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Serum PD-1 Is Elevated after Pembrolizumab Treatment but Has No Predictive Value

Milena Music[1], Marco A.J. Iafolla[2], Annie He Ren[1], Antoninus Soosaipillai[3], Ioannis Prassas[3], and Eleftherios P. Diamandis[1,3,4,5]

Abstract

Immune-checkpoint blockade (ICB) uses antibody targeting of specific inhibitory receptors and ligands. The major limitations of ICB, such as high cost, limited success rate, and immune-related adverse events (irAE), highlight the need for predictive biomarkers. We analyzed pre-immunotherapy and post-immunotherapy serum samples of 24 patients treated with pembrolizumab for changes in PD-1 and over 1,000 additional protein markers using a multiplex proximity extension assay (PEA) to identify potential predictive biomarkers of response and/or toxicity. Candidates were selected based on the criteria that at least 2 patients within any of 3 patient groups (responders without irAEs, responders with irAEs, or nonresponders with irAEs) had either a $\geq$4-fold increase or 4-fold decrease in expression post-immunotherapy. Female and male control samples were used as technical duplicates. A patient group with no response and no irAEs was used to exclude candidates. Following treatment with pembrolizumab, there was a relative increase of PD-1 in the serum of all patients, compared with controls (average 4.4-fold). We identified 7 additional serum proteins that met our candidate selection criteria. These candidate markers did not have any significant association with response or toxicity to pembrolizumab. Overall, we show that serum PD-1 increases post-therapy with pembrolizumab treatment but has no predictive value for response or toxicity in this small set of patients.

Introduction

Immune-checkpoint blockade (ICB) is a form of immunotherapy that has revolutionized the treatment of cancer. This treatment uses antibody targeting of specific inhibitory receptors and ligands, such as cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 (PD-L1; ref. 1). Pembrolizumab is a humanized monoclonal antibody targeting PD-1. In a multicenter, open-label, randomized, phase III trial of patients with advanced melanoma treated with pembrolizumab and ipilimumab, the 24-month overall survival rate was 55% for the pembrolizumab-treated group (2). In September 2014, the FDA approved pembrolizumab for the treatment of patients with previously treated unresectable or metastatic melanoma (3).

The major limitations of ICB agents such as pembrolizumab are high cost, limited success rate, and potential severe toxicity due to immune-related adverse events (irAE; refs. 4–6). These limitations highlight the need for predictive biomarkers that can predict the likelihood of a patient responding favorably to therapy or developing toxicity, and allow for the monitoring of their therapeutic outcome (7, 8). Such biomarkers can help lower the prevalence of irAEs in patients undergoing treatment with ICB and result in higher response rates. Despite the growing need for a personalized approach to cancer treatment (8), there is a paucity of predictive biomarkers of ICB. Currently investigated candidate predictive biomarkers of ICB include PD-L1 expression, mismatch-repair deficiency, increased mutational load, and increased numbers of tumor-infiltrating lymphocytes in the tumor microenvironment (9–12). Several routinely available blood markers, such as lactate dehydrogenase (LDH) and C-reactive protein, have shown some promise in predicting response to ICB (13).

The objective of this study is to utilize the serum samples of 24 patients with cancer treated with pembrolizumab monotherapy in a phase II clinic trial and analyze protein expression changes of PD-1 and other proteins using a proximity extension assay (PEA). A major challenge in the discovery of protein biomarkers is the development of specific and sensitive methods to detect large numbers of low-abundance proteins in biological samples. PEA is suitable for biomarker research, as it consumes only 1 μL of serum sample per analysis of 92 proteins and can detect low-abundance serum proteins with high sensitivity (pg/mL range) and specificity (14). We hypothesize that changes in PD-1 and other serum protein levels during therapy are associated with response and toxicity to pembrolizumab.

[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada. [2]Division of Medical Oncology and Hematology, Princess Margaret Cancer Centre, University Health Network, Toronto, Canada. [3]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Canada. [4]Department of Clinical Biochemistry, University Health Network, Toronto, Canada. [5]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada.

M. Music and M.A.J. Iafolla contributed equally to this article.

Corresponding Author: Eleftherios P. Diamandis, Mount Sinai Hospital, 60 Murray Street (Box 32); ACDC Laboratory Floor 6, Room L6-201-1, Toronto, ON M5T 3L9, Canada. Phone: 416-586-8443; Fax: 416-619- 5521; E-mail: eleftherios.diamandis@sinaihealthsystem.ca

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Materials and Methods

Study population

The Institutional Review Board of the University Health Network approved all protocols, including collection of clinical outcomes and serum samples. All patients provided written informed patient consent prior to study enrollment. The study was carried out in accordance with the Declaration of Helsinki. Patients were treated with single-agent pembrolizumab 200 mg i.v. every 3 weeks in the investigator-initiated phase II clinical trial called INSPIRE (INvestigator-initiated Phase II Study of Pembrolizumab Immunological Response Evaluation; NCT02644369) at Princess Margaret Cancer Centre, University Health Network, Toronto, Canada. Between March 21, 2016, and May 9, 2018, INSPIRE enrolled 106 patients into 5 cohorts ($n \approx 20$ in each cohort): squamous cell cancer of the head and neck, triple-negative breast cancer, high-grade serous epithelial ovarian cancer, malignant melanoma, and mixed solid tumors. Pertinent inclusion criteria were: age $\geq$ 18 years; pathologically proven locally advanced or metastatic solid malignancy that is incurable and without standard treatment options available (with the exception of melanoma); measurable disease based on RECIST 1.1 (15). ECOG 0-1; and adequate organ function. Pertinent exclusion criteria were: prior anti–PD-1/L1/L2 agents (prior anti-CLTA-4 and T-cell costimulatory agents allowed); autoimmune disease; immunodeficiency or immunosuppressive medications (exceeding physiologic corticosteroid replacement); and active central nervous system metastases and/or carcinomatous meningitis. Patients were clinically assessed with comprehensive blood work every 3 weeks and received restaging CT scans every 9 weeks. Clinical outcomes were prospectively annotated.

In this study, 24 patients treated on INSPIRE were selected by one author (M.A. J. Iafolla): 6 patients without response or irAEs from pembrolizumab (group A); 6 patients with irAEs but no response (group B); 6 patients with both response and irAEs (group C); and 6 patients with response but no irAEs (group D). Response is defined as the attainment of RECIST 1.1 complete or partial response. Toxicity is defined as $\geq$ grade 2 CTCAE 4.03 (16) irAEs with at least possible attribution to pembrolizumab and the investigator must attribute the adverse event to be autoimmune in causality as a result of pembrolizumab exposure. Grade 2 irAEs commonly necessitate withholding immunotherapy and augmenting the immune response with first-line corticosteroids (17); this was the reason for justifying this minimum grade cutoff. Only the highest grade of a specific toxicity event was recorded for each patient; the same toxicity event can be recorded more than once per patient if the irAE occurred on a separate occasion. Patients who developed more than one type of toxicity were only counted once. Both response and toxicity data were annotated by INSPIRE data coordinators and then verified by one author (M.A. J. Iafolla). The last clinical outcome update was November 14, 2018. To minimize bias, performance of the PEA and analysis of the results were conducted by investigators blinded to INSPIRE clinical outcomes.

Sample collection

For the purpose of this study, the INSPIRE protocol mandated proteomic research blood samples at 2 time points: within 28 days of first pembrolizumab exposure (pretreatment/baseline) and at cycle 3 pembrolizumab assessment (week 7); the latter point was chosen to precede radiographic assessment yet allow enough time for possible ICB-induced changes in serum- based proteins. Upon collection of blood samples in Vacutainer SST tubes, the blood was allowed to clot for 30 to 60 minutes prior to processing. The tubes were centrifuged at 1,200 $\times$ g for 10 minutes at room temperature. Five hundred microliters of serum was aliquoted into cryogenic vials and frozen at -80°C (range, 6–27 months). Control serum samples from one healthy male and one healthy female were also collected as above and stored at -80°C until analysis.

Protein quantification

Pre- and post-immunotherapy samples were analyzed for relative protein concentration changes using a multiplex PEA (Olink Proteomics). The analysis was performed as a service at Olink facilities in Boston, MA. The technical personnel were blinded to the status of the patient and control samples. Over 1,000 protein markers (available online at https://www.olink.com/products/complete-protein-biomarkers-list/) were assayed across 12 currently available panel types with 92 proteins per panel: cardiometabolic, cell regulation, cardiovascular II, cardiovascular III, development, immune response, inflammation, metabolism, neurology, neuroexploratory, oncology II, and organ damage. One microliter of serum sample per panel was incubated in the presence of a pair of oligonucleotide-labeled antibodies (ProSeek probes; ref. 14). When the antibody pair binds to its corresponding protein antigen in a homogenous assay, a new PCR target sequence is formed by a proximity-dependent DNA hybridization event between the oligonucleotides at the ends of the antibody pair. This sequence is quantified by high-throughput real-time PCR and the generated fluorescence signal correlates directly with the protein abundance in the serum sample. Raw data were provided in terms of a normalized protein expression (NPX) value, which is an arbitrary unit on the $\log_2$ scale, and is used as a relative quantification measure. The higher the NPX value, the higher the protein abundance in the sample. More details about this analytical technology can be found in the link https://www.olink.com/data-you-can-trust/technology/. Patient samples were added in singlicate across all plates, whereas control samples were analyzed as technical duplicates. Additional internal controls were added to each run in order to monitor the quality of individual runs and assay performance. Values that were below the limit of detection (LOD) were reported as the LOD.

Candidate selection

The NPX values were transformed to natural numbers by using the formula 2^{NPX} and the pre- versus post-immunotherapy fold change of each protein was calculated for each patient and for each marker. Candidates were chosen based on the criteria that at least 2 patients within any of patient groups B, C, and D had either a $\geq$ 4-fold increase or 4-fold decrease in expression post-immunotherapy. All candidates were compared with values of the healthy controls as well as to patients in group A (no response/no irAEs). Candidates that had 4-fold or higher change in patients in group A were excluded, except for PD-1 (please see below). Proteins with over 50% difference between duplicates of healthy controls were excluded as candidates.

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Statistical analyses

A paired-sample Wilcoxon test was performed for each protein marker/group combination to test if there is a significant difference between pre- and post-pembrolizumab protein expression values in each group. An unpaired *t* test was used to test for differences in protein expression between subjects with no response (patient groups A and B) and those with response (patient groups C and D), as well as between subjects with no toxicity (patient groups A and D) and those with toxicity (patient groups B and C).

Results

Patient characteristics

The detailed clinicopathologic characteristics of the 24 patients used in this study are summarized in Table 1. Two patients who were initially assigned to group D (+response/−irAEs) at the time of PEA analysis later developed a significant toxicity event; this resulted in 8 patients in group C (+response/+irAEs) and 4 patients in group D. Candidates were selected based on patient grouping at the time of PEA analysis. Gender was approximately evenly distributed (female 54%, male 46%). The median age was 59.7 years at time of first pembrolizumab infusion, but there was a large range in age (27.9–74.7 years). The vast majority of patients were of white ethnicity ($n = 22$; 92%). Melanoma ($n = 9$; 38%), high-grade serous ovarian cancer ($n = 5$; 21%), and triple-negative breast cancer ($n = 4$; 17%) comprised the major tumor types. At the time of last clinical outcome update on November 14, 2018, the median follow-up time was 536 days from the date of first pembrolizumab infusion (range, 133–856 days) and 11 deaths (46%) had occurred. The median number of pembrolizumab infusions was 11, but there was a wide range (2–35 infusions); in total, 3 patients were still receiving treatment at the time of last data update. RECIST progression ($n = 13$; 54%) was the major reason for stopping treatment; the one patient who stopped treatment due to development of intercurrent illness also developed grade 3 colitis. Of the 3 patients who completed the study, 2 developed grade 2 hypothyroidism. Hypothyroidism ($n = 6$) and rash ($n = 5$) were the most common toxicities, and 7 patients developed more than one toxicity. Toxicity necessitated stopping treatment in 3 patients (13%) due to the development of grade 3 colitis in 1 patient with both squamous and basal cell carcinoma, grade 3 pneumonitis in a patient with Merkel cell carcinoma, and a combination of grade 3 lipase elevation, grade 2 hepatitis, and grade 2 rash in a patient with head and neck squamous cell carcinoma; all 3 of these patients attained either complete ($n = 1$) or partial response ($n = 2$) as best response. In total, grade 3 toxicity events occurred in 5 patients (there were no patients who developed more than one grade 3 event) and the rest were grade 2; there were no grade 4 or 5 events. Due to the specific selection of patients chosen for this study, responses are evenly distributed between progressive disease ($n = 12$; 50%) and the sum of partial responses with complete responses ($n = 12$; 50%).

Assay reproducibility

We assessed the reliability and reproducibility of the multiplex assay by analyzing one female and one male serum sample in duplicate. Intra-assay variability was assessed by correlating the concentration of the 1,000+ proteins for the technical duplicates of the female and male control samples (Fig. 1). Concentrations of the analyzed proteins were $\log_{10}$ transformed to best visualize the large range of values. The Pearson correlation coefficients (r) for intra-assay variability between the duplicates of the male and female control samples were 0.972 and 0.952, respectively, confirming the overall good analytical reproducibility of this multiplex platform. We then examined specifically the % difference between duplicate values in both the male and female controls for the 8 candidate biomarker proteins. The calculated % difference between duplicates of healthy controls for our 8 candidate markers ranged from 0.1% to 40.1%. In the male control sample, the maximum % difference was 35.6% (TSHB) whereas in the female control it was 40.1% (PTPN1). In order to avoid false positives, we selected candidate predictive biomarkers whose concentration changed by at least 4-fold between pretherapy and post-therapy samples and had % difference lower than 50% in the technical duplicates of the healthy controls.

PD-1 changes in patient samples

The vast majority of the analyzed proteins did not meet our candidate selection criteria and will not be considered further. Compared with the pretreatment samples, treatment with pembrolizumab was associated with a relative increase of PD-1 in serum samples across all 4 patient groups (Fig. 2). All patients had at minimum a 2.7-fold increase in serum PD-1, with an average increase of 4.4-fold across all patients. The differences between pre- and post-pembrolizumab PD-1 serum expression within each of groups A, B, and C were statistically significant ($P < 0.05$; Fig. 2). By an unpaired *t* test, the change in PD-1 expression levels following treatment with pembrolizumab did not show a significant association with either response or toxicity. There was also no correlation between the number of pembrolizumab infusions and the change in PD-1 levels in the patient sera following treatment.

Other proteins

The observed serum expression levels and changes in 7 other selected candidate proteins are summarized in Table 2 and depicted in Fig. 2. The concentration changes in these proteins after treatment with pembrolizumab did not have any significant association with response or toxicity. The thyroid stimulating hormone beta subunit (TSHB) was over 4-fold decreased in 3 patients with only toxicity and 3 patients with both response and toxicity. One patient who developed toxicity without response had a 19-fold increase; there were no other patients who developed a significant increase in TSHB post-pembrolizumab. Of the 7 patients with a significant change in their TSHB, 6 patients (86%) developed hypothyroidism. Interleukin-17C (IL17C) showed over 4-fold decrease in 2 patients with both response and toxicity and increased in 1 patient who responded to treatment. N-terminal pro b-type natriuretic peptide (NT- proBNP) from the metabolic panel was 4-fold or more decreased in 2 patients with response and toxicity and in 1 patient with response only. On the other hand, 1 patient with toxicity and 1 patient with both response and toxicity had over 4-fold increase in serum NT-proBNP. Caspase-3 (CASP-3) was 4-fold or more increased in 2 patients who responded to treatment, and phosphomevalonate kinase (PMVK) was 4-fold or more increased in 2 patients with toxicity only, as well as in one patient with response only. PMVK was also more than 4-fold decreased in 1 patient with toxicity only

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Table 1. Clinicopathologic characteristics of the 24 INSPIRE patients used in this analysis

Gender	Age at time of first pembrolizumab infusion (years)	Ethnicity	Tumor type	Number of pembrolizumab infusions	Reason for stopping pembrolizumab	irAE		Best response	Length of follow-up (days)	Dead or alive
						Type	Grade			
Group A (−) response (−) toxicity										
Female	50.4	Black or African American	TNBC	5	Progression	None	N/A	PD	631	Dead
Female	35.5	Asian	TNBC	3	Progression	None	N/A	PD	435	Dead
Female	73.5	White	TNBC	3	Progression	None	N/A	PD	142	Dead
Female	66.7	White	HGSO	5	Clinical progression	None	N/A	PD	494	Dead
Female	72.9	White	HGSO	4	Progression	None	N/A	PD	505	Alive
Male	74.1	White	Melanoma	5	Progression	None	N/A	PD	196	Dead
Group B (−) response (+) toxicity										
Male	58.0	White	Melanoma	5	Progression	Hypothyroidism	2	PD	187	Dead
Female	49.4	White	HGSO	3	Progression	Abdominal pain	2	PD	564	Dead
						Arthralgia	2			
						Flu-like symptoms	2			
Female	53.5	White	HGSO	2	Progression	Hypothyroidism	2	PD	461	Dead
Female	44.8	White	HGSO	3	Progression	Diarrhea	2	PD	133	Dead
Male	27.9	White	Sarcoma, granular cell	5	Progression	Rash (on 2 separate occasions)	2	PD	613	Dead
Male	51.5	White	Cholangiocarcinoma	6	Progression	Hypothyroidism	2	PD	289	Alive
Group C (+) response (+) toxicity										
Male	70.5	White	HNSCC	20	Toxicity	ALT elevation	3	CR	856	Alive
						AST elevation	2			
						Hepatitis	2			
						Lipase increase	3			
						Rash	2			
Male	61.4	White	Melanoma	28	Clinical progression	Lipase increased	3	PR	644	Alive
						Pancreatitis	2			
Male	73.1	White	Melanoma	35	Study completion	Hypothyroidism	2	PR	526	Alive
						Rash	2			
Male	70.1	White	Melanoma	21	Intercurrent illness	Colitis	3	PR	731	Alive
						Pneumonitis	2			
						Rash	2			
Female	53.8	White	Melanoma	31	Still on trial	Hypothyroidism	2	PR	630	Alive
Female	54.5	White	Melanoma	23	Still on trial	Hyperthyroidism	2	PR	488	Alive
						Hypothyroidism	2			
Male	70.7	White	Merkel cell carcinoma	16	Toxicity	Pneumonitis	3	PR	578	Dead
Male	50.0	White	Basal cell and squamous cell poorly differentiated carcinoma	16	Toxicity	Colitis	3	PR	621	Alive
Group D (+) response (−) toxicity										
Female	61.3	White	TNBC	27	Still on trial	None	N/A	PR	546	Alive
Female	67.8	White	Melanoma	35	Study completion	None	N/A	PR	782	Alive
Female	45.0	White	Melanoma	25	Progression	None	N/A	PR	517	Alive
Male	74.7	White	Merkel cell carcinoma	21	Progression	None	N/A	PR	578	Alive

Last clinical outcome update November 14, 2018.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CR, complete response; HGSO, high-grade serous ovarian carcinoma; HNSCC, head and neck squamous cell carcinoma; PD, progressive disease; PR, partial response; TNBC, triple-negative breast cancer.

and 1 patient with both response and toxicity. Protein tyrosine phosphatase nonreceptor type 1 (PTPN1) was 4-fold or more increased in 2 patients with toxicity only and decreased in 1 patient with both response and toxicity. Finally, FK506 binding protein 5 (FKBP5) was 4-fold or more increased in 2 patients with toxicity only, whereas it decreased in 1 patient with toxicity only and in 1 patient with both response and toxicity.

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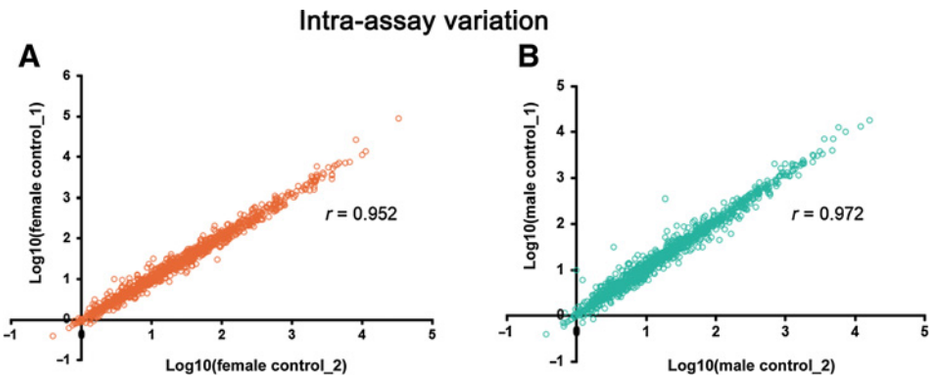

Figure 1.
Olink PEA assay precision based on duplicate measurements of all analyzed proteins. **A,** Strong correlation between the duplicates of a female control serum sample (aliquot 1 vs. aliquot 2) that were assayed in a blinded fashion ($P < 0.001$). **B,** Results for a serum sample from a male control ($P < 0.001$). Pearson correlation coefficient (r) was used for each analysis.

Discussion

In this study, we have used new technology, PEA by Olink, to explore the changes in PD-1 and over 1,000 serum-based proteins in the blood of patients with cancer treated with pembrolizumab monotherapy in a prospective phase II clinical trial. To our knowledge, this is the first exploratory analysis using this novel protein quantification platform for ICB biomarker discovery. According to our calculated % difference values, the reproducibility of this method is inferior to classic single ELISAs, which usually exhibit % coefficients of variation in the range of 10% to 15%. For this reason, we selected stringent cut offs to select our candidates and hopefully avoid false positives.

PD-1 serum concentration was increased in all patients who received therapy (Fig. 2), but it does not appear to be predictive of either response or toxicity to pembrolizumab. This may be a limitation of our small sample size. Although PD-1 is predominantly a transmembrane protein expressed on T cells (18), B cells (19), and NK cells (20), the serum levels of PD-1 were initially investigated in autoimmune conditions (21). Similar to our results, a prospective analysis of serum PD-1 levels in patients with pancreatic cancer failed to show an association with clinical outcome (22). Additionally, there is a lack of correlation between

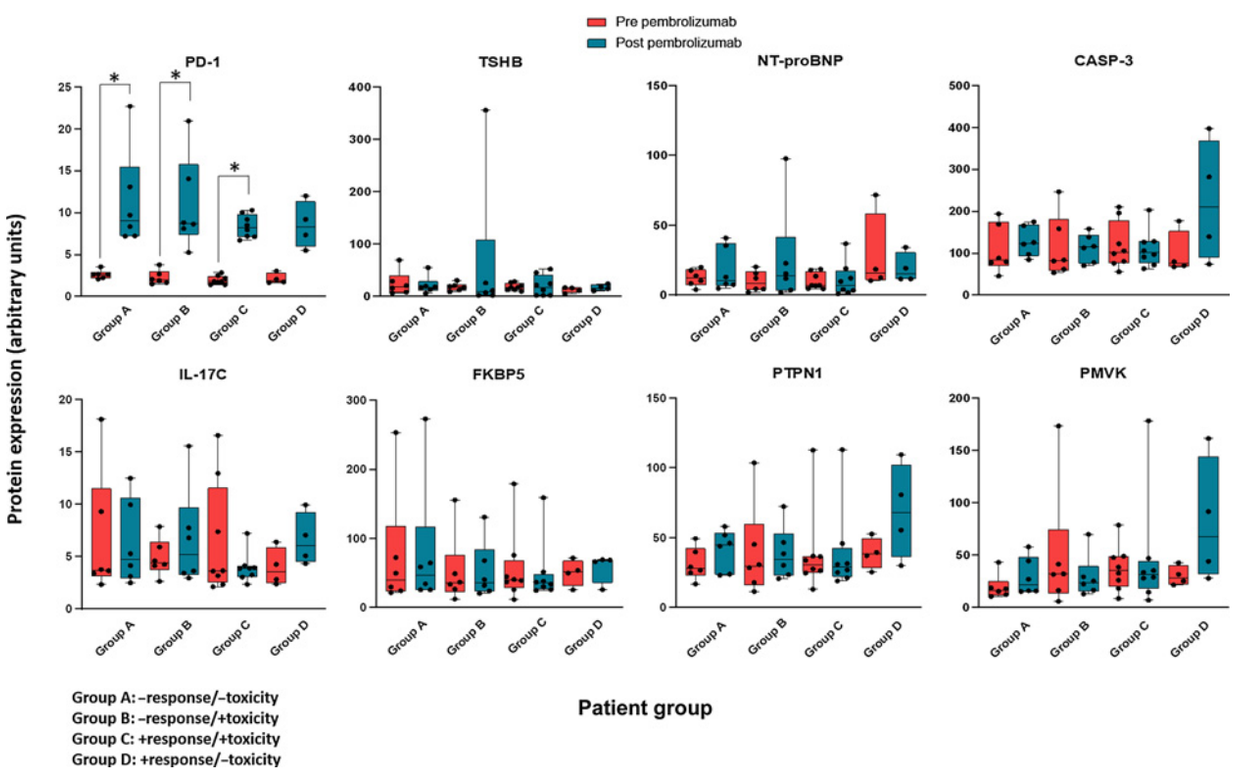

Figure 2.
Pre- and post-pembrolizumab serum protein expression values (arbitrary units) of patients within each patient group for each candidate protein marker identified in this study. Data are shown on a box-and-whisker plot from minimum to maximum with all points shown. For more details about the patient groups and discussion, see text. *, $P < 0.05$ by a paired-sample Wilcoxon test.

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Table 2. Association of candidate protein markers with response and development of irAEs after pembrolizumab treatment[a]

Protein	Group A ($n = 6$) No response No toxicity	Group B ($n = 6$) No response + toxicity	Group C ($n = 8$) +response + toxicity	Group D ($n = 4$) + response No toxicity
PD-1	Increased in 2 patients	Increased in 5 patients	Increased in 4 patients	Increased in 2 patients
TSHB		Decreased in 3 patients Increased in 1 patient	Decreased in 3 patients	
NT-proBNP		Increased in 1 patient	Decreased in 2 patients Increased in 1 patient	Decreased in 1 patient
CASP-3				Increased in 2 patients
Il-17C			Decreased in 2 patients	Increased in 1 patient
PMVK		Increased in 2 patients Decreased in 1 patient	Decreased in 1 patient	Increased in 1 patient
PTPN1		Increased in 2 patients	Decreased in 1 patient	
FKBP5		Increased in 2 patients Decreased in 1 patient	Decreased in 1 patient	

[a]Increased or decreased is defined as a 4-fold or higher concentration change in the post treatment sample, as compared with the pretreatment sample. For more details see text.

the number of pembrolizumab infusions and the change in PD-1 serum levels due to treatment. This finding can also be due to the small sample size, and a larger data set would enable a better evaluation of this effect.

TSHB is one of two subunits of TSH and it is a biomarker of thyroid disease (23). In our study, we found TSHB decreased in 6 patients with irAEs, and 3 of these patients also showed response to treatment. One patient with irAEs had a TSHB increase of 19-fold. Six of 7 patients with a significant change in TSHB developed hypothyroidism after pembrolizumab treatment. It is known that ICB can produce hypothyroidism incident rates of 3.8% to 13.2%, and pembrolizumab may lead to hypothyroidism in 7.9% of patients (24). The notion that a decrease in TSHB may predict hypothyroidism in patients receiving pembrolizumab merits further investigation. However, in these patients, the dimeric form of TSH (alpha and beta subunit complex), measured with a different assay in the clinic (Alinity i TSH Reagent Kit, Abbott Diagnostics), was increased (not decreased) as per the routine INSPIRE clinical tests. Based on these data, we speculate that pembrolizumab treatment may cause disturbances in the pituitary–thyroid axis that affect both the dimeric and monomeric forms of TSH in serum.

IL17C is a cytokine belonging to the IL17 family that is induced in epithelia following inflammatory stimuli and bacterial challenge (25). IL17C is known to be specifically upregulated in colon cancers (26). In a study of 65 patients receiving treatment with ICB and 13 healthy controls, patients who developed irAEs from treatment had lower levels of CXCL9, CXCL10, CXCL11, and CXCL19 at baseline and showed larger increases in CXCL9 and CXCL10 levels at posttreatment compared with patients who did not develop irAEs (27). Although increases in certain cytokines have shown promise as predictors of irAEs from ICB, our study did not observe this increase. NT-proBNP is a protein secreted by cardiac ventricles and is an established marker of cardiac failure (28). None of the patients in our study developed myocarditis. One study found that patients with lung cancer were 7 times more likely to have elevated NT-proBNP and suggested that it may be a diagnostic biomarker of lung cancer (29). A study of metastatic renal cell carcinoma patients treated with sunitinib showed that patients with disease progression had statistically higher plasma levels of NT-proBNP than patients who had clinical benefit (30). The patients with disease progression had a 3-fold increase in plasma NT-proBNP levels, whereas patients with clinical benefit displayed stable levels of the protein. The finding that serum NT-proBNP decreases in some responders to pembrolizumab requires validation with other data sets. Studies have also shown that the activation of CASP-3 is a requirement for apoptosis induction in response to chemotherapeutic drugs such as taxanes and doxorubicin (31). A study evaluated CASP-3 levels in the serum of 60 patients with locally advanced and metastatic breast cancer and found that the 10 complete responders to chemotherapy showed a 1.7-3-fold increase in serum CASP-3, 24 hours after the completion of the first cycle of treatment. An increase in serum CASP-3 following immunotherapy may be a predictor of response, but further validation is necessary. PMVK is an enzyme that catalyzes an essential step in the mevalonate pathway (32), whereas FKBP5 plays a role in the regulation of multiple signaling pathways and has been implicated in both tumorigenesis and chemoresistance (33). PTPN1 is a protein that has been shown to be involved in growth factor signaling as well as being a tumor suppressor (34). Further validation of these proteins in larger data sets is required to determine any significant association with response and/or toxicity to pembrolizumab.

Our study has several limitations. First, the change in some protein levels after pembrolizumab exposure may represent a pharmacodynamic effect. Our sample size is too small to draw more definitive conclusions. This study is a pilot project of 24 patients that balances the high cost of this analysis with the limited patient sera available. Hence, we specifically chose an even distribution of response and toxicity events, and a diversity of irAEs. The large number of proteins screened (>1,000) increases the risk of false-positive signals. Future large-scale prospective biomarker-driven clinical trials are necessary to validate our preliminary findings. Last but not least, the cutoffs used here are arbitrary (although the 4-fold change has been used in another ICB predictive biomarker study; ref. 35) and could exclude potentially useful biomarkers with smaller changes between pre- and post-therapy samples.

Conclusions

We demonstrate that highly multiplexed assays for measuring thousands of serum proteins simultaneously may have utility in identifying biomarkers of response and toxicity in patients receiving immune-checkpoint inhibitors such as pembrolizumab. The biological mechanisms underpinning our results are largely unclear and merit further investigation. We suggest that the candidate biomarkers identified in this study warrant inclusion to larger prospective cohorts with a sufficient sample size to provide more conclusive results.

Disclosure of Potential Conflicts of Interest

Eleftherios P Diamandis is Biochemist-in-Chief at University Health Network and is an unpaid consultant/advisory board member for Abbott Diagnostics. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M. Music, A. Soosaipillai, I. Prassas, E.P. Diamandis
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Music, A.H. Ren, I. Prassas, E.P. Diamandis
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Music, M.A.J. Iafolla, A.H. Ren, A. Soosaipillai, E.P. Diamandis
Writing, review, and/or revision of the manuscript: M. Music, M.A.J. Iafolla, I. Prassas, E.P. Diamandis
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Music, A. Soosaipillai
Study supervision: E.P. Diamandis

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Molecular Cancer Therapeutics

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