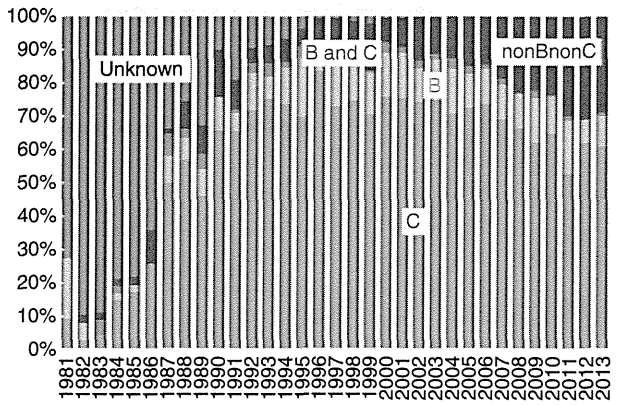


**Figure 2** Annual trends in patients with hepatocellular carcinoma (1981–2013, causes of liver disease [virus], Osaka Red Cross Hospital, Japan).

Next, current annual trends of causes of liver disease are shown in Figure 2. In our hospital, the proportion of non-B, non-C (NBNC) HCC patients has been gradually increasing, while the proportion of HCV-related HCC has been gradually decreasing. It is noteworthy that the proportion of NBNC HCC patients was approximately 30% in 2011, 2012 and 2013 in our hospital. Although most HCC is related to viral infection, there is a substantial population of NBNC HCC patients in Japan and the incidence of NBNC HCC has recently tended to increase.[3,21–25] The background liver diseases of NBNC HCC vary considerably and they include non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcoholic liver disease, autoimmune liver diseases such as autoimmune hepatitis, primary biliary cirrhosis and primary sclerosing cholangitis, congestive liver diseases such as Budd–Chiari syndrome, congenital metabolic liver diseases such as hereditary hemochromatosis and Wilson's disease, occult B infection and aflatoxins as well as liver diseases of unknown etiology.[3] In particular, it is of note that increasing clinical evidences support the fact that NAFLD and NASH can progress to LC and HCC.[26,27] NAFLD or NASH may directly promote liver carcinogenesis independent of the presence of LC.[3,26] Tokushige *et al.* conducted a nationwide survey of 14 530 Japanese HCC patients and demonstrated that alcohol-related HCC accounted for 7.2% of all HCC, followed by unknown HCC (5.1%) and NAFLD-related HCC (2.0%) and the characteristics of these three groups were clearly different (median age, 72 years for NAFLD-related HCC, 68 years for alcohol-related HCC and 73 years for unknown HCC, $P < 0.01$; female sex, 38%, 4% and 37%, respectively, $P < 0.01$) and obesity and lifestyle-related diseases were significantly more frequent in NAFLD-related HCC than in alcohol-related HCC and unknown HCC.[21] On the other hand, one possible reason that the number of HCV-related HCC cases has been recently decreasing is that the rate of HCV eradication has markedly improved due to the progress of treatment for patients with HCV, although antiviral therapies for hepatitis C can prevent but not completely eliminate HCC.[28–30] The estimated risk of HCC is reported to be 15–20-times as high among individuals with HCV as it is among those who are not infected with HCV.[31] In addition, although the number of patients with HCC-related death has steadily increased over the past 50 years, the incidence of HCC has recently started to decrease in Japan, mainly due to the decrease in rates of HCV-related HCC.[19]

Next, current annual trends of proportions of patients with HCC stage I, II, III, IVA and IVB at initial HCC diagnosis in our hospital are shown in Figure 3. The proportion of early stage HCC patients (HCC stage I or II) in the 1980s was 34.1%. However, that in the 1990s increased to 50.6% and that after 2000 increased to over 60%. These improvements may be due to adequate selection of patients at high risk of HCC occurrence and progress in diagnostic imaging technique of HCC such as introductions of contrast-enhanced ultrasonography, multidetector computed tomography and gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) magnetic resonance imaging (MRI).[32] In particular, Gd-EOB-DTPA MRI including the hepatobiliary phase had the highest accuracy with sensitivities for detecting early stage HCC.[32] Success of a surveillance program depends on both target population and surveillance modality.[7]

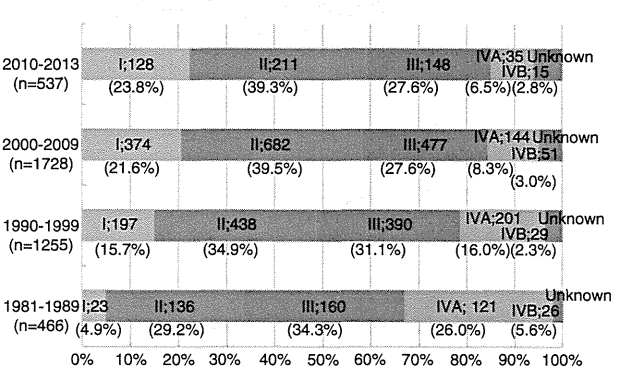


**Figure 3** Hepatocellular carcinoma stage at diagnosis (1981–2013, Osaka Red Cross Hospital, Japan).

## CUMULATIVE OVERALL SURVIVAL FOR ALL HCC CASES FOR THE LAST THREE DECADES AND CUMULATIVE OVERALL SURVIVAL ACCORDING TO CHILD-PUGH STAGE, HCC STAGE AND CAUSES OF LIVER DISEASE

THE CUMULATIVE OVERALL survival (OS) curve for all HCC cases from 1981 to 2013 ($n = 4165$) in our hospital is demonstrated in Figure 4. The 1-, 3-, 5-, 8-, 10- and 15-year survival rates were 79.8%, 55.4%, 37.5%, 21.8%, 14.8% and 6.1%, respectively. One report from the USA showed that the 5-year survival rate in HCC patients in the United States has remained below 12%.[6] As compared with their results of survival, our results are reasonably good, although the reasons for these discrepancies are unclear.

The main predictors affecting survival in HCC patients are liver function and tumor burden.[1–6] Herein, we present the cumulative OS rates according to Child–Pugh stage and HCC stage in Figures 5 and 6. As for Child–Pugh stage, 1-, 3- and 5-year survival rates are 86.3%, 64.6% and 44.8%, respectively, in patients with Child–Pugh A ($n = 2571$), 71.7%, 40.5% and 22.8%, respectively, in patients with Child–Pugh B ($n = 1095$), and 44.0%, 17.1% and 8.3%, respectively, in patients with Child–Pugh C ($n = 265$) (overall significance, $P < 0.001$), suggesting that Child–Pugh stage is closely associated with OS in HCC patients (Fig. 5). These results also indicate that maintaining liver functional reserve is essential for prolonging OS in HCC patients. As shown in our results, prognosis in HCC patients with

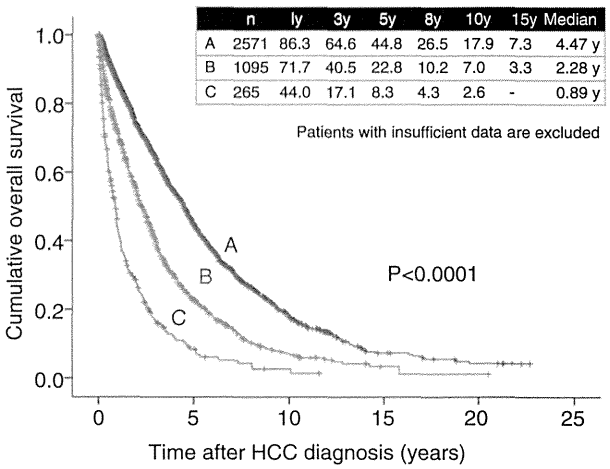


**Figure 5** Cumulative overall survival according to Child–Pugh stage (Osaka Red Cross Hospital, Japan). HCC, hepatocellular carcinoma; y, years.

Child–Pugh C cirrhosis is extremely poor.[33] Thus, in these patients, most of the current HCC practice guidelines recommend LT for patients within the Milan criteria and best supportive care for patients outside the Milan criteria.[4,6] However, in Japan, due to the limited number of brain death donors and advanced age in HCC patients, the Japan Society of Hepatology recommends non-transplant therapies such as transcatheter arterial chemotherapy and ablative therapies even in HCC patients with Child–Pugh C cirrhosis.[34] However, whether HCC patients with Child–Pugh C cirrhosis treated with non-transplant therapies could

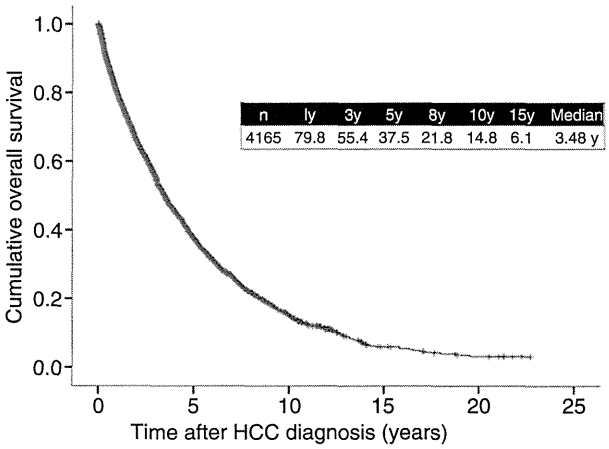


**Figure 4** Cumulative overall survival for hepatocellular carcinoma (HCC) patients (4165 cases, Osaka Red Cross Hospital, Japan). y, years.

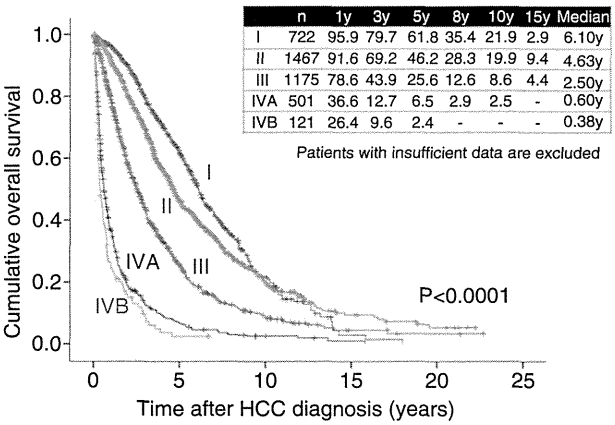


**Figure 6** Cumulative overall survival according to hepatocellular carcinoma (HCC) stage (Osaka Red Cross Hospital, Japan). y, years.

obtain survival benefit remains unclear.[33] To confirm these results, well-characterized studies will be needed in the future.

On the other hand, as for HCC stage, 1-, 3- and 5-year survival rates are 95.9%, 79.7% and 61.8%, respectively, in patients with HCC stage I ($n = 722$), 91.6%, 69.2% and 46.2%, respectively, in patients with HCC stage II ($n = 1467$), 78.6%, 43.9% and 25.6%, respectively, in patients with HCC stage III ($n = 1175$), 36.6%, 12.7% and 6.5%, respectively, in patients with HCC stage IVA ($n = 501$), and 26.4%, 9.6% and 2.4%, respectively, in patients with HCC stage IVB ($n = 121$) (overall significance, $P < 0.001$), demonstrating that HCC stage is also closely associated with OS in HCC patients (Fig. 6). A disease staging system is particularly essential for the management of HCC as it helps to predict prognosis. As shown in Figure 6, patients with advanced stage of HCC have extremely poor prognosis. In this regard, a more adequate surveillance program for early detection of HCC development will be necessary.[35]

As for causes of liver disease, 1-, 3- and 5-year survival rates are 74.3%, 50.7% and 39.1%, respectively, in patients with hepatitis B virus (HBV)-related HCC ($n = 460$), 85.1%, 61.0% and 41.1%, respectively, in patients with HCV-related HCC ($n = 2734$), 78.9%, 56.3% and 38.6%, respectively, in patients with NBNC HCC ($n = 551$) and 72.2%, 52.3% and 35.5%, respectively, in patients with HBV and HCV-related HCC ($n = 83$) (overall significance, $P = 0.620$), demonstrating that prognosis for HCC patients is not affected by causes of liver disease (Fig. 7).

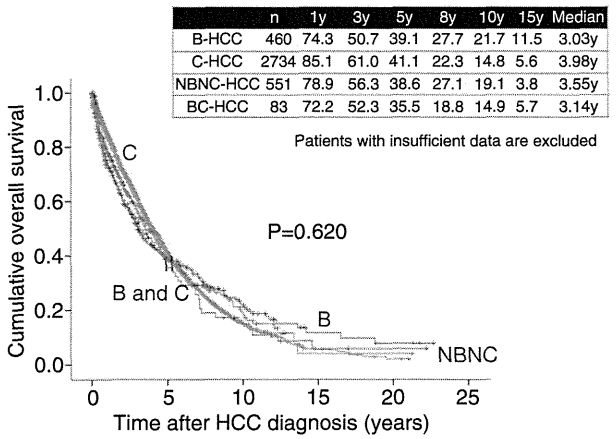


**Figure 7** Cumulative overall survival according to causes of liver disease (Osaka Red Cross Hospital, Japan). HCC, hepatocellular carcinoma; NBNC, non-B, non-C; y, years.

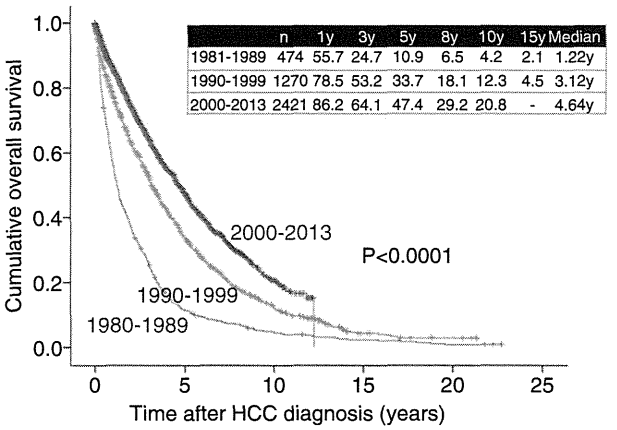


**Figure 8** Cumulative overall survival for cases in the 1980s, 1990s and 2000s (Osaka Red Cross Hospital, Japan). HCC, hepatocellular carcinoma; y, years.

## CUMULATIVE OVERALL SURVIVAL IN THE 1980S, 1990S AND 2000S

AS SHOWN IN Figure 8, 1-, 3-, 5-, 8- and 10-year survival rates were 55.7%, 24.7%, 10.9%, 6.5% and 4.2%, respectively, in HCC patients in the 1980s ($n = 474$), 78.5%, 53.2%, 33.7%, 18.1% and 12.3%, respectively, in HCC patients in the 1990s ($n = 1270$), and 86.2%, 64.1%, 47.4%, 29.2% and 20.8%, respectively, in HCC patients in the 2000s ($n = 2421$) (overall significance, $P < 0.001$), showing that the prognosis for HCC patients has significantly improved in these three decades. As for HCV-related HCC, 1-, 3-, 5-, 8- and 10-year survival rates were 72.1%, 40.1%, 18.4%, 9.5% and 4.8% respectively, in the 1980s ($n = 149$), 81.1%, 56.0%, 35.4%, 17.6% and 11.4%, respectively, in the 1990s ($n = 946$), and 88.8%, 66.6%, 48.5%, 28.8% and 20.7%, respectively, in the 2000s ($n = 1639$) (overall significance, $P < 0.001$) (Fig. 9). As for HBV-related HCC, 1-, 3-, 5-, 8- and 10-year survival rates were 65.9%, 40.7%, 26.7%, 17.9% and 15.6% respectively, in the 1980s and 1990s (from 1981 to 1999) ($n = 178$), and 80.0%, 57.8%, 48.8%, 36.5% and 24.0%, respectively, in the 2000s ($n = 282$) ($P < 0.001$) (Fig. 10). As for NBNC HCC, 1-, 3-, 5-, 8- and 10-year survival rates were 72.5%, 49.7%, 30.8%, 24.0% and 16.5%, respectively, in the 1980s and 1990s (from 1981 to 1999) ($n = 126$), and 81.0%, 58.8%, 42.6%, 27.1% and 20.6%, respectively, in the 2000s ($n = 425$) ($P = 0.074$) (Fig. 11). Significant improvement of survival in hepatitis virus-related HCC may be partly attributed to the progress in antiviral therapies such as nucleoside analogs for

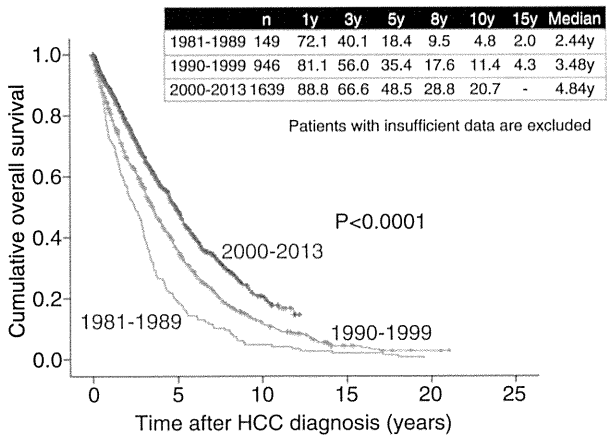


**Figure 9** Cumulative overall survival for patients with hepatitis C virus-related hepatocellular carcinoma (HCC) in the 1980s, 1990s and 2000s (Osaka Red Cross Hospital, Japan). y, years.

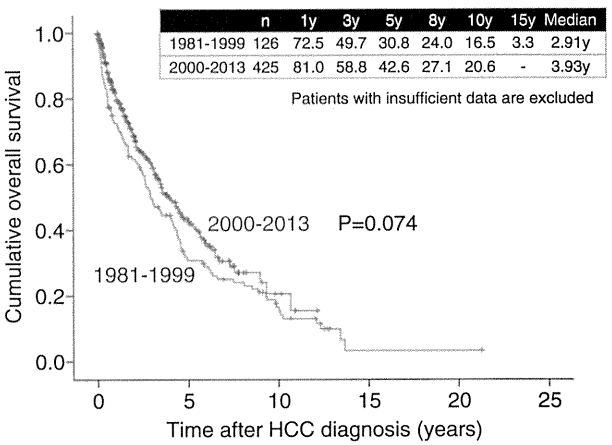


**Figure 11** Cumulative overall survival for patients with non-B, non-C (NBNC)-related hepatocellular carcinoma (HCC) in the 1980s, 1990s and 2000s (Osaka Red Cross Hospital, Japan). y, years.

hepatitis B and interferon (IFN) therapy for hepatitis C.[36–39] Furthermore, detecting populations at high risk populations for development of HCC and close surveillance for HCC occurrence of these populations may contribute to the prolongation of survival in these patients. On the other hand, as compared with hepatitis virus-related HCC, the prognosis in NBNC HCC patients has modestly improved. Possible reasons for this are: (i) detecting populations at high-risk of development HCC and close surveillance for HCC occurrence in patients with NBNC liver disease are difficult; and (ii) performing antiviral therapies is impossible in NBNC HCC patients.[22,23]

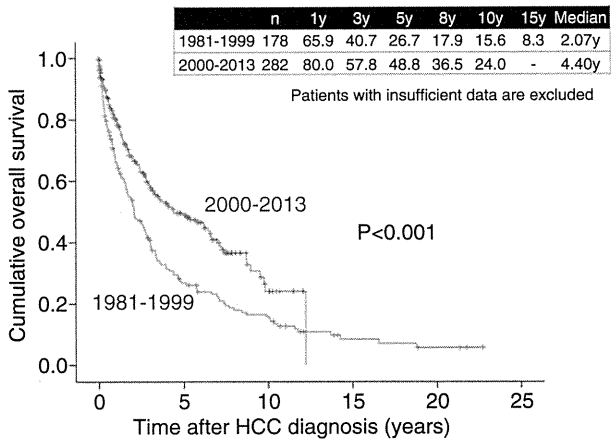


**Figure 10** Cumulative overall survival for patients with hepatitis B virus-related hepatocellular carcinoma (HCC) in the 1980s, 1990s and 2000s (Osaka Red Cross Hospital, Japan). y, years.

## HCC THERAPY: CHANGES FOR THE LAST FEW DECADES

### SR

SURGICAL RESECTION FOR HCC has been performed for more than 40 years. Although several treatment modalities have been proposed, SR is still considered the first-line therapeutic option for the majority of early stage HCC with well-preserved liver function.[40] According to the European Association for the Study of the Liver (EASL) guidelines, SR is indicated in patients with a single tumor not exceeding 2 cm in diameter, performance status (PS) 0, Child–Pugh A and no portal hypertension.[41] In Japan, however, SR is considered in patients with three or less tumors within 3 cm in diameter, no vascular invasion, Child–Pugh A or B, and expected tolerance to surgery, or even in those with four or more tumors larger than 3 cm and vascular invasion if they are expected to tolerate the surgery and that the treatment may improve the patient's prognosis.[34] In our country, large tumor size of HCC is not considered to be an absolute contraindication for SR, although the risk of vascular invasion and dissemination increases with tumor size.[34] For the last few decades, prognosis in HCC patients treated with SR has significantly improved. The establishment of operative guidelines for

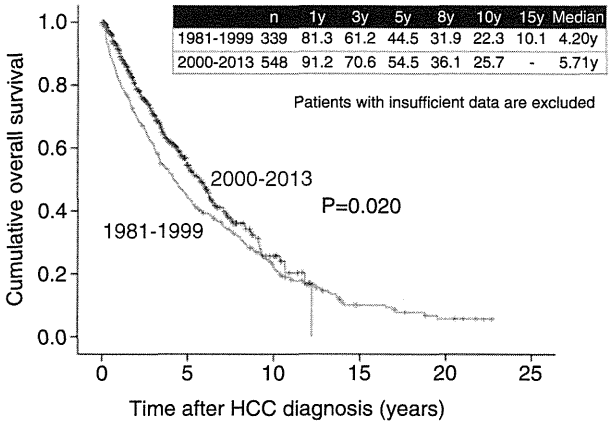


**Figure 12** Cumulative overall survival for hepatocellular carcinoma (HCC) patients treated with surgery in the 1980s, 1990s and 2000s (Osaka Red Cross Hospital, Japan). y, years.

HCC patients with poor hepatic reserve, improved perioperative management and advances in surgical techniques have reduced the risk of postoperative mortality.[42] Taura *et al.* reported that the OS rate in HCC patients treated with SR between 1991 and 2000 ($n = 398$) was significantly better than that in HCC patients treated with SR between 1985 and 1990 ($n = 212$) (58.0% vs 39.1% at 5 years, $P < 0.0001$).[43] However, in our experience in HCC patients treated with SR ($n = 887$), 1-, 3-, 5-, 8- and 10-year survival rates have been 81.3%, 61.2%, 44.5%, 31.9% and 22.3% respectively, in the 1980s and 1990s (from 1981 to 1999) ($n = 339$), and 91.2%, 70.6%, 54.5%, 36.1% and 25.7%, respectively, in the 2000s ($n = 548$) ($P = 0.020$) (Fig. 12).

Traditional surgical management for HCC is open hepatectomy (OH). Laparoscopic hepatectomy (LH), first reported in 1993 as a newly developed procedure, has been performed around the world and has been established as a safe and feasible option for malignant liver tumors. A number of advantages such as less operative morbidity, reductions in postoperative pain and shorter length of hospitalization have been identified when comparing LH to OH from case-matched analyses and case series.[44,45] Advances in surgical techniques are pushing the boundaries of SR for localized disease. On the other hand, several investigators reported that anatomical resection for patients with HCC could improve survival compared with non-anatomical resection.[40,46]

## LT

Among therapies for HCC, the most effective curative option is LT.[4,6] However, LT is not appropriate for all patients, and sufficient preoperative evaluation is needed to prudently allocate the scarce resources available.[4,6] In an attempt to identify the most appropriate patients to undergo LT, the Milan criteria which consider both tumor number and tumor size (a single tumor <5 cm or ≤3 tumors of ≤3 cm) emerged as the international standard by which potential transplant candidates are evaluated in 1990s and this criteria has been validated in several studies.[47,48] In HCC patients within the Milan criteria treated with LT, 5-year survival rates after LT are reported to range 70–80%, and HCC recurrence rates are approximately 10%.[47,48]

On the other hand, several investigators have examined the effect of expanding the Milan criteria on survival, primarily by liberalizing the restrictions on tumor size. Yao *et al.* demonstrated that using University of California San Francisco (UCSF) criteria (single nodule ≤6.5 cm or ≤3 nodules each ≤4.5 cm, with total combined tumor diameter ≤8 cm), the 5-year survival rate after LT in patients within UCSF criteria was 75%, while Kaido *et al.* reported that using the Kyoto criteria (a combination of tumor number ≤10, maximal diameter of each tumor ≤5 cm, and serum des-γ-carboxy prothrombin levels ≤400 mAU/mL), the 5-year survival rate after living donor LT in HCC patients within the Kyoto criteria was 82%, indicating that studies of these expanding criteria showed promising results.[49,50] However, due to the limited number of donors and the scarcity of sufficient available data, current guidelines do not recommend LT for HCC patients outside the Milan criteria.[34,41]

## TACE

Transcatheter arterial chemoembolization is a procedure whereby an embolic agent is injected into the tumor-feeding artery to deprive it of its major nutrient source by means of embolization; this results in ischemic necrosis of the targeted tumor.[11,51] Differences in blood supply to HCC tumors and the liver form the theoretical basis of transcatheter arterial therapy for HCC.[11,51] Transcatheter arterial embolization was initially used to treat HCC by Doyon *et al.* in 1974 and was applied to most unresectable HCC using gelatin sponge particles and anticancer agents by Yamada *et al.* in Japan.[52,53] In the 1980s, TACE was the only non-surgical therapy for unresectable HCC until the introduction of PEI therapy for HCC. In the mid-1990s, lipiodol was newly introduced to enhance mainly the therapeutic effect. It is a substance which is selectively retained within the tumor and increases chemotherapeutic exposure as a drug carrier.[54,55] Thereafter, TACE using lipiodol

emulsion for unresectable HCC has been spread rapidly. The survival benefit of TACE for unresectable HCC was established in two randomized controlled trials (RCT) and in one meta-analysis.[56–58] Thus, TACE plays an important role in treating unresectable HCC. It is clearly defined as a first-line therapy with an improved 2-year survival rate as compared with conservative therapy.[59] The EASL guidelines recommend TACE for unresectable, Child–Pugh A or B multiple HCC with no vascular invasion while in Japan the therapy is recommended even for HCC with vascular invasion if it is Vp1 or Vp2.[34,41]

Takayasu *et al.* conducted a nationwide survey in 8510 HCC patients treated with TACE and reported 1-, 3- and 5-year survival rates of 82%, 47% and 26%, respectively, while in our data, as shown in Figure 13, in 765 HCC patients treated with TACE, 1-, 3- and 5-year survival rates are 72.5%, 36.4% and 17.5%, respectively, which means our results are worse than their results.[60] This is probably due to the differences in baseline tumor characteristics between these two studies. Their study included 927 patients (13%) with stage I HCC and 501 patients (7%) with stage IVA HCC, whereas our study included only 32 patients (4.2%) with stage I HCC and 177 patients (23.1%) with stage IVA or stage IVB HCC.[60]

Although the administration of a chemotherapeutic drug and lipiodol emulsion followed by embolic agents has been the most popular TACE procedure, the recent introduction of an embolic drug-eluting bead (DEB) has provided an attractive alternative to conventional regimens.[2] While several reports have consistently shown a clinical benefit from conventional TACE, its significant adverse effects related to the administrated chemotherapeutic regimens have prevented the development of a clear consensus with regard to the type of chemotherapy that should be used or the optimum frequency of treatment sessions.[2] Clinical trials have demonstrated that DEB loaded with doxorubicin has a safe pharmacokinetic profile with lower systemic drug exposure and significantly reduced liver toxicity in comparison with conventional TACE.[61–63] In Japan, TACE with DEB will likely replace conventional TACE in the near future.

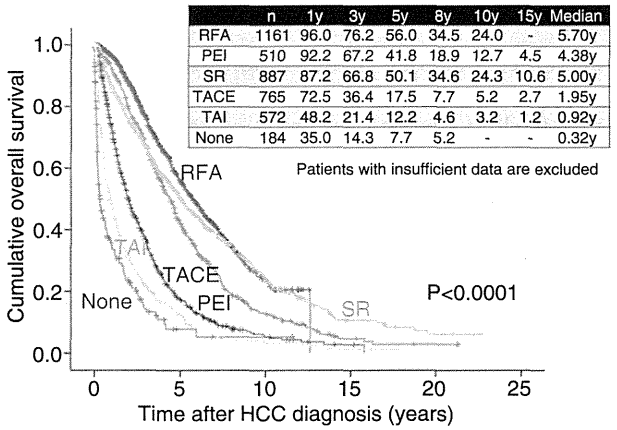


**Figure 13** Cumulative overall survival according to treatment modality at initial hepatocellular carcinoma (HCC) therapy (Osaka Red Cross Hospital, Japan). PEI, percutaneous ethanol injection; RFA, radiofrequency ablation; SR, surgical resection; TACE, transcatheter arterial chemoembolization; TAI, transcatheter arterial infusion chemotherapy; y, years.

## RFA and PEI

Percutaneous ethanol injection, which involves the injection of absolute ethanol directly into targeted tumors through fine needles under guidance of ultrasonography, has been widely used as a standard local ablative therapy for small HCC since its development in Japan in the late 1980s.[64–68] However, in many cases its treatment efficacy is unpredictable because the spread of injected ethanol within the targeted tumor is largely affected by the capsule or septa of the targeted tumor.[64–68] On the other hand, RFA therapy is an alternative technique to PEI that was introduced in Japan in 1999 and RFA therapy heat generated around the electrode tip distributes homogenously in all directions.[64–79] An area of 3 cm or less in diameter can be ablated with a single application of RFA.[64–68] The higher level of local tumor control achieved using RFA as compared with PEI seems to be due to the more expansive coagulative effects of thermal ablation on the targeted HCC nodules and microsatellites surrounding the nodules.[65–68] The survival rate data indicated a significant benefit for RFA over PEI.[65–68] This higher survival rate may be due to the higher rate of complete tumor response using RFA than using PEI; an initial complete response is an independent predictive factor linked to survival.[80] In our experience, as shown in Figure 13, 1-, 3-, 5-, 8- and 10-year survival rates are 92.2%, 67.2%, 41.8%, 18.9% and 12.7% respectively, in HCC patients treated with PEI ($n = 510$) and 96.0%, 76.2%, 56.0%, 34.5% and 24.0%, respectively, in HCC patients treated with RFA ($n = 1161$) ($P < 0.001$). The EASL guidelines recommend percutaneous RFA for HCC with PS 0–2, Child–Pugh A or B, and three or less unresectable tumors of 3-cm diameter or less.[41] In Japan, percutaneous RFA is generally indicated for patients with Child–Pugh A or B and three or less unresectable tumors of 3 cm diameter or less. Even in patients with unresectable tumors larger than 3 cm, percutaneous RFA in combination with

TACE is recommended to expand the ablated area.[34] Furthermore, a bipolar RFA device (CelonPOWER System; Celon Medical Instruments, Teltow, Germany) has been recently introduced in Japan.[81] Unlike a monopolar RFA system which had been already approved in Japan, the primary characteristic of this novel device is its bipolar feature. That is, two electrodes are located on the same RFA needle, allowing electricity flow only between the electrodes at the treatment site of RFA without the need for a grounding pad and the danger of skin burns.[81] We previously demonstrated that using the CelonPOWER System, tumors larger than 3 cm in diameter can be ablated safely.[81] More recently, several investigators have used RFA to treat selected patients with resectable HCC with favorable clinical outcomes, and RFA is gradually gaining popularity in the treatment of resectable HCC in many countries, in addition to Japan.[82,83]

## RFA versus surgery

A continuous improvement of survival outcomes after SR and RFA for HCC was achieved.[7,84–86] Thus, an open question is whether or not RFA can compete with SR as a first-line therapy for patients with small HCC. However, no definite consensus has been reached as to which of these two therapies is the best for small HCC eligible for surgery. Three RCT and several non-RCT that have compared RFA with SR have been reported.[69–79,87–89]

Feng *et al.* reported in their latest RCT that in patients with small HCC with nodular diameters of less than 4 cm and up to two nodules ($n = 168$), percutaneous RFA may provide therapeutic effects similar to those of SR (cumulative OS rates at 1 and 3 years, 96.0% and 74.8% for SR and 90.6% and 76.7% for RFA [$P > 0.05$]; cumulative recurrence free survival (RFS) rates at 1 and 3 years, 93.1% and 61.1% for SR and 86.2% and 49.6% for RFA [$P > 0.05$]).[89] Conversely, Huang *et al.* reported in their RCT that SR may provide better survival and lower recurrence rates than RFA for patients with HCC conforming to the Milan criteria (cumulative OS rates at 1, 3 and 5 years, 98.26%, 92.17% and 75.65% for SR, and 86.96%, 69.57% and 54.78% for RFA [$P = 0.001$]; cumulative RFS rates at 1, 3 and 5 years, 85.22% 60.87% and 51.30% for SR, and 81.74%, 46.08% and 28.69% for RFA [$P = 0.017$]).[88] Zhou *et al.* conducted a meta-analysis to evaluate the efficacy of RFA and SR for the treatment of HCC.[79] They concluded that SR was superior to RFA for patients with small HCC tumors of more than 3 cm that were eligible for surgery; however, for tumors of 3 cm or less, survival levels did not differ significantly between SR and RFA. Furthermore, Cho *et al.* reported that using Markov model analysis, SR was preferable to RFA in terms of OS.[90] Recently, a Japanese nationwide survey revealed that SR ($n = 5361$) results in longer OS and time to recurrence than either RFA ($n = 5548$) or PEI ($n = 2059$) in patients with HCC who had no more than three tumors (≤3 cm) and liver damage of class A or B.[91] In view of these results it is therefore still unclear whether or not SR can achieve higher survival rates than RFA for patients with resectable HCC, although the majority of previous reports indicated that SR had a superior efficacy over RFA in selected HCC patients. In addition, all of the previous three RCT were Chinese based.[87–89] In comparing the results of these RCT with those from Japanese-based studies, the mean age of the Chinese patient population was approximately 10 years younger than that of the patients in the Japanese studies.[75,78,79,87–89,91] In the etiology of liver disease in the Chinese studies, patients with chronic hepatitis B were in the majority.[87–89] However, in the Japanese studies, patients with chronic hepatitis C were in the majority; hence, the study results did not reflect the actual situation in Japan where Japanese HCC patients consist of many aged patients, and the etiology of background liver disease involves chronic hepatitis C, which accounts for approximately 80% of Japanese HCC patients.[75,78,79,91] We also believe that antiviral therapy for background liver diseases (i.e. nucleotide analog therapy for hepatitis B and IFN therapy for hepatitis C) and nutritional supporting therapy for improving liver function such as branched-chain amino acid therapy, as well as tumor-related factors such as HCC stage, tumor size and tumor number should be taken into account when assessing clinical outcomes after initial therapy for HCC.[38,39,92–94] Therefore, caution should be exercised for interpreting these study results. In our country, an RCT (SURF trial) comparing survival between surgery and RFA for patients with resectable HCC of 3 cm or less in size and up to three nodules is underway.[95]

In our experience, in LC patients with HCC with three nodules or less and up to 3 cm in diameter, the 3- and 5-year OS rates in the SR group ($n = 207$) were 76.3% and 55.8%, respectively, and the corresponding OS rates in the RFA group ($n = 666$) were 77.2% and 55.5%, respectively ($P = 0.767$) (Fig. 14). Furthermore, after using propensity score matching (adjusted for possible variables associated with long-term survival of HCC patients: age, sex, tumor number, maximum tumor size, cause of liver disease and Child–Pugh classification), the 3- and 5-year OS rates in the SR group ($n = 179$) were 75.9% and 56.8%, respectively, and the corresponding OS rates in the RFA group ($n = 179$) were 78.6% and

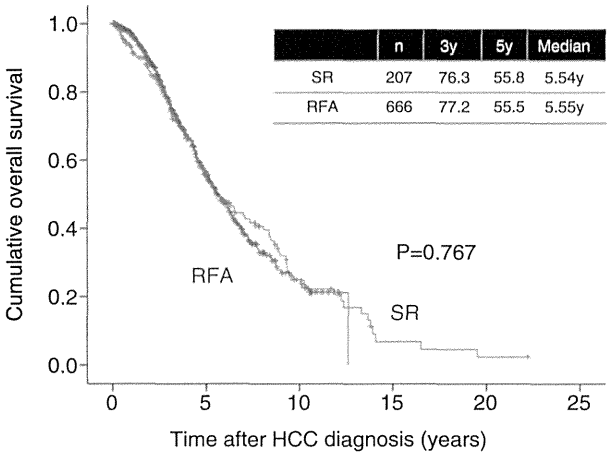


**Figure 14** Cumulative overall survival in hepatocellular carcinoma (HCC) patients with liver cirrhosis, tumor size within 3 cm and up to three nodules treated with radiofrequency ablation (RFA) ($n = 666$) and surgical resection (SR) ($n = 207$). y, years.

60.6%, respectively ($P = 0.266$) (Fig. 15). Our study results indicate that in LC patients with HCC with three nodules or less and up to 3 cm in diameter, patients treated with RFA at initial therapy had prognoses comparable with those treated with SR.

## Chemotherapy

Although systemic chemotherapy such as doxorubicin was not demonstrated to be effective for the treatment

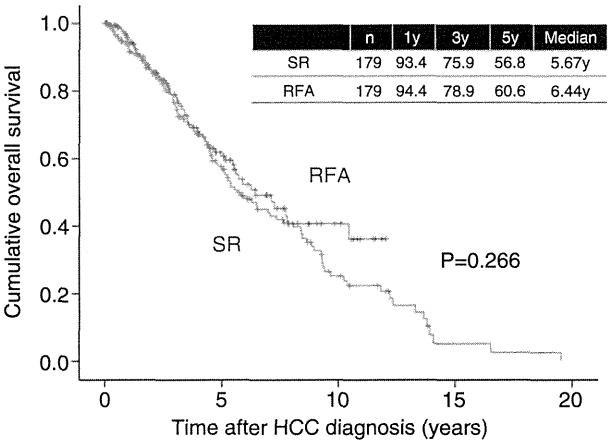


**Figure 15** Cumulative overall survival in hepatocellular carcinoma (HCC) patients with liver cirrhosis, tumor size within 3 cm and up to 3 nodules treated with radiofrequency ablation (RFA) ($n = 179$) and surgical resection (SR) ($n = 179$) after propensity score matching. y, years.

of advanced HCC for several decades, two randomized studies showed that sorafenib therapy obtained survival benefit over the placebo group for patients with unresectable HCC, and molecular-targeted therapy with sorafenib is now approved for use as first-line systemic chemotherapy in these patients.[5,96–98] Sorafenib is a multikinase inhibitor that blocks tumor growth and cell proliferation.[5,96,97] Several clinical trials of molecular targeting agents other than sorafenib have been performed. However, no trials to date have shown that any of these agents have superior efficacy compared with sorafenib for the treatment of unresectable HCC.[99–101] The EASL guidelines recommend sorafenib for unresectable, advanced, Child–Pugh A or B HCC with PS 0–2 and vascular invasion or distant metastasis.[41] According to the Japanese guidelines, on the other hand, sorafenib is recommended for unresectable, advanced, Child–Pugh A HCC with vascular invasion or distant metastasis as well as for patients intolerant to TACE or in whom the procedure is anatomically unsuitable.[34]

Although sorafenib has a significantly higher survival benefit for patients with advanced HCC, the response to sorafenib remains low and the median OS is only extended by approximately 3 months.[5,96–101] Furthermore, predictive factors of responders to sorafenib in patients with HCC have not been well established.[102] To optimize the beneficial effects of sorafenib, combination or sequential therapies comprising sorafenib and other therapies for HCC have been examined. The limited effects of single therapy indicated that the combination therapy would enhance the overall treatment effect. A recent systematic review suggested that sorafenib-based combination with some anticancer agent could be a more effective and tolerable treatment for advanced HCC in the future.[103] Sequential sorafenib therapy after TACE for unresectable HCC is also promising. Sansonno *et al.* reported that a conventional TACE procedure followed by sorafenib therapy resulted in a significantly longer time to progression in patients with intermediate stage HCV-related HCC, with no unexpected side-effects.[104] On the other hand, a clinical trial to study the recurrence-preventing efficacy of sorafenib by administration of it after curative therapy such as SR or RFA is underway (STORM trial).[105]

The success of sorafenib has spurred an explosive increase of clinical studies testing novel molecular targets and other agents for the treatment of HCC. They included sunitinib, brivanib, foretinib, TSU-68, erlotinib, AZD6244, linifanib, regorafenib tivantinib and monoclonal antibodies such as bevacizumab and

glypican-3.[106,107] If favorable results are obtained by these trials, the treatment strategy for HCC will be drastically changed.

On the other hand, hepatic arterial infusion chemotherapy (HAIC) for advanced HCC was originally developed in Japan.[98] Because no RCT regarding efficacy of HAIC for advanced HCC has been conducted and its use is based solely on empirical clinical data, HAIC for advanced HCC is not appreciated in Western countries.[98] However, there are several encouraging reports from Japan. Ando *et al.* reported that in 48 advanced HCC patients with portal vein tumor thrombosis (PVTT) treated with HAIC using low-dose 5-fluorouracil (5-FU) and cisplatin (low-dose FP), the response rate was 48%.[108] Ueshima *et al.* demonstrated that in advanced HCC patients with vascular invasion treated with HAIC using low-dose FP, the response rate was 38.5%, the median time to progression was 4.1 months and the median survival time was 15.9 months, which are superior to those in the SHARP study.[5,109] Obi *et al.* reported that in 116 advanced HCC patients with portal venous invasion treated with combination therapy of intra-arterial 5-FU and systemic IFN-α, 19 patients (16%) showed complete response and 42 (36%) showed partial response.[110] HAIC for advanced HCC may have potential benefit for overcoming drawbacks in sorafenib therapy for advanced HCC, although further well-characterized studies are necessary.

## Radiotherapy

Although radiotherapy for advanced HCC is not recommended in current guidelines, it can be a promising therapy.[34,41,111,112] Xi *et al.* demonstrated that in 41 advanced HCC patients with PVTT or inferior vena cava invasion treated with stereotactic body radiotherapy, 15 (36.6%) achieved complete response, 16 (39.0%) achieved partial response.[111] Nakazawa *et al.* compared the survival benefits of sorafenib ($n = 40$) versus radiotherapy ($n = 57$) in HCC patients with PVTT using propensity score-matching analysis and concluded that radiotherapy is a better first-line therapy than sorafenib in advanced HCC patients with PVTT.[112] However, further prospective studies are warranted to confirm these results.

## Other new emerging therapies for HCC

Recently, new promising therapies for HCC have emerged. For intermediate stage HCC, there is increasing evidence supporting a role for transarterial radioembolization. Radioembolization is a form of brachytherapy in which intra-arterially injected (90)Y-loaded microspheres serve as sources for internal radiation purposes and produces average disease control rates above 80% and is a very well-tolerated therapy in general.[113–115]

Based on the immune system's antitumoral effect, immunotherapy is also a promising new treatment option for HCC. Actually, specific antitumoral T-cell responses can be detected in HCC patients.[13] Clinical trials regarding the effect of the active specific immunotherapy (including dendritic cell vaccine, liver cancer vaccine and HCC genetically engineered vaccine) in HCC patients on survival is still underway.[116]

On the other hand, gene therapy has the potential to provide therapeutic benefits for patients with HCC and has been the subject of intense clinical research in recent years. miRNA are endogenous single-stranded RNA, approximately 22 nucleotides in length. After the discovery of miRNA in 1993, the considerable extent of the gene regulatory capacity of miRNA has been studied. These investigations have shown that specific miRNA have central roles in critical biological processes such as apoptosis, development, cell proliferation and oncogenesis.[117] Clinical studies with regard to the potential clinical uses of miRNA are ongoing, most notably in the early diagnosis and treatment of HCC.[118]

However, all the evidence that support the use of these therapies in HCC is mainly based on retrospective series or non-controlled prospective studies. Hence, further well characterized RCT will be needed to confirm clinical efficacy of these therapies on survival.

## CONCLUSION

OVER THE LAST three decades, prognosis in patients with HCC have markedly improved due to the advances in the treatment for HCC. Furthermore, baseline characteristics in HCC patients have markedly changed. New emerging diagnostic imagings and the adequate selection of high-risk groups for HCC occurrence could enable detection of early stage HCC, potentially improving outcome. Recent new emerging therapies may further improve prognosis in HCC patients.

Finally, we show annual trends for the treatment of HCC in our hospital from 1981 to 2013 in Figure 16.

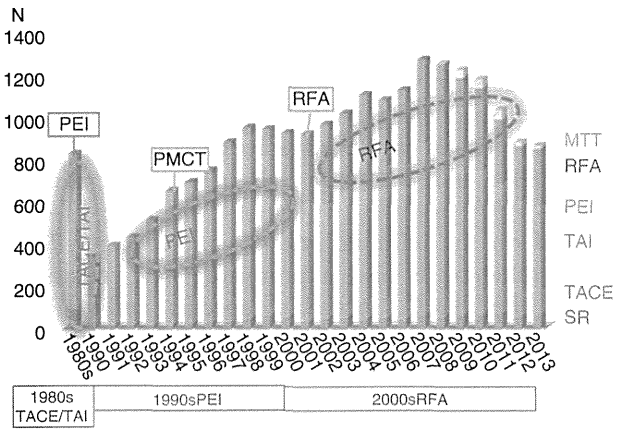


**Figure 16** Annual trends for the treatment of hepatocellular carcinoma (1981–2013, Osaka Red Cross Hospital, Japan). MTT, molecular-targeted therapy; PEI, percutaneous ethanol injection; PMCT, percutaneous microwave coagulation therapy; RFA, radiofrequency ablation; SR, surgical resection; TACE, transcatheter arterial chemoembolization; TAI, transcatheter arterial infusion chemotherapy.

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***Original Article***

# Effect of treatment with branched-chain amino acids during sorafenib therapy for unresectable hepatocellular carcinoma

Haruhiko Takeda, Hiroki Nishikawa, Eriko Iguchi, Yoshiaki Ohara, Azusa Sakamoto, Sumio Saito, Norihiro Nishijima, Akihiro Nasu, Hideyuki Komekado, Ryuichi Kita, Toru Kimura and Yukio Osaki

*Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, Osaka, Japan*

*Aim:* To examine the effect of branched-chain amino acid (BCAA) therapy for patients with unresectable hepatocellular carcinoma (HCC) treated with sorafenib.

*Methods:* Seventy-eight subjects with unresectable HCC with a serum level of albumin of 3.5 g/dL or less treated with sorafenib were evaluated. They were classified into two groups: those receiving BCAA granules ($n = 34$; BCAA group) or a regular diet ($n = 44$; control group). We compared overall survival and administration period of sorafenib, and analyzed absolute changes in serum levels of albumin during sorafenib therapy in 41 patients who continued sorafenib therapy for 1 month or more with a follow up of more than 3 months.

*Results:* Median survival time (MST) in BCAA and control groups was 350 and 143 days ($P = 0.007$), respectively. Median administration period of sorafenib in the two groups was 59 and 41 days ($P = 0.018$). In the 41 patients described above, at 1 month, there was no significant change in the serum level of albumin between the two groups, but at 3 months, the difference in the absolute change in the serum level of albumin in the two groups reached significance ($P = 0.023$). In these subgroup analyses, the administration period of sorafenib as well as the MST in the BCAA group were significantly longer than those in the control group ($P = 0.020$ and $= 0.004$).

*Conclusion:* BCAA treatment during sorafenib therapy in HCC patients is useful for maintaining hepatic functional reserve, which may help to avoid early discontinuance of sorafenib therapy and improve survival.

**Key words:** albumin, branched-chain amino acid, hepatocellular carcinoma, liver dysfunction, sorafenib

## INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common malignancies worldwide.[1] Various therapeutic methods, such as surgery, local ablative therapies, transcatheter arterial chemoembolization (TACE) and radiation therapy, have been applied for HCC patients according to tumor stage and functional reserve in the liver. Nevertheless, effective systemic chemotherapy has not been established for unresectable advanced HCC.[2] In 2008, after two randomized studies – the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) trial and the Asia–Pacific Trial – had shown the efficacy of sorafenib (a multikinase inhibitor that blocks tumor growth and cell proliferation), molecular targeting therapy with sorafenib became first-line systemic chemotherapy for patients with unresectable advanced HCC worldwide.[3–6]

Branched-chain amino acids (BCAA) are three amino acids with branched side chains (i.e. valine, leucine, isoleucine). Patients with liver cirrhosis (LC) have decreased plasma levels of BCAA. This can lead to protein–energy malnutrition (PEM), which comprises impaired glycolysis and glycogenesis, a negative nitrogen balance and hyperlipolysis.[7–9] PEM is associated with high morbidity and mortality due to an increased risk of life-threatening complications, which results in

*Correspondence: Dr Haruhiko Takeda, Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, 5-30 Fudegasaki-cho, Tennoji-ku, Osaka 543-8555, Japan. Email: htakeda511@yahoo.co.jp*

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poorer survival and decrease in quality of life (QOL).[8,9] PEM occurs frequently in patients with LC and represents an important adverse predictive factor for LC patients with HCC.[8–12] Supplementation with BCAA formulas is reported to be useful for improving PEM and QOL in these patients. Several studies have revealed the importance of such nutritional intervention in patients with HCC who have undergone surgery, radiofrequency ablation therapy (RFA) or TACE.[9,10,13–18]

Most subjects with unresectable HCC treated with molecular targeting therapy have concurrent LC with PEM. In such patients, systemic chemotherapy such as sorafenib therapy may further worsen their nutritional condition, resulting in an irreversible outcome. In Japan, many individuals with unresectable HCC treated with sorafenib have developed liver dysfunction such as hypoalbuminemia and increased ascites, which has resulted in discontinuance of sorafenib therapy and, occasionally, liver failure.[19,20] Improvement of underlying PEM should, therefore, be considered a serious matter during sorafenib therapy. However, no studies have assessed the importance of BCAA formulas in subjects with unresectable HCC treated with sorafenib. Here, we ascertained if BCAA treatment can contribute to maintaining hepatic functional reserve and bring survival benefit in subjects with unresectable HCC treated with sorafenib.

## METHODS

### Patients

ONE HUNDRED AND twenty patients with unresectable HCC have been treated with sorafenib at the Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital (Osaka, Japan) from June 2009 to September 2012. Sorafenib therapy was indicated in patients with unresectable HCC determined by dynamic computed tomography (CT); that is: (i) existence of extrahepatic metastases; (ii) refractory to previous HCC therapies such as TACE; (iii) unsuitability for TACE for anatomical reasons; or (iv) vascular invasion such as tumor thrombus in the portal vein. Patients with a performance status of 3 or 4 according to the Eastern Cooperative Oncology Group classification and a pretreatment Child–Pugh score of 8 or more were excluded.

In Japan, BCAA granules are indicated for the treatment of LC patients with a serum albumin level of 3.5 g/dL or less. Hence, we excluded patients with a serum albumin level of 3.6 g/dL or more ($n = 42$) in the present study. Thus, 78 HCC patients with a serum albumin level of 3.5 g/dL or less were evaluated in the current study.

### Diagnosis of HCC

Dynamic CT and ultrasonography of the abdomen were undertaken in all patients. A lesion visualized as a tumor blush in the early-phase scan and as a defect area in the late-phase scan on dynamic CT was diagnosed as typical HCC. It has been verified that such lesions appear as blushes on CT hepatic angiography and as defect areas on CT arterial portography.[21]

Some lesions could not be considered to be typical images of HCC. Hence, we diagnosed HCC with not only imaging but also tumor markers such as α-fetoprotein (normal range, <10 ng/mL) and des-γ-carboxy prothrombin (DCP) (normal range, <10 mAU/mL) using chemiluminescent enzyme immunoassays (Lumipulse PIVKAII; Eisai, Tokyo, Japan). Furthermore, in some patients who presented with atypical liver tumors, we conducted ultrasound-guided tumor biopsy for histological confirmation of HCC. HCC stage was determined using the staging system set by the Liver Cancer Study Group of Japan.[22]

### Initial dose and discontinuance of sorafenib therapy

The recommended initial dose of sorafenib for HCC is 400 mg twice a day.[3–5] Nevertheless, according to the phase-I study by Miller *et al.*, patients with insufficient liver function or renal function (serum total bilirubin >1.5 mg/dL, serum albumin <2.5 g/dL and/or creatinine clearance <40 mg/mL) are recommended to receive a reduced dose of sorafenib.[23] Studies in several countries (including Japan) have reported serious adverse events in several individuals with advanced HCC given an initial dose of sorafenib of 800 mg/day, which led to treatment discontinuation. Moreover, in Japan, the proportion of the population with a body mass index (BMI) of 30 kg/m$^2$ or more has been reported to be 2–3% or less, which is in stark contrast to the 20–30% prevalence in Western countries.[24–26] Taking this information into consideration, the initial dose of sorafenib was determined according to factors such as bodyweight, the BMI, body surface area, age, comorbidity, performance status (PS) and liver function. Hence, the initial sorafenib dose in the present study was 400–800 mg/day. We evaluated the response to sorafenib treatment every 4–8 weeks after the initiation of sorafenib therapy by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1[27,28] and/or tumor markers. Sorafenib treatment continued until disease progression, unacceptable drug-related

toxicity or the patient's wish to discontinue treatment. Follow up consisted of weekly or bi-weekly blood test analyses and physical examination at each visit.

### BCAA granules

Branched-chain amino acid granules (Livact; Ajinomoto, Tokyo, Japan), containing 952 mg of L-isoleucine, 1904 mg of L-leucine and 1144 mg of L-valine per sachet, were administrated p.o. to subjects at one sachet three times daily after meals. Whether BCAA granules were given was determined primarily by each attending physician considering each patient's nutritional status and economic backgrounds.

### Study protocol

The present study comprised retrospective analyses of patient records registered in the database of our hospital. All the protocols were approved by the ethics committee of our hospital. Written informed consent was obtained from each patient before sorafenib therapy was initiated. This retrospective study protocol complied with all provisions of the Declaration of Helsinki.

Patients were classified into two groups: those undergoing BCAA treatment ($n = 34$; BCAA group) or receiving a regular diet ($n = 44$; control group). BCAA therapy had been started 1 day or more before sorafenib was administrated. BCAA treatment had been continued until patients could not take any medicine owing to worsened PS or progressive disease. The primary endpoint was overall survival (OS). The secondary endpoints were the administration period of sorafenib in the two groups and changes in the serum albumin level and Child–Pugh classification.

First, we compared OS and the administration period of sorafenib between the two groups. OS was calculated from the initial date of sorafenib treatment until death from any cause or the last follow up. Next, we carried out subgroup analyses in patients with liver function of Child–Pugh class A ($n = 45$) and in patients who took sorafenib for more than 1 month ($n = 55$).

To ascertain if BCAA therapy contributes to maintaining or improving hepatic functional reserve, we examined 41 patients who took sorafenib for more than 1 month without anticancer therapies other than sorafenib therapy, and who could be observed for 3 months or more after the initiation of sorafenib therapy. The serum level of albumin has been reported to accurately reflect hepatic functional reserve.[9,10] Absolute changes in the serum level of albumin observed at 1 and 3 months after sorafenib administration were compared between the BCAA group and control group. The absolute change was defined as the difference at each assessment time point from the baseline levels before the initiation of sorafenib therapy. Then, the proportion of Child–Pugh class A at 1 and 3 months were compared with that in pretreatment status in both groups. In the same subgroup, we also compared the administration period, total administrated dose of sorafenib and OS between the two groups.

### Statistical analyses

Categorical variables were analyzed by Fisher's exact test. Continuous variables were analyzed by unpaired or paired Student's *t*-tests (if appropriate). OS curves were generated using the Kaplan–Meier method and compared using the log-rank test. The Cox proportional hazard model was used for multivariate analyses of factors with $P < 0.1$ in univariate analyses. Data were analyzed using SPSS ver. 9.0 (SPSS, Chicago, IL, USA). Data are the median value (range). Two-tailed probability values of $P < 0.05$ were considered significant.

## RESULTS

### Baseline characteristics

THE BASELINE CHARACTERISTICS of the two groups are shown in Table 1. The BCAA group comprised 27 males and seven females with a median age of 72 years (range, 55–88). The control group consisted of 37 males and seven females with a median age of 68 years (range, 46–89). Most patients had received previous therapies for HCC: one or more sessions of TACE, RFA or percutaneous ethanol injection and surgery. With regard to pretreatment liver function, 46 patients (59.0%) had liver function of Child–Pugh class A and 32 (41.0%) had liver function of Child–Pugh class B. Although the percentage of stage IV was higher in the control group (31/44 patients; 70%) than in the BCAA group (17/34; 50%), no statistical significant difference was observed between these two groups ($P = 0.135$).

In terms of treatment efficacy, complete response (CR) was obtained in one patient, partial response (PR) in two patients, stable disease (SD) in 28 patients and progressive disease (PD) in 22 patients according to RECIST 1.1, whereas CR was noted in one, PR in six, SD in 26 and PD in 20 patients according to the modified RECIST.[29]

### OS

Median OS was 350 days in the BCAA group and 143 days in the control group (Fig. 1a). OS in the BCAA group was significantly higher than that in the control group ($P = 0.007$).

**Table 1** Baseline characteristics between the BCAA group and control group

| Variable | No. or median value (range) | BCAA ($n=34$) | Control ($n=44$) | $P$ |
|---|---|---|---|---|
| Age (years) | 70 (46–89) | 72 (55–88) | 68 (46–89) | 0.025† |
| Male/female | 64/14 | 27/7 | 37/7 | 0.40‡ |
| Body surface area ($m^2$) | 1.61 (1.13–2.18) | 1.58 (1.31–2.18) | 1.64 (1.13–1.99) | 0.80† |
| Body mass index ($kg/m^2$) | 22.6 (16.1–42.1) | 21.7 (16.1–42.1) | 22.3 (15.2–28.6) | 0.24† |
| Etiology of liver disease | | | | |
| Hepatitis B/hepatitis C/B + C/non-B non-C | 7/52/3/16 | 1/25/2/6 | 6/27/1/10 | 0.29‡ |
| TNM stage | | | | |
| II/III/IVA/IVB | 4/26/14/34 | 2/15/5/12 | 2/11/9/22 | 0.33‡ |
| Tumor invasion into the portal vein, yes/no | 15/63 | 5/29 | 10/34 | 0.40‡ |
| ECOG PS, 0/1/2 | 63/12/3 | 29/3/2 | 34/9/1 | 0.29‡ |
| Child–Pugh classification, A/B | 46/32 | 16/18 | 30/14 | 0.068‡ |
| Pretreatment serum albumin (g/dL) | 3.3 (2.3–3.5) | 3.3 (2.3–3.5) | 3.2 (2.4–3.5) | 0.89† |
| Pretreatment serum ChE (IU/L) | 126 (31–268) | 117 (31–204) | 131 (41–268) | 0.15† |
| Pretreatment serum AFP (ng/mL) | 137.5 (2.3–688 400) | 130 (5.3–431 010) | 98.5 (2–3–688 400) | 0.90† |
| Pretreatment serum DCP (mAU/mL) | 1378 (10–421 210) | 794 (21–98 510) | 2141 (10–421 210) | 0.29† |
| Previous therapies for HCC | | | | |
| TACE, yes/no | 72/6 | 34/0 | 38/6 | 0.033‡ |
| RFA or PEI, yes/no | 43/35 | 18/16 | 25/19 | 0.82‡ |
| Surgery, yes/no | 13/66 | 6/28 | 7/38 | 1.00‡ |
| Initial dose of sorafenib (800 mg/day or 400 mg/day) | 21/57 | 8/26 | 13/31 | 0.22‡ |

Data are the number or median value (range).
†Unpaired Student's $t$-test.
‡Fisher's exact test.
AFP, α-fetoprotein; BCAA, branched-chain amino acid; BCLC, Barcelona Clinic Liver Cancer; ChE, cholinesterase; DCP, des-γ-carboxy prothrombin; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HCC, hepatocellular carcinoma; PEI, percutaneous ethanol injection; RFA, radiofrequency thermal ablation; TACE, transcatheter arterial chemoembolization; TNM, tumor node metastasis.

In the multivariate analysis in terms of OS involving five factors with $P<0.1$ in the univariate analyses, distant metastases ($P=0.018$), liver function of Child–Pugh class A ($P=0.012$), pretreatment serum level of DCP of 1300 mAU/mL or more ($P=0.043$) and BCAA treatment ($P=0.018$) were significant independent factors linked to OS (Table 2). The hazard ratios (HR) and 95% confidence intervals (95% CI) for these independent factors are detailed in Table 2.

## Causes of death in subjects in the BCAA and control groups

Twenty-three patients (68%) in the BCAA group and 34 patients (77%) in the control group died during the follow-up period. The causes of death in the BCAA group were: HCC progression (20 patients); liver failure (two patients); and miscellaneous (one patient). The causes of death in the control group were: HCC progression (27 patients); liver failure (three patients); and miscellaneous (four patients).

## Administration period of sorafenib and total dose of sorafenib

Administration period of sorafenib was defined as the duration from the first day sorafenib was given to the day of discontinuation of sorafenib therapy. Administration period of sorafenib was significantly longer in the BCAA group than in the control group ($P=0.018$) (Fig. 1b). The total dose of administrated sorafenib in the BCAA group tended to be more than that in the control group ($P=0.094$) (Fig. 1c). Between stage II or III HCC patients ($n=30$) and stage IV HCC patients ($n=48$), the administrated dose of sorafenib in the two groups did not differ significantly (38 000 mg vs 35 600 mg, $P=0.783$).

## Subgroup analyses in patients with liver function of Child–Pugh class A

Among patients with a serum level of albumin of 3.5 g/dL or less, 45 patients had liver function of Child–Pugh class A. Of these, 16 received BCAA therapy and 29

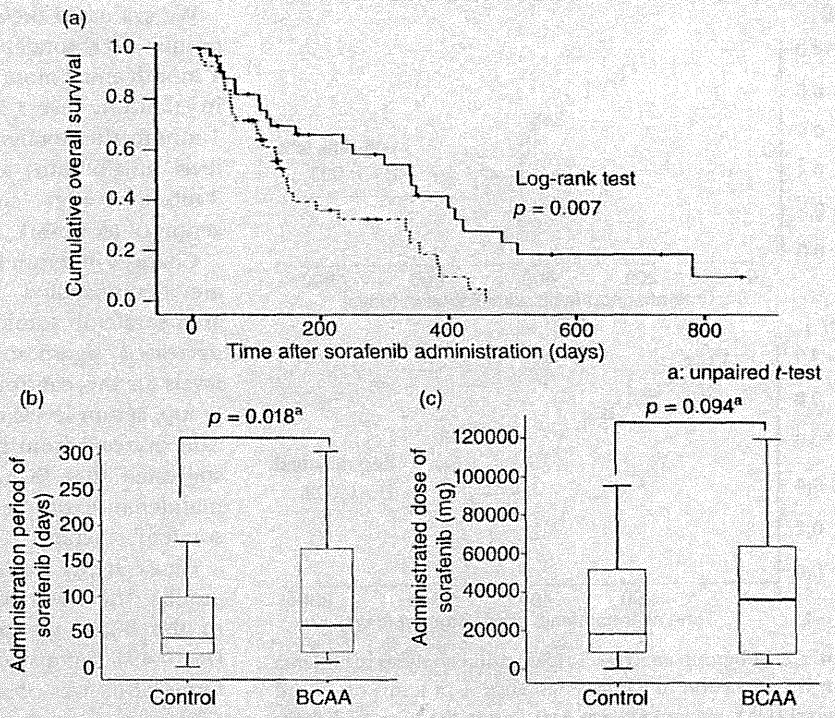


**Figure 1** Analyses for all cases ($n = 78$). (a) Kaplan–Meier overall survival (OS) curve between the branched-chain amino acid (BCAA) group ($n = 34$) and control group ($n = 44$). Median OS was 350 days in the BCAA group and 143 days in the control group ($P = 0.007$). (b) Administration period of sorafenib in the two groups: there is a significant difference ($P = 0.018$). (c) Total administrated dose of sorafenib in the two groups. The dose in the BCAA group tended to be more than that in the control group ($P = 0.098$). ——, BCAA ($n = 34$); ·····, control ($n = 44$).

received regular diets. Median OS was 410 days in the BCAA group and 148 days in the control group ($P = 0.003$) (Fig. 2a). Dosing period of sorafenib was significantly longer in the BCAA group than in the control group (88 days vs 43 days, $P = 0.022$).

## Subgroup analyses in patients who took sorafenib for more than 1 month

Among patients with a serum level of albumin 3.5 g/dL or less, 55 patients could continue receiving sorafenib

**Table 2** Univariate and multivariate analyses contributing to overall survival for all cases ($n = 78$)

| Variable | *n* | Univariate analysis | Multivariate analysis | | |
|---|---|---|---|---|---|
| | | *P*† | HR | 95% CI | *P*‡ |
| Age ≥70 years, yes/no | 40/38 | 0.829 | | | |
| Male/female | 64/14 | 0.308 | | | |
| Child–Pugh classification, A/B | 46/32 | 0.075 | 2.38 | 1.211–4.710 | 0.012 |
| ECOG-PS, 0/1 or 2 | 63/15 | 0.213 | | | |
| Tumor invasion into portal vein, yes/no | 15/63 | 0.740 | | | |
| Distant metastases, presence/absence | 44/34 | 0.018 | 2.45 | 1.168–5.172 | 0.018 |
| Serum AFP >137 ng/mL, yes/no | 39/39 | 0.511 | | | |
| Serum DCP >1300 mAU/mL, yes/no | 40/38 | 0.008 | 0.52 | 0.277–0.979 | 0.043 |
| Serum albumin >3.3 g/dL, yes/no | 39/39 | 0.632 | | | |
| Serum ChE >125 IU/L, yes/no | 39/39 | 0.083 | 1.76 | 0.849–3.651 | 0.129 |
| BCAA treatment, yes/no | 34/44 | 0.007 | 2.36 | 1.160–4.823 | 0.018 |

†Log–rank test.
‡Cox proportional hazard model.
95% CI, 95% confidence interval; AFP, α-fetoprotein; BCAA, branched chain amino acid; ChE, cholinesterase; DCP, des-γ-carboxy prothrombin; ECOG-PS, Eastern Cooperative Oncology Group Performance Status; HR, hazard ratio; TNM, tumor node metastasis.

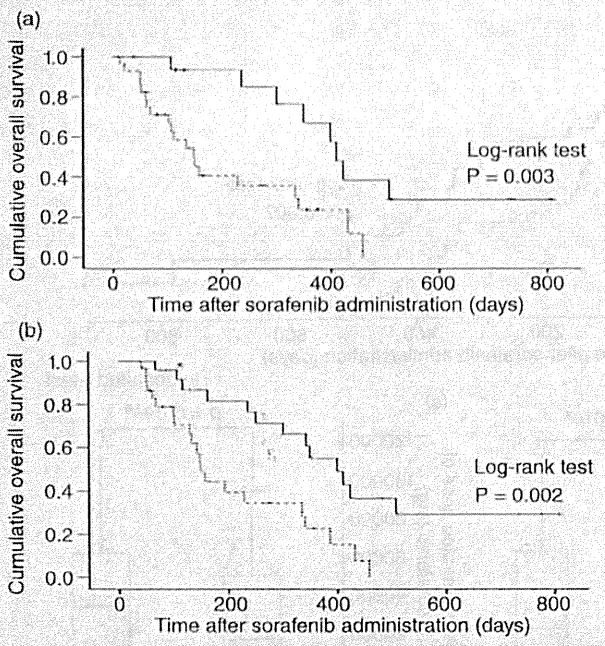


**Figure 2** Subgroup analyses. (a) Subgroup analyses in patients with liver function of Child–Pugh class A in terms of overall survival (OS). Median OS was 410 days in the branched-chain amino acid (BCAA) group ($n = 16$) and 148 days in the control group ($n = 29$) ($P = 0.003$). (b) Subgroup analyses in patients who took sorafenib for >1 month in terms of OS. Median OS was 400 days in the BCAA group ($n = 25$) and 150 days in the control group ($n = 30$) ($P = 0.002$). ——, BCAA ($n = 16$); – – –, control ($n = 29$); ——, BCAA ($n = 25$); – – –, control ($n = 30$).

therapy for more than 1 month. They involved 25 patients in the BCAA group and 30 in the control group. Median OS was 400 days in the BCAA group and 150 days in the control group ($P = 0.002$) (Fig. 2b). Dosing period of sorafenib was longer in the BCAA group than in the control group, and this difference was significant (104 days vs 67 days, $P = 0.015$).

### Comparison of changes in serum levels of albumin and Child–Pugh score, OS and administration period of sorafenib in patients treated with sorafenib for more than 1 month with a follow-up period of more than 3 months

We examined 41 patients who took sorafenib for more than 1 month with no other anticancer therapies during sorafenib treatment and who could be observed for 3 months or more after administration of sorafenib. Among them, 22 received BCAA granules and 19 received regular diets.

We evaluated their serum levels of albumin at three points after sorafenib therapy started: pretreatment, 1 month and 3 months. We defined the absolute change in albumin levels as: $\delta 1$ = (serum level of albumin 1 month after sorafenib therapy) − (pretreatment serum level of albumin) and $\delta 3$ = (serum level of albumin 3 months after sorafenib therapy) − (pretreatment serum of albumin).

Changes in serum levels of albumin in the two groups are demonstrated in Figure 3(a,b). Three months after sorafenib administration, serum levels of albumin decreased significantly compared with pretreatment levels in the control group ($P = 0.009$). In the BCAA group, serum levels of albumin did not show a significant decrease from baseline ($P = 0.76$). These findings suggested that BCAA therapy can contribute to the maintenance of the serum level of albumin during sorafenib therapy.

Figure 3(c,d) are box plots of $\delta 1$ and $\delta 3$ in the two groups. There was no significant difference between $\delta 1$ in the BCAA group and that in the control group ($P = 0.49$), but a significant between the two groups in terms of $\delta 3$ was observed (median $\delta 3$; BCAA group vs control group = −0.1 vs −0.4, $P = 0.023$).

Figure 4 shows the proportion of Child–Pugh classification at the three points, that is, pretreatment, at 1 month and at 3 months in the two groups. Among 15 patients with liver function of Child–Pugh A in the control group, nine patients failed to maintain liver functional reserve of Child–Pugh A at 3 months (Fig. 4a). On the other hand, almost all patients with liver function of Child–Pugh A in the BCAA group maintained their liver function at 3 months (Fig. 4b).

Median OS was 410 days in the BCAA group and 229 days in the control group ($P = 0.004$) (Fig. 5a). Administration period of sorafenib was longer in the BCAA group than in the control group, and this difference was significant (160 days vs 91 days, $P = 0.020$) (Fig. 5b). Total dose of sorafenib in the BCAA group tended to be more than that in the control group ($P = 0.098$) (Fig. 5c).

## DISCUSSION

IN RECENT YEARS, sorafenib therapy for advanced HCC has been employed worldwide. However, there have been several reported cases in which sorafenib therapy was discontinued owing to liver dysfunction related to this therapy.[19,20] Thus, in Japan today, only those with unresectable HCC with pretreatment liver function of Child–Pugh class A are recommended to be