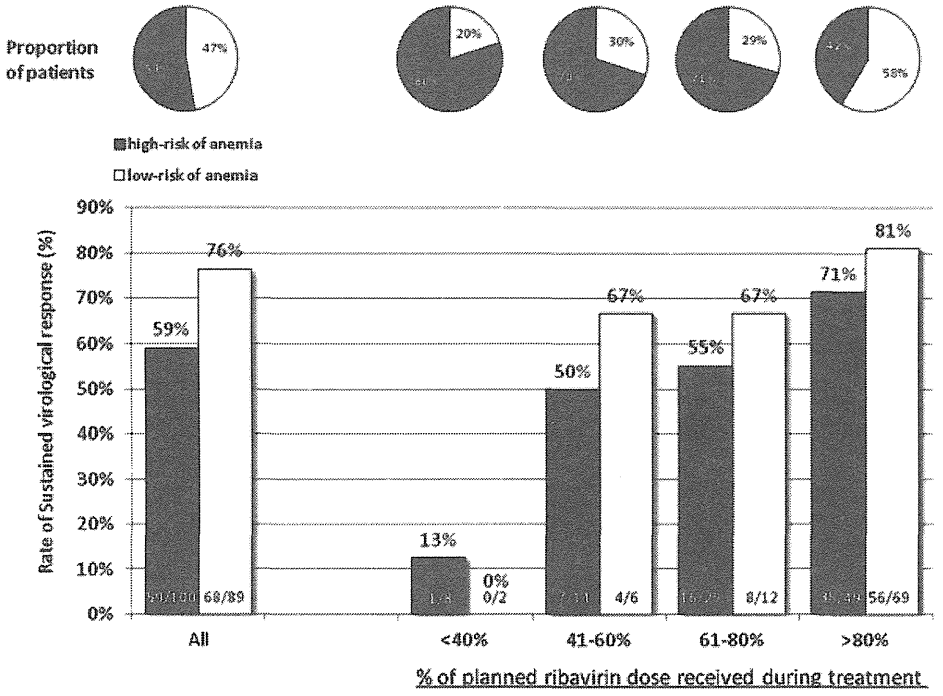

Fig. 4. The impact of risk of anemia and RBV dose on treatment outcome after a complete early virological response. Patients with complete early virological responses were divided into subgroups according to their adherence to RBV: ≤40%, 41–60%, 61–80%, and >80%. For each subgroup, the proportion of patients with a high risk and a low risk of anemia is shown in the upper panel by pie charts, and the rates of sustained virological responses, stratified by high risk and low risk of anemia, are shown in the lower panel by bar graphs. The black and white bars or charts represent patients with high and low risks of anemia, respectively.

high risk of anemia to extend the duration of treatment, even those patients with a complete early virological response, to obtain >80% adherence to RBV.

In conclusion, the combination of the *ITPA* genotype, baseline Hb concentration, and baseline CLcr could be used as a pre-treatment predictor of anemia. The risk of anemia thus identified is associated with adherence to RBV and impacts on the treatment outcome of patients who achieve a complete early virological response. This is in contrast to the major role of the *IL28B* genotype in the prediction of sustained virological responses at baseline and among non-responders at weeks 4 and 12. Patients who achieve a complete early virological response generally have a high probability of a sustained virological response but those who have a high risk of anemia have a high rate of relapse because of reduced adherence to RBV. To improve the rate of sustained virological responses in these patients, it may be postulated that the treatment schedule may be personalized to obtain >80% adherence to RBV. Clearly, this postulate needs to be confirmed in a future study.

REFERENCES

Averbook BJ, Fu P, Rao JS, Mansour EG. 2002. A long-term analysis of 1018 patients with melanoma by classic Cox regression and tree-structured survival analysis at a major referral center: Implications on the future of cancer staging. Surgery 132:589–602.

Azakami T, Hayes CN, Sezaki H, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, Miki D, Tsuge M, Imamura M, Kawakami Y, Takahashi S, Ochi H, Nakamura Y, Kamatani N, Chayama K. 2011. Common genetic polymorphism of ITPA gene affects ribavirin-induced anemia and effect of peg-interferon plus ribavirin therapy. J Med Virol 83:1048–1057.

Baquerizo A, Anselmo D, Shackleton C, Chen TW, Cao C, Weaver M, Gornbein J, Geevarghese S, Nissen N, Farmer D, Demetriou A, Busuttil RW. 2003. Phosphorus ans an early predictive factor in patients with acute liver failure. Transplantation 75:2007–2014.

Breiman LJH, Friedman RA, Olshen CJ, Stone CM. 1980. Classification and regression trees. Calif: Wadsworth.

Cockcroft DW, Gault MH. 1976. Prediction of creatinine clearance from serum creatinine. Nephron 16:31–41.

Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. 2003. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 38:645–652.

Fellay J, Thompson AJ, Ge D, Gumbs CE, Urban TJ, Shianna KV, Little LD, Qiu P, Bertelsen AH, Watson M, Warner A, Muir AJ, Brass C, Albrecht J, Sulkowski M, McHutchison JG, Goldstein DB. 2010. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. Nature 464: 405–408.

Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. 2002. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 347:975–982.

Garzotto M, Park Y, Mongoue-Tchokote S, Bledsoe J, Peters L, Blank BH, Austin D, Beer TM, Mori M. 2005. Recursive partitioning for

risk stratification in men undergoing repeat prostate biopsies. Cancer 104:1911–1917.

Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461:399–401.

Hiramatsu N, Kurashige N, Oze T, Takehara T, Tamura S, Kasahara A, Oshita M, Katayama K, Yoshihara H, Imai Y, Kato M, Kawata S, Tsubouchi H, Kumada H, Okanoue T, Kakumu S, Hayashi N. 2008. Early decline of hemoglobin can predict progression of hemolytic anemia during pegylated interferon and ribavirin combination therapy in patients with chronic hepatitis C. Hepatol Res 38:52–59.

Hiramatsu N, Oze T, Yakushijin T, Inoue Y, Igura T, Mochizuki K, Imanaka K, Kaneko A, Oshita M, Hagiwara H, Mita E, Nagase T, Ito T, Inui Y, Hijioka T, Katayama K, Tamura S, Yoshihara H, Imai Y, Kato M, Yoshida Y, Tatsumi T, Ohkawa K, Kiso S, Kanto T, Kasahara A, Takehara T, Hayashi N. 2009. Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin. J Viral Hepat 16: 586–594.

Hiramatsu N, Kurosaki M, Sakamoto N, Iwasaki M, Sakamoto M, Suzuki Y, Sugauchi F, Tamori A, Kakinuma S, Matsuura K, Izumi N. 2011. Pretreatment prediction of anemia progression by pegylated interferon alpha-2b plus ribavirin combination therapy in chronic hepatitis C infection: Decision-tree analysis. J Gastroenterol 46:1111–1119.

Kim RW. 2002. Global epidemiology and burden of hepatitis C. Microbes Infect 4:1219–1225.

Kurosaki M, Matsunaga K, Hirayama I, Tanaka T, Sato M, Yasui Y, Tamaki N, Hosokawa T, Ueda K, Tsuchiya K, Nakanishi H, Ikeda H, Itakura J, Takahashi Y, Asahina Y, Higaki M, Enomoto N, Izumi N. 2010. A predictive model of response to peginterferon ribavirin in chronic hepatitis C using classification and regression tree analysis. Hepatol Res 40:251–260.

Kurosaki M, Sakamoto N, Iwasaki M, Sakamoto M, Suzuki Y, Hiramatsu N, Sugauchi F, Tamori A, Nakagawa M, Izumi N. 2011a. Sequences in the interferon sensitivity-determining region and core region of hepatitis C virus impact pretreatment prediction of response to PEG-interferon plus ribavirin: Data mining analysis. J Med Virol 83:445–452.

Kurosaki M, Sakamoto N, Iwasaki M, Sakamoto M, Suzuki Y, Hiramatsu N, Sugauchi F, Yatsuhashi H, Izumi N. 2011b. Pretreatment prediction of response to peginterferon plus ribavirin therapy in genotype 1 chronic hepatitis C using data mining analysis. J Gastroenterol 46:401–409.

Kurosaki M, Tanaka Y, Nishida N, Sakamoto N, Enomoto N, Honda M, Sugiyama M, Matsuura K, Sugauchi F, Asahina Y, Nakagawa M, Watanabe M, Sakamoto M, Maekawa S, Sakai A, Kaneko S, Ito K, Masaki N, Tokunaga K, Izumi N, Mizokami M. 2011c. Pretreatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in IL28B and viral factors. J Hepatol 54:439–448.

Kurosaki M, Tanaka Y, Tanaka K, Suzuki Y, Hoshioka Y, Tamaki N, Kato T, Yasui Y, Hosokawa T, Ueda K, Tsuchiya K, Kuzuya T, Nakanishi H, Itakura J, Takahashi Y, Asahina Y, Matsuura K, Sugauchi F, Enomoto N, Nishida N, Tokunaga K, Mizokami M, Izumi N. 2011d. Relationship between genetic polymorphisms of the inosine triphosphatase gene and anemia or outcome after treatment with pegylated-interferon and ribavirin. Antivir Ther 16:685–694.

Kurosaki M, Hiramatsu N, Sakamoto M, Suzuki Y, Iwasaki M, Tamori A, Matsuura K, Kakinuma S, Sugauchi F, Sakamoto N, Nakagawa M, Yatsuhashi H, Izumi N. 2012. Age and total ribavirin dose is an independent predictor of relapse among early virological responders to peg-interferon plus ribavirin therapy in chronic hepatitis C revealed by data mining analysis. Antivir Ther 17:35–43.

Kwo PY, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, Galati JS, Gordon SC, Ravendhran N, Rossaro L, Anderson FH, Jacobson IM, Rubin R, Koury K, Pedicone LD, Brass CA, Chaudhri E, Albrecht JK. 2010. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): An open-label, randomised, multicentre phase 2 trial. Lancet 376:705–716.

LeBlanc M, Crowley J. 1995. A review of tree-based prognostic models. Cancer Treat Res 75:113–124.

Leiter U, Buettner PG, Eigentler TK, Garbe C. 2004. Prognostic factors of thin cutaneous melanoma: An analysis of the Central Malignant Melanoma Registry of the German Dermatological Society. J Clin Oncol 22:3660–3667.

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. Lancet 358:958–965.

McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK. 2002. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 123:1061–1069.

McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ. 2009. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 360:1827–1838.

Miyaki K, Takei I, Watanabe K, Nakashima H, Omae K. 2002. Novel statistical classification model of type 2 diabetes mellitus patients for tailor-made prevention using data mining algorithm. J Epidemiol 12:243–248.

Ochi H, Maekawa T, Abe H, Hayashida Y, Nakano R, Kubo M, Tsunoda T, Hayes CN, Kumada H, Nakamura Y, Chayama K. 2010. Inosine triphosphate pyrophosphatase polymorphism affects ribavirin-induced anemia and outcomes of therapy—A genome-wide study of Japanese hepatitis C virus patients. Gastroenterology 139:1190–1197.

Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Gunthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Mullhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY. 2010. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: A genome-wide association study. Gastroenterology 138:1338–1345.

Sakamoto N, Tanaka Y, Nakagawa M, Yatsuhashi H, Nishiguchi S, Enomoto N, Azuma S, Nishimura-Sakurai Y, Kakinuma S, Nishida N, Tokunaga K, Honda M, Ito K, Mizokami M, Watanabe M. 2010. ITPA gene variant protects against anemia induced by pegylated interferon-alpha and ribavirin therapy for Japanese patients with chronic hepatitis C. Hepatol Res 40: 1063–1071.

Seto WK, Tanaka Y, Liu K, Lai CL, Yuen MF. 2011. The effects of IL-28B and ITPA polymorphisms on treatment of hepatitis C virus genotype 6. Am J Gastroenterol 106:1007–1008.

Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Muller T, Bahlo M, Stewart GJ, Booth DR, George J. 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41:1100–1104.

Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41:1105–1109.

Tanaka Y, Kurosaki M, Nishida N, Sugiyama M, Matsuura K, Sakamoto N, Enomoto N, Yatsuhashi H, Nishiguchi S, Hino K, Hige S, Itoh Y, Tanaka E, Mochida S, Honda M, Hiasa Y, Koike A, Sugauchi F, Kaneko S, Izumi N, Tokunaga K, Mizokami M. 2011. Genome-wide association study identified ITPA/DDRGK1 variants reflecting thrombocytopenia in pegylated interferon and ribavirin therapy for chronic hepatitis C. Hum Mol Genet 20:3507–3516.

Thompson AJ, Fellay J, Patel K, Tillmann HL, Naggie S, Ge D, Urban TJ, Shianna KV, Muir AJ, Fried MW, Afdhal NH, Goldstein DB, McHutchison JG. 2010a. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. Gastroenterology 139:1181–1189.

Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F,

Lawitz EJ, McCone J, Shiffman ML, Galler GW, Lee WM, Reindollar R, King JW, Kwo PY, Ghalib RH, Freilich B, Nyberg LM, Zeuzem S, Poynard T, Vock DM, Pieper KS, Patel K, Tillmann HL, Noviello S, Koury K, Pedicone LD, Brass CA, Albrecht JK, Goldstein DB, McHutchison JG. 2010b. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology 139:120–129 e118.

Thompson AJ, Santoro R, Piazzolla V, Clark PJ, Naggie S, Tillmann HL, Patel K, Muir AJ, Shianna KV, Mottola L, Petruzzellis D, Romano M, Sogari F, Facciorusso D, Goldstein DB, McHutchison JG, Mangia A. 2011. Inosine triphosphatase genetic variants are protective against anemia during antiviral therapy for HCV2/3 but do not decrease dose reductions of RBV or increase SVR. Hepatology 53:389–395.

Valera VA, Walter BA, Yokoyama N, Koyama Y, Iiai T, Okamoto H, Hatakeyama K. 2007. Prognostic groups in colorectal carcinoma patients based on tumor cell proliferation and classification and regression tree (CART) survival analysis. Ann Surg Oncol 14:34–40.

Zlobec I, Steele R, Nigam N, Compton CC. 2005. A predictive model of rectal tumor response to preoperative radiotherapy using classification and regression tree methods. Clin Cancer Res 11:5440–5443.

ISSN 1386-6346

Hepatology Research

JSH

Volume 42 Issue 3 March 2012

The Official Journal of the Japan Society of Hepatology

wileyonlinelibrary.com/journal/hepr

WILEY-BLACKWELL

Hepatology Research

Volume 24, Issue 3, March 2002

Hepatology Research 2012; 42: 226–232 doi: 10.1111/j.1872-034X.2011.00922.x

Review Article

Prediction and prevention of intrahepatic recurrence of hepatocellular carcinoma

Namiki Izumi

Department of Gastroenterology and Hepatology, Musashino Red-Cross Hospital, Musashinoshi, Tokyo, Japan

Hepatocellular carcinoma (HCC) has characteristic features of the coexistence of two life-threatening conditions, cancer and cirrhosis, which makes prognostic assessment difficult. In addition, the high rate of intrahepatic recurrence is a key feature that correlates with poor prognosis and its prevention is an issue for urgent investigation. Gene expression in the tumor and adjacent liver tissue for the prediction of intrahepatic recurrence of HCC has been extensively investigated. Among them, the expression of progenitor cell feature markers in the cancer cells such as epidermal cell adhesion molecule (EpCAM), cytokeratin 19 (CK19) and CD 133 have been shown to be associated with intrahepatic recurrence of HCC. Gene expression patterns from adjacent tissues were shown to predict early and overall recurrence in patients with HCC. Insulin resistance should be included in the analysis for the prevention of recurrence. To suppress or eradicate the replication of hepatitis B and C virus must be the most important issue for prevention. Supplementation by branched chain amino acid-enrichment and administration of vitamin K, acyclic retinoid and chemotherapeutic agents have been examined. There is an urgent need to develop a predictive tool and an effective treatment for prevention. It would be extremely valuable to find a useful biomarker for prediction and to develop new molecular targeting agents for the prevention of HCC recurrence in the near future.

Key words: acyclic retinoid, CD 133, epidermal cell adhesion molecule, hepatocellular carcinoma, CK19, progenitor cell markers, vitamin K2

INTRODUCTION

HEPATOCELLULAR CARCINOMA IS the fourth leading cause of death from malignant neoplastic disease in Japan, and improvement of the prognosis is an important issue to be solved.[1] Because HCC develops from hepatitis B or C virus infection in more than 80% of patients, unlike most solid tumors, the coexistence of two life-threatening conditions, cancer and cirrhosis, makes prognostic assessments difficult. In addition, the high rate of intrahepatic recurrence is a key feature that correlates with poor prognosis, and its prevention is an issue for urgent investigation. The long-term prognosis after surgical resection of HCC remains unsatisfactory compared with other common human cancers because of the high rate of recurrence and lack of effective adjuvant therapy. Poon reported from an extensive analysis of the published reports that the 5-year recurrence rate is more than 70% and this is the main cause of long-term death rather than the underlying cirrhosis.[2] Gene signatures from the tumor and adjacent tissue have been shown to be useful for predicting not only recurrence but also outcome.[3]

Investigation of gene expression in the tumor and adjacent liver tissue is very important for the prediction of intrahepatic recurrence of HCC and its prevention. Following such investigations, there is an urgent need to develop an effective treatment for the prevention of HCC recurrence. To suppress or eradicate the replication of hepatitis B and C virus must be the most important issue for prevention. This will be effective for the prevention of HCC recurrence and improving liver function, when HCC has developed on a background of liver cirrhosis. It would be extremely valuable to find a useful biomarker for prediction and to develop new molecular targeting agents for the prevention of HCC recurrence in the near future.

Correspondence: Dr Namiki Izumi, Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonancho, Musashino-shi, Tokyo 180-8610, Japan. Email: nizumi@musashino.jrc.or.jp

Financial Support: This study was supported by grants from the Japanese Ministry of Welfare, Health, and Labour.
Potential conflict of interest: The author has nothing to declare.
Received 8 September 2011; revision 3 October 2011; accepted 3 October 2011.

FACTORS ASSOCIATED WITH INTRAHEPATIC HCC RECURRENCE, INCLUDING THE TUMOR, SURROUNDING LIVER AND SYSTEMIC COMPLICATIONS

Factors in the tumor associated with HCC recurrence

THE RELATIONSHIP BETWEEN the gene expression signature of HCC and the gross tumor morphology may be investigated using surgically resected liver tissue and the confluent multinodular (CM) type has been shown to be associated with poor prognosis in terms of overall survival and recurrence-free survival.[4] The molecules associated with intrahepatic recurrence of HCC have been investigated in the tumor itself. In particular, a stem-cell epidermal cell adhesion molecule (EpCAM) was shown to be expressed in CM-type HCC, and this was confirmed by immunohistochemical studies of HCC cancer cells of the CM-type by Murakata *et al.* In their analysis, EpCAM was shown to be predictive of poor prognosis in terms of overall and recurrence-free survival.[3] EpCAM is one of the markers of HCC cells derived from cancer stem cells (CSC), which have been shown to have a great capacity for colony formation rate.[5] Recently, EpCAM has been shown to correlate with HCC growth and a new avenue for cancer eradication by targeting Wht/beta-catenin signaling components such as EpCAM was suggested by Yamashita *et al.*[6]

Komuta *et al.* reported that cholangiolocellular carcinoma may be of progenitor cell origin, and that some HCCs also are derived from hepatic progenitor cells, because immunohistological staining of keratin 19 is positive in the cytoplasm.[7] Intermediate hepatobiliary cells that are defined by cytokeratin (CK) 7 and 19 positive immunohistological staining, have been seen in the liver tissue of hepatitis C virus-related cirrhosis and are related independently to HCC occurrence.[8] CK 19 expression was reported to be a useful predictive marker for detecting more aggressive HCCs after curative resection, because tumors with CK 19 expression have a poorer prognosis[9,10] and higher rates of recurrence after curative resection.[11,12] Recently, Tsuchiya *et al.* examined CK 19 expression in tumor biopsy tissue from nodules that were treated completely by radiofrequency ablation (RFA), and positive staining of cytoplasmic CK 19 was related to high recurrence of HCC after curative RFA[13] (Fig. 1).

Sasaki *et al.* reported that cytoplasmic and membranous expression of CD 133 was observed in 22% of HCC patients and cytoplasmic expression of CD 133 was identified as a significant risk factor for the overall survival of the patients.[14] Because CD 133 is a cancer stem-cell marker, other markers of progenitor cells should be investigated as biomarkers of early recurrence and poor prognosis in patients with HCC. Investigation of biomarkers associated with early recurrence of HCC should enable the development of clinically useful molecular targeting agents.

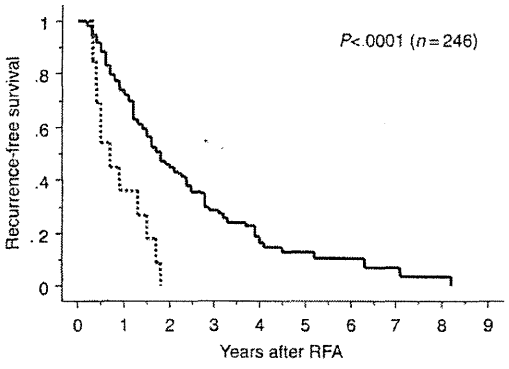

Figure 1 Comparison of recurrence-free survival between K19 positive and negative cases. Recurrence-free survival was lower in patients with positive staining of the biopsied tumor tissue before radiofrequency ablation than in those without K19 staining, as reported by Thsuchiya *et al.*[12] —, CK19 < 1%, $n = 233$; ···, CK19 ≧ 1%, $n = 13$.

Apart from progenitor cell feature markers, the hepatocyte-specific, organic anion transporter peptides (OATP) 1B1 and 1B3 have been shown to be negatively correlated with HCC-related death after recurrence.[15]

HCC recurrence and background liver disease

Liver dysfunction, evaluated by determining serum albumin levels and the branched chain amino-acid to tyrosine ratio (BTR), have been shown by Nakamura *et al.* to be associated with recurrence of HCC after curative resection.[16] Gene expression was investigated in fixed tissue from the liver surrounding HCC following surgical resection and a reproducible gene expression signature that correlated with survival was established in patients with HCC.[17] The same group reported gene expression patterns that predict recurrence of HCC from the tumor itself and adjacent tissues and these gene signatures were shown to predict early and overall recurrence in patients with HCC.[18] Researchers from Germany reported a prognostic model for HCC recurrence, based on gene expression patterns in tumor and

adjacent tissues.[19] They reported that these signatures were useful to predict early and overall recurrence in patients with HCC and that they are helpful for predicting HCC recurrence along with complementary findings from clinical and pathological analysis.

The expression of vascular endothelial growth factor (VEGF) was examined in tumor and non-tumor liver tissue after surgical resection and the tumor/non-tumor ratio of the expression of VEGF was shown to be higher in cases with early recurrence cases than those without and the expression of VEGF was correlated with α-fetoprotein (AFP), protein induced by vitamin K absence-II (PIVKA II) and histological grade.[20]

HCC RECURRENCE AND TUMOR MARKER

PRETREATMENT LEVELS OF tumor markers such as lens culinaris agglutinin A-reactive fraction of AFP (AFP-L3) and des-gamma carboxy prothrombin (DCP) were reported to be useful for the prognostic value in patients treated by locoregional thermal ablation, not in those treated by surgical resection.[21] In the patients treated by surgical resection, postoperative AFP levels were useful for predicting recurrence.[22] However, Yamamoto *et al.* reported that DCP was a more accurate marker for the recurrence than AFP.[23] Positive postoperative AFP-L3 levels were shown to be a marker of prognosis and recurrence in China.[24] In the patients treated by radiofrequency ablation, Beppu *et al.* reported that AFP-L3 fraction levels were significantly predictive of recurrence-free survival by multivariate analysis.[25]

Recently, the micro-total analysis system which is a new sensitive AFP-L3 assay was established in Japan and was reported to provide great utility in determining HCC recurrence, even in the patients with low AFP concentrations.[26,27]

HCC RECURRENCE AND DIABETES MELLITUS OR INSULIN RESISTANCE

COMPLICATION OF DIABETES mellitus (DM) is occasionally observed in patients with HCC and its impact on postoperative recurrence remains controversial. In patients with HCV-related HCC, diabetes was shown to be a risk factor for recurrence and lower rates of overall survival after surgical resection.[28] Insulin resistance as estimated by Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was shown to be an independent risk factor for recurrence of stage 1 HCC after curative RFA.[29] DM also has been shown to be a poor prognostic predictor among those with small HCC with Child–Pugh A reserve.[30]

However, DM did not affect intra-hepatic HCC recurrence and survival of patients with HCC after RFA but persistent hepatitis was shown to affect intra-hepatic HCC recurrence and survival.[31] A meta-analysis of the prognostic role of DM was reported in HCC patients after curative treatment and HCC patients with coexisting DM have a shorter survival time and higher risk of tumor recurrence after curative treatment.[32]

In addition, visceral fat accumulation has been shown to be an independent risk factor for HCC recurrence after curative RFA in patients with non-alcoholic steatohepatitis (NASH).[33] HCC recurrence was shown to be very high in patients with NASH after curative treatment compared with HCC caused by hepatitis C virus infection, which was suggested to be of multicentric origin, similar to HCC associated with HCV infection.[34]

Table 1 Candidate useful agents for the prevention of intrahepatic recurrence of hepatocellular carcinoma (HCC)

Antiviral treatment
Nucleotide analogue for hepatitis B
Interferon plus ribavirin for HCV
Systemic agents
Branched chain amino acid
Vitamin K2
Acyclic retinoid
Chemotherapeutic and molecular targeted agents

SPECIFIC TRIALS FOR THE PREVENTION OF HCC RECURRENCE

TO PREVENT INTRAHEPATIC recurrence of HCC, several important trials including specific antivairal treatment and systemic agents have been reported (Table 1).

Antiviral treatment for hepatitis B virus

In the patients with hepatitis B virus related HCC, a high HBV DNA load was identified as an independent risk factor for HCC recurrence after curative RFA.[35] Lamivudine administration after RFA was investigated in HBV-related HCC but the overall survival and recurrence-free survival did not differ between the patients who received lamivudine treatment and those who did not, although the serum albumin level was shown to improve.[36] However, Chan *et al.* reported that antiviral therapy after surgical resection improved the prognosis, especially disease-free survival in HBV-related HCC.[37]

A meta-analysis of the efficacy of anti-viral therapy was reported by Wong *et al.* who analyzed nine cohort studies with a total of 551 patients. The risk of HCC recurrence was reduced by 41% by the anti-viral treatment and they concluded that anti-viral therapy has potentially beneficial effects after the curative treatment of HBV-related HCC in terms of tumor recurrence, liver-related mortality and overall survival.[38]

Antiviral treatment for hepatitis C virus

Regarding the patients with HCV-related HCC, the long-term outcome was investigated including more than 300 patients after surgical resection and survival was shown to have improved in the period from 2000 to 2006 compared with the period from 1990 to 1999. This improvement in survival is attributable to antiviral therapy with interferon.[39] Viral mutations were investigated and postoperative recurrence of HCC was found to be associated with amino acid (aa) substitutions in the HCV core region, such as aa residue 91. The core mutations were shown to be associated with postoperative recurrence or survival in patients infected with HCV genotype 1b and treated by surgical resection.[40]

Interferon was first shown to be effective for the prevention of recurrence by a randomized study using interferon-β (IFN-β).[41] Thereafter, several reports of the preventive effects of interferon on the recurrence of HCC have been published; interferon did not affect overall prevention of HCC recurrence after resection[42] or RFA[42] but, if the HCV infection had been cured, interferon was effective for preventing the development of HCC and improving survival.[43] A meta-analysis has been reported and IFN-α treatment after curative treatment of primary tumors within the Milan criteria may be effective for the prevention of HCC recurrence, and a higher rate of sustained virological response (SVR) may be associated with a better preventive effect of IFN-α treatment on HCC recurrence.[44,45] To improve the SVR rate, peginterferon treatment after curative treatment of HCC was reported to be closely correlated with the prevention of recurrence.[46]

Branched chain amino-acid supplementation

The backgroud liver dysfunction has been shown to correlate with HCC recurrence, and even after curative resection with small HCC less than 2 cm, postoperative hepatic reserve influences HCC recurrence.[47] Recently, supplementation by branched chain amino acid (BCAA)-enrichment for patients with HCC after RFA has been shown to be effective for the improvement of serum albumin and quality of life[48] and a positive effect on serum albumin by BCAA was noted in patients with Child–Pugh B grade.[49]

Whether BCAA supplementation inhibits HCC recurrence or not is an important issue to be investigated, and recently, the mechanism whereby BCAA is effective for the prevention of the development of HCC has been precisely discussed in detail.[50]

Vitamin K2

In 2004, Habu *et al.*[51] reported that the incidence of development of HCC was reduced among cirrhotic women assigned to receive oral vitamin K2. The incidence of HCC recurrence was clearly shown to be lower than the control group in prospective studies by Mizuta *et al.*[52] and Kakizaki *et al.*;[53] however, conflicting results that HCC recurrence was not reduced by administration of vitamin K2 were reported by Hotta *et al.*[54] Therefore, a large scale multicenter prospective randomized study was conducted in Japan to investigate the preventive effects of vitamin K2 on HCC recurrence. The administration of vitamin K2 at 45 mg per day was not effective in preventing HCC recurrence and, moreover, in the patient group treated with a high dose of vitamin K2 of 90 mg per day, the incidence of HCC was rather higher than the control group.[55] Fortunately enough, severe adverse events were not observed.

Acyclic retinoid

Oral polyprenic acid of an acyclic retinoid was shown to inhibit the development of second primary HCC in a prospective randomized study with a median follow-up of 38 months, reported by Muto *et al.*[56] The overall survival of those receiving the acyclic retinoid was shown to be better than the control group.[57] A large scale multicenter, prospective randomized study has been carried out and oral administration of 600 mg per day of acyclic retinoid was shown to be preventive.[58]

Chemotherapeutic agent and molecular targeted agent

Although adjuvant chemotherapy has been considered for other solid malignancies with a high risk of recurrence, this is difficult in the case of HCC because few conventional chemotherapeutic agents are effective and hepatotoxicity can be of critical significance, because liver function often is already impaired. A randomized trial was performed with uracil-tegaful as postoperative adjuvant therapy, but did not improve the recurrence-free survival, and the overall survival appeared to be reduced.[59]

Whether sofarenib is effective for the prevention of recurrence is now under investigation, including in distinguished centers for treating HCC worldwide, in the so-called STORM trial.[60] A phase III study was conducted to determine whether sorafenib is effective for the prolongation of time to progression after transarterial chemoembolization (TACE), and sorafenib did not significantly prolong the time to progression in patients who responded to TACE.[61]

CONCLUSION

TO IMPROVE THE overall survival of patients with HCC, an important issue is to prevent intrahepatic recurrence. Many significant findings regarding gene expression in the liver and adjacent liver tissue, which relate to intrahepatic recurrence have been reported recently. Following such investigations, there is an urgent need for improved methods of prediction and prevention of intrahepatic recurrence of HCC.

REFERENCES

1 Kudo M, Izumi N, Kokudo N *et al.* HCC expert panel of Japan society of hepatology. *Dig Dis* 2011; 29: 339–64.
2 Poon RT. Prevention of recurrence after resection of hepatocellular carcinoma: a daunting challenge. *Hepatology* 2011; 54: 757–9.
3 Villanueva A, Hoshida Y, Toffanin S *et al.* New strategies in hepatocellular carcinoma: genomic prognostic markers. *Clin Cancer Res* 2010; 16: 4688–94.
4 Murakata A, Tanaka S, Mogushi K *et al.* Gene expression signature of the gross morphology in hepatocellular carcinoma. *Ann Surg* 2011; 253: 94–100.
5 Kimura O, Takahashi T, Ishii N *et al.* Characterization of the epithelial cell adhesion molecule (EpCAM)+ cell population in hepatocellular carcinoma cell lines. *Cancer Sci* 2010; 101: 2145–55.
6 Yamashita T, Ji J, Budhu A *et al.* EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology* 2009; 136: 1012–24.
7 Komuta M, Spee B, Vander Borght S *et al.* Clinicopathological study on cholangiocellular carcinoma suggesting hepatic progenitor cell origin. *Hepatology* 2008; 47: 1544–56.
8 Ziol M, Nault JC, Aout M *et al.* Intermediate hepatobiliary cells predict an increased risk of hepatocarcinogenesis in patients with hepatitis C virus – related cirrhosis. *Gastroenterology* 2010; 139: 335–43.
9 Yang XR, Xu Y, Shi GM *et al.* Cytokeratin 10 and cytokeratin 19: predictive markers for poor prognosis in hepatocellular carcinoma patients after curative resection. *Clin Cancer Res* 2008; 14: 3850–9.
10 Zhuang PY, Zhang JB, Zhu XD *et al.* Two pathologic types of hepatocellular carcinoma with lymph node metastasis with distinct prognosis on the basis of CK 19 expression in tumor. *Cancer* 2008; 112: 2740–8.
11 Uenishi T, Kubo S, Yamamoto T *et al.* Cytokeratin 19 expression in hepatocellular carcinoma predicts early postoperative recurrence. *Cancer Sci* 2003; 94: 851–7.
12 Durnez A, Verslype C, Nevens F *et al.* The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. *Histopathology* 2006; 49: 138–51.
13 Tsuchiya K, Komuta M, Yasui Y *et al.* Expression of keratin 19 is related to high recurrence of hepatocellular carcinoma after radiofrequency ablation. *Oncology* 2011; 80: 278–88.
14 Sasaki A, Kamiyama T, Yokoo H *et al.* Cytoplasmic expression of CD133 is an important risk factor for overall survival in hepatocellular carcinoma. *Oncol Res* 2010; 24: 537–46.
15 Vasuri F, Golifieri R, Fiorentino M *et al.* OATP 1B1/1B3 expression in hepatocellular carcinomas treated with orthotopic liver transplantation. *Virchows Arch* 2011; 459: 141–6.
16 Nakamura Y, Mizuguchi T, Kawamoto M *et al.* Cluster analysis of indicators of liver functional and preoperative low branched-chain amino acid tyrosine ration indicate a high risk of early recurrence in analysis of 165 hepatocellular carcinoma patients after initial hepatectomy. *Surgery* 2011; 150: 250–62.
17 Hoshida Y, Villanueva A, Kobayashi M *et al.* Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N Engl J Med* 2008; 359: 1995–2004.
18 Villanueva A, Hoshida Y, Battiston C *et al.* Combining clinical, pathology, and gene expression data predict recurrence of hepatocellular carcinoma. *Gastroenterology* 2011; 140: 1501–12.
19 Teufel A, Marquardt JU, Galle PR. Novel insight in the genetics of HCC recurrence and advances in transcriptomic data integration. *J Hepatol* 2011 E pub July 23.
20 Amaoka N, Osada S, Kanematsu M *et al.* Clinicopathological features of hepatocellular carcinoma evaluated by vascular endothelial growth factor expression. *J Gastroenterol Hepatol* 2007; 22: 2202–7.
21 Toyoda H, Kumada T, Kaneoka Y *et al.* Prognostic value of pretreatment levels of tumor markers for hepatocellular carcinoma on survival after curative treatment of patients with HCC. *J Hepatol* 2008; 49: 223–32.
22 Nobuoka D, Kato Y, Gotohda N *et al.* Postoperative serum alpha-fetoprotein level is a useful predictor of recurrence after hepatectomy for hepatocellular carcinoma. *Oncol Rep* 2010; 24: 521–8.
23 Yamamoto K, Imamura H, Matsuyama Y *et al.* Significance of alpha-fetoprotein and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma undergoing hepatectomy *Ann Surg Oncol* 2009; 16: 2795–804.

24 Zhang XF, Lai EC, Kang XY *et al.* Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein as a marker of prognosis and a monitor of recurrence of hepatocellular carcinoma after curative liver resection. *Ann Surg Oncol* 2011; **18**: 2218–23.
25 Beppu T, Sugimoto K, Shiraki K *et al.* Clinical significance of tumor markers in detection of recurrent hepatocellular carcinoma after radiofrequency ablation. *Int J Mol Med* 2010; **26**: 425–33.
26 Morimoto M, Numata K, Nozaki A *et al.* Novel lens culinaris agglutinin-reactive fraction of α-fetoprotein: a biomarker of hepatocellular carcinoma recurrence in patients with low α-fetoprotein concentrations. *Int J Clin Oncol* 2011 E pub Aug 26.
27 Kobayashi M, Hosaka T, Ikeda K *et al.* Highly sensitive AFP-L3% assay is useful for predicting recurrence of hepatocellular carcinoma after curative treatment pre-and postoperatively. *Hepatol Res* 2011 E pub Aug 26.
28 Komuta T, Mizukoshi E, Kita Y *et al.* Impact of diabetes on recurrence of hepatocellular carcinoma after surgical treatment in patients with vital hepatitis. *Am J Gastroenterol* 2007; **102**: 1939–46.
29 Imai K, Takai K, Nishigaki Y *et al.* Insulin resistance raises the risk for recurrence of stage 1 hepatocellular carcinoma after curative ablation in hepatitis C virus-positive patients: a prospective, case series study. *Hepatol Res* 2010; **40**: 376–82.
30 Huo TI, Wu JC, Lui WY *et al.* Differential mechanism and prognostic impact of diabetes mellitus on patients with hepatocellular carcinoma undergoing surgical treatment. *Am J Gastroenterol* 2004; **99**: 1479–87.
31 Chen WT, Macatula TC, Lin CC, Lin CJ, Lin SM. Diabetes may not affect outcomes in hepatocellular carcinoma after radio-frequency ablation. *Hepatogastroenterology* 2011; **58**: 551–7.
32 Wang WM, Xu Y, Yang XR, Wang YH, Sun HX, Fan J. Prognostic role of diabetes mellitus in hepatocellular carcinoma patients after curative treatments: a meta-analysis. *Hepatobiliary Pancreat Dis Int* 2011; **10**: 346–55.
33 Ohki T, Tateishi R, Shiina S *et al.* Visceral fat accumulation is an independent risk factor for hepatocellular carcinoma recurrence after curative treatment in patients with suspected NASH. *Gut* 2009; **58**: 839–44.
34 Tokushige K, Hashimoto E, Yatsuji S *et al.* Prospective study of hepatocellular carcinoma in non-alcoholic steatohepatitis in comparison with hepatocellular carcinoma caused by chronic hepatitis C. *J Gastroenterol* 2101; **45**: 960–7.
35 Goto T, Yoshida H, Tateishi R *et al.* Influence of serum HBVDNA load on recurrence of hepatocellular carcinoma after treatment with percutaneous radiofrequency ablation. *Hepatol Int* 2011 E pub Jan 25.
36 Yoshida H, Goto E, Sato T *et al.* Safety and efficacy of lamivudine after radiofrequency ablation in patients with hepatitis B virus-related hepatocellular carcinoma. *Hepatol Int* 2008; **2**: 89–94.
37 Chan AC, Chok KS, Yuen WK, Chan RT, Lo CM, Fan ST. Impact of antiviral therapy on the survival of patients after major hepatectomy for hepatitis B virus-related hepatocellular carcinoma. *Arch Surg* 2011; **146**: 675–81.
38 Wong JS, Wong GL, Tsoi KK *et al.* Meta-analysis: the efficacy of anti-viral therapy in prevention of recurrence after curative treatment of chronic hepatitis B-related hepatocellular carcinoma. *Aliment Pharmacol Ther* 2011; **33**: 1104–12.
39 Shirabe K, Takeishi K, Taketomi A, Uchiyama H, Kayashima H, Maehara Y. Improvement of long-term outcomes in hepatitis C virus antibody-positive patients with hepatocellular carcinoma after hepatectomy in the modern era. *World J Surg* 2011; **35**: 1072–84.
40 Toyoda H, Kumada T, Kaneoka Y, Maeda A. Amino acid substitutions in the hepatitis C virus core region are associated with postoperative recurrence and survival of patients with genotype 1b-associated hepatocellular carcinoma. *Ann Surg* 2011; **254**: 326–32.
41 Ikeda K, Arase Y, Saitoh S *et al.* Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor- A prospective randomized study of hepatitis C virus-related cancer. *Hepatology* 2000; **32**: 228–32.
42 Mazzaferro V, Romito R, Schiavo M *et al.* HCC Italian Task Force. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006; **44**: 1543–54.
43 Shiratori Y, Ito Y, Yokosuka O *et al.* Tokyo-Chiba Hepatitis Research Group. Antiviral therapy for cirrhotic hepatitis C: association with hepatocellular carcinoma development and improved survival. *Ann Intern Med* 2005; **18**: 105–14.
44 Miyake Y, Takaki A, Iwasaki Y, Yamamoto K. Meta-analysis: interferon-alpha prevents the recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *J Viral Hepat* 2010; **17**: 287–92.
45 Shen YC, Hsu C, Chen LT, Cheng CC, Hu FC, Cheng AL. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. *J Hepatol* 2010; **52**: 889–94.
46 Hagihara H, Nouso K, Kobayashi Y *et al.* Effect of pegylated interferon therapy on intrahepatic recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *Int J Clin Oncol* 2011; **16**: 210–20.
47 Kaibori M, Ishizaki M, Saito T, Matsui K, Kwon AH, Kamiyama Y. Risk factors and outcome of early recurrence after resection of small hepatocellular carcinomas. *Am J Surg* 2009; **198**: 39–45.
48 Kuroda H, Ushio A, Miyamoto Y *et al.* Effects of branched-chain amino acid-enriched nutrient for patients with hepatocellular carcinoma following radiofrequency ablation: a one-year prospective trial. *J Gastroenterol Hepatol* 2010; **25**: 1550–5.

49 Ishikawa T, Michitaka I, Kamimura H *et al.* Oral branched-chain amino acids administration improves impaired liver dysfunction after radiofrequency ablation therapy for hepatocellular carcinoma. *Hepatogastroneterology* 2009; **56**: 1491–5.
50 Kawaguchi T, Izumi N, Charlton MR, Sata M. Branched-chain amino acids as pharmacological nutrients in chronic liver disease. *Hepatology* 2011; **54**: 1063–70.
51 Habu D, Shiomi S, Tamori A *et al.* Role of vitamin Ks in the development of hepatocellular carcinoma in women with viral cirrhosis of the liver. *JAMA* 2004; **292**: 358–61.
52 Mizuta T, Ozaki I, Eguchi Y *et al.* The effect of menatetteone, a vitamin K2 analogue, on disease recurrence and survival in patients with hepatocellular carcinoma after curative treatment. *Cancer* 2006; **106**: 867–72.
53 Kakizaki S, Sohara N, Sato K *et al.* Preventive effects of vitamin K on recurrence disease in patients with hepatocellular carcinoma arising from hepatitis C viral infection. *J Gastroenterol Hepatol* 2007; **22**: 518–22.
54 Hotta N, Ayada M, Sato K *et al.* Effect of vitamin K2 on the recurrence in patients with hepatocellular carcinoma. *Hepatogastroenterology* 2007; **54**: 2073–77.
55 Yoshida H, Shiratori Y, Kudo M *et al.* Effect of vitamin K2 on the recurrence of hepatocellular carcinoma. *Hepatology* 2011; **54**: 532–40.
56 Muto Y, Moriwaki H, Nonomiya M *et al.* Prevention of second primary tumors by an acyclic retionid, polyprenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. *N Engl J Med* 1996; **13**: 1561–7.
57 Muto Y, Moriwaki H, Saito A. Prevention of second primary tumors by an acyclic retinoid in patients with hepatocellular carcinoma. *N Engl J Med* 1999; **340**: 1046–7.
58 Okita K, Matsui O, Kumada H *et al.* Effect of peretinoin on recurrence of hepatocellular carcinoma (HCC): results of a phase II/III randomized placebo-controlled trial. *Am Soc Clin Oncol* 2011. ASCO Annual Meeting. Abstract No. 4024.
59 Hasegawa K, Takayama T, Ijichi M *et al.* Uracil-tegaful as an adjuvant for hepatocellular carcinoma: a randomized trial. *Hepatology* 2006; **44**: 891–95.
60 Prints C. Clinical trials of note. Sorafenib as adjuvant treatment for the prevention of disease recurrence in patients with hepatocellular carcinoma (HCC) (STORM). *Cancer* 2009; **115**: 4646.
61 Kudo M, Imanaka K, Chida N *et al.* Phase III study of sorafenib after transarterial chemoembolization in Japanese and Korean patients with unresectable hepatocellular carcinoma. *Eur J Cancer* 2011; **47**: 2117–27.

Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in *IL28B* and viral factors

Masayuki Kurosaki[1], Yasuhito Tanaka[2], Nao Nishida[3], Naoya Sakamoto[4], Nobuyuki Enomoto[5], Masao Honda[6], Masaya Sugiyama[2], Kentaro Matsuura[2], Fuminaka Sugauchi[2], Yasuhiro Asahina[1], Mina Nakagawa[4], Mamoru Watanabe[4], Minoru Sakamoto[5], Shinya Maekawa[5], Akito Sakai[6], Shuichi Kaneko[6], Kiyoaki Ito[7], Naohiko Masaki[7], Katsushi Tokunaga[3], Namiki Izumi[1,*], Masashi Mizokami[2,7]

[1]*Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan;* [2]*Department of Virology, Liver Unit, Nagoya City University, Graduate School of Medical Sciences, Nagoya, Japan;* [3]*Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan;* [4]*Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan;* [5]*First Department of Internal Medicine, University of Yamanashi, Yamanashi, Japan;* [6]*Department of Gastroenterology, Kanazawa University, Graduate School of Medicine, Kanazawa, Japan;* [7]*Research Center for Hepatitis and Immunology, International Medical Center of Japan, Konodai Hospital, Ichikawa, Japan*

Background & Aims: Pegylated interferon and ribavirin (PEG-IFN/RBV) therapy for chronic hepatitis C virus (HCV) genotype 1 infection is effective in 50% of patients. Recent studies revealed an association between the *IL28B* genotype and treatment response. We aimed to develop a model for the pre-treatment prediction of response using host and viral factors.

Methods: Data were collected from 496 patients with HCV genotype 1 treated with PEG-IFN/RBV at five hospitals and universities in Japan. *IL28B* genotype and mutations in the core and IFN sensitivity determining region (ISDR) of HCV were analyzed to predict response to therapy. The decision model was generated by data mining analysis.

Results: The *IL28B* polymorphism correlated with early virological response and predicted null virological response (NVR) (odds ratio = 20.83, $p < 0.0001$) and sustained virological response (SVR) (odds ratio = 7.41, $p < 0.0001$) independent of other covariates. Mutations in the ISDR predicted relapse and SVR independent of *IL28B*. The decision model revealed that patients with the minor *IL28B* allele and low platelet counts had the highest NVR (84%) and lowest SVR (7%), whereas those with the major *IL28B* allele and mutations in the ISDR or high platelet counts had the lowest NVR (0–17%) and highest SVR (61–90%). The model had high reproducibility and predicted SVR with 78% specificity and 70% sensitivity.

Conclusions: The *IL28B* polymorphism and mutations in the ISDR of HCV were significant pre-treatment predictors of response to PEG-IFN/RBV. The decision model, including these host and viral factors may support selection of optimum treatment strategy for individual patients.

Keywords: *IL28B*; ISDR; Peg-interferon; Ribavirin; Data mining; Decision tree.
Received 14 March 2010; received in revised form 22 June 2010; accepted 7 July 2010; available online 19 September 2010

* Corresponding author. Address: Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonan-cho, Musashino-shi, Tokyo 180-8610, Japan. Tel.: +81 422 32 3111; fax: +81 422 32 9551.
E-mail address: nizumi@musashino.jrc.or.jp (N. Izumi).

Introduction

Hepatitis C virus (HCV) infection is the leading cause of cirrhosis and hepatocellular carcinoma worldwide [1]. The successful eradication of HCV, defined as a sustained virological response (SVR), is associated with a reduced risk of developing hepatocellular carcinoma. Currently, pegylated interferon (PEG-IFN) plus ribavirin (RBV) is the most effective standard of care for chronic hepatitis C but the rate of SVR is around 50% in patients with HCV genotype 1 [2,3], the most common genotype in Japan, Europe, the United States, and many other countries. Moreover, 20–30% of patients with HCV genotype 1 have a null virological response (NVR) to PEG-IFN/RBV therapy [4]. The most reliable method for predicting the response is to monitor the early decline of serum HCV-RNA levels during treatment [5] but there is no established method for prediction before treatment. Because PEG-IFN/RBV therapy is costly and often accompanied by adverse effects such as flu-like symptoms, depression and hematological abnormalities, pre-treatment predictions of those patients who are unlikely to benefit from this regimen enables ineffective treatment to be avoided.

Recently, it has been reported through a genome-wide association study (GWAS) of patients with genotype 1 HCV that single nucleotide polymorphisms (SNPs) located near the *IL28B* gene are strongly associated with a response to PEG-IFN/RBV therapy in

Research Article

Table 1. Baseline characteristics of all patients, and patients assigned to the model building or validation groups.

	All patients n = 496	Model group n = 331	Validation group n = 165
Gender: male	250 (50%)	170 (51%)	80 (48%)
Age (years)	57.1 ± 9.9	56.8 ± 9.7	57.5 ± 10.2
ALT (IU/L)	78.6 ± 60.8	78.1 ± 61.4	79.7 ± 59.6
GGT (IU/L)	59.3 ± 63.6	58.9 ± 62.0	60.2 ± 66.9
Platelets (10^9/L)	154 ± 53	153 ± 52	154 ± 56
Fibrosis: F3-4	121 (24%)	80 (24%)	41 (25%)
HCV-RNA: >600,000 IU/ml	409 (82%)	273 (82%)	136 (82%)
ISDR mutation: ≤1	220 (88%)	290 (88%)	145 (88%)
Core 70 (Arg/Gln or His)	293 (59%)/203 (41%)	197 (60%)/134 (40%)	96 (58%)/69 (42%)
Core 91 (Leu/Met)	299 (60%)/197 (40%)	200 (60%)/131 (40%)	99 (60%)/66 (40%)
IL28B: Minor allele	151 (30%)	101 (31%)	50 (30%)
SVR	194 (39%)	129 (39%)	65 (39%)
Relapse	152 (31%)	103 (31%)	49 (30%)
NVR	150 (30%)	99 (30%)	51 (31%)

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; ISDR, interferon sensitivity determining region; Arg, arginine; Gln, glutamine; His, histidine; Leu, leucine; Met, methionine; Minor, heterozygote or homozygote of minor allele; SVR, sustained virological response; NVR, null virological response.

Japanese [6], European [7], and a multi-ethnic population [8,9]. The last three studies focused on the association of SNPs in the *IL28B* region with SVR [7–9] but we found a stronger association with NVR [6]. In addition to these host genetic factors, we have reported that mutations within a stretch of 40 amino acids in the NS5A region of HCV, designated as the IFN sensitivity determining region (ISDR), are closely associated with the virological response to IFN therapy: a lower number of mutations is associated with treatment failure [10–13]. Amino acid substitutions at positions 70 and 91 of the HCV core region (Core70, Core91) also have been reported to be associated with response to PEG-IFN/RBV therapy: glutamine (Gln) or histidine (His) at Core70 and methionine (Met) at Core91 are associated with treatment resistance [4,14]. The importance of substitutions in the HCV core and ISDR was confirmed recently by a Japanese multicenter study [15]. How these viral factors contribute to response to therapy is yet to be determined. For general application in clinical practice, host genetic factors and viral factors should be considered together.

Data mining analysis is a family of non-parametric regression methods for predictive modeling. Software is used to automatically explore the data to search for optimal split variables and to build a decision tree structure [16]. The major advantage of decision tree analysis over logistic regression analysis is that the results of the analysis are presented in the form of flow chart, which can be interpreted intuitively and readily made available for use in clinical practice [17]. The decision tree analysis has been utilized to define prognostic factors in various diseases [18–25]. We have reported recently its usefulness for the prediction of an early virological response (undetectable HCV-RNA within 12 weeks of therapy) to PEG-IFN/RBV therapy in chronic hepatitis C [26].

This study aimed to define the pre-treatment prediction of response to PEG-IFN/RBV therapy through the integrated analysis of host factors, such as the *IL28B* genetic polymorphism and various clinical covariates, as well as viral factors, such as mutations in the HCV core and ISDR and serum HCV-RNA load. In addition, for the general application of these results in clinical practice, decision models for the pre-treatment prediction of response were determined by data mining analysis.

Materials and methods

Patients

This was a multicentre retrospective study supported by the Japanese Ministry of Health, Labor and Welfare. Data were collected from a total of 496 chronic hepatitis C patients who were treated with PEG-IFN alpha and RBV at five hospitals and universities throughout Japan. Of these, 98 patients also were included in the original GWAS analysis [6]. The inclusion criteria in this study were as follows (1) infection by genotype 1b, (2) lack of co-infection with hepatitis B virus or human immunodeficiency virus, (3) lack of other causes of liver disease, such as autoimmune hepatitis, and primary biliary cirrhosis, (4) completion of at least 24 weeks of therapy, (5) adherence of more than 80% to the planned dose of PEG-IFN and RBV for the NVR patients, (6) availability of DNA for the analysis of the genetic polymorphism of *IL28B*, and (7) availability of serum for the determination of mutations in the ISDR and substitutions of Core70 and Core91 of HCV. Patients received PEG-IFN alpha-2a (180 μg) or 2b (1.5 μg/kg) subcutaneously every week and were administered a weight adjusted dose of RBV (600 mg for <60 kg, 800 mg for 60–80 kg, and 1000 mg for >80 kg daily) which is the recommended dosage in Japan. Written informed consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committee. The baseline characteristics are listed in Table 1. For the data mining analysis, 67% of the patients (331 patients) were assigned randomly to the model building group and 33% (165 patients) to the validation group. There were no significant differences in the clinical backgrounds between these two groups.

Laboratory and histological tests

Blood samples were obtained before therapy and were analyzed for hematologic tests and for blood chemistry and HCV-RNA. Sequences of ISDR and the core region of HCV were determined by direct sequencing after amplification by reverse-transcription and polymerase chain reaction as reported previously [4,11]. Genetic polymorphism in one tagging SNP located near the *IL28B* gene (rs8099917) was determined by the GWAS or DigiTag2 assay [27]. Homozygosity (GG) or heterozygosity (TG) of the minor sequence was defined as having the *IL28B* minor allele, whereas homozygosity for the major sequence (TT) was

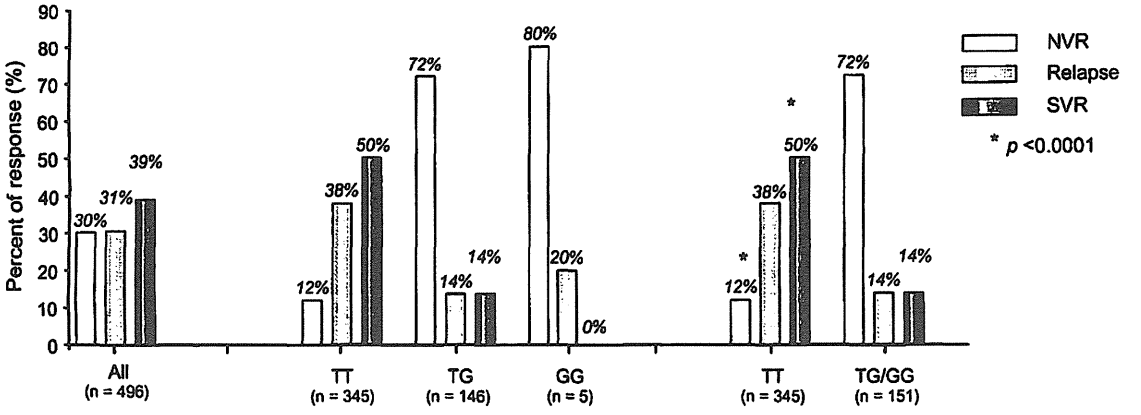

Fig. 1. Association between the *IL28B* genotype (rs8099917) and treatment response. The rates of response to treatment are shown for each rs8099917 genotype. The rate of null virological response (NVR), relapse, and sustained virological response (SVR) is shown. The *p* values are from Fisher's exact test. The rate of NVR was significantly higher ($p < 0.0001$) and the rate of SVR was significantly lower ($p < 0.0001$) in patients with the *IL28B* minor allele compared to those with the major allele.

defined as having the *IL28B* major allele. In this study, NVR was defined as a less than 2 log reduction of HCV-RNA at week 12 and detectable HCV-RNA by qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor, Roche Diagnostic systems, CA) at week 24 during therapy. RVR (rapid virological response) and complete early virological response (cEVR) were defined as undetectable HCV-RNA at 4 weeks and 12 weeks during therapy and SVR was defined as undetectable HCV-RNA 24 weeks after the completion of therapy. Relapse was defined as reappearance of HCV-RNA after the completion of therapy. The stage of liver fibrosis was scored according to the METAVIR scoring system: F0 (no fibrosis), F1 (mild fibrosis: portal fibrosis without septa), F2 (moderate fibrosis: few septa), F3 (severe fibrosis: numerous septa without cirrhosis) and F4 (cirrhosis). Percentage of steatosis was quantified in 111 patients by determining the average proportion of hepatocytes affected by steatosis.

Statistical analysis

Associations between pre-treatment variables and treatment response were analyzed by univariate and multivariate logistic regression analysis. Associations between the *IL28B* polymorphism and sequences of HCV were analyzed by Fisher's exact test. SPSS software v.15.0 (SPSS Inc., Chicago, IL) was used for these analyses. For the data mining analysis, IBM-SPSS Modeler version 13.0 (IBM-SPSS Inc., Chicago, IL) software was utilized as reported previously [26]. The patients used for model building were divided into two groups at each step of the analysis based on split variables. Each value of each variable was considered as a potential split. The optimum variables and cut-off values were determined by a statistical search algorithm to generate the most significant division into two prognostic subgroups that were as homogeneous as possible for the probability of SVR. Thereafter, each subgroup was evaluated again and divided further into subgroups. This procedure was repeated until no additional significant variable was detected or the sample size was below 15. To avoid over-fitting, 10-fold cross validation was used in the tree building process. The reproducibility of the resulting model was tested with the data from the validation patients.

Results

Association between the IL28B (rs8099917) genotype and the PEG-IFN/RBV response

The rs8099917 allele frequency was 70% for TT ($n = 345$), 29% for TG ($n = 146$), and 1% for GG ($n = 5$). We defined the *IL28B* major allele as homozygous for the major sequence (TT) and the *IL28B* minor allele as homozygous (GG) or heterozygous (TG) for the minor sequence. The rate of NVR was significantly higher (72% vs. 12%, $p < 0.0001$) and the rate of SVR was significantly lower (14% vs. 50%, $p < 0.0001$) in patients with the *IL28B* minor allele compared to those with the major allele (Fig. 1).

Effect of the IL28B polymorphism, substitutions in the ISDR, Core70, and Core91 of HCV on time-dependent clearance of HCV

Patients were stratified according to their *IL28B* allele type, the number of mutations in the ISDR, the amino acid substitutions in Core70 and Core91, and the rate of undetectable HCV-RNA at 4, 8, 12, 24, and 48 weeks after the start of therapy were analyzed (Fig. 2A–D). The rate of undetectable HCV-RNA was significantly higher in patients with the *IL28B* major allele than the minor allele, in patients with two or more mutations in the ISDR compared to none or only one mutation, in patients with arginine (Arg) at Core70 rather than Gln/His, and in patients with leucine (Leu) at Core91 rather than Met. The difference was most significant when stratified by the *IL28B* allele type. The rate of RVR and cEVR was significantly more frequent in patients with the *IL28B* major allele compared with those with the *IL28B* minor allele: 9% vs. 3% for RVR ($p < 0.005$) and 57% vs. 11% for cEVR ($p < 0.0001$). These findings suggest that *IL28B* has the greatest impact on early virological response to therapy.

Association between substitutions in the ISDR and relapse after the completion of therapy

Patients were stratified according to the *IL28B* allele, number of mutations in the ISDR, and amino acid substitutions of Core70 and Core91, and the rate of relapse was analyzed (Fig. 3A and B). Among patients who achieved cEVR, the rate of relapse was significantly lower in patients with two or more mutations in the ISDR compared to those with only one or no mutations (15% vs. 31%, $p < 0.005$) (Fig. 3 B). On the other hand, the relapse rate was not different between the *IL28B* major and minor alleles within patients who achieved RVR (3% vs. 0%) or cEVR (28% vs. 29%) (Fig. 3A). Amino acid substitutions of Core70 and Core91 were not associated with the rate of relapse (data not shown).

Factors associated with response by multivariate logistic regression analysis

By univariate analysis, the minor allele of *IL28B* ($p < 0.0001$), one or no mutations in the ISDR ($p = 0.03$), high serum level of

Research Article

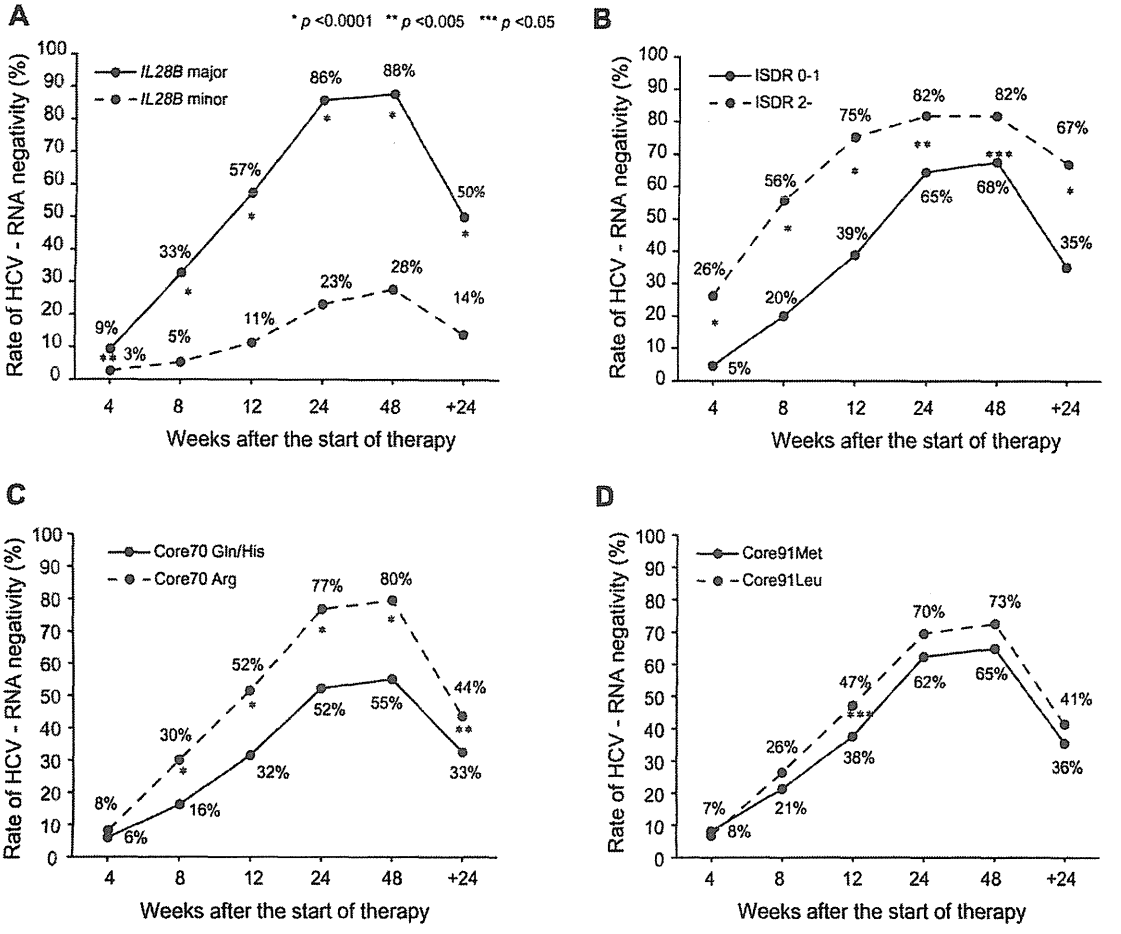

Fig. 2. Effect of *IL28B* mutations in the ISDR, Core70, and Core91 of HCV on time-dependent clearance of HCV. The rate of undetectable HCV-RNA was plotted for serial time points after the start of therapy (4, 8, 12, 24, and 48 weeks) and for 24 weeks after the completion of therapy. Patients were stratified according to (A) the *IL28B* allele (minor allele vs. major allele), (B) the number of mutations in the ISDR (0–1 mutation vs. 2 or more mutations), amino acid substitutions of (C) Core70 (Gln/His vs. Arg), and (D) Core91 (Met vs. Leu). The *p* values are from Fisher's exact test.

HCV-RNA ($p = 0.035$), Gln or His at Core70 ($p < 0.0001$), low platelet counts ($p = 0.009$), and advanced fibrosis ($p = 0.0002$) were associated with NVR. By multivariate analysis, the minor allele of *IL28B* (OR = 20.83, 95%CI = 11.63–37.04, $p < 0.0001$) was associated with NVR independent of other covariates (Table 2). Notably, mutations in the ISDR ($p = 0.707$) and at amino acid Core70 ($p = 0.207$) were not significant in multivariate analysis due to the positive correlation with the *IL28B* polymorphism ($p = 0.004$ for ISDR and $p < 0.0001$ for Core70, Fig. 4).

Genetic polymorphism of *IL28B* also was associated with SVR (OR = 7.41, 95% CI = 4.05–13.57, $p < 0.0001$) independent of other covariates, such as platelet counts, fibrosis, and serum levels of HCV-RNA. Mutation in the ISDR was an independent predictor of SVR (OR = 2.11, 95% CI = 1.06–4.18, $p = 0.033$) but the amino acid at Core70 was not (Table 3).

Factors associated with the IL28B polymorphism

Patients with the *IL28B* minor allele had significantly higher serum level of gamma-glutamyltransferase (GGT) and a higher frequency of hepatic steatosis (Table 4). When the association between the *IL28B* polymorphism and HCV sequences was analyzed, Gln or His at Core70, that is linked to resistance to PEG-IFN and RBV therapy [4,14,15], was significantly more frequent in patients with the minor *IL28B* allele than in those with the major allele (67% vs. 30%, $p < 0.0001$) (Fig. 4). Other HCV sequences with an IFN resistant phenotype also were more prevalent in patients with the minor *IL28B* allele than those with the major allele: Met at Core91 (46% vs. 37%, $p = 0.047$) and one or no mutations in the ISDR (94% vs. 85%, $p = 0.004$) (Fig. 4).

Data mining analysis

Data mining analysis was performed to build a model for the prediction of SVR and the result is shown in Fig. 5. The analysis selected four predictive variables, resulting in six subgroups of patients. Genetic polymorphism of *IL28B* was selected as the best predictor of SVR. Patients with the minor *IL28B* allele had a lower probability of SVR and a higher probability of NVR than those with the major *IL28B* allele (SVR: 14% vs. 50%, NVR: 72% vs.

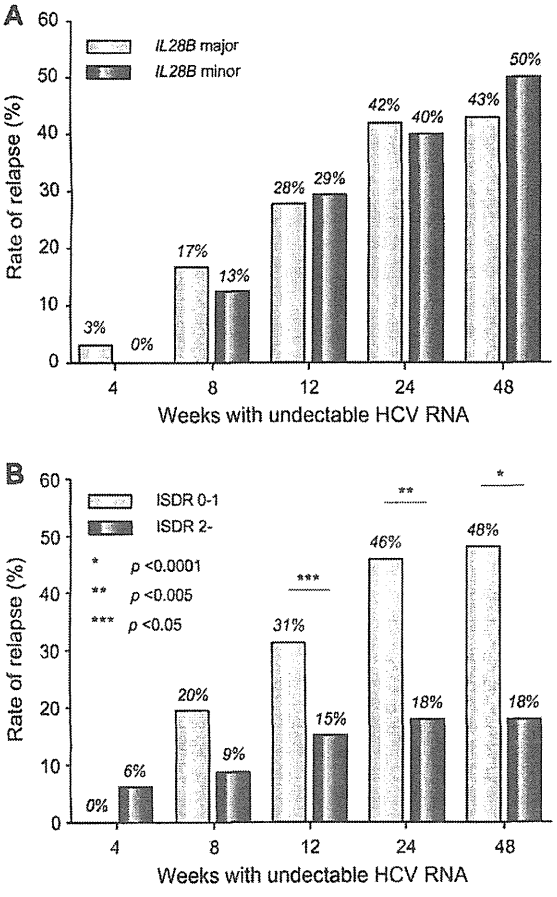

Fig. 3. Association between relapse and the *IL28B* allele or mutations in the ISDR. The rate of relapse was calculated for patients who had undetectable HCV-RNA at serial time points after the start of therapy (4, 8, 12, 24, and 48 weeks). Patients were stratified according to (A) the *IL28B* allele (minor allele vs. major allele) and (B) the number of mutations in the ISDR (0–1 mutation vs. 2 or more mutations). The *p* values are from Fisher's exact test.

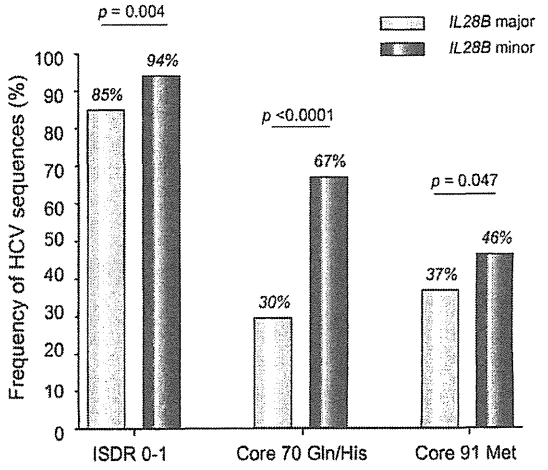

Fig. 4. Associations between the *IL28B* allele and HCV sequences. The prevalence of HCV sequences predicting a resistant phenotype to IFN was higher in patients with the minor *IL28B* allele than those with major allele. (A) 0 or 1 mutation in the ISDR of NS5A, (B) Gln or His at Core70, and (C) Met at Core91. *p* values are from Fisher's exact test.

12%). After stratification by the *IL28B* allele, patients with low platelet counts ($<140 \times 10^9$/L) had a lower probability of SVR and higher probability of NVR than those with high platelet counts ($\geq 140 \times 10^9$/L): for the minor *IL28B* allele, SVR was 7% vs. 19%, and NVR was 84% vs. 62%, and for the major *IL28B* allele, SVR was 32% vs. 66% and NVR was 16% vs. 8%. Among patients with the major *IL28B* allele and low platelet counts, those with two or more mutations in the ISDR had a higher probability of SVR and lower probability of relapse than those with one or no mutations in the ISDR (SVR: 75% vs. 27%, and relapse: 8% vs. 57%). Among patients with the major *IL28B* allele and high platelet counts, those with a low HCV-RNA titer (<600,000 IU/ml) had a higher probability of SVR and lower probability of NVR and relapse than those with a high HCV-RNA titer (SVR: 90% vs. 61%, NVR: 0% vs. 10%, and relapse: 10% vs. 29%). The sensitivity and specificity of the decision tree were 78% and 70%, respectively. The area under the receiver operating characteristic (ROC) curve of the model was 0.782 (data not shown). The proportion of patients with advanced fibrosis (F3-4) was 39% (84/217) in patients with low platelet counts ($<140 \times 10^9$/L) compared to 13% (37/279) in those with high platelet counts ($\geq 140 \times 10^9$/L).

Validation of the data mining analysis

The results of the data mining analysis were validated with 165 patients who differed from those used for model building. Each patient was allocated to one of the six subgroups for the validation using the flow-chart form of the decision tree. The rate of SVR and NVR in each subgroup was calculated. The rates of SVR and NVR for each subgroup of patients were closely correlated between the model building and the validation patients ($r^2 = 0.99$ and 0.98) (Fig. 6).

Discussion

The rate of NVR after 48 weeks of PEG-IFN/RBV therapy among patients infected with HCV of genotype 1 is around 20–30%. Previously, there have been no reliable baseline predictors of NVR or SVR. Because more potent therapies, such as protease and polymerase inhibitor of HCV [28,29] and nitazoxianide [30], are in clinical trials and may become available in the near future, a pre-treatment prediction of the likelihood of response may be helpful for patients and physicians, to support clinical decisions about whether to begin the current standard of care or whether to wait for emerging therapies. This study revealed that the *IL28B* polymorphism was the overwhelming predictor of NVR and is independent of host factors and viral sequences reported previously. The *IL28B* encodes a protein also known as IFN-lambda 3, which is thought to suppress the replication of various viruses including HCV [31,32]. The results of the current study and the findings of the GWAS studies [6–9] may provide the rationale for developing diagnostic testing or an IFN-lambda based therapy for chronic hepatitis C in the future.

Research Article

Table 2. Factors associated with NVR analyzed by univariate and multivariate logistic regression analysis.

	Univariate			Multivariate		
	Odds ratio	95%CI	*p* value	Odds ratio	95%CI	*p* value
Gender: female	0.98	0.67-1.45	0.938	1.29	0.75-2.23	0.363
Age	1.01	0.97-1.01	0.223	0.99	0.97-1.02	0.679
ALT	1.00	1.00-1.00	0.867	1.00	0.99-1.00	0.580
GGT	1.004	1.00-1.01	0.029	1.00	1.00-1.00	0.715
Platelets	0.95	0.91-0.99	0.009	0.92	0.87-0.98	0.006
Fibrosis: F3-4	2.23	1.46-3.42	0.0002	1.97	1.09-3.57	0.025
HCV-RNA: ≥600,000 IU/ml	1.83	1.05-3.19	0.035	2.49	1.17-5.29	0.018
ISDR mutation: ≤1	2.14	1.08-4.22	0.030	0.96	0.78-1.18	0.707
Core 70 (Gln/His)	3.23	2.16-4.78	<0.0001	1.41	0.83-2.42	0.207
Core 91 (Met)	1.39	0.95-2.06	0.093	1.21	0.72-2.04	0.462
IL28B: Minor allele	19.24	11.87-31.18	<0.0001	20.83	11.63-37.04	<0.0001

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; ISDR, interferon sensitivity determining region; Gln, glutamine; His, histidine; Met, methionine; Minor allele, heterozygote or homozygote of minor allele.

Table 3. Factors associated with SVR analyzed by univariate and multivariate logistic regression analysis.

	Univariate			Multivariate		
	Odds ratio	95%CI	*p* value	Odds ratio	95%CI	*p* value
Gender: female	0.81	0.56-1.16	0.253	0.86	0.55-1.35	0.508
Age	0.97	0.95-0.99	0.0003	0.99	0.96-1.01	0.199
ALT	1.00	1.00-1.00	0.337	1.00	[illegible]	0.108
GGT	1.00	1.00-1.00	0.273	1.00	1.00-1.00	0.797
Platelets	1.12	1.01-116	<0.0001	1.13	1.08-1.19	<0.0001
Fibrosis: F0-2	2.64	1.65-4.22	<0.0001	1.87	1.07-3.28	0.029
HCV-RNA: <600,000 IU/ml	2.49	1.55-3.98	0.0001	2.75	1.55-4.90	0.001
ISDR mutation: 2≤	3.78	2.14-6.68	<0.0001	2.11	1.06-4.18	0.033
Core 70 (Arg)	1.61	1.11-2.28	0.012	0.84	0.52-1.35	0.470
Core 91 (Leu)	1.28	0.88-1.85	0.185	1.26	0.81-1.96	0.300
IL28B: Major allele	6.21	3.75-10.31	<0.0001	7.41	4.05-13.57	<0.0001

ALT, alanine aminotransferase; GGT, Gamma-glutamyltransferase; ISDR, interferon sensitivity determining region; Arg, arginine; Leu, leucine; Major allele, homozygote of major allele.

Among baseline factors, *IL28B* was the most significant predictor of NVR and SVR. Moreover, the *IL28B* allele type was also correlated with early virological response: the rate of RVR and cEVR was significantly high for the *IL28B* major allele compared to the *IL28B* minor allele: 9% vs. 3% for RVR and 57% vs. 11% for cEVR (Fig. 2). On the other hand, the relapse rate was not different between the *IL28B* genotypes within patients who achieved RVR or cEVR (Fig. 3). We believe that optimal therapy should be based on baseline features and a response-guided approach. Our findings suggest that the *IL28B* genotype is a useful baseline predictor of virological response which should be used for selecting the treatment regimen: whether to treat patients with PEG-IFN and RBV or to wait for more effective future therapy including direct acting antiviral drugs. On the other hand, baseline *IL28B* genotype might not be suitable for determining the treatment duration in patients who started PEG-IFN/RBV therapy and whose virological response is determined because the *IL28B* genotype is not useful for the prediction of relapse. The duration of therapy should be personalized based on the virological response. Future studies need to explore whether the combination of baseline *IL28B* genotype and response-guided approach further improves the optimization of treatment duration.

The SVR rate in patients having the *IL28B* minor allele was 14% in the present study while it was 23% in Caucasians and 9% in African Americans in a study by McCarthy et al. [33]. On the other hand, the SVR rate in patients having the *IL28B* minor allele was 28% in genotypes 1/4 compared to 80% in genotypes 2/3 in a study by Rauch et al. [9]. These data imply that the impact of the *IL28B* polymorphism on response to therapy may be different in terms of race, geographical areas, or HCV genotypes, and that our data need to be validated in future studies including different populations and geographical areas before generalization.

Table 4. Factors associated with IL28B genotype.

	IL28B major allele n = 345	*IL28B* minor allele n = 151	*p* value
Gender: male	166 (48%)	84 (56%)	0.143
Age (years)	57 ± 10	57 ± 10	0.585
ALT (IU/L)	79 ± 60	78 ± 62	0.842
Platelets (10^9/L)	153 ± 54	155 ± 52	0.761
GGT (IU/L)	51 ± 45	78 ± 91	0.001
Fibrosis: F3-4	76 (22%)	45 (30%)	0.063
Steatosis:			
>10%	16/88 (18%)	13/23 (57%)	0.024
>30%	6/88 (7%)	6/23 (26%)	0.017
HCV-RNA: >600,000 IU/ml	284 (82%)	125 (83%)	1.000

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase.

Four GWAS studies have shown the association between a genetic polymorphism near the *IL28B* gene and response to PEG-IFN plus RBV therapy. The SNPs that showed significant association with response were rs12979860 [8] and rs8099917 [6,7,9]. There is a strong linkage-disequilibrium (LD) between these two SNPs as well as several other SNPs near the *IL28B* gene in Japanese patients [34] but the degree of LD was weaker in Caucasians and Hispanics [8]. Thus, the combination of SNPs is not useful for predicting response in Japanese patients but may improve the predictive value in patients other than Japanese who have weaker LD between SNPs.

Other significant predictors of response independent of *IL28B* genotype were platelet counts, stage of fibrosis, and HCV RVA load. A previous study reported that platelet count is a predictor of response to therapy [35], and the lower platelet count was related with advanced liver fibrosis in the present study. The association between response to therapy and advanced fibrosis independent of the *IL28B* polymorphism is consistent with a recent study by Rauch et al. [9].

There is agreement that the viral genotype is significantly associated with the treatment outcome. Moreover, viral factors such as substitutions in the ISDR of the NS5A region [10] or in the amino acid sequence of the HCV core [4] have been studied in relation to the response to IFN treatment. The amino acid Gln or His at Core70 and Met at Core91 are repeatedly reported to be associated with resistance to therapy [4,14,15] in Japanese patients but these data wait to be validated in different populations or other geographical areas. In this study, we confirmed that patients with two or more mutations in the ISDR had a higher rate of undetectable HCV-RNA at each time point during therapy. In addition, the rate of relapse among patients who achieved cEVR was significantly lower in patients with two or more mutations in ISDR compared to those with only one or no mutations (15% vs. 31%, $p < 0.05$). Thus, the ISDR sequence may be used to predict a relapse among patients who achieved virological response during therapy, while the *IL28B* polymorphism may be used to predict the virological response before therapy. A higher number of mutations in the ISDR are reported to have close association with SVR in Japanese [11–13,15,36] or Asian [37,38] populations but data from Western countries have been controversial [39–42]. A meta-analysis of 1230 patients including 525 patients from Europe has shown that there was a positive correlation between the SVR and the number of mutations in the ISDR in Japanese as well as in European patients [43] but this correlation was more pronounced in Japanese patients. Thus, geographical factors may account for the different impact of ISDR on treatment response, which may be a potential limitation of our study.

To our surprise, these HCV sequences were associated with the *IL28B* genotype: HCV sequences with an IFN resistant phenotype were more prevalent in patients with the minor IL28B allele than those with the major allele. This was an unexpected finding, as we initially thought that host genetics and viral sequences were completely independent. A recent study reported that the *IL28B* polymorphism (rs12979860) was significantly associated with HCV genotype: the *IL28B* minor allele was more frequent in HCV genotype 1-infected patients compared to patients infected with HCV genotype 2 or 3 [33]. Again, patients with the *IL28B* minor allele (IFN resistant genotype) were infected with HCV sequences that are linked to an IFN resistant phenotype. The mechanism for this association is unclear, but may be related to an interaction between the *IL28B* genotype and HCV sequences in the development of chronic HCV infection as discussed by McCarthy et al., since the *IL28B* polymorphism was associated with the natural clearance of HCV [44]. Alternatively, the HCV sequence within the patient may be selected during the course of chronic infection [45,46]. These hypotheses should be explored through prospective studies of spontaneous HCV clearance or by testing the time-dependent changes in the HCV sequence during the course of chronic infection.

How these host and viral factors can be integrated to predict the response to therapy in future clinical practice is an important question. Because various host and viral factors interact in the same patient, predictive analysis should consider these factors in combination. Using the data mining analysis, we constructed a simple decision tree model for the pre-treatment prediction of SVR and NVR to PEG-IFN/RBV therapy. The classification of patients based on the genetic polymorphism of *IL28B*, mutation in the ISDR, serum levels of HCV-RNA, and platelet counts, identified subgroups of patients who have the lowest probabilities of NVR (0%) with the highest probabilities of SVR (90%) as well as those who have the highest probabilities of NVR (84%) with the lowest probability of SVR (7%). The reproducibility of the model was confirmed by the independent validation based on a second group of patients. Using this model, we can rapidly develop an

Research Article

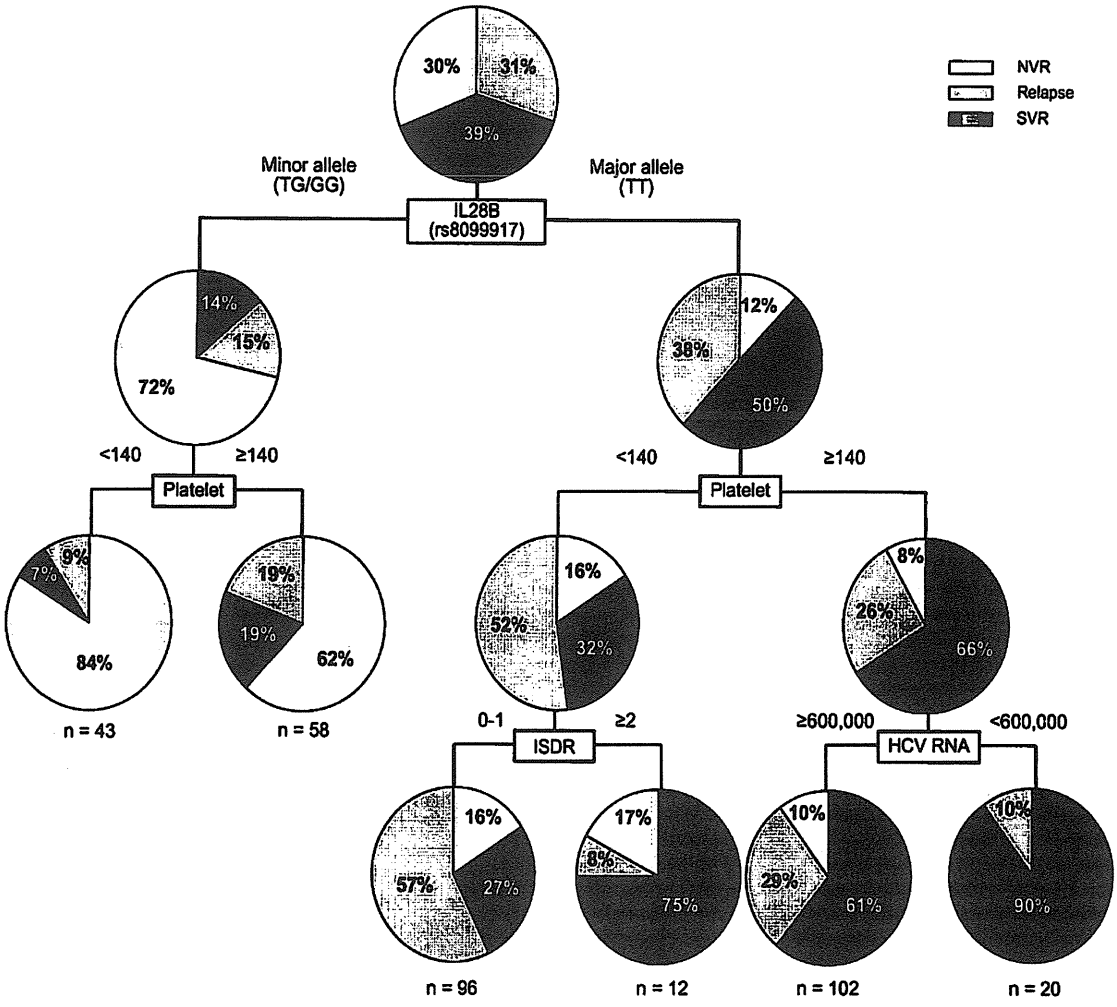

Fig. 5. Decision tree for the prediction of response to therapy. The boxes indicate the factors used for splitting. Pie charts indicate the rate of response for each group of patients after splitting. The rate of null virological response, relapse, and sustained virological response is shown.

estimate of the response before treatment, by simply allocating patients to subgroups by following the flow-chart form, which may facilitate clinical decision making. This is in contrast to the calculating formula, which was constructed by the traditional logistic regression model. This was not widely used in clinical practice as it is abstruse and inconvenient. These results support the evidence based approach of selecting the optimum treatment strategy for individual patients, such as treating patients with a low probability of NVR with current PEG-IFN/RBV combination therapy or advising those with a high probability of NVR to wait for more effective future therapies. Patients with a high probability of relapse may be treated for a longer duration to avoid a relapse. Decisions may be based on the possibility of a response against a potential risk of adverse events and the cost of the therapy, or disease progression while waiting for future therapy.

We have previously reported the predictive model of early virological response to PEG-IFN and RBV in chronic hepatitis C [26]. The top factor selected as significant was the grade of steatosis, followed by serum level of LDL cholesterol, age, GGT, and blood sugar. The mechanism of association between these factors and treatment response was not clear at that time. To our interest, a recent study by Li et al. [47] has shown that high serum level of LDL cholesterol was linked to the *IL28B* major allele (CC in rs12979860). High serum level of LDL cholesterol was associated with SVR but it was no longer significant when analyzed together with the *IL28B* genotype in multivariate analysis. Thus, the association between treatment response and LDL cholesterol levels may reflect the underlining link of LDL cholesterol levels to *IL28B* genotype. Steatosis is reported to be correlated with low lipid levels [48] which suggest that *IL28B* genotypes may be also associated with steatosis. In fact, there were significant correlations between the *IL28B* genotype and the presence of steatosis in the present study (Table 4). In addition, the serum level of GGT, another predictive factor in our previous study, was signif-