ewing's Sarcoma treated with Vigil [21]. Moreover, the correlation of survival to Vigil induction of circulating activated T-cells (ELISPOT) provides further evidence of effective afferent immune induction and effective efferent immune function.

Heretofore, patients treated with Vigil had advanced, recurrent bulky disease. We now demonstrate a prolongation of RFS with maintenance Vigil in patients with stages III/IV ovarian cancer achieving cCR following frontline management; therefore with minimal residual disease (MRD).

2. Methods

2.1. Design

This was a Phase II open-label trial of Vigil vs. SOC maintenance therapy (control) in patients with Stages III/IV serous/endometrioid ovarian/fallopian cancer or primary peritoneal cancer achieving cCR following debulking surgery and chemotherapy. Tumor was harvested at the time of surgical debulking.

Patients achieving cCR (normal CT scan or MRI of the abdomen/pelvis, normal chest X-ray, normal physical examination, CA-125 antigen level ≤35 units/ml, no symptoms suggestive of the presence of active disease) following primary surgical debulking and doublet chemotherapy (carboplatin/paclitaxel) were initially randomized 2 (Vigil): 1 (SOC). Patients received 1.0×10^7 cells/intradermal injection of Vigil (gene transfected autologous tumor cells) once a month for up to 12 doses as long as sufficient material was available. A minimum harvest aliquot to produce 4 monthly injections was required for entry into the study. These patients were managed in an outpatient setting. Hematologic function, liver enzymes, renal function and electrolytes were monitored monthly. Serial immune function analysis including ELISPOT analysis [1,2] of mononuclear cell function to pre-processed autologous tumor cells was monitored at baseline (screening), prior to Vigil injection and at Months 2, 4, 6 and EOT. A positive response was defined as >10 ELISPOTs induced per 10^6 peripheral blood mononuclear cells (PBMC) and twice baseline response. CA-125 was monitored at baseline, monthly for the first year, every 3 months ± 2 weeks for the second and third year. Treatment was continued until disease recurrence or exhaustion of the patient's vaccine supply. Physical examination, performance status, height, weight, temperature, blood pressure, pulse and toxicity (CTCAE v 3.0) were monitored. Additional recordings of the adverse events are shown in the Supplementary.

2.2. Inclusion criteria

Histologically confirmed Stage III/IV papillary serous or endometrioid ovarian cancer was required. A cCR modified to incorporate a CA-125 ≤ 20 U/ml following completion of surgical debulking and chemotherapy was required (based on the predictive impact of pre-maintenance CA-125 [22]) and patients enrolled must have completed at least 5 but no >6 cycles adjuvant carboplatin/paclitaxel or interval debulking + neoadjuvant carboplatin/paclitaxel (per NCCN guidelines, category 1). Methods, objectives and further inclusions are shown in the Supplementary.

2.3. Patient registration and enrollment

Written documentation of full, IRB approval of the protocol and consent document was required before a patient could be registered at all sites. Further registration, ethics and regulatory processes are shown in the Supplementary.

2.4. Tumor procurement/Vigil manufacturing

Gradalis, Inc. manufactured Vigil from the procured tissue. Manufacturing was a 2-day process (product release 3 weeks). The equivalent of a "golf ball size" mass (10–30 g tissue, cumulative) was necessary for vaccine manufacturing, therefore, preoperative radiological scans were required to confirm the presence of a lesion >2 cm. Lesions extending into bowel lumen were excluded due to the risk of bacterial contamination. Further procurement and Vigil manufacturing process are shown in the Supplementary.

2.5. Study treatment administration

2.5.1. Chemotherapy

Patients received 5–6 cycles of chemotherapy as either neoadjuvant or interval debulking + chemotherapy schedule. Recommended regimens are those classified as Level 1 Category of Evidence per NCCN Guidelines Version 3.2012. Patients did not receive maintenance therapy other than Vigil or SOC observation. Patients received Vigil at 1×10^7 cells/ml via intradermal injection for a minimum of 4 doses and a maximum of 12 doses starting ≥3 weeks following completion of chemotherapy (no longer than 2.5 months post chemotherapy). Patients with viable cells in sufficient numbers (i.e. at least 4 doses of 1.0×10^7 cells/injection) received monthly intradermal injections of the tumor cell vaccine for up to 12 months. The sites of injection rotated between the right and left upper arms. If the ipsilateral axillary lymph nodes were radiated or surgically removed during prior therapy, alternative sites (e.g., anterior thigh) were used. The patient was observed for at least 30 min (with a 10% window) following vaccination. During this observation period, vital signs were taken every 10 min. All manufactured vaccine was stored in the vapor phase of liquid nitrogen until ready for use.

2.6. Disease evaluation

Disease recurrence was evaluated in this study at each local site using the Response Evaluation Criteria in Solid Tumors Version 1.1. Disease recurrence was defined as the appearance of any measurable or evaluable lesion or as asymptomatic CA-125 levels >35 U/ml at two consecutive measurements, at least one month apart. The time to recurrence was measured from date of first treatment until the first date that recurrence is objectively documented whether local, regional, or distant.

2.7. 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 [1,2]. Surgically excised tumor was collected on site at the time of the debulking procedure and placed in sterile saline and packaged for transport to the manufacturing facility. The tumor is mechanically and enzymatically dissociated into a single cell suspension. The cells are counted and then transfected with the Vigil plasmid through electroporation. Following plasmid transfer, the cells were washed and irradiated at 10,000 cGy (Blood Bank irradiator). The irradiated cell suspension was then enumerated, aliquoted and frozen (1×10^7 cells). The freeze media consists of 10% DMSO (dimethyl sulfoxide; Cryoserv USP; Bionichepharma US), 1% Human Serum Albumin (ABO Pharmaceuticals) in Plasma-Lyte A at pH 7.4 (Baxter). After freezing the cells were stored in the vapor phase of liquid nitrogen until all release testing is completed. Patients with viable cells in sufficient numbers for at least 1.0×10^7 cells/injection for 4 doses received monthly intradermal injections of the tumor cell vaccine.

2.8. Statistics

The primary objective of this study was to investigate whether maintenance Vigil in patients with Stages III/IV ovarian cancer achieving a cCR following debulking surgery and chemotherapy results in prolongation of RFS compared with SOC observation.

RFS was analyzed from time of surgical procurement and time of treatment/observation start. Patients were censored for survival on the last known date as recurrence free; two [2] patients who were randomized to the control arm and later crossed-over to the Vigil arm were recurrence-free at the time of crossover and censored on the date of crossover.

Analyses of time-to-event variables were performed with the use of log-rank statistics, Kaplan–Meier survival curves, and Cox proportional-hazards models. Hazard ratios with 95% confidence intervals are reported. P-values of <0.05 were considered to indicate statistical significance. All statistical analyses were performed with the use of IBM SPSS Version 22.

3. Results

3.1. Characteristics

Forty-two patients were entered into trial at time of procurement and fulfilled inclusion criteria. Randomization was performed as per initial study design for the first 20 Vigil treated patients and 11 control patients. When preliminary data provided adequate support for a Phase II/III registration trial, the current therapy trial was put on hold. At the time of hold, 10 patients were in chemotherapy consolidation phase of the treatment plan prior to randomization. These 10 patients were de-randomized and all received Vigil. Thus, 31 patients received Vigil and 11 received standard of care. One Vigil patient (1115) received a lower dose, 4×10^6 cells/ml, as exception. At time of relapse patients randomized to SOC (control arm) were eligible to crossover and participate in a sequential trial to receive Vigil in combination with Avastin (platinum resistant) or concurrent with carboplatin/paclitaxel retreatment (platinum sensitive). All patients were followed until disease recurrence. Comparative characteristics are shown in Table 1. Prior to study completion, two patients (1049 and 1074) were permitted crossover to Vigil prior to relapse via study amendment. Patient 1049 crossed over to receive Vigil 14 months after randomization and patient 1074 crossed over 5 months after randomization. Patient 1049 had unresectable <1 cm lung nodules and elevated CA-125 (between 10 and 20 ng/dl) not fulfilling the definition of relapse at time of crossover but did fulfill definition of relapse (related to lung nodule extension) 4 months after crossover. Patient 1074 had not relapsed as of 16 months after Vigil crossover. To be highly conservative in analysis, both were censored as recurrence free in statistical analysis of RFS at time of crossover.

3.2. Safety

Thirty-one patients received 241 intradermal (ID) injections of Vigil, on the monthly schedule. No grade 3/4 product-related adverse effects were identified, 11 control patients received standard of care without early Vigil dosing (exception patients 1049, 1074, see above). No grade 3/4 toxic effects were observed in the control patients. Grade 1, 2 definitely or probably related adverse events are shown in Table 2 comparing Vigil (n = 31) to control (n = 11).

3.3. Immune response

Thirty of 31 Vigil treated patients and 10 of 10 control patients (one unevaluable) demonstrated negative (≤10 ELISPOTs) ELISPOT reactivity (as determined by third party assessment, ZellNet, Ft. Lee, NJ) prior to Vigil treatment at time of randomization. Eight control patients also

Table 1
Demographics.

	Treatment arm (Vigil) n = 31	Control arm (no Vigil) n = 11
Mean age (years)	62	57
Median age (years)	61	53
Age (range)	38–82	39–81
Disease stage		
T3a	0	2 (18%)
T3b	1 (3%)	0
T3c	28 (90%)	9 (82%)
2b	2 (6%)	0
Tumor harvest location		
Pelvis	5 (16%)	2 (18%)
Omentum	10 (32%)	4 (36%)
Ovary	16 (52%)	5 (45%)
GMCSF day 7	2049	2884
TGFb1	99%	90%
TGFb2	85%	81%
Mean cycles of chemo	5.8	6
Range cycles of chemo[a]	2–6	6–7
Ave days between chemo & registration	100	47
Neoadjuvant/adjuvant chemotherapy	4/27	2/9
Debulking		
<1 cm	9	4
≥1 cm	2	–
Microscopic	5	1
None	13	6
Unresectable	1	–
N/A	1	–
Total	31	11
Histo grading		
High grade	27	10
Intermediate grade	1	–
Low grade	1	1
Undetermined	2	–
Total	31	11

1 patient achieved CR with recurrent disease management with Gemcitabine after adjuvant carboplatin/taxol.

[a] 1 patient had chemotherapy related toxicity and received only 2 cycles of adjuvant carboplatin/taxol.

had repeat testing of ELISPOT response prior to crossover after relapse before treatment with Vigil. All 8 samples again showed negative ELISPOT responsiveness. In total, 40/41 patients (98%) had negative ELISPOT reactivity prior to Vigil. Following Vigil injection a positive ELISPOT response as defined (>10 IFNγ spots/10^6 PBMC) was observed in 31 of the 31 initial Vigil treated patients (median ELISPOT reactivity was 134; range, 12–448 spots). The positive responses were first observed at Month 2 (n = 28), 3 (n = 1), 4 (n = 1) and 9 (n = 1). Following crossover, 7 of the 8 patients who relapsed and received late Vigil converted to positive ELISPOT by Month 2. The median ELISPOT reactivity after tumor recurrence and crossover treatment with Vigil was 58; range, 43–105 spots. One patient who did not convert to a threshold >10 ELISPOTs after relapse and crossover with Vigil did increase from

0 (baseline/pre-crossover) to 7. This patient only received 2 vaccinations and demonstrated continued rapid progression. Regarding the 3 patients in the control who did not receive Vigil, one (1080) remained negative for 2 subsequent measurements up to 6 months after randomization. One patient (1046) became positive to ≥10 from within 15 days after subsequent relapse and a third (1056) became positive 4 months after randomization without plausible etiology and remained disease free for 9 months after randomization. In attempt to assess durability of circulating activated T-cells related to Vigil, 9 Vigil treatment group patients underwent ELISPOT assessment after Vigil completion. ELISPOT was demonstrated as positive in 9/9 patients 4 months after last treatment. Two (1033, 1045) maintained positive response out 20 months after last Vigil treatment. Six of these nine remained disease free including the 2 with ELISPOT positive response 20 months after last Vigil dose supportive of functional memory T-cell induction.
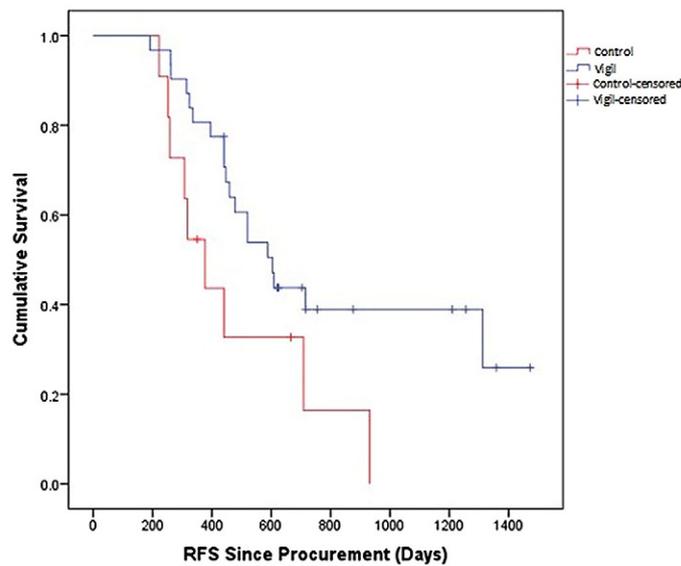
3.4. Recurrence free survival (RFS)

Comparison of RFS from time of procurement between Vigil or control patients following consolidation treatment with carboplatin/paclitaxel is shown in Fig. 1. Two patients were randomized to control (1049, 1074) but were permitted crossover to Vigil prior to relapse at 14 months and 5 months after initial randomization to control. Patient 1049 had non-biopsiable lung nodules (<1 cm) and transient elevated CA-125 (between 10 and 20 ng/dl) not fulfilling the definition of relapse at time of crossover and did fulfill relapse criteria 4 months afterward. Patient 1074 did not demonstrate relapse as of 16 months after Vigil crossover. To be highly conservative, both were censored as recurrence free at time of Vigil crossover. Control RFS is consistent with historical expectation (mean/median RFS 16 months/481/377 days). On the other hand, Vigil response data suggests improved RFS, 27 months/826/604 days by Kaplan Meier analysis (p = 0.033 by the log-rank test). The hazard ratio derived from the Cox model was 0.43 (95% confidence interval [CI], 0.19 to 0.96; p = 0.039). Two Vigil-randomized patients, however, identified as Stage IIB disease received exception for study entry and need for consolidation/maintenance treatment. One patient (1020) had a ruptured tumor (capsule disrupted) within the abdominal peritoneal cavity and tumor cells were noted microscopically within fat and adipose tissue. Her primary tumor was 12.7 × 8.6 cm dimensions. The other patient (1065) had dimensions 13 × 5.5 cm in tumor size with extension to the fallopian tube, rectosigmoid serosa, uterine serosa and perirectal adipose tissue. Mean/Median RFS with exclusion of these patients from analysis was also not different from time of procurement (771/588 days in the Vigil arm, p = 0.064). Analysis of the high median ELISPOT positive population vs. low median ELISPOT population did not reveal a difference in time to relapse duration or overall relapse rate. Additionally, analysis of the time randomized population of 20 Vigil treated patients and 11 control patients prior to derandomization and allowance of all patients prior to maintenance to enter into the Vigil arm also revealed similar results mean/median survival of 516/377 days in the control and 802/588 days in the Vigil arm, p = 0.156 days from procurement. RFS from time of treatment was a

Table 2
AE's definitely or probably related to Vigil™ (n = 31).

Preferred term	Grade	Relation to therapy	Number of subjects	Number of events
Erythema	2	Definitely related	1	1
Injection site reaction	1	Definitely related	5	5
Injection site reaction - bruising	1	Probably related	1	1
Injection site reaction - bruising	1	Definitely related	1	1
Injection site reaction - ecchymosis	1	Definitely related	1	1
Injection site reaction - erythema	1	Definitely related	6	28
Injection site reaction - Induration/fibrosis	1	Definitely related	14	42
Injection site reaction - pain	1	Definitely related	7	10
Injection site reaction - swelling	1	Definitely related	2	4

*No grade 3, 4 AEs definitely or probably related were observed.

Group	N	No. of Recurrences	Mean RFS (days)	Median RFS (days)	p-value
Control	11	9	481	377	0.033
Vigil	31	19	826	604	

Fig. 1. Recurrence free survival of Vigil vs. standard of care control patients. Both groups of patients had debulking surgery, ≥ 5 cycles of carboplatin/paclitaxel and successful vaccine construction (data as of 04/12/2016).

mean/median of 304/195 days in the control arm and 563/352 days in the Vigil arm (p = 0.13 by log-rank test). The hazard ratio derived here from the Cox model was 0.55 (95% CI, 0.24–1.22; p = 0.14).

4. Discussion

The results confirm the safety of Vigil as a maintenance regimen and are consistent with our previous assessments of immune response induction as evaluated by IFNγ ELISPOT assay. Clinical data are also encouraging as evidenced by improvement by >1 year in RFS observed with maintenance Vigil following SOC debulking surgery and adjuvant or neoadjuvant chemotherapy; from time of procurement, mean RFS 27 months/826 days vs. 16 months/481 days. Departures from study design included two patients with complicated IIB disease and one patient not able to receive ≥2 cycles of chemotherapy, both in the Vigil treatment arm. However, a separate analysis excluding these patients who were felt to be at higher risk of recurrence revealed no difference in RFS. As such, although preliminary, evidence of benefit without significant morbidity is encouraging and justifies further randomized double blind testing. The majority of women diagnosed with cancer of the ovary present with advanced stage disease [23] and generally relapse between 13 and 18 months of initial debulking surgery. No effective, minimally toxic therapeutic management for maintenance after achievement of cCR has been accepted as SOC. Results observed with Vigil confirm remarkable safety and do offer preliminary evidence of benefit with a high benefit/risk ratio thereby justifying further investigation of Vigil as maintenance therapy for patients with Stages III/IV ovarian cancer achieving cCR following primary treatment.

Mechanistically, circulating activated T-cells appeared to be readily induced following initiation of Vigil immunotherapy as assessed by modified IFNγ ELISPOT assay. Specifically, whereas 98% of baseline blood samples showed no immune-activity against autologous tumor prior to Vigil, all samples tested demonstrated induction of immune-reactivity following Vigil vaccination in accordance with our prior experience in Phase 1 testing which demonstrated a correlation of Vigil induced ELISPOT response with prolongation of survival [2]. Experience

with sipuleucel-t (Provenge) treatment (prostate acid phosphatase; PAP antigens) in patients with advanced prostate cancer also showed a relationship of sipuleucel-t to induction of ELISPOT reactive T-cells at low levels [24]. In view of the evidence that TGFβ present at initial activation can suppress CD8$^+$ effector function despite activation of effector phenotype, the incorporation of an intrinsic downregulator of TGFβ1 and TGFβ2 in Vigil assumes even greater weight. In addition, secreted TGFβ from ovarian cancer cells transform CD4$^+$CD25$^-$ cells into immunosuppressive Treg cells (CD4$^+$CD25$^+$) [25]. Both Treg tumor infiltration and the granzyme B+/FOX3p+ ratio are associated with outcome in patients with high-grade serous ovarian carcinoma treated with neoadjuvant chemotherapy [26]. Further assessment of the components of induced immune response to Vigil are underway. Interestingly, lack of immune mediated toxic effect to Vigil in a setting of significant clinical benefit and marked functional immune response (high benefit/risk ratio) suggests that the immune response induced by Vigil is focused against cancer specific antigen epitopes as opposed to "self" antigens which would be expected to be associated with autoimmune toxic effect.

Furin, an upstream regulator of TGFβ activity, is a member of the subtilisin-like proprotein convertase family. Proteolytic cleavage by furin is required for TGFβ convertase activation (i.e. pro-TGFβ → TGFβ). High levels of furin mRNA and furin protein are widely expressed in human cancers including carcinoma of the ovary tumors [27] in which the gene is differentially expressed (compared to normal human ovarian surface epithelium cell lines) and the level appears to be inversely correlated with survival [27]. The presence of furin in tumor cells likely contributes significantly to the maintenance of tumor directed, TGFβ-mediated peripheral immune tolerance. Therefore the combination of furin and consequent TGFβ knockdown with intrinsic GMCSF immune stimulatory production and the potential interaction between the two [28] provides the rationale for the initial evaluation of Vigil in ovarian cancer.

The proposed mechanism of action of Vigil is supported by the resultant immune response. Also to be considered is the preliminary evidence of durable, long-term immune responses to Vigil (9 patients

maintaining circulating reactive T-cells ≥4 months after completion of last Vigil dose). These results suggest vaccine induction of IFNγ secreting tumor-specific memory T-cells. A dose-dependent effect of TGFβ on effector memory T-cells (Tem) has previously been shown and may be attributable to the suppressive effect of TGFβ on CD8$^+$ T-bet [29]. This hypothesis needs to be confirmed and this work is underway. Clearly, chemotherapy involving platinum and taxane may also impact immune response. According to the serial immune function analyses by Coleman [30], five of nine patients with Stages III–IV ovarian adenocarcinoma who achieved post-chemotherapy (carboplatin/paclitaxel) remission retained the capacity of CD8$^+$ T-cell responses to a panel of 11 viral peptides restricted by at least six common HLA class I alleles, whereas four patients with disease progression displayed low or reduced responses at different stages of treatment. Chemotherapy produced no apparent effect on naïve (CD45RA$^+$ CCR7$^+$), central memory (CD45RA$^-$ CCR7$^+$), or effector memory (CD45RA$^-$ CCR7$^-$) T-cells, although cisplatin has been shown to transiently reduce immunosuppressive T regulatory cells and downregulate inhibitory ligand PD-L1 and PD-L2 expression [31]. In total, these data support rationale for the use of maintenance immune modulating therapy sequentially after platinum-based chemotherapy.

The immune suppressor functions of TGFβ are likely to play a major role in modulating the effectiveness of cancer cell immunotherapies. TGFβ reduces secretion of GMCSF by activated memory CD8$^+$ T cells, inhibits GMCSF induced maturation of bone marrow derived dendritic cells (DCs) [28] (antigen presentation by immature DCs result in T-cell unresponsiveness) as well as expression of MHC Class II and co-stimulatory molecules [32]. TGFβ also inhibits activated macrophages including their antigen presenting function [33]. Therefore, both the ubiquitous expression of the TGFβ isoforms as well as the inhibitory effects of these isoforms on GMCSF immune modulatory function support a broad based tumor target range for the application of a TGFβ suppressed/GMCSF expressing immune enhancing therapeutic.

Increasing evidence suggests that GMCSF is involved in the augmentation of tumor antigen presentation [34]. Dendritic cells (DCs) mediate a crucial role in priming antigen-specific immune responses [35]. DCs express diverse receptors that allow for recognition and capture of antigens in peripheral tissues, process this material efficiently, albeit by different routes, into the MHC Class I and II presentation pathways, upregulate co-stimulatory molecules upon maturation, and migrate to secondary lymphoid tissues [36]. Following injection of GVAX® in patients, an intense local reaction consisting of dendritic cells, macrophages, and granulocytes was observed [34].

GMCSF has various effects on DCs. First, it induces a subset of DCs that more effectively phagocytose apoptotic tumor cells [37,38]. Second, compared to Flt3 ligand (FL), GMCSF evoked higher levels of co-stimulatory molecules, which is characteristic of greater functional maturation. This enhanced activity results in more efficient T-cell stimulation, thereby broadening the arsenal of induced lymphocyte effector mechanisms. Third, GMCSF promoted uniformly high levels of CD1d on DCs, in contrast to FL, which triggered a more heterogeneous expression. CD1d is a non-classical MHC Class I molecule that presents lipid antigens [39]. The CD1d lipid complex activates natural killer T (NKT) cells, a population of lymphocytes that display a restricted Class I MHC-like receptor. Importantly, NKT cells may play pivotal roles in both endogenous and therapeutic responses to tumors. Product release of each constructed Vigil immunotherapy demonstrated marked product GMCSF upregulation prior to product release and administration for each patient treated with Vigil.

In conclusion, given the high relapse rate of ovarian cancer during maintenance these preliminary results of maintenance Vigil in patients with advanced stage ovarian cancer achieving cCR following induction therapy are encouraging and support further clinical investigation of Vigil. Phase II/III randomized testing is currently underway with Vigil in ovarian cancer as well as in Ewing's sarcoma.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygyno.2016.09.018.

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