Clinical parameters		No. of HCC patient 303	hTERT mRNA p	AFP p	AFP-L3 p	DCP p
Age mean:65 years old (range:22 to 101)			NS	NS	NS	NS
Gender	M	196	NS	NS	NS	NS
	F	107				
Etiology	HBV	97	NS	NS	NS	NS
	HCV	167				
	HBV+HCV	24				
	NBNC	15				
Background lesion	CH	113	NS	NS	NS	NS
	LC	190				
Numbers of tumors	1	199	NS	NS	0.003	0.029
	2	55				
	>3	49				
Tumor size (cm)	< 1	25	<0.001	0.008	NS	NS
	1~2	79				
	2~3	100				
	> 3	99				
Differentiation of tumor (Edmondson grade)	I	24	<0.001	0.019	0.001	NS
	II	43				
	III	33				
	IV	1				
	unknown	101				

hTERT mRNA was independently correlated with tumor size eand differentiation degree. NS: not significant.

Figure 1 Multivariate analysis of tumor markers with clinical parameters in patients with HCC.

bated with the following monoclonal antibodies: anti-hTERT (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-Ki67 (Santa Cruz Biotechnology), anti-TUNEL (Sigma Chemical, MO, USA), HBsAg (Sigma Chemical), and HCV core antibody (Sigma Chemical). Expression degree was confirmed and estimated of hTERT, Ki-67, and TUNEL by the percentage of positively-stained cell number [26-28].

Statistical analysis

Multivariate analysis was performed using SPSS 13.0 (SPSS Corp., Tokyo, Japan). Stratified categories in each clinical parameter were evaluated by One Way ANOVA and multivariate analysis using a logistic regression analysis model. To assess the accuracy of the diagnostic tests, the matched data sets (chronic liver diseases patients and HCC patients) regarding AFP, AFP-L3, DCP, and hTERT-mRNA were analyzed by using receiver operator characteristic (ROC) curve analysis. The correlation of hTERTmRNA between HCC tissue and serum was analyzed using both Paired t test and Spearman's test. The detection rates of HCC in comparison with tumor size were evaluated by Friedman test.

Results

RNA extraction and Real-time quantitative RT-PCR

In each quantitative assay, a strong linear relation was demonstrated between copy number and PCR cycles using RNA controls for concentration ($r^2 > 0.99$; data not shown). hTERTmRNA expression showed stepwise up-regulation with disease progression and the quantification was significantly higher in HCC than in LC, CH and healthy individuals ($P < 0.001$, $P < 0.01$ and $P < 0.001$, respectively, Figure 2A). ROC curve analyses showed that the sensitivity/specificity of hTERTmRNA for HCC were 90.2%/85.4% (Figure 2B). Optimal cut-off values for hTERTmRNA expressions were predicted as 9,332 copies/0.2 ml by stressing the higher specificity. Forty six (15%) of HCC patients, whose AFP, AFP-L3, and DCP were within normal limits, had 4.23 ± 0.32 logarithmic values of hTERTmRNA, and 20 patients of 46 patients were positive for this assay.

Multivariate analysis showed that hTERTmRNA was associated with tumor size and differentiation degree of tumor ($P < 0.001$, each, Figure 1 &3). However, hTERT-mRNA was not associated with age, gender, etiology, background lesion or number of tumor. On the other hand, AFP was related to tumor size and differentiation

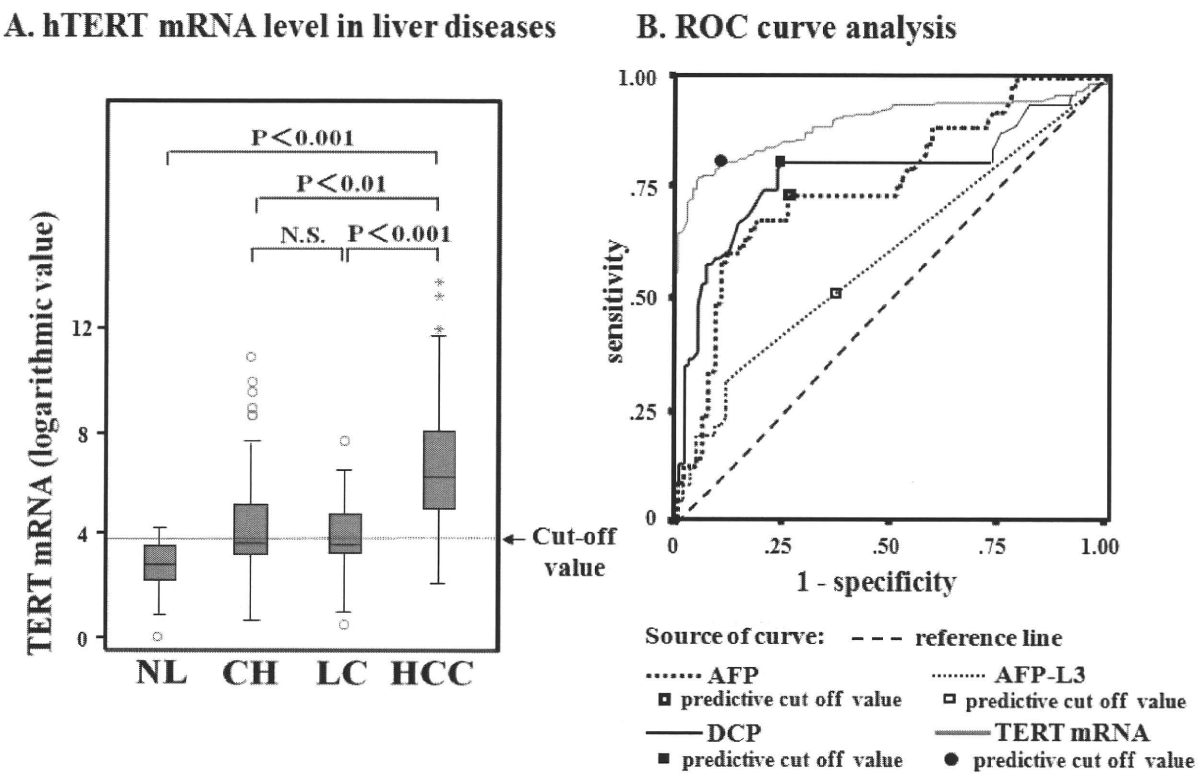

Figure 2 Serum hTERTmRNA and AFP mRNA expression in patients with HCC, LC, and CH, and in healthy individuals and receiver operator characteristics (ROC) curve analyses of hTERTmRNA, AFP, AFP-L3, and DCP. A. hTERTmRNA level in liver diseases. Serum hTERTmRNA levels and AFPmRNA level in patients with HCC, LC, CH, and healthy individuals by real-time RT-PCR were shown. The 95% confidence interval in each group is shown beside the dots. Significant differences between 4 groups are shown in the upper part of the figure. NL, individual with normal liver; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma. Optimal cut-off values for hTERTmRNA expressions were predicted as 9,332 copies/0.2 ml. B. ROC curve analysis of hTERTmRNA, AFP, AFP-L3, and DCP was obtained by importing quantified raw data into SPSS II software. The data were analyzed by Paired t test (P = 0.01) and non-parametric Spearman's test (P = 0.017). AUC of each biomarker in ROC curve analysis is shown. Receiver operator characteristic (ROC) curve analysis of hTERTmRNA, AFP, AFP-L3, and DCP was obtained by importing quantified raw data into SPSS II. The solid line, bold dotted line, dotted line, and bold solid line correspond to DCP, AFP, AFP-L3 and hTERTmRNA, respectively.

(P = 0.008 and P = 0.0199), AFP-L3 was related to number of tumor and differentiation degree (P = 0.003 and P = 0.001), and DCP was associated with only number of tumor (P = 0.029). By Pearson relative test, serum hTERTmRNA significantly associated with tumor size and number of tumors (P < 0.033 and P < 0.003, respectively, Table 1). Importantly, hTERTmRNA was related only to DCP (P = 0.03).

ROC curve analyses showed that the sensitivity/specificity of hTERTmRNA for HCC were 90.2%/85.4% (Table 2). The sensitivity/specificity of AFP, AFP-L3, and DCP were 76.6/66.2, 60.5/88.7, and 83.4/80.3, respectively. Thus, hTERTmRNA was superior to other markers especially in sensitivity. The positive predictive value (PPV)/negative predictive value (NPV) of hTERTmRNA were 83.0/85.9. On the other hand, the PPV/NPV for AFP, AFP-L3, and DCP were 74.6/67.7, 59.6/92.2, and 78.4/73.5, respectively. Consequently, hTERTmRNA was superior to other markers in the diagnosis of HCC. Combinations of hTERTmRNA with AFP level improved the sensitivity/specificity up to 96.0%/87.2%. ROC curve analysis categorized by viruses was examined and sensitivity/specificity in HBV-infected cases was similar to that of HCV-infected cases (additional file 1). hTERT and other markers in LC was not statistically and significantly different in comparison with that in CH.

Estimation of therapeutic effect and the possibility of early HCC detection of hTERTmRNA in comparison with other biomarkers

To examine the significance of hTERTmRNA before and after TAE, serum hTERTmRNA was measured before and 7 days after TAE in 16 HCC patients (Figure 4A). As a result, hTERTmRNA significantly decreased after TAE (P = 0.018), suggesting that changes in hTERTmRNA are indicative of therapeutic effects on HCC. Comparing the follow-up data of hTERTmRNA and AFP (Figure 4B, C),

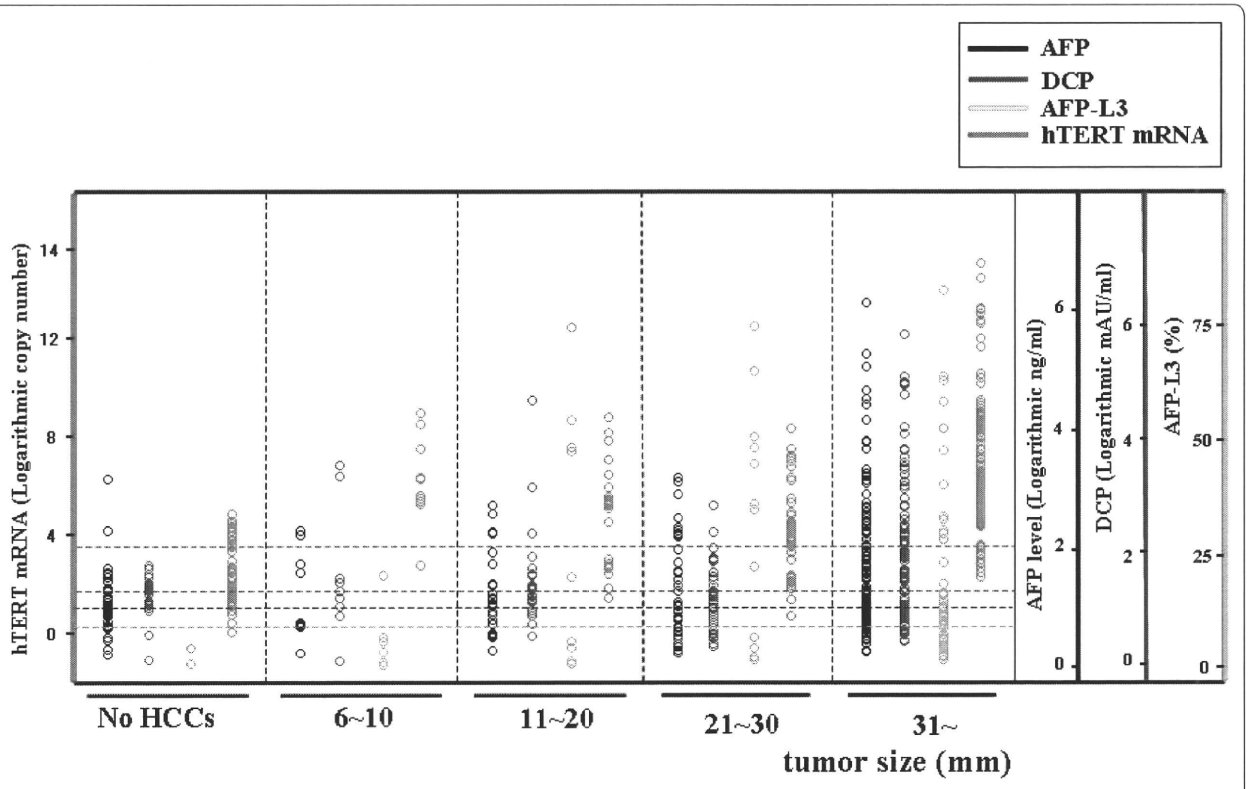

Figure 3 Levels of hTERTmRNA in regard to tumor size (6-10 mm, 11-20 mm, 21-30 mm, and over 31 mm).

the half-life of hTERTmRNA was shorter than that of AFP.

To clarify the significance of hTERTmRNA in monitoring the effect of therapies in comparison with other biomarkers, two representative cases were depicted in Figure 5. The quantification of hTERTmRNA was performed before, 2 and 5 months after RFA in a 73-year-old male patient whose HCC was a single 21 mm-sized (Figure 5A). hTERTmRNA changed similar to AFP, AFP-L3, and DCP, suggesting that hTERTmRNA is useful for monitoring the clinical course of HCC. In a 78-year-old female patient whose HCC was a single 38 mm-sized, a surgical operation was performed (Figure 5B). The values of AFP, DCP, and hTERTmRNA were measured before, 2 and 7 months after the operation. The operation was performed successfully in this patient, however recurrence was found by dynamic CT at 7 months after the operation. Although neither AFP nor DCP detected the recurrence, only hTERTmRNA did. In all the cases that hTERT detected recurrence in the earlier stage, no other imaging modality could detect it at the same time, but when we could find HCC in images such as US, CT, or MR, other markers began to arise.

Finally, we examined the relationship between the positive rates of biomarkers and tumor size. Positive rate of hTERTmRNA was higher than that of the other markers in each category of tumor size; 6-10 mm, 11-20 mm, 21-30 mm, over 31 mm by Friedman test (P = 0.017) (Figure 3). However, the positivity of hTERTmRNA expression tended to reduce slightly in tumors with diameters that exceeded than 51 mm (5.2 ± 1.9 for 56 patients with 31-50 mm of HCC, 5.0 ± 1.8 for 43 patients with HCC over 51 mm; mean ± S.D.) (additional file 2). Dot blot regarding the correlation of hTERT mRNA quantification with tumor differentiation is shown in additional file 3. In a 6 mm HCC case, no marker other than hTERTmRNA was elevated and only abdominal US caught the evidence of HCC (Figure 6(a) A, B).

Immunohistochemistry

Immunohistochemical analysis showed that Ki-67 positivity was observed in the nuclei of cancer cells (Figure 6(b) A). hTERT was observed in both the nuclei and cytoplasm of cancer cells (Figure 6(b) B). Some TUNEL-positive cells were present in cancerous lesions, however the prevalence was low (Figure 6(b) C). hTERT expression was significantly associated with the labeling index of Ki-67 (P = 0.023). When the labeling indices of Ki-67, hTERT and TUNEL were compared with the differentiation degree of HCC, both hTERT and Ki-67 were higher in poorly differentiated HCC than in well and moderately differentiated HCC (Figure 6(b) D).

Table 1: The sensitivity/specificity of each tumor marker for hepatocellular carcinoma and a statistic evaluation of hTERTmRNA level to clinical parameter were shown.

clinical parameter	average ± S.E.	Pearson test P value	Multivariate analysis P value
tumor size (mm)	21.2 ± 0.1	0.033	<0.001
(range: 6-90)			
tumor number	1.8 ± 0.1	0.003	N.S.
tumor differentiation		N.S.	<0.001
AFP (ng/ml)	6146 ± 4554	N.S.	N.S.
(n = 353)			
AFP-L3 (%)	6.7 ± 1.0	N.S.	N.S.
(n = 213)			
DCP (mAU/ml)	18780 ± 1044	0.03	N.S.
	n = 346)		

Discussion

Since HCC has been recently classified as a complex disease with a wide range of risk factors and many cellular signaling pathways have been reported to be involved in hepatocarcinogenesis, a novel biomarker for HCC is required [21]. Since an epoch-making assay to detect telomerase activity was established [11], telomerase has been examined in many kinds of cancers, precancerous lesions and normal tissues using the telomeric repeat amplification protocol and investigated the correlation with telomere length [29,30]. Notwithstanding that telomerase was definitely an unprecedented candidate tumor marker due to its specificity to cancer, it has clinically remained inapplicable because telomerase expres-

Table 2: The sensitivity/specificity of each tumor marker for HCC was depicted.

	Sensitivity	Specificity	OR	PPV/NPV	Cut-off point
hTERTmRNA	90.2	85.4	19.0	83.0/85.9	3.97 (logarithmic copy number)
AFP	76.6	66.2	11.1	74.6/67.7	<10 (ng/ml)
AFP-L3	60.5	88.7	2.2	59.6/92.2	<10 (%)
DCP	83.4	80.3	7.6	78.4/73.5	<40 (mAU/ml)

The sensitivity/specificity values are 90.2%/85.4% for hTERTmRNA, 76.6%/66.2% for AFP, 60.5%/88.7% for AFP-L3, and 83.4%/80.3% for DCP. Regarding a diagnostic assessment in sensitivity and specificity, hTERTmRNA is identified as the most excellent tumor marker. OR: odds ratio, PPV: positive predictive value (%), NPV: negative predictive value (%).

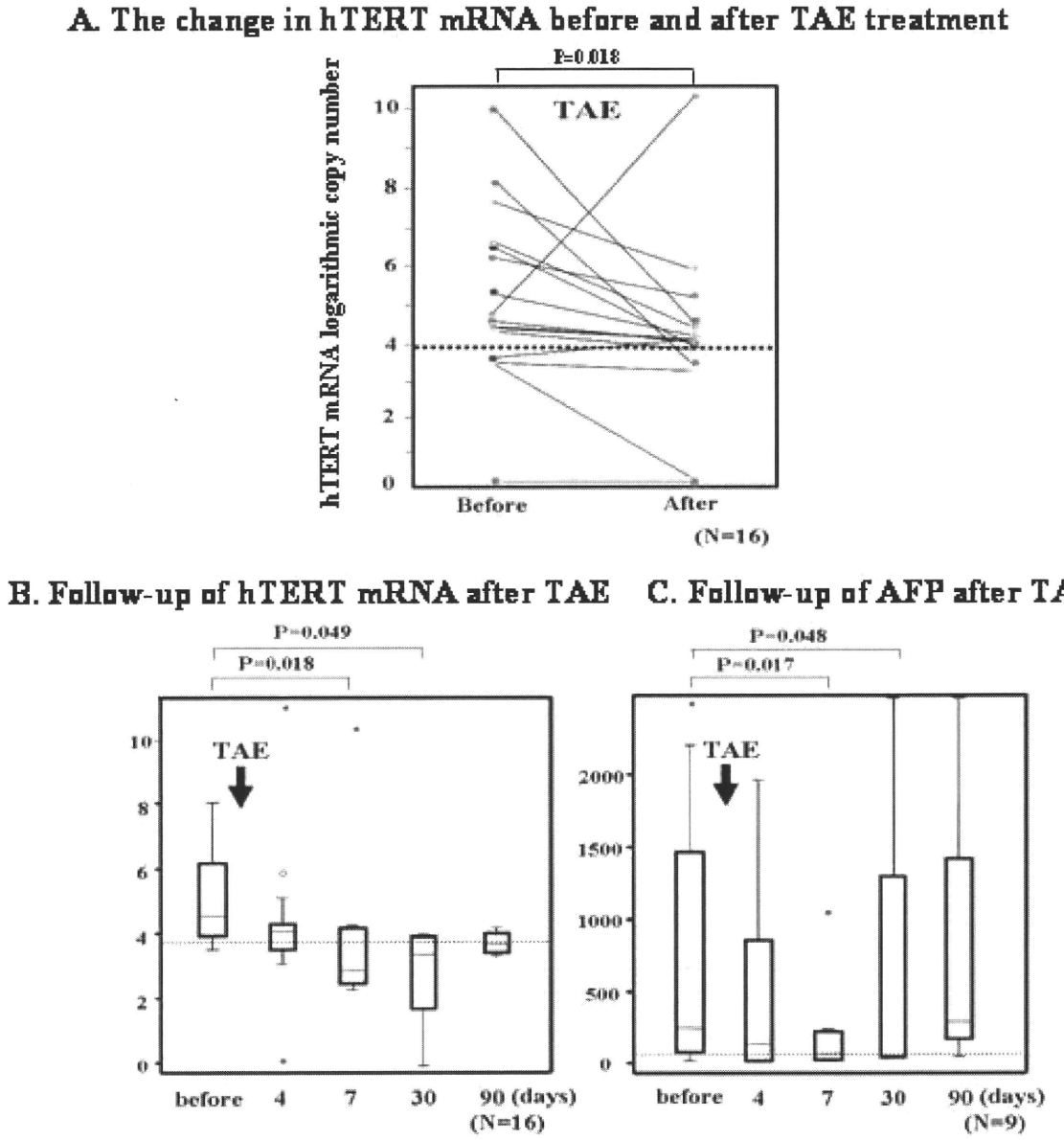

Figure 4 The change of hTERTmRNA before and 7days after TAE. A. Follow-up of serum hTERTmRNA before, 4, 7, 30 and 90 days after TAE. B. Follow-up of serum AFP before, 4, 7, 30 and 90 days after TAE.

sion has not been detected stably in body fluid [12]. In serum, the hTERTmRNA derived from cancer cells seemed to be undetectable because it becomes instable by RNase in blood. Since RNAs in serum are unexpectedly stable within 24 hrs after drawing blood due to particle-associated complex in structure [13,14], it has been suggested that they can be generally detected even in RNase-rich blood. Actually, hTERTmRNA can be detected in serum from breast cancer patients and its maximum sensitivity and specificity are at most 40% and 100%, respectively [4]. The sensitivity in patients with HCC rose to 89.7% in the semi-quantitative assay, and thus compared favorably with the previous findings in which the sensitivity and specificity of AFPmRNA were 69% and 50% for HCC, respectively [31]. Besides, with respect to HCC detection, AFPmRNA was superior to AFP level used routinely in clinic [32]. Recently, in the present study, we reported the sensitivity to detect the nucleotides in blood in the process of RNA extraction, including centrifugation steps less than 1500 × g to remove cellular proteins in serum and a primer set that can detect hTERTmRNA more efficiently than primers in the previous reports (data not shown). We previously reported that hTERT expression was very faint in the

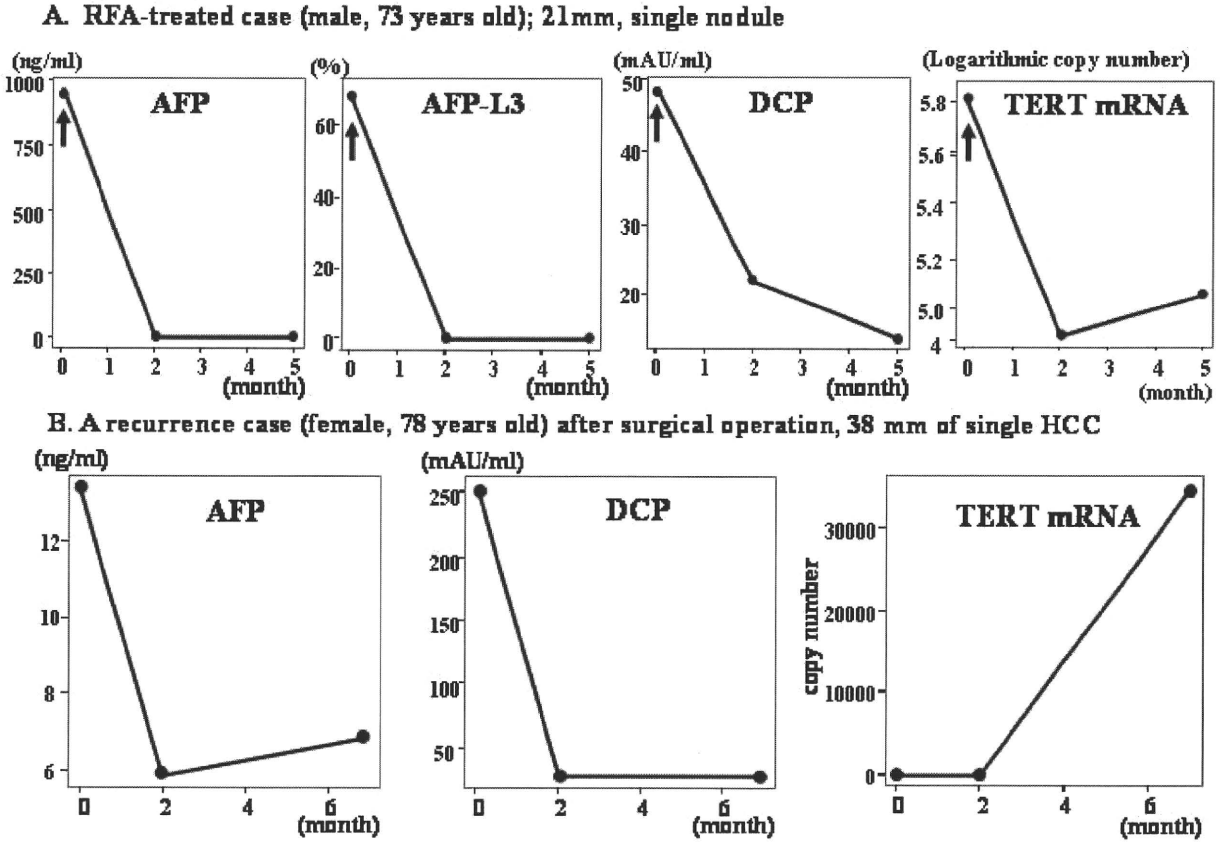

Figure 5 Conventional tumor markers and hTERTmRNA detection during clinical course with therapeutic modalities. A. Changes of AFP, AFP-L3, DCP and hTERTmRNA in RFA-treated case (male, 73 years old), of which HCC was 21 mm-sized and single. B.Changes of AFP, DCP and hTERTmRNA in surgical operation-treated case (female, 78 years old), of which HCC was 38 mm-sized and single.

serum from normal individuals indicating that lymphocytes and circulating normal cells express very low levels of hTERTmRNA [9]. Because hTERTmRNA in lymphocytes is very low, elevated hTERTmRNA levels in serum may mean that hTERTmRNA is derived from cancer cells. Since we could detect negligible amounts of lymphocyte markers after three steps of centrifugation of blood samples, the RNA extraction procedure seemed to remove lymphocytes effectively. In addition, normal or damaged hepatocytes express negligible amounts of hTERT [33,34]. Furthermore, we previously showed the significant correlation of hTERTmRNA expression between tumor tissue and serum [32]. These data suggest that hTERTmRNA detected in serum is derived from tumor cells.

Previously, we reported that qualitative analysis of serum hTERTmRNA was superior to AFP for the purpose of the early detection of HCC, because hTERTmRNA was detectable in HCC patients with normal AFP levels [9]. AFP is being widely used as a reliable marker of HCC not in earlier stage but in the advanced stage [35]. However, in this study, neither AFP was able to distinguish HCC from non-cancerous liver diseases, nor hTERTmRNA was correlated with AFP level ($P = 0.201$), suggesting that quantitative analysis of serum hTERTmRNA was much more sensitive for HCC diagnosis even in the early stage. Because the induction of the abdominal (enhanced-)US, CT, and MRI into the clinical scene enabled us to detect smaller-sized HCC [36], the sensitivity of AFP in the early detection of HCC became less than 70%. Unlike AFP level, AFPmRNA was significantly correlated with hTERTmRNA ($P < 0.001$) and more sensitive than AFP. In the present study, we measured AFP-L3, since AFP-L3 has been reported to be a more HCC-specific marker than AFP [37]. Indeed, the level of AFP-L3 correlated significantly with differentiation and number of HCC although that of AFP was correlated with tumor size and differentiation.

In the present study, of 303 HCC patients, 24 patients were negative below the calculated cut-off value (9,332; 3.97 as logarithmic number) for serum hTERTmRNA. Although the reason why hTERTmRNA was negative in

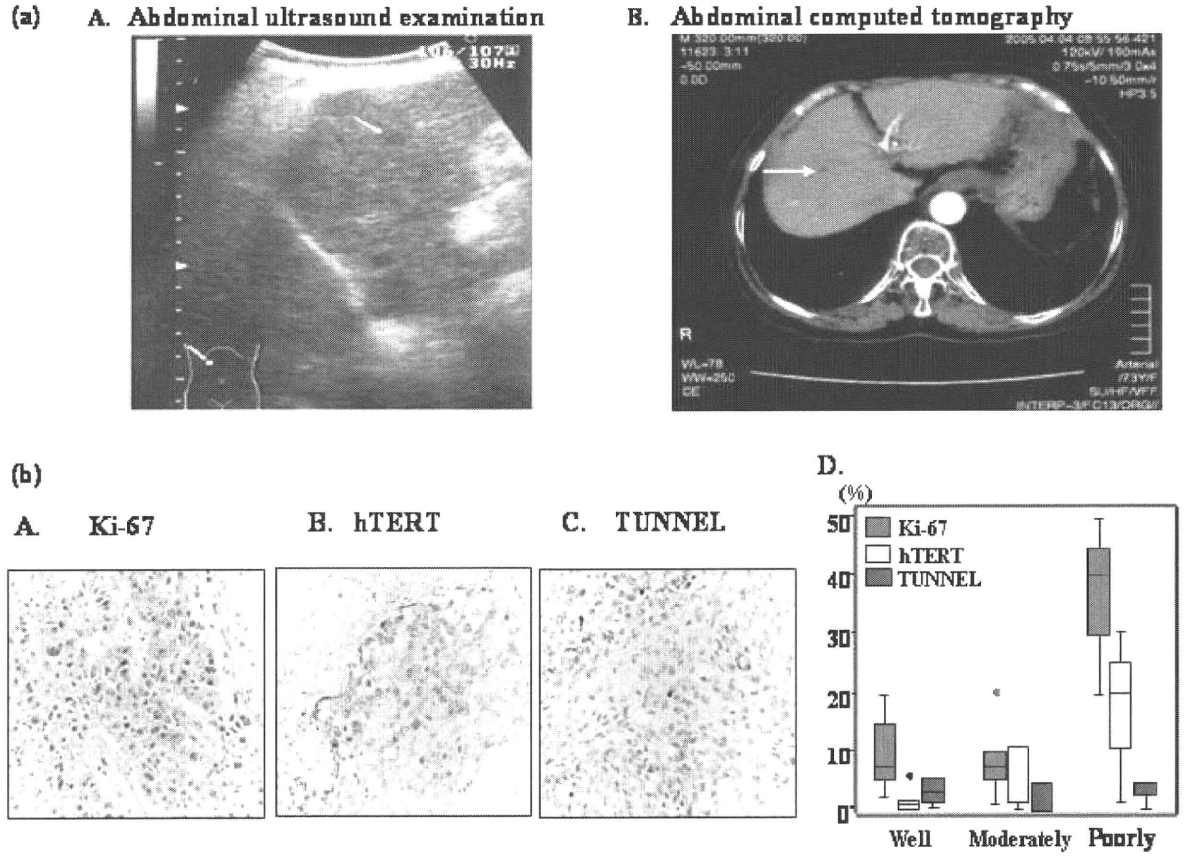

Figure 6 Early detection of small HCC by circulating hTERT mRNA and immunohistochemical analysis of HCC tissues. (a) Imaging diagnosis regarding case with 6 mm HCC. A. Ultrasonography and B. computed tomography of the smallest HCC detected by hTERTmRNA. The diameter of HCC was 6 mm. Left, ultrasonography; right, CT (b) Immunohistochemical analysis of HCC tissues. A. Ki-67 staining (×400), B. hTERT staining (×400), C. TUNEL (×400), D. Labeling indices of Ki-67, hTERT and TUNEL in regard to differential degree of HCC.

these patients is not clear, eleven of 24 hTERTmRNA-negative HCC patients had decompensated liver cirrhosis as the underlying disease. It has been reported that decompensated liver cirrhosis had higher levels of serum TGF-β that promotes apoptosis of immortalized hepatocytes and, in these cases, elevated TGF-β may stimulate apoptosis, resulting in reduction of hTERTmRNA [34,38,39]. hTERT-negative cases had no other common characteristics with age, gender, etiology, child classification etc. than tumor size, ALT, and surrounding lesion. In 23 cases (95.8%), ALT was within 1.5 fold normal limits. In 17 cases (70.8%), surrounding lesion was LC including decompensated situation. Tumor size in 12 cases (50%) was over 30 mm, reflecting on the biological features of cancer itself, as referred in Norton-Simon models regard tumor growth [40]. AFP and DCP were positive in 16 (66.7%) and 11 (45.8%) cases, respectively, suggesting that combinative use of these markers contributes to improve the diagnostic specificity.

Thus, hTERTmRNA is not only improved in both sensitivity and specificity but has a close correlation with tumor size and number in an early stage of HCC. Since HCC repeatedly recurs polyclonally after any treatment as a biological characteristic, the measurement of serum hTERTmRNA makes it possible to recognize recurrence or therapeutic effect in details as well as the usefulness for one-point diagnosis. In this respect, we have to undergo follow-up study after the treatment of HCC [24]. hTERTmRNA expression was closely associated with well to moderate differentiation degree of HCC and was enhanced with the proliferation. We should clarify that serum hTERTmRNA can be detected by what alterations of other molecules during the cancer progression [41-43]. In lower differentiated HCC, tumor cells are proliferating and hTERTmRNA has a tendency to correlate with the differentiation degree and an apoptotic event never reflect on the serum detection of cancer cell-derived mRNAs (Figure 6). Nakashio et al. previously reported the significant correlation of HCC differentiation with

telomerase expression [44]. The results in the present study confirmed their findings. hTERTmRNA showed more sensitivity and specificity compared with AFP-mRNA in HCC patients. However, in liver diseases other than HCC, hTERTmRNA was not correlated with AFP-mRNA. The higher specificity of hTERTmRNA in HCC may be related to fact that AFPmRNA is produced in HCC cells and injured hepatocytes and hTERT is produced mainly in HCC cells. Furthermore, we could detect serum hTERTmRNA expression even in HCC patients with less than 10 mm moderate-differentiated tumor, indicating that hTERT are upregulated during rapid proliferation of tumor at the early phase of oncogenesis, de-differentiation.

Waguri et al. proved that there exist circulating cancer cells derived from original HCC tissues in blood and they can detect hTERTmRNA in blood [45]. The present study suggests that quantification of hTERTmRNAs in serum has diagnostic implications for HCC. Unless apoptosis of cancer cells contributes to the early detection of HCC using serum mRNA, the essence may be immunoreactions [46]. The development of micro vessels may be also involved in the step [47]. We will evaluate the correlation of prognosis with hTERTmRNA and the availability of hTERTmRNA in other cancers by comparison of hTERT-mRNA with other tumor markers [48], and will study its usefulness for inflammatory diseases in which cellular reactions are active [49]. This method depends on RNA stability in each process of RNA purification, storage, and quantification. In the light of its superior positivity to other markers, the assay will be applied for clinical use in the strict condition because it is required to keep the serum RNA as it is in blood and avoid the degradation of RNA quality. Now we are improving RNA stability and PCR condition to better cost/benefit of this assay. In the future, another large-scale study will be required to confirm our results for monitoring HCC and the feasibility for its detection even on a primary care level.

Conclusions

In sum, our results support the suggestion that quantification of circulating hTERTmRNA expression is clinically useful for the early detection of HCC. Furthermore, hTERTmRNA is superior to conventional tumor markers in the diagnosis and recurrence of HCC at the early stage.

Additional material

Additional file 1 TIF ROC curve analysis and AUC in measurement categorized by viruses. ROC curve analysis and AUC in measurement categorized by viruses are demonstrated. Sensitivity/specificity of hTERTmRNA expression in HBV-infected cases is similar to that in HCV-infected cases.

Additional file 2 MS word Positivity of each marker for HCC. Positivity of each marker for HCC was shown, categorized by tumor size.

Additional file 3 TIF Dot blot regarding the correlation of hTERT-mRNA quantification with tumor differentiation. Serum hTERTmRNA quantification in HCC patients (n = 101) diagnosed by liver biopsy was shown, categorized by tumor differentiation. The quantification in serum of HCC patients with well-/moderately-/poorly-/un-differentiation was 4.4 ± 1.4/5.4 ± 2.0/6.3 ± 3.3/5.9 ± 1.8 (mean ± SD).

Abbreviations

(h)TERT: (human) telomerase reverse transcriptase protein; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HBV: hepatitis B virus; LC: liver cirrhosis; CH: chronic hepatitis; AFP: α-fetoprotein; DCP: des-γ-carboxy prothrombin; ALT: alanine aminotransferase; Alb: albumin; CNA: Circulating nucleic acids.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YO analyzed biomedical data and provided blood sample as main researcher in Osaka Red Cross Hospital. MN analyzed biomedical data in Kinki University. MK analyzed biomedical data and provided blood sample as main researcher in Kinki University. KY analyzed biomedical data and provided blood sample in San-in Labor Welfare Hospital. TK analyzed clinical data and the practical analysis in San-in Labor Welfare Hospital. KO analyzed HCC imaging data and the analysis in Saiseikai Gotsu General Hospital. YK was in charge for case study in Saiseikai Gotsu General Hospital. SM analyzed biomedical data and provided blood sample as main researcher in Saiseikai Gotsu General Hospital EN was in charge for case study and biomedical analysis in Saiseikai Gotsu General Hospital. YH analyzed clinical and biomedical data as main researcher in Saiseikai Gotsu General Hospital comprehensively. MK analyzed biomedical data and provided blood sample as main researcher in Matsue City Hospital. SS analyzed biomedical data and provided blood sample as main researcher in Fukuoka University Chikushi Hospital. YH performed biomedical and clinical analysis in surgical case in Tottori University. HK analyzed biomedical data and provided blood sample as chief researcher in San-in Labor Welfare Hospital. JH provided the environment to analyze the data comprehensively. All authors read and approved the final manuscript.

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ORIGINAL PAPER

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Design and rationale for the non-interventional Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and Of its treatment with sorafeNib (GIDEON) study

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SUMMARY

Background: Hepatocellular carcinoma (HCC) is a complicated condition influenced by multiple confounding factors, making optimum patient management extremely challenging. Ethnicity, stage at diagnosis, comorbidities and tumour morphology affect outcomes and vary from region to region, and there is no common language to assess patient prognosis and make treatment recommendations. Despite recent efforts to reduce the incidence of HCC, most patients present with unresectable disease. Non-surgical treatments include ablation, transarterial chemoembolisation and the multikinase inhibitor, sorafenib, but their effects in all patient subgroups are not known and further information is needed to optimise the use of these treatments. **Aims:** The Global Investigation of Therapeutic DEcisions in Hepatocellular Carcinoma and Of its Treatment with SorafeNib (GIDEON) study (ClinicalTrials.gov identifier NCT00812175; http://clinicaltrials.gov/) is an ongoing global, prospective, non-interventional study of patients with unresectable HCC who are eligible for systemic therapy and for whom the decision has been taken to treat with sorafenib under real-life practice conditions. The aim of this study is to evaluate the safety and efficacy of sorafenib in different subgroups, especially Child-Pugh B where data are limited. **Discussion:** This study will recruit 3000 patients from > 40 countries and follow them for approximately 5 years to compile a large and robust database of information that will be used to analyse local, regional and global differences in baseline characteristics, disease aetiology, treatment practice patterns and treatment outcomes, with a view to improve the knowledge base used to guide physician treatment decisions and to improve patient outcomes.

What's known
- HCC is a complex disease influenced by multiple confounding factors that vary from region to region, making optimum patient management extremely complex.
- Sorafenib is an oral multikinase inhibitor with proven efficacy in patients with unresectable HCC, but data in Child-Pugh B are limited.
- There is a need to fully evaluate existing treatments in all patient subgroups to optimise their use.

What's new
- GIDEON will generate data from 3000 patients to evaluate the effects of sorafenib in different patient subgroups, and the resulting large database will be used to analyse local, regional and global differences that influence patient prognosis and management, with a view to refine HCC staging and evaluation and better inform treatment decisions

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, but because of the poor prognosis associated with this disease, it is the third most common cause of cancer-related death (1). Over 80% of patients with HCC are in developing countries, with particularly high incidence rates in sub-Saharan Africa and Southeast Asia (1). There is a low incidence of HCC in developed countries such as the USA, Australia and the UK, but these rates are rising (2).

Risk factors for the development of HCC have been well documented and include the presence of cirrhosis, infection with hepatitis B and C viruses, heavy alcohol intake, diabetes and obesity (1,2). Although surveillance and vaccination programmes have reduced the incidence of HCC in certain populations (3,4), the majority of patients still present with unresectable disease and are unsuitable for surgery. Current treatments for unresectable disease include loco-regional interventions and systemic therapies, although further data on all treatments are required to fully understand their potential, e.g. in patient groups not included in clinical trials.

Non-surgical loco-regional treatment options include ablation therapy and transarterial chemoembolisation (TACE). Ablation therapy is associated with a 5-year survival rate of 40–70%, with best responses seen among patients with single tumours

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and preserved liver function (5). However, TACE is recommended for patients with large/multifocal tumours with no vascular invasion or extra-hepatic spread and is associated with objective response rates of 16–60% (3). Although survival benefits have been reported in only two randomised controlled trials (RCTs) (6,7), a robust meta analysis showed that the treatment with TACE was associated with significant improvements in 2-year survival vs. control (8). However, TACE treatment has a number of important limitations. Residual tumour growth following treatment means that treatment repetition is necessary. Additionally, many of the clinical studies investigating TACE have used a wide range of treatment strategies, including different types of embolic particle, chemotherapy, emulsifying agent and numbers of treatment sessions. For this reason, there is no clear evidence to support an optimum treatment strategy (9). Also, TACE therapy is only possible in patients where the arterial blood supply to the tumour can be isolated, and is not recommended in patients with portal vein thrombosis, those with Child-Pugh C liver function or those with a total serum bilirubin level > 3 mg/ml, as all of these factors have been identified as predictors of poor prognosis in patients treated with TACE (9). Finally, treatment with TACE is associated with considerable side effects; the most commonly reported being postembolisation syndrome that occurs in > 50% of treated patients (3). Other less frequent but more serious complications include hepatic abscess and cholecystitis. Further research is therefore needed to optimise TACE treatment strategy and ensure treatment efforts are directed at patients who will most likely benefit.

Systemic therapies investigated for unresectable HCC have included single-agent and combination chemotherapy regimens, but their efficacy has been disappointing and their use is no longer recommended (9). More recently, the Sorafenib HCC Assessment Randomized Protocol trial, a multicentre, Phase III, double-blind, placebo-controlled trial of 602 Western patients with unresectable HCC, showed that treatment with the oral multikinase inhibitor, sorafenib (Nexavar®; Onyx Pharmaceuticals, Inc., Emeryville, CA, USA; Bayer HealthCare Pharmaceuticals, Inc., Wayne, NJ, USA; Bayer Schering Pharma AG, Berlin, Germany), was associated with a significant improvement in survival compared with placebo [median overall survival (OS) of 10.7 months vs. 7.9 months for sorafenib and placebo respectively, $p < 0.001$] (10). As a result, sorafenib is the first systemic anticancer therapy indicated for treating these patients (9). Similar benefits (median OS of 6.5 months vs. 4.2 months for sorafenib and placebo respectively, $p = 0.014$) were reported in a Phase III, randomised, double-blind, placebo-controlled trial of 226 patients with unresectable HCC from the Asia-Pacific region, thus confirming the efficacy of sorafenib in a broad geographic patient population (11). However, all patients included in these two large RCTs had preserved liver function (Child-Pugh A), and our knowledge regarding the efficacy of sorafenib in patients with hepatic impairment is limited to small subgroups of patients from Phase I and II studies (12,13). Further studies to evaluate the efficacy of sorafenib among all patient groups are therefore needed.

Current treatment guidelines for unresectable disease are therefore based on the best available evidence, including non-randomised trials, case studies and expert opinion; however, significant data gaps exist. Further evidence is needed to fully evaluate current treatment options and optimise their use to improve patient outcomes.

Against this background, the Global Investigation of Therapeutic DEcisions in Hepatocellular Carcinoma and Of its Treatment with SorafeNib (GIDEON) study is an ongoing global, non-interventional study (NIS) of patients with unresectable HCC who are to receive sorafenib as part of their standard clinical care. The study should produce the largest, most robust database of information on factors influencing treatment and outcome of patients with HCC. This manuscript will include details of the GIDEON aims and objectives, study design, target recruitment and timeline. It will also describe the planned analyses and discuss how it is hoped that findings from this study will allow us to gain a detailed understanding of the factors that influence the prognosis and management of these patients, and how this in turn will help us to refine HCC staging and evaluation, better inform treatment choices and ultimately improve outcomes for patients with HCC.

The GIDEON study

Aims, objectives and rationale for GIDEON

Non-interventional studies, or observational studies, are postauthorisation safety studies (PASS) that are usually conducted to gain further information about a licensed product. Observational studies are characterised by the fact that assignment to a particular therapy strategy is not mandated by a study protocol but reflects the participating physician's current practice. The physician alone decides which treatment, if any, is appropriate. In NIS, the decision to include a patient in a study is separate from the treatment decision. Furthermore, no additional diagnostic or monitoring interventions are mandated for the patient as a result of inclusion in an NIS. NIS serve a wide range of purposes but are of particular value

in providing information in wider populations or subgroups not covered in RCTs (14). NIS also enable information to be gathered on other parameters not usually assessed in the clinical trial setting, such as patient acceptance and compliance, physician adherence to information and directions for use and prescription behaviour. The importance of NIS in the literature is increasingly recognised, and guidelines were developed to improve the analysis and reporting of observational studies (15). NIS provide opportunities to enhance the evidence base for established drugs and therapies and increase understanding of the impact of treatments in real-world practice (14).

GIDEON is a global, prospective NIS of patients with unresectable HCC who are candidates for systemic therapy and for whom the decision has been taken to treat with sorafenib. It was initiated to fulfil the postapproval commitment to organisations such as the European Medicines Agency to gather data on the safety and efficacy of sorafenib in patients with Child-Pugh B liver function. Additional goals are to compile a large and robust database of HCC treatment patterns and outcomes among patients with unresectable disease who are candidates for systemic therapy, to answer clinically relevant questions and gain a better understanding of the safety of sorafenib with loco-regional therapies, given either concomitantly or sequentially. Data will also be gathered in the USA and possibly other regions on the characteristics, disease course and treatment outcomes of patients with newly diagnosed HCC or recurring disease after curative treatments, who are not candidates for systemic therapy with sorafenib.

Based on these goals, the primary objective of GIDEON is to evaluate the safety of sorafenib in patients with unresectable HCC in real-life practice conditions. Secondary objectives are to: evaluate the efficacy [OS, progression-free survival (PFS), time to progression (TTP), response rate and stable disease rate] of sorafenib in these patients; determine the duration of therapy according to various patient characteristics; evaluate methods of patient evaluation, diagnosis and follow-up; assess comorbidities and their influence on treatment and outcome in real-life practice rather than a controlled clinical trial setting and evaluate the practice patterns of the physicians involved in the care of these patients.

This study was conducted according to established regulations and recommendations relating to the conduct of NIS; volume 9A of the Rules Governing Medicinal Products in the European Union (16). When required, documented approval from the appropriate ethics committee(s)/institutional review board was obtained for all participating centres prior to the study, according to Good Clinical Practice and local laws, regulations and organisations.

Establishing a global NIS

GIDEON is a Phase IV, international, prospective, open-label, multicentre, non-interventional PASS of patients with unresectable HCC receiving sorafenib under real-life conditions. Approximately, 3000 eligible patients will be recruited by participating physicians from > 40 countries across Europe, Latin America and the Asia-Pacific region and from the USA (Figure 1) and will be observed from the start

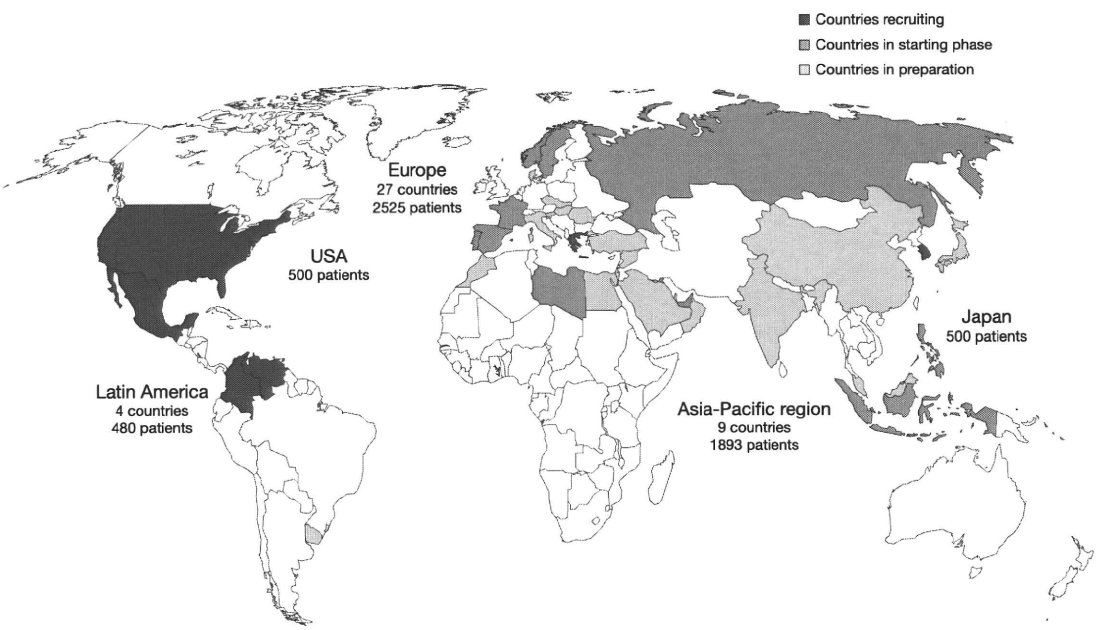


Figure 1 The global reach of GIDEON

of sorafenib therapy to patient withdrawal, loss to follow-up, death or final visit.

An overview of the GIDEON timeline and planned analyses is shown in Figure 2. The first patient's first visit was recorded in January 2009 and the last patient's first visit is anticipated to occur in quarter four of 2012. Interim analyses for safety will be conducted after 500 and 1500 patients have been recruited and followed for 4 months, with the final analysis anticipated in quarter two or three of 2014. The study will end 12 months after enrolment of the 3000th eligible patient, irrespective of whether the final patient dies or not, is lost to follow-up or survives.

Patients to be included in GIDEON

Patients with histologically or cytologically documented or radiographically diagnosed unresectable HCC who are candidates for systemic therapy, and for whom a decision has been made to treat with sorafenib, are eligible for inclusion in GIDEON if they have a life expectancy of > 8 weeks and have provided signed informed consent. Patient exclusion criteria are based on the approved local product information for sorafenib.

Data collection

All data will be collected using case report forms (CRFs). These will be available as paper and electronic versions, with participating countries and their sites able to choose the format of preference. Data will be collected from all enrolled patients at study entry and start of sorafenib, then at intervals normally used by the prescribing physician (estimation: ≥ 6 to ≤ 12 weeks), or until patient death, withdrawal or loss to follow-up, or if significant changes in a patient's disease are observed. All data will be verified through spot site monitoring, which will take place at up to 10% of all sites involved in the study. An overview of the visit schedule and data collected at each visit is shown in Figure 3. Study end-points are summarised in Figure 4. All adverse events (AEs) will be graded according to the National Cancer Institute Common Terminology Criteria version 3.0 (National Cancer Institute, Bethesda, MD, USA), and their likely relationship to sorafenib therapy will be documented. Tumour assessments will be made by computed tomography or other equivalent radiographical method and will be evaluated using the Response Evaluation Criteria in Solid Tumors.

The population of patients who entered this study and received at least one dose of sorafenib will be valid for intent-to-treat safety and efficacy analysis. However, patients who received sorafenib in the past will be excluded from efficacy analysis. All baseline demographic data will be summarised for the intent-to-treat population. AEs and other safety parameters, including blood pressure, Child-Pugh grade and Eastern Cooperative Oncology Group performance status (ECOG PS), will be summarised using the safety population.

Planned subgroup analyses conducted globally, regionally and by country will include: the impact of baseline characteristics on safety, particularly Child-Pugh B; the relationship between baseline characteristics and efficacy; the duration of sorafenib therapy and reasons for discontinuation; the effect of other treatments for HCC on outcome and the impact of different practice patterns on outcome. In addition, subgroup analyses for specific regions may be conducted, such as: an evaluation of common treatments for HCC in Asia; referral and diagnostic patterns in Europe; duration of treatment, tolerability and compliance in Latin America and patient selection for loco-regional therapy in Japan. However, all subgroup analyses performed will depend on the actual data collected.

Statistical considerations for GIDEON

An overall sample size of 3000 patients with unresectable HCC treated with sorafenib is expected to be

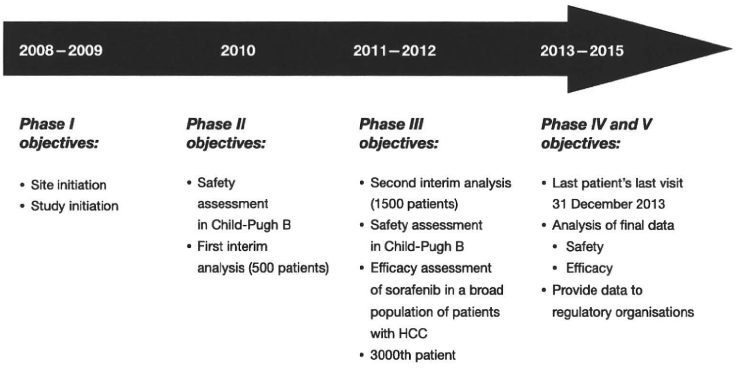
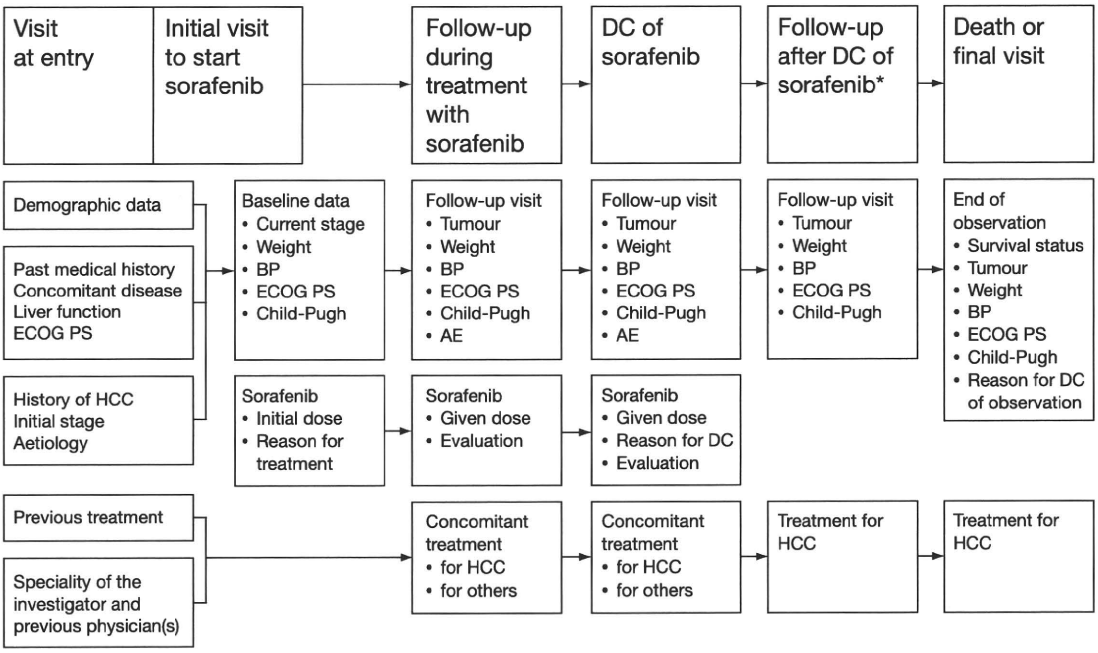
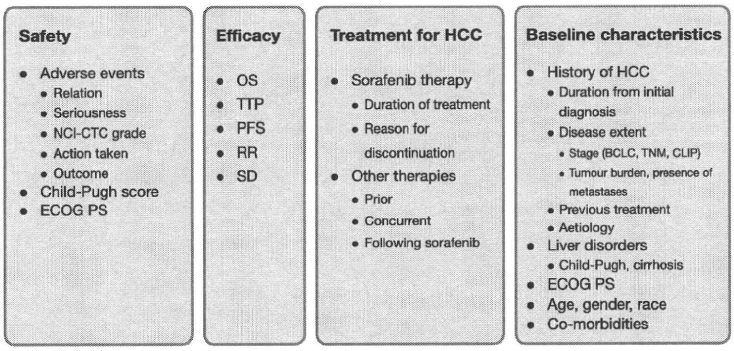


Figure 2 An overview of the GIDEON timeline and planned analyses



*Applicable if patient discontinues therapy, is alive and not lost to follow-up
AE, adverse event; BP, blood pressure; DC, discontinuation;
ECOG PS, Eastern Cooperative Oncology Group performance status; HCC, hepatocellular carcinoma

Figure 3 GIDEON patient assessment schedule



BCLC, Barcelona Clinic Liver Cancer; CLIP, Cancer of the Liver Italian Program; ECOG PS, Eastern Cooperative Oncology Group performance status; HCC, hepatocellular carcinoma; NCI-CTC, National Cancer Institute-Common Toxicity Criteria; OS, overall survival; PFS, progression-free survival; RR, response rate; SD, stable disease; TNM, tumour node metastases; TTP, time to progression

Figure 4 Overview of the GIDEON safety, efficacy, treatment and baseline patient assessments and end-points

sufficient to allow for evaluation of safety of the overall population as well as specific subgroups. With this sample size, there would be an 84% chance of observing an AE with a true incidence of 1% in at least 25 patients.

All baseline, safety and efficacy data were analysed using descriptive statistics. Kaplan–Meier estimates were calculated for the OS, PFS and TTP efficacy end-points. At the time of the analyses, any patient alive or lost to follow-up, without disease progression or death or without documented radiological progression will be censored at the last date of evaluation for OS, PFS and TTP analyses, respectively. Exploratory subgroup analyses of efficacy and safety data may also be performed, stratified by prognostic/predictive baseline factors such as stage, Child-Pugh score, ECOG PS, region and age, as appropriate. Data regarding administration of sorafenib such as duration, given dose, continuation or discontinuation of therapy and dose modification of sorafenib therapy, including reason(s) for discontinuation, will be summarised in a descriptive manner. Treatments for HCC other than sorafenib before, during and after therapy with sorafenib will be summarised descriptively as per available data. The sample size was calculated to collect data to allow for sufficient

evaluation of safety monitoring of all treated patients. Interim analyses are planned during the study primarily for summarising and monitoring safety data, and will be conducted after 500 patients and 1500 patients are enrolled and have been treated on study for at least 4 months.

Discussion

What will GIDEON achieve?

GIDEON is a global PASS initiated to collect more information on the safety and efficacy of sorafenib in patients with unresectable HCC. This is the largest prospective HCC NIS in the world, which will enrol 3000 patients from > 40 countries globally. The compilation of a database of this size in HCC has not previously been undertaken; as no other global registries exist in this area, it is anticipated that the data collected here will be an important contribution to our knowledge and will help to answer important and clinically relevant questions relating to the natural disease course of HCC and liver dysfunction, long-term efficacy and safety of sorafenib therapy, physicians' practice patterns and patients' perspectives.

The number of factors influencing HCC and its disease course make it extremely difficult to accurately assess patient prognosis and make optimum treatment recommendations. The geographical variation of these factors has also prevented the establishment of a universal system to assess all patients. Findings from GIDEON could help to establish a globally applicable staging classification, which could facilitate the accurate and consistent assessment of all patients and help to provide a common language for the broad HCC multidisciplinary team on which to base treatment recommendations. In addition, as GIDEON will collect data regarding the differences in physician treatment practice patterns and outcomes, it may be possible to analyse these data with a view to optimise the role of all members of this large multidisciplinary team and streamline patient care.

Although potentially curative therapy via surgical resection or transplant is possible for some patients with HCC, there is a lack of cadaveric transplants available, and the majority of patients are unsuitable for surgery at presentation. In addition, the recurrence rate of HCC after curative treatment is high and the long-term curable rate is low (17). Thus, non-surgical treatments play a central role in the management of these patients. However, there is still a relative shortage of RCTs to fully evaluate these treatments in all patient subgroups, and more work is needed to fully understand the benefits of each of these treatments and to establish their place in the HCC treatment armamentarium. One of the main focuses of GIDEON is to gain further information on the optimum duration of sorafenib therapy as well as the safety and efficacy of sorafenib in different subgroups of patients, especially patients who are generally excluded from RCTs to minimise errors or confounding factors in the study, i.e. patients who have moderate liver dysfunction (Child-Pugh B) where data are currently limited. However, information is also being gathered regarding the safety and outcomes following other treatments before, during and after sorafenib therapy; thus, it is anticipated that the information gathered in this study will help to improve our understanding of the risks and benefits associated with each of these treatment approaches, which could help us to establish an optimum treatment algorithm for these patients.

In addition to GIDEON, a large clinical trial programme for sorafenib is ongoing that should help us to fully establish its optimum place in therapy. One area of interest is the role of sorafenib as adjuvant therapy to improve survival of patients with HCC. To date, treatments such as radiotherapy and chemotherapy and their combination have been used to reduce tumour size and improve patients' quality of life (18). TACE has recently been shown to be the only palliative treatment that can benefit HCC patients ineligible for curative treatments because of advanced tumour stage or poor hepatic functional reserve; however, the survival gain appears marginal (19) and other effective treatments are urgently needed. Against this background, a large Phase III, randomised, double-blind, placebo-controlled trial of adjuvant sorafenib following either surgical resection or local ablation is currently in progress (STORM study) (20). The primary end-point is recurrence-free survival, with secondary end-points including time to recurrence and OS. Estimated accrual to this trial is 1100 patients and data are due to be reported in 2014.

Another area of interest is the efficacy of sorafenib in combination with, and subsequent to, TACE therapy. A large randomised Phase II trial has been initiated to evaluate the role of sorafenib in combination with TACE in the treatment of patients with intermediate disease (SPACE study) (21). Estimated enrolment to this trial is 350 patients, and final results are expected in late 2010. In addition to this, a Phase III, double-blind, randomised, placebo-controlled trial of sorafenib following TACE in Japanese patients with unresectable, advanced disease is ongoing (Japan post-TACE study) (22). The target recruitment of 414 patients has already been reached and final results are anticipated in early 2010.

Finally, the effects of sorafenib in combination with other targeted agents in HCC are also of

interest, and a large Phase III, randomised, double-blind trial evaluating the efficacy, safety and health-related quality of life of sorafenib plus erlotinib vs. sorafenib plus placebo for the first-line treatment of advanced HCC is in progress (SEARCH study) (23). The target recruitment for this study is 700 patients and the estimated final data collection date is July 2011.

These ongoing studies form a comprehensive and well-integrated clinical trial programme, which will provide data from several points in the treatment pathway. GIDEON is also a key component of this programme, as it will provide data from the entire unresectable patient population treated under real-life conditions, not in a non-restrictive setting and within the approved indication, and will enable the collection of data from populations not commonly included in RCTs, such as those with Child-Pugh B liver function. Thus, in addition to the ongoing interventional studies, the information collected in GIDEON will significantly contribute to the current body of evidence, which helps inform treatment decisions.

What are the limitations of GIDEON?

The information gathered in GIDEON will form a large and robust database that will be analysed to improve our understanding of the global, regional and local differences in patient demographics, disease course and treatment outcomes, with a view to improve our knowledge base and improve patient outcomes. However, as this is an observational NIS, it is associated with a number of limitations. The lack of randomisation to specific treatment arms and the lack of a placebo-control arm will limit any robust evaluation of the efficacy of any of the treatments received by these patients during the course of the study. Comparisons between sorafenib-treated and untreated patients in the USA will be limited because of the small sample size of patients in the USA who will not receive sorafenib. The value of some subgroup analyses may also be limited by small patient numbers, although these analyses may still be hypothesis-generating and could help direct future research. However, given these limitations, it will be important to consider findings from GIDEON together with emerging data from large RCTs, to fully evaluate the safety and efficacy of these treatments and draw any definitive conclusions.

Another possible limitation in GIDEON is that all data will be collected via the completion and submission of CRFs, which could delay data collection and evaluation. However, electronic versions of the CRF have been compiled with a view to facilitate this process and to reduce any lengthy delays in completing and analysing the data collected.

As with any observational study, a number of biases may also exist. The lack of blinding of either the treating physician or the patient to study treatment may introduce a bias in reporting treatment outcomes. It is also possible that physicians engaged in GIDEON may be more likely to choose sorafenib therapy for a larger proportion of their patients than would be representative of normal treatment practice patterns in that area, although all patients receiving sorafenib therapy must be eligible according to the locally approved product information for sorafenib. Finally, while study procedures regarding data collection and verification are in place for GIDEON, it should be noted that in an NIS, potential exists for less robust data than might be expected from an RCT.

Conclusions

GIDEON is the largest global, prospective, open-label NIS ever conducted among patients with unresectable HCC. The study was initiated to further evaluate the safety and efficacy of sorafenib in different patient subgroups, including Child-Pugh B. The collection of information regarding patient baseline demographics, disease aetiology, treatments and outcomes from approximately 3000 patients worldwide, over a period of approximately 5 years, will also enable the compilation of a large and robust database that will be used to analyse local, regional and global differences, with a view to answer some important questions and fill significant data gaps. It is therefore anticipated that findings from GIDEON, together with data from large RCTs, will help improve the knowledge base used to guide physician treatment decisions and may enable physicians to make better informed treatment choices and ultimately improve patient outcomes.

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Author contributions

All authors are members of the Global Steering Committee for the GIDEON study and have been involved in the discussion and modification of the GIDEON protocol. All authors provided critical review of the manuscript and approved the final version for publication.

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REVIEW ARTICLE

Current status of molecularly targeted therapy for hepatocellular carcinoma: clinical practice

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Abstract In recent years, molecular-targeted agents have been used clinically to treat various malignant tumors. In May 2009, sorafenib (Nexavar®) was approved in Japan for "unresectable hepatocellular carcinoma (HCC)", and was the first molecular-targeted agent for use in liver cancer. To date, sorafenib is the only molecular-targeted agent whose survival benefit has been demonstrated in two global phase III randomized controlled trials, and it has now been approved worldwide. Phase III clinical trials are now underway to compare other molecular-targeted agents with sorafenib as first-line treatment agents, and to evaluate other multi-kinase inhibitors of the vascular endothelial growth factor and platelet-derived growth factor receptors, as well as drugs targeting the epidermal growth factor receptor, insulin-like growth factor receptor, and mammalian target of rapamycin, in addition to other molecules targeting other components of the signal transduction pathways. This review outlines the main pathways involved in the development and progression of HCC and the agents that target these pathways.

Keywords Hepatocellular carcinoma · Molecular-targeted agent · Sorafenib · Sunitinib · Brivanib · Complete remission

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Introduction

Advances in molecular cell biology over the last decade have clarified the mechanisms involved in cancer growth, invasion, and metastasis, and enabled the development of molecular-targeted agents, best represented by trastuzumab for breast cancer, imatinib and rituximab for hematopoietic tumors, and gefitinib and erlotinib for lung cancer. These molecular-targeted agents are broadly classified into two categories: drugs targeting cancer cell-specific molecules and nonspecific molecular-targeted drugs for molecular biological abnormalities induced in the host stroma or blood vessels by the presence of cancer. Examples of the former approach include: trastuzumab, which targets HER2, the expression of which is a poor prognostic factor for breast cancer; rituximab, which is used to treat B cell lymphoma, and targets CD20 expressed on normal and neoplastic mature B cells; and imatinib, which binds to the ATP-binding site of Bcr-abl, a protein that causes chronic myelogenous leukemia. However, no critical target molecules responsible for treatment response have been identified in hepatocellular carcinoma (HCC).

In recent years, clinical trials have been conducted for many agents that act on growth factor receptors (for example epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR)) and intracellular signaling pathways. In addition, multi-kinase inhibitors, including sorafenib, have emerged and have been evaluated. Clinical trials are now ongoing to compare drugs with the same mechanism of action and to test the combined efficacy and relative merits of these drugs with existing drugs for many cancers. Because the main treatment option for metastatic, advanced stage cancers, for example breast and colorectal cancer, is systemic chemotherapy, clinical trials are ongoing to investigate how to

combine molecular-targeted agents with standard therapies based on the results of long-term, large-scale clinical trials, and to identify which molecular-targeted agents should be used as initial or second-line therapy. However, for HCC, background liver damage limits the indication for systemic chemotherapy and no anti-cancer drugs were found to be effective in a large-scale randomized controlled trial (RCT). However, now that the usefulness of sorafenib has been demonstrated in clinical trials, the development of drugs that are effective for poor-prognosis advanced HCC with distant metastasis and vascular invasion is eagerly awaited.

Signaling pathways and molecular-targeted agents in HCC

As in other cancers, the molecular mechanisms involved in the development and progression of HCC are complex. It has been shown that, after HBV/HCV infection and alcohol or aflatoxin B1 exposure, genetic and epigenetic changes occur, including oncogene activation and tumor-suppressor gene inactivation, because of an inflammation-induced increase in hepatocyte turnover and oxidative stress-induced DNA damage. Through apoptosis and cell proliferation, these changes lead to the multistep development and progression of a hyperplastic to dysplastic nodule, early HCC, and advanced HCC. A number of studies have reported changes in gene expression, chromosomal amplification, mutations, deletions and copy number alterations (gain/loss), somatic mutations, CpG hypermethylation, DNA hypomethylation, and molecular abnormalities, which can constitute therapeutic targets [1–5].

The binding of growth factors to their receptor proteins activates protein-phosphorylating enzymes, thus activating a cascade of proliferative signaling pathways to transmit proliferative signals into the nucleus. Growth factors, for example epidermal growth factor (EGF), transforming growth factor (TGF)-α/β, insulin-like growth factor (IGF), and vascular endothelial growth factor (VEGF), also function in liver regeneration after injury, whereas fibroblast growth factor (FGF) and the platelet-derived growth factor (PDGF) family are involved in liver fibrosis and HCC growth [6–8]. The receptors for these growth factors are broadly classified into G-protein-coupled receptors and protein kinases. On ligand binding, these receptors activate their downstream intracellular molecules in a cascade fashion. Many of the growth factor receptors and oncogenes have tyrosine kinase activity, and the tyrosine kinases are classified into transmembrane receptor tyrosine kinases such as the EGFR and VEGFR, and cytoplasmic non-receptor tyrosine kinases such as Abl and Src. On the other hand, Raf, MAP kinase/ERK kinase (MEK), and mammalian target of rapamycin (mTOR) are serine/threonine kinases.

In general, the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/Akt/mTOR, c-MET, IGF, Wnt-β-catenin, and hedgehog signaling pathways, and the VEGFR and PDGFR signaling cascades show altered activity in HCC, and agents targeting these pathways are under development (Fig. 1, Table 1) [9]. Many molecular-targeted agents are now under development; the target signaling pathways and growth factors are outlined below.

MAPK pathway (RAS/RAF/MEK/ERK)

The MAPK intracellular signaling pathway, which is mainly involved in cell growth and survival, and regulates cell differentiation, is upregulated in cancer cells. Therefore, this pathway has been extensively studied as a therapeutic target. The MAPK pathway is a common downstream pathway for the EGFR, PDGFR, and VEGFR, and is universally used for signal transduction downstream of cytokine receptors, integrin complexes, and G-protein receptors to Ras. The MAPK pathway also plays an important role in HCC in that its activation is reportedly involved in HCC growth and survival [5]. The downstream extracellular signaling-regulated kinase (ERK) is activated by two upstream protein kinases, which are coupled to growth factor receptors by Ras proteins. Ras, which is activated by ligand binding, activates Raf serine/threonine kinases and MEK (MAP kinase/ERK kinase), whereas MEK phosphorylates and activates ERK, which phosphorylates proteins involved in cell growth, apoptosis resistance, extracellular matrix production, and angiogenesis [10–13].

Raf and Ras inhibitors

Raf and Ras are proto-oncogenes. In particular, K-Ras mutations are commonly observed in many cancers, including pancreatic and colorectal cancers. One study reported that 30% of HCCs have Ras mutations [14]. To our knowledge, no agents targeting Ras are planned to enter clinical trials in the near future. However, because the binding of Ras protein to the cell membrane and its functional activation require farnesylation, several farnesyltransferase inhibitors are being tested for Ras-related tumors. In addition, vaccine therapy for mutant Ras proteins is currently being tested for solid cancers, including HCC.

The Raf family consists of three isoforms, A-Raf, B-Raf, and C-Raf/Raf-1. Genetic abnormalities, for example point mutations and gene rearrangements, have been reported in various cancers [15]; however, in HCC, *ras/raf* mutations