Research Article

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 clinically 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 combination of 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 nivolumab and 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 also highlight the need for further development of novel and/or combinatorial immunotherapies with increased, predictable effectiveness at a lower risk of toxicity. Talimogenealehparepvec(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
Material 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 RNAfurin 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 10^7 cells/injection of 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 effectiveness based 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 minimum dose criterion of 1 x 10^7 cells/ml (and 2.5 x 10^7 survival in both the Phase I melanoma and Phase II patients.

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 immunotherapy. Patient characteristics are shown in table 1.

Vigil induced ELISPOT conversion was defined as ≥10 spots/10^5 cells/ml in Phase I), patients were eligible to receive a maximum of 2.5 x 10^7 cells/ml; 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 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 4 and subsequent time points.

### Table 1. Demographics.

|          | Vigil (n=12) | Intent to Treat (n=15) | Matched Comparator (n=12) |
|----------|-------------|------------------------|---------------------------|
| Age (years) | Mean 61.7 | 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 | 1 | 3 |
|          | Other (BRAF, investigational) 4 | 11 | 9 |

N/A: not applicable

All patients required tissue procurement.

*Matched Comparator: F-025 TNxM0, F-050 T1N0M0. Intent to treat. F-025 T NxM0
for safety and efficacy assessment. The remaining eight were not eligible for treatment for the following reasons: one with ineligible histology and seven with early 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 of 12 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 predominantly 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 3 AEs related to product were observed.

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+ responses after 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 2 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) with a 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 but confirms 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 correlate with ELISPOT activation [19].

| Treatment-Related AEs                       | Grade 1 (n) | Grade 2 (n) | Grade 3 (n) | Grade 4 (n) |
|--------------------------------------------|-------------|-------------|-------------|-------------|
| Injection Site – Erythema                  | 4           | -           | -           | -           |
| Injection Site – Induration                | 4           | -           | -           | -           |
| Rash                                       | 1           | -           | -           | -           |
| Skin Hypopigmentation                      | 1           | -           | -           | -           |
| Fatigue                                    | -           | 1           | -           | -           |
| Irritability                               | -           | 1           | -           | -           |

Table 2. Adverse Events (AEs).

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.

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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           |

* 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 limited number of women in the MC group, in order to address this issue a comparison of survival outcomes was made limited to 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), MHCPepptide/ TCR binding and activation of cytotoxic Tcells (CTC),maturaion 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 1/10^4 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)
counter responses including CTLA4, PD-1, and PD-L1 [33] thereby immune responses, they also have the potential to induce offsetting mutations per Mb of coding DNA are likely to have a low percentage (regardless of histology) with a mean mutational load of >10 somatic patients and found a direct correlation with TMB (p<0.0001). Cancers and frameshift), is important for the activity of anti PD-1 therapy. They formed as a consequence of somatic mutations (particularly missense by hypothesizing (as others have) that recognition of neoantigens, patients [30]. In addition, there was a 40% PR vs. PFS for MMR deficient patients versus 11% in MMR proficient 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 biomarker for 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, and DNA 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 proteosome processing and adequate MHC E 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 axis MAb, 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 withdrawal-regulation of intrinsic tumor cell immunosuppressive TGFβ, will produce 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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