

# Efficacy of pembrolizumab in patients with advanced cancer of unknown primary (CUP): a phase 2 non-randomized clinical trial

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## ABSTRACT

**Background** Cancer of unknown primary (CUP) is an aggressive rare malignancy with limited treatment options. Data regarding clinical activity of immune checkpoint inhibitors in CUP is lacking. Therefore, we evaluated the efficacy of pembrolizumab, a programmed cell death-1 inhibitor, in patients with CUP.

**Methods** The study was designed as a phase 2 basket trial for independent rare tumor cohorts including CUP. Adult patients with CUP who had progressed on previous systemic therapy, performance status 0/1 and measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST V.1.1) were eligible. Patients received pembrolizumab (200 mg) intravenously every 21 days. Twenty-nine patients were enrolled and treated between August 2016 and June 2020. The primary endpoint was non-progression rate (NPR) at 27 weeks (NPR-27) per immune-related RECIST. Key prespecified secondary endpoints were confirmed objective response rate (ORR), safety, duration of response (DoR), progression-free survival (PFS) and overall survival (OS). Pretreatment biopsies were examined for biomarkers of response (programmed cell death ligand-1 (PD-L1) expression and tumor infiltrating lymphocytes (TILs)).

**Results** Among 25 (of 29 enrolled) eligible and evaluable patients, 14 (56%) had poorly differentiated carcinoma. Patients received a median of two lines of therapy prior to enrollment. Median follow-up was 27.3 months. NPR-27 was observed in seven patients (28.0% (95% CI: 12.1 to 49.4)). ORR was 20.0% (95% CI: 6.8 to 40.7) with five patients achieving immune-related partial response with median DoR of 14.7 months (95% CI: 9.8 to 19.6). Median PFS and OS were 4.1 (95% CI: 3.1 to 5.1) and 11.3 (95% CI: 5.5 to 17.1) months, respectively. Treatment-related adverse events of any and grade  $\geq 3$  were seen in 19 (76%) and 4 (16%) patients, respectively. One (4%) patient had grade 3 immune-related acute kidney injury requiring treatment discontinuation. Neither PD-L1 nor TILs were associated with NPR-27. Both positive PD-L1 staining (44.4% vs 6.3%;  $p=0.040$ ) and intense TIL infiltration (44.4% vs 6.3%;  $p=0.040$ ) were associated with response.

**Conclusion** Pembrolizumab showed encouraging efficacy in patients with CUP with acceptable safety profile.

**Trial registration number** NCT02721732.

## Key messages

### What is already known on this topic

⇒ Empirical treatment of carcinoma of unknown primary (CUP) is mostly guided by histopathologic evaluation to identify the tissue of origin. However, the prospect of meaningful treatment outcome remains unclear. In recent years, programmed cell death-1/programmed cell death ligand-1 pathway inhibitors such as pembrolizumab has demonstrated durable responses in certain tumor types and is well tolerated. Thus, we reasoned that these drugs may be effective and safe in patients with other rare cancers, for which treatment options are lacking.

### What this study adds

⇒ This is the first phase 2 study of pembrolizumab in patients with carcinoma of unknown primary. Non-progression rate at 27 weeks (primary endpoint) was 28%. Objective response was seen in 20%, disease control in 44% of evaluable patients and 28% were progression free at 27 weeks.

### How this study might affect research, practice and/or policy

⇒ Pembrolizumab has encouraging clinical activity with an acceptable safety profile in patients with previously treated CUP.

## INTRODUCTION

Cancer of unknown primary site (CUP), a clinically heterogeneous group of metastatic tumors wherein the primary site of origin remains occult after complete clinical evaluation, is a rare cancer with an annual incidence of 4.1/100,000 in the USA.<sup>1 2</sup> Despite advances in treatment of metastatic cancers with known primaries, survival with currently available therapies (empirical or site-specific) in CUP is dismal with 1-year survival rate of <50%.<sup>3-5</sup> Effectiveness of treatments beyond first-line is limited with response rate of

8%–13% and median survival of 4–5 months; and a critical need to develop novel therapies exists.<sup>6–8</sup>

While immune checkpoint inhibitors (ICIs) have shown efficacy for diverse cancers, access for patients with CUP is restricted to those with high microsatellite-instability (MSI-H) and tumor-mutation burden (TMB-H), seen in 1.8% and 11.8% cases, respectively.<sup>5,9</sup> PD-L1 (programmed cell death ligand-1), a predictive biomarker of ICI response in certain cancers, is expressed in 22% of CUP.<sup>9,10</sup> Gene-expression profiling has revealed presence of immune-permissive/sensitive CUP subsets.<sup>11</sup> We performed a phase 2 trial to assess efficacy and safety of pembrolizumab, a humanized anti-programmed cell death 1 (PD-1) monoclonal antibody, in patients with previously treated CUP (subcohort of a large multicohort trial in rare tumors).<sup>12</sup>

## METHODS

### Patients, treatment and assessment

Eligible patients were  $\geq 18$  years old and met clinical and histologic criteria for CUP. All patients had comprehensive work-up performed as per standard CUP guidelines (National Comprehensive Cancer Network and European Society of Medical Oncology to confirm a CUP diagnosis, including uniform clinical, radiographic and pathology review performed at MD Anderson Cancer Center (online supplemental methods).<sup>5,13</sup> All patients were refractory to at least one line of systemic chemotherapy within past 6 months and had Response Evaluation Criteria in Solid Tumors V.1.1 (RECIST V.1.1) measurable disease, Eastern Cooperative Oncology Group performance status of 0/1 and normal organ functions. Patients with prior immunotherapy and autoimmune disease were excluded.

Patients received fixed dose of pembrolizumab (200 mg) intravenously every 3 weeks until disease progression, unacceptable toxicity, completion of 24 months of treatment or withdrawal of consent. No dose modifications were allowed. Imaging studies were performed every 9 weeks or earlier if clinically indicated and response to treatment was assessed using immune-related RECIST (irRECIST).<sup>14</sup> Objective response, defined as immune-related complete (irCR) or partial response (irPR), was confirmed by repeat imaging at least 4 weeks after criteria for response were first met. Treatment-related adverse events (TRAEs) were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events V.4.0.03.12.

Biomarker analysis on pretreatment fresh biopsy or archival tumor sample was done at a central laboratory. PD-L1 expression on tumor and mononuclear inflammatory cells in tumor nests were assessed at a central laboratory by immunohistochemistry using Merck 22C3 antibody. PD-L1 positivity was defined as membranous PD-L1 expression of 3+ staining intensity or 2+ in  $\geq 5\%$  cells. H-scores ranging from 0 to 300 were also calculated using the standard formula:  $(1 \times \% \text{ of cells with } 1+ \text{ staining intensity}) + (2 \times \% \text{ of cells with } 2+ \text{ staining}$

$\text{intensity}) + (3 \times \% \text{ of cells with } 3+ \text{ staining intensity})$ .<sup>15</sup> Tumor infiltrating lymphocytes (TILs) were scored from 0 (no TILs), 1 (few TILs), 2 (moderate TILs) to 3 (intense TILs) infiltration. Details are provided in study protocol (online supplemental protocol and methods).<sup>12</sup>

### Trial design, statistical methods and endpoints

This phase 2 study was an open-label, single-center, multicohort trial (online supplemental figure S1).<sup>12</sup> The primary end point was non-progression rate at 27 weeks (NPR-27), defined as proportion of patients alive and progression-free at 27 weeks per irRECIST. Key prespecified secondary endpoints were objective response rate (ORR), clinical benefit rate (CBR) (percentage of patients with irCR, irPR, or immune-related stable disease  $\geq 4$  months), duration of response (DoR), progression-free survival (PFS), overall survival (OS) and safety. Exploratory objectives were to examine tissue correlates for clinical activity, specifically association of baseline PD-L1 expression and TIL status and ORR and NPR-27. Details are provided in study protocol (online supplemental protocol and methods).

## RESULTS

### Patient characteristics

Between August 29, 2016, and June 29, 2020, 29 patients were enrolled and treated with pembrolizumab and 25 were eligible and evaluable (online supplemental figure S1). Baseline characteristics are shown in [table 1](#). Median age was 59 years (range: 33–78). Most patient were women (72%) and had poorly differentiated carcinoma (56%). Patients had received a median of two lines of therapy prior to enrollment.

### Efficacy analyses

At data cut-off on January 5, 2021, the median follow-up for the evaluable cohort (N=25) was 27.3 months (95% CI: 17.7 to 36.9) and 2 (8%) patients continued treatment. Reasons for discontinuation were disease progression (20 (80%)), toxicity (1 (4%)), and completion of  $>34$  cycles of treatment (2 (8%)). Among 25 evaluable patients, the primary endpoint NPR-27 was 28.0% (95% CI: 12.1 to 49.4) (7 patients) ([figure 1A](#)). Five patients had irPR resulting in ORR of 20.0% (95% CI: 6.8 to 40.7) with median DoR of 14.7 months (95% CI: 9.8 to 19.6) in responders and a CBR of 44.0% (95% CI: 24.4 to 65.1) ([figure 1B](#)). Median PFS and OS were 4.1 (95% CI: 3.1 to 5.1) and 11.3 (95% CI: 5.5 to 17.1) months, respectively ([figure 1C,D](#)). Efficacy outcomes did not differ significantly between key subgroups or for intent-to-treat population (online supplemental tables S1 and S2).

### Safety analyses

TRAEs of any grade were reported by 19 (76%) patients ([table 2](#)). Grade 3 TRAEs occurred in 4 (16%) patients. No grade 4/5 events occurred. One (4%) patient had

**Table 1** Baseline patient characteristics

Characteristics	Patients (N=25)	%
Age at enrollment (years)		
Median (range)	59 (33–78)	
<60 years	13	52
≥60 years	12	48
Sex		
Female	18	72
Male	7	28
Eastern Cooperative Oncology Group performance status		
0	0	0
1	25	100
Tumor histology		
Adenocarcinoma	9	36
Undifferentiated carcinoma	14	56
Squamous cell carcinoma	2	8
Time to trial since first diagnosis (years)		
Median (range)	0.9 (0.3–4.5)	
<6 months	6	24
6 months–1 year	8	32
1 year–2 years	5	20
≥2 years	6	24
Number of previous anticancer lines of treatment		
Median (range)	2 (1–5)	
1	8	32
2	7	28
3	4	16
4/5	6	24
Mismatch-repair (MMR)/microsatellite status (N=11)		
Proficient-MMR/microsatellite-stable	11	100
Deficient-MMR/microsatellite-instability high	0	0
PD-L1 status (H-score)		
0	9	36
1–150	12	48
150–300	4	16
PD-L1 expression status*		
Positive	9	36
Negative	16	64
Tumor infiltrating lymphocytes (TILs) infiltration score		
0 (no TILs)	1	4
1 (few TILs)	11	44
2 (moderate infiltration of TILs)	4	16
3 (intense infiltration of TILs)	9	36

\*Programmed cell death ligand-1 (PD-L1) expression was considered positive if immunohistochemistry was 3+ or 2+ in ≥5% cells.

grade 3 immune-related acute kidney injury and required treatment discontinuation.

### Correlative analyses

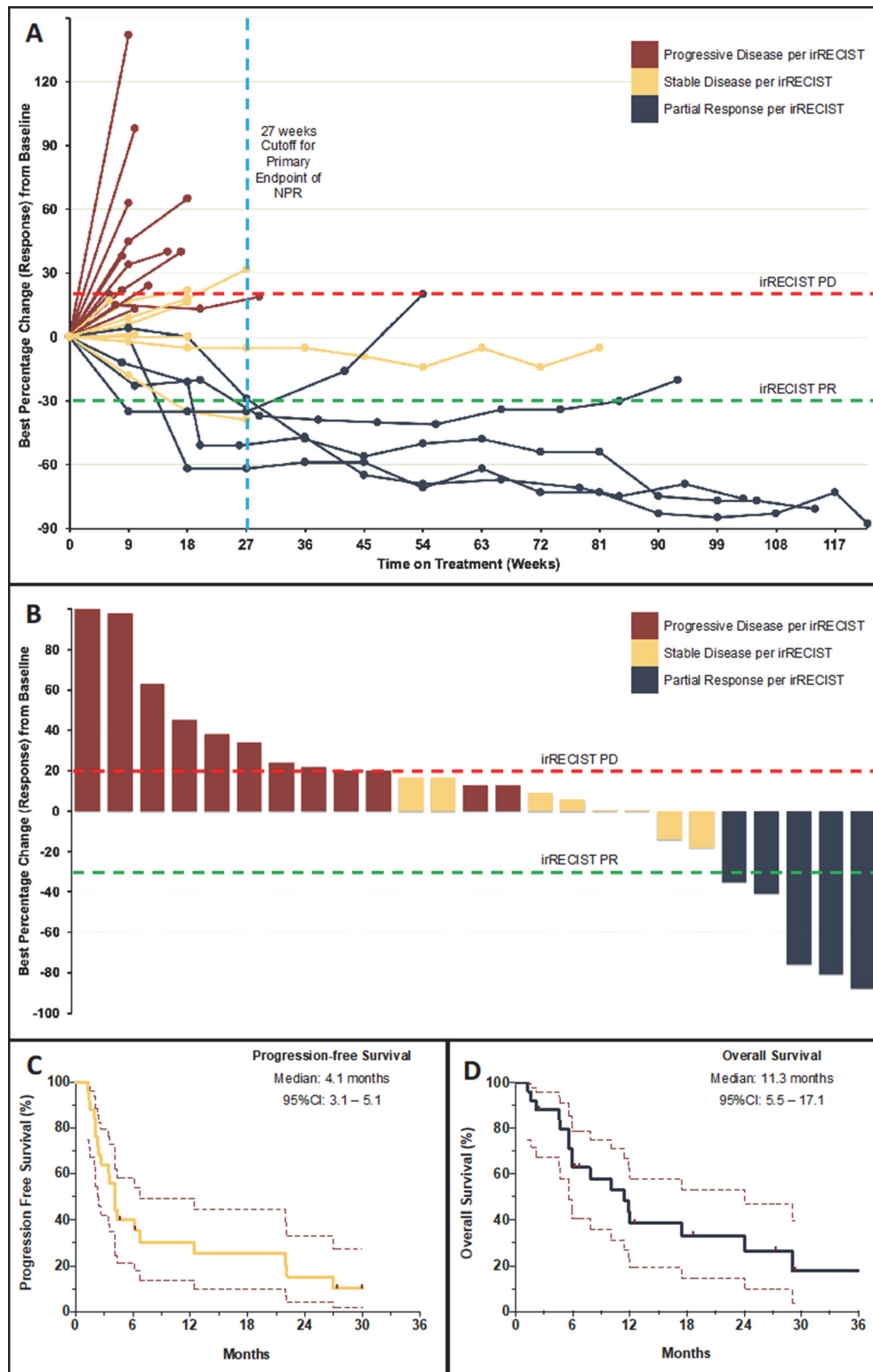
PD-L1 and TIL status was determined in all cases and distribution is shown in [table 1](#). PD-L1 H-score was higher in responders (median 55 vs 1;  $p=0.044$ ) compared with non-responders and a positive PD-L1 staining appeared to be associated with response (44.4% vs 6.3%;  $p=0.040$ ). TIL score of 3 was associated with response (44.4% vs 6.3%;  $p=0.040$ ). Patients with either positive PD-L1 or TIL 3 score had higher ORR (38.5% vs 0.0%;  $p=0.039$ ) compared with those who lacked both these features. Neither PD-L1 nor TILs were associated with NPR-27, PFS or OS (online supplemental figures S2,S3).

### DISCUSSION

CUP is a life-threatening malignancy with limited treatment options. Although role of immunotherapy in CUP is evolving, there is no approved or recommended immunotherapy for this patient population. We report on the first clinical study using single agent pembrolizumab, a PD-L1 inhibitor, in this population. In this study, pembrolizumab demonstrated an NPR-27 and ORR of 28% and 20%, respectively, in treatment refractory CUP with a safety profile consistent with prior reports. Although only five patients had a PR, four of those responses were durable and lasted in excess of 12 months. This activity is similar to that seen with nivolumab in CUP (NivoCUP trial) where the ORR was 24%.<sup>16</sup>

Clinically, CUP is a very heterogenous disease.<sup>2</sup> In our cohort, the seven patients that achieved NPR-27 had undifferentiated carcinoma, adenocarcinoma and squamous cell carcinoma in four, two and one cases, respectively. All but one of these patients had lymph node metastases and none had bone metastases.<sup>17</sup> Notably, of these patients that achieved an irPR, two had immunophenotype consistent with putative Mullerian profile (PAX8 +ER+), one had p16+ (squamous cell carcinoma) and one had a urothelial profile (GATA3+, thrombomodulin+). Although, a small subset of patients with CUP who have MSI-H and high TMB now have The United States Food and Drug Administration (FDA)- approved access to pembrolizumab, there is still a critical need to exploit the role of immunotherapy in a large subset.<sup>5,9,11</sup> Our study is limited in exploring the clinical subtypes of CUP but algorithms integrating clinical, pathological and molecular profiles may indicate putative profiles that are most likely to benefit from immunotherapy. Moreover, genomic-profiling guided assessment of tissue-of-origin, with further refinement, may help selection of patients for immunopermissive tumor types, adding an additional facet to site-directed therapy in CUP.<sup>18</sup>

Our study has certain limitations due to its small sample size and single-center design which lends itself to referral and selection biases that can mar generalizability to an unselected patient population with CUP. Larger



**Figure 1** Tumor response and survival outcomes on pembrolizumab in patients with cancer of unknown primary. (A) (Spider plot) shows the change in sum of target lesion diameters over time in 23 evaluable patients who were treated on the current study and underwent at least one radiological restaging evaluation (two patients had clinical progression prior to first restaging and are reported as default 20% increase). Two patients had unequivocal progression of non-target lesions and were considered as cases with progressive disease (PD). (B) (Waterfall-plot) shows the maximum per cent change from baseline as measured by immune-related Response Evaluation Criteria in Solid Tumors (irRECIST). Partial response (PR) was defined by  $\geq 30\%$  decrease in tumor burden and PD was defined by  $\geq 20\%$  increase in tumor burden, confirmed on a consecutive scan at least 4 weeks apart. (C and D) (Kaplan-Meier curves) show progression-free survival and overall survival of patients on study at the time of data cut-off measured from treatment initiation to disease progression/death and death, respectively. Data from patients without an event were censored at date of last follow-up (marks). NPR, non-progression rate.

**Table 2** Treatment-related adverse events

Adverse event*	All grades† (%)	Grade ≥3 (%)
Fatigue	6 (24)	
Hypothyroidism	5 (20)	
Maculo-papular rash	4 (16)	1 (4)
Aspartate aminotransferase increased	3 (12)	
Alanine aminotransferase increased	2 (8)	
Anorexia	2 (8)	
Diarrhea	2 (8)	1 (4)
Acute kidney injury‡	1 (4)	1 (4)
Arthralgia	1 (4)	
Bullous dermatitis	1 (4)	
Creatinine increased	1 (4)	
Fever	1 (4)	
Hyperthyroidism	1 (4)	
Pneumonitis‡	1 (4)	1 (4)
Skin infection	1 (4)	

\*Treatment-related adverse events (TRAEs) listed here include all those that occurred on study patients regardless of grade and all grade ≥3 events. All TRAEs are coded and graded as per the Common Terminology Criteria for Adverse V.4.0.

†No grade 4 or 5 TRAEs occurred on study.

‡Two (8%) patients had treatment-related serious adverse events.

studies are required to further these findings. However, the single-center enrollment did allow us to ensure the fidelity of CUP diagnosis with rigorous review, which can be a concerning issue with CUP as seen with CUPISCO multicenter trial.<sup>19</sup> Although our exploratory biomarker analyses are limited by size and are hypothesis generating, they lend some important insights. Both PD-L1 and TIL was associated with response. No response was seen with PD-L1 H-score <5 and most patients with a response had intense TIL infiltration. All responders were either PD-L1 positive or had a TIL score 3. While TMB status is currently unknown in this study, its predictive ability for ICI response, especially in light of the accelerated FDA approval of pembrolizumab for treatment of patients with metastatic TMB-High (≥10 mutations/megabase) solid tumors that have progressed following prior treatment in June 2020, makes it important to include these biomarkers in future studies of ICI in CUP.<sup>20</sup> Ongoing correlative studies with whole-exome sequencing for this study will shed further light on biomarkers of response and resistance, in addition to identifying actionable pathways for tumor characterization and other biomarker-directed targeted therapies.

In conclusion, pembrolizumab was well-tolerated and had encouraging (although somewhat limited) efficacy as a single agent in patients with CUP refractory to prior systemic therapy; though patient selection for PD-L1

expression and TILs may enrich for patients more likely to derive clinical benefit. Further trials using combination of ICI and immunomodulators are needed.

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**Patient consent for publication** Not applicable.

**Ethics approval** The protocol was approved by the FDA and the Institutional Review Board at The University of Texas MD Anderson Cancer Center. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. All the study participants provided written informed consent before enrollment.

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**Data availability statement** Data are available upon reasonable request. The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request and approval from study sponsor according to available guidelines at time of request.

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

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**Author note** This paper is dedicated to GRV memory.

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