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ORIGINAL ARTICLE

Immune response and survival of refractory cancer patients who received TGF-β2 antisense/GM-CSF gene modified autologous tumor cell (TAG) vaccine

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TAG vaccine is a novel 'triad vaccine' that involves transfection of autologous tumor with a dual plasmid, TGFβ2 antisense gene and GM-CSF gene. Patients with advanced cancer who failed standard therapy were treated. IFN-γ ELISPOT analysis (Enzyme-Linked Immunospot Assay for Interferon Gamma) using TAG autologous vaccine target cells was performed prior to vaccination and at week 12 after the third vaccination. The purpose of this assessment was to correlate the IFN-γ ELISPOT immune response with long-term survival of advanced cancer patients who received TAG vaccination. Twenty-three of 28 patients received $\geqslant 3$ TAG vaccinations (two patients withdrew consent and three had disease progression prior to the third vaccination). Eleven patients demonstrated a positive ELISPOT response (>10 spots and $\geqslant 2\times$ baseline) at week 12 and 12 patients did not ($P=0.002$). Median survival from time of treatment between ELISPOT-positive and -negative groups was significantly different (550 vs 159 days, $P=0.036$), as was median survival from the time of procurement (627 vs 257 days, respectively, $P=0.043$). In conclusion, the IFN-γ ELISPOT assay may provide an effective measure of immune response following treatment with 'triad vaccines', but additional patient numbers and/or other immune modulatory parameters are necessary for future testing.

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INTRODUCTION

It is known that despite tolerance, tumor cells can retain intrinsic immunogenicity and that tolerance can be antigen-specific rather than global. The TAG vaccine is an irradiated whole cell autologous vaccine that allows for: (1) presentation of tumor-specific and tumor-associated antigen matrix, (2) CD4+ and CD8+ T-cell priming and (3) MHC compatibility. The manufacturing process requires freshly procured tumor tissue (within 48 h of surgery) and is completed within 2 days. The procedure entails the dissection and dissociation of the tumor into a single-cell suspension. Cells are then washed, enumerated and transfected with the TAG expression plasmid. They are incubated overnight to allow expression of the GM-CSF protein and the TGFb2 antisense. On the following day, the cells are harvested, enumerated and then irradiated. Following irradiation (10 000 cGy), the cells are washed, formulated in freeze media and then aliquoted into final containers for freezing and storage.

Our clinical experience with the GVAX (GM-CSF), Lucanix (TGFβ2 antisense) and TAG (TGFβ2 antisense and GM-CSF) vaccines has (1) demonstrated the safety of these modified autologous vaccines, 2) established an effective dose range for each of the individual vaccines and 3) confirmed induction of immune activation.[1–4] Each of these vaccines produced limited but promising clinical outcomes without toxic effects, including multiple durable complete responses (some for >5 years) in advanced melanoma and lung cancer patients refractory to prior standard treatment. To date, 28 patients have received TAG vaccine. Of these, 22 of 26 (73%) evaluable, advanced cancer patients (that is, patients receiving two or more vaccines) achieved stable disease of at least 3 months after receiving the TAG vaccine,[3] including one patient with stage IVb melanoma who achieved complete response as confirmed by imaging studies.[3]

The availability of an immune response biomarker that reflects an *in-vivo* immune response at a designated time point and that correlates with patient survival would facilitate therapeutic development. The ELISPOT is a standardized, cost-effective, functional assay that is both robust and sensitive. In brief, the IFN-γ ELISPOT assay allows visualization of secretory IFN-γ of individual activated or responding cells. Each spot that develops in the assay represents a single reactive cell. Our previously published experience with the TAG vaccine was based on a median follow-up of <1 year from time of tissue procurement and <170 days from initial treatment.[3] We now report an updated 3-year median follow-up of those patients who received TAG vaccine and had, as a minimum, baseline and week 12-activated T-cell assessments, thereby allowing for parallel analysis of IFN-γ ELISPOT (Enzyme-Linked Immunospot) responsiveness and survival duration ($n=23$).

RESULTS

Twenty-three advanced cancer patients, progressing despite prior therapies, received a minimum of three TAG vaccinations and underwent baseline and week 12 ELISPOT assessment.

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Demographics of these 23 patients are shown in Table 1. None of the patients demonstrated adverse effects related to TAG vaccine other than those previously described[3] and no delayed adverse effects were seen.

Immune response

IFN-γ expression with phorbol myristate acetate/ionomycin response was seen in 21 of the 23 patients at baseline and demonstrable by week 12 in the two patients (008 and 041) not exhibiting phorbol myristate acetate/ionomycin response at baseline (data not shown), supporting immune functionality in this patient population. There was no evidence of a week 12 ELISPOT response in 12 of the patients, whereas 11 patients did develop a positive ELISPOT at that time (Table 1). The difference in response between these two groups of patients was statistically significant ($P = 0.002$) at month 3 as seen in Figure 1, which shows results using the patient's non-transfected autologous tumor cells (similar results using irradiated, autologous TAG vaccine cells are not shown). One of the patients exhibiting a negative response at week 12 (037) first demonstrated an ELISPOT response at week 24. Week 24 ELISPOT assay results were available in 8 of the 11 patients with a positive response at week 12, all of which continued to show positive responses at week 24. Patient 013, who achieved a complete response and was the only patient with an ELISPOT assessment beyond week 24, maintained a positive ELISPOT response to original stored tumor cells 92 weeks after discontinuation of vaccine, despite no further vaccination or other anticancer or immune modulatory therapy. Mean and median lymphocyte responses were also monitored. No change in lymphocyte counts over time or between the ELISPOT (+) or (−) groups was observed (see Figure 2).

Survival

In a recent analysis of 182 consecutively seen patients in a Phase I clinic, the median survival from initial consultation was ~264 days.[5] Although there is a suggestion of a pattern of longer duration of survival (median survival, 400 days from procurement) than expected in this group of 23 advanced cancer patients who received three or more TAG vaccinations at 3-year follow-up (Table 1), the limitations of the reported data belie any conclusions. The median time interval from tissue procurement to initiation of therapy was 79 days for all 23 patients, 81 days for ELISPOT responders and 65 days for ELISPOT non-responders. Increased survival from procurement ($P = 0.043$) and from treatment ($P = 0.036$) in those patients with a positive ELISPOT response at week 12 was demonstrated (Figure 3; Table 2). There was no correlation of survival with age, sex, dose, type of cancer, GM-CSF expression or TGFβ2 knockdown level (data not shown).

Table 1. Characteristics and response of treated study patient population receiving at least 3 TAG vaccines ($n = 23$)

Patient ID	*Indication*	*Age*	*Sex*	*No. of prior investigational and/or chemotherapy regimens (single or multiple agent)*	*Tissue site*	*Dose (low/high)*	*No. of vaccines received*	*Best response*	*Survival since tissue procurement (days)*[a]	*Survival since treatment start (days)*[a]	*ELISPOT response at month 3*[b]
008	Neuroendocrine	28	M	0	Pancreas	High	12	SD	1583+	1438+	Positive
009	Neuroendocrine	33	F	3	Adrenal gland	Low	5	PD	880	764	Negative
010	Breast	46	F	10	Metastasis in liver	High	3	NE	318	136	Negative
012	Melanoma	51	M	1	Lung tissue and lymph node	Low	5	SD	807	752	Negative
013	Melanoma	77	M	0	Metastasis in peritoneum	Low	11	CR	1415	1334	Positive
014	Lung	71	F	3	Lung tissue and lymph node	High	3	PD	465	320	Negative
017	Lung	79	F	2	Lung tissue	High	3	PD	137	87	Negative
023	Neuroendocrine	39	F	0	Tumor tissue from liver	High	12	SD	1358+	1295+	Positive
024	Colon	57	F	2	Pelvic lymph node resection	High	4	PD	257	159	Negative
026	Colon	75	F	2	Lymph node deep chest wall	Low	3	PD	515	431	Positive
029	Neuroendocrine	30	F	2	Tumor tissue from liver	High	4	PD	197	135	Negative
031	Breast	64	F	12	Mets from Lung	High	3	SD	168	120	Negative
032	Gastric	59	M	3	Mets from Omentum	Low	6	SD	237	190	Positive
033	Leiomyo-sarcoma	58	F	4	Peritoneal mets	Low	6	PD	627	550	Positive
034	Melanoma	56	M	2	Lymph Node left thigh	High	5	PD	211	162	Negative
035	Bladder	80	F	1	Lung tumors	High	3	NE	132	91	Negative
037[c]	Bladder	56	F	5	Vaginal tumor	Low	11	SD	835	766	Negative
041[c]	Hemangio-pericytoma	65	M	0	Brain	High	4	PD	386+	291+	Negative
043[c]	Cervical	59	F	5	Uterine/cervical	High	3	NE	232	99	Positive
045[c]	Colon	49	M	1	Omentum	Low	4	SD	671+	513+	Positive
048[c]	Prostate	74	M	0	Prostate	Low	7	SD	565+	462+	Positive
049[c]	Colon	34	F	4	Abdomen	Low	5	SD	361	306	Positive
050[c]	Sarcoma	70	M	0	Adrenal Gland	High	9	SD	378	332	Positive

Abbreviations: CR, complete response; NE, not evaluable; PD, progressive disease; SD, stable disease; (+) still alive. [a]Data current as of 18 June 2012. [b]ELISPOT response to non-transfected autologous tumor tissue harvested at time of vaccine procurement. [c]ELISPOT data not reflected in *Clin Can Res*; 17(1): 1 January 2011 publication.

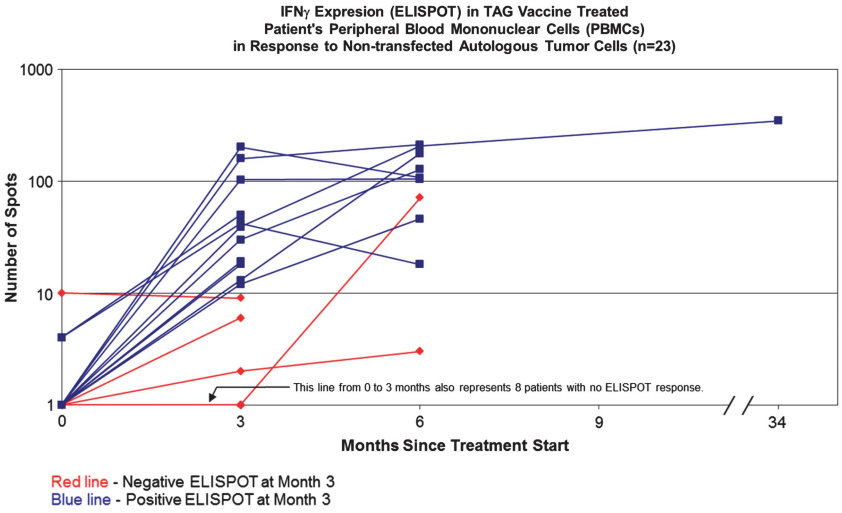

Figure 1. IFN-γ expression (ELISPOT) in TAG vaccine-treated patient's peripheral blood mononuclear cells in response to non-transfected autologous tumor cells ($n = 23$). Blue lines indicate 11 patients (008, 013, 023, 026, 032, 033, 043, 045, 048, 049 and 050) achieving $\geqslant 10$ IFN-γ producing lymphocytes (positive response) at month 3. Red lines indicate 12 patients (009, 010, 012, 014, 017, 024, 029, 031, 034, 035, 037, 041) not achieving positive ELISPOT response at month 3.

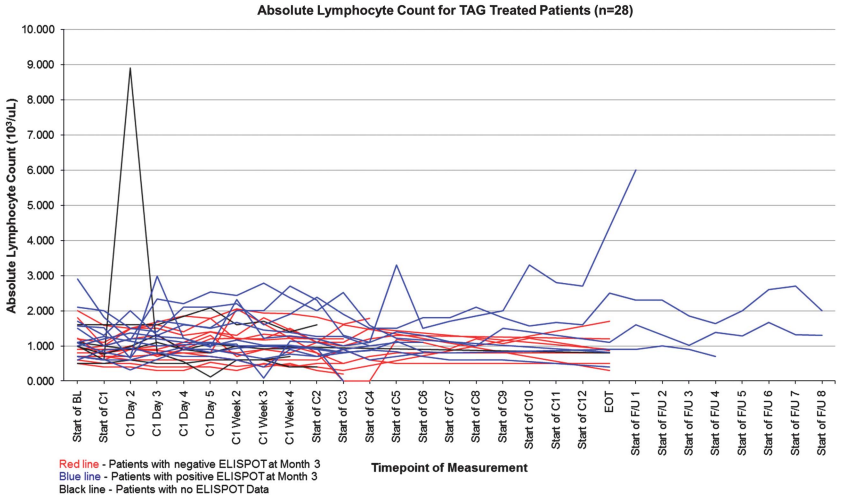

Figure 2. The mean and median (+range) absolute lymphocyte counts at baseline for ELISPOT (+) and ELISPOT (–) groups are 1.36 and 1.10 (0.6–2.9) $\times 10^3$ cells/μl and 1.01 and 0.85 (0.5–2.0) $\times 10^3$ cells per μl. As noted in this figure, the levels remained stable throughout treatment and follow-up; thus, there is no evidence for a differential response in PBMC that would account for the difference in response between the two groups.

DISCUSSION

Development of assays that identify surrogate parameters of immune modulation potentially can be developed as diagnostics to function as early predictors of immune activation and therapeutic response to a relevant cancer vaccine. We utilized a unique ELISPOT assay to monitor response of cancer patients receiving TAG vaccine. Results support further evaluation of the ELISPOT assay as such a predictor and suggest that expanded development with a larger, appropriately powered, number of patients or with more potent triad vaccines may be fruitful. Autologous whole cell vaccines represent the quintessential personalized cancer therapy. Specifically, they express the characterized and uncharacterized tumor antigen mosaic including clonal and antigen spread,[6] are not constrained by HLA type and are a source of both MHC I and II antigens.[7] The availability of an immune response biomarker that reflects an *in-vivo* immune response at a designated time point and that correlates with patient survival would facilitate therapeutic development. The IFN-γ ELISPOT is a validated monoparametric assay.[8–10] The longer term follow-up of these patients with advanced cancer treated with the autologous TAG vaccine allows for the assessment of efficacy of IFN-γ ELISPOT as an early surrogate of survival. A correlation of the month 3 ELISPOT with survival duration was demonstrated in this preliminary assessment. Insofar, as this was a Phase I safety study in patients with advanced solid tumors, it was not designed to be adequately powered for a survival endpoint. It is notable that an ELISPOT-survival correlation has been documented with the FANG vaccine[11] that, rather than using an antisense TGFβ2, incorporates a bifunctional shRNA technology to knockdown furin, the proprotein convertase essential for activation of all immune suppressive isoforms of TGFβ.[12,13] Mean knockdown of TGFβ1 and TGFβ2 with FANG was 93.5 and 92.5%, respectively, at day 7 following vector transfection,[13] whereas previously published mean knockdown of TGFβ2 and TGFβ1 with TAG was 54[12] and ~10%, respectively.

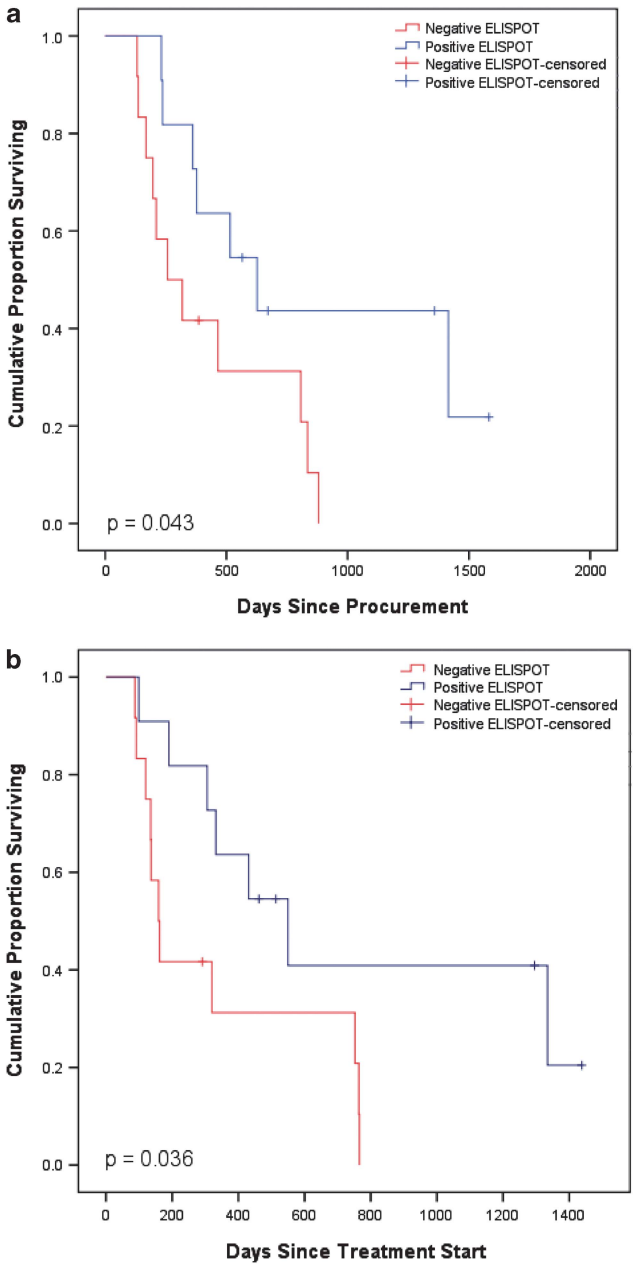

Figure 3. Survival comparison of patients achieving positive ELISPOT response at month 3 (blue) vs those not achieving positive ELISPOT response at month 3 (red) from (**a**) time of procurement and (**b**) time of first vaccination ($n = 23$) (data as of 18 June 2012).

Table 2. Survival by ELISPOT Response

ELISPOT response at month 3	n	*Survival since procurement (days)*		*Survival since treatment start (days)*	
		Mean	*Median*	*Mean*	*Median*
Negative	12	429	257	345	159
Positive	11	879	627	765	550

The combined results of this long-term assessment with those of the recently reported FANG vaccine[13] support further study of 'triad' vaccines[14] as well as continued evaluation of the ELISPOT assay as an early predictor of effectiveness. This could also allow for derivative studies in patients with a non-responding ELISPOT to evaluate early institution of complementary 'immune salvage therapy' to trigger the afferent arm of the immune response, for example, ipilimumab.[15] Additional assays to define immune predictive potential are also recommended as proposed in the FDA draft guidance for therapeutic cancer vaccines (September 2009) and have been incorporated in our development program.

MATERIALS AND METHODS

Study population

All eligible patients were treated and followed up in the outpatient facilities of Mary Crowley Cancer Research Centers (MCCRC) since 2 June 2008. Inclusion criteria, TAG product construction and manufacturing, study design, study population, assessments, tumor response and ELISPOT immune assessment have previously been described.[3] The ELISPOT (Enzyme-Linked Immunospot) assay was performed using Enzyme-Linked Immunospot Assay for Interferon Gamma (BD Biosciences, San Jose, CA, USA). Briefly, tumor cells were harvested from patients undergoing treatment-appropriate excisions and transduced with GM-CSF and TGFβ2 antisense transgenes. Following 100 Gy irradiation, the cells underwent a series of QA assays as defined by established Gradalis Inc. (Carrollton, TX, USA) standard operating procedures. Depending on manufacturing yield, patients received a monthly dose of either 1×10^7 or 2.5×10^7 cells/intradermal injection, up to a maximum of 12 months. *In-vitro* IFN-γ production was determined following phorbol myristate acetate/ionomycin-induced polyclonal T-cell differentiation[16,17] or separate co-incubation with the patient's non-transfected autologous tumor cells and irradiated, autologous TAG vaccine cells. In order to correlate ELISPOT response with survival, all patients with a minimum of baseline and week 12 ELISPOT assessments are included in this long-term follow up analysis ($n = 23$). This includes the 13 patients previously reported with ELISPOT assessment at week 12, 6 patients previously reported but not having been assessed for week 12 ELISPOT response and 4 additional patients who have since been treated with the identical TAG vaccine following the same inclusion and assessment criteria and assessed for week 12 ELISPOT (045, 048, 049 and 050). Of the five patients excluded from analysis, two withdrew consent following their first vaccination and three experienced disease progression prior to their third vaccination.

Statistics

ELISPOT analysis was performed on patients receiving at least three vaccines. Response status at week 12 (and later when available) since (a) treatment start and (b) procurement was compared to baseline using a *t*-test. A positive response was defined as equal to or greater than twice the number of spots at baseline and a minimum of 10 spots.

Survival was analyzed using SPSS to generate Kaplan–Meier curves, and included 23 patients, that compared survival in patients with positive or negative week 12 ELISPOT assessments.

CONFLICT OF INTEREST

The following authors are shareholders in Gradalis Inc.: John Nemunaitis, Neil Senzer and Phillip Maples. All other authors declare no conflict of interest.

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