JBUON 2019; 24(2): 615-621
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Clinical efficacy of DC-CIK combined with sorafenib in the treatment of advanced hepatocellular carcinoma

Zhao Zhou[1], Hui Qin[2], Lixin Weng[1], Yijiang Ni[1]

[1]Department of General Surgery and [2]ICU, the Affiliated Changzhou No.2 People's Hospital of Nanjing Medical University, Changzhou, China.

Summary

Purpose: *To explore the therapeutic efficacy and safety of the combination treatment of dendritic cells and cytokine-induced killers (DC-CIK) and sorafenib in patients with advanced hepatocellular carcinoma (HCC).*

Methods: *Patients diagnosed with advanced HCC and treated with DC-CIK and/or sorafenib in the Affiliated Changzhou No.2 People's Hospital of Nanjing Medical University from January 2015 to January 2016 were retrospectively analyzed. HCC patients were divided into (A): control group (oral administration of sorafenib) and (B): observation group (oral administration of sorafenib combined with DC-CIK). Patients were followed up every 4-8 weeks. Overall survival and adverse events of each patient were recorded. Therapeutic efficacy was evaluated using the modified RECIST criteria.*

Results: *After treatment, ALT and TBIL were remarkably elevated in the control group and decreased in the observation group. No significant change in AFP level was seen in the control group after treatment, whereas it was remarkably decreased in the observation group. The efficacy rate was 16.7% and 51.4% in the control and observation group, respectively. Clinical benefit rate (CBR) was 41.9% and 88.6% in the control group and observation group, respectively. The median survival time of the control and observation group was 13.8 and 18.6 months, respectively. In the observation group there was a significant difference in the survival time between patients with Child-Pugh A and Child-Pugh B, respectively.*

Conclusions: *DC-CIK combined with sorafenib could improve the tumor response rate and prolong overall survival of advanced HCC without increasing the incidence of adverse events. HCC patients achieve a more stable disease condition and longer overall survival with DC-CIK combined with sorafenib than those with individual sorafenib treatment.*

Key words: *DC-CIK, hepatocellular carcinoma, molecular target, sorafenib*

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world, and its incidence ranks fifth and ninth in males and females, respectively. Primary HCC accounts for more than 90% of all HCC cases [1,2]. The morbidity of HCC is second only to lung cancer and severely endangers patients' lives. Currently, liver resection and liver transplantation are the preferred treatment options for HCC. However, only fewer than 20% of HCC patients can be operated due to delayed diagnosis [3]. More seriously, 60% of postoperative HCC patients experience recurrence within 5 years [4]. Abundant blood supply and the lymphatic system in the liver result in frequent metastasis, which is the primary reason for the poor prognosis of this disease. Reports show that the 5-year survival of HCC is only 3-5% [5,6]. Therefore, it is urgent to develop effictive treatments for improving the clinical outcomes of HCC patients.

Sorafenib is a small targeting molecule, a multi-kinase inhibitor that acts on threonine/serine kinases and receptor tyrosine kinases. Sorafenib

Correspondence to: Yijiang Ni, MM. Department of General Surgery, the Affiliated Changzhou No.2 People's Hospital of Nanjing Medical University, 68 Gehu Middle Rd, Wujin District, Changzhou, 213000 Jiangsu, China.
Tel: +86 013776822619, E-mail: 634390337@qq.com
Received: 20/07/2018; Accepted: 11/08/2018

exerts anti-tumor effect in HCC [7,8]. Pharmacological researches have confirmed that sorafenib inhibits tumor neovascularization via inhibiting platelet-derived growth factor receptor (PDGFR) and vascular endothelium growth factor receptor (VEGFR). Moreover, sorafenib directly inhibits tumor growth through inhibiting STAT3, AKT and MAPK pathways [9]. Globally, multicenter studies trials have confirmed that sorafenib could prolong the overall survival time of advanced HCC patients [10]. Although sorafenib has shown satisfying efficacy in the treatment of advanced HCC, the individual difference still remarkably affects the clinical outcome [11,12]. Adverse effects, drug resistance, liver function and HCC stage may influence the therapeutic efficacy of sorafenib [13,14].

Dendritic cells and cytokine-induced killers (DC-CIK) exert anti-tumor activities originating from the functions of both DC and CIK. DC could activate immune functions after recognizing pathogens, and CIK could secret multiple cytokines to kill tumor cells. DC-CIK treatment utilizes both DC and CIK to promote the immune response further, thereby killing tumor cells [15]. DC-CIK has been proved to remarkably prolong the overall survival and improve the quality of life of cancer patients. Currently, the cytotoxic activity of DC-CIK on leukemia, malignant lymphoma and renal cancer cells is present up to 60-90% [16]. However, the specific therapeutic efficacy of DC-CIK on HCC is rarely reported.

Our study aimed to explore the efficacy and safety of sorafenib combined with DC-CIK in the treatment of advanced HCC.

Methods

Clinical data

HCC patients admitted to the Oncology Department of the Affiliated Changzhou No.2 People's Hospital of Nanjing Medical University from January 2015 to January 2016 to be treated with sorafenib were selected. All patients filled in the sorafenib follow-up table. Patients receiving sorafenib were enrolled in the control group and those receiving sorafenib combined with DC-CIK treatment were enrolled in the observation group. This study was approved by the ethics committee of the Affiliated Changzhou No.2 People's Hospital of Nanjing Medical University. All patients signed informed consent before participating in the study.

Inclusion criteria were as follows: 1. Patients with pathologically diagnosed HCC without abnormalities in blood routine tests, cardiopulmonary function, liver, kidney and coagulation functions; 2. Sorafenib treatment lasted for over 2 months; 3. Patients did not receive preoperative anti-tumor treatments; 4. Patients could not be operated or receive second surgery after local therapy due to progressive disease; 5. Patients with Child-Pugh A or B; 6. Patients with Barcelona Clinic Liver Cancer (BCLC) B or C (distant metastasis or portal vein tumor thrombus, considered as BCLC-C); 7. ECOG performance status score of 0-2; 8. Patients did not have other organ dysfunction. Exclusion criteria were as follows: 1. Patients who had other malignancies or non-hepatocyte HCC; 2. Patients with BCLC-A, BCLC-D or Child-Pugh C; 3. Patients who could not have normal oral intake.

Therapeutic regimens

Sorafenib therapy: Oral administration of sorafenib was performed at a dose of 400 mg, bid. One treatment cycle included four-week administration. CT/MRI and sorafenib follow-up were performed every 4 weeks. Sorafenib was discontinued when patients experienced intolerable adverse events or disease progression. Supportive treatments were performed during the sorafenib administration, as needed.

DC-CIK therapy: 50-60 mL of blood were harvested for monocyte extraction. The collected cells were cultured in the laboratory for cell transfusion. DCs were transfused on the first day and CIKs were transfused on the following two days. Intravenous administration of 2 mg dexamethasone was given before cell transfusion. Adverse events, such as fever and rash, were observed during cell transfusion.

Therapeutic efficacy

The therapeutic efficacy in the control and the observation group was evaluated using the Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1) [17]. The therapeutic efficacy was classified as CR (complete

Table 1. Sorafenib follow-up table

Grades	*Clinical description*
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
Grade 4	Life-threatening consequences; urgent intervention indicated
Grade 5	Death related to adverse event

remission), PR (partial remission), SD (stable disease) and PD (progressive disease). Serum levels of ALT, TBIL and AFP before and after treatment were registered and compared.

Adverse events

The adverse events resulting from sorafenib were evaluated using the Common Terminology Criteria Adverse Events, version 4.0 (CTCAE v4.0) [18]. The sorafenib follow-up table recorded all the data (Table 1).

Follow up

Time of death and adverse events were obtained from follow-up data. The follow-up duration ranged from 0.5-2 years, and the last follow-up period ended in January 2018. During the sorafenib treatment, patients were followed-up every 4-8 weeks with AFP, routine blood tests, liver, kidney function and coagulation function and enhanced liver CT or MRI. Patients were required to filling out the sorafenib follow-up table.

Statistics

SPSS 20.0 statistical software package (IBM, Armonk, NY, USA) was used for data analyses. Data were expressed as mean ± standard deviation. Quantitative data and percent data were compared using the t-test and chi-square test, respectively. Kaplan-Meier method and log rank test were also used in this study. P<0.05 was considered statistically significant.

Results

Basic characteristics of HCC patients

A total of 71 HCC patients were enrolled in the study, including 35 patients in the observation group and 36 in the control group. In the observation group, there were 26 males and 9 females. Twenty-nine males and 7 females were enrolled in the control group. No significant differences in gender, age, Child-Pugh classification, AFP level, viral hepatitis history, tumor size and tumor number were found between the two groups (p>0.05, Table 2).

Liver function comparison

Liver function in each patient was evaluated according to levels of ALT (alanine aminotransferase) and TBIL (total bilirubin). After treatment, ALT and TBIL levels were elevated in the control group, whereas no significant differences were found in the observation group (p>0.05, Figure 1A-D). Lower levels of ALT and TBIL were found in the observation group compared with the control group, indicating that DC-CIK combined with sorafenib could effectively improve liver function (Figure 1E and 1F).

Table 2. Basic characteristics of enrolled HCC patients (n=71)

Characteristics	*Control group* *n*	*Observation group* *n*	*p*
Age (years)			0.284
≤60	10	6	
>60	26	29	
Gender			0.527
Male	29	26	
Female	7	9	
ALT(U/L)	36.7±4.1	35.7±3.6	0.157
HBV-infected	33	31	0.248
Tumor diameter	13.1±1.9	12.6±2.1	0.312
Stage of BCLC			0.539
B	8	10	
C	28	25	
Child-Pugh			0.643
A	26	27	
B	10	8	
Distant metastasis	11	9	0.650

BCLC: Barcelona Clinic Liver Cancer, HBV: hepatitis B virus

Table 3. Therapeutic efficacy of HCC patients between control group and observation group

Group	*Control group* *n*	*Observation group* *n*	*p*
CR	1	4	0.154
PR	5	14	0.013
SD	9	13	0.445
PD	11	4	0.048
CR+PR	6	18	0.002

For abbreviations see text

Table 4. Adverse events of HCC patients between control group and observation group

Adverse reactions	*Control group* *n*	*Observation group* *n*	*p*
Hand-foot syndrome	21	19	0.731
Vomit	22	24	0.511
Diarrhea	30	29	0.938
Fatigue	17	20	0.403
Hypertension	18	15	0.546
Leukopenia	7	5	0.562
Anemia	8	7	0.819
Gastrointestinal hemorrhage	2	1	0.574
Hepatic encephalopathy	1	0	0.321

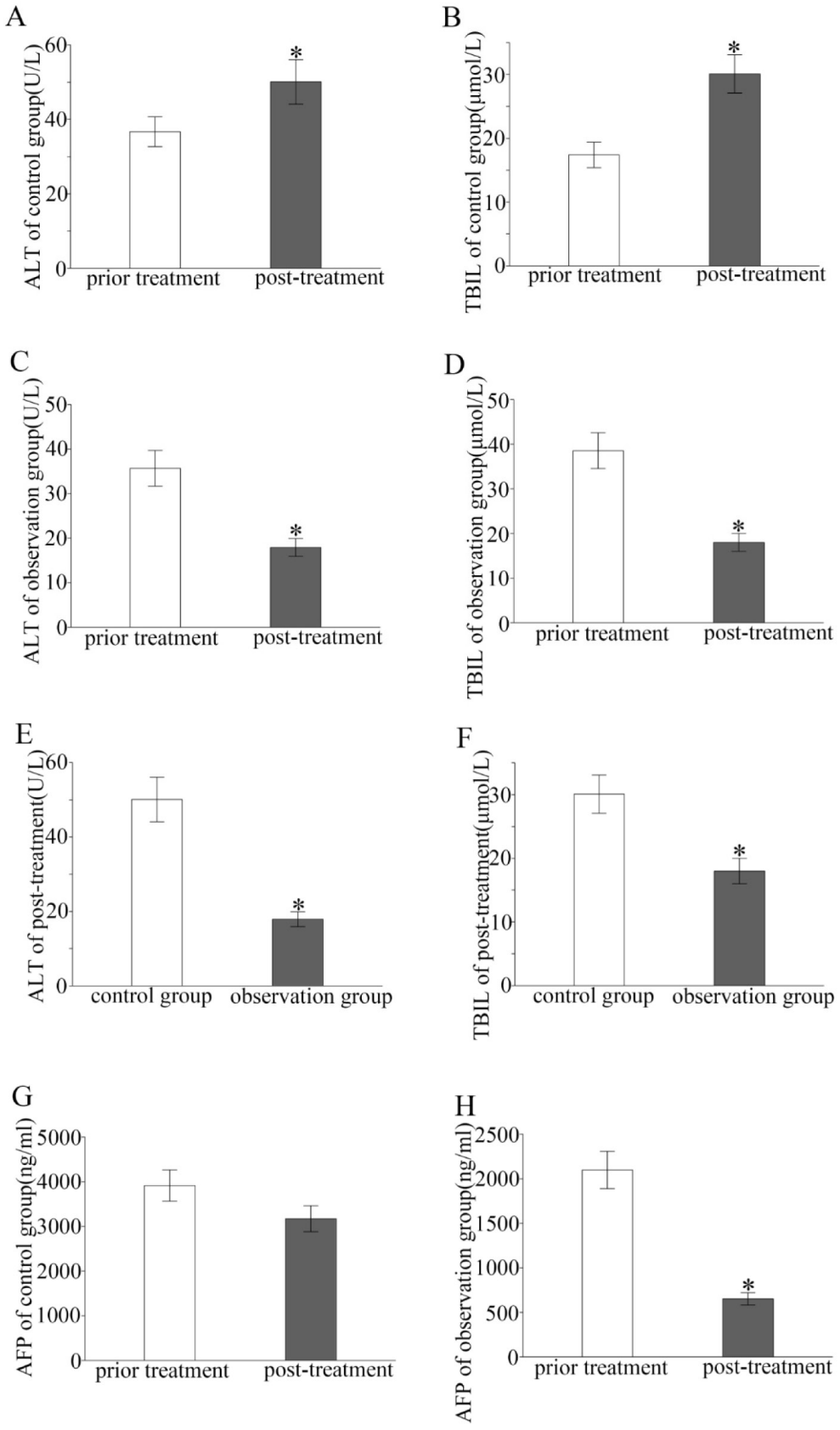

Figure 1. Comparison of blood indexes between the control and the observation group. **(A):** ALT level in control group before and after treatment. **(B):** TBIL level in the control group before and after treatment. **(C):** ALT level in the observation group before and after treatment. **(D):** TBIL level in the observation group before and after treatment. **(E):** Comparison of ALT level before treatment between control group and observation group. **(F):** Comparison of TBIL level before treatment between control group and observation group. **(G):** AFP level in the control group before and after treatment. **(H):** AFP level in the observation group before and after treatment. *p<0.05.

Tumor marker comparison

AFP is considered as a tumor marker for HCC. Oral administration of sorafenib did not markedly decrease AFP level (p>0.05, Figure 1G). However, DC-CIK combined with sorafenib significantly downregulated AFP level in the observation group (p<0.05, Figure 1H).

Comparison of therapeutic efficacy

Six-month follow-up data indicated that the efficacy rate was 16.7% and 51.4% in the control group and observation group, respectively (p<0.05). Clinical benefit rate (CBR) was 41.9% and 88.6% in the control group and the observation group, respectively (p<0.05, Table 3).

Survival analyses

Kaplan-Meier methods with log rank test were used to analyze the overall survival in the two groups. The data showed that the median survival time of the control group and observation group was 13.8 months and 18.6 months, respectively (follow-up duration>24 months, p<0.05, Figure 2).

Subgroup analyses on liver function

In the observation group there was a significant difference in survival time between patients with Child-Pugh A and B, which was 18.4 months and 14.3 months, respectively (p<0.05, Figure 3). No statistical difference in the incidence of adverse events was seen between the control group and observation group (p>0.05).

Adverse events analyses

The main adverse events in this study were hand-foot skin reaction, diarrhea, vomiting, hypertension, fatigue, myelosuppression, and gastrointestinal bleeding. Uncommon adverse events included hair loss and hepatic encephalopathy. No statistical difference in the incidence of adverse events was registered between the control group and observation group (p>0.05, Table 4).

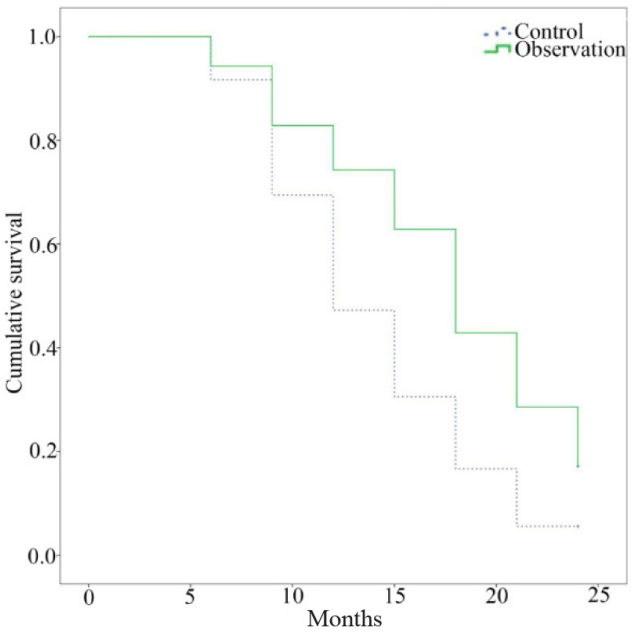

Figure 2. Comparison of overall survival between control group and observation group (p<0.05).

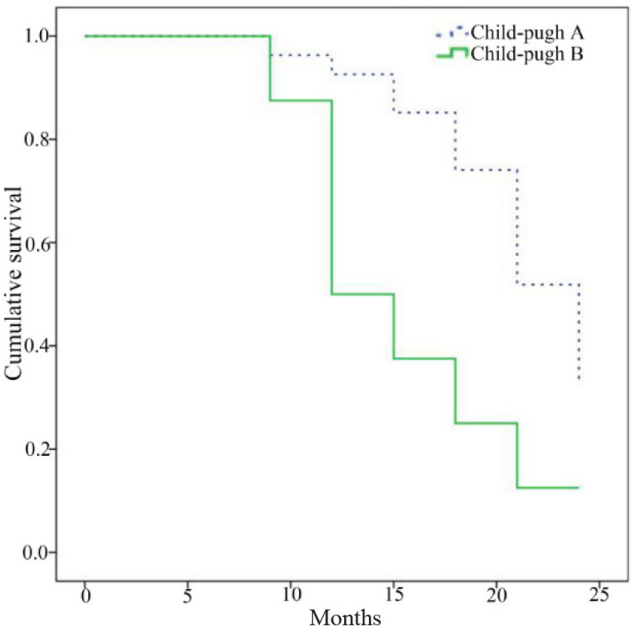

Figure 3. Comparison of overall survival in HCC patients with Child-Pugh A and B (p<0.05).

Discussion

Surgical resection is the optimal treatment for early-stage HCC [19]. However, only 10-30% of HCC patients can be operated, due to early infiltration and metastasis. The overall survival of advanced HCC patients is only 3-6 months [20]. Accumulated evidence has shown that CIK treatment could significantly prevent micrometastases and distant metastasis, thus improving the disease-free survival and overall survival of HCC patients [21-23]. The occurrence, progression and recurrence of primary HCC is closely related to immune escape [24]. In recent years, DC-CIK treatment has been rapidly developed and is used in the clinic. Both CIK and DC-CIK exert anti-tumor effect via producing a large number of cells with antineoplastic activity [25]. Adoptive immune therapy mediated by DC and CIK has shown unique advantages in the tumor treatment [26].

Sorafenib is the first-line drug for advanced HCC approved by EMEA and FDA in 2007. Sorafenib

directly inhibits tumor growth via blocking RAF/MEK/ERK signaling pathway. Besides, sorafenib could indirectly inhibit the proliferation of tumor cells through inhibiting tumor neovascularity [27,28]. In the SHARP phase III clinical trial [10], the median overall survival of HCC patients receiving placebo and sorafenib was 7.9 months and 10.7 months, respectively ($p<0.05$). In addition, the median tumor progression time was 2.8 and 5.5 months ($p<0.05$). The trial by Cheng et al. also confirmed the therapeutic efficacy of sorafenib in treating HCC [29].

In the present study, no significant differences in gender, age, Child-Pugh classification, AFP level, viral hepatitis history, tumor size and tumor number were found between the control group and observation group. Six-month follow-up data indicated that the efficacy rate was 16.7% and 51.4% in the control group and observation group, respectively ($p<0.05$). CBR was 41.9% and 88.6% in the control group and observation group, respectively ($p<0.05$). Survival analysis showed that the median survival time of the control group and observation group was 13.8 months and 18.6 months (follow-up duration> 24 months; $p<0.05$). In the observation group, there was a significant difference in survival time between patients with Child-Pugh A and Child-Pugh B, which was 18.4 months and 14.3 months ($p<0.05$). No statistically significant difference in the incidence of adverse events was seen between the control and the observation group.

In HCC patients with Child-Pugh C, sorafenib may increase the incidence of complications, such as hepatic encephalopathy, gastrointestinal bleeding and even death. Relevant clinical trials have reported that HCC patients with Child-Pugh C present shorter overall survival and rapid disease progression after sorafenib treatment [10]. Hence, cirrhosis accompanied by Child-Pugh C is a contraindication to sorafenib treatment. In our study, there were 27 patients with Child-Pugh A and 8 with Child-Pugh B in the observation group. We found longer survival time in patients with Child-Pugh A compared with those of Child-Pugh B, indicating the significance of Child-Pugh in sorafenib treatment of HCC.

Conclusions

DC-CIK combined with sorafenib could improve the tumor response rate and prolong overall survival in advanced HCC patients without increasing the incidence of adverse events. HCC patients achieve a better stable disease condition and overall survival with DC-CIK combined with sorafenib.

Conflict of interests

The authors declare no conflict of interests.

References

1. Fitzmaurice C, Dicker D, Pain A et al. The Global Burden of Cancer 2013. JAMA Oncol 2015;1:505-27.
2. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. Eur J Cancer 2012;48:599-641.
3. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. Hepatology 2003;37:429-42.
4. Tang Z, Zhou X, Lin Z et al. Surgical treatment of hepatocellular carcinoma and related basic research with special reference to recurrence and metastasis. Chin Med J (Engl) 1999;112:887-91.
5. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
6. Bruix J, Llovet JM. Hepatitis B virus and hepatocellular carcinoma. J Hepatol 2003;39 (Suppl 1):S59-S63.
7. Tai WT, Cheng AL, Shiau CW et al. Signal transducer and activator of transcription 3 is a major kinase-independent target of sorafenib in hepatocellular carcinoma. J Hepatol 2011;55:1041-8.
8. Xu Q, Huang Y, Shi H, Song Q, Xu Y. Sunitinib versus sorafenib plus transarterial chemoembolization for inoperable hepatocellular carcinoma patients. JBUON 2018;23:193-9.
9. Matsuda Y, Fukumoto M. Sorafenib: complexities of Raf-dependent and Raf-independent signaling are now unveiled. Med Mol Morphol 2011;44:183-9.
10. Llovet JM, Ricci S, Mazzaferro V et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378-90.
11. de Stefano G, Farella N, Scognamiglio U et al. Sorafenib after RFA in HCC patients: a pilot study. Hepatogastroenterology 2015;62:261-3.
12. Bruix J, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. Gut 2014;63:844-55.
13. Kuczynski EA, Lee CR, Man S, Chen E, Kerbel RS. Effects of Sorafenib Dose on Acquired Reversible Resistance and Toxicity in Hepatocellular Carcinoma. Cancer Res 2015;75:2510-9.
14. Li J, Wang L, Cong N et al. Efficacy of Sorafenib for Advanced Hepatocellular Carcinoma and Prognostic Factors. Hepatogastroenterology 2014;61:954-7.

15. Qu HQ, Zhou XS, Zhou XL, Wang J. Effect of DC-CIK cell on the proliferation, apoptosis and differentiation of leukemia cells. Asian Pac J Trop Med 2014;7:659-62.
16. Blattman JN, Greenberg PD. Cancer immunotherapy: a treatment for the masses. Science 2004;305:200-5.
17. Ronot M, Bouattour M, Wassermann J et al. Alternative Response Criteria (Choi, European association for the study of the liver, and modified Response Evaluation Criteria in Solid Tumors [RECIST]) Versus RECIST 1.1 in patients with advanced hepatocellular carcinoma treated with sorafenib. Oncologist 2014;19:394-402.
18. Chen AP, Setser A, Anadkat MJ et al. Grading dermatologic adverse events of cancer treatments: the Common Terminology Criteria for Adverse Events Version 4.0. J Am Acad Dermatol 2012;67:1025-39.
19. Tang ZY. Hepatocellular carcinoma surgery--review of the past and prospects for the 21st century. J Surg Oncol 2005;91:95-6.
20. El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011;365:1118-27.
21. Lee JH, Lee JH, Lim YS et al. Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. Gastroenterology 2015;148:1383-91.
22. Hontscha C, Borck Y, Zhou H, Messmer D, Schmidt-Wolf IG. Clinical trials on CIK cells: first report of the international registry on CIK cells (IRCC). J Cancer Res Clin Oncol 2011;137:305-10.
23. Schmeel LC, Schmeel FC, Coch C, Schmidt-Wolf IG. Cytokine-induced killer (CIK) cells in cancer immunotherapy: report of the international registry on CIK cells (IRCC). J Cancer Res Clin Oncol 2015;141:839-49.
24. Willimsky G, Schmidt K, Loddenkemper C, Gellermann J, Blankenstein T. Virus-induced hepatocellular carcinomas cause antigen-specific local tolerance. J Clin Invest 2013;123:1032-43.
25. Schmeel FC, Schmeel LC, Gast SM, Schmidt-Wolf IG. Adoptive immunotherapy strategies with cytokine-induced killer (CIK) cells in the treatment of hematological malignancies. Int J Mol Sci 2014;15:14632-48.
26. Wang QJ, Wang H, Pan K et al. Comparative study on anti-tumor immune response of autologous cytokine-induced killer (CIK) cells, dendritic cells-CIK (DC-CIK), and semi-allogeneic DC-CIK. Chin J Cancer 2010;29:641-8.
27. Nishida N, Kitano M, Sakurai T, Kudo M. Molecular Mechanism and Prediction of Sorafenib Chemoresistance in Human Hepatocellular Carcinoma. Dig Dis 2015;33:771-9.
28. Nagel C, Armeanu-Ebinger S, Dewerth A, Warmann SW, Fuchs J. Anti-tumor activity of sorafenib in a model of a pediatric hepatocellular carcinoma. Exp Cell Res 2015;331:97-104.
29. Cheng AL, Guan Z, Chen Z et al. Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma according to baseline status: subset analyses of the phase III Sorafenib Asia-Pacific trial. Eur J Cancer 2012;48:1452-65.