Journal for ImmunoTherapy of Cancer

Prospective, randomized, double-blind phase 2B trial of the TLPO and TLPLDC vaccines to prevent recurrence of resected stage III/IV melanoma: a prespecified 36-month analysis

Elizabeth Lee Carpenter,[1] Spencer Van Decar ,[2] Alexandra M Adams,[2] Anne E O'Shea,[2] Patrick McCarthy ,[3] Robert Connor Chick,[2] Guy Travis Clifton,[4,5] Timothy Vreeland ,[4,5] Franklin A Valdera,[2] Ankur Tiwari,[6] Diane Hale,[2,4] Phillip Kemp Bohan,[2] Annelies Hickerson,[2] Todd Smolinsky,[2] Katryna Thomas,[2] Jessica Cindass,[2] John Hyngstrom,[7] Adam C Berger,[8] James Jakub,[9] Jeffrey J Sussman,[10] Montaser F Shaheen,[11] Xianzhong Yu,[12] Thomas E Wagner,[13] Mark Faries,[14] George E Peoples[15]

To cite: Carpenter EL, Van Decar S, Adams AM, *et al*. Prospective, randomized, double-blind phase 2B trial of the TLPO and TLPLDC vaccines to prevent recurrence of resected stage III/IV melanoma: a prespecified 36-month analysis. *Journal for ImmunoTherapy of Cancer* 2023;**11**:e006665. doi:10.1136/jitc-2023-006665

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2023-006665).

Accepted 28 June 2023

For numbered affiliations see end of article.

Correspondence to
Dr Spencer Van Decar; spencer.vandecar@gmail.com

ABSTRACT

Background The tumor lysate, particle-loaded, dendritic cell (TLPLDC) vaccine is made by ex vivo priming matured autologous dendritic cells (DCs) with yeast cell wall particles (YCWPs) loaded with autologous tumor lysate (TL). The tumor lysate, particle only (TLPO) vaccine uses autologous TL-loaded YCWPs coated with silicate for in vivo DC loading. Here we report the 36-month prespecified analyses of this prospective, randomized, double-blind trial investigating the ability of the TLPO and TLPLDC (±granulocyte-colony stimulating factor (G-CSF)) vaccines to prevent melanoma recurrence in high-risk patients.

Methods Patients with clinically disease-free stage III/IV melanoma were randomized 2:1 initially to TLPLDC versus placebo (n=124) and subsequently TLPO versus TLPLDC (n=63). All patients were randomized and blinded; however, the placebo control arm was replaced in the second randomization scheme with another novel vaccine; some analyses in this paper therefore reflect a combination of the two randomization schemes. Patients receiving the TLPLDC vaccine were further divided by their method of DC harvest (with or without G-CSF pretreatment); this was not randomized. The use of standard of care checkpoint inhibitors was not stratified between groups. Safety was assessed and Kaplan-Meier and log-rank analyses compared disease-free (DFS) and overall survival (OS).

Results After combining the two randomization processes, a total of 187 patients were allocated between treatment arms: placebo (n=41), TLPLDC (n=103), or TLPO (n=43). The allocation among arms created by the addition of patients from the two separate randomization schemes does not reflect concurrent randomization among all treatment arms. TLPLDC was further divided by use of G-CSF in DC harvest: no G-CSF (TLPLDC) (n=47) and with G-CSF (TLPLDC+G) (n=56). Median follow-up was 35.8 months. Only two patients experienced a related adverse event ≥grade 3, one each in the TLPLDC+G and placebo arms. DFS was 27.2% (placebo), 55.4% (TLPLDC), 22.9% (TLPLDC+G), and 60.9% (TLPO) (p<0.001). OS was 62.5% (placebo), 93.6% (TLPLDC), 57.7% (TLPLDC+G), and 94.6% (TLPO) (p=0.002).

Conclusions The TLPO and TLPLDC (without G-CSF) vaccines were associated with improved DFS and OS in this clinical trial. Given production and manufacturing advantages, the efficacy of the TLPO vaccine will be confirmed in a phase 3 trial.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ While significant improvements in the treatment of advanced melanoma have been made in the last decade, current therapies (checkpoint inhibitor (CPI), BRAF/MEK inhibitors) fail to maintain lasting disease control in most patients or have significant toxicity profiles. Autologous, personalized tumor vaccines inducing or augmenting an immune response to tumor neoantigens are an evolving field in cancer immunotherapy.

WHAT THIS STUDY ADDS

⇒ This study demonstrates that autologous tumor melanoma vaccines (tumor lysate, particle only and tumor lysate, particle-loaded, dendritic cell) used in the adjuvant setting are safe and may improve disease-free survival (DFS) and overall survival (OS) alone or especially in combination with CPI.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study elucidates the optimal production methodology of an autologous tumor vaccine and demonstrates the potential of these vaccines to improve DFS and OS, which needs to be confirmed in a phase 3 trial.

Trial registration number NCT02301611.

INTRODUCTION

While early-stage melanoma is often cured by surgical resection, more advanced stages are associated with a high risk of recurrence and death.[1] Significant advances in melanoma treatments have been made in the last decade with the advent of immune checkpoint inhibitors (CPIs) and pathway BRAF and MEK inhibitors, which are now available as adjuvant therapies in resected disease.[2 3] Unfortunately, BRAF/MEK inhibitors may not offer lasting disease control due to escape mutations.[4] Meanwhile, CPIs may lead to significant toxicity, and efficacy may be limited if there is not a pre-existing immune response to the tumor.[2 3 5 6] An alternative strategy for harnessing the potential of the host immune system is stimulation with a cancer vaccine, which offers the potential for efficacy in the adjuvant setting with less toxicity as a monotherapy than current standard therapies, or may allow CPI therapies to work more effectively by stimulating an endogenous immune response.[2 6 7] Exemplifying this mechanism is sipuleucel-T (Provenge) for castration refractory metastatic prostate cancer, the only Food and Drug Administration (FDA)-approved dendritic cell (DC)-based cancer vaccine, which uses DCs to generate a tumor-specific immune response.[8]

Thus far, there has been little success with such a DC-based vaccine concept in other cancer types.[9–12] However, most melanoma vaccines have been tested in late-stage patients with a large tumor burden, where cancer vaccines may be less effective, than in the adjuvant setting in patients who are clinically disease free.[3 13 14] The tumor lysate, particle-loaded, DC (TLPLDC) vaccine has been previously described by our group and tested in both the adjuvant as well as the metastatic setting in combination with other systemic therapies. The uniqueness of the TLPLDC vaccine is that innate immunity-stimulating yeast cell wall particles (YCWPs) are used to deliver the full antigenic repertoire of a patient's tumor (including unique neoantigens) to isolated autologous immature monocytic-derived DC. The phagocytized YCWPs not only deliver the tumor lysate payload but also induce the final maturation of the DC.[3 15] In an attempt to draw less peripheral blood to isolate sufficient monocytes for DC maturation in our study, we offered as an option a single injection of granulocyte-colony stimulating factor (G-CSF) to elevate the white cell count and half the blood volume drawn at 24–48 hours. In retrospective analysis, we found that the TLPLDC vaccine created with the use of G-CSF, when combined with our expedited DC isolation/maturation process, does not allow for full maturation of the DCs, and subsequently has outcomes comparable with placebo.[16]

Another iteration of the TLPLDC concept, the novel tumor lysate, particle only (TLPO) vaccine, uses similarly tumor lysate (TL)-loaded YCWPs but is capped with silicate for content retention and monocytic attraction and directly inoculated for in vivo DC uptake, thus bypassing multiple steps of TLPLDC production. Our group recently presented results from the embedded bridging portion phase I/IIA of this current phase 2B trial comparing the TLPO with TLPLDC vaccines, which demonstrated equivalent disease-free survival (DFS) and overall survival (OS) and may have important implications in reducing costs and time for vaccine production.[17] We therefore sought in the prespecified analysis of this four-arm study to investigate differences between the TLPLDC vaccine with and without G-CSF, TLPO vaccine, and placebo.

Here, we present the 3-year outcomes of this prospective, randomized, double-blind phase 2B trial comparing the TLPLDC vaccine with G-CSF (TLPLDC+G) and without G-CSF (TLPLDC) with both the TLPO vaccine and placebo. The primary study outcome was 24-month DFS with secondary outcomes of 36-month DFS and OS, as well as safety between the vaccinated arms (TLPLDC, TLPLDC+G, and TLPO) and control groups.

MATERIALS AND METHODS

Trial design and participants

Patients 18 years of age or older with stage III/IV melanoma capable of being disease free after surgery were recruited at the individual study sites. Additional inclusion criteria included: an Eastern Cooperative Oncology Group performance status of 0–1, adequate (1 mg minimum) tumor for vaccine production, and completion of standard adjuvant therapy per National Comprehensive Cancer Network guidelines. Exclusion criteria included the following: evidence of residual disease after surgery and adjuvant treatment, inability to provide sufficient tumor for vaccine production, and immune deficiency or suppression.

Patients were approached and screened by either the research nurse, study coordinator, and/or investigators at individual sites. Patients were consented prior to surgery for tissue collection and additionally after completion of standard therapy prior to vaccination.

Randomization was performed by the clinical research organization via a computer-generated central, permuted block randomization scheme and site-balancing algorithm. Sample size was determined assuming a 60% recurrence rate at 2 years in a mixed population of patients with stage III and IV melanoma, yielding a sample size of 120 required for 80% power to detect a statistical difference in DFS between treatment arms with two-sided α=0.05. In a continuation of the trial, 60 additional patients were randomized 2:1 comparing the TLPLDC and TLPO versions of the vaccine.

The trial commenced in February 2015. Patients were first randomized 2:1 to receive either the TLPLDC vaccine or placebo (n=124), and then randomization transitioned to a 2:1 allocation ratio of TLPO vaccine or TLPLDC vaccine for an additional 63 patients. This resulted in receipt of placebo (n=41), TLPO (n=43), and TLPLDC (n=103) formulations, the latter then subdivided

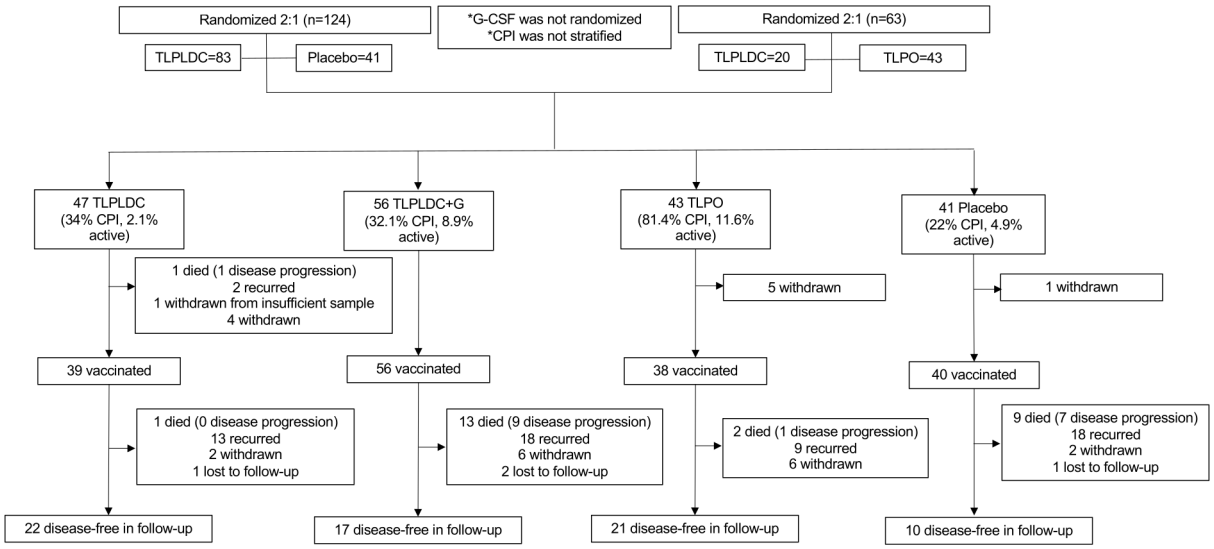


Figure 1 CONSORT diagram. CONSORT, Consolidated Standards of Reporting Trials; CPI, checkpoint inhibitor; G-CSF, granulocyte-colony stimulating factor; TLPLDC, tumor lysate, particle-loaded, dendritic cell; TLPLDC+G, TLPLDC with G-CSF; TLPO, tumor lysate, particle only.

according to use of G-CSF for DC collection (TLPLDC, n=47; TLPLDC+G, n=56) (figure 1). Per the revised statistical plan, this ultimately resulted in four prespecified arms for analysis. It is important to note, the use of G-CSF was not randomized, but rather based on patient and treating physician preference. Additionally, while in the original phase IIB trial, TLPLDC was randomized against placebo, in the continuation bridging trial, TLPO was randomized against TLPLDC only, not placebo. Patients, their treating physicians, study personnel including site principal investigators (PIs), and clinical and medical monitors were blinded to the study arms.

Of note, the protocol was amended during the trial in July of 2017 to allow enrollment of patients on CPI therapy to participate given the change to standard treatment of melanoma. Patients had to tolerate CPI therapy for a minimum of 3 months prior to randomization. The enrollment of patients with active CPI use or a history of CPI use was not stratified between arms.

Vaccine production

Vaccines were produced by Orbis Health Solutions (Greenville, South Carolina, USA). Tumor tissue was collected at the time of surgical resection or pretreatment biopsy and subjected to freeze/thaw cycling in lysis buffer to produce TL. YCWPs are created from *Saccharomyces cerevisiae* via NaOH/HCl digestion of non-cell wall components and washing, producing β-glycan shells. TL is then loaded into YCWP and freeze dried.[15]

To create the TLPO vaccine, TL-loaded YCWPs are capped with silicate for TL retention and attraction of a monocytic infiltrate. The vaccine is then directly inoculated intradermally for DC uptake in vivo.[17] The TLPLDC vaccine and placebo production has been previously described by our research group.[3] The TLPLDC formulation is produced by incubating TL-loaded YCWPs in the presence of the patient's DCs for phagocytosis in vitro. DCs are isolated and prepared from a patient blood draw, either with a single 120 mL collection or 70 mL collection 24–48 hours after a subcutaneous injection of 300 µg G-CSF. The placebo formulation is composed of the patient's DCs after incubation with empty YCWPs.[3 15]

The full vaccination course of the TLPLDC/TLPO vaccines or placebo was produced at a single time after randomization. Tumor samples were maintained for all patients for reproduction/production of the vaccine in the event of recurrence or request at completion of the primary endpoint of the trial.

Interventions and outcomes

Patients received six intradermal vaccinations to the thigh or arm. Initial vaccination was performed at 0, 1, and 2 months followed by a booster series as 6, 12, and 18 months. Patients were followed with history and physical examination every 3–6 months. Additional laboratory and radiographic surveillance were directed by the patient's treating physician. All patients, regardless of randomization, were followed for clinical recurrence and OS for a minimum of 3 years from randomization. If a patient recurred, determined by the patient's treating physicians, he or she was offered participation in an open-label study of TLPLDC or TLPO vaccination in metastatic melanoma and followed for an additional 12 months.[18]

Patients were monitored for 30 min after vaccination for both primary and booster series. Reporting of adverse events (AEs) was performed according to a prespecified Data Safety Monitoring Plan. Standard local and systemic toxicities were collected and graded per the National Cancer Institute CTCAE (Common Terminology Criteria for Adverse Events) V.4.03 graded toxicity scale. AEs were defined as any negative medical occurrence in a subject during the trial regardless of causality. Treatment-related AEs were classified as 'definitely', 'probably', or 'possibly' by the primary investigator of the site. A serious AE (SAE)

was defined as an event meeting the following criteria: death, life-threatening event, inpatient hospitalization, congenital anomaly/birth defect, disability, or required medical or surgical intervention to prevent a listed outcome.

The primary endpoint of this trial was DFS at 24 months, which has been previously reported.[3] The secondary endpoint of the trial, with results reported herein, was 36-month DFS and OS of vaccination arms (TLPLDC, TLPLDC+G, and TLPO) versus placebo. Additional endpoints included safety, measured by total, related, and/or SAEs with associated toxicity grade. Prespecified subgroups included patients receiving CPI therapy.

Protocol-specific therapy was stopped for progressive disease, unacceptable toxicity as measured above, patient decision, or judgment of the treating physician.

Statistical analysis

Statistics were calculated using SPSS (V.22, IBM Corp, Released 2013). Clinicopathologic data and AEs were analyzed via either Student's t-test or Mann-Whitney U test for continuous variables and X^2 test for categorical variables. Median follow-up was calculated by excluding any patient who died prior to 36 months. Kaplan-Meier and log-rank analysis were used to compare DFS and OS. A p value of <0.05 was considered significant for all analyses.

RESULTS

Demographics

A total of 124 patients were randomized 2:1 to TLPLDC (n=83) versus placebo (n=41) initially, and then an additional 63 patients randomized 2:1 to TLPO (n=43) versus TLPLDC (n=20) for a total of 187 patients randomized to these treatment arms (figure 1). Based on prior observation and the revised statistical analysis plan, the TLPLDC vaccination arm was further divided based on G-CSF use in the harvest of the DC resulting in 56 patients who were pretreated with G-CSF (TLPLDC+G) and 47 patients who were not (TLPLDC).

The resulting four treatment arms were largely comparable for disease and treatment-related factors except patients who received the TLPO vaccine were more likely to have stage IV disease than the TLPLDC, TLPLDC+G, or placebo arms, respectively (44.2% TLPO, 10.6% TLPLDC, 28.6% TLPLDC+G, 22.0% placebo, p=0.003). However, TLPO vaccine recipients were also more likely to have received CPI therapy (81.4% TLPO, 34.0% TLPLDC, 32.1% TLPLDC+G, 22% placebo, p<0.001). Clinicopathologic (table 1) and treatment (table 2) comparisons are summarized for each arm.

Safety

In total, 125 of 187 (66.8%) patients experienced any (related and unrelated) AE, with 649 cumulative AEs (112 TLPO, 171 TLPLDC, 210 TLPLDC+G, 156 placebo). Sixty-six patients (35.3%) experienced a related AE of any grade, with a total of 249 related AEs (38 TLPO, 79 TLPLDC, 77 TLPLDC+G, 55 placebo) (table 3).

Thirty-one patients (16.6%) experienced any AE ≥grade 3 for 55 total events, with 17 TLPO, 9 TLPLDC, 17 TLPLDC+Gand 12 placebo (p=0.03). Only two patients experienced a related AE ≥grade 3, one each in the TLPLDC+G (symptomatic anemia, hospitalization) and placebo (infection, hospitalization) arms. There was no significant difference between groups when comparing grade 3 vs 4 vs 5 (p=0.472). Most frequent related AEs were administration site conditions (eg, injection site reaction) and general disorders (eg, fever or fatigue), followed by gastrointestinal disorders (eg, nausea and vomiting) and nervous system disorders consisting of dizziness or headache. There was a significant difference between cumulative local injection site reactions among groups, 18 TLPO, 63 TLPLDC, 19 TLPLDC+G, 34 placebo (p<0.001) (table 4).

Overall, 17 patients (9.1%) experienced 47 SAEs with no differences between groups (11 TLPO, 10 TLPLDC, 18 TLPLDC+G, 8 placebo, p=0.281). The most frequent SAEs were infections, followed by nervous system disorders. Only three patients had a related SAE: pleural effusion (TLPO), symptomatic anemia (TLPLDC+G), and infection (placebo).

Survival

Median follow-up was 35.8 months. The 36-month DFS was 64.0% (95% CI 46% to 77%) for the TLPO vaccination arm, 55.8% (95% CI 39% to 69%) for TLPLDC arm, 24.4% (95% CI 14% to 37%) for TLPLDC+Garm, and 30.0% (95% CI 16% to 45%) for placebo with p<0.001. The 36-month OS was also significantly longer, with 94.8% (95% CI 81% to 99%) for TLPO, 94.2% (95% CI 78% to 99%) for TLPLDC, 69.8% (95% CI 54% to 81%) for TLPLDC+G, and 70.9% (95% CI 52% to 83%) for placebo (p=0.011). The 36-month DFS and OS for the four arms are summarized in figure 2.

Prespecified subgroup analysis

In a prespecified subgroup analysis, limiting analysis to those patients with prior or concurrent CPI therapy, and vaccination with either TLPO or TLPLDC was associated with longer 36-month DFS and OS when compared with placebo or TLPLDC+G (DFS: 62.1% TLPO, 61.9% TLPLDC, 11.1% TLPLDC+G, 37.5% placebo, p=0.008; OS: 93.3% TLPO, 87.5% TLPLDC, 44.4% TLPLDC+G, 58.3% placebo, p=0.02) (figure 3). For patients who did not receive CPI therapy, there was no significant difference in 36-month DFS (p=0.184); however, there was an associated OS benefit (OS: 100% TLPO, 96.0% TLPLDC, 63.9% TLPLDC+G, 62.4% placebo, p=0.048) (figure 4). Demographic and pathologic data for patients by CPI subgroup can be found in supplemental file 1.

Table 1 Demographic and pathologic data for placebo (n=41), TLPLDC (n=47), TLPLDC+G (n=56), and TLPO (n=43) arms

		Treatment arms								P value
		Placebo n=41		TLPLDC n=47		TLPLDC+G n=56		TLPO n=43		
Category		Count	Column %	Count	Column %	Count	Column %	Count	Column %	
Age	Median	58.7		69.5		61.7		63.6		0.07
	Minimum	38.8		22.9		28.4		37.3		
	Maximum	89.9		89.8		89.1		83.6		
Sex	Female	14	34.1	16	34.0	16	28.6	16	37.2	0.83
	Male	27	65.9	31	66.0	40	71.4	27	62.8	
Race	Asian	1	2.4	0	0.0	0	0.0	0	0.0	0.62
	Black	1	2.4	0	0.0	0	0.0	1	2.3	
	Hispanic	1	2.4	1	2.1	2	3.6	0	0.0	
	Native American	0	0.0	0	0.0	1	1.8	0	0.0	
	Other	0	0.0	0	0.0	1	1.8	0	0.0	
	White	38	92.7	46	97.9	52	92.9	41	95.3	
	White, Hispanic	0	0.0	0	0.0	0	0.0	1	2.3	
Breslow (mm)	Median	2		3		3		3		0.68
	Minimum	0		0		0		1		
	Maximum	16		14		50		15		
Ulceration	Missing	22	53.7	29	61.7	35	62.5	25	58.1	0.31
	Absent	7	17.1	11	23.4	11	19.6	13	30.2	
	Present	12	29.3	7	14.9	10	17.9	5	11.6	
	Missing	13	31.7	11	23.4	19	33.9	12	27.9	0.29
Mitotic rate	<1	0	0.0	0	0.0	1	1.8	0	0.0	
	≥1	21	51.2	26	55.3	24	42.9	16	37.2	
	0	2	4.9	4	8.5	6	10.7	2	4.7	
	NA	5	12.2	6	12.8	6	10.7	13	30.2	
AJCC	III	32	78.0	42	89.4	40	71.4	24	55.8	0.003
	IV	9	22.0	5	10.6	16	28.6	19	44.2	
Staging	Missing	1	2.4	0	0.0	0	0.0	0	0.0	0.20
	Primary	18	43.9	19	40.4	22	39.3	10	23.3	
	Recurrent	22	53.7	28	59.6	34	60.7	33	76.7	
T stage	Missing	1	2.4	2	4.3	1	1.8	0	0.0	0.49
	Not Available	7	17.1	4	8.5	6	10.7	5	11.6	
	T0	0	0.0	0	0.0	2	3.6	0	0.0	
	T1	7	17.1	4	8.5	3	5.4	3	7.0	
	T2	4	9.8	10	21.3	11	19.6	3	7.0	
	T3	8	19.5	12	25.5	11	19.6	10	23.3	
	T4	7	17.1	10	21.3	14	25.0	15	34.9	
	Tis	0	0.0	0	0.0	1	1.8	0	0.0	
	TX	7	17.1	5	10.6	7	12.5	7	16.3	

Continued

Table 1 Continued

Category		Treatment arms								P value
		Placebo n=41		TLPLDC n=47		TLPLDC+G n=56		TLPO n=43		
		Count	Column %	Count	Column %	Count	Column %	Count	Column %	
N stage	Missing	1	2.4	2	4.3	2	3.6	0	0.0	0.39
	NA	10	24.4	9	19.1	14	25.0	13	30.2	
	N1	14	34.1	12	25.5	6	10.7	12	27.9	
	N2	9	22.0	11	23.4	15	26.8	8	18.6	
	N3	7	17.1	13	27.7	19	33.9	10	23.3	
M stage	Missing	7	17.1	3	6.4	10	17.9	1	2.3	0.003
	M0	26	63.4	40	85.1	31	55.4	24	55.8	
	M1a	4	9.8	1	2.1	4	7.1	11	25.6	
	M1b	1	2.4	1	2.1	5	8.9	4	9.3	
	M1c	3	7.3	2	4.3	6	10.7	3	7.0	

TLPLDC, tumor lysate, particle-loaded, dendritic cell; TLPLDC+G, TLPLDC with granulocyte-colony stimulating factor; TLPO, tumor lysate, particle only.

DISCUSSION

In this prospective, randomized, double-blind phase 2B trial comparing the TLPO and TLPLDC vaccines (with and without G-CSF pretreatment), both vaccines were well tolerated and shown to be safe. Importantly, 36-month DFS and OS were prolonged among TLPLDC and TLPO vaccination arms when compared with TLPLDC+G or placebo; however, these observations must be viewed in the context of the study limitations most notably the non-randomization of the use of G-CSF in the DC harvest, the non-stratification of CPI therapy, and two different randomization schemes used through the course of the trial.

One of the purported benefits of cancer vaccines has always been low toxicity, and the TLPLDC and TLPO vaccines share this advantage.[19] Across this phase 2B trial,

Table 2 Treatment data for placebo (n=41), TLPLDC (n=47), TLPLDC+G (n=56), and TLPO (n=43) arms

Category		Treatment arm								P value
		Placebo n=41		TLPLDC n=47		TLPLDC+G n=56		TLPO n=43		
		Count	Column %	Count	Column %	Count	Column %	Count	Column %	
CPI (see online supplemental table 3 for types)	No	32	78.0	31	66.0	38	67.9	8	18.6	<0.001
	Yes	9	22.0	16	34.0	18	32.1	35	81.4	
Interferon-α	Missing	26	63.4	35	74.5	40	71.4	23	53.5	0.18
	No	12	29.3	10	21.3	12	21.4	19	44.2	
	Yes	3	7.3	2	4.3	4	7.1	1	2.3	
Interleukin-2	Missing	26	63.4	35	74.5	40	71.4	23	53.5	0.11
	No	12	29.3	11	23.4	14	25.0	20	46.5	
	Yes	3	7.3	1	2.1	2	3.6	0	0.0	
BRAF inhibitor	No	38	92.7	43	91.5	55	98.2	38	88.4	0.27
	Yes	3	7.3	4	8.5	1	1.8	5	11.6	
Radiation	No	30	73.2	33	70.2	44	78.6	33	76.7	0.78
	Yes	11	26.8	14	29.8	12	21.4	10	23.3	

CPI, checkpoint inhibitor; TLPLDC, tumor lysate, particle-loaded, dendritic cell; TLPLDC+G, TLPLDC with granulocyte-colony stimulating factor; TLPO, tumor lysate, particle only.

Table 3 Related adverse events for placebo (n=41), TLPLDC (n=47), TLPLDC+G (n=56), and TLPO (n=43) arms

Related adverse events: all are grade 1 or 2 unless indicated	Treatment arm			
	Placebo n=41	TLPLDC n=47	TLPLDC+G n=56	TLPO n=43
Administration site conditions (local injection site reactions)	34	63	19	18
Blood disorders (ie, anemia, elevated mean corpuscular hemoglobin concentration)	1	0	2 (1 grade 3)	0
Cardiac disorders (ie, pericardial effusion)	0	0	0	1
Vestibular disorder or tinnitus	0	0	2	0
Endocrine disorders (ie, autoimmune thyroiditis)	0	0	0	1
Gastrointestinal disorders (eg, diarrhea, nausea, vomiting, dry mouth)	4	0	7	2
General disorders (eg, fatigue, fever)	6	4	19	5
Infection	1 (1 grade 3)	0	2	0
Injury (ie, radiation recall reaction)	1	0	0	0
Other (eg, weight loss, elevated liver enzymes)	3	0	3	0
Metabolism	0	1	0	0
Musculoskeletal and connective tissue disorders (eg, myalgia, arthralgia, weakness)	2	1	5	1
Nervous system disorders (eg, dizziness, headache)	1	0	9	3
Psychiatric disorders (ie, insomnia)	0	0	1	0
Respiratory, thoracic, and mediastinal disorders (eg, pleural effusion, cough)	0	0	2	3
Skin and subcutaneous tissue disorders (eg, pruritus, rash)	2	10	6	4
	Cumulative placebo=55	Cumulative TLPLDC=79	Cumulative TLPLDC+G=77	Cumulative TLPO=38

TLPLDC, tumor lysate, particle-loaded, dendritic cell; TLPLDC+G, TLPLDC with granulocyte-colony stimulating factor; TLPO, tumor lysate, particle only.

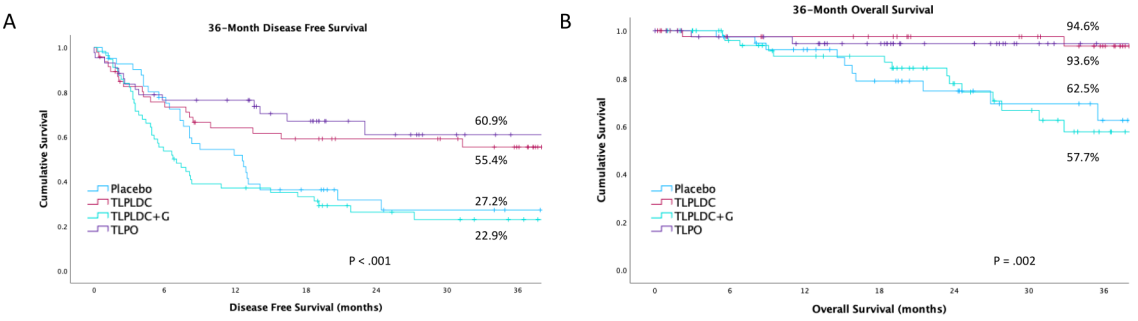
the TLPLDC and TLPO vaccines were extremely well tolerated, with only one related AE ≥grade 3 and two related SAEs in the vaccination arms. While CPIs have changed the landscape of melanoma therapy in the past decade, they can be associated with significant toxicity, particularly when a combination of multiple CPIs is used.[20] Such high toxicity levels may be acceptable in the metastatic setting, where therapeutic options are limited, but less so in the adjuvant setting. As a result, any novel therapy designed to be given in the adjuvant setting in addition to standard therapies must be well tolerated by patients. The addition of the TLPLDC or TLPO vaccine to CPIs as an adjuvant treatment appears to add very limited toxicity, offering promise for these therapies in this setting.

The primary analysis of this study suggested benefit of vaccination, with improved 24-month DFS in a Per

Table 4 Administration site conditions for placebo (n=41), TLPLDC (n=47), TLPLDC+G (n=56), and TLPO (n=43) arms

Administration site conditions	Treatment arm			
	Placebo n=41	TLPLDC n=47	TLPLDC+G n=56	TLPO n=43
Patients with local injection site reactions (eg, erythema, warmth, skin induration, pain)	8	12	8	10
Cumulative local injection site reactions (p<0.001)	34	63	19	18
Local injection site reactions per patient affected	4.25	5.25	2.38	1.80

TLPLDC, tumor lysate, particle-loaded, dendritic cell; TLPLDC+G, TLPLDC with granulocyte-colony stimulating factor; TLPO, tumor lysate, particle only.



Disease Free Survival	Survival Table - Remaining Cases						
	0 mos	6 mos	12 mos	18 mos	24 mos	30 mos	36 mos
Placebo	41	30	20	12	7	5	3
TLPLDC	47	32	26	23	19	17	12
TLPLDC+G	56	30	20	17	9	7	4
TLPO	43	31	29	20	10	5	1

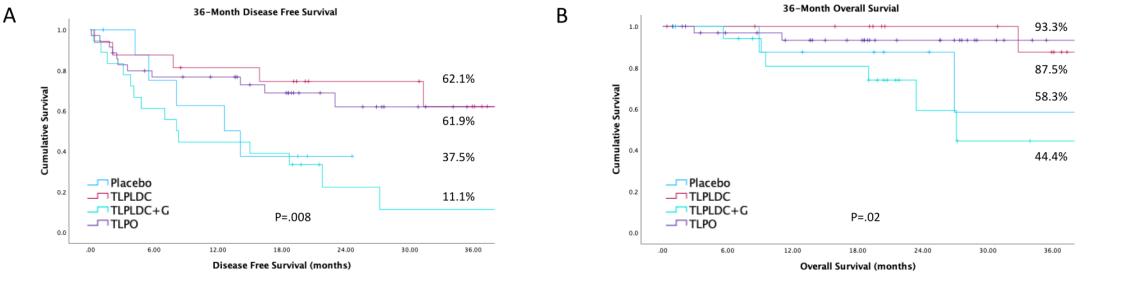
Overall Survival	Survival Table - Remaining Cases						
	0 mos	6 mos	12 mos	18 mos	24 mos	30 mos	36 mos
Placebo	41	37	32	23	18	12	8
TLPLDC	47	38	36	34	29	27	19
TLPLDC+G	56	46	39	36	22	17	8
TLPO	43	35	32	25	15	6	1

Figure 2 Kaplan-Meier curves demonstrating DFS (A) and OS (B) between placebo (n=41), TLPLDC+G (n=56), TLPLDC (n=47), and TLPO (n=43). DFS, disease-free survival; OS, overall survival; TLPLDC, tumor lysate, particle-loaded, dendritic cell; TLPLDC+G, TLPLDC with granulocyte-colony stimulating factor; TLPO, tumor lysate, particle only.

Treatment analysis comparing the TLPLDC vaccine with placebo, but not in the intention-to-treat (ITT) analysis.[3] Subsequently, we found that the use of G-CSF prior to DC harvest negated any potential benefit of the vaccine. Specifically, G-CSF was offered to patients and PIs as an option in the study to facilitate DC harvest using a smaller blood draw. Approximately half the patients in the study elected the G-CSF route. Unfortunately, retrospective RNA-sequencing analysis of DC gene expression suggests that the G-CSF-induced elevated white cell count was composed of many immature cells and that our expedited 72-hour maturation process was insufficient time for the DC to fully mature rendering the TLPLDC+G impotent for T cell stimulation.[21] Prior groups have demonstrated both in vitro and in vivo that immature DCs lack the ability to induce clonal cytotoxic CD8+ T lymphocyte expansion and production of recall antigen-specific CD4+ T lymphocytes.[22 23] Furthermore, in our RNA analysis, the use of G-CSF was associated with higher expression of interleukin 10 producing DCs, which may induce tolerance and regulatory immune T lymphocytes.[24]

After this observation and in discussions with the FDA, the statistical plan was modified and a prespecified analysis was added to the 36-month analysis to assess the outcomes in the TLPLDC group based on the use of G-CSF or not.[16] The TLPLDC+G formulation presented an opportunity to evaluate the relative contribution of components of the vaccines and placebo. TLPLDC+G was composed of TL, YCWPs, and immature DCs, while the placebo had mature DCs, YCWPs, but no TL. TLPLDC had all three. The clinical results suggest that all three components are necessary and DC maturity is essential to the benefit provided by TLPLDC. This difference in maturity between the DC vaccine groups is further evidenced with the observed differences in injection site reactions in TLPLDC versus TLPLDC+G, where the increased immunogenicity of non-G-CSF harvested DC correlated with an increase in cumulative local injection site reactions (table 4). The results of this trial corroborate our prior results regarding the TLPLDC without G-CSF and TLPO vaccines. In this study, the ITT analysis demonstrates prolonged DFS, and most importantly OS, in patients receiving the two active formulations: the TLPLDC (without G-CSF) and TLPO vaccines.

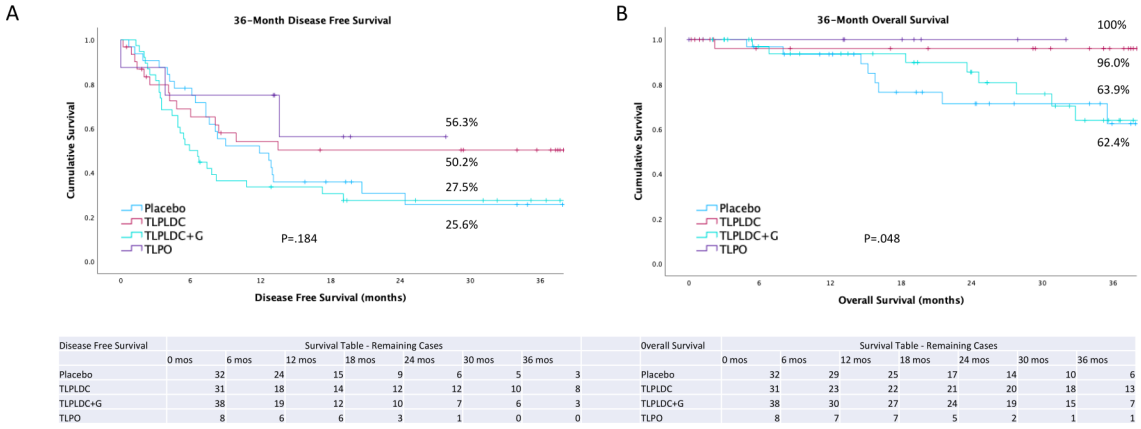
In addition to these overall findings, we performed several prespecified subgroup analyses. The use of the TLPLDC and TLPO vaccines with CPIs, the current standard of care regimen in these high-risk patients, is



Disease Free Survival	Survival Table - Remaining Cases						
	0 mos	6 mos	12 mos	18 mos	24 mos	30 mos	36 mos
Placebo	9	6	5	3	1	0	0
TLPLDC	16	14	12	11	7	7	4
TLPLDC+G	18	11	8	7	2	1	1
TLPO	35	25	23	17	9	5	1

Overall Survival	Survival Table - Remaining Cases						
	0 mos	6 mos	12 mos	18 mos	24 mos	30 mos	36 mos
Placebo	9	8	7	6	4	2	2
TLPLDC	16	15	14	13	9	9	6
TLPLDC+G	18	16	12	12	4	2	1
TLPO	35	28	25	20	13	5	1

Figure 3 DFS (A) and OS (B) for patients receiving CPI therapy (placebo (n=9), TLPLDC (n=16), TLPLDC+G (n=18), and TLPO (n=35)). CPI, checkpoint inhibitor; DFS, disease-free survival; OS, overall survival; TLPLDC, tumor lysate, particle-loaded, dendritic cell; TLPLDC+G, TLPLDC with granulocyte-colony stimulating factor; TLPO, tumor lysate, particle only.



Disease Free Survival	Survival Table - Remaining Cases						
	0 mos	6 mos	12 mos	18 mos	24 mos	30 mos	36 mos
Placebo	32	24	15	9	6	5	3
TLPLDC	31	18	14	12	12	10	8
TLPLDC+G	38	19	12	10	7	6	3
TLPO	8	6	6	3	1	0	0

Overall Survival	Survival Table - Remaining Cases						
	0 mos	6 mos	12 mos	18 mos	24 mos	30 mos	36 mos
Placebo	32	29	25	17	14	10	6
TLPLDC	31	23	22	21	20	18	13
TLPLDC+G	38	30	27	24	19	15	7
TLPO	8	7	7	5	2	1	1

Figure 4 DFS (A) and OS (B) for patients who did not receive CPI therapy (placebo (n=32), TLPLDC (n=31), TLPLDC+G (n=38), and TLPO (n=8)). CPI, checkpoint inhibitor; DFS, disease-free survival; OS, overall survival; TLPLDC, tumor lysate, particle-loaded, dendritic cell; TLPLDC+G, TLPLDC with granulocyte-colony stimulating factor; TLPO, tumor lysate, particle only.

of great interest given the potential synergy between a cancer vaccine that generates a T cell response and CPIs that enhance such a response.[25] A previous subgroup analysis suggested a benefit with combination of the TLPLDC vaccine and CPIs, but this result failed to demonstrate statistical significance.[26] In this analysis, with the benefit of a larger population and longer follow-up, we demonstrate a significantly prolonged DFS and OS in patients treated with TLPLDC or TLPO vaccines who also received CPI therapy, with over 60% of these patients with advanced disease remaining disease free at 3 years. Interestingly, patients receiving the TLPO and TLPLDC vaccines without CPI treatment did not demonstrate an improvement in DFS; however, these patients did show a statistically significant improvement in OS. These results are exploratory in nature and are subgroup analyses only; furthermore, the use of CPI was not stratified, and pertinent details regarding length of CPI therapy were not obtainable for all patients. The results suggest the potential of the TLPO and TLPLDC vaccines to work synergistically with CPIs, which are already standard of care in this setting; however, this will need to be proven in a randomized phase III trial.[25]

This trial shows that the TLPLDC without G-CSF and TLPO are very similar in clinical benefit in both the overall population as well as the subset with vaccine+CPI. However, the TLPO vaccine formulation has significant advantages to the TLPLDC vaccination from both a manufacturing and commercialization perspective. In contrast to the TLPLDC vaccine which requires a patient blood draw, DC harvest and maturation, and ex vivo DC loading, the TLPO vaccine is created from TL-loaded YCWPs capped with silicate and presented to DCs in vivo. The production time for the vaccine series for TLPLDC versus TLPO is 48–72 hours and 14 hours, respectively. No patient blood draws are required for the TLPO formulation, and production labor and cost are substantially reduced. Furthermore, the TLPO vaccine is acellular, translating to other significant benefits such as ease of handling, delivery, and stability and viability of the vaccine. Given these many benefits in combination with our trial results confirming previous findings of equivalent safety and survival outcomes, future trials will proceed with comparison of the TLPO vaccine with placebo in combination with existing standard regimens.

There were several limitations to the study. This is a phase 2B trial with two randomization schemes performed sequentially. The use of G-CSF was non-randomized, and the use of CPIs was not stratified. The resulting four treatments had limited patient numbers. Subsequent phase III trials will be required to test whether the promising findings of TLPO or TLPLDC vaccines is validated in a prospective randomized analysis. As shown in the Consolidated Standards of Reporting Trials, the TLPO and TLPLDC arms had higher rates of withdrawal, but detailed review of patient change of status documentation did not reveal any systematic reasons for this observation. Additionally, the protocol was amended to permit concurrent CPI therapy once FDA approved for use in the adjuvant setting. These concurrent patients were randomized; however, as this was an ongoing study, the ultimate number of patients in each arm with CPI use differed. Additionally, there was a requirement that these patients demonstrate tolerance of the CPI for 3 months prior to randomization which may have introduced some selection bias in the patients later in the study. Given that the TLPO patients were randomized later in the trial, there was a higher proportion of TLPO-treated patients who received CPI compared with the other groups. This was offset to some degree with an over-representation of stage IV patients in the TLPO arm. However, we examined these effects by isolated subgroup analyses, and the benefit of TLPO was confirmed. Additionally, results of the second randomization comparing TLPO and TLPLDC vaccination had no significant differences in receipt of CPI adjuvant therapy between arms and were found to have equivalent outcomes.[17] Nonetheless, these findings must be confirmed in future trials.

CONCLUSION

The final prespecified analysis of this phase 2B trial showed the TLPLDC (without G-CSF) and TLPO vaccines are very well tolerated and were associated with prolonged DFS and OS. Despite the limitations in this study, the results are sufficiently compelling to warrant a phase III trial to prove these benefits. Given the equivalency of the safety and efficacy outcomes between TLPLDC (without G-CSF) and TLPO and the logistical advantages for TLPO, a phase III trial is planned to compare the TLPO vaccine with placebo in combination with current standard of care CPI regimens to prevent melanoma recurrence in high-risk, resected stage III/IV patients.

Author affiliations
[1]Surgery, San Antonio Military Medical Center, Fort Sam Houston, Texas, USA
[2]Department of Surgery, Brooke Army Medical Center, Fort Sam Houston, Texas, USA
[3]General Surgery, Brooke Army Medical Center, Fort Sam Houston, Texas, USA
[4]Surgery, Uniformed Services University, Bethesda, Maryland, USA
[5]Surgical Oncology, Brooke Army Medical Center, Fort Sam Houston, Texas, USA
[6]Department of Surgery, University of Texas Health Sciences Center, San Antonio, Texas, USA
[7]Surgical Oncology, Huntsman Cancer Institute Cancer Hospital, Salt Lake City, Utah, USA
[8]Department of Surgery, Rutgers Cancer Institute of New Jersey, New Brunswick, New Jersey, USA
[9]Surgery, Mayo Clinic, Jacksonville, Florida, USA
[10]Department of Surgery, University of Cincinnati, Cincinnati, Ohio, USA
[11]Medical Oncology, University of Arizona Medical Center-University Campus, Tucson, Arizona, USA
[12]Department of Biological Sciences, Clemson University, Clemson, South Carolina, USA
[13]Orbis Health Solutions, Greenville, South Carolina, USA
[14]Surgical Oncology, Cedars-Sinai Medical Center Angeles Clinic and Research Institute, Los Angeles, California, USA
[15]Cancer Vaccine Development Program, San Antonio, Texas, USA

Twitter Elizabeth Lee Carpenter @elizcarpenter16, Alexandra M Adams @lexyadams16, Robert Connor Chick @connor_chick and James Jakub @DrJamesJakub

Contributors All authors listed have actively participated in the production of this manuscript in any one or more of the following areas: conceptualization, funding acquisition, research, data collection, analysis, methodology, simulation, supervision, verification, draft preparation, revision and editing. GEP serves as guarantor of this manuscript.

Funding This study was sponsored by Elios Therapeutics, Greenville, South Carolina, USA.

Disclaimer The view(s) expressed herein are those of the author(s) and do not reflect the official policy or position of Brooke Army Medical Center, the US Army Medical Department, the Department of the Army, Department of the Air Force, Department of Defense, or the US Government.

Competing interests MF is an advisor for Bristol-Myers Squibb, Sanofi, Array Bioscience and Pulse Bioscience. TEW is an employee of Orbis Health Solutions. GEP is employed by Orbis Health Solutions and Cancer Insight; is a consultant for Rapamycin Holdings, Heat Biologics, Abexxa Biologics, and Pelican Therapeutics; and has received funding from the above as well as Sellas Life Sciences and Genentech. JJ served on a Novartis Melanoma Surgical Oncology Advisory Board. GTC is employed by Parthenon Therapeutics. All remaining authors have declared no conflicts of interest.

Patient consent for publication Not required.

Ethics approval This study involves human participants and was approved by the Western Institutional Review Board (IRB) and site IRBs (protocol 20141932). Participants gave informed consent to participate in the study before taking part. The voluntary, fully informed consent of the subjects used in this research was obtained as required by 32 CFR 219 and DODI 3216.02_AFI40-402.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplemental information.

ORCID iDs
Spencer Van Decar http://orcid.org/0000-0001-5370-8991
Patrick McCarthy http://orcid.org/0000-0002-8805-8148
Timothy Vreeland http://orcid.org/0000-0003-4593-7502

REFERENCES

1 Davar D, Tarhini AA, Kirkwood JM. Adjuvant Immunotherapy of Melanoma and development of new approaches using the Neoadjuvant approach. *Clin Dermatol* 2013;31:237–50.
2 Testori AAE, Chiellino S, van Akkooi ACJ. Adjuvant therapy for Melanoma: past, current, and future developments. *Cancers (Basel)* 2020;12:1994.
3 Vreeland TJ, Clifton GT, Hale DF, *et al*. A phase IIb randomized controlled trial of the TLPLDC vaccine as adjuvant therapy after surgical resection of stage III/IV Melanoma: A primary analysis. *Ann Surg Oncol* 2021;28:6126–37.
4 Eroglu Z, Ribas A. Combination therapy with BRAF and MEK inhibitors for Melanoma: latest evidence and place in therapy. *Ther Adv Med Oncol* 2016;8:48–56.
5 Wahid M, Jawed A, Mandal RK, *et al*. Recent developments and obstacles in the treatment of Melanoma with BRAF and MEK inhibitors. *Crit Rev Oncol Hematol* 2018;125:84–8.
6 Marin-Acevedo JA, Soyano AE, Dholaria B, *et al*. Cancer Immunotherapy beyond immune Checkpoint inhibitors. *J Hematol Oncol* 2018;11:8.
7 Kleponis J, Skelton R, Zheng L. Fueling the engine and releasing the break: combinational therapy of cancer vaccines and immune Checkpoint inhibitors. *Cancer Biol Med* 2015;12:201–8.
8 Handy CE, Antonarakis ES. Sipuleucel-T for the treatment of prostate cancer: novel insights and future directions. *Future Oncology* 2018;14:907–17.
9 Bol K, Bloemendal M, van Willigen W, *et al*. 1078Mo MIND-DC: A randomized phase III trial to assess the efficacy of adjuvant Dendritic cell vaccination in comparison to placebo in stage IIIB and IIIC Melanoma patients. *Annals of Oncology* 2020;31:S732.
10 Ahmed MS, Bae YS. Dendritic cell-based therapeutic cancer vaccines: past, present and future. *Clin Exp Vaccine Res* 2014;3:113–6.
11 Figlin RA, Tannir NM, Uzzo RG, *et al*. Results of the ADAPT phase 3 study of Rocapuldencel-T in combination with Sunitinib as first-line therapy in patients with metastatic renal cell carcinoma. *Clin Cancer Res* 2020;26:2327–36.
12 Ribas A, Camacho LH, Lee SM, *et al*. Multicenter phase II study of matured Dendritic cells pulsed with Melanoma cell line Lysates in patients with advanced Melanoma. *J Transl Med* 2010;8:89.
13 Diane F H, G Travis C, Alan K S, *et al*. Cancer vaccines: should we be targeting patients with less aggressive disease? *Expert Rev Vaccines* 2012.
14 Sahin U, Türeci Ö. Personalized vaccines for cancer Immunotherapy. *Science* 2018;359:1355–60.
15 Herbert GS, Vreeland TJ, Clifton GT, *et al*. Initial phase I/IIa trial results of an Autologous tumor Lysate, particle-loaded, Dendritic cell (TLPLDC) vaccine in patients with solid tumors. *Vaccine* 2018;36:3247–53.
16 O'Shea A, Chick R, Clifton G, *et al*. 310 The effect of pretreatment with G-CSF prior to dendritic cell collection during the phase iib trial of an

autologous DC-based vaccine for advanced, resectable melanoma. 35th Anniversary Annual Meeting (SITC 2020); November 2020
17 Carpenter EL, Adams AM, Chick RC, *et al*. *Society for Melanoma Research 2021 Annual Meeting Abstract: Tumor Lysate Particle Only Vaccine (TLPO) vs. Tumor Lysate Particle-loaded, Dendritic Cell (TLPLDC) Vaccine to Prevent Recurrence in Resected Stage III/IV Melanoma Patients: Results of a Phase I/IIa Trial*. 2021.
18 Adams AM, Chick RC, Vreeland TJ, *et al*. Safety and efficacy of Autologous tumor Lysate particle-loaded Dendritic cell vaccination in combination with systemic therapies in patients with recurrent and metastatic Melanoma. *Melanoma Res* 2021;31:378–88.
19 DeMaria PJ, Bilusic M. Cancer vaccines. *Hematol Oncol Clin North Am* 2019;33:199–214.
20 Kennedy LB, Salama AKS. A review of cancer Immunotherapy toxicity. *CA Cancer J Clin* 2020;70:86–104.
21 Adams AM, Carpenter EL, Clifton GT, *et al*. Divergent clinical outcomes in a phase 2B trial of the TLPLDC vaccine in preventing Melanoma recurrence and the impact of Dendritic cell collection methodology: a randomized clinical trial. *Cancer Immunol Immunother* 2023;72:697–705.
22 Jonuleit H, Giesecke A, Kandemir A, *et al*. Induction of tumor peptide-specific cytotoxic T cells under serum-free conditions by mature human Dendritic cells. *Arch Dermatol Res* 2000;292:325–32.
23 Jonuleit H, Giesecke-Tuettenberg A, Tüting T, *et al*. A comparison of two types of Dendritic cell as Adjuvants for the induction of Melanoma-specific T-cell responses in humans following Intranodal injection. *Int J Cancer* 2001;93:243–51.
24 Sittig SP, de Vries IJM, Schreibelt G. Primary human blood Dendritic cells for cancer Immunotherapy-Tailoring the immune response by Dendritic cell maturation. *Biomedicines* 2015;3:282–303.
25 Vreeland TJ, Clifton GT, Herbert GS, *et al*. Gaining ground on a cure through synergy: combining Checkpoint inhibitors with cancer vaccines. *Expert Rev Clin Immunol* 2016;12:1347–57.
26 Chick RC, Faries MB, Hale DF, *et al*. Multi-institutional, prospective, randomized, double-blind, placebo-controlled phase IIb trial of the tumor Lysate, particle-loaded, Dendritic cell (TLPLDC) vaccine to prevent recurrence in high-risk Melanoma patients: A subgroup analysis. *Cancer Med* 2021;10:4302–11.