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ORIGINAL ARTICLE

Open-label phase II study of the efficacy of nivolumab for cancer of unknown primary

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Background: Cancer of unknown primary (CUP) has a poor prognosis. Given the recent approval of immune checkpoint inhibitors for several cancer types, we carried out a multicenter phase II study to assess the efficacy of nivolumab for patients with CUP.

Patients and methods: Patients with CUP who were previously treated with at least one line of systemic chemotherapy constituted the principal study population. Previously untreated patients with CUP were also enrolled for exploratory analysis. Nivolumab (240 mg/body) was administered every 2 weeks for up to 52 cycles. The primary endpoint was objective response rate in previously treated patients as determined by blinded independent central review according to RECIST version 1.1.

Results: Fifty-six patients with CUP were enrolled in the trial. For the 45 previously treated patients, objective response rate was 22.2% [95% confidence interval (CI), 11.2% to 37.1%], with a median progression-free survival and overall survival of 4.0 months (95% CI, 1.9-5.8 months) and 15.9 months (95% CI, 8.4-21.5 months), respectively. Similar clinical benefits were also observed in the 11 previously untreated patients. Better clinical efficacy of nivolumab was apparent for tumors with a higher programmed death-ligand 1 expression level, for those with a higher tumor mutation burden, and for microsatellite instability-high tumors. In contrast, no differences in efficacy were apparent between tumor subgroups based on estimated tissue of origin. Adverse events were consistent with the known safety profile of nivolumab. No treatment-related death was observed.

Conclusions: Our results demonstrate a clinical benefit of nivolumab for patients with CUP, suggesting that nivolumab is a potential additional therapeutic option for CUP.

Key words: nivolumab, primary unknown cancer, CUP

INTRODUCTION

Cancer of unknown primary (CUP) is defined as histologically confirmed metastatic cancer for which identification of the primary site is not possible after an appropriate diagnostic approach.[1] CUP accounts for 2%-5% of all diagnosed cancers, with an incidence of 5.3 to 19 cases per 100 000 people worldwide.[2] The prognosis of such patients is poor, with a median survival time of 6-12 months. Most (∼80%) patients with CUP are categorized into an unfavorable subset and receive empirical chemotherapy, including platinum-taxane regimens.[2-8] Given that CUP is a heterogeneous clinical entity, its treatment remains problematic and not well developed.

Cancer immunotherapy including the administration of immune checkpoint inhibitors (ICIs) has markedly changed the treatment paradigm for many types of cancer, including malignant melanoma, non-small-cell lung cancer, gastroesophageal cancer, genitourinary cancer, and head and neck cancer.[9] Our recent immune profiling of CUP with the use of immunohistochemistry (IHC) and analysis of gene expression suggested that individuals with CUP might receive clinical benefit from ICI treatment because their immune

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profiles are similar to those of patients with ICI-responsive malignancies.[10] Limited data, however, have been available regarding the clinical efficacy of ICIs for patients with CUP.[10-12] We here present the results of an investigator-initiated phase II trial of nivolumab, an antibody to programmed cell death protein 1 (PD-1), in patients with CUP.

PATIENTS AND METHODS

Study design and patients

The trial was designed as a nonrandomized, open-label, multicenter, investigator-initiated phase II study of nivolumab in patients with CUP (UMIN database registration number UMIN000030649). Eligibility criteria included a diagnosis of CUP with mandatory pathological evaluation including IHC as well as chest-abdomen-pelvis computed tomography, positron emission tomography, gastroscopy, colonoscopy, and medical examination before study enrollment. Medical examination included gynecology consultation for women; breast examination in women with adenocarcinoma; urological examination in patients with a main lesion in the peritoneum, retroperitoneum, or inguinal region; and otolaryngological examination in those with squamous cell carcinoma. Pathological analysis was carried out to exclude malignant melanoma, malignant lymphoma, sarcoma, and neuroendocrine tumor. Additional key inclusion criteria were measurable disease according to RECIST version 1.1;[13] an Eastern Cooperative Oncology Group performance status of 0 or 1; a tissue sample obtained no more than 1 year before enrollment; at least one prior line of systemic therapy including platinum-containing regimens for previously treated patients; and adequate organ function as defined by a hemoglobin level of $\geq$8.0 g/dl, an absolute neutrophil count of $\geq$1000/mm^3, a platelet count of $\geq$100 000/mm^3, serum transaminase levels of $\leq$3.0 times the upper limit of normal, a serum total bilirubin concentration of $\leq$1.5 mg/dl, and a serum creatinine level of $\leq$1.5 times the upper limit of normal. Key exclusion criteria included concurrent autoimmune disease or a history of chronic or recurrent autoimmune disease; a history of ICI [anti-PD-1, anti-programmed death-ligand 1 (anti-PD-L1), or anti-cytotoxic T-lymphocyte antigen 4] therapy or treatment with any T cell-stimulatory antibody; and CUP of the favorable subset, including extragonadal germ cell syndrome, neuroendocrine carcinoma, adenocarcinoma restricted to axillary lymph nodes (women), peritoneal carcinomatosis (women), or squamous carcinoma limited to cervical, supraclavicular, or inguinal lymph nodes. Patients for whom curative surgery or radiation therapy was suitable were also excluded.

The protocol was approved by the institutional review board at each site (Supplementary material, available at https://doi.org/10.1016/j.annonc.2021.11.009), and the study was conducted in accordance with the provisions of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. All patients provided written informed consent before study entry.

Procedures

All enrolled patients received intravenous nivolumab 240 mg every 2 weeks for up to 52 cycles ($\sim$2 years) or until disease progression, unacceptable toxicity, or study withdrawal. Patients who discontinued treatment for reasons other than progression were followed until progression, initiation of a new anticancer therapy, withdrawal of consent, or loss to follow-up. Patients could continue to receive treatment beyond disease progression if they met prespecified criteria, as described in Supplementary material, available at https://doi.org/10.1016/j.annonc.2021.11.009.

Radiologic imaging was carried out every 6 weeks. Confirmatory imaging was conducted at least 4 weeks after the initial documentation of a response. Tumor response was assessed according to RECIST version 1.1. Adverse events (AEs) were monitored throughout the study and for 28 days after discontinuation of study treatment, and they were graded by investigators according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.

Prediction of primary site and assessment of tumor characteristics

Primary site prediction was carried out as previously described.[14] Microsatellite instability (MSI) testing with an assessment method based on the quasi-monomorphic variation range was carried out as previously described.[15,16] MSI status was classified as MSI-high on the basis of the presence of two or more unstable markers, as MSI-low for only one unstable marker, and as microsatellite stable with no unstable markers.[17] The tumor mutation burden (TMB) scores computed by the workflow of the Ion Reporter 5.10 using the Oncomine Tumor Mutation Load w2.0 workflow (Thermo Fisher Scientific, Waltham, MA). Methods for IHC and sequencing analysis are described in Supplementary methods, available at https://doi.org/10.1016/j.annonc.2021.11.009.

Outcomes

The primary endpoint was objective response rate (ORR) in previously treated patients as determined by blinded independent central review (BICR) according to RECIST version 1.1. ORR was defined as the proportion of patients with a confirmed complete response (CR) or partial response (PR), with confirmation by repeat consecutive assessment $\geq$4 weeks from the date the response was first documented. Key secondary endpoints included ORR according to RECIST version 1.1 as determined by investigator assessment, disease control rate (proportion of patients with a CR, a PR, or stable disease), duration of response (DoR, time from response to progression), progression-free survival (PFS, time from the date of the first dose of study medication to disease progression or death from any cause), overall survival (OS, time from the date of the first dose of study medication to death), and PFS and OS rates at 6, 12, 18, and 24 months, all in previously treated patients and in the overall population, as well as safety and tolerability among all treated patients. These endpoints were also

evaluated for previously untreated patients. Additional exploratory endpoints included outcome according to the level of PD-L1 expression (cut-offs of 1%, 10%, and 50%) or tumor-infiltrating lymphocyte (TIL) density in tumor samples.

Statistical analysis

The required sample size was calculated to be 38 subjects, under the assumptions that the expected ORR was 20% and the threshold ORR was 5%, with a significance level of 2.5% (one-sided) and power of 80%, according to the exact method based on the binomial distribution. The target sample size was thus set to 45 subjects who had been treated previously. For exploratory analysis, an additional 11 patients who had not been treated previously were enrolled in the study. The analysis set for efficacy and safety included all enrolled patients who received at least one dose of nivolumab. Kaplan—Meier survival curves were constructed for PFS and OS. Hazard ratios and associated 95% confidence intervals (CIs) were calculated by Cox proportional hazards models. Tumor responses were compared according to PD-L1 tumor proportion score (TPS) or combined positive score (CPS) at cut-offs of 1%, 10%, and 50%, as well as according to median TIL density, with the use of Fisher's exact test. Statistical analysis was carried out with the use of SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Patients

Between 19 February 2018 and 9 August 2019, a total of 56 patients with CUP, including 45 previously treated and 11 previously untreated individuals, were enrolled at 10 sites in Japan. All enrolled patients were included in efficacy and safety analysis. Patient and disease characteristics are summarized in Table 1 and Supplementary Tables S1-S3, available at https://doi.org/10.1016/j.annonc.2021.11.009. Most previously treated patients had received carboplatin plus paclitaxel, with nivolumab constituting second-line treatment for 57.8% of all previously treated individuals. At the data cut-off for this analysis (9 February 2020), the median follow-up duration was 8.4 months (range, 1.1-21.6 months) for previously treated patients and 17.2 months (range, 0.1-21.3 months) for previously untreated patients. Treatment was ongoing in seven previously treated patients (15.6%). A total of 38 previously treated patients and all 11 untreated patients had discontinued nivolumab, largely as a result of disease progression (Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2021.11.009).

Table 1. Baseline demographics and disease characteristics

	Previously treated *n* (%)	Previously untreated *n* (%)
Median age (range), years	66.0 (39-80)	64.0 (52-80)]
Sex		
Male	17 (37.8)	5 (45.5)
Female	28 (62.2)	6 (54.5)
ECOG performance status		
0	10 (22.2)	6 (54.5)
1	35 (77.8)	5 (45.5)
Histology		
Well or moderately differentiated adenocarcinoma	6 (13.3)	2 (18.2)
Poorly differentiated adenocarcinoma	11 (24.4)	2 (18.2)
Adenocarcinoma (differentiation unknown)	12 (26.7)	3 (27.3)
Squamous cell carcinoma	6 (13.3)	1 (9.1)
Undifferentiated carcinoma	5 (11.1)	0 (0.0)
Other	5 (11.1)	3 (27.3)
Number of metastatic sites		
1	20 (44.4)	6 (54.5)
2	8 (17.8)	4 (36.4)
≥3	17 (37.8)	1 (9.1)
Metastatic sites		
Liver	12 (26.7)	0 (0.0)
Lung	14 (31.1)	4 (36.4)
Bone	9 (20.0)	0 (0.0)
Pleura	5 (11.1)	1 (9.1)
Peritoneum	10 (22.2)	1 (9.1)
Skin	1 (2.2)	1 (9.1)
Brain	3 (6.7)	0 (0.0)
Adrenal gland	4 (8.9)	0 (0.0)
Lymph node	33 (73.3)	9 (81.8)
Other	11 (24.4)	2 (18.2)
Lymph nodes only		
Yes	14 (31.1)	5 (45.5)
No	31 (68.9)	6 (54.5)
Location of positive lymph nodes		
Neck	4 (8.9)	5 (45.5)
Supraclavicular	9 (20.0)	6 (54.5)
Axillary	5 (11.1)	1 (9.1)
Mediastinal	12 (26.7)	6 (54.5)
Intraperitoneal (abdominal)	23 (51.1)	6 (54.5)
Inguinal	7 (15.6)	2 (18.2)
Retroperitoneal	4 (8.9)	0 (0.0)
Other	3 (6.7)	0 (0.0)
Prior lines of chemotherapy		
1	26 (57.8)	
2	9 (20.0)	
≥3	10 (22.2)	
Prior radiotherapy		
Yes	10 (22.2)	0 (0.0)
No	35 (77.8)	11 (100.0)

ECOG, Eastern Cooperative Oncology Group.

Efficacy

Among the 45 previously treated patients, 2 (4.4%) individuals had a confirmed CR and 8 (17.8%) had a confirmed PR, yielding an ORR of 22.2% (95% CI, 11.2% to 37.1%), according to RECIST version 1.1 by BICR, which met the primary endpoint (Figure 1A and Table 2). The median DoR was 12.4 months (range, 2.8 to >8.4 months). Among the 11 previously untreated patients, the ORR was 18.2% (95% CI, 2.3% to 51.8%), with 1 confirmed CR (9.1%) and 1 confirmed PR (9.1%) by BICR (Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2021.11.009 and Table 2). The median DoR was 3.7 months (range, 2.3-5.1 months). For the total patient population, the ORR was thus 21.4% (12 of 56 patients, with a 95% CI of 11.6% to 34.4%). A long-lasting reduction in tumor size was apparent in a proportion of responders (Figure 1B and Supplementary Figure S3, available at https://doi.org/10.

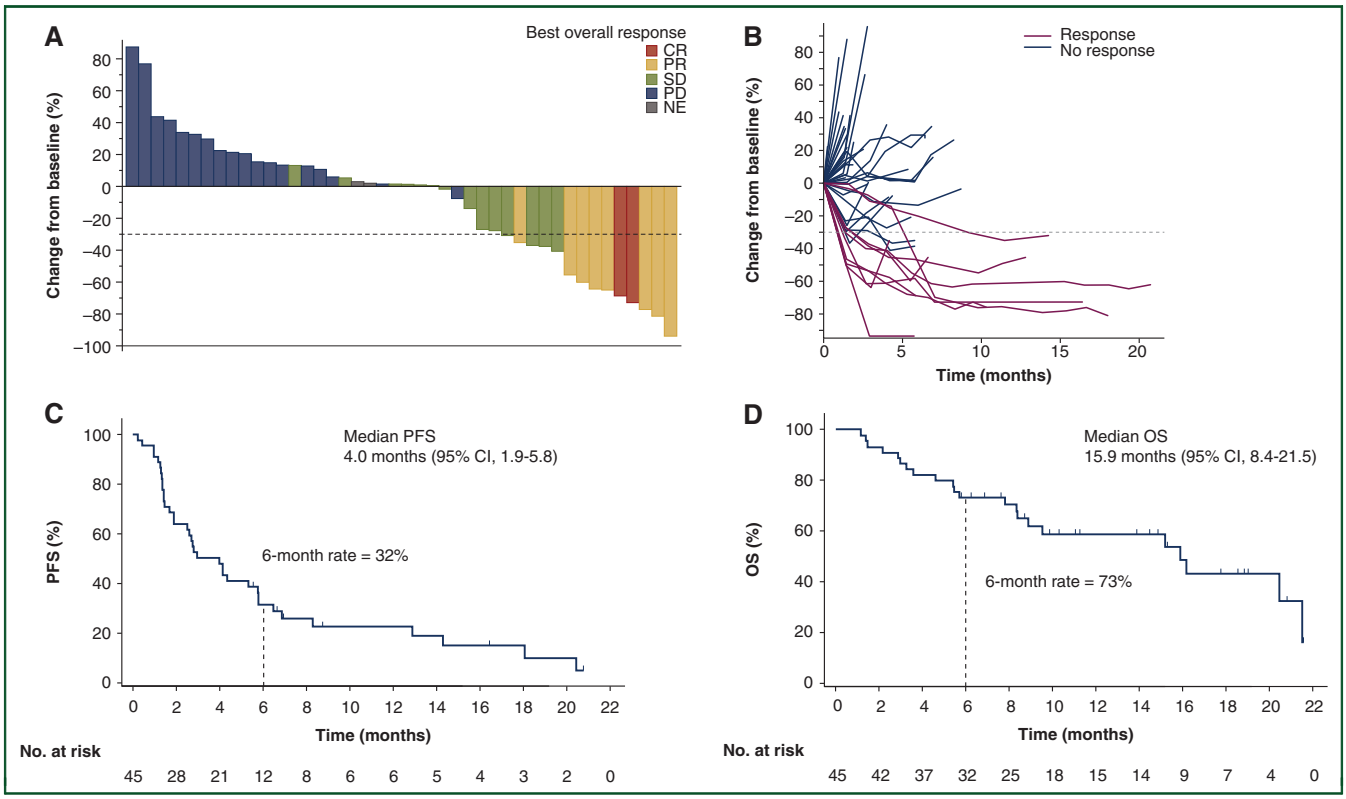


Figure 1. Clinical efficacy of nivolumab treatment in previously treated patients with CUP as assessed by blinded independent central review (RECIST version 1.1).
(A) The best percentage change in target lesion size relative to baseline. The values represent the largest percentage change in the sum of the longest diameters for each patient with a measurable tumor. Patients are color-coded according to response. (B) Longitudinal changes in target lesion size from baseline. (C), (D) Kaplan–Meier curves for PFS and OS, respectively. Six-month survival rates are also indicated.
CI, confidence interval; CR, complete response; CUP, cancer of unknown primary; NE, not evaluable; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

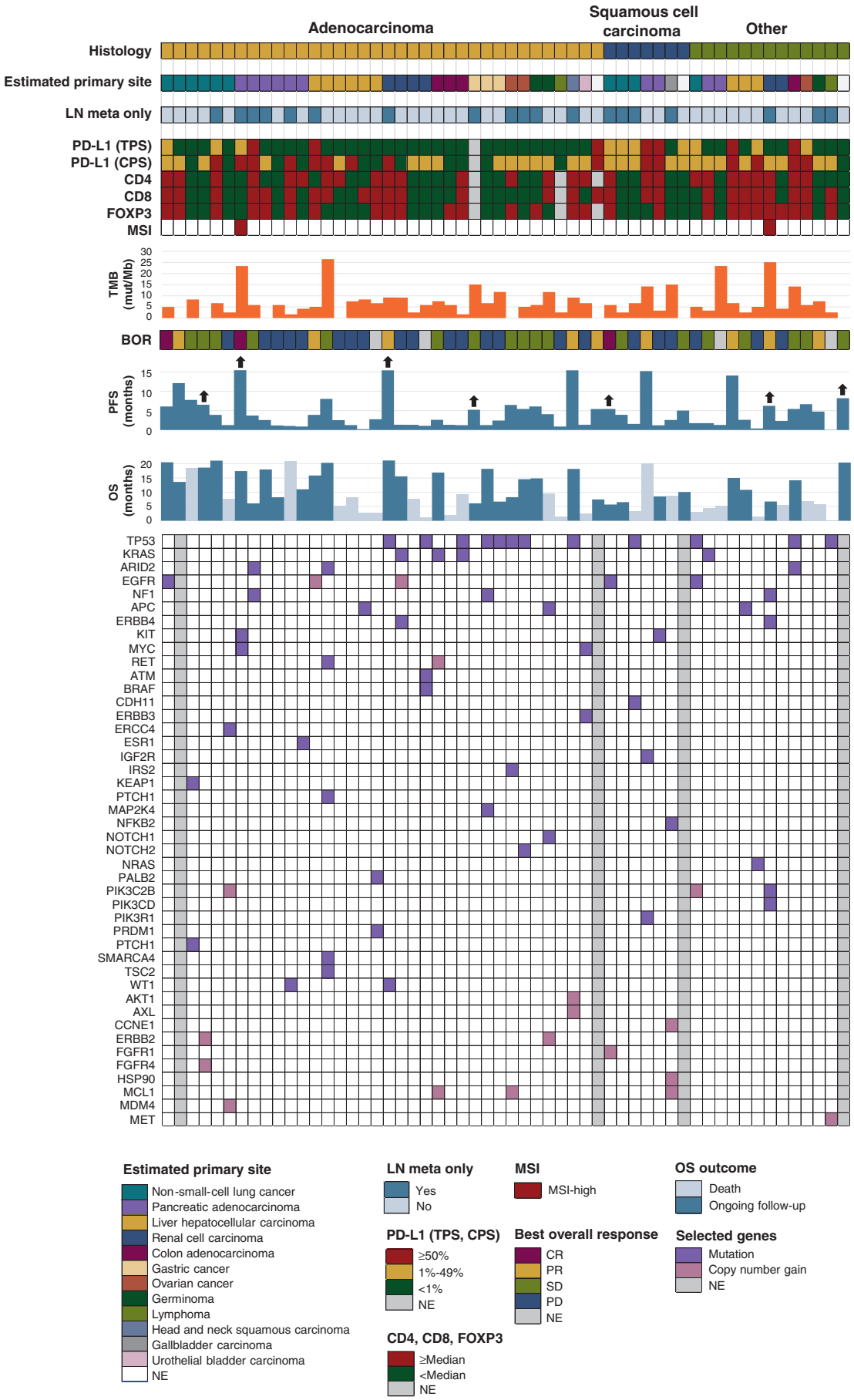
1016/j.annonc.2021.11.009). Investigator assessment and central assessment of response were highly concordant (Table 2 and Supplementary Figure S4, available at https://doi.org/10.1016/j.annonc.2021.11.009).

Among the 45 previously treated patients, there were 37 (82.2%) PFS events and 22 (48.9%) overall OS events (according to RECIST version 1.1 by BICR) at the data cut-off of 9 February 2020. The median PFS was 4.0 months (95% CI, 1.9-5.8 months), with an estimated 6-month PFS rate of 32% (Figure 1C). The median OS was 15.9 months (95% CI, 8.4-21.5 months), with an estimated 6-month OS rate of 73% (Figure 1D). Among the 11 previously untreated patients, there were 11 (100%) PFS events and 4 (36.4%) OS events at the data cut-off. The median PFS was 2.8 months (95% CI, 1.1-6.5 months), with an estimated 6-month PFS rate of 27% (Supplementary Figure S5, available at https://doi.org/10.1016/j.annonc.2021.11.009). The median OS was not reached (95% CI, 2.6 months to not reached), with an estimated 6-month OS rate of 73% (Supplementary Figure S5, available at https://doi.org/10.1016/j.annonc.2021.11.009). PFS analysis based on investigator assessment is shown in Supplementary Figure S6, available at

Table 2. Best overall response according to RECIST version 1.1

	All (n = 56)		Previously treated (n = 45)		Previously untreated (n = 11)	
	BICR	Investigator assessment	BICR	Investigator assessment	BICR	Investigator assessment
Objective response rate, n (%)	12 (21.4)	12 (21.4)	10 (22.2)	11 (24.4)	2 (18.2)	1 (9.1)
(95% CI for %)	(11.6–34.4)	(11.6–34.4)	(11.2–37.1)	(12.9–39.5)	(2.3–51.8)	(0.2–41.3)
Best overall response, n (%)						
Complete response	3 (5.4)	2 (3.6)	2 (4.4)	2 (4.4)	1 (9.1)	0 (0.0)
Partial response	9 (16.1)	10 (17.9)	8 (17.8)	9 (20.0)	1 (9.1)	1 (9.1)
Stable disease	18 (32.1)	20 (35.7)	14 (31.1)	14 (31.1)	4 (36.4)	6 (54.5)
Progressive disease	22 (39.3)	20 (35.7)	18 (40.0)	17 (37.8)	4 (36.4)	3 (27.3)
Nonevaluable	4 (7.1)	4 (7.1)	3 (6.7)	3 (6.7)	1 (9.1)	1 (9.1)
Disease control rate, n (%)	30 (53.6)	32 (57.1)	24 (53.3)	25 (55.6)	6 (54.5)	7 (63.6)
(95% CI for %)	(39.7–67.0)	(43.2–70.3)	(37.9–68.3)	(40.0–70.4)	(23.4–83.3)	(30.8–89.1)

BICR, blinded independent central review; CI, confidence interval.


Adenocarcinoma
Squamous cell carcinoma
Other
Histology
Estimated primary site
LN meta only
PD-L1 (TPS)
PD-L1 (CPS)
CD4
CD8
FOXP3
MSI
TMB (mut/Mb)
BOR
PFS (months)
OS (months)
TP53
KRAS
ARID2
EGFR
NF1
APC
ERBB4
KIT
MYC
RET
ATM
BRAF
CDH11
ERBB3
ERCC4
ESR1
IGF2R
IRS2
KEAP1
PTCH1
MAP2K4
NFKB2
NOTCH1
NOTCH2
NRAS
PALB2
PIK3C2B
PIK3CD
PIK3R1
PRDM1
PTCH1
SMARCA4
TSC2
WT1
AKT1
AXL
CCNE1
ERBB2
FGFR1
FGFR4
HSP90
MCL1
MDM4
MET
Estimated primary site
Non-small-cell lung cancer
Pancreatic adenocarcinoma
Liver hepatocellular carcinoma
Renal cell carcinoma
Colon adenocarcinoma
Gastric cancer
Ovarian cancer
Germinoma
Lymphoma
Head and neck squamous carcinoma
Gallbladder carcinoma
Urothelial bladder carcinoma
NE
LN meta only
Yes
No
MSI
MSI-high
OS outcome
Death
Ongoing follow-up
PD-L1 (TPS, CPS)
≥50%
1%-49%
<1%
NE
Best overall response
CR
PR
SD
PD
NE
Selected genes
Mutation
Copy number gain
NE
CD4, CD8, FOXP3
≥Median
<Median
NE

https://doi.org/10.1016/j.annonc.2021.11.009. Analysis of the clinical efficacy of nivolumab for the total patient population is provided in Supplementary Figure S7, available at https://doi.org/10.1016/j.annonc.2021.11.009.

Treatment outcome according to tumor characteristics

We next evaluated the clinical efficacy of nivolumab according to tumor characteristics, including histology, gene alterations and predicted tissue of origin based on next-generation sequencing (NGS) results,[14] metastatic pattern (lymph node only or other), as well as known biomarkers for ICIs such as PD-L1 expression, MSI, and TMB (Figure 2 and Supplementary Figure S8, available at https://doi.org/10.1016/j.annonc.2021.11.009).

The ORR, PFS, and OS according to histological subgroup are shown in Supplementary Table S4 and Supplementary Figure S9, available at https://doi.org/10.1016/j.annonc.2021.11.009, with nivolumab efficacy being apparent across histological types. Patients with nodal metastasis only have previously been found to show a more favorable prognosis than those with extranodal metastasis.[10,18] Among our study population, 19 individuals (33.9%) showed a metastatic pattern in which lesions were limited to multiple lymph nodes. A higher ORR and longer median PFS and OS were evident in patients with this metastatic pattern than in those without it (Supplementary Figure S10 and Supplementary Figure S5, available at https://doi.org/10.1016/j.annonc.2021.11.009).

Molecular tumor profiling is a developing diagnostic technique that allows prediction of a tumor site of origin for CUP. Several methods, including those based on microarray analysis or reverse transcription and real-time polymerase chain reaction analysis, have shown potential for such identification of original sites.[19] We carried out a tissue of origin analysis based on gene expression and gene alteration data obtained by NGS, which predicted a tissue of origin for 53 patients, with 12 different sites predicted (Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2021.11.009). On the basis of the predicted tumor types, these patients were categorized into subgroups with tumors that are relatively sensitive or responsive to site-directed treatment (colorectal, breast, ovarian, kidney, prostate, bladder, non-small-cell lung, and germ cell cancers as well as lymphoma) or with less responsive tumor types (biliary tract, pancreatic, gastroesophageal, liver, cervical, endometrial, and head and neck cancer), with such categorization of CUP having previously been found to predict patient survival.[14,20] No clear difference in survival or response was detected between these subgroups (Figure 3A and B and Supplementary Table S7, available at https://doi.org/10.1016/j.annonc.2021.11.009), suggesting that nivolumab is of benefit even in individuals diagnosed with a type of CUP for which site-directed therapy is expected to have a limited efficacy.

We next categorized patients according to whether ICI monotherapy had been approved for their predicted tissue of origin by the Japanese Ministry of Health, Labor, and Welfare, the European Medicines Agency, or the United States Food and Drug Administration (FDA) as of 31 March 2020.[21-23] The ICI-approved group included patients whose estimated primary sites were non-small-cell lung, liver, kidney, gastroesophageal, ovarian, head and neck, or bladder cancer or lymphoma. Survival outcome for nivolumab treatment did not differ according to such ICI approval status (Supplementary Figure S11, available at https://doi.org/10.1016/j.annonc.2021.11.009). ORR according to these subgroups is shown in Supplementary Table S7, available at https://doi.org/10.1016/j.annonc.2021.11.009.

Tumor samples from 55 of the total of 56 patients were available for evaluation of the relation between nivolumab efficacy and PD-L1 expression as determined by IHC. The proportion of patients with a PD-L1 TPS or CPS of $\geq$1% was 30.9% and 78.2%, respectively. The ORR as determined by BICR for these patients was 41.2% and 27.9%, respectively, whereas that for patients with a PD-L1 expression level lower than this cut-off was 13.2% and 0%, respectively (Supplementary Tables S8 and S9, available at https://doi.org/10.1016/j.annonc.2021.11.009). ORR as well as PFS and OS according to all the prespecified PD-L1 TPS and CPS cut-offs are provided in Figure 3C and D, Supplementary Tables S8 and S9, and Supplementary Figures S12 and S13, available at https://doi.org/10.1016/j.annonc.2021.11.009. Supplementary Tables S10 and Supplementary Figure S14, available at https://doi.org/10.1016/j.annonc.2021.11.009, show ORR, PFS, and OS according to CD4+, CD8+, or FOXP3+ TIL density. Higher CD4+ and CD8+ TIL levels tended to be associated with a better response and survival.

TMB was evaluated in 50 patients, with the median value being 7.75 mutations/megabase (mut/Mb). Patients with a high TMB ($\geq$7.75 mut/Mb) were found to have a better ORR as well as longer PFS and OS compared with those with a low TMB (Figure 3E and F and Supplementary Table S11, available at https://doi.org/10.1016/j.annonc.2021.11.009). Among 42 patients whose tumors were available for assessment of MSI, 2 (4.8%) had MSI-high tumors. The estimated primary sites of these patients were pancreatic adenocarcinoma and renal cell carcinoma, and they had TMB values of 23.4 and 25.1 mut/Mb, respectively. These two patients experienced a PR, with a DoR of 461 and 117 days, respectively, and both remained on the study

Figure 2. Individual treatment outcome and response for all 56 patients according to tumor characteristics.
From top to bottom are presented: histology; estimated primary site; metastatic pattern of multiple lymph nodes (LN meta) only; expression of PD-L1 (TPS and CPS) as well as CD4+, CD8+, or FOXP3+ TIL density; MSI status; TMB (mutations/Mb); best objective response by blinded independent central review (RECIST version 1.1); PFS; OS color-coded by OS outcome; and distribution of selected gene alterations. Black arrows for PFS indicate ongoing response.
BOR, best objective response; CPS, combined positive score; CR, complete response; MSI, microsatellite instability; NE, not evaluable; OS, overall survival; PD, progressive disease; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PR, partial response; SD, stable disease; TIL, tumor-infiltrating lymphocyte; TMB, tumor mutation burden; TPS, tumor proportion score.

treatment at the time of data cut-off. Among 43 previously treated patients with microsatellite stable (MSS) tumors, ORR was 18.6% (95% CI, 8.4% to 33.4%) (Supplementary Table S12, available at https://doi.org/10.1016/j.annonc.2021.11.009). Survival benefit of nivolumab was also seen in such MSS tumors (Supplementary Figure S15, available at https://doi.org/10.1016/j.annonc.2021.11.009).

The mutation frequencies of the top three genetic abnormalities detected were alterations of *TP53*, *EGFR*, and *KRAS*, respectively (Supplementary Figure S8, available at https://doi.org/10.1016/j.annonc.2021.11.009), consistent with previous findings.[14,24] The presence of these genetic alterations was not associated with clinical benefit of nivolumab in our study cohort (data not shown).

Safety

Among all patients, including both previously treated and untreated, 53 (94.6%) individuals experienced AEs of any grade, with 34 (60.7%) experiencing AEs of grade 3 or 4. Serious AEs were observed in 27 (48.2%) patients, and 4 patients (7.1%) discontinued nivolumab because of AEs. No treatment-related death occurred (Table 3). The most common AEs of any grade were constipation ($n = 13$, 23.2%), anemia ($n = 10$, 17.9%), diarrhea ($n = 10$, 17.9%), hypothyroidism ($n = 9$, 16.1%), and rash ($n = 9$, 16.1%). Immune-related AEs or infusion reactions occurred in 32 (57.1%) of the 56 patients, with most such events being of grade 1 or 2. Immune-related AEs of grade 3 or 4 occurred in four patients (7.1%), including two patients with acute kidney injury and one patient each with hepatitis and rash (Table 3).

DISCUSSION

As far as we are aware, our investigator-initiated phase II study is the first to show that nivolumab has clinical activity with manageable toxicity in a statistically assessable number of patients with CUP. Treatment with nivolumab thus showed an ORR of 22.2% in previously treated patients as determined by BICR, which met the primary endpoint. Our observations are in line with responses to ICIs in patients with various tumor types.[9] Among the total of 12 patients who achieved a PR or CR, 5 individuals experienced a DoR of >6 months. Compared with the limited survival benefit of conventional systemic chemotherapy,[7,8,10] the median OS of 15.9 months in previously treated patients of the present study suggests that nivolumab has the potential to change the therapeutic framework for the treatment of CUP, for which no evidence-based standard of care currently exists. Furthermore, the ORR was 21.4% and the median OS was 16.2 months for all patients, including both previously treated and chemotherapy-naive individuals, suggestive of a potential clinical benefit of nivolumab for the entire CUP patient population.

Only a few studies have investigated the immune profile of CUP, although an understanding of immune characteristics is important for various cancer types because of their possible association with the clinical response to immunotherapy.[25,26] We recently showed that CUP has immune characteristics that are similar to those of ICI-responsive solid cancers and which therefore render it suitable for ICI treatment.[10] About one-third of patients with CUP were thus found to harbor tumors with a PD-L1 TPS of ≥1%, and antitumor immunity-related gene expression in CUP was similar to that in ICI-responsive malignancies. In the present study, 30.9% of patients with CUP had a PD-L1 TPS of ≥1%, consistent with our previous finding.[10] We here found for the first time that the ORR as well as the PFS and OS for nivolumab in patients with CUP were associated with the PD-L1 expression level in tumor cells.

Recent studies have identified several additional predictive markers for ICI treatment, including a high TMB and MSI or mismatch repair deficiency.[27,28] Studies of these markers in CUP are limited, however.[26] In our study, two patients whose tumors were found to be MSI-high experienced a durable response. Furthermore, tumors with a TMB higher than the median value of 7.75 mut/Mb, including the two MSI-high cases, were associated with a higher nivolumab efficacy. Our data thus suggest that both MSI and TMB are appropriate predictive biomarkers for ICIs which could be also used in CUP. In this study, we used an NGS panel which was different from the FoundationOne CDx assay. Even though pembrolizumab, another antibody to PD-1, has received FDA approval for TMB-high solid tumors as defined by a TMB of ≥10 mut/Mb measured by the FoundationOne CDx assay, a recent study showed the possibility that a single TMB threshold cannot identify patients in a pan-cancer fashion who may benefit from ICIs.[29] Further evaluation of TMB cut-offs will be needed.

Some patients experience benefit from nivolumab even if their tumors are neither MSI-high nor TMB-high. An immune-inflamed profile, characterized by the presence of both CD8+ and CD4+ T cells within the tumor, is considered to be another predictive biomarker candidate for ICIs, given that such tumors have a preexisting antitumor immune response.[30-32] Indeed, in the present study, a better clinical efficacy of nivolumab was observed for patients with CUP whose tumors had a higher density of CD4+ or CD8+ TILs. Furthermore, an increased benefit of nivolumab was apparent in patients presenting with lymph node-only metastasis. Various organ-specific responses to ICIs have been described, with lymph nodes predominating as sites of responsive lesions.[33,34] Their role as essential source organs of immune cells may contribute to the observed greater response of lymph nodes, although the precise underlying mechanisms remain unclear. Our results thus indicate the impact of the tumor microenvironment, including various aspects of tumor-host interactions, on the outcome of immunotherapy for CUP.

One view of CUP is that it merely represents disseminated disease derived from a primary tumor that cannot be identified because its size or anatomic location renders it difficult to detect by imaging techniques.[35] Molecular profiling for primary site prediction has therefore been developed for CUP.[20,36,37] Algorithms that we established in a previous

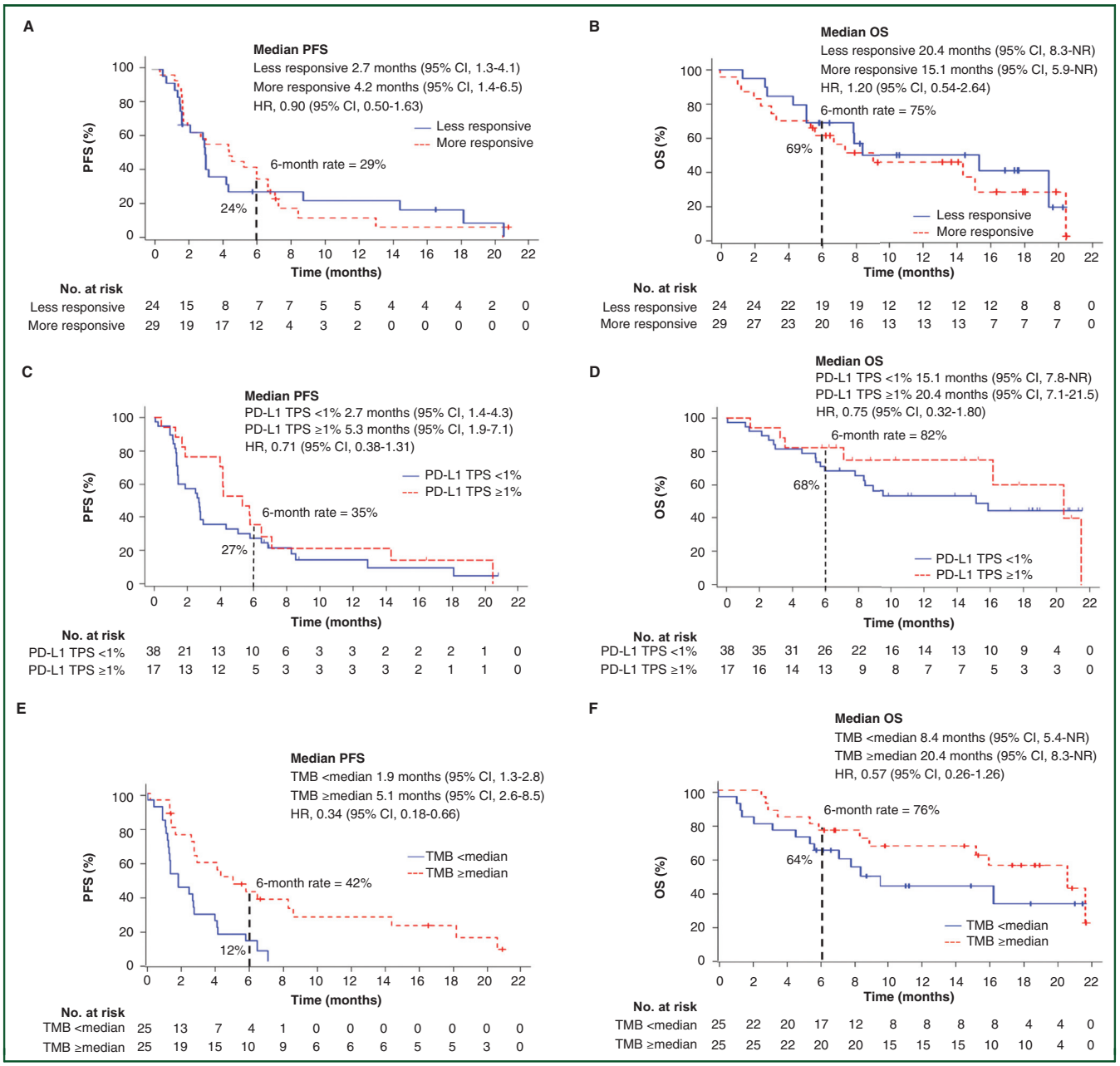


Figure 3. Clinical efficacy of nivolumab treatment according to more or less responsive tumor types, PD-L1 expression, or TMB for all study patients.
Kaplan—Meier curves for (A) PFS and (B) OS as determined by blinded independent central review for patients with more responsive versus less responsive tumor types. Kaplan—Meier curves for (C) PFS and (D) OS as determined by blinded independent central review for patients with CUP classified according to a cut-off for PD-L1 TPS of 1%. Kaplan—Meier curves for (E) PFS and (F) OS as determined by blinded independent central review for patients with CUP classified according to TMB. Six-month survival rates are also indicated.
CI, confidence interval; CUP, cancer of unknown primary; HR, hazard ratio; NR, not reached; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival; TMB, tumor mutation burden; TPS, tumor proportion score.

study[14] were applied for prediction of the primary tissue of origin in the present study. Importantly, this is the first study to show that the efficacy of nivolumab was not associated with whether the predicted primary origin was categorized as responsive to site-directed systemic chemotherapy.

For several cancer types, ICIs have recently been added as standard treatments.[9] Some of these cancer types were among the predicted primary sites for the present study cohort. Although it is difficult to draw any conclusion due to a limited sample size, such ICI-approved primary sites did not predict the response of CUP to nivolumab in our study cohort. Given the unique features of CUP and the difficulty in establishing an optimal treatment strategy for each patient, further investigation of prediction of tissue of origin and characterization of the tumor microenvironment are warranted.

Limitations of the present study include the lack of a comparator and the limited sample size. Despite its heterogeneity, CUP has traditionally been treated as a single entity, making it difficult to conduct phase III trials in order to establish new therapeutic options. Although historical empiric chemotherapy for patients with CUP results in an

Table 3. Incidence of adverse events (AEs) for all patients (n = 56)

	Overall, n (%)	
All AEs	53 (94.6)	
Grade 3 or 4 AEs	34 (60.7)	
Serious AEs	27 (48.2)	
AEs leading to treatment discontinuation	5 (8.9)	
Treatment-related AEs leading to death	0 (0.0)	
AEs with a frequency of ≥10%	Any grade	Grade 3 or 4
Constipation	13 (23.2)	0 (0.0)
Anemia	10 (17.9)	8 (14.3)
Diarrhea	10 (17.9)	0 (0.0)
Hypothyroidism	9 (16.1)	0 (0.0)
Rash	9 (16.1)	0 (0.0)
Decreased appetite	6 (10.7)	1 (1.8)
Dry skin	6 (10.7)	0 (0.0)
Pruritus	6 (10.7)	0 (0.0)
Immune-related AEs		
Diarrhea/colitis	10 (17.9)	0 (0.0)
Diarrhea	10 (17.9)	0 (0.0)
Colitis	1 (1.8)	0 (0.0)
Hepatitis	6 (10.7)	1 (1.8)
AST increased	4 (7.1)	0 (0.0)
ALT increased	3 (5.4)	0 (0.0)
Hepatic failure	2 (3.6)	1 (1.8)
Hypersensitivity	1 (1.8)	0 (0.0)
Infusion-related reaction	1 (1.8)	0 (0.0)
Hyperthyroidism	3 (5.4)	0 (0.0)
Hypothyroidism	9 (16.1)	0 (0.0)
Nephritis/renal impairment	6 (10.7)	2 (3.6)
Hypercreatininemia	4 (7.1)	0 (0.0)
Acute kidney injury	2 (3.6)	2 (3.6)
Tubulointerstitial nephritis	1 (1.8)	0 (0.0)
Pneumonitis	3 (5.4)	0 (0.0)
Rash	15 (26.8)	1 (1.8)
Rash	9 (16.1)	0 (0.0)
Rash maculopapular	4 (7.1)	0 (0.0)
Rash papular	2 (3.6)	0 (0.0)
Drug eruption	1 (1.8)	1 (1.8)

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

ORR of 10% to 40%, the survival benefit of such treatment is limited, with a PFS of 3-6 months and an OS of 8-13 months.[3,4,20,36,38-41] By comparison, the survival benefit observed in the present study suggests the potential of nivolumab to confer a better outcome in such patients. A recent multicohort phase II study of pembrolizumab in advanced rare cancers included 22 patients with CUP. Among the 13 of these patients assessable for objective response, the ORR according to immune-related RECIST was 23% (3/13 patients, with a 95% CI of 5% to 54%).[12] These results provide additional support for the use of ICIs in CUP treatment. Another limitation of our study is the difficulty associated with standardization of CUP diagnosis. A lack of standardized applied definitions, classifications, and diagnostic workup for CUP tumors limits generalization of the conclusions from a single study to the real world.

In conclusion, our results demonstrate a definite clinical benefit of nivolumab in patients with CUP. Benefits were more apparent in patients with known biomarkers for ICIs, such as PD-L1 expression, TMB, and MSI status, while some patients responded well regardless of such biomarker status, implying the need for further biomarker evaluation in this unique disease population. They support the potential of nivolumab to become an additional therapeutic option for CUP, a disease with limited treatment options.

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REFERENCES

1. Pavlidis N, Pentheroudakis G. Cancer of unknown primary site. *Lancet*. 2012;379:1428-1435.
2. Pavlidis N, Fizazi K. Carcinoma of unknown primary (CUP). *Crit Rev Oncol Hematol*. 2009;69:271-278.
3. Briasoulis E, Kalofonos H, Bafaloukos D, et al. Carboplatin plus paclitaxel in unknown primary carcinoma: a phase II Hellenic Cooperative Oncology Group Study. *J Clin Oncol*. 2000;18:3101-3107.
4. Greco FA, Erland JB, Morrissey LH, et al. Carcinoma of unknown primary site: phase II trials with docetaxel plus cisplatin or carboplatin. *Ann Oncol*. 2000;11:211-215.
5. Greco FA, Pavlidis N. Treatment for patients with unknown primary carcinoma and unfavorable prognostic factors. *Semin Oncol*. 2009;36:65-74.
6. Pavlidis N, Khaled H, Gaafar R. A mini review on cancer of unknown primary site: a clinical puzzle for the oncologists. *J Adv Res*. 2018;6:375-382.
7. Travis WD, Brambilla E, Nicholson AG, et al. The 2015 World Health Organization classification of lung tumors: Impact of genetic, clinical and radiologic advances since the 2004 classification. *J Thorac Oncol*. 2015;10:1243-1260.
8. Fizazi K, Greco FA, Pavlidis N, Daugaard G, Oien K, Pentheroudakis G. ESMO Guidelines Committee. Cancers of unknown primary site: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2015;26(suppl 5):v133-v138.
9. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018;359:1350-1355.
10. Haratani K, Hayashi H, Takahama T, et al. Clinical and immune profiling for cancer of unknown primary site. *J Immunother Cancer*. 2019;7:251.
11. Groschel S, Bommer M, Hutter B, et al. Integration of genomics and histology revises diagnosis and enables effective therapy of refractory cancer of unknown primary with PDL1 amplification. *Cold Spring Harb Mol Case Stud*. 2016;2:a001180.
12. 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:e000347.
13. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45:228-247.
14. Hayashi H, Takiguchi Y, Minami H, et al. Site-specific and targeted therapy based on molecular profiling by next-generation sequencing for cancer of unknown primary site: a nonrandomized phase 2 clinical trial. *JAMA Oncol*. 2020;6:1931-1938.
15. Akagi K, Oki E, Taniguchi H, et al. Real-world data on microsatellite instability status in various unresectable or metastatic solid tumors. *Cancer Sci*. 2021;112:1105-1113.
16. Bando H, Okamoto W, Fukui T, Yamanaka T, Akagi K, Yoshino T. Utility of the quasi-monomorphic variation range in unresectable metastatic colorectal cancer patients. *Cancer Sci*. 2018;109:3411-3415.

17. Boland CR, Thibodeau SN, Hamilton SR, et al. A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res*. 1998;58:5248-5257.
18. Hemminki K, Bevier M, Hemminki A, Sundquist J. Survival in cancer of unknown primary site: population-based analysis by site and histology. *Ann Oncol*. 2012;23:1854-1863.
19. Handorf CR. Gene expression analysis and immunohistochemistry in evaluation of cancer of unknown primary: time for a patient-centered approach. *J Natl Compr Canc Netw*. 2011;9:1415-1420.
20. Hayashi H, Kurata T, Takiguchi Y, et al. Randomized phase II trial comparing site-specific treatment based on gene expression profiling with carboplatin and paclitaxel for patients with cancer of unknown primary site. *J Clin Oncol*. 2019;37:570-579.
21. U.S. Food and Drug Administration. Drugs. Oncology (Cancer)/Hematologic Malignancies Approval Notifications. 2021. Available at https://www.fda.gov/drugs. Accessed March 31, 2020.
22. Ministry of Health, Labour and Welfare. 2021. Available at https://www.mhlw.go.jp/english/. Accessed March 31, 2020.
23. European Medicines Agency. Medicines. Available at https://www.ema.europa.eu/en/medicines. Accessed March 31, 2020.
24. Conway AM, Mitchell C, Kilgour E, Brady G, Dive C, Cook N. Molecular characterisation and liquid biomarkers in Carcinoma of Unknown Primary (CUP): taking the 'U' out of 'CUP'. *Br J Cancer*. 2019;120:141-153.
25. Hainsworth JD, Greco FA. Cancer of unknown primary site: new treatment paradigms in the era of precision medicine. *Am Soc Clin Oncol Educ Book*. 2018;38:20-25.
26. Gatalica Z, Xiu J, Swensen J, Vranic S. Comprehensive analysis of cancers of unknown primary for the biomarkers of response to immune checkpoint blockade therapy. *Eur J Cancer*. 2018;94:179-186.
27. Marabelle A, Fakih M, Lopez J, et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *Lancet Oncol*. 2020;21:1353-1365.
28. Andre T, Shiu KK, Kim TW, et al. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer. *N Eng J Med*. 2020;383:2207-2218.
29. McGrail DJ, Pilié PG, Rashid NU, et al. High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. *Ann Oncol*. 2021;32:661-672.
30. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014;515:563-567.
31. Tumeh PC, Harview CL, Yearle JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature*. 2014;515:568-571.
32. Haratani K, Hayashi H, Tanaka T, et al. Tumor immune microenvironment and nivolumab efficacy in EGFR mutation-positive non-small-cell lung cancer based on T790M status after disease progression during EGFR-TKI treatment. *Ann Oncol*. 2017;28:1532-1539.
33. Nishino M, Ramaiya NH, Chambers ES, et al. Immune-related response assessment during PD-1 inhibitor therapy in advanced non-small-cell lung cancer patients. *J Immunother Cancer*. 2016;4:84.
34. Schmid S, Diem S, Li Q, et al. Organ-specific response to nivolumab in patients with non-small cell lung cancer (NSCLC). *Cancer Immunol Immunother*. 2018;67:1825-1832.
35. Kamposioras K, Pentheroudakis G, Pavlidis N. Exploring the biology of cancer of unknown primary: breakthroughs and drawbacks. *Eur J Clin Invest*. 2013;43:491-500.
36. Hainsworth JD, Rubin MS, Spigel DR, et al. Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon research institute. *J Clin Oncol*. 2013;31:217-223.
37. Varadhachary GR, Raber MN. Cancer of unknown primary site. *N Engl J Med*. 2014;371:757-765.
38. Park YH, Ryoo BY, Choi SJ, Yang SH, Kim HT. A phase II study of paclitaxel plus cisplatin chemotherapy in an unfavourable group of patients with cancer of unknown primary site. *Jpn J Clin Oncol*. 2004;34:681-685.
39. Huebner G, Link H, Kohne CH, et al. Paclitaxel and carboplatin vs gemcitabine and vinorelbine in patients with adeno- or undifferentiated carcinoma of unknown primary: a randomised prospective phase II trial. *Br J Cancer*. 2009;100:44-49.
40. Hainsworth JD, Spigel DR, Raefsky EL, et al. Combination chemotherapy with gemcitabine and irinotecan in patients with previously treated carcinoma of an unknown primary site: a Minnie Pearl Cancer Research Network Phase II trial. *Cancer*. 2005;104:1992-1997.
41. Hainsworth JD, Burris HA, Calvert SW, et al. Gemcitabine in the second-line therapy of patients with carcinoma of unknown primary site: a phase II trial of the Minnie Pearl Cancer Research Network. *Cancer Invest*. 2001;19:335-339.