

Vigil: Personalized Immunotherapy Generating Systemic Cytotoxic T Cell Response

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Received: 25 August 2020; Accepted: 19 October 2020

Citation: Herron J, Smith N, Stanbery L, et al. Vigil: Personalized Immunotherapy Generating Systemic Cytotoxic T Cell Response. Cancer Sci Res. 2020; 3(1): 1-4.

ABSTRACT

Recent research has focused on ways to activate and sustain an immune response. Vigil, an autologous tumor vaccine has shown both safety and efficacy in solid tumors. Here we show that advanced cancer patients exhibit reduced CD3⁺ T cells compared to healthy donor controls. Following vaccination with Vigil, the number of CD3⁺ and CD3⁺CD8⁺ T cells increases to levels comparable to healthy controls. This data is suggestive that Vigil is able to generate a systemic cytotoxic T lymphocyte response.

Keywords

Cytotoxic T cell, T cell, Vigil, IFN gamma.

Introduction

Cell mediated immunity (CMI) or lack of, plays a significant role in cancer progression. During CMI, natural killer (NK) cells and phagocytes work to control tumorigenesis. Phagocytes recognize and engulf cells that express non-self antigens, while NK cells secrete perforin and granzyme which induces apoptosis [1]. CD8⁺ cytotoxic T lymphocytes (CTLs) also play a key role in CMI and are predictive of improved clinical outcomes in many solid tumors [2-9]. In order to generate memory T cells, CD4⁺ helper cells are required for effective T cell priming [10].

Recent research has focused on ways to generate an anticancer immune response by increasing the number of tumor infiltrating lymphocytes, using immune modulatory therapy including vaccines, cellular therapy and chimeric antigen receptor therapy (CAR-T) [11]. An autologous cell immunotherapy, sipuleucel-T has proven clinically effective in prostate cancer [12]. Similarly, CAR-T cell therapy, which utilizes an engineered receptor to bind specific tumor antigens, has shown significant improved clinical outcomes in hematologic malignancies [13]. Although, CAR-T use has suggested limited results in targeting solid tumors [14,15].

Vigil is an autologous vaccine constructed from harvested tumor tissue and transfected ex vivo with a plasmid containing a bi-functional short hairpin (bi-shRNA) construct targeting furin and the GM-CSF gene. Activating an immune response is critical for effective anti-tumor control. Previous results support the safety of Vigil in various solid tumors such as Ewing's sarcoma, ovarian cancer, colorectal cancer and melanoma [16-21]. Benefit of Vigil over placebo as maintenance therapy in Stage III/IV ovarian cancer was recently demonstrated in somatic BRCA wild type expressive patients, with significant improvement in RFS and OS [21]. Vigil also demonstrated improved clinical outcomes in correlation with IFN γ -ELISPOT positive response of circulating mononuclear cells to autologous (self) tumor in solid tumors [20]. Subsequent three year follow up continued to demonstrate improved outcomes and relationship to IFN γ -ELISPOT response which suggests evidence of long term immune memory activation [22]. Here, we report circulating cytotoxic T cell response to Vigil in four patients with advanced solid tumors.

Methods

Patient Population

Patient EW 167-3006 was a 22 year old Caucasian male with metastatic recurrent Ewing's sarcoma who received three prior lines of systemic treatment before tumor resection and Vigil

construction on 12/2016. The patient was randomized and received gemcitabine/docetaxel on the control arm of protocol CL-PTL-121 part 1 [Phase 2 Clinical Trial in Third-Line or Greater Ewing's Sarcoma (NCT02511132)] and remained recurrence free for 7 months from the time of procurement. After relapse, EW 167-3006 crossed over to CL-PTL-121 part 2 (Phase 2 Clinical Trial in Third-Line or Greater Ewing's Sarcoma) and received 10 cycles of combination Vigil (1×10^7 cells/ml)/Irinotecan/Temozolomide. From the time of treatment start on CL-PTL-121 part 2, RFS was 15 months and based on the last known date alive (LKDA) OS was 29.5 months. Peripheral blood mononuclear cells (PBMCs) were analyzed from the time of tissue procurement and the end of Vigil treatment on CL-PTL-121 part 2.

Patient EW 167-3014 was a 26 year old Caucasian male with metastatic recurrent Ewing's sarcoma who received 12 prior lines of systemic treatment before Vigil construction on 02/2018. The patient received 12 cycles of combination Vigil (1×10^6 cells/ml)/Irinotecan/Temozolomide under CL-PTL-121 part 2. From treatment start RFS was 8.2 months and OS was 20.7 months. PBMCs of this subject were analyzed from the time of tissue procurement and treatment cycle four.

Patient 136_0024 was a 72 year old Caucasian female with *BRC1/2*-wt, platinum-sensitive, Stage IIIC recurrent high grade serous cancer of the fallopian tube who received one prior systemic treatment before tissue procurement for Vigil construction on 10/2016. The patient was randomized and received placebo on the control arm of the VITAL study (NCT02346747) and remained recurrence free for 13.6 months from the time of procurement. After relapse, patient 136_0024 crossed over to a Phase 1 study of Vigil and atezolizumab (CL-PTL-126, NCT03073525) and received two cycles of Vigil (1×10^7 cells/ml) followed by 10 cycles of combination Vigil and atezolizumab (1200mg), and an additional 3 cycles of atezolizumab alone until disease progression. From the time of treatment start on CL-PTL-126, RFS was 11.9 months and based on the LKDA OS was 12 months. PBMCs from this subject were analyzed from the time of tissue procurement and treatment cycle six.

FANG-CLM-1001 was a 50 year old Caucasian male with stage IVA colorectal cancer who received one prior systemic chemotherapy regimen before tissue procurement for Vigil construction on 09/2012. The patient enrolled on a Phase 2 study of Vigil/FOLFOX (CL-PTL-107, NCT01505166) and received 12 doses of Vigil (1×10^7 cells/ml) in combination with modified FOLFOX (oxaliplatin/l-leucovorin/fluorouracil bolus/fluorouracil) for 7 cycles. From the time of treatment start RFS was 32.2 months and based on the LKDA OS was 37.5 months. PBMCs were analyzed from treatment cycle three.

All patients were monitored for safety during study enrollment.

PBMC Storage and Processing

PBMCs were harvested at the time of tissue procurement and after a minimum of three cycles of Vigil. One patient did not have a

baseline sample (FANG-CLM-1001). PBMCs were drawn in green top tubes and isolated via Ficoll density gradient centrifugation, placed into cryotubes at a concentration of 1×10^6 cells/mL and stored at -80C. Following thaw, PBMCs were counted and viability assessed by Lonza using the Luminex Muse system and Viacount reagent.

Flow Cytometry

Flow cytometry was performed by Lonza. Patient PBMCs were stained with monoclonal anti-human antibodies against CD3, CD4, CD8 (Miltenyi Biotech) for 10 min at 4°C in PBS/0.1% BSA buffer and dead cells were excluded using Sytox A Advanced viability dye (Invitrogen). Samples were acquired on a Guava EasyCyte 8HT flow cytometer (Luminex) instrument utilizing Guava InCyte software for post-acquisition analysis.

Results

Phenotypic Characterization of PBMCs

The percentage of CD3+, CD3+CD4+ and CD3+CD8+ was determined from PBMC samples at time of tissue procurement and after Vigil treatment with a minimum of 3 cycles. PBMC samples from pretreatment exhibited a lower percentage of viable PBMC CD3+ cells compared to previously harvested healthy donors (Figure 1). However, post treatment, CD3+ percentage levels rose to levels close to healthy donors (Table 1). The level of CD3+CD8+ cells as a percentage of viable CD3+ cells rose significantly post treatment. No change in CD3+CD4+ cells was observed.

Discussion

Previous studies with Vigil in advanced solid tumors found a survival benefit in IFN γ -ELISPOT positive patients [23]. IFN γ is a central regulator of anticancer T cell activity and drives differentiation into memory T cell function or to undergo apoptosis [24]. Further, presentation of relevant neoantigens by dendritic cells modulates the formation of CTLs. However, tumors have evolved mechanisms to escape this effect including production of immune suppressive cytokines which decrease tumor infiltrating lymphocytes (TILs) into the tumor microenvironment (TME) [25].

Here we examined the activation of CD3+ T cells following vaccination with Vigil which would effectively prime naïve CD8+ T cells to the neoantigen repertoire. CD3+ cells denote mature T cells that have differentiated into T helper cells (CD3+CD4+) or CTLs (CD3+CD8+). Consistent with previously published work, we found that the advanced cancer patients presented here had a low percentage of CD3+ cells at baseline [26]. The three patients with baseline samples had received multiple lines of previous cytotoxic therapy, which likely also contributed to reduced baseline CD3+ T cell counts.

Following vaccination with Vigil, there was a corresponding increase in CD3+ T cells, specifically, CD3+CD8+ T cells. This suggests that Vigil is able to prime, activate and promote the generation of tumor specific CTLs. Increased levels of TILs (including CTLs) within the TME is associated with improved

clinical outcomes in a variety of cancers, including melanoma, ovarian, breast, cervical and non-small cell lung cancer [2-9]. Previously, peptide based cancer vaccines have been investigated as a way to prime CTLs with limited success likely due to several factors, including poor immunogenicity of the antigen, immune suppressive environment within the TME, and intratumor heterogeneity [27,28]. Vigil administration may overcome several of these factors.

Vaccination with Vigil educates T cells to the relevant tumor neoantigens as shown by IFN γ -ELISPOT response and has demonstrated suggestive evidence of durable clinical benefit [16-20,22]. Vigil activity measured by CD3+CD8+ T cell expansion as shown here, may provide evidence to explain clinical benefit demonstrated in recently published Phase 2b trial involving Vigil in maintenance front line stage III/IV ovarian cancer [21]. These results combined with robust safety data support rationale for use of Vigil in combination with immune checkpoint inhibitors or other immune modulating therapy to increase fraction of self neoantigen targeting CD3+CD8+ T cells.

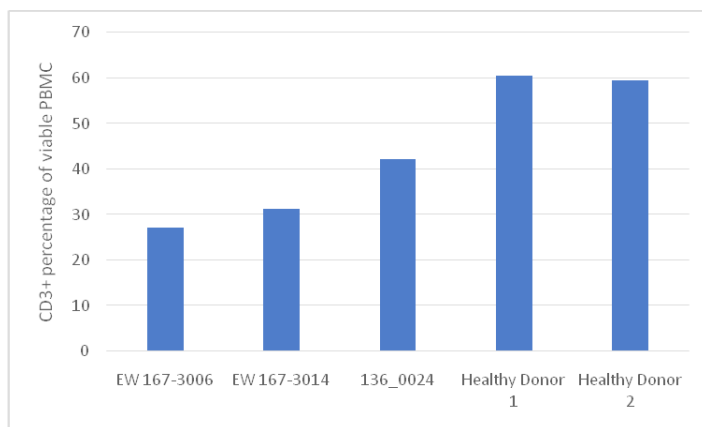


Figure 1: CD3+ cell counts in advanced cancer patients prior to treatment start compared to healthy donor controls. A pre-Vigil sample was not available for FANG-CLM-1001.

Subject ID	At Time of Procurement		Post Treatment	
	CD3+ (%)	CD3+/CD8+ (%)	CD3+ (%)	CD3+/CD8+ (%)
EW 167-3006	27.2	43.7	44.1	55.1
EW 167-3014	31.3	35.6	42.2	41.9
136_0024	42.2	13.5	55.7	21.5
FANG-CLM-1001*	N/A	N/A	70.9	41.3

*no baseline sample for this patient.

Table 1: Change in T cell population pre and post treatment.

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