

## Original Article

# Effect of sorafenib combined with CIK cell treatment on immunity and adverse events in patients with late-stage hepatocellular carcinoma

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**Abstract:** We investigated the effect of sorafenib combined with cytokine-induced killer (CIK) cell treatment on immunity and adverse events in patients with late-stage hepatocellular carcinoma (HCC). From March 2011 to July 2013, 24 patients with late-stage HCC receiving sorafenib treatment were included. Among them, 11 patients also received CIK cell treatment. The data on cellular immune indicators in venous blood before and after sorafenib treatment and before and after autologous CIK cell transfusion were reviewed along with drug-related adverse events. Data comparisons were done using statistical software. CD<sub>3</sub> and CD<sub>4</sub> counts and CD<sub>4</sub>/CD<sub>8</sub> ratio in patients treated by sorafenib alone decreased after treatment (P<0.05), while CD<sub>8</sub> count increased (P<0.05). However, opposite variations were observed in combined treatment group; the adverse events such as fatigue and diarrhea in patients after CIK cell treatment were alleviated substantially. Sorafenib combined with CIK cell treatment for patients with HCC can improve cellular immunity and reduce drug-related adverse events.

**Keywords:** Ultrasound guidance, catheter ablation, hepatocellular carcinoma, targeted therapy, immunity

## Introduction

Primary hepatocellular carcinoma (HCC) is among the malignancies that have the highest incidence and poorest prognosis in China. Although surgery remains the first choice for the treatment of HCC, it only applies to early-stage patients. As hepatocellular carcinoma is relatively insensitive to chemotherapy and radiotherapy, the 5-year survival of the patients is still very low (<5%) [1]. It is greatly urgent to look for effective means to treat hepatocellular carcinoma. Along with the recent developments of molecular targeted drugs, their applications to primary hepatocarcinoma are expanding. The targeted drugs only kill the tumor cells with little damage to other organs, thereby improving the patients' life quality. Sorafenib is the first multi-targeting drug, which is also the first drug to be approved by FDA for treating hepatocellular carcinoma due to its anti-tumor effect [2]. Sorafenib (4-(4-(3-(4-Chloro-3-(trifluorome-

thyl)phenyl)ureido)phenoxy)-n-methylpicolinamide) is a novel oral multikinase inhibitor. It has an inhibitory effect on Raf-1 kinase, B-Raf, vascular endothelial growth factor receptor-2 (VEGFR-2), platelet-derived growth factor receptor (PDGFR), FMS-like tyrosine kinase-3 (Flt-3) and C-kit [3]. Sorafenib can not only inhibit tumor cell proliferation by targeting KIT, Flt-3 and Raf kinases, but also suppress tumor angiogenesis by inhibiting VEGFR, PDGFR and Raf kinases. Several international multi-center clinical trials have demonstrated the efficacy of sorafenib against late-stage hepatocarcinoma and its effect in prolonging survival [4]. However, the patients treated by sorafenib for late-stage hepatocarcinoma may suffer from immunosuppression and drug-related adverse events. For example, the skin symptoms include hand-foot skin reaction, erythema and desquamation [5]; gastrointestinal symptoms include decreased appetite and diarrhea; the resulting bone marrow damage may lead to leukopenia and ane-

mia [6]. These adverse events have restricted the applications of sorafenib and cause great pains to patients. Cytokine-induced killer (CIK) cell treatment is another major treatment after surgery, chemotherapy and radiotherapy, marking a milestone in the fight against tumors in the 21<sup>st</sup> century [7]. CIK cells refer to a group of cytotoxic lymphocytes whose tumor cell killing activities are induced by various stimulating factors [8]. Already applied extensively in clinic, CIK cell treatment has shown good effect against many tumors, bringing new hope as another immunotherapy besides adoptive immunotherapy [9, 10]. CIK cell treatment can improve cellular immunity and reduce chemotherapy-related adverse events. However, there are few reports concerning sorafenib combined with CIK cell treatment in late-stage HCC [7], especially in relation to the improvement of cellular immunity and adverse events. We collected 24 patients with late-stage HCC treated by sorafenib from March 2011 to July 2014. The cellular immune indicators in venous blood were measured before and after targeted therapy with sorafenib and before and after autologous CIK cell transfusion. Moreover, these cases were followed up to observe the adverse events. The data were compared using statistical software.

### Materials and methods

#### General information

**Clinical data:** From March 2011 to July 2013, 24 patients pathologically confirmed as late-stage HCC by hepatic puncture at General Hospital of Shenyang Military Region were collected. These patients were not suitable for surgical resection according to the surgeons and imaging findings, with Karnofsky Performance Status (KPS) score >70. All of them were treated by sorafenib, and 11 of them were treated in combination with CIK cell treatment; all cases were followed up for 6 months. Patients treated by sorafenib alone and those in combination with CIK cell treatment did not differ in terms of gender, age, clinical staging and metastasis ( $P>0.05$ ) (see **Table 1**). They met the following inclusion criteria: Pathologically diagnosed as late-stage HCC (typical imaging findings and alpha-fetoprotein  $\geq 500$  ng·ml<sup>-1</sup>); Treated by sorafenib for at least 3 months; Child-Pugh class A; At least one measurable tumor with diameter  $\geq 1$  cm; No severe dysfunction of main organs. Cases were excluded if they met any of

the following criteria: KPS below 70; Complicated by severe cardiovascular diseases and diabetes; other types of hepatocarcinoma.

**Reagents:** Sorafenib tablets were manufactured by Bayer Pharma AG, with the brand name Nexavar and dose of 200 mg/tablet.

#### Methods

**Treatment scheme:** All patients took Nexavar orally (200 mg, bid) with no dose adjustment during the treatment for at least 3 months. Sorafenib was discontinued if any of the following conditions occurred: Acute gastrointestinal bleeding; Severe hepatic dysfunction; Hypertension (above stage III); Recurrent proteinuria ( $>2$  g·d<sup>-1</sup>). Adoptive immunotherapy with CIK cells was simultaneous with sorafenib treatment.

**Procedures of CIK cell treatment:** Peripheral blood mononuclear cells (PBMCs) were collected and induced into CIK cells through in vitro culture, which were then transfused back. Transfusion was performed twice during each course of treatment (once every two days) with cell count not less than  $2 \times 10^9$ /L each time. The total cell count was not less than  $5 \times 10^9$ /L for each course. Transfusion was performed once a month, for 3 consecutive months.

**Detection of CD<sub>3</sub>, CD<sub>4</sub>, CD<sub>8</sub> and CD<sub>4</sub>/CD<sub>8</sub>:** Venous blood samples were drawn before targeted therapy and after treatment for 3 months for patients receiving monotherapy or combination therapy. The blood samples were detected by Department of Laboratory Medicine.

**Statistical analysis:** SPSS18.0 software was used for statistical analyses, and all data were expressed as  $\bar{x} \pm s$ . For intergroup comparison of count data,  $\chi^2$  test was adopted. For intergroup comparison of measurement data, independent samples t test was used. For intragroup comparison of measurement data before and after treatment, paired-samples t test was used.  $P<0.05$  indicated significant difference.

### Results

#### Comparison of clinical data before treatment between the two groups

The monotherapy group and the combination therapy group did not differ significantly in terms of baseline data ( $P>0.05$ ) (see **Table 1**).

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**Table 1.** Comparison of baseline data between the two groups [*n* (%), *n*=24]

Group	Gender		Age	TNM staging		Site of metastasis		
	Male	Female	( $\bar{x} \pm s$ , years)	III B	Stage IV	Bone	Lung	Lymph nodes
Sorafenib monotherapy group	7 (53.85%)	6 (46.15%)	58.51±3.43	5 (38.46%)	8 (61.54%)	2 (15.38%)	3 (23.08%)	8 (61.54%)
Combination therapy group	7 (63.64%)	4 (36.36%)	57.17±2.98	4 (36.36%)	7 (63.64%)	1 (9.09%)	1 (9.09%)	9 (81.82%)

Note: The two groups did not differ significantly in terms of gender, age, TNM staging and site of metastasis, *P*<0.05.

**Table 2.** Comparison of cellular immune indicators after treatment between the two groups ( $\bar{x} \pm s$ , *n*=24)

	CD <sub>3</sub>	CD <sub>4</sub>	CD <sub>8</sub>	CD <sub>4</sub> /CD <sub>8</sub>
Sorafenib monotherapy group	53.62±4.62	30.37±3.09	30.25±3.01	1.01±0.11
Combination therapy group	63.37±5.37	39.51±4.71	27.52±3.09	1.43±0.15

Note: The differences between the two groups were statistically significant, *P*<0.05.

**Table 3.** Comparison of incidence of drug-related adverse events between the two groups ( $\bar{x} \pm s$ , *n*=24)

Adverse events	Monotherapy group	Combination therapy group	<i>P</i> value
Fatigue	8 (61.54%)	3 (27.27%)	0.0932
Skin rash	10 (76.93%)	8 (72.73%)	0.8130
Pain	5 (38.46%)	4 (36.36%)	0.9158
Insomnia	6 (46.15%)	1 (9.09%)	0.0465
Decreased appetite	7 (53.85%)	1 (9.09%)	0.0205
Diarrhea	9 (69.23%)	3 (27.27%)	0.0405

Note: The two groups differed significantly in the incidence of adverse events. The significance was tested by chi-square test, and *P*<0.05 indicated significant difference.

### Comparison of cellular immune indicators after treatment between the two groups

CD<sub>3</sub> and CD<sub>4</sub> counts and CD<sub>4</sub>/CD<sub>8</sub> ratio in combination therapy group were obviously higher than those of monotherapy group after treatment (*P*<0.05); CD<sub>8</sub> count in combination therapy group was significantly lower than that of monotherapy group (*P*<0.05). The results are shown in **Table 2**.

### Comparison of incidence of drug-related adverse events between the two groups

The incidence of adverse events including insomnia, fatigue, nausea & vomiting and decreased appetite reduced considerably in combination therapy group, showing statistically significant difference compared with monotherapy group (*P*<0.05). However, the incidence of skin rash and pains was not significantly improved. The incidence of insomnia, fatigue and decreased appetite in combination therapy group was much lower compared with

the monotherapy group (*P*<0.05). The results are shown in **Table 3**.

### Safety evaluation

No single case was found to have recurrence or metastasis during the 6-month follow-up. In combination therapy group, 1 case (9.1%) suffered from mild to moderate fever during autologous CIK cell transfusion. Normal body temperature was restored within 24 h after treatment with nonsteroidal anti-inflammatory drugs or physical cooling; 2 cases (18.2%) complained of symptoms similar to the common cold including aching and soreness of the lower extremities, which were alleviated spontaneously within 48 h; 1 case (9.1%) presenting with feebleness showed spontaneous improvement without special treatment.

### Discussion

Hepatocellular carcinoma (HCC) is a common primary malignancy of the liver. It ranks the fifth

in male population and the seventh in female population in Europe and USA in terms of incidence, which is the third leading cause of cancer-related deaths. In China, hepatocarcinoma ranks the second, only after lung carcinoma in terms of mortality. The survival rate of HCC in the USA and developing countries which keep cancer registry is only 3%-5%. Meanwhile, the incidence of HCC worldwide is also increasing [11]. Sorafenib is the most widely used targeted drug and the only drug approved by FDA for non-resectable HCC. However, there are no effective treatments for those insensitive to sorafenib or showing progression after sorafenib treatment [4]. Some patients may suffer from immunosuppression and adverse events such as diarrhea and skin rash after sorafenib treatment. After drug discontinuance for less tolerant patients, immunosuppression will further lead to tumor recurrence and metastasis. Therefore, looking for treatment that can improve immunity and reduce sorafenib-related adverse events for HCC patients is of crucial importance to increase patients' life quality, expand the scope of applicability of sorafenib and inhibit tumor recurrence and metastasis.

CIK cells are a group of heterogeneous cells induced by cytokines in vitro culture using PBMCs. They have a direct killing activity on tumor cells through secretion of various cytokines which can regulate the immune system. Meanwhile, CIK cells mediate the apoptosis of tumor cells via ligand-receptor binding. CIK cell treatment is a powerful adoptive immunotherapy after chemotherapy, radiotherapy and targeted molecular therapy for cancers, which possesses the characteristics of strong proliferative activity, high tumor killing activity and wide spectrum of anti-tumor activity [12]. Though many reports have been published in recent years concerning CIK cell treatment in tumors, little is known about the efficacy of CIK cell treatment combined with sorafenib in late-stage HCC. We carried out a retrospective analysis and found that  $CD_3$  and  $CD_4$  counts and  $CD_4/CD_8$  ratio in the combination therapy group were obviously higher than those in sorafenib monotherapy group ( $P<0.05$ );  $CD_8$  count in the combination therapy group was much lower compared with sorafenib monotherapy group ( $P<0.05$ ). These differences were all statistically significant. The anti-tumor mechanisms work through cellular immune response and humoral immune response. In the former, T cell

subsets ( $CD_3$ ,  $CD_4$ ,  $CD_8$ ) are mainly involved, but their activity is severely inhibited in late-stage hepatocarcinoma [13], which is further aggravated by targeted therapy [14]. This is manifested as a reduction of  $CD_3$  and  $CD_4$  cells and the increase of  $CD_8$  cells as well as a decrease of  $CD_4/CD_8$  ratio. Our experiment showed that  $CD_3$  and  $CD_4$  counts were increased in the combination therapy group compared with the monotherapy group. This indicated that the combination therapy can improve the immunity of patients and alleviate immunosuppression caused by sorafenib. Moreover, in the combination therapy group, the incidence of adverse events decreased considerably compared with the monotherapy group, including insomnia, fatigue, nausea & vomiting and decreased appetite ( $P<0.05$ ).

To conclude, sorafenib combined with CIK cell treatment can effectively improve the immunity of HCC patients and relieve the drug-related adverse events. The life quality and treatment effect are improved correspondingly. However, the follow-up duration was limited, and the positive effect of the combination therapy on overall survival and progression-free survival should be determined by long-term observations. The purpose is to provide ideal indicators of treatment efficacy of the combination therapy for late-stage HCC.

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### Disclosure of conflict of interest

None.

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