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Follow-up of bi-shRNAfurin /GM-CSF Engineered Autologous Tumor Cell (EATC) Immunotherapy Vigil® in patients with advanced melanoma

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Abstract

Over the last decade, management of melanoma has dramatically evolved from chemotherapy through targeted molecular therapy (BRAF V600E signaling) and, currently, immunotherapy (checkpoint inhibitors, immunogenic oncolytic viruses). Response, time to progression and survival has improved for many melanoma patients undergoing targeted therapy, but insensitive population subsets, adaptive resistance and toxic side effectslimit therapeutic benefit. Previous studies have shown a correlation between Vigil® engineered autologous tumor cell (EATC) immunotherapy induced circulating activated T-cells responsive against autologous tumor cells and survival prolongation. We now assess the safety and response to Vigil (1 x 10^7 cells/ intradermal injection monthly x 4-12) in 12 patients with advanced metastatic melanoma in comparison with 12 who underwent similar standard of careautologous tumor harvest but received other treatment regimens,not Vigil. None of the patients experienced≥ Grade 3 treatment-related toxicity. Two Grade 2 adverse events (AE) (fatigue, irritability) and local regionalGrade 1 AE (injection site erythema, induration, rash, skin hypopigmentation)in 19 of 63 injections were observed. IFN-γ ELISPOT analysis (PBMC) showed the induction of T-cell activation from 0-1 at baseline to 78 spots/10^6 cells post first cycle of Vigil. Median survival of Vigil treated patients was 20 months compared to 7 months (KaplanMeier analysis, log rank p=0.00009). In conclusion, preliminary evidence of safety and activity of Vigil supportsfurther clinical evaluation in advanced melanoma.

Introduction

MAGE-A3. MAGE-A1, NY-ESO-1 and SSX-2), a high tumor mutation burden (TMB) leading to an increased number of tumor-specific epitopes, and clinicallya reproducible response rate to immunotherapies [1-4] particularly to the recently FDA approved immune checkpoint inhibitors. One of these inhibitors is ipilimumab (Yervoy; a human monoclonal antibody (hMAb) CTLA-4 inhibitor), which was FDA approved in 2011 for patients with advanced, unresectable Stage III and IV melanoma [5]. Results show improvement in recurrence-free survival (RFS) as compared to placebo in the EORTC trial 18071 (HR 0.75, 95% CI 0.64 – 0.90), [6]. Pembrolizumab (Keytruda), ahMAbPD-1 inhibitor, subsequently demonstrated response rates of 36% [7] and has proven to be superior to chemotherapy and single agent ipilimumab in patients with advanced melanoma [8-10] as has nivolumab (Optivo) [11]. However, >60% of melanoma patients do not achieve an optimal response to a single agent checkpoint inhibitor and subsets of patients (i.e. PD-L1$^-$; low TMB) predictively respond less favorably. Although the combination of mechanistically different immune checkpoint inhibitors elicits higher response rates, in a randomized trial of nivolumab alone,ipilimumab alone, or the combinationof the two in treatment-naïve patients with unresectable stage III or IV melanoma,the combination achieved an ORR of 57.6% (compared to 43.7% with nivolumaband 19% with ipilimumab) with a durable response of 11.5 months,but with 55% treatment-related Grade 3 or higher toxicities. Furthermore, in 36.4% of patients the combination leads to treatment-related discontinuation[9]. Although these data confirm the effectiveness of immunotherapy in advanced melanoma, they alsohighlight the need for further development of novel and/or combinatory immunotherapies with increased, predictable effectiveness at a lower risk of toxicity. Talimogenelaherparepvec(T-VEC), a genetically-modified, immune-enhanced H. simplex type I virus, is systemically effective in advanced melanoma [12] but the FDA indication is limited to Stages IIIb, IIIc or IVM1a disease that are unresectable based on regional efficacy shown in Phase III testing [13,14].

Vigil is a DNA engineered autologous tumor cell (EATC) immunotherapy. It contains a dual vector; a bi-shRNA targeting furinthe pro-protein convertase that activates the immunosuppressive TGF-beta 1 and 2 and the gene encoding hGM-CSF. A phase I clinical

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trial of Vigil in patients with heavily pretreated advanced solid tumors showeda significant survival benefit which correlated with induction of an immune response measured by the interferon gamma (IFN-γ) ELISPOT assay. We now update the results of Vigil clinical activity in patients with advanced melanoma.

Materials and methods

The method and mechanism of construction and manufacturing of Vigil (formerly known as FANG) has previously been described [15,16]. The Vigil vector encodes for GM-CSF expressive cDNA and the bi-sh RNA^{furin} in autologous tumor cells. Following protocol-specific informed consent, tumor tissue is harvested, placed in sterile media and delivered to the Gradalis, Inc. manufacturing facility (Carrollton, TX, USA). Vigil is manufactured over 2 conservative days. Subsequent manufacturing, following FDA discussion, now utilizes Gentamicin in the sterile media in order to reduce contamination risk. First, autologous tumor cells are dissociated into a single-cell suspension, followed by electroporation(which allows cell transfection with the plasmid), and overnight incubation. Then the cells are irradiated, placed into the final vials, cryopreserved, and undergo release testing. Following product release by Quality Assurance compliance, patients are registered for treatment every 4 weeks with 1.0 x 10^7cells/injectionof Vigil.

Study design

This follow-up includes all Vigil treated melanoma patients enrolled in both the Phase I solid tumor trial [15] and a Phase II trial of Vigil in patients with advanced or recurrent melanoma. The primary objective of the Phase I trial was to determine safety following the administration of Vigil (EATC). The primary objective of the non-randomized Phase II open label trial was to evaluate the effect of Vigil on immune stimulation in patients with melanoma and to assess survival efficacy in comparison with historical data.

Secondary objectives were to expand the Phase I safety evaluation of Vigil immunotherapy in patients with advanced solid tumors without alternative standard therapy options and to evaluate effectivenessbased on IFN-γ ELISPOT induction/conversion and on survival in both the Phase I melanoma and Phase II patients.

Depending on the manufacturing cell yield from the harvested tumor for a minimumdose criterionof 1 x 10^7cells/ml (and 2.5 x 10^7 cells/ml in Phase I), patients were eligible to receive a maximum of 12 intradermal injections. Each injection was administered monthly, alternating between the right and left upper arms. Safety assessment included physical examination, performance status, weight, height, temperature, blood pressure and pulse, as well as toxicity profile. Laboratory assessment, blood immune biomarker assessment, response rate [RECIST 1.1 and irRC (Phase II)] and survival were used for efficacy assessments. The treatment was continued until documentation of progressive disease or to a maximum of 12 injections. The trials were performed after approval by a local Human Investigations Committee and in accordance with an assurance filed and approved by the Department of Health and Human Services. This included approval for a long-term follow-up of the melanoma patients analyzed in this review.

Patient population

All eligible patients were treated in the outpatient facility of Mary Crowley Cancer Research (MCCR; Dallas, TX, USA).

IFN-γ ELISPOT assay

The ELISPOT (enzyme-linked immunospot) assay as previously described [17] was performed using the enzyme-linked immunospot assay for IFN-γ, (BD Biosciences, San Jose, CA, USA). Tumor and mononuclear cells were applied on an antibody coated microplate reacting with IFN-γ. Quantitative results in form of reactive spots to IFN-γ, secreted by cytotoxic CD8+ T cells, were measured and used for immune response function analysis. The reading of the ELISPOT plates was performed independently by ZellNet Consulting, Inc. (Fort Lee, NJ, USA). A value of ≥10 spots and 2x baseline was considered as positive ELISPOT response status. Serial ELISPOT analyses were performed at baseline, Month 2,Month 4and subsequent time points. Vigil induced ELISPOT conversion was defined as ≥10 spots/10^5 cells and 2x baseline. All patients were ELISPOT negative at baseline.

Statistical evaluation

Survival was analyzed from time of surgical procurement. Patients were censored for survival on the last known date alive. Analyses of time-to- event variables were performed with the use of log-rank statistics and Kaplan–Meier survival curves. P-values of less than 0.05 were considered to indicate statistical significance. All statistical analyses were performed with the use of IBM SPSS Version 22.

Results

Patient characteristics

A total of 27 patients with advanced melanoma were enrolled in the Phase I and Phase II studies (BB-IND14205: CL-PTL-101, CL-PTL-114). All patients underwent tumor procurement as part of the standard medical management for palliative control of disease, which allowed for Vigil manufacture. Patient characteristics are shown in table 1.

Successful manufacturing of Vigil with 2.5 x 10^7 cells/ml (Phase I) or 1 x 10^7 cells/ml (Phase I, Phase II) was performed in 20 out of 27 patients. The other 7 products could not be released because of insufficient cell dose (n=1) or contamination (n=6). Twelve of the 20 patients received Vigil at 1 x 10^7 cells/ml dose and all were evaluable

Table 1. Demographics.

	Vigil (n=12)	Intent to Treat (n=15)	Matched Comparator (n=12)
Age (years)			
Mean	63.7	60.7	60.5
Range	32-89	39-80	49-80
Gender			
Female	6	2	2
Male	6	13	10
Ethnicity			
Caucasian	12	15	12
Stage*			
IIIa-c	3	1	1
IV	9	12	10
Prior Systemic Therapy			
Chemo	2	4	3
Radiation	2	3	2
Checkpoint Inhibitor	1	3	1
Other (BRAF, investigational)	4	11	9
Vigil Dose			
1 x 10^7cells/ml	12	N/A	N/A

N/A: not applicable

All patients required tissue procurement.
*Matched Comparator: F-025 TxNxM0, F-050 T1N0M0. Intent to treat. F-025 TxNxM0

for safety and efficacy assessment.The remaining eight were not eligible for treatment for the following reasons: one with ineligible histology and seven withearly mortality (<42 days after surgery) prior to planned treatment with Vigil. Thus, 15 patients (7 ineligible, 8 product non-released)who signed consent and underwent surgery for Vigil construction (the intent to treat population; ITT) were not treated with Vigil. They received other standard of care/experimental treatment. In our previous experience, Vigil release was generally within 21-28 days, therefore in order to allow for a conservative assessment,the 3 patients not receiving Vigil who failed to survive 42 days were excluded from a second MC analysis. Thus the conservative MC analysis consists of12 patients that underwent palliative surgical procedures, had Vigil successfully manufactured and survived ≥42 days.These patients were identified as the MC group.

Safety

Nineteen Grade 1 treatment-related adverse events(AE) were observed in the 12 Vigil treated patients. These adverse events were predominately limited to the intradermal injection site in the skin (*i.e.,* erythema, induration and bruising). There were two Grade 2 treatment-related AEs observed (Table 2). No ≥ Grade 3AEs related to product were observed.

Table 2. Adverse Events (AEs).

	Vigil (n=12)			
Treatment-Related AEs	Grade 1	Grade 2	Grade 3	Grade 4
	(n)	(n)	(n)	(n)
Injection Site – Erythema	4	-	-	-
Injection Site – Induration	13	-	-	-
Rash	1	-	-	-
Probably Treatment-Related AEs				
Skin Hypopigmentation	1	-	-	-
Possibly Treatment-Related AEs				
Fatigue	-	1	-	-
Irritability	-	1	-	-

Immune response

Using serial PBMC from each patient, ELISPOT induction/conversion was demonstrated in 10 of 10 evaluable patients after treatment with Vigil by Month 3. Seven of 10 patients showed an ELISPOT+ response by Month 2, two patients by Month 3 and one patient at the end of treatment (6.5 months after start of treatment) (Figure 1). The ELISPOT+ responsesafter first dose reflected an increase from 1 spot baseline to a median 78 spots (n=7). Five patients were followed and assessed for ELISPOT reactivity after completion of Vigil dosage and all 5 achieved ELISPOT+response (Figure 1). In three, reassessment was limited to two months post treatment initiation, but in two repeat ELISPOT reactivity was demonstrated for more than 1 year after Vigil discontinuation.

Clinical response and survival

Vigil treated patient response is shown in table 3. The median survival from procurement of patients treated with Vigil was 20 months (616 days, range 137-1660 days) compared to both the MC cohort (n=12, not including 3 early mortality patients (<42 days)) who had a median survival of 7 months (208 days; p-value 0.00009) (Figure 2) and the ITT population (n=15 patients)witha median survival of 4 months (122 days). .Eighty-three percent (10/12) of the Vigil treated patients survived ≥1 year from procurement (Table 3).

Discussion

This evaluation of Vigil engineered autologous tumor cell therapy in patients with advanced melanoma is preliminary butconfirms safety and provides evidence of immune responsiveness (by IFN-γ ELISPOT) in melanoma patients comparable to that previously reported in heavily pretreated patients with other advanced solid tumors and in patients with advanced, recurrent Ewing's Sarcoma [17,18]. It is encouraging that 8 of the 12 treated patients experienced SD for ≥6 months and that the survival difference was greater than 1 year between Vigil treated and similar ITT and MC patients. These results are consistent with long-term follow-up results of Vigil in prior trials, where survival advantage was observed to correlatewith ELISPOT activation [19].

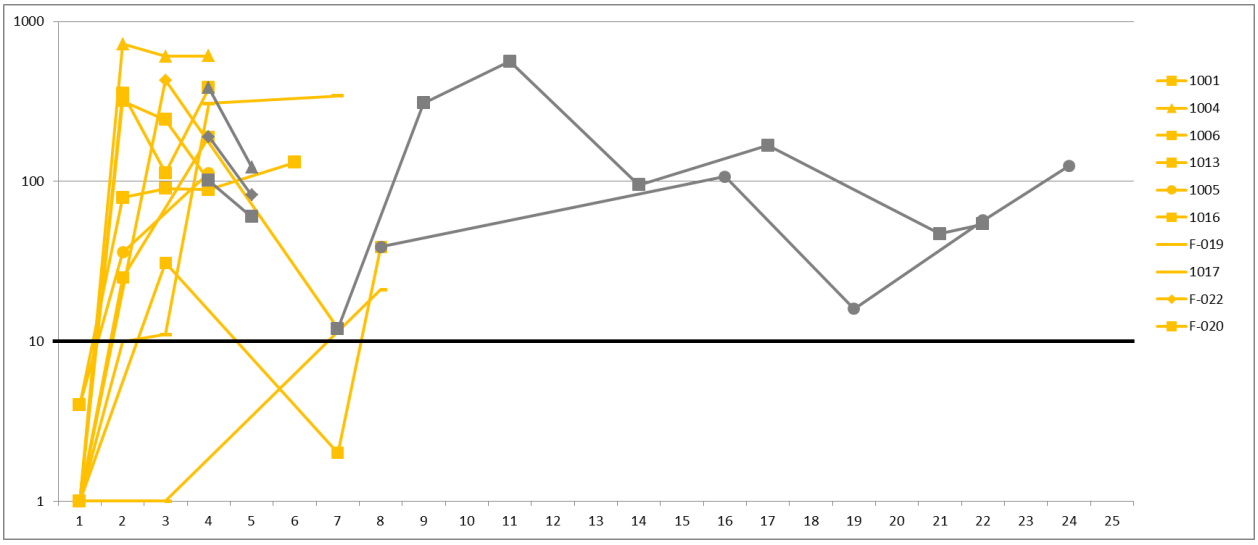
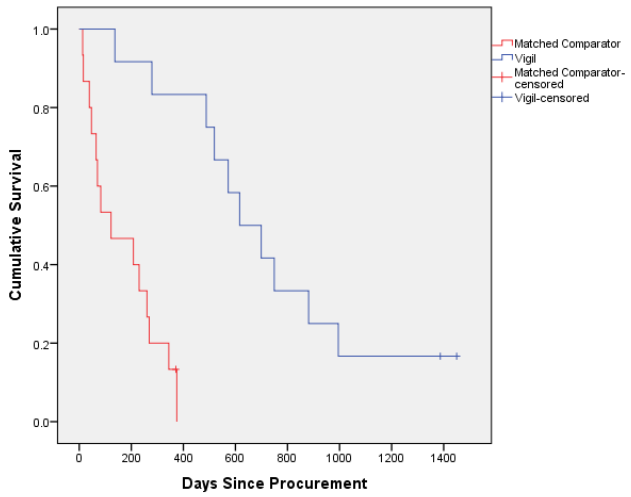


Figure 1. IFN-γ ELISPOT Response to vigil

Melanoma ELISPOT + response graph of patients that received Vigil of Phase I and II. Ten patients are represented by two colors i) yellow: on treatment with Vigil and ii) dark gray: off-treatment/follow-up. The y-axis represents the reactive spots on the IFN-γ ELISPOT assay. The x-axis represents different time point of assessments. All patients start out with a negative ELISPOT response status and overcome the threshold of ≥10 spots by Month 3 as the latest. All patients show consistent positive response status at end of treatment with Vigil. Long-term follow-up in two of the patients (F-020, F-022) demonstrate long-term immune response to cancer cells.

Table 3. Response of vigil treated patients.

Patient ID	Vigil Cycles Received	Days Alive Since Procurement	Days Alive Since Treatment	Months Since Treatment Start	Reason for Discontinuation	Survival Status
1001	4	279	117	3.9	Disease Progression	Dead
1004	4	1660	1142	38.1	Normal Completion	Alive
1005	6	498	456	15.2	Disease Progression	Alive
1006	4	1632	1156	35.5	Normal Completion	Alive
1008	1	137	11	.37	Disease Progression	Dead
1013	4	699	644	21.5	Disease Progression	Dead
1016	7	616	552	18.4	Disease Progression	Alive
1017	8	488	385	12.8	Disease Progression	Dead
F-005	3	749	560	18.7	Disease Progression	Dead
F-019	7	572	490	16.3	Normal Completion	Dead
F-020	7	881	835	27.8	Normal Completion	Dead
F-022	8	995	942	31.4	Normal Completion	Dead



Group	N	No. of Deaths	Mean (days)	Median (days)	p-value
Matched Comparator*	12	11	204	208	0.00009
Vigil	12	10	736	616	
* Excludes 3 patients with survival data <40 days (1009, 1012, F-050)					

Figure 2. Vigil *vs.*matched comparator survival since procurement
Kaplan Meier Survival Curve of patients with advanced melanoma in Phase I and II of Vigil. The y-axis shows survival rate and the x-axis represents time in days since procurement. The red is the control group (Matched Comparator, n=12) and blue is the Vigil patient cohort (n=12).

Although the MC patient group fulfilled the same inclusion and exclusion criteria as the Vigil treated patients, the gender imbalance, 83:17% *vs.*50:50% respectively (Table 1) in this concurrently accrued but non-randomized study, suggests an alternative interpretation of the survival results. In a number of studies, including a pooled analysis of gender as an independent prognostic variable for survival in advanced melanoma [20], gender has been shown to be a significant variable with a female to male survival advantage of approximately 30%. Given the limited number of women in the MC group, in order to address this issue a comparison of survival outcomes was made limited to the men in the Vigil and MC groups. The six Vigil treated men achieved a median survival (dated from procurement) of 657.5 days (range 488-995 days)with a mean of 674.2 days whereas the 10 men in the MC group had a median survival of 238.5 days (range 47-375 days) with a mean of 195 days. Thus, even in a gender specific comparison (within the limits of the data pool, retrospective combined protocol update), the survival advantage of Vigil over SOC appears to be sustained.

There are several key mechanisms of immune-modulating activity that must be considered for development of effective cancer immunotherapeutics [21]. These include the processing and presentation of cancer related antigens, the specificity of those antigens, antigen presentation through antigen presenting cells (APC, e.g., dendritic cells), MHCpeptide/ TCR binding and activation of cytotoxic Tcells (CTC),maturation of these T cells into effector and memory subsets, circulation of CTC to target tumor cells, and infiltration into the tumor microenvironment and the recognition of the cancer antigens with consequent cytolysis. Vigil is a unique combinatorial immunotherapeutic that allows for an enhancedimmune effector arm by presenting the full panoply of tumor-associated antigens and neoantigens, enhanced activation and attraction of mature dendritic cells by local GM-CSF expression and suppression of TGF-beta related immune suppression, and facilitated acquisition of T cell effector memory function represented by long-term ELISPOT responsiveness post Vigil immunotherapy treatment.

By utilizing the full matrix of patient cancer-related tumor associated antigens (TAAs) and neoantigensthe autologous tumor cell Vigil immunotherapy avoids the necessities of epitope identification and HLA matching. Lack of toxic effects in the setting of marked elevation of total body circulating activated T cells (median $1/10^6$ mononuclear cells baseline to $78/10^6$ mononuclear cells post Vigil) suggests that the T cell receptor response was generated to high affinity TAA and neoantigens and, if produced, to below affinity thresholdself antigens. Other approaches such as CAR-T with limited antigen repertoires have thus far shown limited responses in patients with non-hematologic malignancies and potentially lethal side effects such as cytokine release syndrome. Vigil, on the other hand, appears to induce a modulated, relevanttumor-related antigen T cell activation that correlates with survival in patients with19 different advanced solid tumor types.

Recent progress in molecular immunologyhas resultedin the dramatic and oftentimes durable clinical responses seen with immune checkpoint inhibitors in immunogenic melanoma and other supposedly non-immunogenic cancers. The clinical effectiveness of PD-1/PD-L1 axis checkpoint inhibitor therapy (as evidenced by FDA approval in melanoma, NSCLC, renal cell carcinoma, and bladder cancer) indicates that potentially effective tumor-targeting cytotoxic T-lymphocytes (CTLs) are present in the tumor microenvironment, however either 1)

are unable to override intrinsic or adaptive resistance, 2) are subject to T cell exhaustion, or 3) are no longer interactive with initially sensitive tumors due to epitope drift and/or evolving somatic mutations and consequent neoantigen formation.

As noted, PD-1/PD-L1 can be either constitutively or inducibly expressed. Further, induction can be either oncogene-driven [22] or T cell-driven (via IFN-γ and STAT3), the latter being the presumptive mechanism of adaptive (tumor cell) resistance [23,24]. There is evidence of vaccine enhanced PD-L1 expression in response to systemic treatment. Similar to Vigil, GVAX, is a GM-CSF producing autologous whole cell tumor vaccine but without intrinsic immunosuppressive TGFβ knockdown. A recent study showed PD-L1 IHC positivity in 12.5% (3 of 25) of resected specimens from unvaccinated patients with pancreatic cancer [25]. Two weeks following GVAX vaccine, specimen membranous PD-L1 expression was increased to 25% (10 of 40) and, in addition, was found in vaccine induced intratumoral tertiary nodules in >80% of patients. In the same report, cyclophosphamide + GVAX (Cy/GVAX) treatment of Panc02 xenografts in C57B16 mice resulted in a 12.5% cure rate compared to 38% with the combination of Cy/GVAX and monoclonal antibody (MAb) targeting PD-1. Likewise the combination significantly increased overall survival (OS) to 81.5 days compared to MAb PD-1 alone, 50 days. Furthermore, in the presence of chronic viral infection or cancer, the persistent exposure of CTLs to high antigen concentrations can result in CD8+ T cell dysfunction; a phenomenon called T cell exhaustion [26]. Treatment with PD-L1/PD-1 axis inhibitors can restore T cell functionality [27]. These data provide a rationale for combining Vigil and an immune checkpoint inhibitor in patients with advanced melanoma and thus provide a basis for both salvage immune checkpoint inhibitor therapy in patients progressing after Vigil in patients who demonstrated an immune response as well as for *de novo* therapy.

The tumor mutation burden(TMB), not otherwise associated with a survival advantage,has emerged as a potential biomarkerfor effective PD-L1/PD-1 axis checkpoint inhibitor therapy [28]. Melanoma, in part due to the significant impact of an external mutagen (UV light), is one of the highest TMB expressing cancers. The analysis of immune checkpoint inhibitor responses in patients with high mutation rates reveals a correlation with a limited number of mutations involving specific DNA repair genes; i.e. POLD1, POLE, andDNA mismatch repair (MMR) defects, which play a prominent role in the biogenesis of colorectal cancer [29]. In an analysis of responses to PD-L1 blockade (pembrolizumab), Le and colleagues reported a 78% immune related PFS for MMR deficient patients versus 11% in MMR proficient patients [30]. In addition, there was a 40% PR *vs.*0% PR, in the two groups, respectively. Rizvi et al addressed the underlying mechanism by hypothesizing (as others have) that recognition of neoantigens, formed as a consequence of somatic mutations (particularly missense and frameshift), is important for the activity of anti PD-1 therapy. They then characterized the neoantigen tumor landscape on these same patients and found a direct correlation with TMB ($p<0.0001$). Cancers (regardless of histology) with a mean mutational load of >10 somatic mutations per Mb of coding DNA are likely to have a low percentage capable of proteasome processing and adequate MHC I:peptide binding affinity to produce epitopes recognizable by T cells [31,32]. However, insofar as these neoantigenic epitopes elicit antitumor immune responses, they also have the potential to induce off-setting counter responses including CTLA4, PD-1, and PD-L1 [33] there by accounting, at least in part, for the benefit derived from checkpoint inhibitors.

In conclusion, given 1) the apparent effectiveness of the engineered autologous tumor cell Vigil immunotherapy, 2) oncogene or immunotherapy mediated IFNγ-induced expression of the PD-1/PD-L1 axis components (adaptive resistance), 3) the enhanced effectiveness of GM-CSF secreting autologous tumor cell therapies combined with anti-PD-1/PD-L1 axisMAb, 4) PD-1/PD-L1 blockade reversion of T cell exhaustion [34,35], and 5) the limited response activity to monomodal anti-PD-1/PD-L1 in PD-L1 negative populations, it is our contention that Vigil immunotherapy upregulation of activated T cell populations, as a result of combining both local GMCSF local immune enhancement withdown-regulation of intrinsic tumor cell immunosuppressive TGFβ,willproduce an additive if not synergistic combinatorial immunotherapeutic regimen in conjunction with immune checkpoint inhibition. Such a study is in progress.

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Disclosure/Conflict of interest

The following authors are shareholders in Gradalis, Inc. and Strike Bio: Jeffrey Lamont, Padmasini Kumar, GladiceWallraven, Neil Senzer and John Nemunaitis. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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