Supplemental Material

Efficacy of Pembrolizumab in Patients with Advanced Cancer of Unknown Primary (CUP): A Phase 2 Nonrandomized Clinical Trial

Table of Contents

Supplemental Methods

1. Study Design and Patients

a. *Patients, Treatment and Assessment*

Eligible patients were ≥18 years old, had histologically confirmed advanced rare cancer whose disease had progressed while on standard therapies (if available) within the previous 6 months. Patients were enrolled in ten cohorts: (1) squamous cell carcinoma (SCC) of the skin, (2) anaplastic thyroid carcinoma, later revised to small cell malignancies of non-pulmonary origin, (3) adrenocortical carcinoma (ACC), (4) medullary renal cell carcinoma, (5) carcinoma of unknown primary (CUP), (6) penile carcinoma, (7) thymic carcinoma, later revised to vascular sarcoma, (8) testicular cancer, later relabeled as germ cell tumor, (9) paraganglioma–pheochromocytoma, and (10) other rare tumor histologies (excluding tumor types included in NCT02054806).[1] Patient had to have Response Evaluation Criteria in Solid Tumors-version1.1 (RECISTv1.1) measurable disease except for patients in cohorts 9 and 10 who could have evaluable disease, Eastern Cooperative Oncology Group performance status (ECOG PS) of 0/1 and normal organ functions. Patients with prior immunotherapy, active autoimmune disease, concurrent malignancy, and active/untreated brain metastases were excluded. Details are provided in study protocol (Supplement-Protocol).

- *Diagnostic Criteria for CUP:* All patients in the CUP cohort met clinical and histologic criteria for CUP.

These patients were required to have had a comprehensive work-up performed as per standard CUP guidelines (National Comprehensive Cancer Network [NCCN] and European Society of Medical Oncology [ESMO]) to confirm a CUP diagnosis, including uniform clinical, radiographic and pathology review performed at MD Anderson Cancer Center. Since molecular profiling for tissue-of-origin in not routinely recommended for CUP, this was not a mandate on the protocol. Prior treatments were allowed as per discretion of treating physicians as per guidelines. Similarly, biomarker therapies based on tumor sequencing were allowed, except immunotherapy, as per discretion of treating physicians.

All eligible patients received 200 mg of pembrolizumab intravenously every 3 weeks until documented disease progression, unacceptable adverse event(s), investigator's decision to stop treatment, withdrawal of consent, or completion of 24 months of treatment with pembrolizumab. No dose modifications were allowed, but in the event of adverse events dose delays were permitted. Patients who had radiographic progression but were clinically stable were allowed to continue pembrolizumab until progressive disease (PD) was confirmed by imaging at least 4 weeks after the first PD assessment.

Tumor radiographic assessment was performed 9 weeks for the first 6 months then every 12 weeks thereafter and at discretion of treating physician if patients had had complete response (CR), partial response (PR), or stable disease (SD) for >27 weeks. Tumor measurements were performed by Institutional Quantitative Imaging Analysis

Core. Radiographic assessment was performed as per immune-related RECIST (irRECIST) and was applied to direct clinical management.[2] The irRECIST criteria were used to capture immunotherapy specific response patterns. Due to possibility of pseudo-progression, irRECIST requires confirmation for PD by repeat consecutive assessment at least 4 weeks after first documentation of PD. New lesions which define progression by RECISTv1.1 do not define progression by irRECIST and are instead included in the sum of measurements to assess changes of tumor burden. In case of confirmation of PD, the day of first documentation of PD was considered as the actual date of PD. Additionally, patients who achieved either CR or PR needed confirmation of this response by repeat radiographic assessment in at least 2 consecutive scans. Patients with confirmed CR could discontinue pembrolizumab after receiving at least 24 weeks of treatment. No dose modifications were allowed. Adverse events (AEs) were assessed from the date of initiation of protocol therapy until ≥28 days after last dose and were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events-version4.0.03.12 (CTCAEv4.0). Details are provided in study protocol (Supplement-Protocol).[1]

b. Trial Design, Statistical Methods and Endpoints

This phase 2 study was designed as an open-label, single-center, multicohort trial. The protocol and all amendments were approved by the University of Texas M.D. Anderson Institutional Review Board (IRB). The study was conducted in accordance with Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines. Patients provided written informed consent before study enrollment.

The primary end point was non-progression rate (NPR) at 27 weeks, defined as proportion of patients who were alive and progression-free at 27 weeks. The study used Simon's optimal two-stage design. In the first stage, if at ≥3 of first 12 patients had NPR at 27 weeks, an additional 13 were allowed to enroll. If >7 of the 25 total evaluable patients in a cohort were alive and progression-free at 27 weeks, the drug would have been considered worthy of further study in that cohort. The null success rate was set at 20% and the alternative at 40% for each cohort. This design had a 10% Type I error rate, 82% power and 56% probability of stopping after the first stage if the true 27-week PFS rate was 20%. Details are provided in study protocol (Supplement-Protocol). Key pre-specified secondary endpoints were objective response rate (ORR), clinical benefit rate (CBR) [percentage of patients with irCR, irPR, or irSD (stable disease) ≥4 months], duration of response (DoR), progression-free (PFS), overall survival (OS) and safety. Exploratory objectives were to examine tissue correlates for clinical activity.

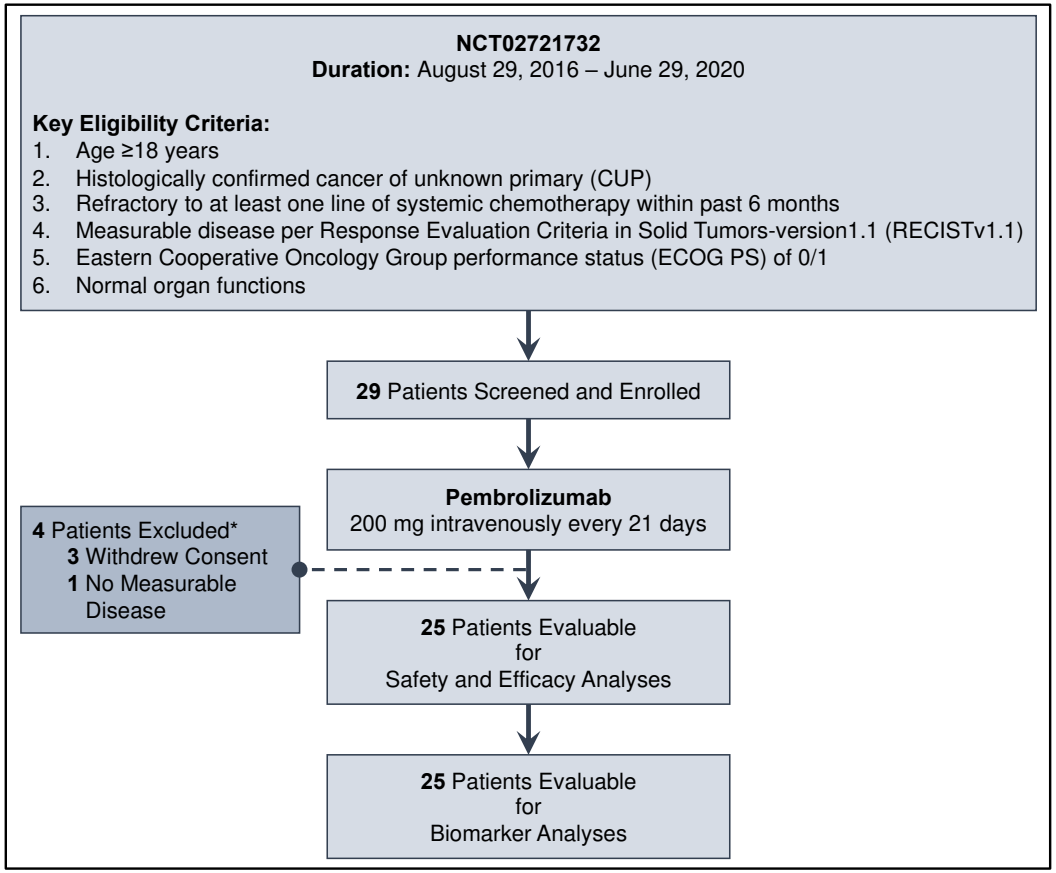
Patient who met all eligibility criteria and received at least one dose of pembrolizumab were included in the primary safety and efficacy analysis. Patients who withdrew consent were excluded from any analyses. Data are reported as of January 5, 2021. Descriptive statistics were used. Clopper and Pearson method was used to calculate exact 95% confidence intervals (95%CI) for proportions. Time to event outcomes (PFS, OS) were estimated using Kaplan-Meier method. Fisher's-exact or Chi-squared test (when appropriate) were used for comparing proportions across groups. Unpaired non-parametric Mann-Whitney (Wilcoxon rank sum) test was used to compare means (and medians) between two distinct groups. Statistical analysis was performed using GraphPad Prism version 8.0.0

(GraphPad Software, San Diego, CA USA) and SPSS Statistics version 25.0 (IBM SPSS Software, Armonk, NY USA).

c. Biomarker Analyses

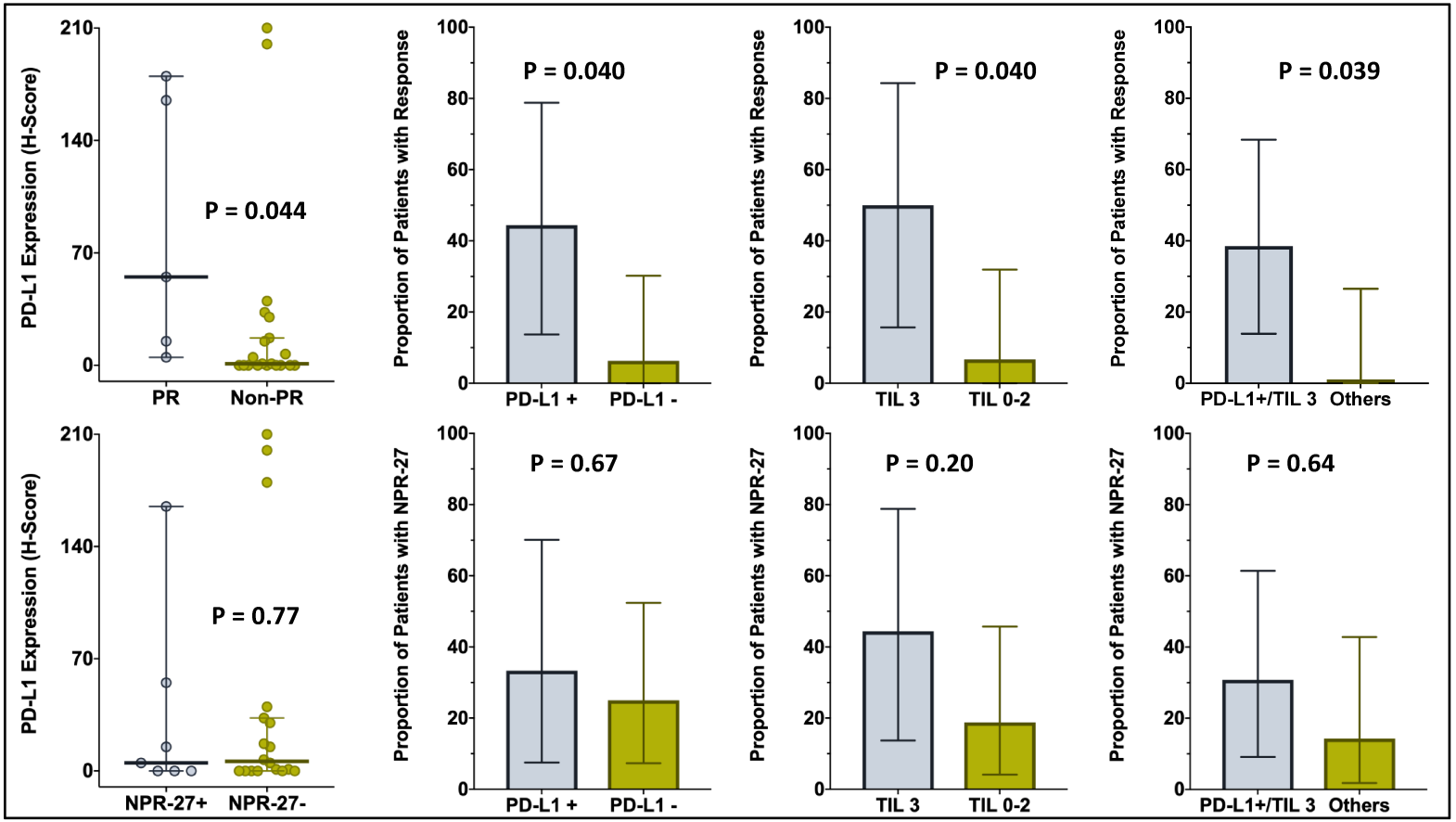
Biomarker analyses were performed on fresh tissue sample obtained at baseline or an archival tissue sample. Programmed cell death ligand-1 (PD-L1) expression was assessed using immunohistochemistry (IHC) at a central laboratory on formalin-fixed paraffin-embedded (FFPE) tissue sections using Merck 22C3 antibody. The intensity of PD-L1 membranous staining on tumor and mononuclear inflammatory cells in tumor nests were assessed. Tissue sections with membranous PD-L1 staining of 3+ or 2+ in ≥5% cells were considered PD-L1-positive. PD-L1 expression was also reported as H-score (sum of product of percentage and intensity of membrane staining) and ranged from 0 to 300. Tumor infiltrating lymphocytes (TILs) within tumor nests were scored on a scale from 0 to 3 with 0 being absence of TILs, 1 being a few TILs, 2 being moderate number of TILs and 3 being intense intratumoral lymphocytic infiltration. For dichotomous distinction (positive vs. negative), TIL was considered positive if TIL score was 3. Details are provided in study protocol (Supplement-Protocol).

Figure S1. Study flowchart



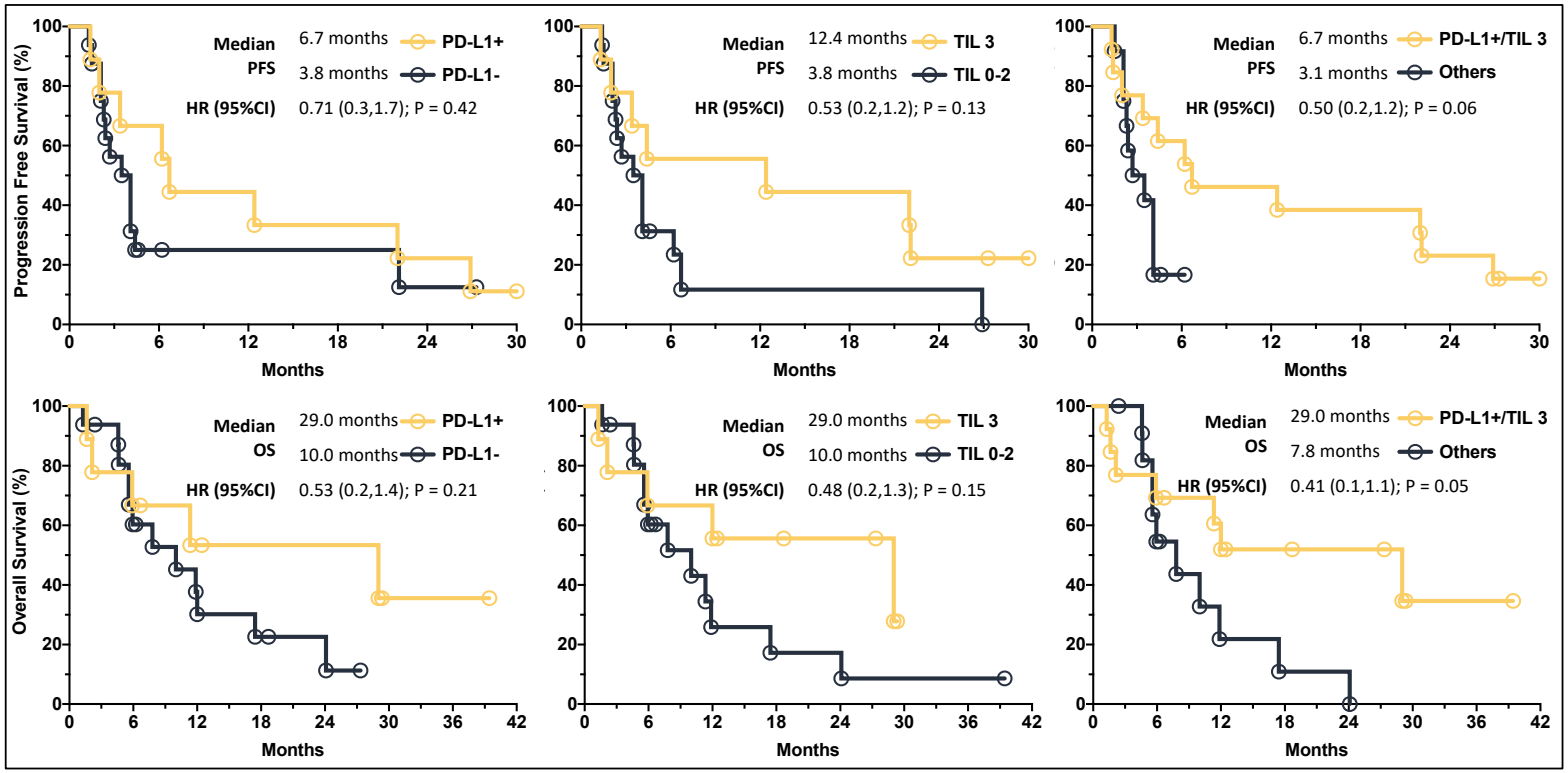
*** Note:** Among 4 patients that were excluded from the analyses: 1 patient had no measurable disease (only evaluable disease – small FDG avid lymph node metastases) was considered an eligibility failure. This patient remained on therapy for 38 months with stable disease and then progressed. Among 3 patients that withdrew consent, 2 withdrew consent after first dose (reason: patient 1 – unknown as patient lost to follow-up and patient 2 – burden and cost of travel) and were unevaluable for any efficacy outcomes. The remaining 1 patient withdrew consent after 4 doses with first scan after dose 3 showing irPD (unconfirmed) for reason of burden of travel. This patient was unevaluable for efficacy outcomes due to loss to follow-up. None of these patients reported grade ≥3 AEs and 2 of these patients had grade 1 (constipation, hyponatremia, nausea, hyperuricemia, hyperkalemia, increased creatinine, diarrhea, maculo-papular rash) and grade 2 (hypothyroidism) AEs. Also see eTable 1.

Figure S2. Biomarker analysis showing association between PD-L1 and TILs and response outcomes.



Abbreviations: *NPR-27, non-progression rate at 27 weeks; PD-L1, programmed cell death ligand-1; PR, partial response; +, positive; -, negative; TIL(s), tumor infiltrating lymphocytes score*

Figure S3. Biomarker analysis showing association between PD-L1 and TILs and survival outcomes.



Abbreviations: *95%CI, 95% confidence interval; HR, hazard ratio; OS, overall survival; P, p-value; PFS, progression-free survival; PD-L1, programmed cell death ligand-1; +, positive; -, negative; TIL(s),* t*umor infiltrating lymphocytes score*

Table S1. Key clinical trial outcomes and analyses for intent-to-treat population (N = 29) (including patients excluded from primary analysis due to ineligibility or withdrawal of consent).

Outcome	Evaluable Population (N)	Events of Interest	Results	Reason for Exclusion
Efficacy				
NPR-27	29	8	27.6% (95%CI: 12.7-47.2)	• None excluded
ORR per irRECIST	26	5	19.2% (95%CI: 6.5-39.4)	• No measurable disease per RECIST (N = 1) • No second scan for restaging (N = 2)
CBR per irRECIST	26	11	42.3% (95%CI: 23.4-63.1)	• No measurable disease per RECIST (N = 1) • No second scan for restaging (N = 2)
PFS (median)	29	23	4.1 m (95%CI: 2.9-5.2)	• None excluded
OS (median)	29	19	11.3 m (95%CI: 5.6-17.1)	• None excluded
Safety				
All TRAEs	29	21	72%	• None excluded
TRAEs ≥ Grade 3	29	4	14%	• None excluded

Abbreviations: *95%CI, 95% confidence interval; HR, hazard ratio; m, months; N, number of patients; NPR-27, non-progression rate at 27 weeks; ORR, objective response rate as per irRECIST (immune-related RECIST); OS, overall survival; P, p-value; PFS, progression-free survival*

Table S2. Subgroup analysis showing association between key clinical subgroups and outcomes.

Characteristics	N (25)	NPR-27 (%)	P	ORR (%)	P	mPFS (m)	PFS HR (95%CI; P)	mOS	OS HR (95%CI; P)
All eligible and evaluable	25	28.0		20.0		4.1	(3.1 – 5.1)	11.3	(5.5 – 17.1)
Age at enrollment									
< 60 years	13	23.1	0.67	23.1	>0.99	4.1	1.08	11.3	0.72
≥ 60 years	12	33.3		16.7		4.1	(0.5,2.5; 0.85)	8.9	(0.3,1.9; 0.49)
Sex									
Female	18	27.8	>0.99	22.2	>0.99	4.2	0.58	11.9	0.69
Male	7	28.6		14.3		2.7	(0.2,1.6; 0.22)	5.9	(0.2,2.2; 0.47)
Tumor histology									
Adenocarcinoma	9	22.2	0.73	11.1	0.24	4.1	0.88	7.8	0.38
Undifferentiated carcinoma	14	28.5		21.4		5.3		12.0	
Squamous cell carcinoma	2	50.0		50.0		14.1		20.4	
Time from diagnosis to trial									
< 6 months	6	0.0	0.20	16.7	0.90	5.2	0.33	11.3	0.58
6 months – 1.5 years	9	33.3		22.2		3.5		5.9	
≥ 1.5 years	10	40.0		20.0		4.2		12.0	
Prior lines of therapy									
1	8	25.0	0.94	25.0	0.69	6.4	0.72	17.4	0.80
2/3	11	27.3		18.2		2.7		5.6	
4/5	6	33.3		16.7		4.2		11.9	

Abbreviations: *95%CI, 95% confidence interval; HR, hazard ratio; mOS, median overall survival; mPFS, median progression-free survival; N, number of patients; NPR-27, non-progression rate at 27 weeks; ORR, objective response rate as per irRECIST; P, p-value*

Supplemental References

1. Naing A, Meric-Bernstam F, Stephen B, et al. Phase 2 study of pembrolizumab in patients with advanced rare cancers. *J Immunother Cancer* 2020;8(1) doi: 10.1136/jitc-2019-000347 [published Online First: 2020/03/20]

2. Nishino M, Tirumani SH, Ramaiya NH, et al. Cancer immunotherapy and immune-related response assessment: The role of radiologists in the new arena of cancer treatment. *Eur J Radiol* 2015;84(7):1259-68. doi: 10.1016/j.ejrad.2015.03.017 [published Online First: 2015/05/06]