Efficacy and safety of immune checkpoint therapy in hepatocellular carcinoma: meta-analysis

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Abstract

Aim: Immune checkpoint inhibitors (ICIs) are proven to be an effective way to treat the disease of hematologic malignancies. But there is still plenty of uncertainty about the effectiveness of ICIs on hepatocellular carcinoma. The Meta-analysis was conducted to evaluate the efficacy and safety of ICIs treatment in patients with HCC.

Methods: Four electronic databases, including PubMed, Embase, Cochrane database, and ClinicalTrials.gov, were systematically retrieved for relevant observational studies published before November 1, 2018. The objective response rate (ORR) and adverse events were analyzed. Meta and Metafor Packages in R were utilized to accomplish meta proportion analysis.

Results: A total of 462 patients from 7 studies were included in this meta-analysis. The pooled estimated ORR of ICIs was 19.8% (95% CI 16.4% to 23.7%). No substantial heterogeneity was observed among studies (Q = 2.0427, P = 0.92, I² = 0.0%). The common adverse events on any grade were saw in increased AST (22.7%, 95%CI 13.8% to 35.2%), fatigue (20.9%, 95%CI 10.9% to 36.3%), rash (18.5%, 95%CI 8.9% to 34.4%) and pruritus (17.3%, 95%CI 13.5% to 21.8%). Increased AST (9.9%, 95%CI 4.4% to 21.0%) and increased ALT (5.8%, 95%CI 3.7% to 8.9%) were the most common adverse events on grade greater than 3.

Conclusion: Although ICIs treatment has a certain efficacy on liver cancer, it also causes some adverse events which should be noticed by clinicians.

Keywords: Hepatocellular, immune-checkpoint inhibitor, CLAT-4, PD1/PD-L1

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INTRODUCTION
Liver cancer is the fourth most common cause of cancer-related death worldwide. Among all liver cancer type, hepatocellular carcinoma (HCC) is the most common neoplasm, accounting for approximately 90% cases\(^1\). The common risk factors of HCC are cirrhosis, hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, alcohol abuse and metabolic syndrome\(^3\). The median overall survival of untreated HCC was 7 months, suggesting that its poor prognosis is attributable to advanced stages of diagnosis\(^4\). First line usage of multi-kinase inhibitor such as sorafenib was able to increase survival in advanced HCC from 7.9 months to 10.7 months (hazard ratio, 0.69). Unfortunately, this benefit was usually restricted by high resistance\(^5\). It's obviously that other approaches are still needed in treatment with advanced HCC. Immune checkpoints inhibitors (ICIs) therapy aiming to restore anticancer immunity has emerged as a promising therapy in liver cancer. Both clinical and preclinical studies revealed that there was a highly immunosuppressive tumor microenvironment and defective T cell recruitment in advanced HCC\(^7\). Exhaustion of CD4\(^+\) T cells has also been reported as a mechanism of immune evasion in HCC\(^6\). ICIs are monoclonal autoantibodies (mAbs) specifically targeting the inhibitory receptors on T cells (the so-called immune checkpoints). The most common types of ICIs are the cytotoxic T lymphocyte antigen 4 (CTLA4) and the programmed death 1 (PD-1) and its ligand PD-L1. Those all act as negative co-regulators to limit further T cell activation, which are normally responsible for limiting the escalated and chronic immune responses with deleterious autoimmune effects\(^9\). ICIs have been evaluated in a series of clinical trials for melanoma, non-small cell lung cancer (NSCLC) and renal cell carcinoma, and they have yielded favorable outcomes\(^11\). Some of the clinical trials with ICIs in liver cancers have been conducting in recent years, and more studies are still in the stage of recruiting. During the 2nd phase of clinical trials, ORR is an important outcome to evaluate the efficacy of anticancer drugs, which is also an essential factor to determine the carrying out of the 3rd phase of clinical trials. In this system review, we will retrieve studies about ICIs on liver cancer with outcome of ORR and analysis of the efficacy and safety.

METHODS
We carried out a comprehensive systematic search to identify studies about immune checkpoint inhibitor conducted on patients with HCC. The study was performed with adherence to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines\(^14\).

Literature search strategy
We mainly searched four databases (PubMed, Embase, Cochrane database, and ClinicalTrials.gov) for articles published before November 1st, 2018. Controlled vocabulary and text word for synonymous terminology were both used in the search strategies. The following keywords were combined with Boolean logistical strategy for search: nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, ipilimumab, tremelimumab, checkpoint inhibitors, PD1, programmed death 1, PD-L1, programmed cell death ligand 1, CTLA-4, cytotoxic T lymphocyte associate protein 4, and hepatocellular carcinoma, liver cancer, liver neoplasm, hepatic cancer, hepatic tumor. The search strategy in pubmed was as follow: (nivolumab OR pembrolizumab OR atezolizumab OR avelumab OR durvalumab OR ipilimumab OR tremelimumab OR "checkpoint inhibitors" OR "PD1" OR "programmed death 1" OR "PD-L1" OR "programmed cell death ligand1" OR "CTLA-4" OR "cytotoxic T lymphocyte associate protein 4") and ("hepatocellular carcinoma" OR "liver cancer" OR "liver neoplasm" OR "hepatic cancer" OR "hepatic tumor").

Selection criteria
Inclusion criteria was as follow: (1) randomized controlled clinical trials (RCTs) or non-randomized controlled clinical trials (n-RCTs); (2) patients pathologically diagnosed with hepatocellular carcinoma; (3) patients treated with PD1/PD-L1 or CTLA-4 monoclonal antibody; (4) studies with an outcome of objective response rate. Studies were excluded if they met the following criteria: (1) reviewer or case-report; (2) duplications with early publications from same authors or institutions; (3) unable to obtain full test.
Study selection
Two investigators (Tang WN, Deng Y) independently screened the titles and abstracts of retrieved articles to choose potential relevant articles. Disagreement about particular studies were discussed and resolved by consensus.

Data extraction
Data extraction was carried out independently by the two reviewers (Tang WN, Deng Y). The following information was extracted from the eligible studies: information of the articles (author, published year, and study design), patient characteristics (number, age, area or nationality, race, and gender), liver disease condition (hepatitis virus infection, Eastern Cooperative Oncology Group (ECOG) performance scale, Child-Pugh stage, prior therapy), intervention in patients (agent, target, dosage, duration of dosing), outcome of efficacy (ORR).

Statistical analysis
The data analysis process was initially conducted by the third author (Ma LT). The pooled estimated ORRs and their 95%CI were derived. Meta and Metafor Packages in R were utilized to accomplish meta proportion analysis. Logit transformation of raw proportion was performed before further analyses to increase validity. The ratio of between-study heterogeneity to total heterogeneity was quantified by $I^2$ and $P$ value. The assumption of homogeneity was considered invalid for $I^2 > 25\%$ and $P < 0.10$. A chi-square test ($Q$-test) was performed to test whether the heterogeneity between studies existed or not. If between-study heterogeneity were not significant, a fixed model would be applied to get a summarized proportion; otherwise a DerSimonian and Laird random effects model would be adopted. Two side $P < 0.05$ was considered statistically significant.

RESULTS

Eligible studies
A total of 545 related articles were identified by the initial search strategy. After screening titles and abstracts, 525 studies were excluded because of irrelevant topics, review articles, molecular mechanism studies, experiments on animals, and clinical trials on recruiting. We then carefully reviewed the full texts of the remaining 20 potentially eligible papers. And then, 13 articles were excluded, because one of them was not able to obtain full test, two of them did not have the outcome of ORR, and ten papers were case-reports. Finally, seven studies were chosen for the following analysis. Figure 1 shows the study selection flowchart. Data from all eligible studies were obtained from published manuscripts.

Study characteristics
A total of 462 populations from seven studies were included in this meta-analysis. There was one paper published in 2013, five papers in 2017 and one paper in 2018. Six studies were carried out with multi-center clinical trials and one study was carried out with mono-center in China. Most of studies were in phase 1 or phase 2 of clinical trials. There were two studies conducting with CTLA-4 inhibitor (Tremelimumab), four studies with PD1/PD-L1 inhibitor (Pembrolizumab, Nivolumab, Durvalumab) and one study with combination of PD1 inhibitor and CTLA inhibitor (durvalumab and tremelimumab). Table 1 shows the main characteristics of the eligible trials.

Efficacy of immune checkpoint inhibitors in HCC
Overall, the pooled estimated ORR of patients treated with ICIs was 19.8% (95%CI: 16.4%-23.7%). No substantial heterogeneity was observed among single-study ($Q = 2.0427, P = 0.92, I^2 = 0.0\%$). Study of El-Khoueiry et al. \textsuperscript{[16]} weighed most with estimated proportion of 19.6% (95%CI: 14.8%-25.5%). And the second weighted article was Zhu et al. \textsuperscript{[17]} with estimated proportion of 17.3% (95%CI: 11.2%-25.8%). See Figure 2.
Publication bias

A funnel plot was made to investigate potential publication bias. Unweighted Egger regression test was performed in each analysis to test whether the funnel plot is symmetrical or not [Figure 3].

Adverse events of ICIs

If an adverse event was mentioned in more than 4 published papers, the incident rate of adverse event was estimated by R software. The pooled estimated incident rate and its 95% confident interval were calculated on all grades and on grade greater than 3 respectively. The adverse events included in our study were fatigue, pruritus, rash, diarrhoea, nausea, asthenia, pulmonary toxicity, increased AST and increased ALT. The increased ALT was the most common adverse event whose pooled estimated incident rate was 22.7% (95%CI: 13.8%-35.2%), which was followed by fatigue 20.9% (95%CI: 10.9%-36.3%), rash 18.5% (95%CI:...
8.9%-34.4%), and pruritus 17.3% (95%CI: 13.5%-21.8%). The most common adverse event on grade greater than 3 was increased AST, whose pooled estimated incident rate was 22.7% (95%CI: 13.8%-35.2%). The second was increased ALT 13.9% (95%CI: 8.8%-21.3%). The remaining adverse events of grade greater than 3, such as fatigue, pruritus, rash, diarrhoea, nausea, pulmonary toxicity, showed a small difference among pooled estimated incident rates, which were around 1%-2%. The incident rate of Asthenia was only 0.9%, as shown in Table 2.

**DISCUSSION**

This meta-analysis was performed to investigate the ORR published by papers which aimed to analyze the effectiveness of ICIs in patients with HCC. The derived overall estimated ORR reported on these non-heterogeneity papers is 19.8% (95%CI: 16.4%-23.7%, \( P < 0.001 \)). The result of this study is different from the investigation of other tumors. The difference maybe mainly caused by the heterogeneity among tumors. High response rate (50%-90%) of ICIs can be obtained with classical Hodgkin lymphoma, desmoplastic melanoma, Merkel cell carcinoma and microsatellite instability carcinoma. But the response rate of ICIs is reduced to 15%-25% when treating solid tumors such as non-small cell lung cancer, head and neck cancer, gastroesophageal cancer, bladder and urothelial cancer \(^{22}\). Compared to hematological malignancies, HCC as a solid tumor, shows not only a more complicated tumor microenvironment but the unique immune escape. All of these reasons may cause the low ORR in HCC patients treated with ICIs. One of the effective ways to enhance the drug response to ICIs is to obtain specific markers of liver cancer \(^{23}\). A high rate of ORR (> 30%) can be regard as a proper goal in the single arm clinical trial aiming at groundbreaking treatment \(^{24}\). There are currently five anti-PD-1/PD-L1 antibodies and two CTLA-4 blocking antibodies approved by the United States Food and Drug Administration (FDA). But only Nivolumab and Tremelimumab were approved to treat with HCC, with clinical trials of ICI agents currently ongoing \(^{25}\). The overall estimated ORR is only 19.8% based on the current study, which needs to be verified with multicenter randomized controlled studies.
<table>
<thead>
<tr>
<th>No</th>
<th>Author</th>
<th>Year</th>
<th>Clinical trial phase</th>
<th>Area</th>
<th>Target</th>
<th>Agent</th>
<th>Male, n (%)</th>
<th>Dosage</th>
<th>Duration of dosing</th>
<th>Patient number</th>
<th>Age in year (range)</th>
<th>Viral status</th>
<th>ECOG</th>
<th>Child-Pugh score</th>
<th>Child-Pugh stage</th>
<th>Alpha-fetoprotein</th>
<th>BCLC stage</th>
<th>Prior antitumor therapy</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Sangro et al.</td>
<td>2013</td>
<td>Phase 2</td>
<td>Multi-center</td>
<td>NG</td>
<td>CTLA-4</td>
<td>Tremelimumab</td>
<td>15 (71.4%)</td>
<td>15 mg/kg</td>
<td>360 days</td>
<td>21</td>
<td>65.2 (48-79)</td>
<td>HCV (100%)</td>
<td>0</td>
<td>1 (28.6%)</td>
<td>6.5 (100%)</td>
<td>A (57%)</td>
<td>B (43%)</td>
</tr>
<tr>
<td>2</td>
<td>Duffy et al.</td>
<td>2017</td>
<td>Phase 1</td>
<td>Multi-center</td>
<td>NG</td>
<td>CTLA-4</td>
<td>Tremelimumab</td>
<td>28 (87.5%)</td>
<td>3.5 mg/kg; 10 mg/kg</td>
<td>6 months</td>
<td>32</td>
<td>61 (36-76)</td>
<td>HCV (90%)</td>
<td>0 (25%)</td>
<td>5 (44%)</td>
<td>NG</td>
<td>NG</td>
<td>B (25%) Other systemic therapies (32%) TACE (39%) Resection (28%) Ablation (28%)</td>
</tr>
<tr>
<td>3</td>
<td>Zhu et al.</td>
<td>2018</td>
<td>Phase 2</td>
<td>Multi-center</td>
<td>White</td>
<td>PD-1</td>
<td>Pembrolizumab</td>
<td>86 (3%)</td>
<td>200 mg intravenous injection</td>
<td>2 years</td>
<td>104</td>
<td>68 (62-73)</td>
<td>HBV (21%)</td>
<td>0 (61%)</td>
<td>NG</td>
<td>A (94%)</td>
<td>C (6%)</td>
<td>B (24%) C (76%)</td>
</tr>
<tr>
<td>4</td>
<td>El-Khoueiry et al.</td>
<td>2017</td>
<td>Phase 1/2</td>
<td>Multi-center</td>
<td>White</td>
<td>PD-1</td>
<td>Nivolumab</td>
<td>171 (80%)</td>
<td>3 mg/kg</td>
<td>Depend on disease progression</td>
<td>214</td>
<td>64 (56-70)</td>
<td>HBV (83%) HCV (23%)</td>
<td>5 (70%)</td>
<td>NG</td>
<td>6 (29%)</td>
<td>7-9 (2%)</td>
<td>NG</td>
</tr>
<tr>
<td>5</td>
<td>Feng et al.</td>
<td>2017</td>
<td>NG</td>
<td>China</td>
<td>Asian</td>
<td>PD-1</td>
<td>Nivolumab</td>
<td>8 (72.7%)</td>
<td>4 mg/kg</td>
<td>6 cycles</td>
<td>11</td>
<td>54.8 (42-70)</td>
<td>HBV (100%)</td>
<td>0 (81.8%)</td>
<td>NG</td>
<td>NG</td>
<td>40.5 (26-70)</td>
<td>NG</td>
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<tr>
<td>6</td>
<td>Wainberg et al.</td>
<td>2017</td>
<td>Phase 1/2</td>
<td>Multi-center</td>
<td>White</td>
<td>PD-L1</td>
<td>Durvalumab</td>
<td>32 (80.0%)</td>
<td>10 mg/kg</td>
<td>Q2W</td>
<td>12 months</td>
<td>40</td>
<td>61.5 (20-77)</td>
<td>HBV (25%)</td>
<td>NG</td>
<td>NG</td>
<td>0 (57.5%)</td>
<td>NG</td>
</tr>
<tr>
<td>7</td>
<td>Kelley et al.</td>
<td>2017</td>
<td>Phase 1</td>
<td>Multi-center</td>
<td>NG</td>
<td>PD-1</td>
<td>Durvalumab</td>
<td>NG</td>
<td>8 months</td>
<td>40</td>
<td>NG</td>
<td>NG</td>
<td>HBV (28%)</td>
<td>NG</td>
<td>A (93%)</td>
<td>NG</td>
<td>NG</td>
<td>Systemic therapy (70%)</td>
</tr>
</tbody>
</table>

AFP: alpha fetoprotein; BCLC: barcelona clinic liver cancer; CTLA-4: cytotoxic T-lymphocyte associated antigen 4; ECOG: the eastern cooperative oncology group; PD1: programmed death 1; PD-L1: programmed death ligand 1; HCV: hepatitis C virus; HBV: hepatitis B virus; NG: not given; RFA: radiofrequency ablation; TACE: transcatheter arterial embolization
and clinical trials with other endpoints (such as overall survival, OS).

ICIs targeting CTLA-4 and PD-1/PD-L1 have dramatically changed the outcomes of patients with advanced-stage malignancies. However, ICIs may cause unique side effects, known as immune-related adverse events (irAEs). These side effects are mostly transient and mild, but can occasionally be fatal. Our analysis indicated that the most common AEs associated with ICIs treatment in HCC patients was increased AST (22.73%, 95%CI: 13.8%-35.2%), which was also the most common AEs of grade greater than 3 (9.94%, 95%CI: 4.4%-21.0%). This result is inconsistent with previous studies on other cancers. Respectively, the most common AEs and severe AEs (grade 3-4) were fatigue in NSCLC [26,27], fatigue and lipase elevation or fatigue and rash in melanoma [28,29], low appetite and asthenia in urothelial carcinoma [30], rash and lipase elevation in Hodgkin's lymphoma [31], and neutropenia in lymphoma [32]. Patients with HCC treated with ICIs presented adverse event of fatigue in the second common place followed by rash and fatigue. Rash and fatigue are high incidence skin AEs. Skin AEs are the most original irAE, taking place every 3.6 weeks after treatment [33]. The pooled estimated incident rate of diarrhea is 12.46% (95%CI: 7.9%-19.1%), which was the most common reported gastrointestinal toxicity. Other gastrointestinal toxicities such as abdominal pain, constipation, vomiting, were rarely reported and not taken into consideration here. Result from recent research showed that the incidence rate of diarrhea was higher with CTLA-4 blocked than the PD-1/PD-L1 blocked [34]. Adverse events of instance nausea, asthenia and pulmonary toxicity were less commonly reported in this study.

There are still some limitations in this study. Firstly, the final 7 studies included were all non-randomized controlled clinical trials; it might produce bias and downgrade the level of evidence. Secondly, some factors such as the origin of the HCC and patients’ race might produce bias on outcomes, which can not be controlled in this meta analysis. However, the ORR is a straightforward index in evaluating the effectiveness of immunotherapy, and result from this meta analysis can be referred in clinical application.

DEclarations
Authors’ contributions
Conception and design: Zhang HW, Tang WN
Date analysis and interpretation: Tang WN, Ma LT, Deng Y, Wang W
Manuscript preparation: Tang WN, Wang W
Critical revision and finalizing of the manuscript: Zhang HW

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Not applicable.

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Not applicable.

Consent for publication
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