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Phase Ib study of patients with metastatic castrate-resistant prostate cancer treated with different sequencing regimens of atezolizumab and sipuleucel-T

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ABSTRACT

Background Combining an immune checkpoint inhibitor with a tumor vaccine may modulate the immune system to leverage complementary mechanisms of action that lead to sustained T-cell activation and a potent prolonged immunotherapeutic response in metastatic castration resistant prostate cancer (mCRPC).

Methods Subjects with asymptomatic or minimally symptomatic mCRPC were randomly assigned in a 1:1 ratio to receive either atezolizumab followed by sipuleucel-T (Arm 1) or sipuleucel-T followed by atezolizumab (Arm 2). The primary endpoint was safety, while secondary endpoints included preliminary clinical activity such as objective tumor response and systemic immune responses that could identify key molecular and immunological changes associated with sequential administration of atezolizumab and sipuleucel-T.

Results A total of 37 subjects were enrolled. The median age was 75.0 years, median prostate specific antigen (PSA) was 21.9 ng/mL, and subjects had a median number of three prior treatments. Most subjects (83.8%) had at least one treatment-related adverse event. There were no grade 4 or 5 toxicities attributed to either study drug. Immune-related adverse events and infusion reactions occurred in 13.5% of subjects, and all of which were grade 1 or 2. Of 23 subjects with Response Evaluation Criteria in Solid Tumors measurable disease, only one subject in Arm 2 had a partial response (PR) and four subjects overall had stable disease (SD) at 6 months reflecting an objective response rate of 4.3% and a disease control rate of 21.7%. T-cell receptor diversity was higher in subjects with a response, including SD. Immune response to three novel putative antigens (SIK3, KDM1A/LSD1, and PIK3R6) appeared to increase with treatment.

Conclusions Overall, regardless of the order in which they were administered, the combination of atezolizumab with sipuleucel-T appears to be safe and well tolerated with a comparable safety profile to each agent administered as monotherapy. Correlative immune studies may suggest the combination to be beneficial; however, further studies are needed.

Trial registration number NCT03024216.

INTRODUCTION

Prostate cancer is the most common non-cutaneous malignancy in North American men accounting for 29% of new cancer cases. More importantly, metastatic prostate cancer is the third-leading cancer-related cause of death in the USA.[1] Up to 80% of patients with metastatic prostate cancer demonstrate objective and symptomatic responses with androgen deprivation.[2] However, castration-resistant prostate cancer (CRPC) inevitably develops,[3–5] and at this stage, median overall survival (OS) is approximately 3.5 years.[6] Thus, additional treatment strategies are needed.

In the past decade, six drugs with different mechanisms of action have been shown to prolong OS in patients with CRPC. These include the tubulin targeting chemotherapy cabazitaxel,[7] the immunotherapy sipuleucel-T,[8] the androgen biosynthesis inhibitor abiraterone,[9] the second generation androgen receptor antagonists enzalutamide, apalutamide and darolutamide,[10–12] the alpha-emitting radiopharmaceutical radium-223,[13] and the PARP inhibitors olaparib and rucaparib in cancers expressing *BRCA1, BRCA2* or *ATM*.[14 15] However, these treatments only extend survival by a few months, and patients with metastatic CRPC (mCRPC) still have a poor prognosis and are in need of treatments that provide a durable benefit.

It is known that sipuleucel-T prolongs survival in patients with mCRPC previously treated with chemotherapy. The first

randomized, placebo-controlled, phase III trial for sipuleucel-T (D9901) enrolled 127 men with asymptomatic mCRPC randomly assigned at a 2:1 ratio of treatment versus control. Median OS was improved by 4 months compared with placebo (25.9 vs 21.4 months; p=0.01).[16] Subsequent real-world studies, IMPACT study, and the PROCEED registry have confirmed and extended the finding that sipuleucel-T is beneficial in chemotherapy naïve mCRPC.[8 17–19]

Immune checkpoint inhibitors have demonstrated some activity in prostate cancer, but not enough as a monotherapy to warrant approval for the treatment of mCRPC. Ipilimumab, an immune checkpoint inhibitor targeting CTLA-4, demonstrated activity when administered in combination with radiotherapy in patients with mCRPC who had disease progression after docetaxel.[20] However, in phase III trials, ipilimumab failed to prolong OS in an unselected patient population. Furthermore, a phase II study investigating pembrolizumab monotherapy, an immune checkpoint inhibitor targeting the programmed death-1 (PD-1) protein, demonstrated antitumor activity in <10% of men with mCRPC previously treated with docetaxel and androgen targeted therapy, with an acceptable safety profile.[21] Similarly, a phase III study investigating atezolizumab, an immune checkpoint inhibitor targeting programmed death-ligand 1 (PD-L1), in combination with enzalutamide did not show an improvement in OS compared with enzalutamide alone, leading to the early termination of the study.[22] Though generally considered to be an immunologically "cold" tumor, mCRPC exhibits distinct immune cell infiltrates, with variable immune cell density[23] and cellular phenotypes[24] depending on genomic alterations such as PTEN loss and TP53 mutation. However, markers predicting benefit to immune checkpoint inhibitor therapy, such as PD-L1 expression and tumor mutational burden, are typically low.[25–28] Atezolizumab, which targets human PD-L1, has been shown to have antitumor activity in a wide variety of cancers including urothelial cancer, lung cancer, triple negative breast cancer, hepatocellular carcinoma, and melanoma. It is currently being investigated both as a monotherapy and in combination with other immunotherapies in other types of malignancies.

Combining sipuleucel-T with atezolizumab was conceived as a rational strategy that could show augmented antitumor activity in mCRPC as compared with each agent as monotherapy. Sipuleucel-T has been shown to attract immune infiltration in the neoadjuvant setting.[29] We hypothesized that the activity of the immune cells induced by sipuleucel-T could be negatively regulated by immune checkpoints; therefore, adding an immune checkpoint inhibitor could augment the objective response observed with sipuleucel-T. However, the optimal sequence for this combination and the safety was still unknown. In this context, we performed a randomized phase Ib study comparing the sequential administration of atezolizumab followed by sipuleucel-T to sipuleucel-T followed by atezolizumab in patients with asymptomatic or minimally symptomatic mCRPC. Immune activation was evaluated using general immune function assays (T-cell receptor (TCR) clonality and cytokine levels) and also sipuleucel-T product parameters including CD54 upregulation, cumulative final product CD54+ cell numbers, and cumulative total nucleated cell counts, since these have been associated with OS.[30]

METHODS

Study design and subjects

This was an open-labeled randomized, dual-arm, phase Ib study assessing the safety and preliminary efficacy of sequential administration of atezolizumab and sipuleucel-T in subjects with asymptomatic or minimally symptomatic mCRPC. Subjects were randomly assigned in a 1:1 ratio to receive either atezolizumab followed by sipuleucel-T (Arm 1) or sipuleucel-T followed by atezolizumab (Arm 2). The primary endpoint was safety and tolerability of the combination treatment. Preliminary efficacy was a secondary objective. Correlative studies investigating changes in immune activation in response to treatment including characterization of molecular changes, immunogenicity profile, immune responses, and cytokine profile were also objectives in this trial.

Men with confirmed metastatic adenocarcinoma of the prostate and castrate levels of testosterone with sequentially rising prostate specific antigen (PSA) levels were eligible for this study provided they met the definition of asymptomatic or minimally symptomatic (ie, no use of opiate analgesia for cancer-related pain as identified on a patient reported brief pain inventory). Eligible subjects had to meet the following criteria: serum creatinine <1.5× the upper limit of normal (ULN), total bilirubin <1.5× ULN and serum aspartate aminotransferase <2.5× ULN, white blood cells ≥2500/µL, an absolute neutrophil of ≥1500, and platelets >100,000). Subjects were excluded if they had received prior immune therapy or were simultaneously undergoing treatment with any chemotherapy, radiotherapy, immunotherapy, or hormonal therapy besides medical or surgical castration. Subjects with any history of autoimmune diseases, clinically significant cardiac or pulmonary disease, uncontrolled infection, diseases of the central nervous system, active secondary malignancy, HIV, or hepatitis were excluded. Subjects currently receiving chronic corticosteroids, whose dose cannot be decreased to 10 mg or less of prednisone equivalent, were excluded from this study. All subjects signed an Institutional Review Board (IRB) approved informed consent.

Treatments and assessments

Treatment in both arms consisted of an induction phase lasting 12 weeks followed by a maintenance phase beginning at week 13 and continuing until subjects experienced disease progression, unacceptable toxicity, or loss of clinical benefit. Subjects in Arm 1 received treatment in the induction phase as follows: atezolizumab 1200 mg

by intravenous infusion on weeks 1 and 4 followed by sipuleucel-T administered intravenously on weeks 6, 8, and 10. Subjects in Arm 2 received induction treatment as follows: sipuleucel-T administered intravenously on weeks 1, 3, and 5 followed by atezolizumab 1200 mg intravenously on weeks 7 and 10. Subjects with an objective response, defined as either a complete response (CR) or partial response (PR), or stable disease (SD) at the end of week 12 could receive atezolizumab 1200 mg intravenously every 3 weeks until disease progression or a loss of clinical benefit occurred (maintenance phase). Subjects were categorized into one of three Halabi risk groups (Low, Group 1; Intermediate, Group 2; High, Group 3) based on the following variables: Eastern Cooperative Oncology Group (ECOG) performance status (0, 1, or 2), baseline PSA, lactate dehydrogenase, alkaline phosphatase, albumin, hemoglobin, presence of bone metastases, and presence of lymph node metastasis.[31] Once categorized into one of the three risk groups, subjects were stratified and randomized using a block design. Subjects who discontinued or withdrew from the induction phase of the study were replaced.

Samples for hematology, serum chemistries, coagulation, and urinalysis were obtained prior to each infusion. Furthermore, these blood-based analyses along with serum PSA were evaluated at week 12 and subsequently every 12 weeks thereafter until disease progression.

Sipuleucel-T infusions were prepared from peripheral blood mononuclear cells (PBMCs) as previously reported.[8] The dose level of atezolizumab in this study was 1200 mg (equivalent to an average body weight–based dose of 15 mg/kg) administered by intravenous infusion over 30 (±10) min every 3 weeks (±2 days). The initial dose of atezolizumab was delivered over 60 (±15) min.

Response

Tumor assessments were conducted at baseline, every 12 weeks thereafter, and at the end of treatment by CT and bone scanning. Efficacy outcome measures included radiographic progression-free survival (rPFS), objective response rate (ORR), and disease control rate (DCR) by Prostate Cancer Working Group 3-modified Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1,[32] and OS. PSA levels were not used to determine disease progression or to trigger radiographic evaluations. At the conclusion of the study, a blinded, independent radiological review was used to confirm the time to objective disease progression.

Adverse events

All treatment-emergent adverse events were reported until the time of objective disease progression. Thereafter, only events that were determined by the investigators to be at least possibly related to sipuleucel-T and/or atezolizumab were reported. Monitoring for treatment-emergent adverse events (AEs) and survival occurred at 2 and 6 months after disease progression and at intervals of 6 months or less thereafter. All subjects who had undergone at least one leukapheresis procedure or atezolizumab infusion were included in the safety population. Adverse events and laboratory values were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events, V.4.0. Immune-related AEs (irAEs) were defined as per previous report.[33] Multiple occurrences of specific events were counted once per patient; the event with the greatest severity was summarized. Additional anticancer interventions and causes of death were collected for all subjects. The cut-off date for data presented here was October 15, 2020.

Immunohistochemical staining

A diagnostic antihuman PD-L1 monoclonal antibody (Clone 22C3, Agilent Technologies/Dako, Carpinteria, California, USA) was used to detect PD-L1 expression on formalin-fixed, paraffin-embedded tumor tissue by immunohistochemistry (IHC), as previously described.[34] PD-L1 positivity was defined as a combined positive score (CPS) of >10. The CPS was calculated by: (1) For an area of 200 tumor cells, count the number of PD-L1 positive cells (tumor cells, lymphocytes, and macrophages) and divide by 200 and then multiply by 100 to generate a score for this 200 tumor cell area. (2) Repeat this for a total of four 200 tumor cell areas. (3) Calculate the average of the scores from these four areas to calculate the final CPS. A board certified pathologist (OTMC) reviewed all slides.

Correlative immune testing

Correlative testing was performed to assess immune response associated with study drug administration. Whole blood samples were collected at baseline and weeks 12, 16, 20, 32, 45, and 58 (Arm 1) or weeks 7, 11, 15, 27, 40, and 53 (Arm 2). Immune assays, as detailed below, were performed at Dendreon (Seal Beach, California, USA).

T-cell responses

Antigen-specific memory T-cell responses were evaluated by an interferon (IFN)-γ enzyme-linked immunospot (ELISpot) assay. PVDF ELISpot plates (Millipore, Burlington, Massachusetts, USA) were coated with an anti-IFN-γ antibody (clone D1K, MabTech, Cincinnati, Ohio, USA) overnight, then plates were blocked and rinsed with phosphate-buffered saline (PBS)/Tween. Cryopreserved PBMCs were defrosted and rested overnight in media then aliquoted at 3×10^5 PBMC/well in a volume of 200 µL/well with media alone or with media containing antigen (PA2024: PAP-GMCSF fusion protein, PAP, or CEFT peptide pool (control)) in triplicate. Plates were incubated for 40–48 hours then washed and incubated with Streptavidin conjugated anti-IFN-γ antibody (clone B6-1, MabTech). After incubation, plates were rinsed with PBS/Tween and incubated with biotin conjugated with alkaline peroxidase for another hour. Afterwards, the plates were rinsed with PBS/Tween and incubated with BCIP (5-bromo-4-chloro-3-indolyl phosphate) to visualize IFN-γ secreting cells. ELISpot data are depicted with the

median of triplicates minus background (PBMCs incubated with media).

T-cell proliferation

Sipuleucel-T infusions are associated with a T cell proliferative response to PA2024 and prostatic acid phosphatase (PAP). Thus, antigen-specific T-cell proliferation to PA2024, PAP, and phytohaemagglutinin (PHA, control) were tested via a tritiated thymidine incorporation assay deploying 96-well plates. Cryopreserved PBMCs were defrosted and rested overnight in media then plated at 1×10^5 PBMC/well in a total volume of 200 µL/well with either media alone or with media containing antigen (PA2024: PAP-GMCSF fusion protein, PAP, or PHA) in triplicate. Plates were incubated for a total of 5 days, then the wells were pulsed with 0.5 µCi of ^{3}H-thymidine overnight. The amount of ^{3}H-thymidine incorporated into the cell was quantified by a γ-radiation counter with the degree of proliferation (ie, stimulation index (SI)) being defined as the amount of ^{3}H-thymidine incorporation divided by ^{3}H-thymidine incorporation with media alone.

Humoral response

Antibody responses against PAP and PA2024 were assessed by ELISA. First, 96-well plates were coated overnight with either PAP, PA2024, or Tetanus (control). Subsequently, plates were blocked with PBS/casein and rinsed with PBS/Tween. Serially diluted serum was then aliquoted in duplicate to each set of plates and incubated at room temperature for 2 hours followed by rinsing the plates with PBS/Tween and incubated with a mixture of anti-IgG and anti-IgM antibodies (Jackson Immunoresearch, West Grove, Pennsylvania, USA) for 1 hour. Plates were then rinsed with PBS/Tween and incubated for another 1 hour with horseradish peroxidase (HRP)-conjugated anti-IgM and anti-IgG. Afterwards, plates were rinsed with PBS/Tween and O-phenylenediamine dihydrochloride (Sigma, St. Louis, Missouri, USA) for 15 min. Next, the developing reaction was stopped with the addition of 2 N HCl (50 µL/well; Sigma, St. Louis, Missouri, USA). Plates were read on a spectrophotometer (Synergy HT, BioTEK, Winooski, Vermont, USA) at 492 nm and the endpoint titer was calculated as being the last dilution of serum that yielded an optical density reading comparable to assay background.

Serum antibody-epitope profiling

The serum epitope repertoire analysis (SERA) assay has been previously published in detail.[35] Briefly, serum samples at a 1:25 dilution were incubated with a fully random 12-mer bacterial display peptide library with 1×10^{10} diversity and 10-fold oversampling in 96-well, deep-well plates. Cells displaying peptides bound to serum IgG antibodies were captured by magnetic separation using Protein A/G Sera-Mag SpeedBeads (GE Life Sciences, 17152104010350) and selected cells were grown overnight at 37°C. The samples were prepared for next generation sequencing by isolating plasmids from each pool of propagated cells, followed by two rounds of PCR; the first amplifying the peptide-encoding DNA, and the second adding barcodes with well-specific indices. Samples were normalized to 4 nM, pooled, and sequenced on the Illumina NextSeq500 (Illumina, San Diego, California, USA).

Protein-based immunome-wide association study (PIWAS)

Using the prostate cancer samples as cases and samples from 2514 healthy individuals as controls, we ran a PIWAS analysis against a modified human proteome.[36] PIWAS was parameterized to have a window size of 5, the number of SD approach, and the maximum peak signal. A PIWAS value was calculated per sample per antigen and the outlier sum false discovery rate as defined previously was used to prioritize antigens.[36] The reference human proteome was downloaded from Uniprot on March 20, 2020. A modified human proteome was then assembled by masking regions on the reference human proteome that may reflect atezolizumab binding signals due to high sequence similarity.

Serial sample PIWAS analysis

For a subject with multiple samples at different time points, serial sample PIWAS analysis was applied to identify antigens with significant signal increase. A PIWAS value at each time point was calculated per subject. A Z-score was then calculated based on the difference between two PIWAS values when compared with a reference null distribution indicating no biological variability. Using these Z-scores, antigens with significantly increased PIWAS values were identified with Bonferroni adjusted $p<0.05$. Significant and shared antigens were then identified among subjects.

Sum-of-PIWAS approach

As an individual PIWAS value indicates immune response per sample per antigen, a sum-of-PIWAS value for all antigens per sample was calculated to estimate the overall autoantigen immune response of extreme signals per sample. Specifically, for each sample, all PIWAS values that were 6 or greater were added to obtain a sum-of-PIWAS value.

TCR repertoire analysis

DNA was extracted from PBMCs collected at baseline and at weeks 11 or 12 using the Qiagen AllPrep DNA/RNA Micro Kit (Hilden, Germany). RNA library preparation, sequencing, and preliminary data processing of all samples was performed at Cedars-Sinai Genomics Core using the immunoSEQ platform (TCRβ survey level). The TCRβ CDR3 sequences were calculated as described previously.[37]

Serum cytokines

Serum samples collected at baseline and at week 11 or 12 were analyzed for 22 cytokines (MCP-1/CCL2, MIP-1 α /CCL3, MIP-1 β/CCL4, IP-10/CXCL10, IL-8/CXCL8, G-CSF, GM-CSF, IFN-γ, IL-10, IL-13, IL-17A, IL-1β/IL-1F2, IL-1ra/IL-1F3, IL-2, IL-4, IL-5, IL-6, CCL5/RANTES,

TNF-α, VEGF, FGF-basic, IL1α/IL-1F1) using a customized Luminex assay (Cat # FCSTM18-22 R&D Systems, Minneapolis, MN). Measurements were performed using a Luminex 200 instrument (Luminex, Austin, Texas, USA) and were analyzed using a standard curve for each molecule (xPONENT software, Luminex).

Peripheral blood mononuclear cell analysis

PBMCs were stained and analyzed by flow cytometry as previously described.[38] The following antibodies were used for cell surface staining: FITC-conjugated antihuman CD45 (Biolegend, Catalog #368508), PerCP-Cyanine5.5-conjugated antihuman CD3 (Tonbo Biosciences, Catalog #65–0037), PE-conjugated antihuman CD4 (Tonbo, Catalog #50–0048), APC-conjugated antihuman CD8 (Tonbo, Catalog # 20–0087), Brilliant Violet 421-conjugated antihuman CD279 (PD-1, Biolegend, Catalog #329920). Ghost Dye UV 450 (Tonbo) was used to assess live versus dead status of cells. Samples were acquired on a LSRII analyzer (BD Biosciences) and analyzed with FlowJo software (Treestar). For all FACS, experiments debris and dead cells were excluded from the analyzed gates.

Statistical analysis

This study was designed to obtain preliminary safety and clinical activity information in subjects with asymptomatic or mildly symptomatic mCRPC. Analyses were based on all subjects who received any amount of study treatment (safety evaluable population). The ORR and DCR with corresponding 95% CIs were calculated using the Clopper-Pearson method. The rPFS and OS were assessed using the Kaplan-Meier method, with 95% CIs for median rPFS and OS estimated using the Brookmeyer-Crowley method. While this study was not powered to detect a survival difference between the two treatment arms, there was a protocol-specified requirement to follow each patient for survival (and treatment-related AEs) for up to 12 months after randomization.

RESULTS

Subjects

From January 15, 2017 through October 30, 2019, 37 subjects were enrolled in this study (n=19 Arm 1, n=18 Arm 2). All 37 subjects were included in the safety population and thus analyzed for both safety and efficacy (online supplemental figure 1). Notably, three subjects did not complete induction treatment. These three subjects were replaced (two in Arm 1 and one in Arm 2); otherwise, all subjects received three doses of sipuleucel-T and two doses of atezolizumab. The most common reason for treatment discontinuation was disease progression. Median duration of treatment was 4.9 months (range, 0–9.9 months) for Arm 1 and 5.1 months (range, 1.4–13.7 months) for Arm 2. Baseline characteristics were generally as expected for a sipuleucel-T eligible mCRPC population (table 1). Median age for the total population was 75.0 years (range, 53–86 years), 73.0% of subjects had an ECOG performance status 0, 18.9% had received prior docetaxel-based chemotherapy, and 62.2% had received two or more previous antiandrogen therapies. Median PSA level for the total population was 21.9 ng/mL at study entry

Safety

At least one treatment-related AE was reported in 31 subjects (83.8%), including 7 (18.9%) with at least one grade 3 treatment-related AE. The most common treatment-related AEs were diarrhea, nausea, fatigue, and hypertension in Arm 1 and fatigue, pain, joint pain, platelet decrease, constipation, and anemia in Arm 2 (table 2A). irAEs, which were based on a list of common terms, occurred in 5 (13.5%) subjects with similar incidences between the two arms and all of which were grade 1 or 2 (table 2B). There were no grade 4 or five toxicities attributed to either study drug and no irAE required systemic steroid therapy.

Antitumor activity

In total, 23 subjects were evaluable for response (table 3). No patient in either arm had a CR and only one patient in Arm 2 had a PR. Three subjects (30%) in Arm 1 and one patient (7.7%) in Arm 2 had SD. Thus, for the overall population the ORR was 4.3% and the DCR was 21.7%. For the individual arms, the DCR was 30% for Arm 1 and 15.4% for Arm 2. These subjects are subsequently noted as responders. Among the 12 subjects who had at least one measurable target lesion by CT at baseline, 4 (33.3%) had a decrease from baseline in the sum of target lesions, including 1 (8.3%) with a PR and 2 (16.7%) subjects with SD (figure 1). Among the five subjects with radiographic response, one remained on treatment at data cut-off, while the remaining three had experienced subsequent disease progression (figure 2A). Overall, the median rPFS was 3.0 months (95% CI 2.8 to 5.6 months). Median rPFS was 3.3 months (95% CI 2.7 to 7.8 months) for Arm 1 and 2.9 months (95% CI 2.6 to 5.5 months) for Arm 2 (figure 2B). Median OS was not reached (NR; 95% CI 11.1 to NR) in Arm 1 and 21.4 months (95% CI 16.6 to NR) in Arm 2 (figure 2C). The estimated 12-month survival rates were 70.6% in Arm 1 and 83.3% in Arm 2. For both arms, median OS was 23.6 months (95% CI 16.6 to NR) and the estimated 12-month survival rate was 77.2%. A total of 15 subjects (40.5%) out of 37 had some decrease in PSA level, including 1 patient (5.3%) in Arm 1 and 3 subjects (16.7%) in Arm 2 who had a >50% decrease (table 3 and online supplemental figure 2). PD-L1 expression in patient samples is shown in online supplemental figure 3). Only one subject, who was classified as a non-responder in Arm 2, had CPS >10. Furthermore, non-responders in both arms had numerically higher levels of PD-L1 expression compared with responders.

Table 1 Patient characteristics

Parameters	Total (n=37) No.	%	Arm 1 (n=20) No.	%	Arm 2 (n=17) No.	%	p-value
Age, years							0.67
Median	75		75		74		
Range	53–86		53–86		55–84		
Race: white	4	10.80	2	10.00	2	11.80	0.94
Disease location							0.55
Bone only	19	51.40	9	45	10	58.80	
Soft tissue only	5	13.50	2	10	3	17.60	
Bone and soft tissue	11	29.70	7	35	4	23.50	
Visceral	2	5.40	2	10	0		
Number of bone mets							0.51
0	7	18.90	3	15	4	23.50	
1-10	17	45.90	11	55	6	35.30	
>10	13	35.10	6	30	7	41.20	
ECOG performance status							0.57
0	27	73	14	70	13	76.50	
1	9	24.3	6	30	3	17.60	
2	1	2.70	0		1	5.90	
Median PSA, ng/mL Range	21.9 0.33–636.8		20.2 2.1–636.8		26.3 0.33–529		0.71
Median alkaline phosphatase, U/L Range	76 41–741		68.5 41–321		92 46–741		0.28
Median hemoglobin, g/dL Range	13 9.8–15.3		12.9 10.2–15.3		13 9.8–14.9		0.98
Median LDH, U/L Range	181 114–677		180 114–677		181 130–229		0.32
Gleason Score							1
≤7	17	45.90	9	45	8	47.10	
≥8	15	40.50	8	40	7	41.20	
Unknown	5	13.50	3	15	2	11.80	
Patients with prior chemotherapy for mCRPC	7	18.90	3	15	4	23.50	0.68
Patients receiving docetaxel-based chemotherapy subsequent to study treatment	11	29.70	6	30	5	29.40	1
Patients with two or more previous anti-androgen therapies	23	62.20	14	70	9	52.90	0.33

Immune activity

First, we performed TCR repertoire analysis using whole blood samples from 16 subjects (n=9 Arm 1 and n=7 Arm 2) to assess the functional phenotype associated with each treatment. TCR clonotype analysis revealed that the main population consists of (T Cell Receptor Alpha Constant) TRAC and (T Cell Receptor Beta Constant) TRBC subtypes (figure 3A, online supplemental figure 4). Interestingly, the TRAC population tended to decrease and TRBC population tended to increase after the treatment in Arm 1, but not in Arm 2. In addition, we found that TCR diversity was relatively higher in the one responder in Arm 2 compared with non-responders in both arms (figure 3B).

Next, using a panel of T-cell related cytokines, we compared cytokine expression in serum pretreatment and post-treatment using specimens from 22 subjects (11 in each arm). Overall, the heatmap shows that cytokine

Table 2 (A) Summary of Treatment related AEs occurring in ≥ 5% of Patients in Either Treatment, (B) Immune-related adverse events (irAEs)

(A)

	Total	%	Arm 1 Grade1-2	%	Arm 1 Grade3-4	%	Arm 2 Grade1-2	%	Arm 2 Grade3-4	%
Any AEs	31	83.8	13	68.4	3	15.8	11	61.1	4	22.2
Fatigue	9	24.3	3	15.8	0	0	6	33.3	0	0
Diarrhea	6	16.2	4	21.1	0	0	2	11.1	0	0
Hypertension	5	13.5	3	15.8	0	0	1	5.6	1	5.6
Joint Pain	5	13.5	2	10.5	0	0	3	16.7	0	0
Nausea	5	13.5	4	21.1	0	0	1	5.6	0	0
Platelet count decreased	5	13.5	2	10.5	0	0	3	16.7	0	0
Pain	5	13.5	0	0	0	0	4	22.2	1	5.6
Anemia	4	10.8	1	5.3	0	0	2	11.1	1	5.6
Chills	4	10.8	2	10.5	0	0	2	11.1	0	0
Constipation	4	10.8	1	5.3	0	0	3	16.7	0	0
Fever	3	8.1	1	5.3	0	0	2	11.1	0	0
Hypoglycemia	3	8.1	1	5.3	0	0	2	11.1	0	0
Increased Alkaline Phosphatase	3	8.1	2	10.5	0	0	1	5.6	0	0
Shortness of breath	3	8.1	1	5.3	0	0	2	11.1	0	0
Chest pain	2	5.4	0	0	0	0	2	11.1	0	0
Dizziness	2	5.4	1	5.3	0	0	1	5.6	0	0
Flu like symptoms	2	5.4	0	0	0	0	2	11.1	0	0
Headache	2	5.4	0	0	0	0	2	11.1	0	0
Hyperglycemia	2	5.4	1	5.3	0	0	1	5.6	0	0
Hypermagnesemia	2	5.4	1	5.3	0	0	1	5.6	0	0
Hypernatremia	2	5.4	1	5.3	0	0	1	5.6	0	0
Hypotension	2	5.4	0	0	1	5.3	0	0	1	5.6
Infusion related reaction	2	5.4	1	5.3	0	0	1	5.6	0	0

(B)

	All	%	Arm 1 Grade1-2	%	Arm 1 Grade3-4	%	Arm 2 Grade1-2	%	Arm 2 Grade3-4	%
Any	5	13.5	2	10.5	0	0	3	16.7	0	0
Dermatologic	3	8.1	2	10.5	0	0	1	5.6	0	0
Endocrine	1	2.7	0	0	0	0	1	5.6	0	0
Hepatitis	1	2.7	0	0	0	0	1	5.6	0	0
Infusion related reaction	2	5.4	1	5.3	0	0	1	5.6	0	0

levels in the post-treatment responder is higher than those in baseline (figure 3C and online supplemental figure 5) compared with non-responder changes from baseline, suggesting that T-cell activation by sipuleucel-T may be associated with response.

Further analysis showed mean PA2024-specific ELISpot counts (averaged over time) were similar in both arms (52.3 spots in Arm 1 vs 45.4 spots in Arm 2; p=0.62). In both arms, PA2024-specific ELISpot counts were significantly increased compared with baseline at each postbaseline visit (Arm 1: baseline vs week 12; p=0.022 and Arm 2: baseline vs week 11; p<0.0001) (figure 3D and online supplemental table 1). When averaged across all time points, PA2024-specific T-cell proliferation responses were numerically but not statistically higher in Arm 1 compared with Arm 2 (p=0.34; figure 3D and online supplemental table 1). At all time points through week 32, PA2024-specific T-cell proliferation responses were

Table 3 Summary of responses

Variable	All	Arm-1	Arm-2	p-value
Response assessed per RECIST criteria	23	10	13	0.38
No. of patients who can be assessed by RECIST				
PR	1 (4.3%)	0	1 (7.7%)	
SD	4 (17.4%)	3 (30%)	1 (7.7%)	
Non-CR/Non-PD	5 (21.7%)	1 (10%)	4 (30.8%)	
PD	13 (56.5%)	6 (60%)	7 (53.8%)	
ORR, No (%)	1 (4.3%)	0	1 (7.7%)	1
DCR,* No (%)	5 (21.7%)	3 (30%)	2 (15.4%)	0.62
Response assessed per PCWG3	27	16	11	1
No. of patients who can be assessed by RECIST				
PR	0	0	0	
SD	10 (37%)	6 (37.5%)	4 (36.4%)	
PD	17 (63%)	10 (62.5%)	7 (63.6%)	
ORR, No (%)	0	0	0	1
DCR, No (%)	10 (37%)	6 (37.5%)	4 (36.4%)	1
rPFS	37	20	17	0.27
No. of patients who can be assessed median (95% CI)	3.0 months (2.8 to 5.6)	3.3 months (2.6 to 7.8)	2.9 months (2.6 to 5.6)	
PSA response † in patients with baseline PSA measurement No. of patients who can be assessed	30	20	17	
PSA decline 50% †	4 (10.8%)	1 (5%)	3 (17.6%)	0.32

*Define as the percentage of patients with confirmed complete or partial response or stable disease. Patients who died without evidence of disease progression before death were considered to have stable disease.
†Define as the percentage of patients with a reduction in PSA level from baseline by 50% or greater as confirmed on an additional PSA evaluation performed > 3 weeks later.
CR, complete response; DCR, disease control rate; NE, not evaluable; ORR, overall response rate; PCWG3, Prostate Cancer Working Group 3; PD, progressive disease; PR, partial response; PSA, prostate specific antigen; rPFS, radiographic progression free survival.

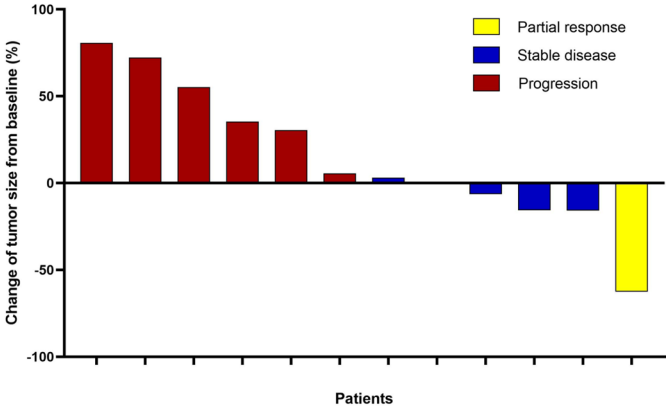

Figure 1 Waterfall plot of antitumor activity. Percentage change from baseline in the sum of longest diameter of target lesions as assessed by RECIST V.1.1 by central review. Thirteen subjects assigned to Arm 1 and 12 subjects assigned to Arm 2 were not evaluable for change from baseline in tumor size because they did not have one or more evaluable postbaseline imaging assessments or did not have any target lesions. RECIST, Response Evaluation Criteria in Solid Tumors.

significantly higher compared with baseline in both arms (Arm 1; p=0.034; figure 3D, baseline vs week 12; p=0.0097) (Arm 2; p=0.019; figure 3D, baseline vs week 11; p=0.011, baseline vs week 11; p=0.049, and baseline vs week 15; p=0.014). All subjects developed PA2024-specific T-cell responses after sipuleucel-T treatment. PA2024 antibody titers after sipuleucel-T treatment in Arm 1 and Arm 2 were 16.8 times (p=0.00066) and 12.8 times (p=0.00025) higher, respectively, on average compared with baseline, and similar between arms, remaining significantly elevated through week 32 (figure 3 and online supplemental table 1).

Exploratory analysis of PA2024 T-cell stimulation index was performed in subjects for whom cells could be processed within 24 hours of collection, thus precluding the need to freeze the cells before analysis. The median ratio of the T-cell stimulation index at 12 weeks in Arm 1 and 11 weeks in Arm 2 was 32.9 times (p=0.00033) and 24.0 times (p=0.0014) higher, respectively, on average compared with baseline (preinfusion). Increased IgG levels to secondary antigens such as PSA, PAPi, and PAPm were observed in both arms at all time points through

Figure 2

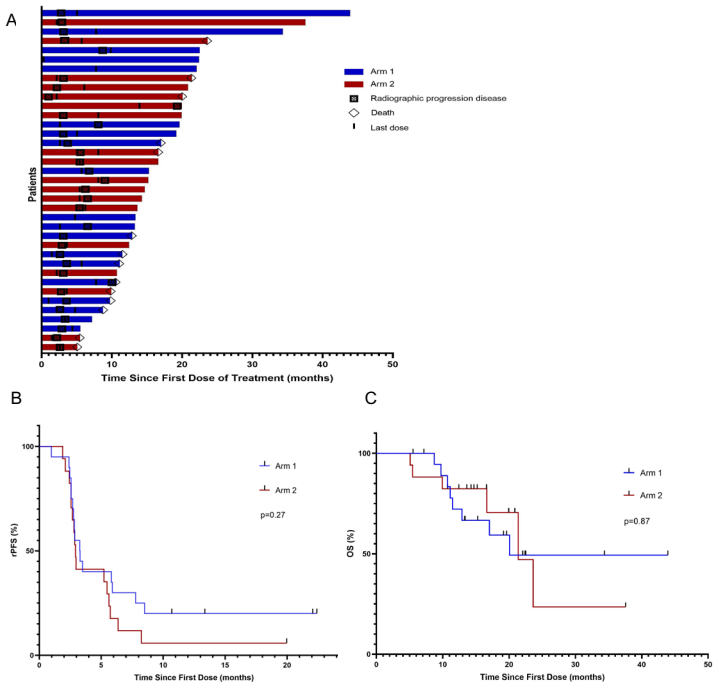

Figure 2 Antitumor activity. (A) Swimmer plot showing subjects with confirmed response as assessed by PCWG3-modified RECIST. (B) Kaplan-Meier plot of rPFS by treatment arm. (C) Kaplan-Meier plot of OS by treatment arm. OS, overall survival; PCWG3, Prostate Cancer Working Group 3; RECIST, Response Evaluation Criteria in Solid Tumors; rPFS, radiographic progression-free survival.

week 32 (online supplemental table 1); however, there was no significant difference between responders and non-responders in either arm.

In addition, to assess the T-cell population, PBMCs were collected and analyzed by flow cytometry for CD45+ (general leucocyte marker) and CD4+ and CD8+ T cells expression. The percentage of total T cells (Arm 1 29.4% vs Arm 2 30.3%, p=0.86), CD4+ T cells (Arm 1 13.2% vs Arm 2 12.5%, p=0.85), and CD8+ T cells (Arm 1 14.4% vs Arm 2 14.8%, p=0.92) were similar in both arms (representative results are shown in figure 3E). Interestingly, the percentage of total T cells was significantly higher in responders versus non-responders in Arm 1 (p=0.044) but not in Arm 2 (p=0.51). However, CD4+ or CD8+ T cells were numerically but not statistically higher in responders compared with non-responders in both arms (CD4+ T cell; p=0.72 for Arm 1 and p=0.92 for Arm 2, CD8+ T cell; p=0.15 for Arm 1 and p=0.46 for Arm 2).

The SERA assay was performed on samples from 37 subjects to assess autoantigen signal in subjects pretreatment and post-treatment in both arms. SERA uses a large random bacterial peptide display library with the PIWAS method to assess outlier antigens in samples. At baseline, a sum-of-IWAS calculation identifies a non-significant difference in the baseline number of outlier antigens in subjects with prostate cancer relative to a large cohort of healthy controls that becomes significant over time (online supplemental figure A,C). No differences were observed either at baseline or after therapy between responders and non-responders (online supplemental figure B,D). PIWAS identified 92 outlier antigens that were significantly different in responders versus. non-responders at baseline (online supplemental table 2).[39–45] Serial PIWAS analysis also identified 40 antigens that increased between baseline and week 7 from Arm 2 (online supplemental table 3) and 18 antigens that increased in responders versus non-responders in either Arm 1 or Arm 2 (online supplemental table 4). Arms were combined for this analysis given the limited numbers of responders. The trajectories of response to three putative antigens, SIK3, KDM1A/LSD1, and PIK3R6, that were shared in at least two subjects in response to sipuleucel-T at (week 7, Arm 2) and also in response to combined therapy (week 12, either Arm 1 and Arm 2) are shown in figure 4.

DISCUSSION

This is the first report of the safety, clinical activity, and immune activity associated with combination immunotherapy using sipuleucel-T and atezolizumab in subjects with mCRPC. Sipuleucel-T and atezolizumab each target the immune system by two distinct mechanisms and each, independently, has modest activity in mCRPC. The rationale for this trial is predicated on the hope that by combining the different mechanisms of action of these

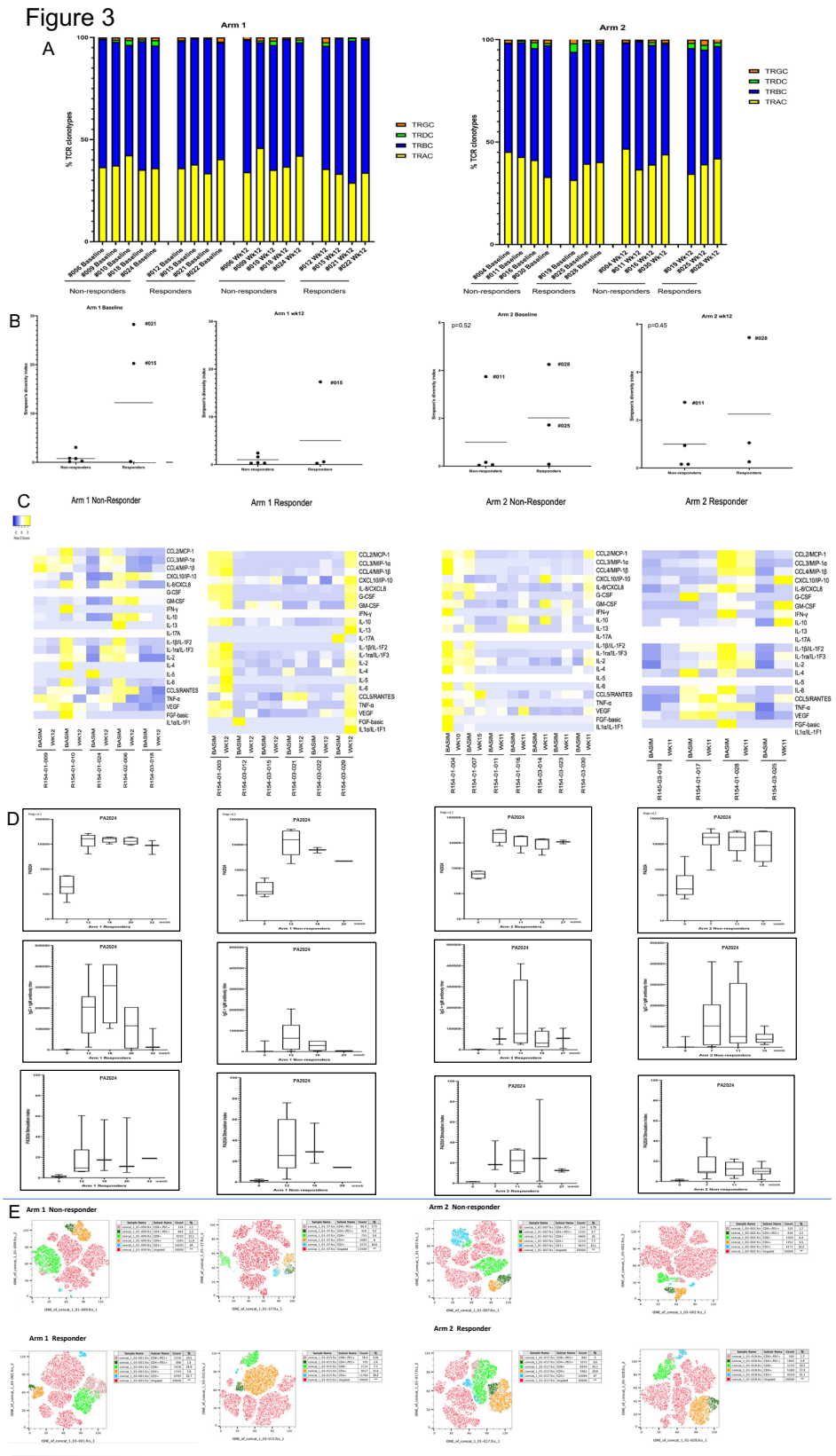

Figure 3 On study immunological landscape. (A) TCR clonotype analysis. (B) TCR DI. (C) Heatmap illustrating differences in 22 serum cytokines. The scale represents SD from the mean after a *Z*-transformation of signal values of a cytokine across all samples. *Yellow* represents a higher level of cytokine expression and *blue* a lower level, relative to the mean across all samples for each cytokine. (D) ELISpot analysis of PA2024. (E) Flow cytometry analysis of T cell population. Each central bar in the box indicates median, and the two whisker boundaries indicate the 5th and 95th percentiles. DI, diversity index; ELISpot, enzyme-linked immunospot; TCR, T-cell receptor.

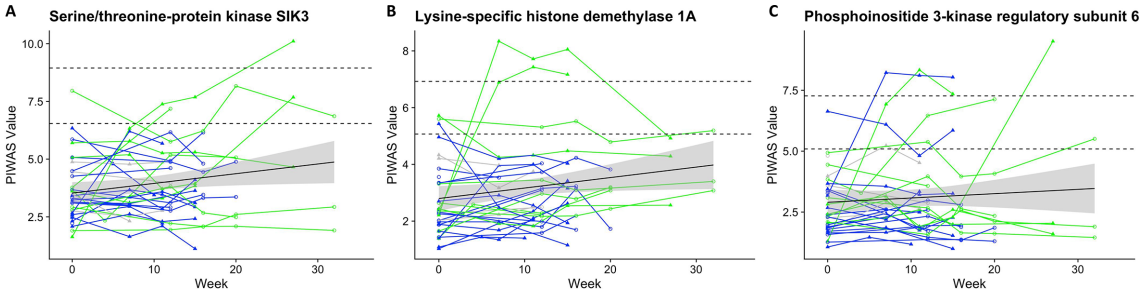

Figure 4 Candidate shared antigens among 6-month responders. (A) SIK3, (B) KDM1A/LSD1, and (C) PIK3R6 are candidate shared antigens with significantly increased signals from baseline to week 12 in at least two 6-month responders. These antigens also have increased signals from baseline to week 7 in Arm 2 subjects treated with Sipuleucel-T prior to Atezolizumab treatment. Horizontal dashed lines indicate 95th and 99th percentile of control PIWAS values. Green dots: responders, n=10; Blue dots: non-responder, n=19; gray dots: missing response information, n=5. Circle: a sample is from Arm 1; triangle: a sample is from Arm 2. Black line is trend line by linear regression using PIWAS values of all samples, and gray area indicates the 95% CI of the trend line. PIWAS, protein-based immunome-wide association study.

two agents would lead to increased anticancer activity without increased toxicity. Overall, the combination of sipuleucel-T and atezolizumab was well-tolerated in this study, regardless of which was administered first. There were no grade 4 or 5 AEs observed in this study. The most reported AE, fatigue, was observed in 24.3% of subjects overall, which was similar to a previous study using atezolizumab as monotherapy for advanced solid tumors.[46] There were no unexpected AEs and limited irAEs, and the safety profile of the present study was consistent with what has been published with each agent as monotherapy.[8 16]

During a time when there are many other treatment options, a median OS of 23.6 months was noted, which is similar to the 25.8 months previously reported by Kantoff *et al* for sipuleucel-T monotherapy.[8] Of note, in the study by Kantoff *et al*, nearly three-quarters of the subjects had a Gleason score of 7 or less, while in our study approximately only 50% had Gleason score of 7 or less. Although the observed ORR was a modest 4.3%, it is similar to that seen with immune checkpoint inhibitor monotherapy in mCRPC.[19] Notably, the responses that did occur were rather durable, and eight subjects (21.6%) had SD lasting more than 6 months. These data add to a growing body of evidence suggesting that despite its more immunosuppressive microenvironment, certain patients with mCRPC may benefit from immunotherapy. Although the combination studied did not demonstrate clear superiority over monotherapy, immune activation appeared to be greater in Arm 1.

Identification of predictive biomarkers for cancer immunotherapy has been a significant challenge. Our exploratory biomarker research assessed changes in cytokines, neoantigens, TCRs, and PBMCs. Exploratory biomarker analysis hinted at a relationship between response and a higher TCR diversity, and an increased putative immune response to three antigens, SIK3, KDM1A/LSD1, and PIK3R6. Furthermore, outcomes were not dependent on PD-L1 status (online supplemental figure 5). TCR diversity has been associated with clinical benefit in immune therapy for cancer.[47–49] In the present study, we can also confirm the trend of increased TCR diversity. Interestingly, the patient with the highest TCR diversity in Arm 2 (patient #028) had radiological SD at 15 months and a dramatic reduction in PSA levels (96.8% reduction). Note that serum PSA at diagnosis was 236 ng/dL, serum PSA at study entry was 12.3 ng/dL, and bony metastatic disease was noted in spine and pelvic girdle.

Furthermore, we observed a difference in autoantigen signal at baseline between subjects with cancer and healthy controls that increased over time. The difference is most notable in non-responders and is likely due to an increase in tumor burden and tumor antigens as has been previously described for known tumor antigens.[50] A limited number of autoantigens that are shared between at least two subjects increased in response to sipuleucel-T, and a limited number of these are also increased at 6 months in responders versus non-responders. Interestingly, all three antigens are members of cell-signaling kinase pathways associated with tumorigenesis,[51–53] and it could be postulated that an increase in humoral response against these antigens might lead to decreased oncogenic signaling. Exploration of the significance and prevalence of humoral signaling against these antigens should thus be considered with future studies.

In summary, this study suggests that the combination of sipuleucel-T and atezolizumab is safe and well tolerated with a comparable safety profile to each agent administered as monotherapy. Furthermore, correlative immune studies suggest that the combination may be beneficial; nevertheless, objective responses were rare. Better predictive biomarkers to select patients who may respond and additional combination strategies are needed for immunotherapy to make a greater impact on mCRPC.

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