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Clinical Research

Anti-EGFR chimeric antigen receptor-modified T cells in metastatic pancreatic carcinoma: A phase I clinical trial

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ABSTRACT

The current clinical outcome for patients with metastatic pancreatic carcinoma (PC) remains poor. Epidermal growth factor receptor (EGFR) is detectable in PC, suggesting that EGFR is a rational target in PC. We conducted a phase I clinical trial to evaluate the safety and efficacy of autologous anti-EGFR chimeric antigen receptor–modified T (CAR T–EGFR) cells in patients with metastatic PC. The expression levels of EGFR on tumor cells detected by immunohistochemistry were required to be more than 50%. Sixteen patients were enrolled and received one to three cycles of the CAR T–EGFR cell infusion within 6 months (median dose of CAR T cells: $3.48 \times 10^6/\text{kg}$; range, 1.31 to $8.9 \times 10^6/\text{kg}$) after the conditioning regimen with 100 to 200 mg/m² nab-paclitaxel and 15 to 35 mg/kg cyclophosphamide. Grade ≥ 3 adverse events included fever/fatigue, nausea/vomiting, mucosal/cutaneous toxicities, pleural effusion and pulmonary interstitial exudation and were reversible. Of 14 evaluable patients, four achieved partial response for 2–4 months, and eight had stable disease for 2–4 months. The median progression-free survival was 3 months (range, 4–months) from the first cycle of CAR T–EGFR cell treatment, and the median overall survival of all 14 evaluable patients was 4.9 months (range, 2.9–30 months). Decreased EGFR expression on tumor cells was observed in patients who achieved stable disease with shrinkage of metastatic lesions in the liver, and enrichment of central memory T cells in infused cells improved the clinical response. In conclusion, the treatment with CAR T–EGFR cells is safe and effective in patients with metastatic PC. This trial was registered at www.clinicaltrials.gov (identifier no: NCT01869166).

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Introduction

Pancreatic carcinoma (PC) is one of the most lethal and common cancers and is rarely diagnosed in young persons under 40 years of age [1]. It is the 12th most common cancer and has the fifth highest mortality rate worldwide; the 5-year survival rate is 9.3% (data derived from statistics from the National Cancer Institute). In China this year 2015, 90 100 new cases are expected to be diagnosed, and 79 400 people are expected to die from PC [2]. Although surgical resection is the only potentially curative treatment for PC [1], approximately 50–60% of patients with PC cannot undergo surgery because of the late stage of their disease (often with metastasis) at diagnosis [3], resulting in poor prognosis and a median overall survival rarely

exceeding 2.3 to 10.3 months [4]. FOLFIRINOX or a combination of gemcitabine and nab-paclitaxel is considered standard treatment for patients with metastatic PC, and the 2-year survival is improved in approximately 10% of patients who have received either FOLFIRINOX or gemcitabine and nab-paclitaxel [1,5,6]. Even so, a more effective treatment strategy is needed to treat patients with metastatic PC.

Recent immunotherapy approaches using chimeric antigen receptor–modified (CAR) T cells have shown remarkable outcomes in the field of cancer treatment; in particular, great success has been achieved in hematological malignancies treated with anti-CD19 CAR T cells [7,8]. In addition, early-phase clinical trials of CAR T–cell treatment have recently been launched and addressed safety and feasibility in solid tumors. Nevertheless, the clinical outcome data are still limited [9,10]. In our previous clinical studies, we designed a CAR against epidermal growth factor receptor (EGFR) and demonstrated the safety and efficacy of infusion of anti-EGFR CAR T (CAR T–EGFR) cells in human patients with advanced non–small cell lung cancer

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and biliary tract cancer [11,12]. EGFR expression has been described in approximately 70–90% of patients with PC and is associated with a poor prognosis, indicating that EGFR represents a suitable therapeutic target for the treatment of metastatic PC [13–16]. Therefore, in this study, we expanded a parallel clinical trial (clinicaltrials.gov identifier no: NCT01869166) to evaluate the safety and efficacy of CAR T–EGFR cells in patients with metastatic PC.

Methods

Study design

This study was approved by the ethics committee of the Chinese PLA General Hospital and was registered at www.clinicaltrials.gov (identifier no: NCT01869166). Consent was obtained from eligible patients according to the Declaration of Helsinki. No commercial sponsors were involved in the study. This study was designed to assess the safety and efficacy of treatment with autologous CAR T–EGFR cell infusion in patients with metastatic PC. The primary objective was to evaluate the safety and efficacy of CAR T–EGFR cells in treating metastatic PC patients, and the secondary outcomes included overall response rate, progression-free survival (PFS), overall survival (OS) and disease control rate, which was defined according to the patients who achieved a complete response (CR), partial response (PR) and stable disease (SD) in all evaluable patients.

Patients

Eligible patients were older than 18 years, had an Eastern Cooperative Oncology Group performance status of 0 to 2, had histologically or cytologically confirmed metastatic PC with EGFR expression of more than 50% as determined by immunohistochemistry (IHC) and

had measurable lesions. All enrolled patients had to have adequate bone marrow reserve and adequate cardiac, pulmonary, hepatic and renal function. Exclusion criteria included obstructive jaundice, pregnancy and lactation, HIV infection and primary immunodeficiency, which were indicated in detail in our previous study [12].

Clinical protocol

The detailed clinical protocol is shown in the schema in Figure 1A. Patients received a conditioning regimen with nab-paclitaxel to destroy the tumor stroma at a total dose of 100 to 200 mg/m² on day –7 and a dose of 15–35 mg/kg cyclophosphamide on days –3 and –2 to deplete lymphocytes. Next, patients received CAR T–EGFR cell infusion with escalating cell doses, which were described in a previous study [12]. All patients were encouraged to receive the next cycle of CAR T–EGFR cell treatment when the CAR gene copy number in their peripheral blood detected by quantitative real-time polymerase chain reaction (Q-PCR) declined to close to baseline, and all patients were encouraged to receive three cycles of CAR T–EGFR cell treatment within 6 months unless new tumor lesions or intolerable toxicities appeared. In addition, patients received palliative radiotherapy to relieve tumor-associated pain or other symptoms. All enrolled patients received contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography/CT to evaluate the baseline overall tumor burden before the conditioning regimen and CAR T–EGFR infusion. The clinical responses were evaluated according to the Response Evaluation Criteria in Solid Tumors version 1.0 (RECIST 1.0), and the immune-related response criteria and adverse events were documented and graded based upon the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE 4.0) [11,12]. The clinical response evaluation was observed

Figure 1. Clinical protocol scheme. (A) The procedure of CAR T–EGFR cell treatment. The patients were encouraged to receive the next cycle of CAR T–EGFR cell treatment when CAR gene copies declined to close to baseline and to receive three cycles of CAR T–EGFR cell treatment within 6 months unless new tumor lesions or intolerable toxicities appeared. CTX, cyclophosphamide; PBMC, peripheral blood mononuclear cells. (B) CONSORT diagram. Thirty-two patients with metastatic PC were screened for EGFR expression, and 16 patients whose EGFR expression level was more than 50% were eligible for treatment with CAR T–EGFR cells. Fourteen patients were excluded, seven because EGFR expression was negative and seven because they lacked IHC results. Sixteen patients received CAR T–EGFR cell treatment, 14 patients were evaluated clinical response and 2 were lost to follow-up.

for at least 1 month in all evaluable patients. All eligible patients were enrolled to receive CAR T–EGFR cell infusion and were followed up between April 2015 and May 2019. The detailed methods for the generation of CAR T–EGFR cells, the administration of CAR T–EGFR cells, flow cytometry, Q-PCR and ELISA are presented in supplementary files.

Statistical analysis

GraphPad Prism version 5.0 for Windows was used to generate the statistics. Descriptive statistics were used to summarize the data in multiplex analyses. PFS and OS were determined by the Kaplan–Meier method. The relationships between CAR gene copies and the number of central memory T cells (Tcm) in infused CAR T–EGFR cell products were analyzed by linear regression models. The significance of differences between groups was determined by using a *T*-test. A *P* value <0.05 was considered statistically significant.

Results

Patient characteristics

The detailed patient screening is presented in Figure 1B. Sixteen patients who had EGFR-positive and metastatic PC were enrolled to receive treatment with CAR T–EGFR cell infusion. The EGFR expression on tumor cells determined by IHC is presented in supplementary Table 1, and the detailed patient clinical characteristics are listed in Table 1. Their median age was 58 years (range, 36–76 years), and nine patients were men. The primary tumor lesions of 16 patients were found in the pancreatic head in three patients, the pancreatic tail in eight patients and the pancreatic body in five patients; 10 of 16 patients had more than two metastases. One PC patient with liver metastasis received no treatment before enrollment in this clinical study.

Table 1
Patient clinical characteristics.

Patient no.	Gender/age (years)	Primary lesion	Metastatic status	Prior therapies	Conditioning nab-P (mg/m ²), CTX (mg/kg)	Cycles of CAR T cell infusion	Total no. infused CAR T cells (× 10 ⁶ /kg)	Best response/PFS (months)
1	M/36	Pancreas tail	Liver, retroperitoneal bulky lesions, lumbar vertebra	Gem+N-p × 6; RT; RFA; micro-HSCT × 4	nab-P: 175, CTX: 25	1	2.00	SD/3
2	M/53	Pancreas body	Omentum, retroperitoneal lymph nodes	Operation	nab-P: 175, CTX: 35	2	4.43	SD/3
3	F/58	Pancreas body	Retroperitoneal lymph nodes	N-p × 8;	nab-P: 175, CTX: 15	2	5.77	PR/4
4	M/63	Pancreas body	abdominal multiple metastasis, Retroperitoneal lymph nodes	FOLFIRINOX × 1; Operation; Gem+S1 × 5;	nab-P: 175, CTX: 15	2	2.24	SD/4
5	Fe/58	Pancreas head	Liver	S1 × 3	nab-P: 125, CTX: 15	1	2.42	PD
6	F/76	Pancreas tail	Liver	None	nab-P: 125, CTX: 25	2	3.64	PR /2
7	M/72	Pancreas tail	Liver, spleen, omentum, retroperitoneal lymph nodes	Gem+S1 × 2; S1 × 1	nab-P: 175, CTX: 15	1	1.31	Loss of follow-up
8	F/47	Pancreas tail	Omentum	Gem × 2	nab-P: 175, CTX: 35	2	4.74	PR/2
9	F/72	Pancreas body	Liver	None	nab-P: 175, CTX: 25	1	1.57	PD
10	M/60	Pancreas head	Liver	Gem+S1 × 1	nab-P: 125, CTX: 25	1	1.92	SD/2
11	F/61	Pancreas tail	Liver	Gem+S1 × 4	nab-P: 175, CTX: 25	2	4.83	SD/4
12	M/60	Pancreas tail	Liver, spleen, omentum, retroperitoneal lymph nodes	Gem × 2; RFA	nab-P: 175, CTX: 25	1	2.03	Loss of follow-up
13	M/56	Pancreas head	Liver, spleen, omentum, Retroperitoneal lymph nodes	Operation, nimotuzuma, RT, S1 × 2	nab-P: 175, CTX: 25	3	5.96	PR/4
14	M/41	Pancreas tail	Liver, spleen, omentum, retroperitoneal lymph nodes	Gem+N-p × 2	nab-P: 125, CTX: 25	1	3.75	SD/3
15	M/57	Pancreas tail	Liver, retroperitoneal lymph nodes	Operation, Gem+N-p × 2	nab-P: 175, CTX: 25	1	3.32	SD/3
16	F/44	Pancreas body	Liver, retroperitoneal lymph nodes	Gem+N-p × 2; operation	nab-P: 175, CTX: 25	2	8.90	SD/2

CTX, cyclophosphamide; F, female; HSCT, hematopoietic stem cell transplantation; Gem, gemcitabine; M, male; nab-P, nab-paclitaxel; PD, progression of disease; PR, partial response; RFA, radiofrequency ablation; RT, radiotherapy; S1, tegafur, gimeracil and oteracil potassium; SD, stable of disease.

CAR T–EGFR cell phenotypes

All 16 patients' CAR T–EGFR cells, including cells infused in the second and third cycles, were successfully generated after 10 days of culture and ready for infusion. A mean of 97.1% of the infused cells were CD3⁺ T cells, containing a mean of 41.43% CD4⁺ and 59.01% CD8⁺ T cells. In addition, a mean of 35.79% of the infused cells were central memory T cells (CD3⁺CD45RO⁺CD62L⁺CCR7⁺), and other cell phenotypes, including CD56, CD45RO, CD62L and CCR7, are presented in supplementary Table 2.

CAR T–EGFR cell administration

All 16 patients received a total of 25 cycles of CAR T–EGFR cell treatment within 6 months, including eight patients who received one cycle, seven patients who received two cycles and one patient who was administered three cycles. After conditioning regimens with nab-paclitaxel and cyclophosphamide, patients received a median of 3.48×10^6 /kg (range, 1.31 – 8.9×10^6 /kg) CAR T–EGFR cells (Table 1).

Adverse events

The 16 patients' adverse events after receiving CAR T–EGFR cell treatment were categorized according to CTCAE V4.0 and are summarized in Table 2. All adverse events could be reversed after intensive medical interventions, and no treatment-related deaths occurred in this study. Hematological toxicities, including neutropenia, lymphocytopenia and thrombocytopenia, were observed, which were mainly associated with conditioning regimens. It cannot be determined whether the hematological toxicities were related to CAR T–EGFR cell infusion. The CAR T–EGFR cell infusion was tolerated though grade ≥3 fever/fatigue, nausea/vomiting, and mucosal/cutaneous toxicities occurred in several patients (supplementary Figure 1). In

Table 2
Adverse events in 16 patients after receiving CAR T-EGFR cell infusion.

Adverse Events	Any Grade		Grade 3–4	
	N	%	N	%
Mucosal/cutaneous				
Mucositis oral	7	43	1	6
Oral mucosal edema	10	63	1	6
Oral mucosal hemorrhage	1	6	0	0
Gastrointestinal hemorrhage	1	6	1	6
Dry skin	7	43	1	6
Dermatitis herpetiformis	4	25	2	13
Hematologic				
Neutropenia	2	13	1	6
Lymphocytopenia	14	88	6	38
Thrombocytopenia	6	38	0	0
Others				
Fever/fatigue	8	56	1	6
Pleural effusion	5	31	2	13
Pulmonary interstitial exudation	3	19	2	13
Diarrhea	4	25	0	0
Nausea/vomiting	4	25	1	6
Myalgia/arthralgia	3	19	0	0

addition, Patients 10 and 11 suffered grade ≥ 3 pleural effusion and pulmonary interstitial exudation toxicities accompanied by a significant increase in cytokines (Figure 2). Patient 11 experienced dyspnea

and hypoxemia within half an hour of the second cycle of CAR T-EGFR cell infusion; and CT scans showed the appearance of pulmonary interstitial exudation and pleural effusion, and the levels of serum interleukin (IL)-6 and C-reactive protein (CRP) were rapidly elevated (Figure 2A,B). Subsequently, 1 mg/kg methylprednisolone was administered immediately, leading to the patient's recovery with pleural effusion and pulmonary interstitial exudation 4 days after methylprednisolone administration, as well as restoration of normal serum IL-6 and CRP levels. Patient 10 experienced pleural effusion and pulmonary interstitial exudation without any disease symptoms, and pleural effusion, pulmonary interstitial exudation and the levels of serum IL-6 and CRP recovered without any treatment on day 15 after the second cycle cell infusion (Figure 2B,C).

Persistence of CAR T-EGFR cells *in vivo*

The persistence of CAR T-EGFR cells was analyzed by Q-PCR to detect the CAR gene copy number in patient peripheral blood (Figure 3A). Rapid increases in CAR gene copy numbers were observed after CAR T-EGFR cell infusion, and CAR gene copy numbers gradually decreased to the verge of baseline levels within 1 month. In addition, the correlation between the number of Tcm in infused CAR T-EGFR cells and CAR gene copies was analyzed by

Figure 2. Lung toxicities after CAR T-EGFR cell treatment. (A) CT scans showed the appearance of pulmonary interstitial exudation and pleural effusion in patient 11 within half an hour after the second cycle of CAR T-EGFR cell infusion and recovery 4 days after immediate administration of 1 mg/kg methylprednisolone (MP). (B) There was rapid elevation of IL-6 and CRP in patients 10 and 11 after CAR T-EGFR cell infusion and recovery after methylprednisolone administration in patient 11 and no intensive medical interventions in patient 10. (C) CT scans showed pulmonary interstitial exudation and pleural effusion in patient 10 after the second cycle of CAR T-EGFR cell infusion and recovery without any treatment on day 15 after cell infusion. Red arrow, pulmonary interstitial exudation; black arrow, pleural effusion.

Figure 3. Persistence of CAR T-EGFR cells. (A) Persistence of infused CAR T-EGFR cells in patients peripheral blood. The level of CAR T-EGFR cells was assessed using Q-PCR to detect the CAR gene copies (no. μg^{-1} DNA) in genomic DNA. Eight patients received 1 cycle, seven patients received two cycles and one patient received three cycles of CAR T-EGFR cell infusion. NA, no assay. (B) CAR gene copies at day 29 after CAR T-EGFR cell infusion were significantly correlated with the number of central memory T cells (Tcm) in infused cells according to linear regression analysis. (C) A significant difference in Tcm number in infused CAR T-EGFR cells between patients who achieved PR/SD and those who had PD was identified by the *t*-test.

linear regression models, and it indicated that there was a significant correlation between them ($P = 0.0399$; Figure 3B).

Clinical response

Two of the 16 enrolled patients were lost to follow-up after receiving the first cycle of CAR T-EGFR cell treatment. Of the 14 evaluable patients who received the first cycle of CAR T-EGFR cell treatment, four achieved PR for 2–4 months, and eight had SD for 2–4 months, including four patients whose target lesions were decreased compared with baseline (Table 1 and Figure 4A,B). In all evaluable patients, the overall objective response rate was four of 14 (28.6%), the disease control rate was 12 of 14 (85.7%), the median PFS time was 3 months (range, 2–4 months; Figure 4C) and the median OS time was 4.9 months (range, 2.9–30 months, Figure 4D) from the first cycle of CAR T-EGFR cell treatment. The CT scans showed that patient 13 achieved PR after CAR T-EGFR cell treatment (Figure 4E), and the MRI scans of patients three and six who achieved PR are presented in supplementary Figure 2.

In addition, the relationship between the number of Tcm in infused CAR T-EGFR cells and clinical response was analyzed by *T*-test, indicating that the Tcm number in the infused cells in patients who achieved PR and SD after CAR T-EGFR cell infusion was higher than that in patients who had PD ($P = 0.0468$; Figure 3C).

Patient 1 was a 36-year-old man was diagnosed with metastatic PC with liver, retroperitoneal and lumbar vertebra metastases (Table 1). Transient disease degradation was observed in the early stage of chemotherapy and radiotherapy; however, the disease was in rapid progression during the late stage of treatments, including intensive chemotherapies. After receiving one cycle of CAR T-EGFR cell infusion,

the patient achieved SD accompanied by shrinkage of metastatic lesions in the liver (Figure 5A). In addition, IHC analysis showed decreased levels of EGFR-positive tumor cells and remarkable necrosis in liver metastatic lesions, and CD3-positive lymphocytes were enriched in the biopsied tissue (Figure 5B and supplementary Figure 3A).

Patient 16, a 44-year-old woman, was diagnosed with PC with liver and retroperitoneal lymph node metastases (Table 1). The patient achieved SD after 2 cycles of chemotherapy with nab-paclitaxel and gemcitabine. IHC analysis showed EGFR overexpression on tumor cells in pancreatic lesions. Then, the patient was enrolled to receive CAR T-EGFR cell treatment. After two cycles of CAR T-EGFR cell infusion, the patient achieved SD accompanied by a reduction of metastatic lesions in the liver by MRI evaluation at 2 months (Figure 5C). IHC analysis showed that the patient had significantly decreased levels of EGFR-positive tumor cells in liver metastatic lesions; in addition, CD3-positive lymphocytes were enriched and infiltrated into metastatic lesions (Figure 5D).

Discussion

Because EGFR overexpression has been described in PC, it is considered a suitable target antigen for PC immunotherapy. This study, which followed our prior phase I clinical trials on patients with advanced non-small-cell lung cancer and biliary tract cancer by treated with CAR T-EGFR cells [11,12], evaluated the related toxicities and clinical efficacy of CAR T-EGFR cell treatment in metastatic PC patients. The results showed that 12 of the 14 metastatic PC patients achieved different degrees of clinical disease control, whereas several patients suffered from grade ≥ 3 adverse events. Our results provided further support for the idea that CAR T-EGFR cells

Figure 4. Clinical response after CAR T-EGFR cell treatment. (A) Duration of clinical response evaluated from the first cycle of CAR T-EGFR cell treatment. Patients 7 and 12 lost follow-up after the treatment with CAR T cells, and patients 5 and 9 had PD evaluated from 1 month after the first cycle of CAR T cell treatment. PR indicates a partial response, SD indicates a stable disease, and PD indicates progression disease. (B) The best clinical response following CAR T-EGFR cell treatment. Clinical response as the sum of the longest diameters of target lesions as assessed by RECIST 1.1 in patients compared with baseline. Zero indicates baseline; the range between two black dotted lines indicates stable disease. (C) Progression-free survival in all evaluable patients was analyzed by the Kaplan-Meier method ($N = 14$). The median progression-free survival time was 3 months. (D) Kaplan-Meier curve of all evaluable patients ($N = 14$). The median overall survival time was 4.9 months. (E) CT scans showed a reduction of primary tumor lesion (red line) before (baseline) and after CAR T-EGFR cell treatment in patient 13.

can infiltrate into tumor lesions and eradicate EGFR-positive tumor cells, and the clinical response was positively related to the enrichment of central memory T cells in infused cells.

In our previous study of CAR T-EGFR cells as a treatment for biliary tract cancer, it was indicated that the persistence of CAR T-EGFR cells and clinical response were deeply associated with the number of Tcm in CAR T-EGFR cells [12]. Similarly, in this study, we also demonstrated the positive correlation of CAR T-EGFR cell persistence with the Tcm number in infused CAR T-EGFR cells, and the enrichment of Tcm in infused cells improved the clinical response. In addition, no difference was observed in the numbers of Tcm in the first and second cycles of cell infusion or in CAR gene copies after the first and second cycle cell infusions (Figure 3A and supplementary Figure 4); in addition, the single chain fragment variable targeting EGFR was derived from a human source in this study, suggesting that no antibody against CAR T-EGFR cells was generated after the first cycle of cell infusion.

The infiltration of T cells, including CAR T cells, is critical in solid tumor treatment [11,17,18]. In this study, an enrichment of CD3⁺ T cells as assessed using IHC, was observed in liver metastatic lesions in two patients who achieved SD, and significantly decreased levels

of EGFR-positive tumor cells were observed. Further, the CAR gene signal could be detectable using Q-PCR in these two patients' tumor biopsies (supplementary Figure 3B). Therefore, this study indicates that CAR T-EGFR cells can infiltrate into the tumor lesions and present the effective and specific anti-tumor ability in metastatic PC. In future studies, we could modify CAR T cells to express chemokine receptors or enzymes that can degrade the extracellular matrix to promote the infiltration of CAR T cells in tumor lesions and directly analyze CAR T cells using RNA-scope technology to label and detect CAR genes in tumor biopsies [19–22].

MRI scans showed that two patients achieved SD with shrinkage of metastatic lesions in the liver after CAR T-EGFR cell treatment. Interestingly, current IHC data indicate that the expression level of EGFR on tumor cells decreased, especially in patient 16, whose tumor cells were EGFR-negative after CAR T-EGFR cell treatment. Because of the heterogeneity of solid tumor cells, the expression of human epidermal growth factor receptor-2 (HER2) on tumor cells was detected [23,24]. Tumor cells expressed HER2, and its expression level was decreased after CAR T-EGFR cell treatment (supplementary Figure 3C). The pathological results showed that EGFR-positive tumor cells were eliminated, whereas HER2-positive tumor cells remained,

Figure 5. Clinical outcome in patients 1 and 16 after CAR T-EGFR cell treatment. (A) MRI scans showed a reduction of metastatic lesions in the liver 1 month after CAR T-EGFR cell treatment in patient 1. Red arrows indicate metastatic lesions in the liver. (B) IHC of metastatic lesions in the liver in patient 1. Hematoxylin and eosin (HE), CD3 and EGFR staining (upper panels) were performed on the biopsy sample (red arrow), which was obtained from one of the metastatic lesions in the liver 1 month after CAR T-EGFR cell treatment (lower panels). No tumor biopsy was obtained from patient 1 before CAR T-EGFR cell treatment. (C) Shrinkage of the liver metastatic lesion (red arrow) in patient 16 as shown by MRI scans after CAR T-EGFR cell treatment. (D) IHC of metastatic lesions in the liver in patient 16. The biopsy sample was obtained from liver metastatic lesions at 1 month after CAR T-EGFR cell treatment and is presented in Figure 5C. The expression levels of EGFR (upper panels) and CD3 (lower panels) in the biopsy sample are presented before and after CAR T-EGFR cell treatment.

causing the patients to only achieve SD as evaluated by MRI after CAR T-EGFR cell treatment. However, we are unable to determine whether the site bias of biopsy contributed to the disappearance of EGFR-positive tumor cells.

EGFR is detectable in most epithelial cells except those in epithelium-derived malignancies, and the occurrence of on-target/off-tumor toxicity on EGFR-expressing normal cells has been noted, which is one of the lethal risks for solid tumor patients after CAR T-EGFR cell infusion [25]. Grade 3 to 4 mucosal/cutaneous adverse events, pulmonary interstitial exudation and pleural effusion in several patients, similar to our previous clinical studies, were observed after administration of CAR T-EGFR cell treatment [11,12]. In particular, patient 11 experienced pulmonary interstitial exudation and pleural effusion accompanied by dyspnea and hypoxemia and a rapid elevation of serum IL-6 and CRP levels, but the levels returned to normal after methylprednisolone administration. The occurrence of on-target/off-tumor toxicity is still an unavoidable problem in recent and ongoing studies, and the following solutions could reduce the risk: (i) select a specific antigen or an antigen with high expression and frequency on tumor cells [26,27]; (ii) explore novel CAR designs to enhance the specificity for tumor cells, for example, bispecific CAR T cells that express two CARs on the cell surface [28,29]; and (iii) optimize the CAR affinity [30]. Even so, on-target/off-tumor toxicity still requires close attention and observation in solid tumor patients after CAR T cell treatment.

Although the patients achieved a high disease control rate after CAR T-EGFR cell treatment, the median PFS and OS time in this trial were not desirable. Improvement in the OS of patients with solid tumors is needed after CAR T-EGFR cell treatment. Herein, patient 16 achieved SD for 2 months after two cycles of CAR T-EGFR cell treatment, and her disease progressed. Subsequently, multiple cycles of chemotherapy with FOLFIRINOX, nab-paclitaxel, gemcitabine, cyclophosphamide and bevacizumab, radiotherapy and checkpoint

blockade with anti-PD-1 antibody were administered, and her disease was controlled. Unfortunately, the patient died approximately 2.5 years after receiving CAR T-EGFR cell treatment. Similar to the bridge-to-transplantation strategy that demonstrated therapeutic potential in patients with hematological malignancies after CAR T cell treatment [31–33], bridging to chemotherapy or other therapies, such as immune checkpoint blockade and radiotherapy, may derive unexpected clinical benefits and improve overall survival in solid tumor patients after CAR T cell treatment.

In summary, current data support that CAR T-EGFR cell treatment is safe and effective in metastatic PC patients. On the basis of our findings, further clinical studies for improving the clinical response and reducing the risk of on-target/off-tumor toxicity remain needed to explore solid tumor treatment with CAR T cells, and altering the tumor environment with a conditioning regimen and overcoming tumor heterogeneity with bispecific CAR T cells may improve the clinical outcome. Although the current clinical efficacy of CAR T cells is not satisfactory in solid tumor treatment, it is still promising and encouraging.

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Declaration of Competing Interest

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

Author Contributions

WH conceived and designed the experiment; YG, ZW, CT, HD and YW performed the experiments; YL, KF, FS and QY provided management of patient (patient enrollment, treatment, data collection); YG and YL analyzed the data, and YG wrote and revised the manuscript. All authors read and approved of final manuscript.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.jcyt.2020.04.088](https://doi.org/10.1016/j.jcyt.2020.04.088).

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