Heliyon 10 (2024) e37503

Contents lists available at ScienceDirect

Heliyon

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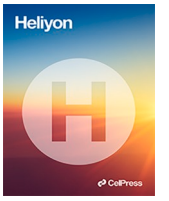

Research article

Efficacy of PD-1/PD-L1 blockade immunotherapy in recurrent/metastatic high-grade neuroendocrine carcinoma of the cervix: A retrospective study

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ARTICLE INFO

Keywords:
Efficacy
PD-1/PD-L1 inhibitors
High-grade neuroendocrine carcinoma of the cervix

ABSTRACT

Although high-grade neuroendocrine carcinoma of the cervix (HGNECC) accounts for less than 1 % of all cervical cancers, it exhibits marked aggressiveness and resistance to radiation and chemotherapy. We retrospectively investigated the efficacy of immunotherapy for recurrent/metastatic HGNECC in a real-world setting. From September 2016 to December 2022, a total of 29 patients with HGNECC accepted PD-1/PD-L1 inhibitors; of these, six cases (20.7 %) were PD-L1 positive (combined positive score ≥1). According to their primary treatment, the patients were assigned to either a surgery group (n = 14) or a non-surgery group (n = 15). In the surgery group, four patients received anti-PD-1 therapy immediately after surgery, while six, two, one, and one patients started immunotherapy after the first, second, third, and forth recurrence, respectively. In the non-surgery group, seven patients started immunotherapy as part of their primary treatment, while the other four, two, and two patients received anti-PD-1 drugs as the second, third, and forth lines of treatment, respectively. The seven-patient group showed longer progression-free survival after immunotherapy (PFSi) and overall survival than those of their counterparts (P = 0.085 and 0.08, respectively), while this benefit was not observed in other subgroups. No significant correlation was observed between PD-L1 and PFSi expression. Interestingly, one patient with a high tumor mutation burden (TMB-H) had a long PFSi of 26 months and experienced no recurrence until the last follow-up. Based on these findings, we propose that PD-1/PD-L1 inhibitors may prolong the survival of patients with HGNECC who start immunotherapy as the first-line of treatment. This indicates that early immunotherapy may be a better choice for this challenging malignancy. Moreover, the predictive role of TMB-H in immunotherapeutic outcomes requires further investigation.

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https://doi.org/10.1016/j.heliyon.2024.e37503
Received 4 March 2024; Received in revised form 3 September 2024; Accepted 4 September 2024
Available online 7 September 2024

1. Introduction

According to the World Health Organization classification system, neuroendocrine carcinoma of the cervix (NECC) is classified into two main types: poorly differentiated neuroendocrine carcinomas (also known as high grade NECC [HGNECC]) and well-differentiated neuroendocrine tumors [1]. There are two primary subtypes of HGNECC: small-cell neuroendocrine carcinoma (SCNEC, accounting for 80 % of all cases) and large-cell neuroendocrine carcinoma (LCNEC, accounting for 12 % of all cases). Although extremely rare, HGNECC is characterized by significant aggressiveness and a high incidence of recurrence and distant metastasis [2,3]. Moreover, most patients present resistant to currently available chemotherapeutic drugs and radiation [4]. These issues lead to clinical difficulties in treating HGNECC, and its prognosis is much poorer than that of common cervical cancers (such as squamous cell cancer and adenocarcinoma).

In the last decade, immunotherapies targeting key immune checkpoints, such as CTLA-4 and PD-1/PD-L1, have shown promising efficacy against a panel of human cancers [5]. To date, several PD-1/PD-L1 inhibitors have been approved for clinical use based on their favorable performance in clinical trials [6]. Thus, it is hypothesized that immunotherapy may be beneficial for patients with HGNECC, especially those who experience tumor recurrence or metastasis even after comprehensive treatment. Considering the significant challenges of conducting a prospective trial, we retrospectively evaluated the real-world efficacy of immunotherapy for HGNECC at our center, aiming to uncover further evidence to guide clinical practice.

2. Materials and methods

2.1. Patient selection

For this retrospective study, we recruited 29 patients with recurrent/metastatic HGNECC who were treated with PD-1/PD-L1 inhibitors at our center between September 2016 and December 2022. Another 35 patients with recurrent/metastatic HGNECC who did not receive immunotherapy were designated as controls (Fig. 1). Experienced pathologists confirmed pathological diagnoses, including histology, lymphovascular space invasion, and lymph node metastasis. By screening the medical record system, we extracted information regarding patient diagnosis, treatment, and outcomes, and the depth of invasion was determined by our pathologist using a microscope. Tumor stage was determined according to the 2018 Federation International of Gynecology and Obstetrics (FIGO) staging system for cervical cancer. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (approval no. 2022-KY-1433). The ethics committee approved the consent waiver because many patients died or were lost to contact during the last follow-up. All procedures were performed strictly in accordance with the relevant guidelines and regulations, including the standards of the Declaration of Helsinki.

2.2. Treatment-response evaluations and follow-up

Response evaluations were performed by experienced gynecologic oncologists and radiologists in strict accordance with the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) and immune-related RECIST. After treatment completion, patients were followed up every 2 months in the 1st year, every 3 months in the 2 nd year, and every 6 months in the 3rd year. If disease progression

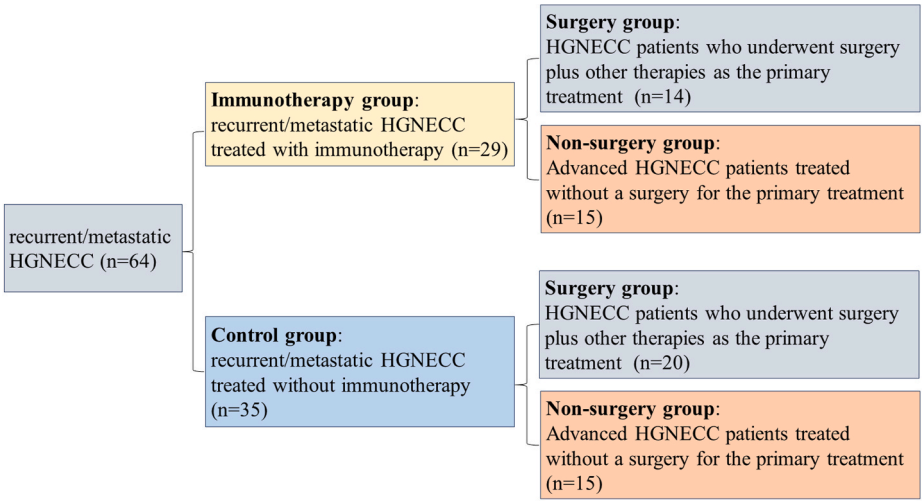

Fig. 1. Study flowchart of the study. This study enrolled 64 recurrent/metastatic patients with HGNECC, of which 29 cases received immunotherapy (Immunotherapy group) and 35 did not (Control group). In the immunotherapy group, 14 patients underwent surgery as part of the primary treatment, while the other 15 patients did not. In the control group, 20 patients were treated with surgery in the primary treatment, and the other 15 cases were not suitable for an operation owing to advanced disease.

was suspected, follow-up was immediately performed. Follow-up included pelvic examination, high-risk Human Papilloma Virus (HPV) testing, cervical cytology, and imaging tests (ultrasonography, CT, MRI, PET/CT, and SPECT). Progression-free survival (PFS) was defined as the interval between HGNECC diagnosis and disease progression. PFS after immunotherapy (PFSi) was defined as the progression-free period achieved after the first use of PD-1/PD-L1 inhibitors, the interval from the start of immunotherapy to later recurrence/metastasis, or the last follow-up if no recurrence or metastasis occurred. Overall survival (OS) was defined as the interval from the pathological diagnosis of HGNECC to patient death or the last follow-up.

2.3. Determination of PD-L1 expression, microsatellite instability (MSI), and tumor mutation burden (TMB)

Immunohistochemical staining for PD-L1 (clone 22-C3; DAKO, Germany) was performed on formalin-fixed paraffin-embedded tissues from all 29 cases. We scored the expression of PD-L1 using the combined positive score (CPS) as described previously [7]. Next-generation sequencing (NGS) was conducted using a Genetic Analyzer 3500DX (Applied Biosystems, CA, USA) to assess MSI and TMB in seven patients. High TMB (TMB-H) was defined as more than 10 mutations per megabase.

2.4. Statistical analysis

Chi-square tests or Fisher's exact probability tests were used to compare the clinical and pathological baseline characteristics of patients with HGNECCs from different groups. Kaplan–Meier analysis was applied to evaluate the PFSi and OS benefits of different treatments and PD-L1 status (CPS $\geq$1 was defined as PD-L1 positive). All statistical analyses were performed using SPSS 22.0 (IBM, NY, USA), and $P < 0.05$ was considered statistically significant.

3. Results

Baseline characteristics were comparable between patients with HGNECC who received immunotherapy and those who did not.

As shown in Table 1, the median age was 50 years (range, 26–67 years) and 52 years (range, 26–70 years) in the two groups, respectively. Regarding the FIGO stage at diagnosis, 20.7 %, 13.8 %, 30.9 %, and 34.5 % of the patients were at stages I, II, III, and IV, respectively. In the non-immunotherapy group, these rates were 22.9 %, 14.4 %, 37.2 %, and 25.7 %, respectively.

A flowchart illustrating the process of patient inclusion is displayed in Fig. 1. In the immunotherapy group, 48.3 % of the patients underwent surgery as part of the primary treatment (surgery group), whereas the other 15 patients with advanced tumors did not undergo surgery (non-surgery group). In the surgery group, 11 patients (78.6 %) received chemotherapy plus radiotherapy as post-operative adjuvant therapy, while the other three patients were only treated with chemotherapy. In the non-surgery group, the percentage of patients who received chemotherapy plus radiotherapy was as high as 73.3 %.

Similarly, in the control group, patients with HGNECC (n = 35) who did not receive immunotherapy were further divided into two subgroups: surgery (n = 20) and non-surgery (n = 15). In the surgery group, 55 % of patients received a combination of chemotherapy and radiotherapy as postoperative adjuvant therapy.

No significant differences in patients age, FIGO stage, or treatment were noted between the immunotherapy and non-immunotherapy groups ($P = 0.407$, 0.892, and 0.454, respectively; Table 1).

3.1. Clinical and pathological parameters of patients with HGNECC who received immunotherapy

The clinical and pathological parameters of the patients with HGNECC who underwent surgery and immunotherapy are listed in

Table 1
The baseline characteristics of HGNECC patients.

	Immunotherapy group (n = 29)	Non-immunotherapy group (n = 35)	P value
Age at diagnosis mean (range)	50 (26–67)	52 (26–70)	0.407
Stage at diagnosis n (%)			0.892
IB1	2 (6.9 %)	2 (5.7 %)	
IB2	4 (13.8 %)	5 (14.3 %)	
IB3	0	1 (2.9 %)	
IIA1	2 (6.9 %)	3 (8.6 %)	
IIA2	0	1 (2.9 %)	
IIB	2 (6.9 %)	1 (2.9 %)	
IIIB	1 (3.4 %)	1 (2.9 %)	
IIIC1	5 (17.2 %)	8 (22.9 %)	
IIIC2	3 (10.3 %)	4 (11.4 %)	
IVA	0	2 (5.7 %)	
IVB	10 (34.5 %)	7 (20.0 %)	
Primary treatments n (%)			0.454
surgery + chemotherapy	3 (10.3 %)	9 (25.7 %)	
surgery + chemotherapy + radiotherapy	11 (37.9 %)	11 (31.4 %)	
chemotherapy	4 (13.8 %)	5 (14.3 %)	
chemotherapy + radiotherapy	11 (37.9 %)	10 (28.6 %)	

Table 2
The baseline parameters of HGNECC who received surgery and immunotherapy (n = 14).

case	HPV	stage	histology	LVSI	LNM
1	16	IIIC1p	SCNEC	Pos	Pos
2	NK	IIIC1p	SCNEC	Pos	Pos
3	16	IIA1	SCNEC	Neg	Neg
4	18	IIA1	SCNEC/AC	Pos	Neg
5	Neg	IB2	SCNEC/LCNEC	Pos	Neg
6	Neg	IIB	SCNEC	Neg	Neg
7	NK	IB1	SCNEC	Pos	Neg
8	Neg	IB2	SCNEC/AC	Pos	Neg
9	18	IIIC1p	SCNEC	Pos	Pos
10	18	IIIC1p	SCNEC	Pos	Pos
11	18	IB2	SCNEC/LCNEC/AC	Neg	Neg
12	16	IB2	SCNEC	Pos	Neg
13	Neg	IB1	SCNEC/AC	Neg	Neg
14	18	IIIC2p	SCNEC/SCC/AC	Pos	Pos

SCNEC: small cell neuroendocrine carcinoma; LCNEC: large cell neuroendocrine carcinoma; SCC: squamous cell carcinoma; AC: adenocarcinoma; LVSI: lymphovascular space invasion; LNM: lymph node metastasis; Neg: negative; Pos: positive; NK: not known.

Table 2. Among them, five and three patients were HPV18- and HPV16-positive, respectively, while four patients were negative for high-risk HPV. Two patients did not undergo HPV testing. Histological analysis showed that eight women had pure SCNEC, while the other six cases reflected a mixture of SCNEC and other pathologic subtypes. Regarding the FIGO stage, six patients were at stage I (two at IB1 and four at IB2), three at stage II (two at IIA1 and one at IIB), and five at stage III (four at IIIC1P and one at IIIC2P).

Table 3 shows the baseline characteristics of the nonsurgical group. Eight and one patients were HPV18- and HPV59-positive, respectively, and three patients were negative for high-risk HPV. Three patients did not undergo HPV testing. Histologically, 13 cases exhibited pure SCNEC; one case, pure LCNEC; one case, a mixture of SCNEC and LCNEC. Regarding the FIGO stage, one patient was at stage IIB, four were at stage III (one at IIIB, one at IIIC1r, and two at IIIC2r), and ten were at stage IVB.

3.2. Molecular features of patients with HGNECC in the immunotherapy group

To determine the molecular alterations associated with immunotherapy, we evaluated PD-L1 protein levels using IHC. As shown in Table 4 and Fig. 2(A–D), PD-L1 was positive (CPS $\geq$1) in 20.7 % of the 29 patients with HGNECC. Moreover, all seven patients who underwent NGS were microsatellite stable (MSS), and TMB-H was detected in only one of the six cases (Table 4). Regarding common gene mutations, two patients presented mutations in the BRCA1/2 genes, one patient in the KRAS gene, one in the P53 gene, one in the ARID1A gene, and one in the RICTOR gene, respectively (Table 4).

3.3. Earlier use of immunotherapy may benefit advanced patients with HGNECC

In the surgery group, four patients received immunotherapy as part of their adjuvant treatment after radical surgery, achieving PFSi values of 12, 10, 8, and 12 months (Table 5, Fig. 3). Six patients started immunotherapy after the first recurrence and had a median PFSi of 3 months (range, 1–21 months). The remaining two, one, and one patients initiated immunotherapy after the second, third, and forth recurrences, respectively, with a PFSi of approximately 1–5 months (Table 5, Fig. 3).

To determine the efficacy of immunotherapy for recurrent/metastatic HGNECC, we enrolled 20 patients who did not receive

Table 3
The baseline parameters of HGNECC who received immunotherapy without a surgery (n = 15).

case	HPV	stage	histology	Metastatic sites
15	18	IIIC1r	SCNEC	pelvic LN
16	18	IVB	SCNEC	distant LN
17	18	IVB	LCNEC	liver/bone/distant LN
18	Neg	IVB	SCNEC	lung/distant LN
19	Neg	IVB	SCNEC	bone/liver/distant LN
20	NK	IVB	SCNEC	distant LN
21	18	IIIC2r	SCNEC/LCNEC	distant LN
22	18	IVB	SCNEC	liver/distant LN
23	18	IVB	SCNEC	lung/liver
24	NK	IVB	SCNEC	bone/liver
25	18	IIIC2r	SCNEC	distant LN
26	Neg	IIB	SCNEC	none
27	NK	IVB	SCNEC	distant LN
28	59	IVB	SCNEC	lung/distant LN
29	18	IIIB	SCNEC	none

Neg: negative; NK: not known; SCNEC: small cell neuroendocrine carcinoma; LCNEC: large cell neuroendocrine carcinoma; LN: lymph node.

Table 4
Molecular alterations in HGNECC patients from the immunotherapy group.

Case	CPS	TMB	MSI	Gene mutations
1	<1	NK	NK	NK
2	<1	NK	NK	NK
3	5	NK	NK	NK
4	<1	NK	NK	NK
5	<1	NK	NK	NK
6	<1	NK	NK	NK
7	<1	NK	NK	NK
8	<1	NK	NK	NK
9	<1	0.88	MSS	GNAS/LIFR
10	<1	4.99	MSS	**BRCA1**/YES1/RICTOR/BCL6/
11	<1	NK	NK	NK
12	5	NK	NK	NK
13	<1	5.76	MSS	KRAS/HGF/TP73/
14	<1	NK	NK	NK
15	20	NK	NK	NK
16	<1	8.15	MSS	TP53/AXIN1/CARD11
17	<1	NK	NK	NK
18	<1	NK	NK	NK
19	<1	NK	NK	NK
20	<1	NK	NK	NK
21	5	2.51	MSS	FANCA/FBXO11/FBWX7/KDM6A
22	<1	NK	NK	NK
23	<1	NK	MSS	NK
24	<1	NK	NK	NK
25	<1	NK	NK	NK
26	<1	33.3	MSS	**BRCA2**/ARID1A/BMPR1A/BRAF
27	5	NK	NK	NK
28	3	NK	NK	NK
29	<1	NK	NK	NK

CPS: combined positive score; TMB: tumor mutation burden; MSI: microsatellite instability; MSS: microsatellite stability; NK: not known.

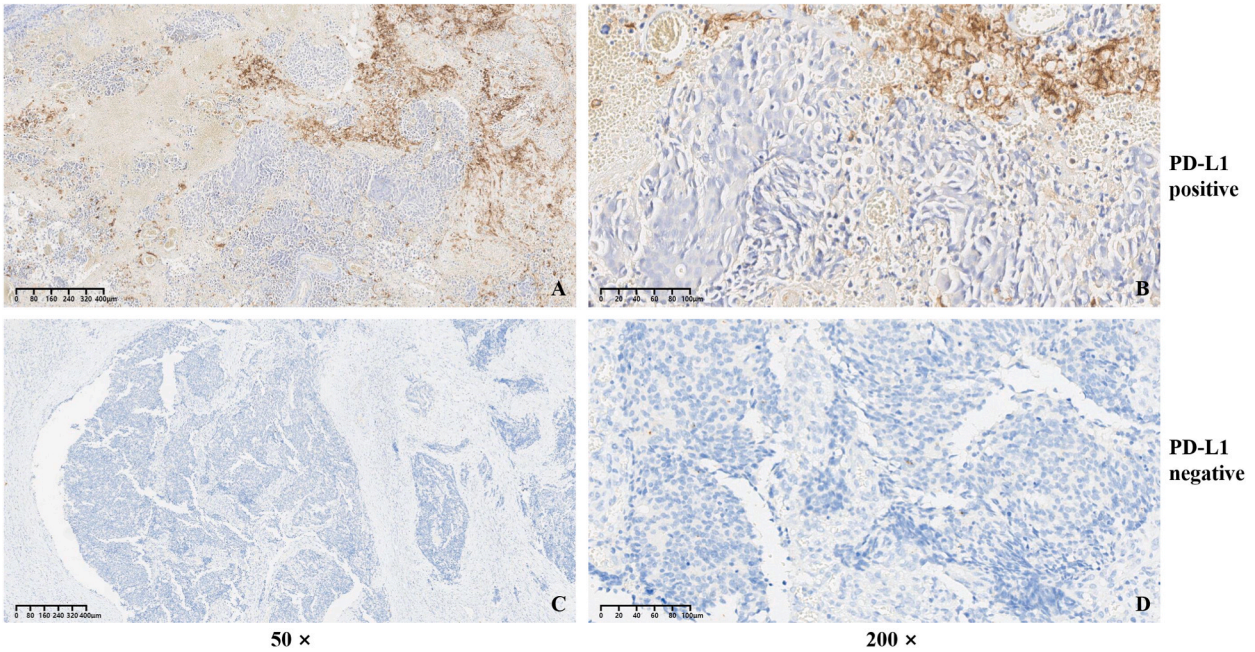

Fig. 2. Representative IHC staining of PD-L1 in patients with HGNECC. (A and B) PD-L1 was positively stained in both tumor cells and immune cells; CPS = 20. (Magnification: A, 50 × ; B, 200 ×). (C and D) Negative staining of PD-L1 was observed; CPS = 0. (Magnification: C, 50 × ; D, 200 ×).

Table 5
Treatment details for HGNECC patients.

Case	status	1st line	2nd line	3rd line	4th line	5th line
1	R	Su + Rad + EP + **Sin**	Top + T + Bev			
2	R	Su + Rad + EP + **Sin**	Rad + **Env**	Rad + **Env**		
3	R	Su + EC + **Tis**	Iri + P	Iri + Sur	Sur + **Pen**	
4	R	Su + Rad + TC	**Sin** + Bev + Su	Rad		
5	R	Su + Rad + TP	Su + Rad	TP	TC	Iri + **Cam**
6	R	Su + Rad + EP	EP + **Cam**			
7	R	Su + Rad + TC	Rad + EP + Ap	T + **Sin**		
8	NR	Su + Rad + EP	a-T + **Tor**			
9	R	Su + TC	EP	Ap + **Tis**		
10	R	Su + Rad + EP	EP + **Cam**	Iri + An	a-T + P	Ge
11	NR	Su + Rad + EP	EP + **Sin**			
12	R	Su + Rad + TC + Bev	EP + **Tis** + Bev			
13	R	Su + EP	EP	Top + Ap	**Cam** + Bev	Top + C
14	R	Su + TP + Rad + **Tis**	TP + **Sin**			
15	R	EP + Rad + **Tis**				
16	R	EP + Rad + **Sin** + Bev	**Cam** + An			
17	R	EC + An + **Cam**	a-T + C			
18	R	EP + Rad + **Tis**	EC + Bev			
19	NR	EP + Bev + **Sin**				
20	R	EP + Rad + **Sin**				
21	NR	EP + Rad + **Sin**				
22	R	EP + Rad	TC	Iri	T + **Cam**	
23	R	EP	Iri + **Sin**			
24	R	EP	Rad + An	Iri + C	Ap + **Sin**	
25	R	Rad + EC	EP + **Tis**			
26	R	EP + Rad	EP + **Sin**			
27	R	EP + Rad	Iri + P	An + **Sin** + Cap		
28	R	Rad + TC	EP + **Tis**			
29	R	Rad + TC	EP	Ap + **Sin**		

PFSi: progression free survival after immunotherapy; R: recurrence; NR: no recurrence; Su: surgery; Rad: radiation; E: etoposide; P: cisplatin; Top: topotecan; T: taxel; Bev: bevacizumab; C: carboplatin; Iri: irinotecan; Sur: surufatinib; Ap: apatinib; a-T: albumin-Bounded paclitaxel; Ge: gemcetabine; An: anlotinib; Cap: capecitabine; Sin: sintilimab; Env: envafolimab; Tis: tislelizumab; Pen: penpulimab; Cam: camrelizumab; Tor: toripalimab.

immunotherapy after the first recurrence/metastasis as the control group (Table 6). Compared to the six cases from the immunotherapy group, they obtained similar PFS (3 months vs. 3.5 months, Fig. 4A; P = 0.271). Additionally, no benefits for OS were noted in the two groups (median OS: 22.5 months vs. 15 months, Fig. 4B, P = 0.105).

In the non-surgery group, 15 patients with advanced HGNECC received combined treatments including radiotherapy, chemotherapy, and immunotherapy. Among them, seven patients started immunotherapy as the first-line treatment, and four, two, and two patients received immunotherapy after the first, second, and third tumor progression, respectively (Table 5, Fig. 3). Seven cases who received first-line immunotherapy showed a median PFSi of 9.5 months (range, 3–12 months), which was longer than those of their counterparts without immunotherapy (median PFS = 6 months, range: 2–18 months; Table 7 and Fig. 4C, P = 0.085). A similar trend was consistently observed for OS (median OS: 17 months vs. 13 months, Fig. 4D, P = 0.08).

3.4. PD-L1 status failed to predict the response to immunotherapy

The Kaplan–Meier curve showed no correlation between PD-L1 status and PFSi (Fig. 4E, P = 0.89). Owing to the small number of patients with MSI-H (0/7) and TMB-H (1/6) in our cohort, we were unable to conduct a statistical analysis to investigate their relationship with PFSi. Interestingly, we noted that one patient with TMB-H (33.3 mutations/Mb) achieved a PFSi of 26 months, which was superior than that of other patients from the same group. The treatment details for this patient were presented in Fig. 5(A–L).

4. Discussion

The treatment of HGNECC is significantly challenging, especially in patients with recurrent or metastatic cancers [8,9]. Various treatment strategies have been implemented over the last few decades; however, none have achieved satisfactory results [10]. In recent years, PD-1/PD-L1 inhibitors have demonstrated significant efficacy in a large group of human cancers, including common gynecological cancers such as cervical cancer and endometrial cancer [11,12]. Thus, we explored whether PD-1/PD-L1 inhibitors could benefit patients with HGNECC. To date, several case reports have been published on this topic. For example, in a patient with recurrent/metastatic SCNEC (PD-L1 negative), the single use of nivolumab resulted in complete remission, and the favorable effects continued even after the cessation of nivolumab (the therapy was halted owing to severe adverse effects) [13]. Consistently, another patient with metastatic LCNEC achieved a PFS longer than 10 months after combined treatment with radiotherapy and nivolumab. Moreover, both TMB-H and deficient-mismatch repair were detected in this patient, both of which have been previously proven to predict a superior response to immunotherapies [14]. In contrast, treatment with pembrolizumab (the first commercial

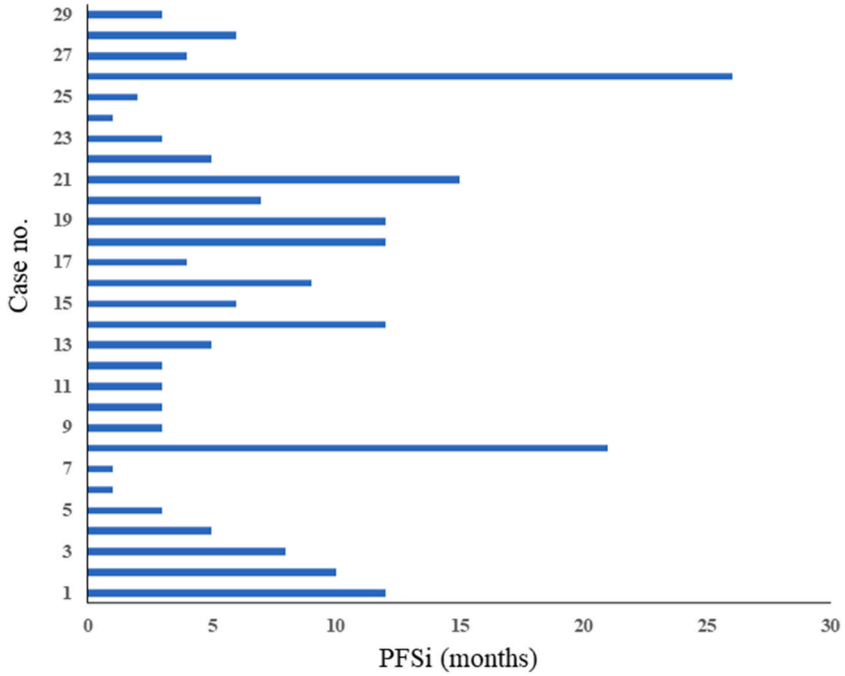

Fig. 3. PFSi of each patient in the immunotherapy group (n = 29). In the surgery group (case 1–14), four patients received immunotherapy as part of their adjuvant treatment post-surgery, achieving PFSi values of 12, 10, 8, and 12 months (case 1, 2, 3, and 14). Immunotherapy was initiated for six patients following first recurrence; they had a median PFSi of 3 months (range, 1–21 months, case 4, 6, 8, 10–12). Immunotherapy was started for the remaining patients (two, one, and one patients) after the second, third, and forth recurrences, respectively, with a PFSi of approximately 1–5 months (case 5, 7, 9, and 13). In the non-surgery group (case 15–29), seven patients received immunotherapy as part of their first-line treatment (case 15–21) and had a median PFSi of 9.5 months (range, 3–12 months). The other four (case 23, 25, 26, 28), two (case 27 and 29), and two (case 22 and 24) patients received immunotherapy after the first, second, and third tumor progression.

Table 6
Baseline characteristics of recurrent/metastatic HGNECC who accepted surgery as preliminary treatments while did not accept immunotherapy after 1st recurrence.

Case	Recurrent/metastatic sites	Treatments	PFS (months)	status
1	pelvic cavity	EP + Rad	1	PD
2	lung/bone	EP	2	PD
3	lung	Pem + P	3	PD
4	bone	EP + Rad	3	PD
5	lung	Ir + P	2	PD
6	breast/bone	TP + Rad	4	PD
7	liver	EP	3	PD
8	lung	EP + Rad	6	PD
9	breast/bone	TP	3	PD
10	pelvic cavity	EP + Rad	10	PD
11	lung	EP	7	PD
12	bone	EP + Rad	5	PD
13	lung/skin	EP	3	PD
14	lung/pelvic cavity	TC + Rad	3	PD
15	lung/bone/liver	EP	6	PD
16	lung	TC + Rad	1	PD
17	lung/bone	Ir	5	PD
18	lung/liver	EP + Rad	4	PD
19	pelvic cavity	TP + Rad	1	PD
20	lung/pelvic cavity	EP + Rad	3	PD

E: etoposide; P: platinum; Pem: Pemetrexed; Ir: Iritecan; T: taxel; C: carboplatin; Rad: radiotherapy; PD: progressive disease.

anti-PD-1/PD-L1 inhibitor) only induced a short response in one of seven patients with HGNECC in a phase II clinical trial [15]. Owing to the limited number of cases, this result should be interpreted with caution and warrants further validation. In the present study, a minimal benefit was only seen in patients with advanced HGNECC who received immunotherapy as a first-line treatment. Thus, we propose that the early use of PD-1/PD-L1 inhibitors might be a better choice for advanced HGNECC.

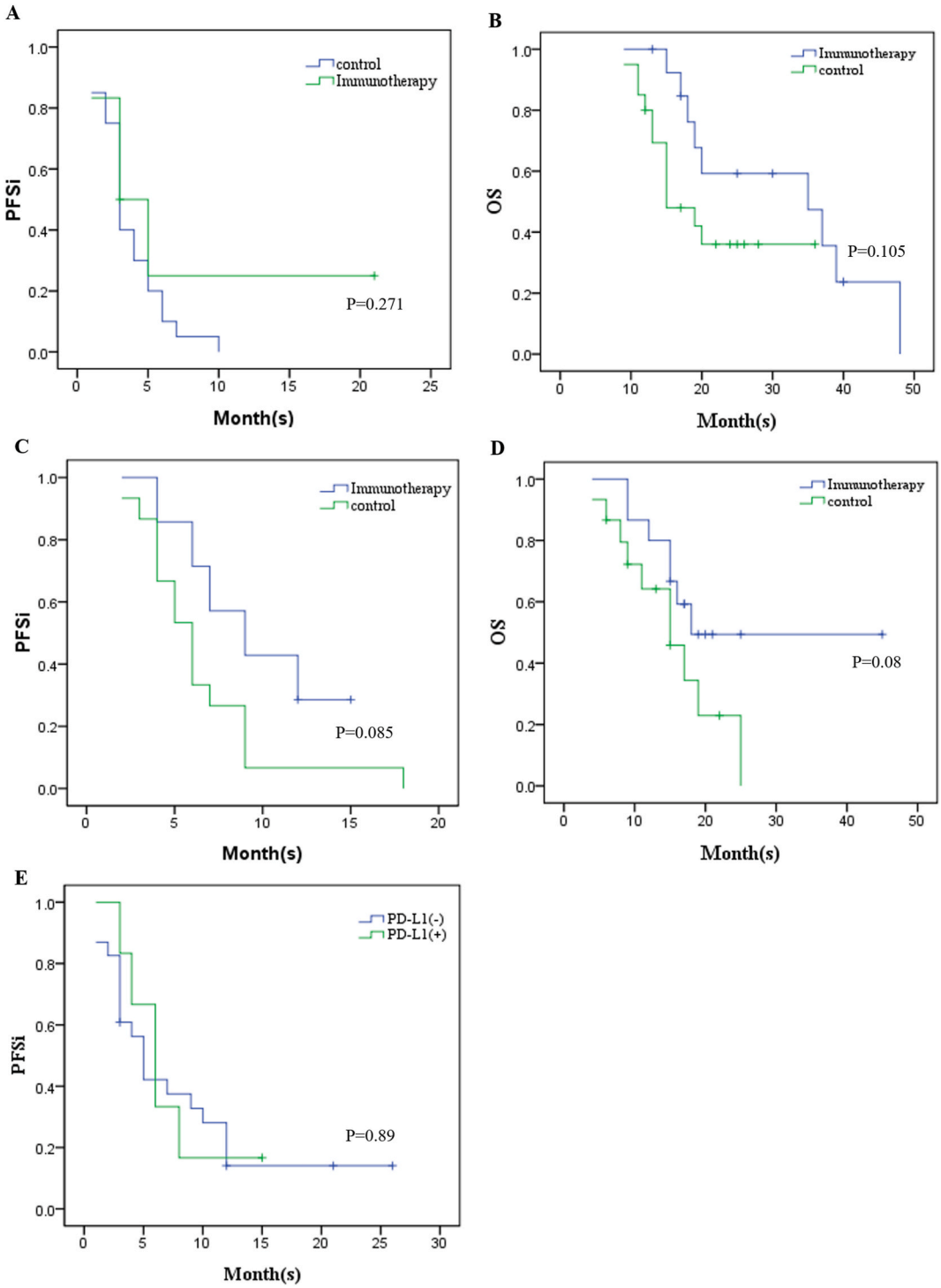

Fig. 4. Earlier use of immunotherapy might benefit patients with advanced HGNECC. The results of Kaplan–Meier analysis for evaluating the efficacy of immunotherapy in these: (A and B), six patients (the surgery group) who received immunotherapy following the first recurrence showed poor PFSi or OS compared to their counterparts (P = 0.271 and 0.105, respectively); (C and D), in the non-surgery group, seven patients receiving immunotherapy as part of their primary treatment had longer PFSi and OS (P = 0.085 and 0.08, respectively); (E), there was no correlation between PD-L1 status and PFSi in both groups (P = 0.89).

Table 7
Baseline characteristics of advanced HGNECC patients without immunotherapy.

case	stage	primary treatments	PFS (months)	status
1	IIIC2r	Rad + EP	7	PD
2	IVB	EP	2	PD
3	IVB	Rad + TP	9	PD
4	IVB	EP	5	PD
5	IIIC1r	Rad + TC	5	PD
6	IIIC2r	Rad + EP	9	PD
7	IVA	Rad + EP	18	PD
8	IVB	EC	3	PD
9	IVA	TP	4	PD
10	IVB	TP	6	PD
11	IIIB	Rad + EP	4	PD
12	IVB	Rad + EP	6	PD
13	IVB	Rad + TC + Bev	4	PD
14	IIIC1r	Rad + TP	6	PD
15	IIIC1r	Rad + EP	9	PD

Rad: radiotherapy; E: etoposide; T: taxol; P: platinum; C: carboplatin; Bev: bevacizumab. PD: progressive disease.

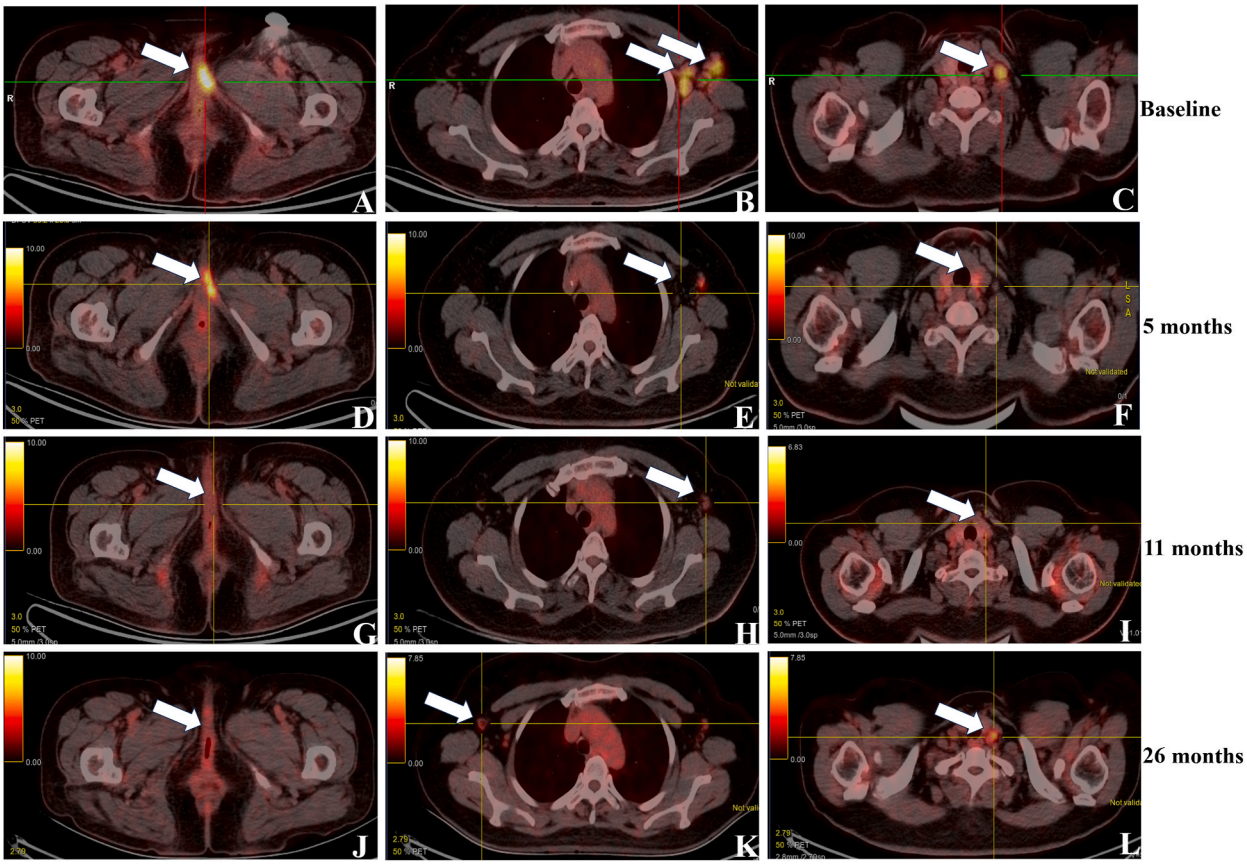

Fig. 5. Representative images of a patient with TMB-H who had a PFSi as long as 26 months. This patient was diagnosed with HGNECC (FIGO stage IIB) in March 2018 and treated with radiotherapy plus chemotherapy (completed in September 2018). During the regular follow-up (July 2019), PET-CT indicated a widespread metastasis in the left vulva (A), left axillary lymph nodes (B), and left supraclavicular lymph nodes (C). This diagnosis was confirmed by pathology via biopsy, and the patient was treated with chemotherapy plus a PD-1/PD-L1 inhibitor (completed in November 2019). Subsequently, PET-CT was performed again to evaluate the efficacy. As shown in (D–F), a notable shrinkage was observed in all the three metastasis sites. Approximately 0.5 year later (April 2020), PET-CT indicated minimal metabolic activities in these sites (G–I). Ten months later (September 2021), the right axillary lymph nodes (K) and left supraclavicular lymph nodes (L) showed disease progression, while no change was noted in the left vulva (J). *: the metastasis sites are indicated by white arrows.

In clinical practice, PD-L1 is the most commonly used biomarker for predicting the efficacy of anti-PD-1/PD-L1 regimens [16]. However, the expression of PD-L1 in HGNECC tissues remains controversial as previous reported. In 2009, Morgan et al. reported that PD-L1 was positive in seven of 10 SCNEC tissues [17]. Additionally, in another study, 56 % of HGNECC cases showed positive staining for PD-L1 (CPS $\geq$1), with 24 % cases exhibiting notably high expression (CPS $\geq$10) [18]. Moreover, positive PD-L1 expression was observed in 22 of the 43 patients with HGNECC, with an average CPS of 6.82 [19]. Consistently, the positive rates of PD-L1 (CPS $\geq$1) were as high as 70 % (n = 20) and 68.5 % (n = 89) in two Chinese cohorts [20,21]; one of these studies reported that higher PD-L1 expression correlated with favorable outcomes in patients with SCNEC [21]. In contrast, Cimic et al. reported that only 10 % of HGNECC cases were PD-L1 positive [22]. In our study, all 29 patients were evaluated for PD-L1 expression, and the positivity rate was 20.7 %, which was not as high as that previously reported in two Chinese cohorts [20,21]. Several issues might contribute to these variances, such as disparate assessment criteria, tumor heterogeneity, and racial/ethnic diversity. For example, Schultheis et al. reported that a higher number of PD-L1 positive cases were detected using RNA sequencing than with IHC (37 % vs. 19 %), suggesting that more precise tests can identify additional PD-L1 positive cases in HGNECC [23]. In a study by Carrol et al., although PD-L1 was positive in only one of 22 pure SCNEC cases, as many as three of six mixed HGNECC specimens exhibited positive staining for PD-L1, indicating significant heterogeneity in HGNECC [24].

Although a large amount of evidence supports a close association between the efficacy of PD-1/PD-L1 inhibitors and PD-L1 status, our data did not corroborate this finding in patients with HGNECC. Through stratification analysis, we determined the efficacy of immunotherapy in these two subgroups. In group 1, six patients with early-stage HGNECC who were amenable to radical surgery and accepted PD-1/PD-L1 inhibitors after the first recurrence achieved a median PFSi of 3 months, which was not superior to that of the control. Interestingly, in group 2, which comprised seven patients with advanced HGNECC who started immunotherapy as the first-line treatment, minimal benefits were observed in terms of PFS and OS, although they did not reach statistical significance. As our sample size was small, these results need to be addressed in future studies.

In addition to PD-L1 status, MSI-H and TMB-H are two other biomarkers used to predict response to immunotherapy [25]. Owing to their failure to repair DNA replication errors, MSI-H tumors express high levels of neoantigens, making the tumor cells immunogenic, which sensitizes them to PD-1/PD-L1 inhibitors [26]. Interestingly, the reported MSI-H incidence rates in HGNECC are inconsistent across six studies: 33 % (n = 9), 0 % (n = 43), 30 % (n = 20), 0 % (n = 28), 0 % (n = 31), and 3.9 % (n = 51) [17,19,20,22,24,27]. We propose that this bias may result from the different technologies used to detect MSI (such as IHC, next-generation sequencing, or PCR). TMB-H was detected in only one of 31 (3 %) and 18 of 97 (18.6 %) cases of NECC in two previous studies [22,28]. In our cohort, the incidences of MSI-H and TMB-H were 0 % (0/6) and 14.2 % (1/7), respectively. Collectively, our results showed that all three predictors (PD-L1 expression, MSI, and TMB-H) were rare in HGNECC, strongly implying that immunotherapy with PD-1/PD-L1 inhibitors may not provide a significant survival benefit. Interestingly, one patient with TMB-H (MSS and PD-L1 negative) achieved a much longer PFSi than the others in their group. This phenomenon warrants further investigation in larger HGNECC populations.

Although gene mutations in HGNECC are not as frequent as those in common cervical cancers [22,27,29], there have been several cases in which precision treatment was administered, depending on their unique molecular features. For example, Rose et al. reported satisfactory outcomes in a patient with stage IV HGNECC (genetic background: BRCA2 mutation, MSS, and TMB-low) treated with rucaparib after chemotherapy. Until the last follow-up, PFS as long as 15 months was achieved, and no signs of disease progression were detected [30]. Thus, these two studies indicated that PARP inhibition may be a candidate strategy for the treatment of HGNECC. In our cohort, BRCA1/2 mutations were detected in two of the seven patients (28.6 %). Similarly, in a study by Xing et al., BRCA1/2 mutations were uncovered in two of 10 patients with SCNEC [31], and in Pei's study, BRCA2 mutations were found in two of 49 (4.1 %) patients. Additionally, alterations in homologous recombination repair genes (BRCA2, ATM, PALB2, FANCA, FANCL, and FANCF) have been demonstrated in more than 14.2 % of patients with SCNEC [27]. In two other studies, IHC results proved that PARP1 was positive in 75 % (n = 20) and 91 % (n = 11) of SCNEC patients [20,24]. Collectively, these findings suggest a potential role for PARP inhibitors in patients with HGNECC having specific genetic signatures; hence, further exploration is warranted.

In addition to their roles in DNA repair, PARPs participate in the manipulation of DNA methylation and transcription factors. Jiao et al. found that PARP inhibitors increased PD-L1 expression in breast cancer cell lines and xenograft tumors [32]. Furthermore, the combination of PD-L1 blockers and PARP inhibitors in these models appeared to act synergistically, improving efficacy over the use of either therapy alone. Similarly, Sen et al. showed that PARP inhibition significantly potentiated the effectiveness of PD-L1 inhibitors in small cell lung cancer and that their combination produced complete responses in mouse models [33]. Thus, we propose that the dual blockade of PD-L1 and PARP-1 might be an alternative strategy for patients with HGNECC who manifest negative or low PD-L1 expression.

Although this is the first case series of patients with HGNECC who underwent immunotherapy, several limitations must be addressed. First, all our immunotherapeutic regimens were administered as a combination of chemotherapy, radiation, or anti-angiogenic drugs, which might introduce a concern as to whether anti-PD-1/PD-L1 drugs played a dominant role in the combined therapy. Second, seven brands of anti-PD-1/PD-L1 drugs were administered to our patients, which may have resulted in an unpredictable bias. Third, a mixture of HGNECC and other histological types (such as squamous cell cancer and adenocarcinoma) is common. Especially for patients who do not undergo surgery, tiny tissue samples obtained via biopsy may not fully reveal the exact heterogeneity of the entire tumor mass. This might also introduce uncertainty in evaluating the expression of PD-L1 and other related biomarkers, eventually affecting the assessment of tumor response to treatment.

In conclusion, we present the first case series of patients with HGNECC treated with PD-1/PD-L1 inhibitors. We demonstrated that immunotherapy, as a first-line treatment, might assist in prolonging survival in patients with advanced HGNECC. Moreover, patients with TMB-H may benefit more from immunotherapy and warrant further investigation. Considering the extreme rarity of HGNECC, our findings will facilitate the precise selection of patients with HGNECC for immunotherapy.

Ethics statement

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (approval no. 2022-KY-1433). The ethics committee approved the consent waiver because many patients died or were lost to contact during the last follow-up. All procedures were performed strictly in accordance with the relevant guidelines and regulations, including the standards of the Declaration of Helsinki.

Fundings

None.

Data availability statement

All data generated or analyzed during this study are included in this article. This information is available from the corresponding author upon request.

CRediT authorship contribution statement

Haifeng Qiu: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Min Wang:** Writing – original draft, Methodology, Formal analysis, Data curation. **Dian Wang:** Methodology, Formal analysis, Data curation. **Yulu Wang:** Project administration, Methodology, Investigation. **Ning Su:** Project administration, Methodology. **Shuping Yan:** Validation, Methodology. **Liping Han:** Writing – review & editing, Project administration, Methodology, Investigation. **Ruixia Guo:** Writing – review & editing, Project administration, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Elsevier for the language editing service during the preparation of this manuscript.

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