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Influence of Sorafenib on Host Immunity in Patients with Liver Cirrhosis With Advanced Hepatocellular Carcinoma Stratified by Etiology

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Abstract. *Aim: We previously reported that sorafenib induces Th1 [interferon-γ (IFNγ)-positive interleukin 4 (IL4)-negative] dominance which prevents tumor cells from escaping the host immune system in patients with liver cirrhosis (LC) and advanced hepatocellular carcinoma (aHCC). However, in that study we did not assess the influence of sorafenib on host immunity according to the etiology of LC. Therefore, this study was retrospectively performed to evaluate the impact of sorafenib therapy for aHCC on host immunity in patients stratified according to the etiology of LC: Patients and Methods: A total of 116 adult Japanese patients with LC and aHCC received sorafenib therapy at our hospital. Blood samples were collected before and after treatment for 4 weeks. Results: Twenty-two patients had hepatitis B virus (HBV)-related LC, 62 patients had hepatitis C virus (HCV)-related LC, 22 patients had alcoholic LC, and 10 patients had LC without these causative factors. In patients receiving sorafenib at a dose of 400 mg/day, patients in Child–Pugh class A, and patients with stage IVA aHCC, Th2 (IFNγ-negative/IL4-positive) cells decreased significantly after treatment, although there was no significant impact on the tumor response. In addition, Th2 cells decreased significantly in patients with HCV-related LC after treatment, while there were no significant changes in the other groups. Conclusion: Sorafenib might prevent tumor cells from escaping the host immune system in patients with aHCC and HCV-related LC, although it does not seem to do so in those with LC of other etiologies.*

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Key Words: Sorafenib, Th1/Th2 balance, host immunity, etiology, hepatocellular carcinoma, liver cirrhosis.

Liver cancer was reported to be the sixth most common cancer diagnosed worldwide (749,000 new cases annually) and the third frequent causes of cancer-related death overall (1), with hepatocellular carcinoma (HCC) being the second leading cause of cancer-related death for men and the sixth highest cause for women worldwide (2). The oral multikinase inhibitor sorafenib is the first systemic agent approved for HCC and has revolutionized treatment of advanced HCC (aHCC) in patients with liver cirrhosis (LC). Sorafenib targets the rapidly accelerated fibrosarcoma (RAF)/mitogen-activated protein kinase/extracellular signal-related kinase signaling pathway, and shows strong *in vivo* antitumor activity, and it has been used to treat patients with aHCC (Barcelona Clinic Liver Cancer stage C) not responding to transcatheter arterial chemoembolization (3-6). As a multikinase inhibitor, sorafenib shows activity against vascular endothelial growth factor receptor (VEGFR) 2, platelet-derived growth factor receptor (PDGFR), c-KIT receptor, (v-raf murine sarcoma viral oncogene homolog B1 (b-RAF), and p38 (7), which are important molecules in signal transduction pathways that may contribute to the pathogenesis of HCC (8). Sorafenib prevents tumor growth by inhibiting several components of the RAF-mitogen-activated protein kinase kinase (MEK)/extracellular signal–regulated kinase (ERK) signaling pathway, and it also suppresses angiogenesis by acting on VEGFR-1, VEGFR-2, VEGFR-3, and PDGFR-B (9). A phase III study of regorafenib (RESORCE) (10), another multikinase inhibitor, suggested that it may be a promising treatment option for patients who show disease progression on sorafenib therapy

(11). However, sorafenib still has an important role in treating aHCC and its influence on host immunity in patients with LC with this cancer is of interest.

Th1 [interferon-γ (IFNγ)-positive interleukin 4 (IL4)-negative] and Th2 (IFNγ-negative/IL4-positive) cells cross-regulate their own development. It has been reported that Th2 type cytokines down-regulate antitumor immunity (12), while activation of Th1 responses promotes antitumor immunity (13-16). When treating aHCC in patients with LC, the influence of tumor-related factors, the characteristics of anticancer drugs or molecular-targeting agents, and the role of host immunity all need to be considered. We previously reported that sorafenib reduced peripheral blood Th2 cells in patients with LC with aHCC and that it might induce Th1 dominance and prevent tumor cells from escaping the immune system in these patients (17). However, that study did not examine whether the etiology of LC had any influence on the efficacy of sorafenib therapy for aHCC: Accordingly, the present study was retrospectively performed to evaluate the effects of sorafenib therapy for aHCC on host immunity in patients with LC stratified according to the etiology of their liver disease.

Patients and Methods

Patients. Between 2009 and 2017, a total of 116 adult Japanese patients with LC received sorafenib therapy for aHCC at our hospital. Sorafenib was administered for 4 weeks at a dose of 200-800 mg/day, depending on the patient's body size and age. Data for this study were obtained from blood samples collected early in the morning before and after sorafenib treatment for 4 weeks. Written informed consent was obtained from each patient after the potential complications of sorafenib treatment were fully explained (3).

Analysis of CD4-positive T-cell subsets. Blood samples were kept at room temperature and analyzed within 6 hours. Subsets of CD4-positive T-cells in peripheral blood were analyzed after nonspecific stimulation with phorbol 12-myristate 13-acetate (PMA), ionomycin, or brefeldin A (Sigma Chemical Co., St. Louis, MO, USA), according to the modified method of Jung *et al*. (18). Flow cytometry was used to detect cytoplasmic expression of IFNγ and IL4 by CD4-positive T-cells from peripheral blood after culture and staining, as reported previously (17). Then the CD4-positive T-cell population was divided into IFNγ-positive/IL4-negative (Th1) cells and IFN-γ-negative/IL4-positive (Th2) cells. Regulatory T-cells (Treg cells) were identified as $CD25^{high}/CD127^{low}$ cells (17).

Evaluation of tumor response. For each patient, the tumor response was assessed according to the modified Response Evaluation Criteria in Solid Tumors (RECIST) (19, 20).

Statistical analysis. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 11.0; SPSS, Chicago, IL, USA). Results are expressed as the mean±standard deviation. Wilcoxon's signed-rank sum test was used to compare patient characteristics within each group. In all analyses, $p \leq 0.05$ was considered to indicate statistical significance.

Table I. *Clinical characteristics of the 116 patients with liver cirrhosis and advanced hepatocellular carcinoma*.

Etiology of cirrhosis	HBV	HCV	Alcoholic	Non-ABC
No. of patients	22	62	22	10
Age, years				
Mean	66.5±8	72.5±6	66.2±6	74.4±7
Gender, n				
M	19	49	21	7
F	3	13	1	3
Child–Pugh classification, n				
A	5	45	16	7
B	17	16	6	3
C	0	1	0	0
Stage of tumor, n				
III	0	3	0	0
IVA	18	46	18	8
IVB	4	13	4	2
Dose of sorafenib, n				
200 mg/day	2	8	0	0
400 mg/day	16	51	17	9
800 mg/day	4	3	5	1

HBV: Hepatitis B virus; HCV: hepatitis C virus; non-ABC: non-HBV-/non-HCV liver cirrhosis with no alcohol exposure.

This study was approved by the Ethical Review Board of Toho University Medical Center, Omori Hospital (number 27-141).

Results

The 116 patients were divided into four groups. Twenty-two patients had hepatitis B virus (HBV-)-related LC (HBV group), 62 patients had hepatitis C virus (HCV-)-related LC (HCV group), 22 patients had alcoholic LC (alcohol group), and 10 patients had non-B-/non-C LC with no alcohol exposure (non-ABC group). We excluded patients with LC due to autoimmune diseases such as autoimmune hepatitis or primary biliary cirrhosis. Patients characteristics according to group are given in Table I: Significant differences were noted in age, Child–Pugh class and sorafenib dosage among the groups (Table I).

Changes of serum characteristics. In the HBV group, the serum level of creatinine and the platelet count decreased significantly after sorafenib treatment compared with before treatment. In the HCV group, serum levels of total bilirubin, direct bilirubin, aspartate transaminase, alanine amino-transferase, alpha-fetoprotein (AFP)-L3, and *des*-gamma carboxyprothrombin (DCP) all increased significantly after sorafenib treatment, while serum albumin and the platelet count decreased after treatment. In the alcohol group, the serum level of direct bilirubin increased after sorafenib treatment, while serum albumin, the platelet count, and the prothrombin time

Table II. *Comparison of clinical characteristics for 116 patients with liver cirrhosis and advanced hepatocellular carcinoma.*

Etiology of cirrhosis	HBV		HCV		Alcoholic		Non-ABC	
	Pre Tx	Post Tx	Pre Tx	Post Tx	Pre Tx	Post Tx	Pre Tx	Post Tx
Ammonia (mg/dl)	49.4±41	53.7±42	52.0±37	60.1±29	46.2±21	47.9±17	36.8±13	49.3±11
Total bilirubin (g/dl)	1.0±0.6	1.0±0.4	1.0±0.4	1.3±0.7**	1.0±0.4	1.2±0.6	0.7±0.2	0.7±0.3
Direct bilirubin (g/dl)	0.3±0.3	0.3±0.2	0.4±0.2	0.6±0.4**	0.3±0.2	0.4±0.3*	0.2±0.1	0.3±0.1
Albumin (mg/dl)	3.7±0.5	3.5±0.7	3.3±0.5	3.0±0.5**	3.6±0.5	3.2±0.6**	3.3±0.8	3.4±0.5
AS (IU/l)	52.5±28	51.8±28	77.5±53	99.0±58***	76.0±102	86.1±98	37.8±10	52.8±19
ALT (IU/l)	26.3±14	32.3±25	49.6±41	61.3±38.9***	35.1±36	36.9±26	17.8±10	27.8±19
Total cholesterol (mg/dl)	178.1±52	160.4±60	150.8±59	140.3±45	178.2±67	174.0±72	149.8±40	141.4±39
BUN (mg/dl)	14.7±5	13.9±5	17.7±7	16.1±6	16.4±7	18.2±12	19.2±5	20.3±7
Creatine (mg/dl)	0.8±0.2	0.7±0.2**	0.9±0.3	0.8±0.3***	0.9±0.3	0.9±0.4	0.8±0.4	0.8±0.4
WBC (n/mm^3)	5,018.8± 2,710	7,537.5± 10,227	4,491.1± 1,329	5,520.5± 6,407	6,215.0± 2,358	5,700.8± 1,738	5,316.7± 1,613	5,816.7± 2,667
Lymphocytes (n/mm^3)	1,148.3±592	1,060.5±594	1,197.1±420	1,130.0±400	1,389.0±518	1,238.3±579	1,113.5±381	1,213.2±400
Monocytes (n/mm^3)	395.2±169	361.4±191	335.9±130	307.1±142	487.3±228	425.7±175	379.5±236	109.2±40*
Platelets (×10^4/mm^3)	18.9±14	15.4±12**	12.7±5	11.1±5**	16.7±8	14.2±6.4**	23.2±14	18.9±10*
Prothrombin time (%)	82.9±15	82.1±15	80.4±14	76.2±17	81.1±11	74.5±15*	88.2±12	87.7±12
AFP (ng/ml)	38,736.9± 119,184	57,454.5± 196,855	37,798.7± 196,443.4	23,307.4± 88,205.8	10,039.6± 21,241	15,390.1± 27,707.9	39.5±76	77.0±162
AFP-L3 (%)	41.6±25	40.3±25	26.7±25	30.4±27***	36.3±26	37.4±25	15.8±18	18.2±25
DCP (AU/ml)	27,708.7± 61,601	157,539.0± 516,603	7,523.9± 24,186	21,627.7± 40,437****	21,884.8±5 3,207	30,912.6± 60,049	4,594.8± 8,180	22,910.7± 45,695*

HBV: Hepatitis B virus; HCV: hepatitis C virus; non-ABC: non-HBV-/non-HCV liver cirrhosis with no alcohol exposure; AST: aspartate transaminase; ALT: alanine aminotransferase; AFP: alpha-fetoprotein; alpha-fetoprotein-L3; BUN: blood urea nitrogen; DCP: des-gamma carboxyprothrombin; Tx: sorafenib therapy; WBC: white blood cells. Significantly different from pre-therapy value at: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

decreased after treatment. In the non-ABC group, the serum level of DCP increased after sorafenib treatment, while the monocyte and platelet counts decreased significantly after treatment (Table II).

Response. Table III summarizes the response to sorafenib treatment. No patient achieved a partial response (PR) in the alcohol-associated LC group and the non-ABC group, although patients in the HBV or HCV group achieved PR.

Peripheral blood Th1, Th2, and Treg cells. There were no significant differences in Th1 cells or Treg cells between before and after sorafenib treatment at any of the three dose levels. In contrast, there was a significant decrease in Th2 cells after treatment (3.8±2% *vs*. 3.5±2%; p=0.0398, by Wilcoxon's signed-rank sum test) in the patients receiving sorafenib at 400 mg/day, although there were no significant changes in Th2 cells after treatment at dose of 200 and 800 mg/day. With regard to the response to sorafenib, no significant changes in Th1, Th2, and Treg cells were noted after treatment according to response category. There were also no significant changes in Th1 and Treg cells after treatment in patients from each Child–Pugh class. However, a significant decrease in Th2 cells occurred in patients with Child–Pugh class A LC after sorafenib treatment (3.2±1% *vs*. 2.9±1%; p=0.0493, by Wilcoxon's signed-rank sum test), although there was no significant change in those with Child–Pugh class B: Regarding the influence of tumor, no significant changes in Th1 and Treg cells occurred after treatment in patients with HCC of any stage. Interestingly, a significant decrease in Th2 cells was noted in patients with stage IVA disease after sorafenib treatment (3.7±2% *vs*. 3.4±2%; p=0.0174, by Wilcoxon's signed-rank sum test), although there was no significant change in Th2 cells after treatment in those with stage IVB disease (Table IV).

Table III. *Objective responses after sorafenib treatment for 4 weeks of patients with liver cirrhosis and advanced hepatocellular carcinoma according to etiology.*

	CR	PR	SD	PD	Response rate (%)
HBV n=22	0	1	14	7	4.5
HCV n=62	0	5	39	18	8.1
Alcoholic n=22	0	0	9	13	0.0
Non-ABC n=10	0	0	8	2	0.0

HBV: Hepatitis B virus; HCV: hepatitis C virus; non-ABC: non-HBV-/non-HCV liver cirrhosis with no alcohol exposure; CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease. Data are the number of patients.

Table IV. *Comparison of CD4-positive T-cells before and after sorafenib treatment in patients with liver cirrhosis and advanced hepatocellular carcinoma.*

	Cell type	Pre Tx, %	Post Tx, %	*p*-Value
Dose of sorafenib				
200 mg/day	Th1	24.8±8	24.7±8	0.8137
	Th2	3.2±1	2.9±1	0.4485
	Treg	9.8±2	9.1±3	0.3882
400 mg/day	Th1	26.2±10	25.8±12	0.2846
	Th2	3.8±2	3.5±2	0.0398
	Treg	8.9±3	9.1±3	0.9975
800 mg/day	Th1	24.1±13	23.4±15	0.5292
	Th2	4.1±3	4.1±2	0.4415
	Treg	8.1±2	8.3±3	0.8613
Response				
PR	Th1	28.2±18	28.8±21	0.6858
	Th2	3.9±2	3.6±2	0.4652
	Treg	8.9±3	8.7±4	0.7003
SD	Th1	26.2±10	26.4±11	0.9869
	Th2	3.6±2	3.6±2	0.6191
	Treg	8.7±3	8.7±3	0.8365
PD	Th1	25.1±10	24.2±11	0.1217
	Th2	4.0±2	3.7±2	0.0855
	Treg	9.4±3	9.2±3	0.6858
Child–Pugh class				
A	Th1	25.3±10	24.0±11	0.1145
	Th2	3.2±1	2.9±1	0.0493*
	Treg	9.8±2	9.1±3	0.8234
B	Th1	27.4±12	28.6±13	0.7316
	Th2	3.8±2	3.5±2	0.3751
	Treg	8.9±3	9.1±3	0.0850
Stage				
IVA	Th1	25.3±11	25.1±12	0.2467
	Th2	3.7±2	3.4±2	0.0174*
	Treg	8.9±3	9.0±3	0.9850
IVB	Th1	25.6±9	26.0±10	0.7226
	Th2	3.8±2	3.9±2	0.5131
	Treg	9.4±3	8.7±3	0.7112

Th1: CD4-positive T-cells [interferon-γ (IFNγ)$^+$/interleukin 4(IL4)$^-$]; Th2: CD4-positive T-cells (IFNγ$^-$/IL-4$^+$), Treg: CD4-positive T-cells (CD25$^+$/CD127$^-$). Significantly different from pre-therapy value at: *p<0.05.

Table V. *Comparison of CD4-positive T-cells before and after sorafenib treatment (Tx) in patients with liver cirrhosis and advanced hepatocellular carcinoma according to etiology.*

Etiology	Cell type	Pre Tx, %	Post	*p*-Value
HBV	Th1	24.9±12	23.4±14	0.1578
	Th2	3.9±2	3.5±2	0.6980
	Treg	9.1±3	9.1±3	0.3794
HCV	Th1	27.2±10	25.6±12	0.0886
	Th2	3.3±2	2.9±1	0.0496
	Treg	9.2±3	9.1±3	0.8655
Alcohol	Th1	22.7±7	23.5±8	0.4811
	Th2	4.7±3	4.6±2	0.9518
	Treg	9.3±3	9.2±3	0.6874
Non-ABC	Th1	25.8±11	25.9±10	0.4631
	Th2	3.6±2	4.0±1	0.8927
	Treg	7.9±2	8.3±2	0.6002

HBV: Hepatitis B virus; HCV: hepatitis C virus; non-ABC: non-HBV-/non-HCV liver cirrhosis with no alcohol exposure; Th1: CD4-positive T-cells [interferon-γ (IFNγ)$^+$/interleukin 4(IL4)$^-$]; Th2: CD4-positive T-cells (IFNγ$^-$/IL-4$^+$), Treg: CD4-positive T-cells (CD25$^+$/CD127$^-$). Significantly different from pre-therapy value at: *p<0.05.

Changes in Th1, Th2, and Treg cells according to the etiology of LC. No significant changes in Th1, Th2 and Treg cells were detected in the HBV group after sorafenib. In contrast, a significant decrease in Th2 cells occurred in the HCV group after sorafenib treatment (3.3±2% *vs*. 2.9±1%; p=0.0496 by Wilcoxon's signed-rank sum test), although there were no significant changes in Th1 and Treg cells. No significant changes in these cell types occurred in the alcohol and non-ABC groups (Table V).

Changes in Th1, Th2, and Treg cells in the HCV group. We further investigated the effects of sorafenib treatment on host immunity in patients with aHCC with HCV-related LC (the HCV group), because only these patients showed significant changes in immune parameters in the above analyses. When sorafenib was administered at 200 mg/day or 800 mg/day, there were no significant changes after treatment with respect to Th1 cells, Th2 cells, and Treg cells. However, in patients receiving sorafenib at 400 mg/day, Th2 cells significantly decreased after treatment (3.0±2% *vs*. 3.4±2%; p=0.0372 by Wilcoxon's signed-rank sum test), while there were no significant changes in Th1 cells and Treg cells (Figure 1). When the influence of the response to sorafenib was assessed, no significant differences were noted in Th1 cells or Treg cells after treatment according to response category. Although Th2 cells decreased after sorafenib treatment, the changes were not significant (Figure 2). In patients from the HCV group classified as Child–Pugh class B, there were no significant changes in Th1, Th2 or Treg cells with sorafenib treatment. In patients classified as Child–Pugh class A, Th2 cells significantly decreased after sorafenib treatment (3.1±1% *vs*. 3.5±2%; p=0.0366, by Wilcoxon's signed-rank sum test), although there were no significant changes in Th1 and Treg cells (Figure 3). In patients from the HCV group with stage III disease or stage IVB disease, no significant differences were noted in Th1, Th2 and Treg cells after sorafenib treatment, although Th2 cells decreased after treatment in both subgroups. In contrast, Th2 cells significantly decreased after treatment (2.8±1% *vs*. 3.2±1%; p=0.0494 by Wilcoxon's signed-rank sum test) in patients with stage IVA disease, although there were no significant changes in Th1 cells and Treg cells (Figure 4).

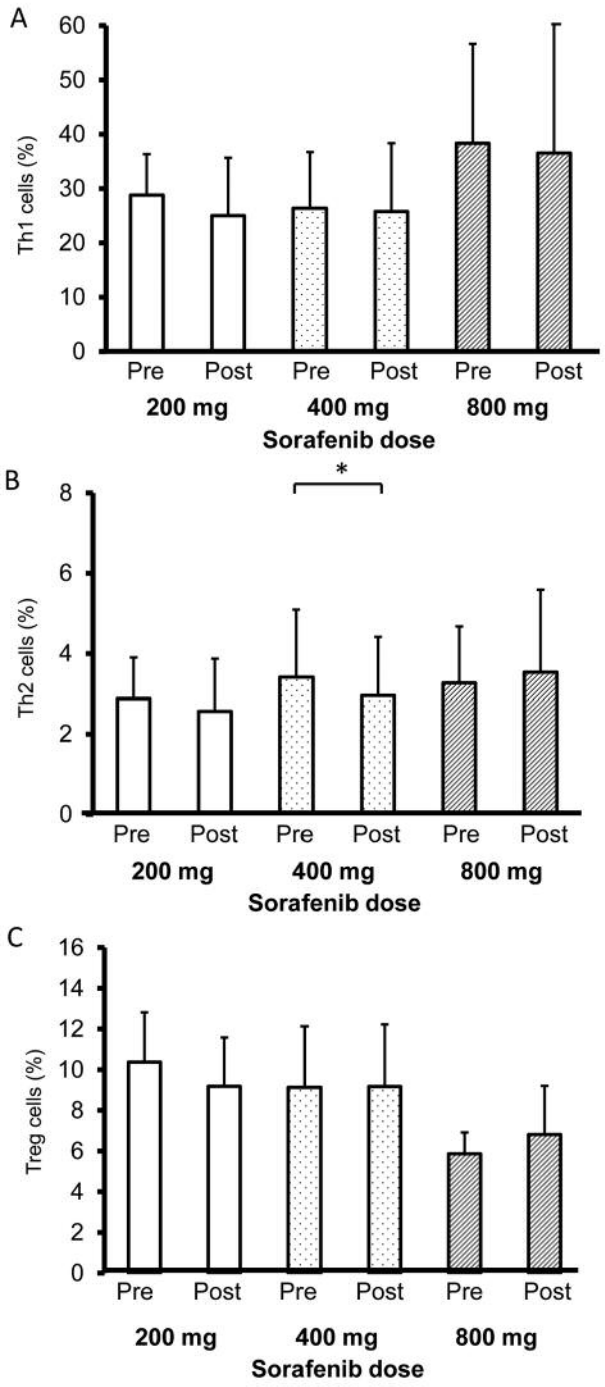

Figure 1. *Effects of dose level of sorafenib on host immunity as reflected by the proportion of Th1 [interferon-γ (IFNγ)-positive interleukin 4 (IL4)-negative] (A) and Th2 (IFNγ-negative/IL4-positive) (B), and regulatory (Treg) (C) T-cells in peripheral blood from patients with hepatitis C virus-related liver cirrhosis and advanced hepatocellular carcinoma. At sorafenib doses of 200 mg/day and 800 mg/day, no significant differences were noted between pre- and post-treatment Th1 (A), Th2 (B), and Treg (C) cell levels. At a dose of 400 mg/day, Th2 cells significantly decreased after sorafenib treatment (*p=0.0372 by Wilcoxon's signed-rank sum test), while there were no differences in Th1 and Treg cells. Data are the mean±standard deviation.*

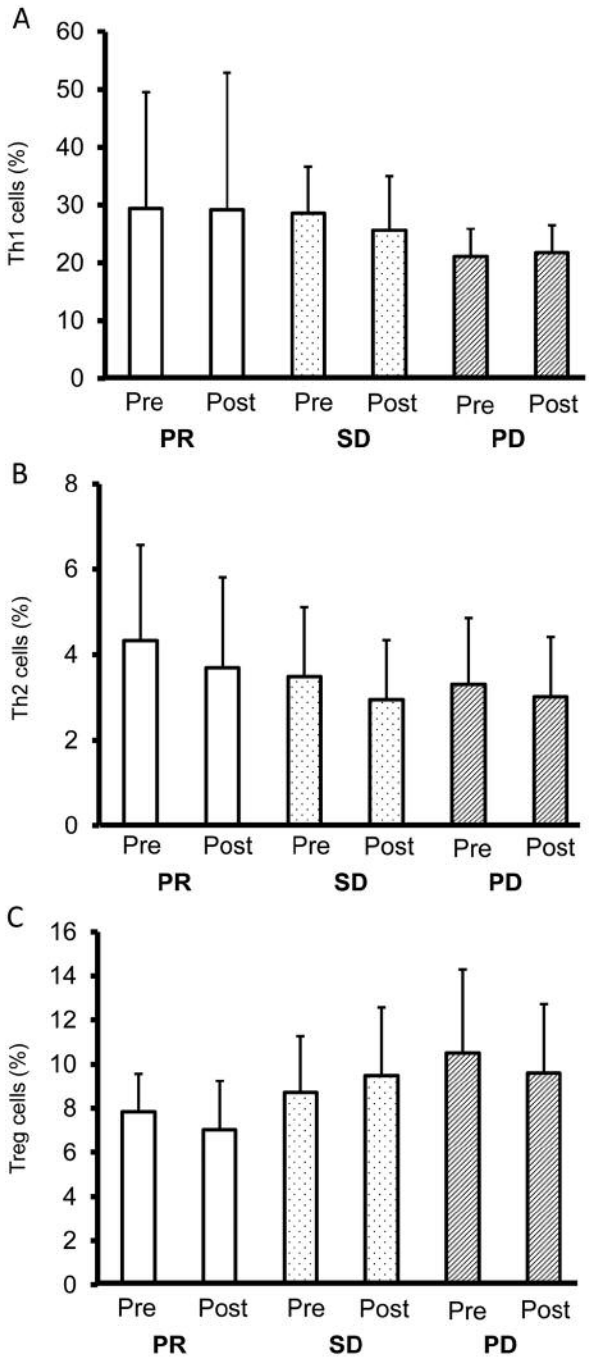

Figure 2. *Effects of sorafenib on host immunity as reflected by the proportion of Th1 [interferon-γ (IFNγ)-positive interleukin 4 (IL4)-negative] (A) and Th2 (IFNγ-negative/IL4-positive) (B), and regulatory (Treg) (C) T-cells in peripheral blood in patients with advanced hepatocellular carcinoma and hepatitis C virus-related liver cirrhosis in relation to tumor response. There were no significant differences in Th1 cells and Treg cells pre- and post-treatment in each response category. There were also no significant changes in Th2 cells, although Th2 cells decreased after treatment in each response category (Wilcoxon's signed-rank sum test). Data are the mean±standard deviation. CR: Complete response, PR: partial response, SD: stable disease, PD: progressive disease.*

Discussion

The present study demonstrated that the effects of sorafenib on host immunity in patients with aHCC differed according to the etiology of their underlying LC: We previously reported that sorafenib treatment caused a decrease in peripheral blood Th2 and Treg cells in patients with LC with aHCC, which might induce Th1 dominance and prevent tumor cells from escaping the host immune system (17). However, we did not examine whether the etiology of LC influenced the effects of sorafenib on host immunity in that study. Therefore, we carried out the present investigation in patients receiving sorafenib for aHCC who were stratified according to the etiology of their underlying LC, and we identified significant effects of sorafenib on host immunity in patients with HCV-related LC: Th1 cells and Th2 cells cross-regulate each other during development. It was reported that cytokines released by Th2 cells inhibit antitumor immunity (12), while Th1 cytokines promote antitumor immunity (13-16). We previously demonstrated that Th1 dominance is lost due to an increase in Th2 cells in patients with HCC, and that carcinogenesis may be more likely to occur in patients who have chronic HCV infection and an increase in Th2 cells (21). We also previously suggested that Th2 dominance might induce carcinogenesis in patients with HCV-related LC, rather than carcinogenesis leading to Th2 dominance (22).

In the present study, we found that Th2 cells were significantly reduced by sorafenib in patients with aHCC with LC receiving treatment at a dose of 400 mg/day, patients with Child–Pugh class A LC, and patients with stage IVA disease. In particular, this change was significant in patients with aHCC with HCV-related LC: Thus, liver injury in patients with HCV-related LC with aHCC receiving sorafenib treatment might be caused by inducing Th1 dominance from Th2 dominance. Moreover, switching from Th2 dominance to Th1 dominance by sorafenib treatment did not occur in patients with HBV-related LC, patients with alcoholic LC, or non-HBV-/non-HCV-related LC without alcohol exposure. Sorafenib may not have affected host immunity in patients with aHCC with alcoholic LC because of immunosuppression due to continuation of alcohol drinking. However, it is unclear why there was a different response of host immunity to sorafenib between patients with aHCC with underlying LC due to HBV or HCV infection. It was reported that the gene expression profile of HCC differs between patients with HBV and those with HCV infection (23, 24). In addition, the histological severity of HCV-related liver disease is closely correlated with the risk of HCC (25), while HCC occasionally develops in healthy HBV surface antigen carriers who have persistent HBV infection with normal liver function and no necroinflammation (26). HBV X oncoprotein (HBx) has been implicated in hepatocarcinogenesis mediated by HBV (27, 28), and its persistent high expression in the livers of transgenic mice resulted in hyperplasia that led to HCC without prior inflammation (29). Such differences between HBV and HCV may help to explain why the response of host immunity to sorafenib depends on the etiology of LC: Proteomic analysis has shown that the heat-shock protein (HSP) 70 family is overexpressed in HCC associated with HCV infection compared with normal liver tissues (30), while therapy using HSP70-expressing dendritic cells (DCs) was shown to be both safe and feasible in patients with HCV-related HCC (31). It was also reported that vaccination using DCs co-pulsed with the HSP70/HBx antigen complex was an effective immunotherapy strategy for HBV-related HCC (32). Sorafenib treatment might normalize DCs in patients with aHCC with HCV-related LC: In addition, the different responses of host immunity to sorafenib in patients with HBV-or HCV-related HCC in the present study might be related to differences in DC number or function. However, it is unknown whether there are differences in the effects of sorafenib on DCs between patients with aHCC with HBV-or HCV-related LC, and we were unable to examine this point in the present study.

Zhao *et al*. demonstrated that sorafenib inhibited T-cell proliferation and induced T-cell apoptosis, and they suggested that it may interfere with T-cell-related immunity by inducing apoptosis (33). We have also reported that sorafenib treatment can promote tumor necrosis factor (TNF)-related or FAS-related apoptosis by increasing the circulating level of TNFα or reducing that of soluble FAS (34). Furthermore, Hipp *et al*. reported that sorafenib significantly reduced the induction of antigen-specific T cells, impaired intracellular signaling cascades in DCs, and induced apoptosis of DCs. They concluded that sorafenib interferes with the maturation and functioning of monocyte-derived DCs (35). Kohga *et al*. demonstrated that a disintegrin and metalloproteinase 9 (ADAM9) was overexpressed in human HCC tissues, while *ADAM9* knockdown increased the expression of membrane-bound major histocompatibility complex class I-related chain A (MICA), reduced production of soluble MICA, and increased the sensitivity of human HCC cells to natural killer cells. They also indicated that sorafenib enhanced the sensitivity of HCC to natural killer cells *via* inhibition of ADAM9 protease activity and modification of MICA expression (36). However, it is unclear how sorafenib affects MICA expression. It is also unknown whether the effects of sorafenib on ADAM9 differ in patients with aHCC with HBV-related or HCV-related LC, and we did not address this issue in the present study.

In conclusion, our results indicate that sorafenib treatment might induce Th1 dominance by reducing Th2 cells in patients receiving a dose of 400 mg/day, patients with Child–Pugh class A LC, and patients with stage IVA HCC: Sorafenib may

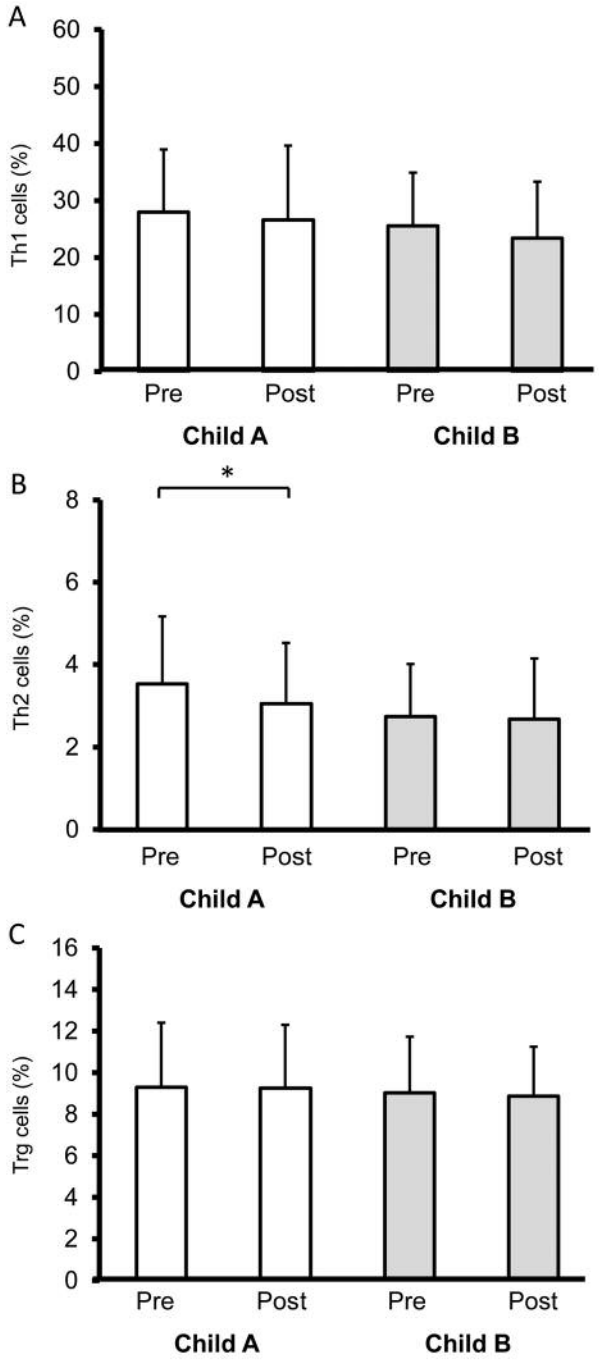

Figure 3. *Effects of sorafenib on host immunity as reflected by the proportion of Th1 [interferon-γ (IFNγ)-positive interleukin 4 (IL4)-negative] (A) and Th2 (IFNγ-negative/IL4-positive) (B), and regulatory (Treg) (C) T-cells in peripheral blood in patients with advanced hepatocellular carcinoma and hepatitis C virus-related liver cirrhosis in relation to the Child-Pugh class. In patients from Child-Pugh class B, there were no significant differences between pre- and post-sorafenib treatment levels of Th1, Th2, and Treg cells. In patients from Child–Pugh class A, Th2 cells showed a significant decrease after sorafenib treatment (*p=0.0366 by Wilcoxon's signed-rank sum test), although there were no significant changes in Th1 cells or Treg cells. Data are the mean±standard deviation.*

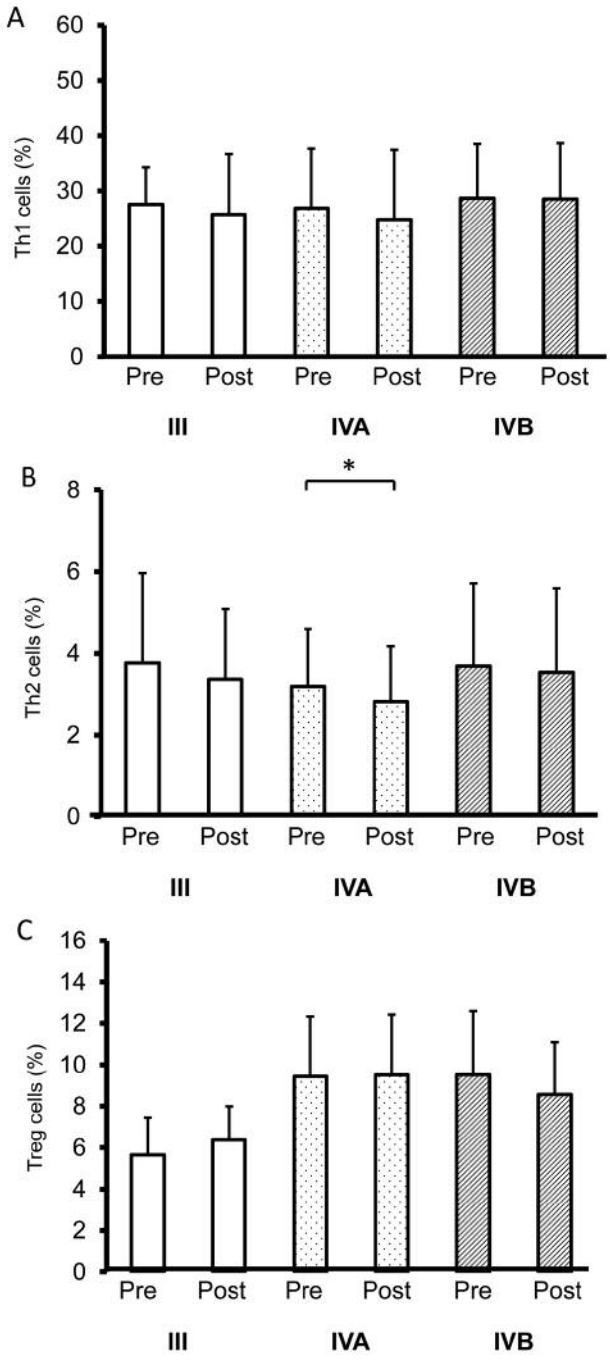

Figure 4. *Effects of sorafenib on host immunity as reflected by the proportion of Th1 [interferon-γ (IFNγ)-positive interleukin 4 (IL4)-negative] (A) and Th2 (IFNγ-negative/IL4-positive) (B), and regulatory (Treg) (C) T-cells in peripheral blood in patients with advanced hepatocellular carcinoma and hepatitis C virus-related liver cirrhosis in relation to the tumor stage. In patients with stage III or stage IVB disease, there were no significant changes in Th1 cells and Treg cells pre and post sorafenib treatment, although Th2 cells decreased after treatment in both groups. In patients with stage IVA disease, Th2 cells showed a significant decrease after sorafenib treatment (*p=0.0494 by Wilcoxon's signed-rank sum test), although there were no significant changes in Th1 cells and Treg cells. Data are the mean±standard deviation.*

prevent tumor cells from escaping the host immune system in patients with aHCC with HCV-related LC, although it does not seem to have this effect in patients with LC of other etiologies. However, it is still unclear whether the effects of sorafenib on host immunity differ between patients with aHCC with HBV-or HCV-related LC: Further studies will be needed to assess the influence of sorafenib on DCs and ADAM9 expression in patients with aHCC with LC, as well as the relation between host immunity and HBx or MICA.

Data Availability

The data used to support the findings of this study are available from the corresponding Author upon request.

Conflicts of Interest

The Authors declare that they have no conflicts of interest in regard to this study.

Authors' Contributions

NH performed all the experiments, data analysis and finalized the article. TN, KK, and KH were performed to analysis of CD4-positive T-cells by using of flow cytometry. MA, NY, YO, DM, YD, YM, TM, NW, KM, and MS collected blood samples and performed data analysis. IY approved the final article.

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References

1 Greten TF, Duffy AG and Korangy F: Hepatocellular carcinoma from an immunologic perspective. Clin Cancer Res *19*: 6678-6685, 2013. PMID: 24030702. DOI: 10.1158/1078-0432.CCR-13-1721

2 Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin *61*: 69-90, 2011. PMID: 21296855. DOI: 10.3322/caac.20107

3 Llover JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J and SHARP Investigators Study Group: Sorafenib in advanced hepatocellular carcinoma. N Engl J Med *359*: 378-390, 2008. PMID: 18650514. DOI: 10.1056/NEJMoa 0708857

4 Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D and Guan Z: Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: A phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol *10*: 25-34, 2009. PMID: 19095497. DOI: 10.1016/S1470-2045 (08)70285-7

5 Kudo M, Izumi N, Sakamoto M, Matsuyama Y, Ichida T, Nakashima O, Matsui O, Ku Y, Kokudo N, Makuuchi M and Liver Cancer Study Group of Japan: Survival analysis over 28 years of 173,378 patients with hepatocellular carcinoma in Japan. Liver Cancer *5*: 190-197, 2016. PMID: 27493894. DOI: 10.1159/000367775

6 Kudo M: Locoregional therapy for hepatocellular carcinoma. Liver Cancer *4*: 163-164, 2015. PMID: 27330497. DOI: 10.1159/000367741

7 Wilhelm SM, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, Schwartz B, Simantov R and Kelley S: Discovery and development of sorafenib: A multikinase inhibitor for treating cancer. Nat Rev Drug Discov *5*: 835-844, 2006. PMID: 17016424. DOI: 10.1038/nrd2130

8 Avila MA, Berasain C, Sangro B and Prieto J: New therapies for hepatocellular carcinoma. Oncogene *25*: 3866-3884, 2006. PMID: 16799628. DOI: 10.1038/sj.onc.1209550

9 Wilhelm SM, Crter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G and Trail PA: BAY 43-9006 exhibits broad-spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res *64*: 7099-7109, 2004. PMID: 15466206. DOI: 10.1158/0008-5472.CAN-04-1443

10 Bruix J, Qin S, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, Yokosuka O, Rosmorduc O, Breder V, Gerolami R, Masi G, Ross PJ, Song T, Bronowicki JP, Ollivier-Hourmand I, Kudo M, Cheng AL, Llovet JM, Finn RS, LeBerre MA, Baumhauer A, Meinhardt G, Han G and RESORCE Investigators: Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): A randomised, double-blind, placebo-controlled, phase 3 trial. Lancet *389(10064)*: 36, 2017. PMID: 27932229. DOI: 10.1016/S0140-6736(16)32453-9

11 Kudo M: Regorafenib as second-line systemic therapy may change the treatment strategy and management paradigm for hepatocellular carcinoma. Liver Cancer *5*: 235-244, 2016. PMID: 27781196. DOI: 10.1159/000449335

12 Kobayashi M, Kobayashi H, Polland RB and Suzuki F: A pathogenic role of Th2 cells and their cytokine products on the pulmonary metastasis of murine B16 melanoma. J Immunol *160*: 5869-5873, 1998. PMID: 9637498.

13 Zitvogel L, Mayordomo JI, Tjandrawan T, DeLeo AB, Clarke MR, Lotze MT, Storkus WJ: Therapy of murine tumors with tumor peptide-pulsed dendritic cells: Dependence on T-cells, B7 co-stimulation, and T-helper cell 1-associated cytokines. J Exp Med *183*: 87-97, 1996. PMID: 8551248.

14 Tsung K, Meko JB, Peplinski GR, Tsung YL and Norton JA: IL-12 induces T-helper 1-directed antitumor response. J Immunol *158*: 3359-3365, 1997. PMID: 9120294.

15 Weiner GJ, Liu HM, Wooldridge JE, Dahle CE and Krieg AM: Immunostimulatory oligodeoxynucleotides containing the CpG motif are effective as immune adjuvants in tumor antigen immunization. Proc Natl Acad Sci USA *94*: 10833-10837, 1997. PMID: 9380720.

16 Hu H-M, Urba WJ and Fox BA: Gene-modified tumor vaccine with therapeutic potential shifts tumor-specific T-cell response from type 2 to a type 1 cytokine profile. J Immunol *161*: 3033, 1998. PMID: 9743368.

17 Nagai H, Mukozu T, Matsui D, Kanekawa T, Kanayama M, Wakui N, Momiyama K, Shinohara M, Iida K, Ishii K, Igarashi Y and Sumino Y: Sorafenib prevents escape from host immunity in liver cirrhosis patients with advanced hepatocellular carcinoma. Clin Dev Immunol *2012*: 607851, 2012. PMID: 22666283. DOI: 10.1155/2012/607851

18 Jung T, Schauer U, Heusser C, Neumann C and Rieger C: Detection of intracellular cytokines by flow cytometry. J Immunol Methods *159*: 197-207, 1993. PMID: 8445253.

19 Llovet JM1, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, Schwartz M, Lotze M, Talwalkar J, Gores GJ and Panel of Experts in HCC-Design Clinical Trials: Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst *100*: 698–711, 2008. PMID: 18477802. DOI: 10.1093/jnci/djn134

20 Lencioni R and Llovet JM: Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Smin Liver Dis *30*: 52-60, 2010. PMID: 20175033. DOI: 10.1055/s-0030-1247132

21 Matsui T, Nagai H, Sumino Y and Miki K: Relationship of peripheral blood CD4-positive T cells to carcinogenesis in patients with HCV-related chronic hepatitis and liver cirrhosis. Cancer Chemother Pharmacol *62*: 401-406, 2008. PMID: 17932673. DOI: 10.1007/s00280-007-0618-1

22 Kogame M, Nagai H, Shinohara M, Igarashi Y, Sumino Y and Ishii K: Th2 Dominance might induce carcinogenesis in patients with HCV-related liver cirrhosis. Anticancer Res *36*: 4529-4536, 2016. PMID: 27630292. DOI: 10.21873/anticanres.11000

23 Okabe H, Satoh S, Kato T, Kitahara O, Yanagawa R, Yamaoka Y, Tsunoda T, Furukawa Y and Nakamura Y: Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: Identification of genes involved in viral carcinogenesis and tumor progression 1. Cancer Res *61*: 2129-2137, 2001. PMID: 11280777.

24 Iizuka N, Oka M, Yamada-okabe H, Mori N, Tamesa T, Okada T, Takemoto N, Tangoku A, Hamada K, Nakayama H, Miyamoto T, Uchimura S and Hamamoto Y: Comparison of gene expression profiles between hepatitis B virus-and hepatitis C virus-infected hepatocellular carcinoma by oligonucleotide microarray data on the basis of a supervised leaning method. Cancer Res *62*: 3939-3944, 2002. PMID: 12124323.

25 Di Bisceglie AM: Hepatitis C and hepatocellular carcinoma. Hepatology *26(Suppl)*: 34S-38S, 1997. PMID: 9305661. DOI: 10.1002/hep.510260706

26 Popper H, Shafritz DA and Hoofnagle JH: Relation of the hepatitis B virus carrier state to hepatocellular carcinoma. Hepatology *7*: 764-772, 1987. PMID: 3038725.

27 Kim CM, Koike K, Saito I, Miyamura T and Jay G: HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature *351*: 317-320, 1991. PMID: 2034275. DOI: 10.1038/351317a0

28 Yu DY, Moon HB, Son JK, Jeong S, Yu SL, Yoon H, Han YM, Lee CS, Park JS, Lee CH, Hyun BH, Murakami S and Lee KK: Incidence of hepatocellular carcinoma in transgenic mice expressing the hepatitis B virus X-protein. J Hepatol *31*: 123-132, 1999. PMID: 10424292.

29 Koike K, Moriya K, Iino S, Yotsuyanagi H, Endo Y, Miyamura T and Kurokawa K: High-level expression of hepatitis B virus HBx gene and hepatocarcinogenesis in transgenic mice. Hepatology *19*: 810-819, 1994. PMID: 8138251.

30 Takashima M, Kuramitsu Y, Yokoyama Y, Iizuka N, Toda T, Sakaida I, Okita K, Oka M and Nakamura K: Proteomic profiling of heat shock protein 70 family members as biomarkers for hepatitis C virus-related hepatocellular carcinoma. Proteomics *3*: 2487-2493, 2003. PMID: 14673798. DOI: 10.1002/pmic.200300621

31 Maeda Y, Yoshimura K, Matsui H, Shindo Y, Tamesa T, Tokumitsu Y, Hashimoto N, Tokuhisa Y, Sakamoto K, Sakai K, Suehiro Y, Hinoda Y, Tamada K, Yoshino S, Hazama S and Oka M: Dendritic cells transfected with heat-shock protein 70 messenger RNA for patients with hepatitis C virus-related hepatocellular carcinoma: a phase 1 dose escalation clinical trial. Cancer Immunol Immunother *64*: 1047-1056, 2015. PMID: 25982372. DOI: 10.1007/s00262-015-1709-1

32 Wang H, Feng F, Wang XP, Wang RS, Wu Y, Zhu MG, Zhang H and Zhuang ZX: Dendritic cells pulsed with Hsp70 and HBxAg induce specific antitumor immune responses in hepatitis B virus-associated hepatocellular carcinoma. Mol Med Rep 13: 1077-1082, 2016. PMID: 26647961. DOI: 10.3892/mmr.2015.4654

33 Zhao W, Gu YH, Song R, Qu BQ and Xu Q: Sorafenib inhibits activation of human peripheral blood T cells by targeting LCK phosphorylation. Leukemia *22*: 1226-1233, 2008. PMID: 18337760. DOI: 10.1038/leu.2008.58

34 Nagai H, Kanekawa T, Kobayashi K, Mukozu T, Matsui D, Matsui T, Kanayama M, Wakui N, Momiyama K, Shinohara M, Ishii K, Igarashi Y and Sumino Y: Changes of cytokines in patients with liver cirrhosis and advanced hepatocellular carcinoma treated by sorafenib. Cancer Chemother Pharmacol *73*: 223-229, 2014. PMID: 24220937. DOI: 10.1007/s00280-013-2344-1

35 Hipp MM, Hilf N, Walter S, Werth D, Brauer KM, Radsak MP, Weinschenk T, Singh-Jasuja H and Brossart P: Sorafenib, but not sunitinib, affects function of dendritic cells and induction of primary immune responses. Blood *111*: 5610-5620, 2008. PMID: 18310500. DOI: 10.1182/blood-2007-02-075945

36 Kohga K, Takehara T, Tatsumi T, Ishida H, Miyagi T, Hosui A and Hayashi N: Sorafenib inhibits the shedding of major histocompatibility complex class I-related chain A on hepatocellular carcinoma cells by down-regulating a disintegrin and metalloproteinase 9. Hepatology *51*: 1264-1273, 2010. PMID: 20099300. DOI: 10.1002/hep.23456

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