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An analysis report on the application of immune checkpoint inhibitors after liver transplantation

Man Xie, Zhi-ping Dang, Xue-guo Sun, Bei Zhang, Qun Zhang, Qiu-ju Tian, Jin-zhen Cai and Wei Rao

***Abstract*:** Up to now, a variety of immune checkpoint inhibitors (ICIs) have been proved to have good therapeutic effects in the treatment of hepatocellular carcinoma (HCC). However, the effects of their applications in liver transplant (LT) recipients are still unclear. In this analysis report, the clinical applications and therapeutic effects of ICIs on LT recipients with hepatic tumor recurrence or *de novo* carcinoma based on eight databases, including PubMed, EMBASE, Web of Science, Google Scholar, China National Knowledge Infrastructure, Wanfang Data, and CQVIP, were investigated. And the prior treatment, disease response, adverse reactions, and prognosis of patients with malignant tumors after LT and receiving ICI treatments were analyzed. After screening, a total of 28 articles with 47 recipients on the application of ICIs after LT were included. In these patients, their median age was 57 (14–71) years and the main type of tumor after LT was HCC (59.6%). The overall remission rate following ICI treatment was 29.8% (14/47) and the disease progression rate was 68.1% (32/47). Among all these patients, 31.9% (15/47) of patients had immune rejection; the median survival time was 6.5 (0.3–48) months, and the fatality rate was 61.7% (29/47). Considering that the therapeutic effect of ICIs in LT recipients with HCC recurrence or *de novo* carcinoma is not ideal, ICI treatment should be carefully considered for LT patients, and further research is needed.

Keywords: hepatocellular carcinoma, immune checkpoint inhibitors, liver transplantation, rejection

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Correspondence to:
Wei Rao
Division of Hepatology, Liver Disease Center, Department of Organ Transplantation, The Affiliated Hospital of Qingdao University, No. 59 Hai'er Road, Laoshan District, Qingdao City 266600, Shandong Province, China
qdfy_raowei@qdu.edu.cn

Man Xie
Zhi-ping Dang
Xue-guo Sun
Department of Gastroenterology, The Affiliated Hospital of Qingdao University, Qingdao, China

Bei Zhang
Department of Immunology, Medical College of Qingdao University, Qingdao, China

Qun Zhang
Qiu-ju Tian
Jin-zhen Cai
Division of Hepatology, Liver Disease Center, The Affiliated Hospital of Qingdao University, Qingdao, China

Department of Organ Transplantation, The Affiliated Hospital of Qingdao University, Qingdao, China

Introduction

Liver transplantation (LT) is an effective treatment for the various end-stage liver diseases such as decompensated cirrhosis and hepatocellular carcinoma (HCC), and the postoperative survival rate of patients is continuously improving.[1] However, due to long-term use of immunosuppressants, the incidence of postoperative HCC recurrence in LT recipients is significantly higher than in the general population,[2–5] which is an important factor affecting the long-term survival of recipients after surgery. In recent years, immune checkpoint inhibitors (ICIs), a new type of anti-tumor drugs, have shown significant survival benefits in a variety of tumor types.[6–9] Nevertheless, there are few studies in the field showing that ICIs are effective in malignant tumors after LT. This study collected data from LT patients using ICIs to treat malignant tumors through literature search and performed a preliminary analysis of the safety and effectiveness of the clinical application of ICIs after LT.

Methods

Literature search

In this study, eight databases including PubMed, EMBASE, Web of Science, Google Scholar, China National Knowledge Infrastructure,

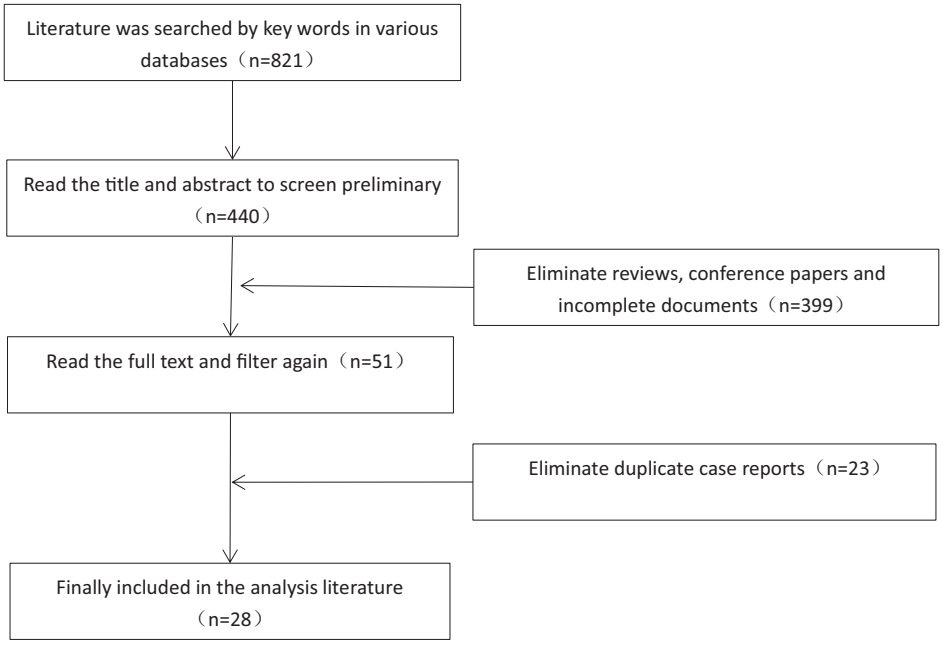

Figure 1. The screening process of the literature analysis.

Wanfang Data, and CQVIP, were used to search relevant literature in Chinese and English from the establishment of the databases to 1 February 2021. The searching term included '((Immune Checkpoint Inhibitors) OR (ICIs) OR (Immunocheckpoint Inhibitors)) OR ((Nivolumab) OR (Pembrolizumab) OR (Camrelizumab) OR (Ipilimumab) OR (Avelumab) OR (Atezolizumab) OR (Daratumumab) OR (Durvalumab) OR (SHR-1210) OR (Cemiplimab) OR (Toripalimab) OR (Camrelizumab) OR (Sintilimab))OR ((PD-1) OR (PD-L1) OR (CTLA-4)) AND ((Liver Transplantation) OR (Liver Transplant) OR (LT)) AND (Cancer) OR (Neoplasm) OR (Carcinoma) OR (Malignance)' and the screening process was listed in Figure 1.

The conduct of our study was approved by the Ethical Affairs Committee of the Affiliated Hospital of Qingdao University (the ethics approval number: QYFYWZLL 26944).

Inclusion and exclusion criteria

Inclusion criteria of this study were (1) detailed reports on the specific process and follow-up results of ICI treatments for recipients after LT, including patient gender, age, tumor type, ICI type and usage, immunosuppressive regimen, rejection, tumor response, treatment effect, and prognosis and (2) articles on ICI application to treat LT patients with new or recurring malignant tumors. The exclusion criteria of this study were articles with (1) repetitive content, (2) incomplete data and reports, and (3) patients who had graft failure before ICI treatment.

Data extraction

The data extracted from the included literature were median age, gender, malignant tumor type, ICI type, time from LT to immunotherapy, immunosuppressive regimen during ICI treatment, occurrence of graft rejection, clinical effects, and survival time.

Statistical analysis

SPSS software (24.0, IBM, Armonk, NY) was used for statistical analysis in this study. The numerical variables conforming to the normal distribution are expressed by mean ± SD (minimum ~ maximum) and analyzed by *t*-test; The numerical variables that do not conform to the normal distribution are represented by M (Q1, Q3) and Mann–Whitney test; The utilization rate of counting data is statistically described, and Fisher exact test is used for statistical analysis, and Kaplan–Meier method was used for survival

analysis. The value $p < 0.05$ indicated a statistically significant difference.

Results

Based on the inclusion and exclusion criteria described above, a total of 28 articles[10–37] including 27 articles in English and 1 article in Chinese that met the criteria were identified. A total of 47 patients who received ICI treatments after LT were reported in this study (Table 1).

Description of demographics and disease characteristics

The 28 articles that met the inclusion criteria included a total of 47 patients who received ICIs after LT, including 37 males and 10 females, with a mean age of 57 (14–71) years (Table 2). The main tumor types that occurred after transplantation were HCC recurrence (28 cases), followed by malignant melanoma (11 cases), non-small cell lung cancer (3 cases), with colorectal cancer, cholangiocarcinoma, squamous cell carcinoma, hypopharyngeal squamous cell carcinoma, and post-transplant lymphoproliferative disease (PTLD) for 1 case each.

In these 47 cases, the ICIs were applied as first-line therapy after LT in only 6 cases (two studies), and was used along with other locoregional therapy or systemic therapy in the other 41 cases (Table 3).

The immunotherapy regimens used included 42 cases of programmed cell death protein 1 (PD-1) monoclonal antibodies alone (23 cases of nivolumab, 11 cases of pembrolizumab, 5 cases of toripalimab, and 3 cases of camrelizumab), 3 cases of cytotoxic T lymphocyte-associated antigen (CTLA-4) monoclonal antibodies (ipilimumab) alone, and 2 cases of combined regimen (pembrolizumab plus ipilimumab).

Among the 47 patients, immunosuppressive regimen included steroids in 6 cases, mammalian target of rapamycin (mTOR) inhibitors in 14 cases (sirolimus in 11 cases, everolimus in 3 cases), calcineurin inhibitors in 19 cases (tacrolimus in 17 cases, cyclosporine in 2 cases) and mycophenolate mofetil in 9 cases.

The follow-up time of 47 patients was 37.9 (20.5~84.7) months, and the median interval from transplantation to ICIs was 3 (0.5~20) years; The median survival time after treatment was 6.5 (0.3~48) months; The overall remission rate of malignant tumors after LT treated with ICIs was 29.8% (14/47), and the case fatality rate was 61.7% (29/47).

Evaluation of safety and treatment effectiveness of ICIs

Among the 47 patients who were treated with ICIs after LT, 15 patients (31.9%) had graft rejection, and 29 patients (61.7%) died of organ failure(37.9%, 11/29) and primary disease progression (62.1%, 18/29) as shown in (Table 4). Of the 42 LT patients treated with PD-1 monoclonal antibodies, 14 (32%) had rejection, and the median survival time of these patients was 8 (0.3–24) months. Among them, the probability of rejection in the patients treated with nivolumab, pembrolizumab, camrelizumab, and toripalimab was 35% (8/23), 54% (6/11), 0%, and 0%, respectively. Rejection occurred in one (33.3%) of three LT patients treated with CTLA-4 monoclonal antibodies, and the median survival time of these patients was 4 (3–48) months. Two patients with malignant tumors after LT and with the combination therapy of pembrolizumab plus ipilimumab did not experience rejection, and the median survival time of the patients was 16.5 (9–24) months.

The patients with malignant tumors after LT were treated with ICIs and had a disease remission rate of 29.8% (14/47), a disease progression rate of 68.1% (32/47), and mortality was 61.7% (29/47). Among them, the remission rates of treatment with nivolumab, pembrolizumab, camrelizumab, and toripalimab were 13% (3/23), 45.5% (5/11), 0%, and 60% (3/5), respectively. In addition, the disease progression cases of the four drug treatments were 86.9% (20/23), 36.4% (4/11), 100% (3/3), and 40% (2/5), and the mortality rates of these four ICI treatments were 86.9% (20/23), 36.4% (4/11), 100% (3/3), and 0%. Among the patients with CTLA-4 monoclonal antibodies, the disease remission rate was 66.7% (2/3), the disease progression rate was 66.7% (2/3), and the mortality rate was 66.7% (2/3). Among the patients treated with the combination therapy of pembrolizumab plus ipilimumab, the remission rate was 50% (1/2); the disease progression rate was 50% (1/2), and mortality was 0% (0/2).

Table 1. Application of immune checkpoint inhibitors (ICIs) in patients after liver transplantation (LT).

Authors	Publication year	Age (years)	Gender	Primary disease	Time from LT to ICI therapy (years)	IS therapy before ICI treatments	IS therapy during ICI treatments	Malignant tumor	ICIs, cycle	Organ rejection	Organ failure	Outcomes (time)	Survival time (months)
DeLeon *et al.*[10]	2018	56	Male	HCC	2.7	NA	Tac	HCC	Nivo, 3×	No	–	Progressive disease	1.2
DeLeon *et al.*[10]	2018	55	Male	HCC	7.8	NA	Rap + MMF	HCC	Nivo, 4×	No	–	Progressive disease	1.1
DeLeon *et al.*[10]	2018	34	Female	HCC	3.7	NA	Tac	HCC	Nivo, 5×	No	–	Progressive disease	1.3
DeLeon *et al.*[10]	2018	63	Male	HCC	1.2	NA	Tac	HCC	Nivo, 2×	No	–	NA	0.3
DeLeon *et al.*[10]	2018	68	Male	HCC	1.1	NA	Rap	HCC	Nivo, 2×	Yes	NA	NA	0.9
Friend *et al.*[11]	2017	20	Male	FL-HCC	3	NA	Rap (2 mg)	HCC	Nivo, 2×	Yes	Yes	Death due to organ failure (4 weeks)	0.9
Friend *et al.*[11]	2017	14	Male	FL-HCC	3	NA	Tac (4 mg)	HCC	Nivo, 1×	Yes	Yes	Death due to organ failure (5 weeks)	1.1
De Toni and Gerbes[12]	2017	41	Male	HCV, HCC	1	NA	Tac (1 mg)	HCC	Nivo, 15×	No	–	Progressive disease after separate response (7 months)	10
Varkaris *et al.*[13]	2017	70	Male	Cryptogenic cirrhosis, HCC	8	Tac	Low-dose (50%) Tac	HCC	Pemb	No	–	Progressive disease	3
Gassmann *et al.*[14]	2018	53	Female	HCV, HCC	3	Pred + MMF + Eve	Eve + MMF	HCC	Nivo, 1×	Yes	Yes	Death due to organ failure (4 weeks)	0.9
Rammohan *et al.*[15]	2018	57	Male	HBV, HCC	4	Tac + MMF + Pred	Tac + MMF + Pred + Rap	HCC	Pemb, 10×	No	–	Complete remission as determined by imaging	>10
Wang *et al.*[16]	2016	48	Male	HBV, HCC	1	Tac + Rap	Tac + Rap	HCC	Pemb, 1×	Yes	No	NA	>8
Zhuang *et al.*[17]	2020	54	Male	HBV, HCC	2.7	Tac	Tac	HCC	Nivo, 31×	No	No	Progressive disease	20
Amjad *et al.*[18]	2020	62	Female	HCV, HCC	1.3	Tac + MMF	Tac + MMF	HCC	Nivo	No	–	Complete remission	>20
Qiu J *et al.*[19]	2020	54	Male	HCC	4.3	Tac + MMF + Pred	Rap	HCC	Camrelizumab	No	No	Progressive disease	9
Morales *et al.*[20]	2015	67	Male	HCV, HCC	8	Tac + MMF/ Rap + MMF	Low-dose Rap (1 mg)	Mel	Ipil, 4×	No	–	Partial remission (3 months)	>3
Ranganath and Panella[21]	2015	59	Female	α1-AT	8	Tac	Tac	Mel	Ipil, 4× plus Pemb	No	–	Progressive disease	>9
Kuo *et al.*[22]	2018	62	Female	ALD, HCC	5	Pred + Tac + MMF	Low-dose Rap (1 mg) + MMF	Mel	Ipil, 4× plus Pemb, 25×	No	–	Partial remission (3 months)	>24
Schvartsman *et al.*[23]	2017	35	Male	BA	20	Tac	Tac	Mel	Pemb, 2×	No	–	Complete remission	>6
Tio *et al.*[24]	2018	63	Female	NA	NA	NA	Cyclosporine	Mel	Pemb, 1×	Yes	Yes	Death due to organ failure (3 weeks)	0.6
Dueland *et al.*[25]	2017	67	Female	Melanoma	1.5	Rap + MMF	Pred (10 mg)	Mel	Ipil	Yes	No	Progressive disease	4
DeLeon *et al.*[10]	2018	54	Male	Melanoma	5.5	NA	MMF + Eve	Mel	Pemb	No	–	Complete remission	>21

(Continued)

Table 1. (Continued)

Authors	Publication year	Age (years)	Gender	Primary disease	Time from LT to ICI therapy (years)	IS therapy before ICI treatments	IS therapy during ICI treatments	Malignant tumor	ICIs, cycle	Organ rejection	Organ failure	Outcomes (time)	Survival time (months)
DeLeon *et al.*[10]	2018	63	Male	Melanoma	3.1	NA	MMF + Pred	Mel	Pemb, 1×	Yes	No	NA	0.7
Abdel-Wahab *et al.*[26]	2020	46	Female	NA	5	NA	Rap (5 mg)	Mel	Pemb	Yes	–	Progressive disease	–
Biondani *et al.*[27]	2018	54	Male	HCV	13	Tac + Pred + MMF	Eve + Tac + Pred (60 mg/day tapered to 5 mg/day)	Non-small cell lung cancer	Nivo	No	–	Progressive disease	15
Gomez*et al.*[28]	2016	61	Male	HCV, HCC	2	NA	NA	HCC	Nivolumab, 2×	Yes	No	Remission	1
Anugwom and Leventhal[29]	2020	62	Male	ALD, HCV, HCC	1.2	Tac	NA	Lung cancer	Nivolumab	No	Yes	Died of cholestatic hepatitis	2
Braun *et al.*[30]	2020	66	Male	Cryptogenic liver disease	3	Tac	Pred	Lung cancer	Nivolumab, 1×	Yes	Yes	Progressive disease	2
Pandey and Cohen[31]	2020	54	Female	HCV, HCC	7.2	Tac + Eve	Tac	HCC	Ipilimumab, 7×	No	No	Remission	48
Owoyemi *et al.*[32]	2020	57 (41–64)	7 Male/ 1 Female	NA	1.6 (0.7–3.5)	1 case: Tac + Pred; 5 cases: Tac; 1 case: Cyclosporine + Pred; 1 case: Aza + Pred	2 cases: Tac + MMF + Pred; 3 cases: Tac; 2 cases: Rap; 1 case: Eve	5 cases: HCC; 2 cases: Mel; 1 case: squamous cell carcinoma	6 cases: Nivolumab 2 cases: Pembrolizumab	2 graft rejection cases	No	5 cases: Progressive disease Mann–Whitney; 1 case: stable; 1 case: partial remission; 1 case: complete remission	2.8 (0.7–6.6)
Dai *et al.*[33]	2021	42	Male	HBV, HCC	0.5	Tac + MMF	Tac + Rap	HCC	Camrelizumab, 7×	No	No	Died of lung infection and respiratory failure	6
Dai *et al.*[33]	2021	62	Male	HCC	2.1	Tac + MMF	Tac + Rap + MMF	HCC	Camrelizumab, 10×	No	No	Progressive disease	18
Kondo *et al.*[34]	2021	52	Male	ALD	3	Cyclosporine + MMF	Cyclosporine + MMF	Squamous cell carcinoma of pharynx	Nivolumab, 4×	No	No	Progressive disease	2
Bittner *et al.*[35]	2019	71	Male	ALD	11.9	NA	NA	PTLD	Nivolumab, 15×	Yes	No	Progressive disease	15
Chen *et al.*[36]	2019	61	Male	ALD	3.5	Eve + MMF	Tac + Pred	Colorectal cancer	Pembrolizumab, 15×	Yes	No	Remission	>11
Shi *et al.*[37]	2021	59	Male	ICC	1.3	NA	NA	ICC	Toripalimab, 8×	No	No	Progressive disease	>5
Shi *et al.*[37]	2021	46	Male	HCC	0.7	NA	NA	HCC	Toripalimab, 7×	No	No	Progressive disease	>4
Shi *et al.*[37]	2021	46	Male	HCC	1.2	NA	NA	HCC	Toripalimab, 3×	No	No	Stable	>2
Shi *et al.*[37]	2021	62	Male	HCC	1	NA	NA	HCC	Toripalimab, 2×	No	No	NA	>1
Shi *et al.*[37]	2021	66	Male	HCC	1	NA	NA	HCC	Toripalimab, 1×	No	No	NA	>1

ALD, alcoholic liver disease; Eve, everolimus; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; ICI, immune checkpoint inhibitor; Ipil, ipilimumab; IS, immunosuppressive; Mel, melanoma; MMF, mycophenolate mofetil; NA, not available; Nivo, nivolumab; Pemb, pembrolizumab; Pred, prednisone; PTLD, post-transplant lymphoproliferative disease; Rap, rapamycin; Tac, tacrolimus.

Table 2. Characteristics of LT patients and malignant tumors.

Demographics and disease characteristics	Total, *n* = 47 (100%)	With rejection, *n* = 15 (32%)	Without rejection, *n* = 32 (68%)
Median age (range, year)	57 (14–71)	61 (14–71)	55.5 (34–70)
Gender			
Male	37	10 (27%)	27 (73%)
Female	10	5 (50%)	5 (50%)
Types of malignant tumor			
Hepatocellular carcinoma	28	NA	NA
Melanoma	11	NA	NA
Non-small cell lung cancer	3	1 (33%)	2 (67%)
Post-transplant lymphoproliferative disease	1	1 (100%)	0
Colorectal cancer	1	1 (100%)	0
Cholangiocarcinoma	1	NA	1 (100%)
Squamous cell carcinoma	1	NA	NA
Squamous cell carcinoma of pharynx	1	0	1 (100%)
Immune checkpoint inhibitors			
PD-1 monoclonal antibodies	44	NA	NA
Nivolumab	23	NA	NA
Pembrolizumab	13	NA	NA
Camrelizumab	3	0 (0%)	3 (100%)
Toripalimab	5	0 (0%)	5 (100%)
CTLA-4 monoclonal antibodies	5	1 (20%)	4 (80%)
Ipilimumab	3	1 (33%)	2 (67%)
Pembrolizumab + ipilimumab	2	0 (0%)	2 (100%)
Median time from transplantation to immunotherapy (year)	3 (0.5–20)	3 (1–11.9)	2.85 (0.5–20)
Immunosuppressive regimens			
Steroid	6	3 (50%)	3 (50%)
mTOR inhibitors	14	7 (50%)	7 (50%)
Sirolimus	11	6 (55%)	5 (45%)
Everolimus	3	1 (33%)	2 (67%)
Calcineurin inhibitor	19	4 (21%)	15 (79%)
Tacrolimus	17	3 (18%)	14 (82%)
Cyclosporine	2	1 (50%)	1 (50%)
Mycophenolate mofetil	9	2 (22%)	7 (78%)

CTLA, cytotoxic T lymphocyte-associated antigen; LT, liver transplant; mTOR, mammalian target of rapamycin; PD-1, programmed cell death protein 1.

Table 3. Treatments applied before ICIs used in these 47 cases.

Reference	Cases	Malignant tumor	Were ICIs used as first-line therapy?	Other therapy before ICIs
DeLeon *et al.*[10]	7	HCC	No	All HCC patients had previously received sorafenib and the median number of previous therapies was two
Friend *et al.*[11]	2	HCC	No	One case received capecitabine, while the other patient was treated with gemcitabine and oxaliplatin for 18 cycles, before capecitabine monotherapy with rapid progression of metastatic disease
De Toni and Gerbes[12]	1	HCC	No	Transarterial chemoembolization (TACE) and microwave ablation
Varkaris *et al.*[13]	1	HCC	No	Sorafenib, chemoradiation therapy with capecitabine and external beam radiation
Gassmann *et al.*[14]	1	HCC	No	Sorafenib
Rammohan *et al.*[15]	1	HCC	No	Sorafenib
Wang *et al.*[16]	1	HCC	NA	NA
Zhuang *et al.*[17]	1	HCC	No	Sorafenib, three times of mFolfox-6 chemotherapy, one gemcitabine plus S-1 therapy and one pulmonary TACE
Amjad *et al.*[18]	1	HCC	No	TACE
Qiu J *et al.*[19]	1	HCC	NA	NA
Morales *et al.*[20]	1	Mel	No	Paclitaxel, five cycles of chemotherapy as well as 14 of 20 planned fractions of palliative radiotherapy
Ranganath and Panella[21]	1	Mel	No	Adjuvant interferon and local radiation
Kuo *et al.*[22]	1	Mel	No	Pazopanib
Schvartsman *et al.*[23]	1	Mel	No	A wide local excision and adjuvant radiation; four cycles of carboplatin and paclitaxel
Tio *et al.*[24]	1	Mel	NA	NA
Dueland *et al.*[25]	1	Mel	Yes	No
Abdel-Wahab *et al.*[26]	1	Mel	NA	NA
Biondani *et al.*[27]	1	Non-small cell lung cancer	No	A lobectomy with lymph node dissection (pT2aN1) and received adjuvant chemotherapy with cisplatin–vinorelbine. Stereotactic radiotherapy was performed on brain metastasis

(Continued)

Table 3. (Continued)

Reference	Cases	Malignant tumor	Were ICIs used as first-line therapy?	Other therapy before ICIs
Gomez *et al.*[28]	1	HCC	No	Sorafenib
Anugwom and Leventhal[29]	1	Lung cancer	No	Several chemotherapy regimens including sorafenib, carboplatin/gemcitabine, combination folinic acid, fluorouracil, and oxaliplatin. Approximately 2 months before presentation, he was started on systemic nivolumab, with palliative intent and radiation therapy for the abdominal wall metastasis
Braun *et al.*[30]	1	Lung cancer	No	Carboplatin–pemetrexed was initiated before he was treated with trastuzumab emtansine
Pandey and Cohen[31]	1	HCC	No	Nanoknife ablation and sorafenib, ethanol ablation, and regorafenib
Owoyemi *et al.*[32]	8	5 cases: HCC; 2 cases: melanoma; 1 case: squamous cell carcinoma	No	Sorafenib, radiation in combination with surgery or platinum-based chemotherapy
Dai *et al.*[33]	2	HCC	No	One case received lenvatinib, and the other received resection, TACE, and radiofrequency ablation
Kondo *et al.*[34]	1	Squamous cell carcinoma of pharynx	No	Undergo right neck dissection, concurrent chemoradiotherapy (cisplatin + radiotherapy), radiotherapy, two courses of the EXTREME regimen, comprising cisplatin (CDDP), 5-fluorouracil (5-FU), and cetuximab
Bittner *et al.*[35]	1	PTLD	No	Eight cycles of rituximab, two cycles of high-dose methotrexate, three cycles of intrathecal rituximab, cytarabine, and dexamethasone
Chen *et al.*[36]	1	Colorectal cancer	No	Right hemicolectomy, seven cycles of FOLFOX (5-FU/leucovorin/oxaliplatin), five cycles of irinotecan and cetuximab
Shi *et al.*[37]	5	1 case: ICC; 4 cases: HCC	Yes	No

HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; ICI, immune checkpoint inhibitor; Mel, melanoma; NA, not available; PTLD, post-transplant lymphoproliferative disease.

Table 4. Immune checkpoint inhibitors (ICIs) and treatment response.

ICIs	Rate of rejection in %	Median survival time (months)	Rate of disease remission[a] in %	Rate of disease progression in %	Mortality in %
PD-1/PD-L1	32 (14/42)	8 (0.3–24)	26 (11/42)	69 (29/42)	64 (27/42)
Nivolumab	35 (8/23)	1.15 (0.3–20)	13 (3/23)	87 (20/23)	87 (20/23)
Pembrolizumab	54 (6/11)	8 (0.6–24)	45 (5/11)	36 (4/11)	36 (4/11)
Camrelizumab	0 (0/3)	9 (6–18)	0 (0/3)	100 (3/3)	100 (3/3)
Toripalimab	0 (0/5)	2.1 (0.7–6)	60 (3/5)	40 (2/5)	0 (0/5)
CTLA-4					
Ipilimumab	33 (1/3)	4 (3–48)	67 (2/3)	67 (2/3)	67 (2/3)
Combined regimen					
Pembrolizumab + ipilimumab	0 (0/2)	16.5 (9–24)	50 (1/2)	50 (1/2)	0 (0/2)
Total	32 (15/47)	6.5 (0.3–48)	30 (14/47)	68 (32/47)	62 (29/47)

CTLA, cytotoxic T lymphocyte-associated antigen; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.
[a]Disease remission included complete remission and partial remission.

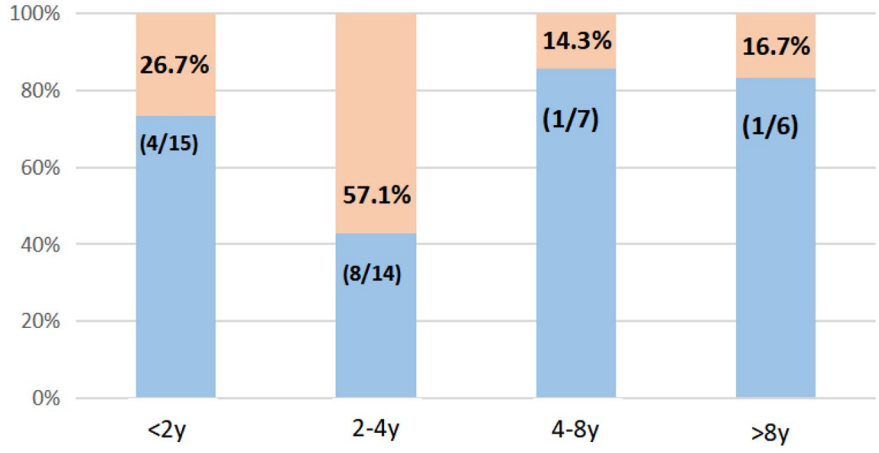

Figure 2. Relationship between the rate of graft rejection and the time to start immunotherapy.

In this study, five patients with partial data missing were excluded. The probability of graft rejection in the remaining 42 patients receiving ICI therapy at 2 years, 4 years, 8 years, and 20 years after LT was 26.7% (4/15), 57.1% (8/14), 14.3% (1/7), and 16.7% (1/6), respectively, indicating that the rate of rejection in patients gradually reduced as the median time to starting immunotherapy increased (Figure 2).

Impact of immunosuppressive therapy on the safety and effectiveness of ICI treatments

Except for some literatures that failed to provide immunosuppressive regimen, a total of 31 patients with immunosuppressive regimens were included for analysis (Table 5). During ICI treatment, the graft rejection rate in patients receiving steroid monotherapy was 100% (2/2) and both of them died of disease progression; the rejection rate of

Table 5. Immunosuppressive regimen, graft rejection, and tumor response.

Immunosuppressive regimen	Rate of rejection in %	Median survival time (months)	Rate of disease remission in %	Mortality in %
Single-agent immunosuppressive therapy	38 (7/18)	3 (0.3–48)	17 (3/18)	72 (13/18)
Steroid	100 (2/2)	4 (2–4)	0 (0/2)	100 (2/2)
Sirolimus	60 (3/5)	1.95 (0.9–9)	20 (1/5)	60 (3/5)
Tacrolimus	10 (1/10)	3 (0.3–48)	20 (2/10)	70 (7/10)
Cyclosporine	100 (1/1)	0.6	0 (0/1)	100 (1/1)
Combined immunosuppressive regimen	31 (4/13)	11 (0.7–24)	38 (5/13)	69 (9/13)
2-drug combination	40 (4/10)	8 (0.7–24)	40 (4/10)	70 (7/10)
3-drug combination	0 (0/2)	15	0 (0/2)	100 (2/2)
4-drug combination	0 (0/1)	10	100 (1/1)	0 (0/1)
Total	35 (11/31)	6 (0.3–48)	26 (8/31)	71 (22/31)

rapamycin (mTOR) inhibitor and sirolimus monotherapy was 60% (3/5), one case had remission, and three cases died of disease progression finally. Among the 10 patients treated with tacrolimus alone, 1 case had rejection, 2 cases had remission, 7 cases died of disease progression, and the only patient treated with cyclosporine alone also had rejection and finally died. For the combined immunosuppressive regimen, of the 10 recipients treated with two immunosuppressive agents, 4 had rejection, 4 had remission, and a total of 7 died. Two patients who were treated with three drugs did not have rejection, but eventually died of disease progression. One patient who was treated with four drugs had remission.

Survival curve analysis of patients with ICI treatments

Among the 47 cases in this study, 29 cases (61.7%) died, including 15 cases of rejection and 11 cases (73.3%); no rejection occurred in 32 cases and 18 cases died (56.3%). Except for 16 cases with partial missing data, the survival curve of the remaining 31 cases was analyzed. The results showed that the overall survival time of those without rejection (21 cases) was 5.5 months, which was higher than that of those with rejection (10 cases). The difference was statistically significant ($p = 0.002$, Log Rank = 9.164, Figure 3).

Discussion

Graft rejection and ICIs after transplantation

In this study, we conducted the latest and largest case report of ICI treatments in patients with tumors after LT. The results showed that among the 47 patients treated with ICIs, 31.9% of patients had graft rejection and the median survival time was 6.5 (0.3–48) months. In malignant melanoma, ICI-associated transplant rejections were mostly reported in LT recipients, and the mortality of liver transplant recipients was more than 36.5%.[38] Rejection was often accompanied by high mortality, and 44% of all patients died of graft failure. Abdel-Wahab *et al.*[26] analyzed 39 organ transplant recipients, including LT. About 41% of the recipients had graft rejection after ICI treatment, of which 81% had graft loss and 46% died. Kumar *et al.*[39] analyzed the clinical data of 64 organ transplant patients including 37 cases of melanoma, 10 cases of HCC, 7 cases of lung cancer, and 10 cases of other tumors, which showed that overall allograft rejection rate was 40.6% (26/64 cases) in organ transplant recipients following ICI therapy. The graft rejection rate was 44% (17/39 cases) for renal, 31.6% (6/19 cases) for liver, and 20% (1/5 cases) for cardiac allografts. Among LT recipients, the rejection rate of patients treated with nivolumab was the highest (33%, 3/9 cases), the second was pembrolizumab treatment (20%, 2/5 cases), while the lowest

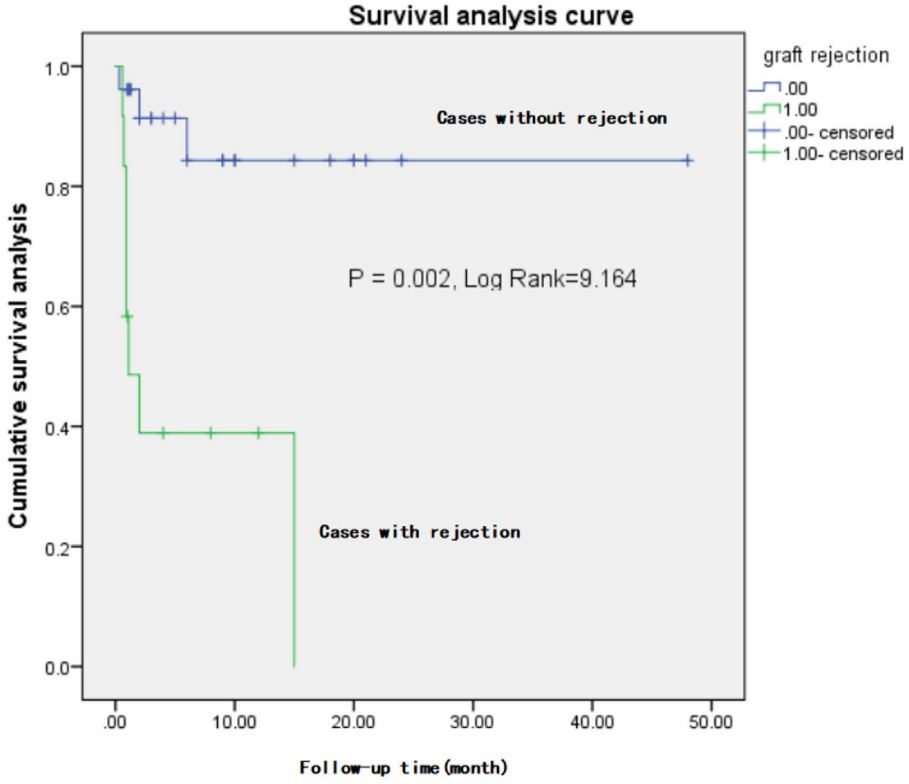

Figure 3. Relationship between graft rejection and patient survival time.

incidence was ipilimumab treatment (12.5%, 1/8 cases). These results indicated that after LT, patients with tumors that were treated with ICIs had a higher rate of graft rejection, and their overall prognosis was limited.

The mechanism of graft immune rejection is similar to tumor immune rejection. The response rate of tumors to PD-1 inhibitors is higher than that of CTLA-4 inhibitors.[40] In addition, the positive expression of PD-1 and programmed death-ligand 1 (PD-L1) proteins in some graft biopsies also suggests that the PD-1 pathway may be involved in the pathogenesis of transplant tolerance and immune rejection.[38] Therefore, the rate of graft rejection may be higher when using PD-1/L1 pathway blockers. In the prospective, single-arm study of Shi *et al.*[37] all the five patients without PD-L1 expression in their grafts received anti-PD1 therapy without developing graft-related immune-related adverse events. Besides, one off study patient with positive graft PD-L1 expression suffered graft rejection. The prospective, single-arm research showed that graft PD-L1 expression may be a promising marker for transplant recipients' organ rejection following anti-PD1 immunotherapy, although needed to be further investigated in patients with solid organ transplantation.

Safety of ICI treatments in LT. In comparing the safety of different ICI treatment regimens, studies have shown that the rejection rate of patients receiving CTLA-4 inhibitors is lower than that of patients receiving PD-1 inhibitors. The rejection rates of CTLA-4 inhibitors and PD-1 inhibitors were 11% and 30%, respectively.[19] Other studies have shown that CTLA-4 helps induce but fails to maintain transplant tolerance. Organ transplant patients receiving CTLA-4 inhibitor treatment may have relatively low graft rejection in the early stages after transplantation.[41,42] The data from this study showed that the risk of rejection of pembrolizumab and nivolumab was higher than that of other ICIs. The rejection rate of CTLA-4 monoclonal antibodies was lower than that of PD-1 monoclonal antibodies. The median survival time of patients with CTLA-4 monoclonal-antibody therapy was longer than those with PD-1 monoclonal-antibody therapy, which was

consistent with the findings in another study.[39] Thus, CTLA-4 monoclonal-antibody therapy may be superior to PD-1 monoclonal-antibody therapy in terms of safety in LT recipients. Another study has shown that CTLA-4 monoclonal antibodies are the first-line drugs for the treatment of melanoma in organ transplant recipients and is safer and more desirable than PD-1 monoclonal antibodies.[43] In addition, this study showed that two patients treated with a combined regimen (pembrolizumab plus ipilimumab) did not experience rejection. A recent study reported that a liver LT patient was treated with atezolizumab and bevacizumab without any signs of rejection, suggesting a new breakthrough standard treatment option for HCC.[44] However, because the number of patients receiving the combined regimen was small, the authors could not conclude that the combined regimen was safer than the others.

Effectiveness of ICI treatments in LT. In the evaluation of the effectiveness of ICI treatments, the results of Kumar *et al.* showed that the disease remission rate of patients treated with nivolumab or pembrolizumab was 26% and 53%, respectively. The disease remission rate of ipilimumab was 20%.[39] In this study, the disease remission rates of patients treated with PD-1 monoclonal antibodies and CTLA-4 monoclonal antibodies were 32% and 67%, the disease progression rates were 73% and 67%, and mortality rates were 66% and 67%, respectively. However, due to the small sample size and the retrospective design of this study, it is impossible to infer which ICIs are more effective. A large sample, prospective study is needed for further clarification.

This study showed that the time interval between immunotherapy and LT was a potential factor affecting the risk of graft rejection. The longer the time interval between immunotherapy and LT, the lower the risk of graft rejection after ICI treatment, which was consistent with the viewpoint of Qiu *et al.*[19] In contrast, if ICIs are used too early following LT, the risk of transplant rejection may be increased. However, delay in the start of immunotherapy may result in a significant reduction in the effectiveness of ICIs. Therefore, in patients who have received LT and are considered for ICI treatment, close follow-up is recommended during first-line conventional treatment to identify signs of disease progression as early as possible and to carefully weigh the start time of immunotherapy.[45]

Immunosuppressants in ICI treatments after LT. Drugs used for immunosuppression after LT include four categories: major steroids, mTOR inhibitors (sirolimus, everolimus), calcineurin inhibitors (tacrolimus, cyclosporine), and mycophenolate mofetil. Since different immunosuppressants work at different stages of the cell cycle, they are often used in combination to achieve optimal results. This study showed that during ICI treatment, patients receiving different immunosuppressive regimens had different rates of graft rejection. Patients treated with steroids had a higher rate of rejection than patients treated with other immunosuppressive regimens. Patients treated with calcineurin inhibitors had a lower probability of rejection, which was consistent with the results of the study of Abdel-Wahab *et al.*[26] In this study, among the LT patients treated with ICIs, three out of five patients (60%) who were treated with sirolimus alone (single-agent immunosuppressive therapy) had graft rejection, and one out of nine patients (11%) who were treated with tacrolimus had graft rejection. Existing data suggest that patients using tacrolimus may have a relatively low risk of rejection. However, due to the limited data in this study, we cannot definitively infer which immunosuppressive regimens interfere less with immunotherapy. Further verification is needed *via* future clinical trials.

Although there is concern that immunosuppressive therapy may alter the effectiveness of ICI treatment, clinical studies have shown that LT patients treated with ICIs and immunosuppressive therapy simultaneously responded to immunotherapy.[19] This study showed that patients receiving steroid and tacrolimus treatments had a disease remission rate of 25% and 23%, respectively. The disease remission rate of the patients on a combined immunosuppressive regimen was 44%. These results indicated that a combined immunosuppressive regimen for the initiation of ICI treatment may be more conducive to disease response than single-agent immunosuppression.

The survival analysis results showed that the median overall survival of patients with graft rejection was significantly lower than that of the patients without graft rejection. Among the 25 LT patients treated with ICIs, 64% of the patients

died during the follow-up period. The main cause of death was graft rejection or the progression of primary malignant tumors. Among them, four patients (16%) died of transplant organ failure caused by rejection. Therefore, for patients after LT, the occurrence of graft rejection significantly affected overall survival.

By blocking the inhibitory receptors of immune checkpoints, ICIs restore antigen initiation, proliferation, T cell migration and effector function, and stimulate the host immune response. However, initiation of immunity to tumor cells may also lead to fatal transplant rejection.[39] Thus, for patients with LT who have recurrent, refractory, and metastatic malignancies under long-term immunosuppression, immunotherapy with ICIs may be effective. Nevertheless, the risk of graft rejection that may result from this should not be ignored. Thus, we believe that for patients with recurrence or new malignant tumors after LT, the indications for ICI treatment should be carefully considered.

Limitations

The data in this retrospective study were from published case reports that did not represent most of the population but were used to infer the overall situation. Besides, the potential selection and reporting bias might affect the conclusion of this study.

Conclusion

The pooled analysis of 47 recipients in the application of ICIs after LT published in literature showed that the overall remission rate following ICI treatment was 29.8% and the disease progression rate was 68.1%. Among all patients, 31.9% of patients had immune rejection; the case fatality rate was 61.7%, which showed the experience of ICI therapy in LT was still limited and far from rosy.

To further improve the therapeutic effects of ICIs in LT patients, there are still lots of work to be done in the future, including but not limited to preferential selection of recipient and immunosuppressants, careful consideration of risk–benefit in ICIs therapy, combination therapies or monotherapy of ICIs regimen, identifying best predictive biomarkers of response or graft rejection, and more molecular mechanisms or prospective studies are needed to explore the complex interactions between the immune system, tumor antigens, and transplant antigens.

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Author contributions

Man Xie: Data curation; Investigation; Methodology; Project administration; Writing – original draft.
Zhi-ping Dang: Data curation; Methodology.
Xue-guo Sun: Data curation; Project administration.
Bei Zhang: Conceptualization; Investigation; Software; Supervision.
Qun Zhang: Data curation; Investigation; Methodology.
Qiu-ju Tian: Data curation; Methodology; Software.
Jin-zhen Cai: Conceptualization; Writing – review & editing.
Wei Rao: Conceptualization; Formal analysis; Project administration; Supervision; Writing – review & editing.

Ethics approval and consent to participate

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ORCID iD

Wei Rao https://orcid.org/0000-0002-6559-583X

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