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Randomized phase II trial of sipuleucel-T immunotherapy preceded by sensitizing radiation therapy and sipuleucel-T alone in patients with metastatic castrate resistant prostate cancer☆

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A B S T R A C T

Background: Sipuleucel-T is an autologous cellular immunotherapy indicated for patients with asymptomatic or minimally symptomatic metastatic castration resistant prostate cancer (mCRPC). Since radiation therapy (RT) can suppress bone marrow function and immune responses, previous studies evaluating sipuleucel-T excluded patients who received RT less than or equal to 28 days prior to sipuleucel-T therapy. Recent evidence suggests that RT may act synergistically with immunotherapy to enhance and broaden antitumor immune response.

Methods: Patients who met standard criteria for sipuleucel-T were randomized to receive sipuleucel-T alone (Arm A) or sipuleucel-T initiated 1 week after completing sensitizing RT to single metastatic site (Arm B). RT was delivered at 300cGy/day to 3000 cGy total. The primary endpoint was the ability to safely combine sipuleucel-T preceded by RT and generate sipuleucel-T with adequate product immune activation parameters. Secondary endpoints included the measurement of systemic immune responses to prostatic acid phosphatase (PAP), a target for sipuleucel-T immune therapy and PA20204 (recombinant fusion protein utilized in the generation of sipuleucel-T).

Results: 51 pts were enrolled, 2 did not receive any sipuleucel-T because of vascular access problems and were excluded. 24 were treated on Arm A, 25 on Arm B. 47/49 patients received all 3 sipuleucel-T infusions. Median age was 66 yrs (range 45–90). Sipuleucel-T product parameters including: total nucleated cell (TNC) count, antigen presenting cell (APC) count were similar in both groups. Cumulative APC upregulation was higher in Arm A. 1 patient in Arm A demonstrated PSA response. Median progression free survival (PFS) was 2.46 months on Arm A and 3.65 months on Arm B ($p = 0.06$). Both arms showed similar increases in humoral responses to PA2024 and PAP. IFN-γ ELISPOT T-cell activation responses to PA20204 were observed in both arms, but were more robust in the Arm A ($p = 0.028$). Both arms were well-tolerated, with fatigue as the most common grade 2 adverse event (1 patient in Arm A and 3 patients in Arm B).

Conclusions: Sensitizing RT completed 1 week before generation of sipuleucel-T did not affect the majority of product parameters and the ability to deliver sipuleucel-T therapy. RT did not enhance the humoral and cellular responses associated with sipuleucel-T therapy.

Background

Prostate cancer is the most common noncutatenous malignancy and the second leading cause of cancer-related death in men in the United States [1]. Androgen deprivation therapy is effective in the initial management of advanced prostate cancer but eventually cancer evolves into the aggressive phenotype referred to as castration resistant prostate cancer (CRPC) which is usually fatal within 12–36 months [2]. Sipuleucel-T is an active cellular immunotherapy consisting of autologous, peripheral blood mononuclear cells (PBMC's), including antigen presenting cells (APC's) that have been activated in vitro with a recombinant fusion protein (PA2024). PA2024 consists of a prostate antigen, prostatic acid phosphatase (PAP) fused to granulocyte-macrophage colony-stimulating factor (GMCSF), an immune cell activator [3]. Sipuleucel-T was evaluated in a randomized placebo-controlled Phase 3 Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT) trial in patients with asymptomatic and minimally symptomatic metastatic CRPC. The results of that trial demonstrated statistically significant median survival advantage of 4.1 months (25.8 months in the sipuleucel-T group vs 21.7 month in placebo group) leading to FDA approval [4]. Peripheral blood immune responses and cumulative product parameters reflected by APC number, APC activation (CD54 upregulation) and total nucleated cell number correlated with overall survival in patients treated with sipuleucel-T [5]. Radiotherapy has

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traditionally been thought of as immunosuppressive and therefore not appropriate for combination with treatments that activate the immune system. In fact the eligibility for the IMPACT trial stipulated at least 28 day interval between external beam radiation and sipuleucel-T therapy. However, a growing body of evidence suggests that effects of ionizing radiation on immune function are complex and in some aspects can be immunostimulatory [6,7]. Radiation can synergize with immunotherapy by broadening the immune repertoire in T cells (vaccination effect), by attracting T cells to the irradiated site (homing effect) and by rendering irradiated cells more vulnerable towards T-cell-mediated cell kill (vulnerability effect) [7]. Radiation can induce a proinflammatory microenvironment within tumors that provide antigen presenting cells (APCs) with maturation-inducing stimuli [8]. Cell death caused by irradiation results in exposure of the lymphatic system to tumor antigens through necrosis or apoptosis of tumor cells and the subsequent release of cellular debris [9]. Irradiation of APCs has been shown to enhance antigenic peptide presentation [10]. Radiation therapy can potentially enhance the effect of immunotherapy and induce tumor regression at sites distant from the primary site of radiotherapy (so called abscopal effect) [11,12]. The abscopal effect involving immune response has been reported in cases of metastatic melanoma treated with immune checkpoint inhibitor ipilimumab and localized radiotherapy [13,14]. Various radiation doses and schedules (stereotactic versus conventional) have been proposed to induce maximum immunostimulatory effect, but so far there is no clinical evidence of an advantage for a particular radiation regimen [7].

Based on the hypothesis of potential immune-sensitizing effect of radiotherapy we performed a multi-institution randomized phase II clinical trial of sipuleucel-T versus sipuleucel-T preceded by radiation therapy to single site of metastatic disease in patients with metastatic castration resistant prostate cancer.

Methods

Patients

Eligible men had asymptomatic or mildly symptomatic metastatic castration-resistant prostate cancer, with at least one metastasis amenable to external beam radiation therapy. Additional eligibility criteria included ECOG PS ≤ 2, no history of chemotherapy within last 3 months, no prior radiation therapy for metastatic disease, no systemic steroids within last 28 days, no liver or brain metastases.

Randomization and treatment

Patients were randomly assigned in a 1:1 ratio to receive either sipuleucel-T alone (Arm A) or sipuleucel-T preceded by a course of external beam radiation therapy (EBRT) to single metastatic site (Arm B). Radiation therapy was given in 10 fractions of 300 cGy for a total dose of 3000 cGy. The radiation target was chosen based on safety and the presence or absence of symptoms. Eligibilty criteria allowed for the presence of mildly symptomatic lesions and those were preferentially selected for EBRT. For patients assigned to EBRT the radiation therapy was completed 7 days prior to 1st leukapheresis. Patients were scheduled for three leukapheresis procedures (at weeks 0, 2 and 4), each followed approximately 3 days later by infusion of sipuleucel-T. Sipuleucel-T was prepared in a standard fashion at a central manufacturing facility by culturing antigen presenting cells (APCs) for 36–44 at 37 °C with media containing PA2024. Each dose of sipuleucel-T contained a minimum of 50 million activated CD54+ cells, (increased expression of CD54 is a surrogate marker of APC activation) [5]. After premedication with acetaminophen and an antihistamine, patients received sipuleucel-T intravenously over a period of approximately 60 min and were then observed for at least 30 min.

Table 1
Patient characteristics.

Patient characteristic	Sip-T (Arm A) N = 24[a]	Sip-T + RT (Arm B) N = 25[a]
Age	64 (45–90)	67 (54–81)
PSA Median (Range)	20.35 (0.38–130.87)	13.05 (0.42–186)
Gleason score Median (Range)	8 (6–10)	7 (7–9)
Metastatic site		
Bones only	11 (46%)	17 (68%)
Bones and Lung	2 (8%)	1 (4%)
Bones and Lymph Nodes	7 (29%)	3 (12%)
Lungs	1 (4%)	0 (0%)
Other	3 (13%)	4 (16%)

[a] 1 pt in each arm did not receive any sip-T because of IV access and were excluded from analysis.

Table 2
Adverse events.

Adverse event	Sip-T (Arm A) N = 24[a]		Sip-T + RT (Arm B) N = 25[a]	
	Grade 2	Grade 3	Grade 2	Grade 3
Chills	3 (13%)	0	0	0
Fatigue	1 (4%)	0	2 (8%)	0
Infusion Reaction	1 (4%)	0	1 (4%)	0
Decreased Lymphocyte Count	1 (4%)	0	0	0
Nausea	0	0	1 (4%)	0
Pain in Extremity	0	0	1 (4%)	0
Anemia	0	0	1 (4%)	1 (4%)
Anorexia	0	0	1 (4%)	0
Headache	1 (4%)	0	1 (4%)	0
Hypertension	0	0	3 (12%)	0

[a] 1 pt in each arm did not receive any sip-T because of IV access and were excluded from analysis.

Table 3
Treatment summary.

	Sip-T (Arm A) N = 24[a]	Sip-T + RT (Arm B) N = 25[a]
Completed all 3 infusions	24 (100%)	23 (92%)
PSA response rate (>50% decline)	1 (4%)	0
Best Radiographic Response Rate		
SD (Stable Disease)	7 (29%)	8 (32%)
PD (Progressive Disease)	12 (50%)	12 (48%)
Site of RT		
Rib/Sternum/Clavicle	NA	8 (32%)
Vertebral column	NA	7 (28%)
Long bone	NA	3 (12%)
Pelvic bone	NA	3 (12%)
Lymph node	NA	1 (4%)
Skull	NA	1 (4%)
Other	NA	2 (8%)

[a] 1 pt in each arm did not receive any sip-T because of IV access and were excluded from analysis.

Correlative studies

In order to assess the effect of radiation therapy on parameters of immune response to sipuleucel-T, blood samples were collected at baseline (week 0), week 4, 12 and 24 and analyzed utilizing INFγ ELISPOT, T cell proliferation and humoral response assays. In addition the effect of radiation on sipuleucel-T product parameters including APC number, APC activation (CD54 upregulation) and total nucleated cell number was analyzed. Immune assays were performed at Dendreon Corporation, the maker of sipuleucel-T.

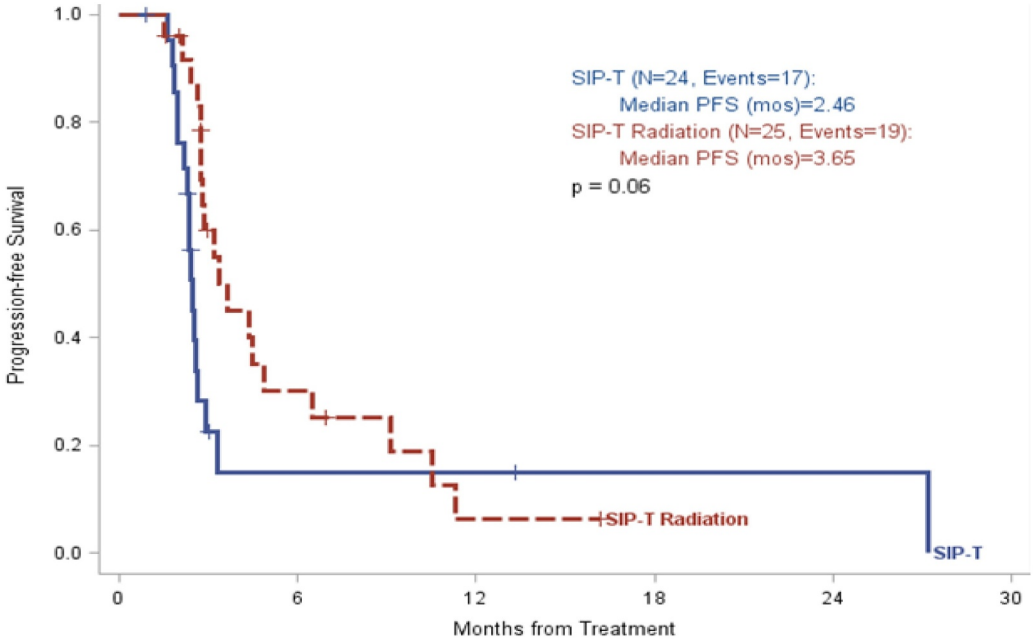

Fig. 1. Progression free survival.

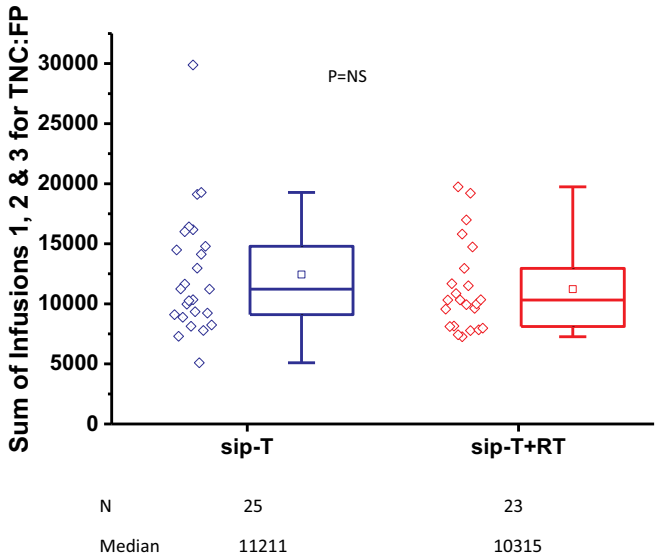

Fig. 2. Total nucleated cells (TNC) in sipuleucel-T final product (FP).

ELISPOT

Antigen-specific memory T cell responses were assessed by means of IFNγ ELISPOT assay. PVDF ELISPOT (enzyme-linked immunoSPOT) plates (Millipore) are coated with an anti-IFNγ monoclonal antibody (mAb) (clone D1K, MabTech) overnight, after which time plates are blocked and washed with phosphate-buffered saline/Tween (PBST). Cryopreserved PBMC, thawed and rested overnight in medium are then plated in triplicate at 3×10^5 PBMC/well in a total volume of 200μL/well with either medium alone or with medium containing antigen (PA2024: PAP-GMCSF fusion protein, PAP or CEFT peptide pool, an assay control). Plates are then incubated for 40–48 h after which time plates are washed then incubated with Strepatavidin conjugated anti-IFNγ mAb (clone B6-1, MabTech). After incubation plates are further washed with PBST and then incubated biotin conjugated with alkaline peroxidase for a further hour. Afterwards plates are then washed with PBST and then incubated with a substrate, BCIP (5-Bromo-4-chloro-3-indolyl phosphate) to visualize IFNγ secreting cells. ELISPOT data are presented as the median of triplicates with background (PBMCs incubated with media) IFNγ spots subtracted.

T cell proliferation

Antigen-specific T cell proliferation to PA2024, PAP and PHA are assayed by means of a standard tritiated thymidine (^{3}H-thymidine) incorporation assay using round bottom 96 well tissue culture plates. Cryopreserved PBMC, thawed and rested overnight in medium are then plated in triplicate at 1×10^5 PBMC/well in a total volume of200 μL/well with either medium alone or with medium containing antigen (PA2024: PAP-GMCSF fusion protein, PAP or PHA – Phytohaemagglutinin). Plates are incubated for 5 days, after which the wells are pulsed with 0.5 μCi of ^{3}H-thymidine overnight, and the amount of ^{3}H-thymidine incorporation into the nucleus Is quantified by means of a γ-radiation counter with the degree of proliferation expressed as a stimulation index (SI), defined as

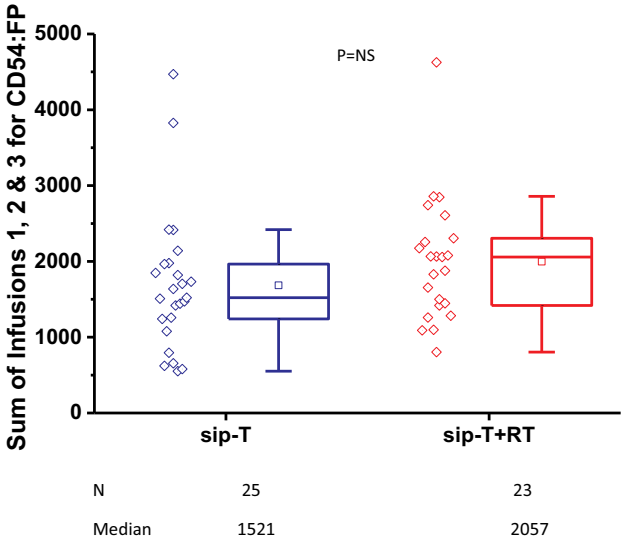

Fig. 3. CD54 + cells in sipuleucel-T final product (FP).

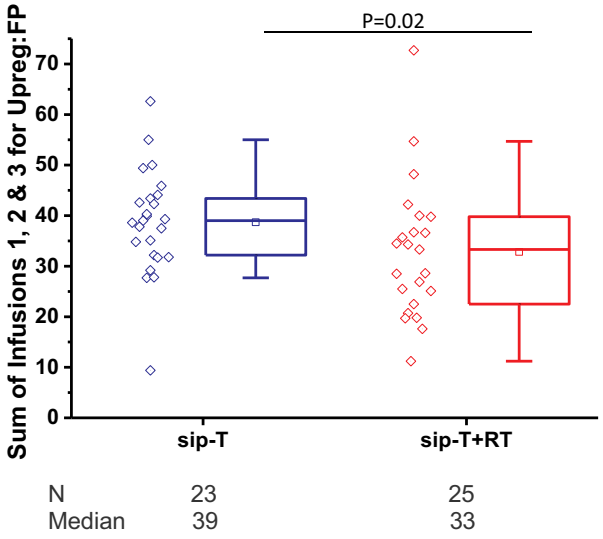

Fig. 4. Cumulative upregulation of CD54 + cells in sipuleucel-T final product (FP).

^{3}H-thymidine incorporation in the presence of antigen divided by ^{3}H-thymidine incorporation with media alone.

Humoral response

Antibody responses against PA2024 and PAP are determined by means of standard antibody ELISA (enzyme-linked immunosorbent assay). 96 well flat bottomed plates are coated with either PA2024, PAP or Tetanus (an assay control, and measure of immunocompetence) overnight, after which time plates are blocked with PBS/casein and then washed with PBST (phosphate buffered saline tween). Serially diluted serum is then added in duplicate to each set of plates and incubated at room temperature for 2 h, after which time plates are washed with PBST and then incubated with a mixture of anti-IgM and anti-IgG (Jackson Immunoresearch) for an hour. After this time plates are washed again with PBST and then incubated for an hour with horse radish phosphatase conjugated anti IgG + anti-IgM. Plates were further washed with PBST and O-phenylenediamine dihydrochloride substrate (Sigma) was then added for 15 min after which the developing reaction was stopped by the addition of 50 µl/well of 2 N HCL (Sigma). Plates were read on a Synergy HT spectrophotometer (BioTEK) at 492 nm and the endpoint titer was determined as being the last dilution of serum that yielded an O.D. reading equivalent to assay background.

Objectives

The primary objective of a study was to assess the ability to safely combine sipuleucel-T with radiation therapy and generate adequate sipuleucel-T product parameters for infusion of 3 doses of immunotherapy. Secondary objectives included assessment of the effect of radiation therapy to single metastatic sites on immune responses generated by sipuleucel-T including antibody and T-cell proliferation, antigen-specific memory T cell responses to prostatic acid phosphatase (PAP) and fusion protein PA2024, and clinical parameters including PSA and radiographic responses, progression free survival and toxicity.

Statistical methods

The primary endpoint was the percent of patients able to receive all three infusions of sipuleucel-T, which reflects the ability to collect adequate numbers of CD54 + cells for the generation of the vaccine. This also incorporates the willingness and ability to receive the three infusions. There were early stopping rules for failure to receive all three infusions, where 8% failing to receive all three infusions on Arm B, consistent with historical data would be acceptable, whereas 33% would be unacceptable (the study had 82% chance of stopping early if the true rate of failure was 33% and a 4% chance of stopping early with a true 8% rate). There was also an interim stopping rule for failure to initiate sipuleucel-T therapy on Arm B. The secondary endpoints considered evaluation of PAP, PA2024, toxicity, radiological responses and progression-free survival. Survival statistics include Kaplan-Meier estimates and plots, and the log-rank test. Radiological responses were compared with Fisher's Exact test, and the biological correlates were assessed using a *t*-test with a two-sided level of significance presented.

Results

Fifty one patients were accrued, (25 to sipuleucel-T (Arm A) and 26 to sipuleucel-T and radiation (Arm B). One patient in each group did not receive any sipuleucel-T because of difficulties with intravenous access and these patients were excluded from analysis. Among evaluable patients 100% completed all 3 sipuleucel-T infusions in Arm A and 92% in Arm B. Patient characteristics are summarized in Table 1. Median PSA values reflected relatively favorable subset of metastatic castration resistant prostate cancer patients treated with sipuleucel-T. The majority of patients had bone metastases with or without lymph node involvement. Treatment was well tolerated and only 1 patient in Arm B experienced treatment related grade 3 event (anemia, Table 2).

One patient in Arm B experienced PSA partial response but there were no radiographic responses. There was a trend in median progression free survival (PFS) favoring Arm B (3.65 months) over Arm A (2.46 months) but the difference was not statistically significant ($p = 0.06$, Fig. 1). There was no difference in the majority of sipuleucel-T product parameters including total nucleated cells (Fig. 2) and CD54+ cells (Fig. 3) between Arm A and Arm B. However the cumulative upregulation of CD54 + cells (ratio of CD54+ cells in sipuleucel-T product to baseline) favored sipuleucel-T arm alone. (Fig. 4). This was related to higher upregulation of CD54+ cells at the time of 1st infusion (Fig. 5). There were no differences between arms for the prime-boost phenomenon, with both arms showing increased upregulation of CD54+ at the 2nd and 3rd infusions compared to the 1st infusion (Fig. 5). Up to 20 samples per time point were available for immune response assays. T cell responses by INFγ ELISPOT at week 12 was greater in Arm A ($p = 0.028$, Fig. 6). There were no differences in T cell proliferation and humoral responses at various time points between two treatment arms (Figs. 7 and 8).

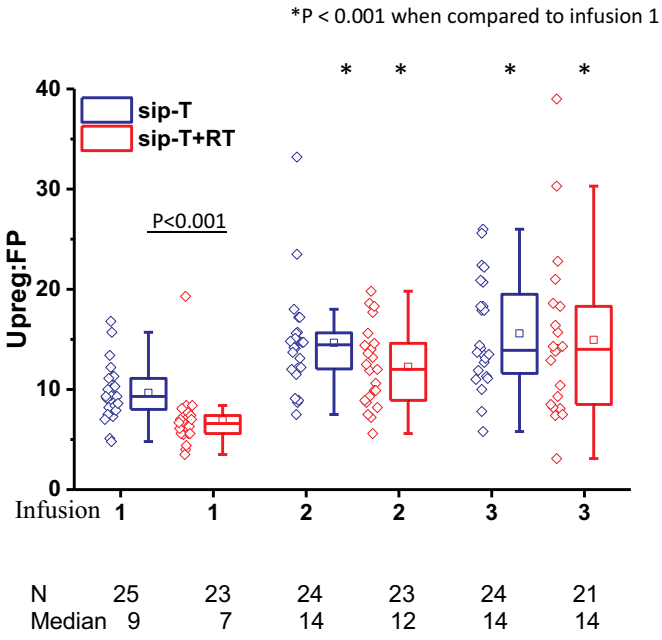

Fig. 5. Upregulation of CD54 + cells in sipuleucel-T final product (FP) by individual infusions.

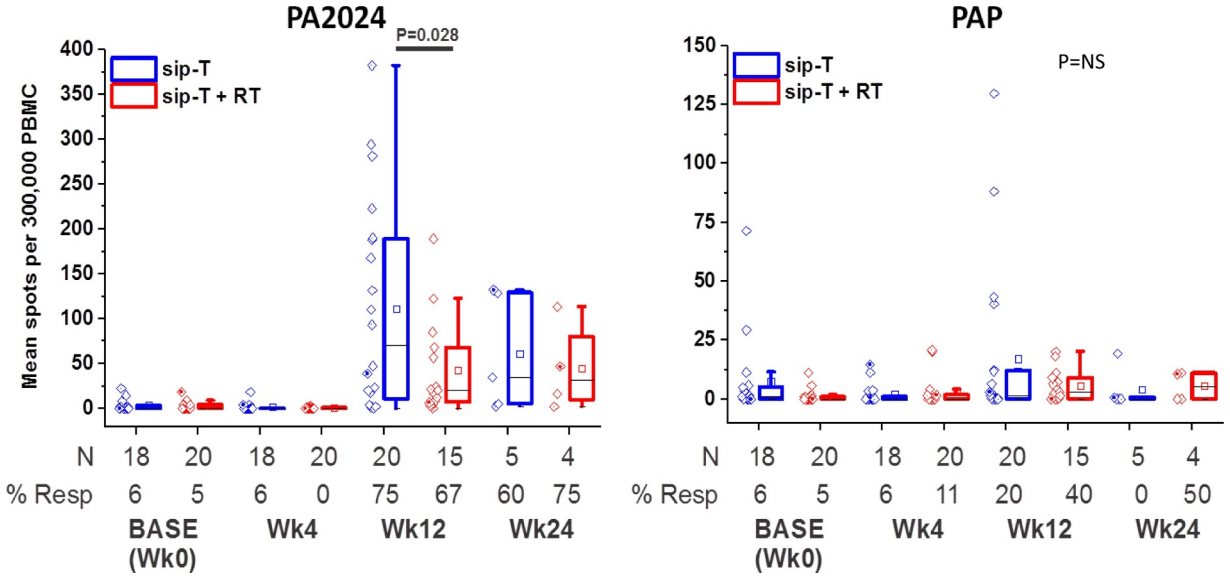

Fig. 6. IFNγ ELISPOT responses.

Conclusions

Radiation therapy to single metastatic CRPC site delivered at 300cGy/day up to 3000 cGy and completed 1 week before initiation of sipuleucel-T was safe and feasible. Radiation therapy did not impact the majority of sipuleucel-T product parameters including total nucleated cells (TNC) and CD54+ cells. However, cumulative CD54+ cell upregulation was lower in patients treated with the sipuleucel-T plus radiation (Arm B) versus patients treated with sipuleucel-T alone (Arm A). This effect was primarily influenced by decreased CD54 upregulation at the time of 1st sipuleucel-T infusion since CD54 upregulation at the time of subsequent infusions was equal between treatment arms suggesting that suppressive effect of radiation therapy was short-lived. Radiation therapy did not induce significant effects on majority of measured immune response parameters including antibody response and T-cell proliferation generated by sipuleucel-T to prostatic acid phosphatase (PAP), and fusion protein PA2024. However IFNγ ELISPOT response to PA2024 antigen at week 12 favored sipuleucel-T alone (Arm A). Despite that radiation therapy combined with sipuleucel-T (ARM B) was associated with a trend towards prolonged PFS as compared to sipuleucel-T alone, however the difference was not statistically significant and may be explained by more favorable characteristics in sipuleucel-T plus radiation (Arm B) patients, including lower median PSA and lower Gleason score. The concept of radiation therapy to oligometastatic sites is also being considered on its own merits as a component of multimodality therapy of metastatic prostate cancer [15]. In that context metastasis directed radiation therapy may provide benefit by decreasing metastatic tumor burden independent of interaction with other treatment modalities. Whether that ultimately translates into meaningful clinical benefits requires larger confirmatory studies. It appears though that radiation of oligometastases is becoming increasingly adopted in the management of prostate cancer and our study provides evidence that the length of interval between radiation and sipuleucel-T treatment is unlikely to impact its effectiveness.

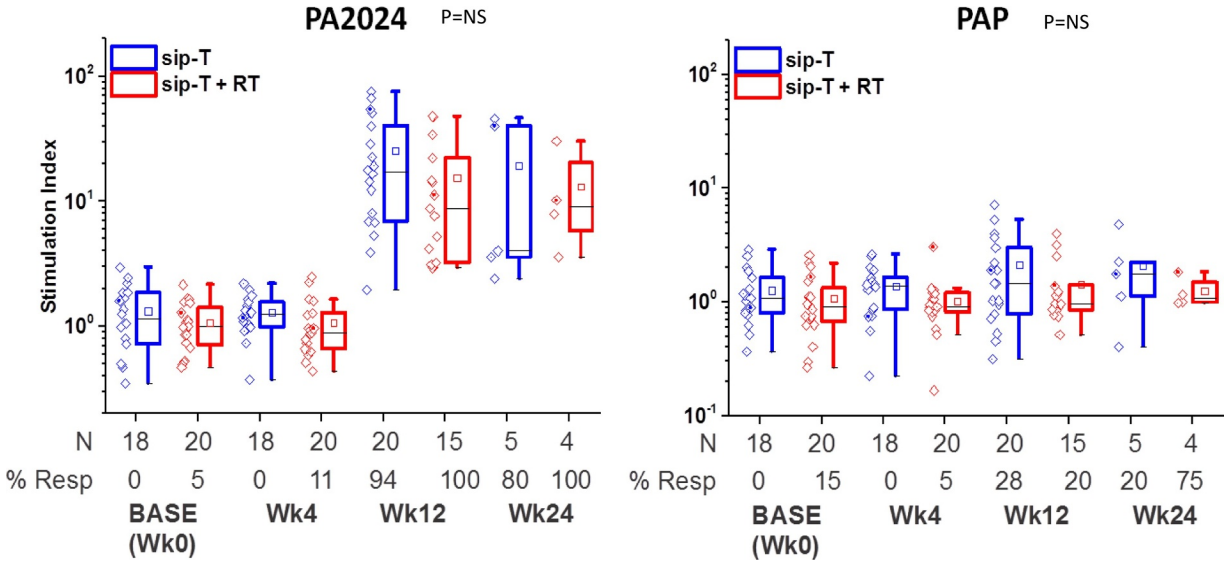

Fig. 7. Cellular proliferative responses.

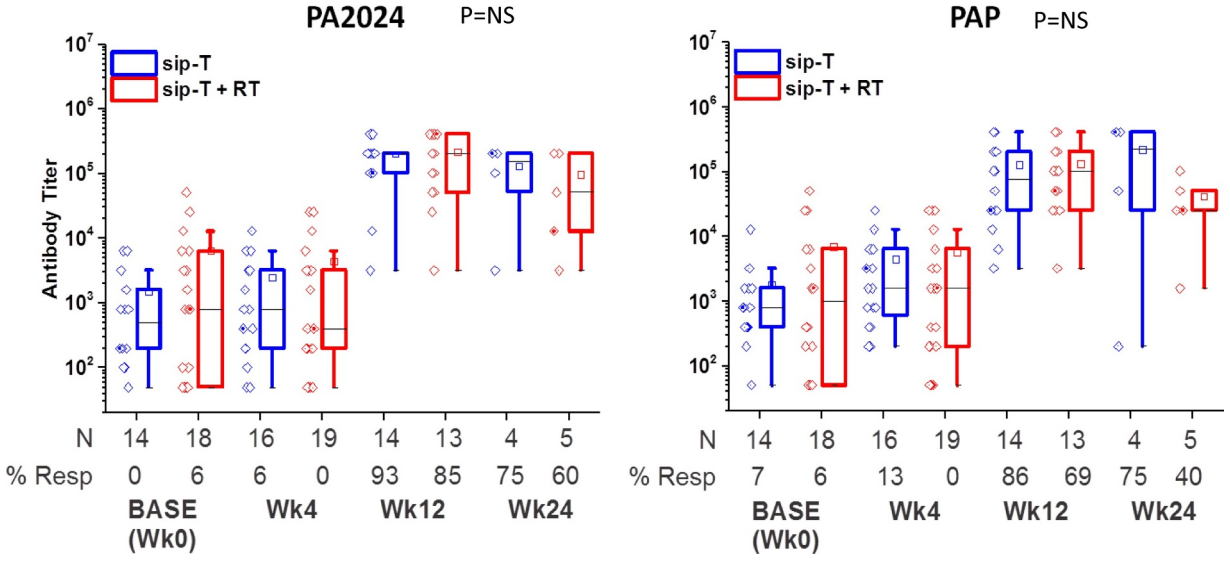

Fig. 8. IgG + IgM humoral responses.

In summary our trial did not support the hypothesis that radiation therapy as administered in this study exerts any favorable effect on immune response in patients with mCRPC treated with sipuleucel -T. Nevertheless radiation-induced immune modulation continues to be an area of interest but radiation doses, schedule, types of immunotherapy and specific combinational strategies and clinical validity of that concept itself remains to be elucidated.

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