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Assessment of Low Dose Vigil® Engineered Autologous Tumor Cell (EATC) Immunotherapy in Patients with Advanced Solid Tumors

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Abstract

Previously we demonstrated not only safety but also provided evidence of clinical benefit to Vigil® vaccine (1 x 10^7cells/injection 1x/month for 1-8 injections). In addition, we identified a relationship between survival and Vigil® induced circulating activated T-cells against autologous, preprocessed tumor cells (γIFN-ELISPOT) [1,2]. Here we review 15 patients with advanced, heavily pretreated progressive metastatic disease who underwent autologous tumor harvest and subsequent Vigil® construction but in whom manufacturing was only able to construct low-dose Vigil® (1 x 10^6 – 8.3 x 10^6 cells/injection 1x/month for 1-8 injections). Of the 12 patients for whom sequential γIFN-ELISPOT assessment was available, all were γIFN-ELISPOT response negative (<10 spots) at baseline and subsequently developed a positive response. Specifically, 11 converted after 1 cycle of Vigil® immunotherapy and one after 2 cycles. The median (range) γIFN-ELISPOT response was 143.5 (6-474) spots post Vigil® compared to 1 (0-2) pre Vigil®. Median overall survival for these 12 patients was 28.7 months. The three patients without γIFN-ELISPOT assessment had a median survival of 25.3 months. No ≥ grade 1 Vigil® related toxicity was observed. These data which suggest comparable immunological and clinical effectiveness of low-dose Vigil® imply that a smaller harvest tumor volume may be adequate for Vigil® construction, possibly allowing for an image guided core needle biopsy procedure rather than excisional resection for tumor acquisition.

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Introduction

The absence of antitumor T-cell activity in advanced cancer patients indicates not only the results of central and peripheral processing, but also one or more of a variety of afferent immune response pathway defects such as impaired antigen processing and presentation, altered cell-to-cell and co-stimulatory signals, secreted tumor and micro environment immunosuppressive cytokines, alterations in effector cell signal transduction and chronic antigen exposure negatively influencing T-cell differentiation and resulting in T-cell exhaustion [3]. Restoration and/or revitalization of T effector cell function is a promising approach for immunotherapy. Vigil® is a DNA plasmid based immunotherapy which utilizes autologous irradiated tumor cells transfected with a DNA plasmid, encoding for GMCSF expression and furin knockdown mediated TGFβ 1 and 2 downregulation.

In a previously reported Phase I clinical trial of Vigil® administered at ≥ 1 x 10^7 cells/injection 1x/month for 1-12 injections in patients with heavily pretreated advanced solid tumors, in addition to demonstrated safety the study suggested a survival advantage as well as a correlation of survival duration with an elicited positive γIFN-ELISPOT response (25.7 months γIFN-ELISPOT + *vs.* 11.6 months γIFN-ELISPOT-)[1,2].

The safety, effective induction of an immune response (γIFN-ELISPOT) and clinical benefit of Vigil® has been shown in a variety of advanced cancer types [4-7]. However, heretofore there has no data available to characterize the effectiveness of lower dose-range Vigil® (i.e., < 1 x 10^7 cells/injection) including both the quantitative γIFN-ELISPOT response and correlation to survival.

We now report on 15 patients with less than optimal accessible disease volume for harvest or without sufficient tumor harvest for higher dose, in whom only lower dose Vigil® was able to be constructed and administered under protocol.

Methods

Study design

These lower dose patients were participants in an expanded cohort of an ongoing open-label, non-randomized, single-arm Phase 1 study [1,2]. It was established in order to assess lower dose Vigil® above 1 x 10^6 cells/injection x 4 injections. The standard dose for other Vigil® dosing has been ≥ 1 x 10^7 cells/injection x 4 injections. Patients with solid tumors following prior standard of care cancer treatment were grouped into 1 of 3 lower dose (1 x 10^6, 4 x 10^6, 8 x 10^6 cells/intradermal injection) cohorts of plasmid transfected autologous tumor cells once a month for up to 12 doses as long as sufficient material was available (minimum of 4 injections). Selection of patients for each dose cohort was dependent on the amount of tumor cell yield following harvest and processing of patients entered into the Phase 1 study.

Patients were followed for safety, sequential γIFN-ELISPOT response assessment and survival. Written documentation of full IRB approval of the protocol and consent document was required before a patient could be registered at the site. All patients were treated at a single site, The Mary Crowley Cancer Research Center (Dallas, TX).

Inclusion criteria

Histologically confirmed advanced or metastatic deemed non-curable with standard of care therapy (if limited to a single lesion may not be a candidate for curative surgery or radiation therapy) was required. Successful vaccine manufacture from one or more tissue sites or fluid obtained from the following major organ systems: digestive, endocrine, reproductive, respiratory and urinary was allowed. Clinically indicated surgical procedure to collect viable tumor for Vigil® EATC manufacturing was required for enrollment. All patients were required to have signed IRB approved informed consent.

Vigil® manufacture

The construction and GMP manufacturing of Vigil® immunotherapy have previously been described [1,2]. Vigil® cellular immunotherapy was constructed for every patient after surgical collection of autologous tumor tissue, dissociation into single-cell suspension, plasmid transfection, incubation and irradiation.

γIFN-ELISPOT assay

The γIFN-ELISPOT (enzyme-linked immunospot) assay as previously described [4] was performed using the enzyme-linked immunospot assay for IFN-γ, (BD Biosciences, San Jose, CA, USA). Target (Tumor) cells and Effector (mononuclear) cells were applied in a 3:1 ratio (7.5 x 10^3 : 2.5 x 10^3) 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 γIFN-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 γIFN-ELISPOT response status. Serial γIFN-ELISPOT analyses were performed at baseline, month 2, month 4 and subsequent time points. Vigil® induced γIFN-ELISPOT conversion was defined as ≥ 10 spots and 2x baseline. All patients were γIFN-ELISPOT negative at baseline.

Table 1: The Groups of Low Dose Vigil® Treated Patients.

	Vigil® (n=15)
Age (years)	
Mean	44
Range	13-72
Gender	
Female	10
Male	5
*Ethnicity**	
Caucasian	11
African American	1
Hispanic / Latino	1
Asian	1
Stage	
Stage 4	15
Prior Therapies	
Prior Systemic Chemotherapy /Patient (range)	8-Jan
Prior Radiation Ablation of Disease Sites/Patient (range)	3-Jan
Other Prior anticancer Rx/Patient* - investigational Rx, GVAX, GMCSF (range)	3-Jan
Tumor Type	
Ewing's Sarcoma	6 (40%)
Ovarian Cancer	6 (40%)
NSCLC	1 (0.67%)
Renal Cell Cancer	1 (0.67%)
Thyroid Cancer	1 (0.67%)
Vigil Dose	
4-8.3 x 10^6 cells/ml	13 (86.7%)
1 x 10^6 cells/ml	2 (13.3%)
Vector Efficacy (Mean Values)	
TGFβ1 knockdown	99%
TGFβ2 knockdown	90%
GMCSF Expression Day 7	1202.47

N/A: Not Applicable
All patients required tissue procurement
*Ethnicity: 1 patient without ethnicity.

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.

Results

Patient population

Fifteen patients with advanced solid tumors received at least 1 dose of Vigil® (1.0 – 8.3 x 10^6 cells via ID injection). Demographics are shown in Table 1. All patients underwent tumor procurement as part of the standard medical management for palliative control of disease and qualified for Vigil® immunotherapy. Time of Vigil® treatment was a median of 166 days (range 45-369 days) after tissue procurement.

Safety

A total of 71 Vigil® autologous tumor cell administrations were given to the 15 patients. All treatment-related AE's were limited to

Table 2: Treatment Related AE's.

AE's Preferred Term	Grade 1 (Number of Events)	Grade 2 (Number of Events)	Grade 3 (Number of Events)	Grade 4 (Number of Events)	Grade 5 (Number of Events)
Definitely Related	**n=70**	**n=0**	**n=0**	**n=0**	**n=0**
• Injection Site Erythema	16				
• Injection Site Induration	46				
• Injection Site Pain	5				
• Injection Site Swelling	1				
• Injection Site Tenderness	2				

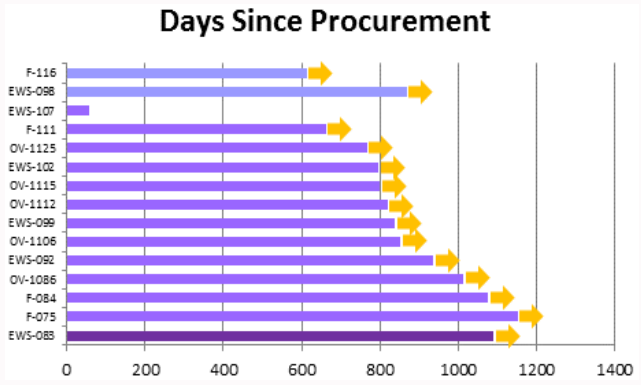

Figure 1: Individual survival by dose. All 15 patients are represented by purple bars.Light purple graph represents $1x10^6$ cells, purple represents $4x10^6$ cells and dark purple represents $8x10^6$. The y-axis represents each patient's (#116, #098, #107, #111, #OV-1125, #102, #OV-1115, #OV-1115, #099, #OV1106, #092, #OV1086, #084, #075, #083) survival organized by dose level. The x-axis represents time in days since procurement.The orange arrows represent survival "Alive" status.

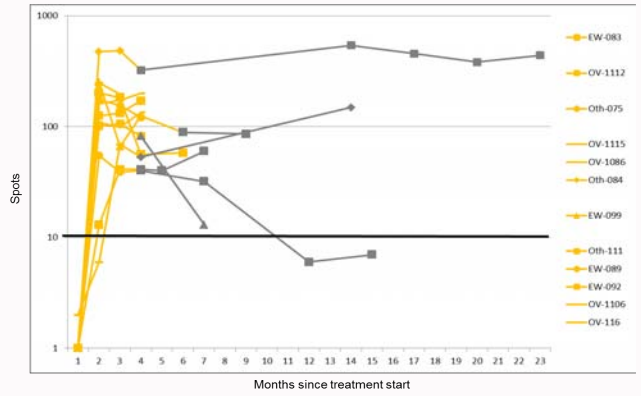

Figure 2: γIFN-ELISPOT response curve. Positive is defined as a threshold of ≥10 reactive spots. 12 patients are represented by color code of i) yellow: on treatment with Vigil®, or ii) grey: off-treatment. The y-axis represents the reactive spots on the γIFN-ELISPOT assay. The x-axis represents time in months since treatment start. All patients were observed to have a negative ELISPOT response prior to Vigil® (≤10 spots).

Grade 1 injection site reactions such as erythema, swelling, induration, tenderness, or pain (Table 2). Other non-treatment related AE's were observed: 31 grade 1, 8 grade 2 and 4 ≥ grade 3 [2 due to disease progression, 1 grade 3 transient hypoglycemia and 1 grade 4 transient hypoglycemia].

Survival

We observed the mean Kaplan-Meier survival for all 15 patients treated with "lower-dose" Vigil® as 968 days from time of tissue procurement. Separate survival of the γIFN-ELISPOT assayed (n = 12), (n = 3 not assayed for ELISPOT) showed similar results (data not shown). Actual survival of 93% was observed at 1 year. Survival of individual patients is shown by dose (Figure 1).

ELISPOT response

Twelve patients were analyzed for γIFN-ELISPOT assay at baseline (pre Vigil®) and post treatment with Vigil® engineered autologous tumor cells. All patients were baseline negative with a median of 1 spot (range 0 to 2 spots). γIFN-ELISPOT reactivity demonstrated a significant induction of systemic immune response in 12 of 12 evaluable patients after treatment start with Vigil®. Eleven patients had a positive γIFN-ELISPOT response status by month 2 (after 1 cycle of Vigil®) and one patient by month 3 (after 2 cycles of Vigil®) (Figure 2). The median value of ELISPOT "+" response status after one cycle of Vigil® was 143.5 (6 to 474) spots. One patient had responses below threshold of 10 spots prior to subsequent elevation. In addition, three patients (F-075, F-084, Ov-116) were followed for long-term ELISPOT assessments after completion of treatment with Vigil® immunotherapy. Two of these patients maintain positive ELISPOT status for 15 months (F-075) and 23 months (F-084) and had prolonged survival of ≥ 1075 days.

Discussion

This evaluation of lower-dose Vigil® immunotherapy in patients with advanced solid tumors demonstrates preservation of immune responsiveness (by γIFN-ELISPOT) to a similar degree as previously reported in similar heavily pretreated patients who received higher Vigil® doses (≥ 1 x 10^7 cells/ml) [1,2,5]. Importantly, there also appears to be comparable survival duration. These results are encouraging and suggest durable immune activity induced at up to a 1log lower dose. This is of interest in further development of Vigil® technology as the quantity of harvested tumor required to achieve such a dose level could be obtained by image guided core biopsy rather than surgical resection. As previously reported, a ≥ 2cm nodule has been required to achieve manufacture of ≥ 4 vials of Vigil® at 1 x 10^7 cells/injection. The potential for a clinically effective lower dose Vigil® as demonstrated in these patients justifies further study in an effort to increase the number of patients eligible for tumor harvest and to minimize the potential for discomfort and side effects that can be associated with a more extensive surgical resection.

The autologous tumor cell approach utilized by Vigil® provides the full spectrum of tumor associated antigens, differentiation antigens, tumor-germ cell antigens, and unique neoantigens that are expressed on tumor cells [8]. The latter, specific to cancer cells and exempt from central and peripheral processing, have emerged as highly immunogenic immunotherapeutic targets [9-11].

A phase II study of a TGFβ2 antisense allogeneic tumor cell vaccine (Belagenpumatucel-L) suggested a vaccine dose-response relationship to survival; a 581 day median survival of those receiving

≥ 2.5 x 10^7 cells/injection compared to 252 days in those receiving 1.25 x 10^7 cells/injection (p = 0.0186) [12]. Unlike Vigil® the allogeneic Belagenpumatucel-L would not have presented tumor specific neoantigens, effected only a > 35% downregulation of TGFβ2 rather than a 93.5% and 92.5% downregulation of TGFβ1 and TGFβ2, respectively [1], and did not incorporate the GMCSF transgene. Microenvironment TGFβ1, 2 is a multifariously immunosuppressive cytokine affecting CD8 T-cells, CD4 T-cells (including induction of Tregs) and dendritic cell proliferation, migration and activity [13]. In addition, the downregulation of TGFβ in murine pancreatic cancer models in combination with allogeneic GMCSF secreting GVAX shows significant benefit in survival and Treg depletion [14]. Likewise, TGFβ1 gene silencing in ovarian cancer cells demonstrated enhanced immune response by downregulation of CD4CD25+FoxP3 Tregs, ovarian cancer antigen-specific immune response targeting mesothelin and HE4, and increased numbers of CD8+ interferon-gamma secreting T effector cells [15].

It is hypothesized that γIFN-ELISPOT assessment, considering its correlation with survival, provides an early indicator of Vigil® enhanced immunogenicity and clinical activity and, via an interaction of multifactorial functional pathways, allows for effectiveness even at lower doses. A broader in-depth assessment of the active T effector γIFN-ELISPOT positive fraction and determination of the individual neoantigen signal[s] induced are underway. Further evaluation of lower dose Vigil® utilizing image guided biopsy is justified to determine relationship to direct patient benefit parameters (i.e. safety, comfort, response, time to progression and survival).

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Disclosure/Conflict of Interest

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References

1. Senzer N, Barve M, Kuhn J, Melnyk A, Beitsch P, Lazar M, et al. Phase I trial of "bi-shRNAi(furin)/GMCSF DNA/autologous tumor cell" vaccine (FANG) in advanced cancer. Mol Ther. 2012;20(3):679-86.
2. Senzer N, Barve M, Nemunaitis J, Kuhn J, Melnyk A, Beitsch P, et al. Long Term Follow Up: Phase I Trial of "bi-shRNA furin/GMCSF DNA/Autologous Tumor Cell" Immunotherapy (FANG™) in Advanced Cancer. Journal of Vaccines and Vaccination. 2013;4(8):209.
3. Jiang Y, Li Y, Zhu B. T-cell exhaustion in the tumor microenvironment. Cell Death Dis. 2015;6:e1792.
4. Nemunaitis J, Barve M, Orr D, Kuhn J, Magee M, Lamont J, et al. Summary of bi-shRNA/GM-CSF augmented autologous tumor cell immunotherapy (FANG) in advanced cancer of the liver. Oncology. 2014;87(1):21-9.
5. Ghisoli M, Barve M, Mennel R, Lenarsky C, Horvath S, Wallraven G, et al. Three-year Follow up of GMCSF/bi-shRNA(furin) DNA-transfected Autologous Tumor Immunotherapy (Vigil®) in Metastatic Advanced Ewing's Sarcoma. Mol Ther. 2016;24(8):1478-83.
6. Oh J, Barve M, Matthews CM, Koon EC, Heffernan TP, Fine B, et al. Phase II study of Vigil® DNA engineered immunotherapy as maintenance in advanced stage ovarian cancer. Gynecol Oncol. 2016;143(3):504-510.
7. Barve M, Kuhn J, Lamont J, Beitsch P, Manning L, Pappen B, et al. Follow-up of bi-shRNA furin / GM-CSF Engineered Autologous Tumor Cell (EATC) Immunotherapy Vigil® in Patients with Advanced Melanoma. Biomedical Genetics and Genomics. 2016;1(3):81-86.
8. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348(6230):69-74.
9. Castle JC, Kreiter S, Diekmann J, Lower M, van de Roemer N, de Graaf J, et al. Exploiting the mutanome for tumor vaccination. Cancer Res. 2012;72(5):1081-91.
10. Gubin MM, Artyomov MN, Mardis ER, Schreiber RD. Tumor neoantigens: building a framework for personalized cancer immunotherapy. J Clin Invest. 2015;125(9):3413-21.
11. Lu YC, Robbins PF. Targeting neoantigens for cancer immunotherapy. Int Immunol. 2016;28(7):365-70.
12. Nemunaitis J, Dillman RO, Schwarzenberger PO, Senzer N, Cunningham C, Cutler J, et al. Phase II study of belagenpumatucel-L, a transforming growth factor beta-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. J Clin Oncol. 2006;24(29):4721-30.
13. Santarpia M, Gonzalez-Cao M, Viteri S, Karachaliou N, Altavilla G, Rosell R. Programmed cell death protein-1/programmed cell death ligand-1 pathway inhibition and predictive biomarkers: understanding transforming growth factor-beta role. Transl Lung Cancer Res. 2015;4(6):728-42.
14. Soares KC, Rucki AA, Kim V, Foley K, Solt S, Wolfgang CL, et al. TGF-beta blockade depletes T regulatory cells from metastatic pancreatic tumors in a vaccine dependent manner. Oncotarget 2015;6(40):43005-15.
15. Wei H, Liu P, Swisher E, Yip YY, Tse JH, Agnew K, et al. Silencing of the TGF-beta1 gene increases the immunogenicity of cells from human ovarian carcinoma. J Immunother. 2012;35(3):267-75.