Case Report: Immune-mediated Complete Response in a Patient With Recurrent Advanced Ewing Sarcoma (EWS) After Vigil Immunotherapy

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**BACKGROUND**

Ewing sarcoma (EWS) is an aggressive, poorly differentiated tumor of the bone and soft tissue driven by the EWS/FLI1 (t(11;22) [q24;q12]) gene fusion in 85% of cases with an incidence of 2.9 per million cases in the United States. Approximately 30% to 40% of patients develop recurrent local or metastatic disease after frontline treatment with aggressive chemotherapy. Recurrent disease is associated with a poor prognosis with a 5-year survival of 10% to 25%.1,2 Prognosis is particularly poor when recurrence develops within 2 years of chemotherapy initiation (5y survival <10%).

Recently, we have published clinical phase I data that provided evidence of immune biomarker and associated clinical response to Vigil, a novel bi-shRNA-furin (bi-shRNAfurin) DNA autologous tumor immunotherapy (serially manufactured from first and second recurrences with ELISPOT assay correlation. Results support justification of further testing of Vigil with ELISPOT assay as a biomarker to assess level of immune response and correlation with disease control.

**Key Words:** Ewing sarcoma, Vigil, immunotherapy

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**Summary:** Ewing sarcoma is a highly resistant disease with a <10% chance of survival at 5 years after failure of frontline chemotherapy. This is a case report of an Ewing sarcoma patient with metastatic disease recurrence <2 years after standard chemotherapy/radiation who achieved a durable and sustained complete response after 2 series of treatments with Vigil (GMCSF/bi-shRNAfurin DNA autologous tumor immunotherapy) serially manufactured from first and second recurrences with ELISPOT assay correlation. Results support justification of further testing of Vigil with ELISPOT assay as a biomarker to assess level of immune response and correlation with disease control.

**MATERIALS AND METHODS**

**Design**

This analysis involves a case report of a patient (#062) entered into a phase I trial3 of intradermal Vigil at 1 × 10^7 cells/injection administered monthly for a minimum of 4 months. #062 was managed in an outpatient setting at Mary Crowley Cancer Research. Hematologic function, liver enzymes, renal function, and electrolytes were monitored monthly. Serial immune function analysis including interferon-γ ELISPOT analysis3,5 of peripheral blood mononuclear cell function to preprocessed autologous tumor cells was performed at baseline (screening), before Vigil injection and at months 2, 4, 6, end of treatment, and at follow-up. A positive response was defined as >10 ELISPOTs induced per 5 × 10^3 peripheral blood mononuclear cells and ≥2 × baseline. Physical examination, performance status, height, weight, temperature, blood pressure, pulse, and toxicity were monitored.

**Tumor Procurement/Vigil Manufacturing**

Gradalis Inc. (Carrollton, TX) manufactured Vigil from tumor procured during resection of a 1.0 × 0.8 cm lung mass. Manufacturing was a 2-day process (product release 3 wk). Preoperative radiologic scans were required to confirm the presence of a lesion before resection.5

**Investigational Product**

Vigil is made up of irradiated autologous tumor cells previously electroporated ex vivo with the Vigil plasmid expressing rhGMCSF and a bifunctional shRNA targeting the pro-protein convertase furin so as to simultaneously suppress expression of both transforming growth factor (TGF)β1 and TGFβ2 immunosuppressive proteins.5 Tumor was collected on site at the surgical debulking procedure to confirm recurrence and placed in sterile saline then packaged for transport to the manufacturing facility

dendritic cell vaccine) by demonstrating clinical response and improved outcome.4 Herein we report a unique case of immune induction and presumptive prolonged memory cell persistence following second-line and third-line treatment with Vigil, a GMCSF/bi-shRNAfurin DNA expressive autologous tumor immunotherapy, in a high-risk patient with widespread metastatic EWS who experienced recurrence within 2 years of combined modality chemotherapy and radiation.
where the tumor was mechanically and enzymatically dissociated into a single cell suspension. The cells were counted and then transfected with the Vigil plasmid through electroporation. After plasmid transfer, the cells were washed and irradiated within 24 hours at 10 Gy (iradiator). The irradiated cell suspension was then enumerated, aliquoted, and frozen (1 x 10^7 cells per vial). The freeze media consists of 10% 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. The product is released for administration once release criteria are met (generally within 3 wk).

**CASE PRESENTATION**

#062 presented with left hip and leg pain at age 15. Computerized tomography (CT) scan identified a mass in her pelvic region. Staging by magnetic resonance imaging scan, bone scan and positive FDG-PET (maximum SUV 3.6) showed widely metastatic disease involving the lower abdomen (8 cm pelvic mass), bone (pelvis, T11, and L4), and lung. A core tumor biopsy showed tumor composed of small blue cell tumor with necrosis consistent with EWS and chromosomal analysis confirmed the presence of the pathognomonic EWS/FLI1 t(11;22) translocation. The patient was treated with vincristine/actinomycin and cyclophosphamide/ifosfamide/etoposide and local intensity-modulated radiation therapy to her left pelvis and L4, as well as whole lung radiation with boost (35 Gy) to T11. During frontline chemotherapy she experienced several grade 3 adverse events and was hospitalized multiple times for chemotherapy-related toxicity. Imaging assessment at completion of treatment demonstrated no evidence disease confirming a complete response. However, <24 months after initial treatment, follow-up imaging identified recurrent disease in multiple lobes of the lung. Shortly afterwards, following Mary Crowley Cancer Research Investigational Review Board approved informed consent, she underwent thoracoscopic surgery with removal of a progressive lesion from which 8 vials of Vigil with a unit dose of 1 x 10^7 cells/mL were manufactured (Gradalis Inc.). A tissue specimen also was sent for EWS/FLI1 type 1 sequence analysis, which identified the EWS exon 7-FLI1 exon 6 fusion gene (Strike Bio, Carrollton, TX). Immune response was assessed by modified interferon-γ ELISPOT assays at baseline before Vigil and at sequential time points during and after completion of Vigil. Her baseline ELISPOT assay was negative. She received her first cycle of Vigil as part of a phase I trial 1 month later. A positive ELISPOT response (from a baseline of 0 to 93 spots/5 x 10^3 cells read independently by ZellNet Consulting Inc., NJ) was first noted at the end of month 2. All 8 cycles of Vigil were well tolerated and there were no ≥ grade 3 adverse events. By the end of treatment she achieved stable disease (SD) by RECIST 1.1 criteria. A CT scan at 1-year follow-up demonstrated SD of the target lesions in her lungs and immunosassay showed a durable positive ELISPOT response. These results persisted for over 18 months (Fig. 1) at which time CT imaging revealed increase in size of multiple lung lesions. In addition, serial immune biomarker testing had shown a significant decrease in ELISPOT count (Fig. 1) just before imaging confirmation of progressive disease.

Twenty months after her first Vigil immunotherapy construction she underwent a second tissue procurement of an expanding lung lesion as part of phase I trial for manufacture of a second Vigil immunotherapy. Six doses were produced. Pathology reconfirmed EWS identifying malignant cells (small blue cell tumor) and CD99, FLI1, NXK2.2 positive immunohistochemistry identical to the original pathology. Next generation sequencing analysis (Foundation Medicine, Boston, MA) of this specimen showed a low tumor mutation burden (TMB) of 3 mutations per Mb and reconfirmed the EWSR1 fusion that was also evaluated by reverse transcription-polymerase chain reaction. Fluorescent in situ hybridization for each end of the EWSR1 gene in chromosome band 22q12 (192 of 200 [96%] nuclei showed separation of the signals; consistent with chromosome rearrangement at band 22q12) (ProP- ath, Dallas, TX) and a new molecular signal involving CAD rearrangement at intron 35 was identified. However, mutations of CIITA, FBXO11, FLYWCH1, HIST1H1C, MKI67, and PLCG2 identified in the original Ewing sample were not present in the second Vigil immunotherapy sample.

#062 then received a second series of 6 treatments of Vigil without adverse event other than grade 1 to 2 erythema and swelling at the injection site at cycle 4. The baseline ELISPOT before the second treatment series was 15 spots/5 x 10^3 cells. One month after cycle 1 the ELI-
SPOT increased to 115 spots/5 x 10^3 cells against the pre-
processed sarcoma cells from the second harvest and has remained above threshold throughout her subsequent follow-up visits (Fig. 1). Her first postsecond series Vigil follow-up CT scan (1 mo after end of treatment) showed no evidence of active disease with only persistent, stable, subcentimeter nodules (1 to 2 mm) that were present at her original presenting scans and consistent with benign granulomas. At 10 months after the second tissue procurement and 3.5 years from her first recurrence #062 continues without evidence of active disease by both CT scan and PET scan (Fig. 2). She has not received any anticancer treatment other than Vigil.

In summary, we present a patient with EWS/FLI1 type 1 EWS who sequentially achieved a rarely seen immunotherapy induced classical defined complete responses (to both first and second recurrences) following Vigil. Circulating ELISPOT reactive T cells were induced by treatment with Vigil and serial assays correlated with the patient’s clinical course.

**DISCUSSION**

The case history of #062 illustrates the effectiveness of a unique immunotherapeutic and the potential utility of immune biomarker patterns. After initial treatment with Vigil, the patient experienced a SD status more durable than what would have been predicted based on historical data. Then, nearly a year after completion of the second series Vigil treatments, she achieved a clinical and CT/PET complete response that correlated with serial ELISPOT assays, a biomarker response that has previously been shown to correlate with survival following Vigil treatment. Moreover, this response was achieved without toxic side effects. The persistence of activated T-cell levels for 13 months after completion of Vigil treatment #1 and for 10 months after completion of Vigil treatment #2 is suggestive of and consistent with the presumptive induction of durable memory T cells. In the absence of T-cell phenotyping the kinetics of memory T-cells subtypes cannot be delineated nor the relative numbers of central memory (Tcm; CD62L+/
CCR7+ and effector memory (Tem) (CD62L−/CCR7−) T cells be ascertained. However, in so far as TGFβ1 has been shown to suppress the acquisition of T-cell effector memory functionality at initial antigen activation,8 induction of Tem consequent to the >90% TGFβ1, TGFβ2 downregulation effected by the bi-shRNAfurin component of Vigil is a reasonable hypothesis. It is likely that the dominant neoantigen target of the first Vigil vaccine was related to the EWS/FLI1 type 1 fusion protein, as suggested by Peng et al9 in the EWS/FLI1 peptide-pulsed dendritic cell-treated xenograft response and in vitro analysis, particularly given the low TMB of EWS patients in general and of #062 in particular.10–12 It is typically a high TMB that is associated with a higher probability of functional neoantigens which have not been subject to central processing. CT scan and PET scan reversion to negative status following the second series Vigil therapy suggests a complete immunologic clearance of EWS/FLI1 expressive cells. The emergence of an EWS/FLI1 subclone with loss of the CHIA, FBXO11, FLYWCH1, HIST1H1C, MKI67, and PLCG2 mutations and emergence of CAD rearrangement as a result of clonal competition or allopatric speciation in a therapeutically altered immune environment13,14 or the occurrence of clonal divergence before diagnosis may explain the altered genotype following the first Vigil course. This could also be consistent with shifting neoantigen landscape dynamics under T-cell pressure15 or intertumoral molecular heterogeneity as has been previously reported in EWS (albeit infrequently).16 These considerations will inform future studies with Vigil.

To our knowledge this is the first reported case of an immunotherapy-mediated complete response with biomarker correlate in an EWS patient with progressive and metastatic recurrent disease. ELISPOT assays have been used by others as well and have shown correlation with clinical benefit.17–20 The current results accord with these reports and emphasize the need to monitor both the somatic genetic changes in cancers, particularly at the time of postimmunotherapy recurrence, and the necessity of expanding immune biomarker

![FIGURE 1. Interferon-γ ELISPOT expression of #062 treated with Vigil from baseline and after the first (yellow curve representing treatment, gray line for off-treatment) and second (yellow curve representing treatment, gray line for off-treatment) treatment course with Vigil. The squares demonstrate each injection of first treatment with Vigil; circles for second treatment with Vigil. The y-axis represents the (mean) number of spots counted on the ELISPOT microplate after exposure of cancer cells to mononuclear cells from patient. Disease responding computerized tomography (CT) scan and CT/PET scan are labeled as progressive disease (PD), standard disease (SD), or complete response (CR). *Multiple expanding lung lesions <20%, largest lesion harvested for vaccine.](image1)

![FIGURE 2. Computerized tomography (CT) scan highlighting one of several progressive pulmonary lesions before first Vigil treatment compared with first CT scan representing complete response 10 months after second Vigil treatment was initiated.](image2)
assays to further define those immune effectors associated with effective, durable immune responses. Clearly, such information could contribute to the design of more effective event-specific immunotherapeutics.

We are currently exploring further molecular/immune testing methodology to delineate the mechanics and cellular phenotype of Vigil-induced immune response and to determine the patterns of immune response kinetics in relation to shifting antigen landscape dynamics so as to better optimize therapy. Randomized testing of Vigil in advanced EWS is ongoing at several sites across the country (NCT02511132).

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