

- Obinata K, Maruyama T, Hayashi M, Watanabe T, Nittono H (1996) Effect of taurine on the fatty liver of children with simple obesity. *Adv Exp Med Biol* 403:607–613
- Ogasawara M, Nakamura T, Koyama I, Nemoto M, Yoshida T (1993) Reactivity of taurine with aldehydes and its physiological role. *Chem Pharm Bull (Tokyo)* 41:2172–2175
- Pacioretty L, Hickman MA, Morris JG, Rogers QR (2001) Kinetics of taurine depletion and repletion in plasma, serum, whole blood and skeletal muscle in cats. *Amino Acids* 21:417–427
- Parildar-Karpuzoglu H, Dogru-Abbasoglu S, Balkan J, Aykac-Toker G, Uysal M (2007) Decreases in taurine levels induced by beta-alanine treatment did not affect the susceptibility of tissues to lipid peroxidation. *Amino Acids* 32:115–119
- Parsons RB, Ramsden DB, Waring RH, Barber PC, Williams AC (1998) Hepatic localisation of rat cysteine dioxygenase. *J Hepatol* 29:595–602
- Pasantes MH, Wright CE, Gaull GE (1985) Taurine protection of lymphoblastoid cells from iron-ascorbate induced damage. *Biochem Pharmacol* 34:2205–2207
- Penttila KE (1990) Role of cysteine and taurine in regulating glutathione synthesis by periportal and perivenous hepatocytes. *Biochem J* 269:659–664
- Perez Tamayo R (1983) Is cirrhosis of the liver experimentally produced by CCl<sub>4</sub> and adequate model of human cirrhosis? *Hepatology* 3:112–120
- Pushpakiran G, Mahalakshmi K, Anuradha CV (2004) Protective effects of taurine on glutathione and glutathione-dependent enzymes in ethanol-fed rats. *Pharmazie* 59:869–872
- Refik Mas M, Comert B, Oncu K, Vural SA, Akay C, Tasci I, Ozkomur E, Serdar M, Mas N, Alcigir G, Yener N (2004) The effect of taurine treatment on oxidative stress in experimental liver fibrosis. *Hepatal Res* 28:207–215
- Reid WD (1972) Mechanism of allyl alcohol-induced hepatic necrosis. *Experientia* 28:1058–1061
- Reymond I, Sergeant A, Tappaz M (1996) Molecular cloning and sequence analysis of the cDNA encoding rat liver cysteine sulfinate decarboxylase (CSD). *Biochim Biophys Acta* 1307:152–156
- Serafini-Cessi F (1972) Conversion of allyl alcohol into acrolein by rat liver. *Biochem J* 128:1103–1107
- Shaffer JE, Kocsis JJ (1981) Taurine mobilizing effects of beta alanine and other inhibitors of taurine transport. *Life Sci* 28:2727–2736
- Sjovall J (1959) Dietary glycine and taurine on bile acid conjugation in man; bile acids and steroids 75. *Proc Soc Exp Biol Med* 100:676–678
- Slater TF, Cheeseman KH, Ingold KU (1985) Carbon tetrachloride toxicity as a model for studying free-radical mediated liver injury. *Philos Trans R Soc Lond B Biol Sci* 311:633–645
- Smith KE, Borden LA, Wang CH, Hartig PR, Brancheck TA, Weinshank RL (1992) Cloning and expression of a high affinity taurine transporter from rat brain. *Mol Pharmacol* 42:563–569
- Sohn OS, Ishizaki H, Yang CS, Fiala ES (1991) Metabolism of azoxymethane, methylazoxymethanol and *N*-nitrosodimethylamine by cytochrome P450IIE1. *Carcinogenesis* 12:127–131
- Sturman JA (1993) Taurine in development. *Physiol Rev* 73:119–147
- Sturman JA, Messing JM (1991) Dietary taurine content and feline reproduction and outcome. *J Nutr* 121:1195–1203
- Tappaz M, Bitoun M, Reymond I, Sergeant A (1999) Characterization of the cDNA coding for rat brain cysteine sulfinate decarboxylase: brain and liver enzymes are identical proteins encoded by two distinct mRNAs. *J Neurochem* 73:903–912
- Uchida S, Kwon HM, Yamauchi A, Preston AS, Marumo F, Handler JS (1992) Molecular cloning of the cDNA for an MDCK cell Na(+)- and Cl(-)-dependent taurine transporter that is regulated by hypertonicity. *Proc Natl Acad Sci USA* 89:8230–8234
- Vermeulen NP, Bessems JG, Van de Straat R (1992) Molecular aspects of paracetamol-induced hepatotoxicity and its mechanism-based prevention. *Drug Metab Rev* 24:367–407
- Warskulat U, Flögel U, Jacoby C, Hartwig HG, Thewissen M, Merx MW, Molojavyi A, Heller-Stilb B, Schrader J, Haussinger D (2004) Taurine transporter knockout depletes muscle taurine levels and results in severe skeletal muscle impairment but leaves cardiac function uncompromised. *FASEB J* 18:577–579
- Warskulat U, Borsch E, Reinehr R, Heller-Stilb B, Monnighoff I, Buchczyk D, Donner M, Flögel U, Kappert G, Soboll S, Beer S, Pfeffer K, Marschall HU, Gabrielsen M, Amiry-Moghaddam M, Ottersen OP, Dienes HP, Haussinger D (2006) Chronic liver disease is triggered by taurine transporter knockout in the mouse. *FASEB J* 20:574–576
- Watanabe A, Hobara N, Nagashima H (1985) Lowering of liver acetaldehyde but not ethanol concentrations by pretreatment with taurine in ethanol-loaded rats. *Experientia* 41:1421–1422
- Waterfield CJ, Turton JA, Scales MD, Timbrell JA (1991) Taurine, a possible urinary marker of liver damage: a study of taurine excretion in carbon tetrachloride-treated rats. *Arch Toxicol* 65:548–555
- Waterfield CJ, Turton JA, Scales MD, Timbrell JA (1993) Reduction of liver taurine in rats by beta-alanine treatment increases carbon tetrachloride toxicity. *Toxicology* 29:7–20
- Waters E, Wang JH, Redmond HP, Wu QD, Kay E, Bouchier-Hayes D (2001) Role of taurine in preventing acetaminophen-induced hepatic injury in the rat. *Am J Physiol Gastrointest Liver Physiol* 280:G1274–G1279
- Weltman MD, Farrell GC, Liddle C (1996) Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. *Gastroenterology* 111:1645–1653
- Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C (1998) Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. *Hepatology* 27:128–133
- Wilson AS, Davis CD, Williams DP, Buckpitt AR, Pirmohamed M, Park BK (1996) Characterisation of the toxic metabolite(s) of naphthalene. *Toxicology* 114:233–242
- Wu C, Kennedy DO, Yano Y, Otani S, Matsui-Yuasa I (1999) Thiols and polyamines in the cytoprotective effect of taurine on carbon tetrachloride-induced hepatotoxicity. *J Biochem Mol Toxicol* 13:71–76
- Wu G, Yang J, Sun C, Luan X, Shi J, Hu J (2009) Effect of taurine on alcoholic liver disease in rats. *Amino Acids* 643:313–322
- Yamamoto S (1994) Oral taurine therapy for painful muscle cramp in liver cirrhosis. *Am J Gastroenterol* 89:457–458
- You JS, Chang KJ (1998) Taurine protects the liver against lipid peroxidation and membrane disintegration during rat hepatocarcinogenesis. *Adv Exp Med Biol* 442:105–112

# グリチルリチン製剤

商品名 強力ネオミノファーゲンシー, ミノフィット, ヒシファーゲン, グリチロン, ネオファーゲンC

松崎 靖司

## 静注用グリチルリチン製剤

### 同種薬剤

強力ネオミノファーゲンシー<sup>®</sup>, ミノフィット<sup>®</sup>\*, ヒシファーゲン<sup>®</sup>\* (\*はナショナルセンター採用の後発品)

### 作用機序

抗アレルギー作用(抗アレルギー作用, アラキドン酸代謝系酵素の阻害作用), 免疫調節作用, 肝細胞傷害抑制作用, 肝細胞増殖促進作用, ウイルス増殖抑制・不活化作用などが実験的に確認されている。

### 代表的な病態に応じた使い方

強力ネオミノファーゲンシー<sup>®</sup>(SNMC)は, 国内 36 施設における慢性肝炎 133 症例を対象に, 1 日 40 ml, 1 カ月間連日投与の二重盲検比較試験が行われた。その結果, 本剤投与群 67 例中の有効率は 68.7%, プラセボ群 27.3% に比し, 明らかな有意の差をもって有効であること, 肝機能検査項目別では, AST, ALT および  $\gamma$ -GTP 値の改善が有意の差をもって認められた。これにより, 1979 年「慢性肝疾患における肝機能異常の改善」が追加承認された。

SNMC100 ml を 8 週間投与して治療前後の

肝組織を採取した。肝生検標本は投与前後をブラインド化。その判定の結果は, 投与後の肝組織は明らかに改善していた。これによって, トランスアミナーゼ値の改善された症例は, 組織像も良くなっていることが証明された。

さらに 1 日投与量 40 ml では効果の不十分な症例もあることから, 国内 11 施設における慢性肝炎, 肝硬変 178 症例を対象に, 1 日 40 ml, 3 週間連日静注投与を行い, 2 週目の ALT 値が正常値上限値の 1.5 倍以上に改善しなかった症例 93 例を対象に, 40 ml 継続投与群と 100 ml 増量投与群との用量別比較試験が行われた。その結果, 100 ml 増量投与群が 40 ml 継続投与群に比し, 有意に ALT 値を改善することが認められ, 40 ml で ALT 値改善不十分な症例に 100 ml 増量投与は有用であることが認められた。これにより, 1994 年, 慢性肝疾患の用法・用量に関する承認事項の一部変更が承認され, 1 日 100 ml を限度とした, 大量投与が可能となった。

- ① **薬剤名**: 強力ネオミノファーゲンシー<sup>®</sup>, ミノフィット<sup>®</sup>, ヒシファーゲン<sup>®</sup>
- ② **用法・用量**: 通常, 成人には 1 日 1 回 5~20 ml を静脈内に注射する。なお, 年齢, 症状により適宜増減する。慢性肝疾患に対しては 1 日 1 回 40~60 ml を静脈内に注射または点滴静注する。年齢, 症状により適宜増減する。な

### 肝底療法は肝細胞癌の発癌予防に有効か？

Ikedaらは表1に示す如く、342名の慢性C型肝炎患者のうち、SNMC投与を行った群での肝発癌率は5年で13.3%、10年で21.5%、非投与群では5年で26%、10年で35.5%であったと報告した。比例ハザードモデルを用いた解析では、SNMC注射の有無が有意に発癌率を低下させる要因として抽出された。

SNMCの肝発癌予防については、本邦における後ろ向き研究であるが、投与群が非投与群に比べ有意に肝発癌率が低下したとする報告がある。SNMC投与継続群84例、非投与群109例をレトロスペクティブに15年間追跡した。その結果、SNMC継続投与によりほぼ半数に発癌率の低下がみられ、ALT値が正常値の2倍以下に下降した症例では明らかな発癌抑制がみられた。この報告からC型慢性肝炎後の発癌予防には、ウイルスの排除が第一であるが、炎症を抑制してトランスアミナーゼ値を落ち着かせておくことも重要との認識が得られた。ただし、この報告では長期予後の遅いについては言及されていない。この報告からC型慢性肝炎後の発癌予防には、炎症を抑制してトランスアミナーゼ値を落ち着かせておくことも重要との認識が得られた。

お、増量する場合は1日100mlを限度とする。

③効果：慢性肝疾患における肝機能異常の改善

④使用上の注意点：高齢者の投与：低カリウム血症などの副作用の発現率が高い傾向が認められるので、患者の状態を観察しながら慎重に投与する。さらに、ショックなどの発現を予測するため、十分な問診を行う、ショック発現時に救急処置のとれる準備をしておく、投与後、患者を安静な状態に保たせ、十分な観察を行うなどの基本的注意が必要である。また、甘草を含有する製剤との併用は、本剤に含まれるグリチルリチン酸が重複し、偽アルドステロン症が現れやすくなるので注意する。妊婦への投与に関する安全性は確立していないので、これらの患者には治療上の有益性が危険性を上回ると判断される場合にのみ投与する。

注射速度：静脈内投与は、患者の状態を観察しながらできるだけ投与速度を緩徐にする必要

【表1】強力ミノファージェン注は肝発癌のリスクを低下させる(文献3より抜粋)

Factors	Category	Risk Ratio(95% CI)	p
線維化レベル	F1	1	
	F2~3	2.94(1.20~7.21)	.018
	F4	9.21(3.73~22.8)	<.001
性別	1: Female	1	
	2: Male	2.80(1.35~5.81)	.006
SNMC注の有無	1: No	1	
	2: Yes	0.49(0.27~0.86)	.014

がある。グリチルリチン酸または甘草を含有する製剤の経口投与により、横紋筋融解症が現れたとの報告がある。

### 注意すべき副作用・相互作用

ループ利尿薬などを併用した場合、カリウム排泄が増強し血清カリウム低下が現れやすくなる。重大な副作用として、ショック、アナフィラキシーショック、アナフィラキシー様症状、偽アルドステロン症(頻度不明)：低カリウム血症、血圧上昇、ナトリウム・体液の貯留、浮腫、尿量減少、体重増加などの偽アルドステロン症が現れることがある。また低カリウム血症のため、脱力感、筋力低下、などの症状が現れることがある。

### 適応外使用

中等度以上の肝細胞障害型薬物性肝障害例(ALT 300 IU/ml以上)においては、SNMCの静注を行いALT改善に努める。SNMCは1回、20~100mlの静注をする。

### 経口グリチルリチン製剤

#### 同種薬剤

グリチロン®錠

## 作用機序

前記 SNMC と同様に抗アレルギー作用、免疫調節作用、肝細胞傷害抑制作用、肝細胞増殖促進作用、などが実験的に確認されている。

## 代表的な病態に応じた使い方

慢性肝炎についての二重盲検比較試験が 1980 年代に施行された。その結果、国内 19 施設における慢性肝炎 224 例に対して本剤 1 日 9 錠、連日 12 週間経口投与を行われ、本剤投与群はプラセボ群に比し有意に肝機能の改善が認められた。

①薬剤名：グリチロン<sup>®</sup>、ネオファーゲン C<sup>®</sup>錠

②用法・用量：通常、成人には 1 回～3 錠、小児には 1 錠を 1 日 3 回食後経口投与する。なお、年齢、症状により適宜増減する。

③効果：慢性肝疾患における肝機能異常の改善

④使用上の注意点：高齢者の投与には、低カリウム血症などの副作用の発現率が高い傾向が認められるので、患者の状態を観察しながら慎重に投与する。そのほか、前記 SNMC の内容と

同様である。

## 注意すべき副作用・相互作用

ループ利尿薬などを併用した場合、カリウム排泄が増強し血清カリウム低下が現れやすくなる。重大な副作用として、偽アルドステロン症（頻度不明）：低カリウム血症、血圧上昇、ナトリウム・体液の貯留、浮腫、尿量減少、体重増加などの偽アルドステロン症が現れることがある。また、脱力感、筋力低下、筋肉痛、四肢痙攣・麻痺などの横紋筋融解症の症状が現れることがあるので注意を要する。

## 文献

- 1) Iino S, et al : Therapeutic effects of stronger neominophagen C at different doses on chronic hepatitis and liver cirrhosis. *Hepato Res*, 19 : 31-40, 2001
- 2) Arase Y, et al : The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 79 : 1494-1500, 1997
- 3) Ikeda K, et al : A long-term glycyrrhizin injection therapy reduces hepatocellular carcinogenesis rate in patients with interferon-resistant active chronic hepatitis C : a cohort study of 1249 atients. *Dig Dis Sci* 51 : 603-609, 2006
- 4) 松崎靖司, 他 : インターフェロン療法が行えない場合の対応. *medicina* 47 : 446-450, 2010

MEDICAL BOOK INFORMATION

医学書院

# 今日の救急治療指針 第2版

監修 前川和彦・相川直樹  
編集 杉本 壽・堀 進悟・行岡哲男・山田至康・坂本哲也

臨床の第一線で活躍している執筆陣による救急に特化した治療指針。救急外来で遭遇する症候・傷病に関して、「緊急度」と「重症度」を重視して編集。初療時の考え方や対応の仕方（最初にすること、重症度を見分けるポイント、入院の判断基準）など、救急の現場で役立つ知識が満載。

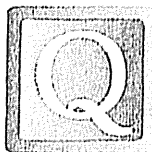
●A5 頁984 2012年  
定価 13,650円(本体13,000円+税5%)  
[ISBN978-4-260-01218-8]

▶ 回 答

久留米大学医学部内科学講座消化器内科部門・

消化器疾患情報講座 1) 講師 2) 教授

1) 川口 巧 2) 佐田通夫



## IFN 治療不応・高齢者 C 型慢性肝炎難治例の肝庇護療法

70 歳以上高齢者の C 型慢性肝炎で、PEG-IFN + リバビリン療法で治療が完結せず、SNMC、UDCA 併用療法でも ALT 値が十分低下しない難治例について、最も効果が期待される肝庇護療法（組み合わせ、投与法）のデータがあれば併せて。  
(長野県 H)

# A

**UDCA、SNMC などの肝庇護剤は安全性も高いが、患者個人の反応によってテーラーメイド化して施行すべきであり、きめ細やかな対応ができるかかりつけ医に向けた治療法であると言える**

SNMC + UDCA は我が国においては肝庇護療法のスタンダードであり、多くの医師がこれを使用している。しかし、それらの ALT 値改善効果は限定されており、これらのみでは ALT 値を正常化することはなかなか難しい。UDCA の投与量は、慢性 C 型肝炎には 1 日 600mg の投与が推奨されており<sup>1)</sup>、この効果は濃度依存性であり、保険診療上は 1 日 900mg までは認められている。下痢などの副作用が問題ない用量までこれを増量することが可能である。

SNMC の使用量、使用間隔については、通常は 1 回 40mL・週 3 回投与が一般的であるが、1 回量 100mL まで増量可能であり、また、回数も連日まで増やすことが可能である。血圧上昇や低カリウム血症 (hypokalemia) に注意を払う必要があること、来院が頻繁になることなどから患者の利便性が低いことなどの問題があるが、検査データを見ながら患者それぞれに合わせて行う。

これらの組み合わせで ALT 値がまだ高い場合、IFN に対する副作用などで治療が中止になった例以外で、本人の理解が得られる場合は、IFN 少量長期投与も検討対象となる。以前の IFN 治療で、SVR には至らなかったものの ALT が正常化した症例がよい適応となる。PEG-IFN  $\alpha$ -2a (ペガシス<sup>®</sup>) 90  $\mu$ g を 1~2 週に 1 回の割合、あるいは自己注射が可能な IFN  $\alpha$  (スミフェロン<sup>®</sup>DS) を 1 回量 300 万 IU、週 3 回投与する。いずれの場合も効果・副作用により適宜増減し、治療は可及的に長期に行う。通常の IFN 治療と同様、血小板数の減少や開始当初のインフルエンザ症状などの副作用はあるが、投与量が少なく、また治療間隔も空くため、比較的耐容性がよいことが多いが、一般の診療所で行

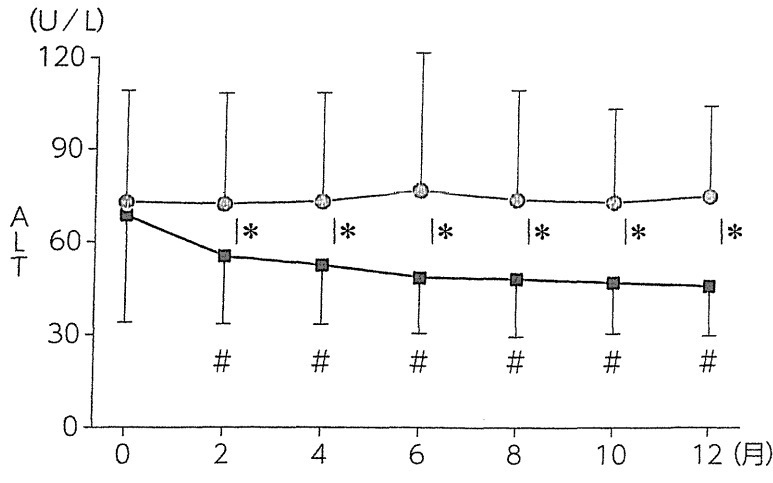


図1 SNMC+少量瀉血療法 (n=36) 群 (■) とSNMC療法 (n=36) 群 (●) でのALTレベルの変化(平均値 ± 標準偏差)

\* P<0.05 同時期のSNMC群に比べて有意差あり。

# P<0.05 ベースラインのALT値に比べて有意差あり。

(文献<sup>4)</sup>より改変)

う場合は、導入期は肝臓専門医の意見を聞きながら施行したほうが安全である。ウイルス量の減少が得られなくても、ALT値が正常化することが多いが、投与開始後6カ月でALT値あるいはAFP値が改善しない場合は中止を検討すべきである。

IFNが使用できない症例に対して考えるべき肝庇護療法としては、瀉血療法がある。瀉血は一般的には2~4週ごとに200~400mL行い、血清フェリチン値10ng/mLあるいはHb値10~11g/dLを目標とする<sup>2)</sup>。70歳以上の高齢者では、Hbもあまり高くないことが多く、貧血に伴う合併症(心不全や脳梗塞など)も懸念されるため、慎重に行うべきである。瀉血はUDCAまたはSNMCとの併用で相加的な効果が認められ、UDCAと瀉血療法の併用ではUDCA単独療法よりもALT値の低下が見られること<sup>3)</sup>、またSNMCとの併用では、SNMCを投与する際に少量の瀉血を併用することによってALT値がさらに改善することが報告されている(図1)<sup>4)</sup>。これはSNMCの注射前に週60mL程度の血液を瀉血する方法で、高齢者や代償性肝硬変(compensated cirrhosis)症例にも安全に施行できたという。例えば、週3回注射に来た場合は、1回20mLの瀉血を行う。

◎文献

- 1) Omata M, et al: Gut 56: 1747, 2007.
- 2) Hayashi H, et al: Am J Gastroenterol 89: 986, 1994.
- 3) Wakusawa S, et al: Hepatol Res 18: 54, 2000.
- 4) Tanaka N, et al: J Gastroenterol 44: 577, 2009.

▶回答

東京医科大学茨城医療センター消化器内科

1) 准教授 2) 教授

1) 池上 正 2) 松崎靖司

## 内科



## γ-GTPとLDHのみ高値の理由

65歳，男性．臨床検査で20年ほど前からγ-GTP 130 IU/L前後（基準値75 IU/L），LDH 280 IU/L前後（基準値115～245 IU/L）と高値が続いている．AST 29 IU/L，ALT 23 IU/Lと常に正常．全身状態は正常．飲酒はほとんどしない．心，肝，腎その他疾患なし．中肉中背，肥満なし．γ-GTP，LDHが常に高い理由について．

（埼玉県 S）

### A

まずAST，ALTが完全に正常値である間隔，異常値の継続期間を明確にした上でγ-GTP，LDHともに疾患を鑑別する．本例のような場合，病的に問題となるケースは少ないことが多い

γ-GTP (γ-glutamyl transpeptidase) は，ペプチドのN末端のグルタミン酸を他のペプチドまたはアミノ酸に転移する酵素である．解毒に関与している物質であるグルタチオンを作るのに重要な役割を果たしている．グルタチオンは肝ミクロソームにおける薬物代謝などに重要な役割を持つため，γ-GTPは肝細胞に多量に含まれる．ある種の薬物（ジアゼパム，フェニトイン，フェノバルビタールなどの向精神薬）などにより，γ-GTPはミクロソーム酵素としての誘導を受け血中濃度は上昇する．

さらにγ-GTPは胆道から分泌される酵素でもある．また，生体内には腎臓に最も多く含まれ，次いで膵臓，肝臓，脾臓，精巣，小腸などに多く含まれるが，血液中に存在するγ-GTPのほとんどが肝臓由来のため，血液中のγ-GTPは主に肝臓・胆道系の異常を反映する．つまり，γ-GTPは肝臓などの組織が障害を受けることによって，組織内のγ-GTPが血液中に流れ出てしまう場合（逸脱）と，アルコールや薬物などによって組織の酵素量が増える（酵素誘導）ことによって異常値を示す．またγ-GTP値には性差があり，女性ホルモンがγ-GTPの生成を抑制してい

ると考えられ，女性に比べて男性のほうが高値を示しやすいとされる．また，年齢とともに軽度の上昇傾向があるとされている．

γ-GTPの存在は毛細胆管側や胆管上皮細胞などに多く見られるので，高値になる場合は，胆汁うっ滞を呈する薬物性，ウイルス性肝炎，原発性胆汁性肝硬変（primary biliary cirrhosis；PBC），原発性硬化性胆管炎（primary sclerosing cholangitis；PSC）などの胆管病変を呈するもの，胆道閉塞などや，アルコール性肝障害，脂肪肝といった肝細胞障害でも認められる．胆石，肝臓癌などでも上昇する．γ-GTPのみ高値を示すケースでは，その原因の多くはアルコール摂取によるものである．最近，アルコールとは無関係に，栄養過剰や肥満がもとでγ-GTPやALTが上昇する，非アルコール性脂肪性肝疾患（non-alcoholic fatty liver disease；NAFLD），非アルコール性脂肪性肝炎（non-alcoholic steatohepatitis；NASH）と言われる病気が増えてきている．これらの場合，γ-GTPのみが高い場合もある．

以上のような異常値を示す機序を勘案し，病態を考えることになる．乳酸脱水素酵素（lactate dehydrogenase；LDH）は，細胞内

で糖をエネルギーに変える際に必要な酵素で、あらゆる臓器に含まれているが、特に心臓、肺、腎臓、脳、肝臓、骨格筋、血球（赤血球、白血球）に多く含まれる。よって、それらの細胞が障害（破壊）を受けると細胞外に出てくるため、血液中のLDHは高値を示す。高値の場合、肝疾患、心疾患、筋疾患、その他（悪性貧血、白血病、溶血性貧血など）が考えられる。

食事による変動はないが、運動によって上昇することがある。ジョギングなどの日常の軽い運動でも高値となり、時には1週間近く続くこともある。検査数日前はなるべく運動を控えたほうがよい。基準値は検査方法や測定方法、測定機器、用いる試薬、単位などにより値が異なる。また、個人個人の生理的変動によってかなり左右されることもあるため、少し値が基準値から外れていたからといって神経質にならないことである。

これらの機序を考えると、本例の場合、まず両検査値とも20年前から同じ施設で同じ測定法でチェックされているか、また数値はどのくらいの値で、どのくらいの間隔で検査を行い、どのくらいの期間異常値を指摘されていたかなどを、まず明確にしなくてはならないことが前提である。またLDHに関しては、同施設で連続的に異常値を示しているかは重要な点である。採血の時に溶血してもLDHは高値となるため、よく吟味することが必要である。

$\gamma$ -GTPに関しては、上記のようにAST、ALTが完全に正常値であることがどのくらいの間隔で、どのくらいの期間、同施設で異常値が継続しているか、明確にすることが肝

要である。 $\gamma$ -GTP、LDHともに前記のような疾患を鑑別することが第一であろう。次に画像検査などでも異常もなく、諸検査によりすべての疾患が否定された場合、どのように考えればよいか問題となる。

明確な文献、EBMはないのであるが、筆者らの経験を述べる。LDHが異常値である点に関しては、上記の鑑別診断を施行することで比較的容易に判断できるものと考えて。 $\gamma$ -GTPの異常値に関しての考察が重要であろう。現在、比較的多く見られる疾患は、飲酒が完全に否定されている場合、栄養過剰や肥満が原因で起こる単純な脂肪肝、特にNAFLDが多い。ただ、肝生検を施行しないと完全には診断できるものではない。

筆者らの経験例では、同様の事例に肝生検を施行してもほとんど異常を認めないことがあり、診断が明確につかない場合があった。肝生検は肝臓すべての場所をチェックできるものではなく、小さな組織片であるので、サンプリング・エラーもあり一概にすべて正常かは明確にできない。また、薬物、健康食品、食品添加物などで異常を来す場合もあり、原因の特定には大変苦勞する。また、過去に薬物性肝障害を起こし、正常化せず $\gamma$ -GTPの異常値だけが長期にくすぶる場合もある。

以上の通りであり、本例のような場合で病的に問題となるケースは少ないことが多いことを最後に記す。

◆回答

東京医科大学茨城医療センター長  
松崎靖司



## FGF3/FGF4 Amplification and Multiple Lung Metastases in Responders to Sorafenib in Hepatocellular Carcinoma

Tokuzo Arai,<sup>1\*</sup> Kazuomi Ueshima,<sup>2\*</sup> Kazuko Matsumoto,<sup>1\*</sup> Tomoyuki Nagai,<sup>1,2</sup> Hideharu Kimura,<sup>1</sup> Satoru Hagiwara,<sup>2</sup> Toshiharu Sakurai,<sup>2</sup> Seiji Haji,<sup>3</sup> Akishige Kanazawa,<sup>4</sup> Hisashi Hidaka,<sup>5</sup> Yukihiko Iso,<sup>6</sup> Keiichi Kubota,<sup>6</sup> Mitsuo Shimada,<sup>7</sup> Tohru Utsunomiya,<sup>7</sup> Masashi Hirooka,<sup>8</sup> Yoichi Hiasa,<sup>8</sup> Yoshikazu Toyoki,<sup>9</sup> Kenichi Hakamada,<sup>9</sup> Kohichiroh Yasui,<sup>10</sup> Takashi Kumada,<sup>11</sup> Hidenori Toyoda,<sup>11</sup> Shuichi Sato,<sup>12</sup> Hiroyuki Hisai,<sup>13</sup> Teiji Kuzuya,<sup>14</sup> Kaoru Tsuchiya,<sup>14</sup> Namiki Izumi,<sup>14</sup> Shigeki Arai,<sup>15</sup> Kazuto Nishio,<sup>1</sup> and Masatoshi Kudo<sup>2</sup>

The response rate to sorafenib in hepatocellular carcinoma (HCC) is relatively low (0.7%–3%), however, rapid and drastic tumor regression is occasionally observed. The molecular backgrounds and clinico-pathological features of these responders remain largely unclear. We analyzed the clinical and molecular backgrounds of 13 responders to sorafenib with significant tumor shrinkage in a retrospective study. A comparative genomic hybridization analysis using one frozen HCC sample from a responder demonstrated that the 11q13 region, a rare amplicon in HCC including the loci for *FGF3* and *FGF4*, was highly amplified. A real-time polymerase chain reaction–based copy number assay revealed that *FGF3/FGF4* amplification was observed in three of the 10 HCC samples from responders in which DNA was evaluable, whereas amplification was not observed in 38 patients with stable or progressive disease ( $P = 0.006$ ). Fluorescence *in situ* hybridization analysis confirmed *FGF3* amplification. In addition, the clinico-pathological features showed that multiple lung metastases (5/13,  $P = 0.006$ ) and a poorly differentiated histological type (5/13,  $P = 0.13$ ) were frequently observed in responders. A growth inhibitory assay showed that only one *FGF3/FGF4*-amplified and three *FGFR2*-amplified cancer cell lines exhibited hypersensitivity to sorafenib *in vitro*. Finally, an *in vivo* study revealed that treatment with a low dose of sorafenib was partially effective for stably and exogenously expressed *FGF4* tumors, while being less effective in tumors expressing *EGFP* or *FGF3*. **Conclusion:** *FGF3/FGF4* amplification was observed in around 2% of HCCs. Although the sample size was relatively small, *FGF3/FGF4* amplification, a poorly differentiated histological type, and multiple lung metastases were frequently observed in responders to sorafenib. Our findings may provide a novel insight into the molecular background of HCC and sorafenib responders, warranting further prospective biomarker studies. (HEPATOLOGY 2013;57:1407–1415)

Abbreviations: 5FU, 5-fluorouracil; CGH, comparative genomic hybridization; DMEM, Dulbecco's modified Eagle's medium; EGFR, epidermal growth factor receptor; FBS, fetal bovine serum; FFPE, formalin-fixed, paraffin-embedded; FISH, fluorescence *in situ* hybridization; HCC, hepatocellular carcinoma; IC<sub>50</sub>, 50% inhibitory concentration; mRNA, messenger RNA; PCR, polymerase chain reaction; PIVKA-II, protein induced by vitamin K absence or antagonist-II; RPMI-1640, Roswell Park Memorial Institute 1640; RT-PCR, reverse-transcription PCR.

From the <sup>1</sup>Department of Genome Biology, <sup>2</sup>Department of Gastroenterology and Hepatology, and <sup>3</sup>Department of Surgery, Kinki University Faculty of Medicine, Osaka, Japan; the <sup>4</sup>Department of Hepato-Biliary-Pancreatic Surgery, Osaka City General Hospital, Miyakojima-hondori, Miyakojima-ku, Osaka, Japan; the <sup>5</sup>Department of Gastroenterology, Internal Medicine, Kinsato University East Hospital, Sagamihara, Japan; the <sup>6</sup>Second Department of Surgery, Dokkyo Medical University, Mibu, Japan; the <sup>7</sup>Department of Surgery, The University of Tokushima, Tokushima, Japan; the <sup>8</sup>Department of Gastroenterology and Metabolism, Ehime University Graduate School of Medicine, Ehime, Japan; the <sup>9</sup>Department of Gastroenterological Surgery, Hiroaki University Graduate School of Medicine, Hiroaki, Japan; the <sup>10</sup>Department of Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan; the <sup>11</sup>Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan; the <sup>12</sup>Department of Gastroenterology and Hepatology, Shimane University, Faculty of Medicine, Luno, Japan; the <sup>13</sup>Department of Gastroenterology, Japan Red Cross Date General Hospital, Date, Japan; the <sup>14</sup>Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan; and the <sup>15</sup>Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University, Graduate School of Medicine, Tokyo, Japan.

Received October 7, 2011; accepted June 25, 2012.

Supported by the Third-Term Comprehensive 10-Year Strategy for Cancer Control (K. N.), a National Cancer Center Research and Development Fund (H22-015, to M. K.), and a Grant-in-Aid for Scientific Research (23650627, to K. N.).

\*These authors contributed equally to this work.

treatment. The present study was approved by the institutional review boards of all the centers involved in the study, and informed consent was obtained from the patients.

**Isolation of Genomic DNA.** Genomic DNA samples were extracted from deparaffinized tissue sections preserved as FFPE tissue using a QIAamp DNA Micro kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA samples were extracted from surgical frozen sections using a QIAamp DNA Mini kit (Qiagen) according to the manufacturer's instructions. The DNA concentration was determined using the NanoDrop2000 (Thermo Scientific, Waltham, MA).

**Comparative Genomic Hybridization Analysis.** The Genome-wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA) was used to perform array comparative genomic hybridization (CGH) on genomic DNA from HCC and paired liver samples according to the manufacturer's instructions. A total of 250 ng of genomic DNA was digested with both Nsp I and Sty I in independent parallel reactions, subjected to restriction enzymes, ligated to the adaptor, and amplified using polymerase chain reaction (PCR) with a universal primer and TITANIUM Taq DNA Polymerase (Clontech, Palo Alt, CA). The PCR products were quantified, fragmented, end-labeled, and hybridized onto a Genome-wide Human SNP6.0 Array. After washing and staining in Fluidics Station 450 (Affymetrix), the arrays were scanned to generate CEL files using the GeneChip Scanner 3000 and GeneChip Operating Software version 1.4. In the array CGH analysis, sample-specific copy number changes were analyzed using Partek Genomic Suite 6.4 software (Partek Inc., St. Louis, MO).

**Copy Number Assay.** The copy numbers for *FGF3* and *FGF4* were determined using commercially available and predesigned TaqMan Copy Number Assays according to the manufacturer's instructions (Applied Biosystems, Foster City, CA) as described.<sup>10</sup> The primer IDs used for the *FGFs* were as follows: *FGF3*, Hs06336027\_cn; *FGF4*, HS01235235\_cn. The *TERT* locus was used for the internal reference copy number. Human Genomic DNA (Clontech) and DNA from noncancerous FFPE tissue were used as a normal control.

**Real-Time Reverse-Transcription PCR.** Real-time reverse-transcription PCR (RT-PCR) was performed as described.<sup>11</sup> In brief, complementary DNA was prepared from the total RNA obtained from each surgical frozen section using a GeneAmp RNA-PCR kit (Applied Biosystems). Real-time RT-PCR amplification

was performed using a Thermal Cycler Dice (TaKaRa, Otsu, Japan) in accordance with the manufacturer's instructions under the following conditions: 95°C for 5 minutes, followed by 50 cycles of 95°C for 10 seconds and 60°C for 30 seconds. The primers used for the real-time RT-PCR were as follows: *FGF3*, 5'-TTT GGA GAT AAC GGC AGT GGA-3' (forward) and 5'-CGT ATT ATA GCC CAG CTC GTG GA-3' (reverse); *FGF4*, 5'-GAG CAG CAA GGG CAA GCT CTA-3' (forward) and 5'-ACC TTC ATG GTG GGC GAC A-3' (reverse); *GAPD*, 5'-GCA CCG TCA AGG CTG AGA AC-3' (forward) and 5'-ATG GTG GTG AAG ACG CCA GT-3' (reverse). *GAPD* was used to normalize expression levels in the subsequent quantitative analyses.

**Fluorescence In Situ Hybridization Analysis.** Fluorescence *in situ* hybridization (FISH) was performed as described.<sup>10</sup> Probes designed to detect the *FGF3* gene and *CEN11p* on chromosome 11 were labeled with fluorescein isothiocyanate or Texas red and were designed to hybridize to the adjacent genomic sequence spanning approximately 0.32 Mb and 0.63 Mb, respectively. The probes were generated from appropriate clones from a library of human genomic clones (GSP Laboratory, Kawasaki, Japan).

**Immunoblotting.** Western blot analysis was performed as described.<sup>11</sup> The following antibodies were used: monoclonal FGF3 (R&D Systems, Minneapolis, MN), FGF4 and FGFR2 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), and phosphorylated FGFR and horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology, Beverly, MA). NIH-3T3 cells were exposed to the indicated concentrations of sorafenib for 2 hours and were then stimulated with FGF4-conditioned medium for 20 minutes.

**Cell Growth Inhibitory Assay.** To evaluate growth inhibition in the presence of various concentrations of sorafenib, we used an MTT assay as described.<sup>12</sup>

**Plasmid Construction, Viral Production, and Stable Transfectants.** The methods used in this section have been described.<sup>12</sup> The complementary DNA fragment encoding human full-length *FGF3* or *FGF4* was isolated using PCR and Prime STAR HS DNA polymerase (TaKaRa, Otsu, Japan) with following primers: *FGF3*, 5'-GG GAA TTC GCC GCC ATG GGC CTA ATC TGG CTG CTA-3' (forward) and 5'-CC CTC GAG GCC CAG CTA GTG CGC ACT GGC CTC-3' (reverse); *FGF4*, 5'-GG GAA TTC GCC GCC ATG TCG GGG CCC GGG ACG GCC GCG GTA GCG C-3' (forward) and 5'-CC CTC GAG

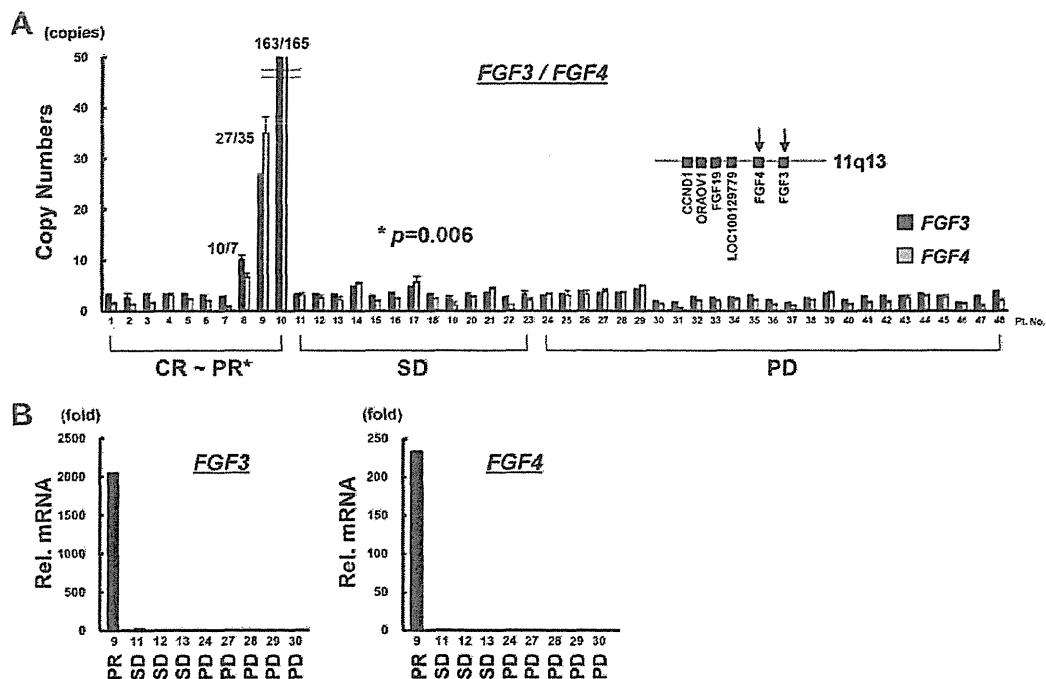


Fig. 2. *FGF3/FGF4* gene amplification is frequently observed in responders to sorafenib in HCC. (A) *FGF3/FGF4* gene amplification was determined using the TaqMan copy number assay in DNA samples obtained from 48 HCC samples that had been treated with sorafenib. *FGF3* amplification of >5 copies was observed in three of the sorafenib responders. \*Complete response + partial response versus stable disease + progressive disease. (B) *FGF3/FGF4* gene amplification mediates the overexpression of *FGF3/FGF4* mRNA. The mRNA expression levels of *FGF3* and *FGF4* were examined in nine HCC samples that were available as frozen samples among 48 HCC samples that were treated with sorafenib. Rel. mRNA, target gene/*GAPD*  $\times 10^6$ .

The 11q13 locus is known to be a frequently amplified region in several human cancers except HCC.<sup>13</sup> Thus, we hypothesized that the amplification of 11q13 may be involved in a marked response to sorafenib.

***FGF3/FGF4* Gene Amplification Is Frequently Observed in Responders to Sorafenib.** To address the question of whether *FGF3/FGF4* gene amplification is also found in the HCC of other responders to sorafenib, we examined HCC specimens collected from 11 other medical centers in Japan. Because most of the HCC samples were collected as FFPE samples, we used a TaqMan Copy number assay.<sup>10</sup> A copy number assay revealed that *FGF3/FGF4* amplification was observed in three of the 10 (30%) HCC samples that responded to sorafenib, whereas no amplification was observed in the 38 specimens from patients with stable or progressive disease ( $P = 0.006$ , Fig. 2A). The copy numbers for *FGF3/FGF4* were  $10.2 \pm 0.8/6.7 \pm 0.8$ ,  $26.7 \pm 0.4/35.1 \pm 3.1$ , and  $162.5 \pm 9.0/165.0 \pm 12.5$  copies in the amplified samples, whereas the copy numbers of *FGF3* for all the other samples were below 5 copies. The correlation between the *FGF3* locus and the *FGF4* locus copy numbers was very high ( $R = 0.998$ ), indicating that the DNA copy number assay

for *FGF3/FGF4* was a sensitive and reproducible method.

***FGF3/FGF4* Gene Amplification Mediates the Overexpression of *FGF3/FGF4* Messenger RNA.** We examined the messenger RNA (mRNA) expression levels of *FGF3/FGF4* in nine HCC samples that were available as frozen samples among the 48 sorafenib-treated samples, as shown in Fig. 2A. One amplified sample expressed extremely high mRNA levels of *FGF3/FGF4* compared with nonamplified samples (Fig. 2B). The results demonstrated that *FGF3/FGF4* gene amplification mediates the overexpression of *FGF3/FGF4* mRNAs and proteins (Figs. 2B and 1D).

***FISH Analysis Confirmed *FGF3/FGF4* Gene Amplification.*** We used FISH analysis to examine *FGF3/FGF4* amplification and to verify the results of the above-described PCR-based DNA copy number assay. All *FGF3/FGF4*-amplified clinical samples were confirmed as exhibiting high-level *FGF3* amplification using FISH analysis (Fig. 3). One patient showed multiple scattered signals, whereas two patients showed large clustered signals. Nonamplified HCC yielded a negative result for gene amplification. These results clearly demonstrate the presence of *FGF3/FGF4*

**Table 2. Clinicopathological Characteristics and *FGF3/FGF4* Gene Amplification in Responders and Nonresponders to Sorafenib**

Characteristic	Responders (n = 13)	Nonresponders (n = 42)	P Value*
Age, years (range)	63 (47-84)	66 (22-89)	0.98
Sex, M/F	10/3	30/12	0.97
Viral status, no.			0.69
HBV	5	10	
HCV	6	16	
B+C	0	1	
Non-B, non-C	2	15	
AFP, ng/mL (range)	378 (8-404,100)	56 (2-114,248)	0.33
PIVKA-II, mAU/mL (range)	728 (14-847,000)	81 (11-147,000)	0.78
Clinical stage, no.			0.73
II	0	1	
III	3	13	
IV	10	28	
Primary tumor, cm (range)	5 (0-14)	3 (0-15)	0.20
Lung metastasis, no.			0.13
(−)	6	31	
(+)	7	11	
Multiple lung metastases, no.			0.006
<5	8	40	
≥5	5	2	
Other metastases, no.			0.24
(−)	11	26	
(+)	2	16	
Histological type, no.			0.13
Well	1	7	
Moderate	6	26	
Poor	5	6	
Combination†	1	3	
Response, no.			ND
Complete response	6	—	
Partial response	7	—	
Stable disease	—	16	
Progressive disease	—	24	
Not evaluable	—	2	

Abbreviations: AFP, alpha-fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; ND, not done.

\*P values of viral status and histological type were calculated between HBV versus HCV and poorly differentiated versus nonpoorly differentiated.

†HCC with cholangiocarcinoma component.

more common among responders to sorafenib (responders, 5/13 [38%]; nonresponders, 6/42 [14%];  $P = 0.13$ ). These results suggest that multiple lung metastases and a poorly differentiated histology may be clinical biomarkers for sorafenib treatment in patients with HCC.

**Sorafenib Potently Inhibits Cellular Growth in *FGF3/FGF4*-Amplified and *FGFR2*-Amplified Cell Lines.** We examined the growth inhibitory effect of sorafenib in various cancer cell lines to evaluate whether activated FGFR signaling is involved in the response to sorafenib. Among 26 cell lines, KYSE220 was the only *FGF3/FGF4*-amplified cell line (data not shown), and HSC-43, HSC-39, and KATOIII were the only *FGFR2*-amplified cell lines.<sup>14</sup> Sorafenib

potently inhibited cellular growth in these four cell lines at a sub- $\mu$ M 50% inhibitory concentration ( $IC_{50}$ ) (Fig. 5A). The  $IC_{50}$  values were as follows: HSC43, 0.8  $\mu$ M; HSC39, 0.6  $\mu$ M; KATOIII, 0.4  $\mu$ M; and KYSE220, 0.18  $\mu$ M. These results suggest that activated FGFR signaling may be involved in the response to sorafenib.

**Sorafenib Inhibits Tumor Growth in *FGF4*-Introducing Cell Lines In Vivo.** Finally, we established cancer cell lines stably overexpressing *EGFP*, *FGF3*, or *FGF4* to examine the relationship between the gene function of *FGF3* or *FGF4* and drug sensitivity to sorafenib *in vivo*. Western blotting confirmed that exogenously expressed *FGF3* and *FGF4* were secreted into the culture medium (Fig. 5B). Sorafenib inhibited the *FGF4*-conditioned, medium-mediated expression levels of phosphorylated FGFR (Figure 5C). A similar result was obtained using recombinant *FGF4* (data not shown). Mice inoculated with these cell lines were treated with a low dose of oral sorafenib (15 mg/kg/day) or without sorafenib (vehicle control). *FGF3* overexpression did not increase the tumor volume compared with *EGFP* tumors; however, *FGF4* overexpression aggressively increased tumor volume and clearly enhanced the malignant phenotype (Fig. 5D). Notably, the low-dose sorafenib treatment significantly inhibited the growth of the A549/*FGF4* tumors, whereas it was not effective against A549/*EGFP* and A549/*FGF3* tumors (Fig. 5D). These results suggest that overexpression of *FGF4* is partially involved in the response to sorafenib.

## Discussion

The *FGF3* gene was first identified and characterized based on its similarity to the mouse *fgf3/int-2* gene, which is a proto-oncogene activated in virally induced mammary tumors in mice.<sup>15</sup> Meanwhile, the *FGF4* gene was first identified in gastric cancer as an oncogene *HST*, which has the ability to induce the neoplastic transformation of NIH-3T3 cells upon transfection.<sup>16</sup> These genes were initially regarded as proto-oncogenes. *FGF3* and *FGF4* genes are located side-by-side and are also closely located to the *FGF19* and *CCND1* genes (within 0.2 Mb of the 11q13 region).<sup>13</sup> The 11q13 region is known as a gene-dense region, and gene amplification of this region is frequently observed in various solid cancers (including breast cancer, squamous cell carcinoma of the head and neck, esophageal cancer, and melanoma) at frequencies of 13%-60%.<sup>13</sup> On the other hand, the frequency of *FGF3/FGF4* amplification in HCC remains

sorafenib is necessary. We are presently undertaking a prospective molecular translational study (2010-2012) in a cohort of Japanese patients with sorafenib-treated HCC.

Multiple lung metastases were frequently observed among responders to sorafenib (38%) but were less common among nonresponders (5%). Based on a Japanese follow-up survey of patients with primary HCC, lung metastasis was observed in 7% (169/2355) of the patients at the time of autopsy.<sup>20</sup> Another study demonstrated that 15% of patients were found to have extrahepatic metastases, and lung metastasis was detected in 6% of 995 consecutive HCC patients.<sup>21</sup> When compared with these data from large-scale studies, the frequency of lung metastasis among responders to sorafenib seems quite high. In addition, a poorly differentiated histological type tended to be more common among responders, although the correlation was not significant.

In conclusion, we found that *FGF3/FGF4* gene amplification, multiple lung metastases, and a poorly differentiated histological type may be involved in the response to sorafenib.

## References

- Jemal A, Murray T, Samuels A, Tiwari RC, Ghafoor A, et al. Cancer statistics, 2005. *Ca Cancer J Clin* 2005;55:10-30.
- Yamamoto J, Kosuge T, Takayama T, Shimada K, Yamasaki S, Ozaki H, et al. Recurrence of hepatocellular carcinoma after surgery. *Br J Surg* 1996;83:1219-1222.
- Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res* 2004;64:7099-7109.
- Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378-390.
- Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009;10:25-34.
- So BJ, Bekaii-Saab T, Bloomston MA, Patel T. Complete clinical response of metastatic hepatocellular carcinoma to sorafenib in a patient with hemochromatosis: a case report. *J Hematol Oncol* 2008;1:18.
- Nakazawa T, Hidaka H, Shibuya A, Koizumi W. Rapid regression of advanced hepatocellular carcinoma associated with elevation of des-gamma-carboxyprothrombin after short-term treatment with sorafenib—a report of two cases. *Case Rep Oncol* 2010;3:298-303.
- Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497-1500.
- Lynch TJ, Bell DW, Sordella R, Gurubhagavata S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129-2139.
- Matsumoto K, Arai T, Hamaguchi T, Shimada Y, Kato K, Oda I, et al. FGFR2 gene amplification and clinicopathological features in gastric cancer. *Br J Cancer* 2012;107:727-732.
- Matsumoto K, Arai T, Tanaka K, Kaneda H, Kudo K, Fujita Y, et al. mTOR signal and hypoxia-inducible factor-1 alpha regulate CD133 expression in cancer cells. *Cancer Res* 2009;69:7160-7164.
- Kaneda H, Arai T, Tanaka K, Tamura D, Aomatsu K, Kudo K, et al. FOXQ1 is overexpressed in colorectal cancer and enhances tumorigenicity and tumor growth. *Cancer Res* 2010;70:2053-2063.
- Ormandy CJ, Musgrove EA, Hui R, Daly RJ, Sutherland RL. Cyclin D1, EMS1 and 11q13 amplification in breast cancer. *Breast Cancer Res Treat* 2003;78:323-335.
- Takeda M, Arai T, Yokote H, Komatsu T, Yanagihara K, Sasaki H, et al. AZD2171 shows potent antitumor activity against gastric cancer over-expressing fibroblast growth factor receptor 2/keratinocyte growth factor receptor. *Clin Cancer Res* 2007;13:3051-3057.
- Peters G, Brookes S, Smith R, Dickson C. Tumorigenesis by mouse mammary tumor virus: evidence for a common region for provirus integration in mammary tumors. *Cell* 1983;33:369-377.
- Sakamoto H, Mori M, Taira M, Yoshida T, Matsukawa S, Shimizu K, et al. Transforming gene from human stomach cancers and a noncancerous portion of stomach mucosa. *Proc Natl Acad Sci U S A* 1986;83:3997-4001.
- Takeo S, Arai H, Kusano N, Harada T, Furuya T, Kawauchi S, et al. Examination of oncogene amplification by genomic DNA microarray in hepatocellular carcinomas: comparison with comparative genomic hybridization analysis. *Cancer Genet Cytogenet* 2001;130:127-132.
- Nishida N, Fukuda Y, Komeda T, Kita R, Sando T, Furukawa M, et al. Amplification and overexpression of the cyclin D1 gene in aggressive human hepatocellular carcinoma. *Cancer Res* 1994;54:3107-3110.
- Chochi Y, Kawauchi S, Nakao M, Furuya T, Hashimoto K, Oga A, et al. A copy number gain of the 6p arm is linked with advanced hepatocellular carcinoma: an array-based comparative genomic hybridization study. *J Pathol* 2009;217:677-684.
- Ikai I, Arai S, Ichida T, Okita K, Omata M, Kojiro M, et al. Report of the 16th follow-up survey of primary liver cancer. *Hepatol Res* 2005;32:163-172.
- Uka K, Aikata H, Takaki S, Shirakawa H, Jeong SC, Yamashina K, et al. Clinical features and prognosis of patients with extrahepatic metastases from hepatocellular carcinoma. *World J Gastroenterol* 2007;13:414-420.

# Baseline Factors and Early Viral Response (Week 4) to Antiviral Therapy With Peginterferon and Ribavirin for Predicting Sustained Virologic Response in Patients Infected With Hepatitis C Virus Genotype 1: A Multicenter Study

Hidenori Toyoda,<sup>1</sup> Takashi Kumada,<sup>1\*</sup> Noritomo Shimada,<sup>2</sup> Koichi Takaguchi,<sup>3</sup> Tatsuya Ide,<sup>4</sup> Michio Sata,<sup>4</sup> Hiroyuki Ginba,<sup>5</sup> Kazuhiro Matsuyama,<sup>5</sup> and Namiki Izumi<sup>6</sup>

<sup>1</sup>Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan

<sup>2</sup>Division of Gastroenterology and Hepatology, Shinmatsudo Central General Hospital, Matsudo, Japan

<sup>3</sup>Department of Internal Medicine, Kagawa Prefectural Central Hospital, Takamatsu, Japan

<sup>4</sup>Department of Digestive Disease Information and Research, Kurume University School of Medicine, Kurume, Japan

<sup>5</sup>Department of Life Cycle Management, Roche Diagnostics Japan K.K., Tokyo, Japan

<sup>6</sup>Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Musashino, Japan

Both baseline predictive factors and viral response at week 4 of therapy are reported to have high predictive ability for sustained virologic response to peginterferon and ribavirin combination therapy in patients with hepatitis C virus (HCV) genotype 1. However, it is not clear how these baseline variables and week 4 response should be combined to predict sustained virologic response. In this multicenter study, the authors investigated the impact of baseline predictive factors on the predictive value of week 4 viral response. Receiver-operating characteristic curve analyses were performed to evaluate the ability of week 4 reduction in HCV RNA levels to predict sustained virologic response in 293 Japanese patients infected with HCV genotype 1b. Analyses were performed in all patients and in patient subgroups stratified according to baseline variables. Overall, week 4 viral reduction demonstrates a high predictive ability for sustained virologic response. The sensitivity, specificity, positive predictive value (PPV), negative predictive value, and accuracy were higher than those of viral reduction at week 12. However, the best cut-off levels differ depending on the baseline factors and they were lower in patients with unfavorable baseline predictors. When patients had the TG/GG rs8099917 genotype, the best cut-off was markedly low with low PPV. Week 4 viral response can be a predictor of sustained virologic response in patients with HCV genotype 1 and is better than week 12 viral response. However, the cut-off

levels should be modified based on the baseline predictive variables. **J. Med. Virol.** 85:65–70, 2013. © 2012 Wiley Periodicals, Inc.

**KEY WORDS:** chronic hepatitis C; peginterferon and ribavirin; week 4 viral response; baseline predictive factors, genetic polymorphism near the *IL28B* gene

## INTRODUCTION

Although the combination antiviral therapy with peginterferon (PEG-IFN) and ribavirin has increased markedly the rate of patients with a sustained virologic response, that is, the eradication of hepatitis C virus (HCV), only 50% of patients infected with HCV genotype 1 had achieved a sustained virologic response, approximately. Several studies reported that early HCV viral dynamics during therapy have a high

This study was supported by Roche Diagnostics Japan, K.K. There is no competing interest on this study. The employment status of H. Ginba and K. Matsuyama did not influence the data and the interpretation of the study.

\*Correspondence to: Takashi Kumada, Department of Gastroenterology, Ogaki Municipal Hospital, 4-86 Minaminokawa, Ogaki, Gifu 503-8502, Japan.  
E-mail: takashi.kumada@gmail.com

Accepted 30 July 2012

DOI 10.1002/jmv.23428

Published online in Wiley Online Library  
(wileyonlinelibrary.com).

predictive value for a sustained virologic response in HCV genotype 1-infected patients. Previous studies reported that the response of HCV during combination therapy, that is, the changes in serum HCV RNA levels after starting therapy, has been shown to be an important predictor of the treatment outcome [Zeuzem et al., 2001; Buti et al., 2002; Berg et al., 2006]. Several recent reports have emphasized the importance of evaluating the viral dynamics at 4 weeks after starting therapy to predict a sustained virologic response. A rapid virologic response, in which serum HCV RNA is undetectable at 4 weeks after starting therapy, has been a strong predictive factor of a sustained virologic response reportedly [Martinez-Bauer et al., 2006; Poordad et al., 2008; Martinot-Peignoux et al., 2009; de Segadas-Soares et al., 2009]. In addition to a rapid virologic response, reduced serum HCV RNA levels at 4 weeks after starting therapy has also been reported to have a strong predictive value for the likelihood of achieving sustained virologic response to PEG-IFN and ribavirin combination therapy in patients infected with HCV genotype 1 [Yu et al., 2007; Huang et al., 2010; Toyoda et al., 2011; Marcellin et al., 2012]. These studies suggested that a reduction in HCV RNA levels at week 4 is closely associated with the probability of achieving sustained virologic response.

Aside from early viral response to therapy, several baseline host and viral factors are associated with treatment outcome. Genetic polymorphism near the *IL28B* gene (rs12979860 or rs8099917) is the strongest baseline factor associated with treatment outcome in patients with HCV genotype 1 reportedly [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; McCarthy et al., 2010; Rauch et al., 2010]. In addition, studies from Japan have reported that amino acid substitutions at residue 70 of the HCV core region and amino acids 2,209–2,248 of the NS5A region of HCV (i.e., interferon sensitivity-determining region, ISDR) are viral factors associated with treatment outcome in patients with HCV genotype 1b [Enomoto et al., 1996; Akuta et al., 2005, 2007a; Donlin et al., 2007; Maekawa and Enomoto, 2009; Hayes et al., 2011]. Given these various predictors for a sustained virologic response, that is, week 4 viral response and baseline variables, how should they be combined to predict treatment outcome more precisely? In the present study, the authors investigated how to incorporate week 4 viral response to PEG-IFN and ribavirin combination therapy with baseline predictive factors to predict a sustained virologic response.

## MATERIALS AND METHODS

### Patients and Analyses

In this multicenter study, 682 patients who underwent PEG-IFN alpha-2b and ribavirin combination therapy in a standard treatment regimen at one of the participating institutions, (Musashino Red Cross Hospital, Kurume University Hospital, Shin-Matsudo

Central General Hospital, Kagawa Prefectural Central Hospital, and Ogaki Municipal Hospital) between December 2004 and January 2010 were initially included into the retrospective analyses. All patients were infected with HCV genotype 1b; patients with HCV genotype 1a are usually not found in the Japanese general population. Pretreatment HCV RNA levels were  $\geq 5.0 \log_{10}$  IU/ml, based on a quantitative real-time PCR-based method (COBAS AmpliPrep/COBAS TaqMan HCV Test; Roche Molecular Systems, Pleasanton, CA; lower limit of quantification,  $1.7 \log_{10}$  IU/ml; lower limit of detection,  $1.0 \log_{10}$  IU/ml) [Colucci et al., 2007; Pittaluga et al., 2008], because the use of ribavirin along with PEG-IFN is not approved by Japanese National Medical Insurance System for patients with pretreatment HCV RNA levels  $< 5.0 \log_{10}$  IU/ml. No patients had co-infection with hepatitis B virus or human immunodeficiency virus. All patients had 100% medication adherence for both PEG-IFN and ribavirin during the initial 4 weeks of therapy and 80% or more throughout the treatment period. Among these 682 patients, three baseline factors, genetic polymorphism near the *IL28B* gene, amino acid substitution at residue 70 of the HCV core region, and ISDR sequence had been measured prior to treatment in 405 patients. We excluded 112 of these 405 patients with extended treatment duration up to 72 weeks because the extension of treatment duration might influence outcomes, leaving 293 patients who underwent 48-week standard regimen included in the final sample (Fig. 1).

Receiver-operating characteristic (ROC) analyses were performed to evaluate the value of week 4 reduction in HCV RNA levels in predicting sustained virologic response and an area under the ROC curve (AUROC) was generated. Best cut-off levels were determined based on the sensitivity and specificity. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were also calculated using these cut-off levels. Analyses were performed for all patients and subgroups according to baseline variables. The same analyses were performed on the reduction in HCV RNA levels at week 12 after starting therapy.

The study protocol was in compliance with the Helsinki Declaration and was approved by the ethics committee of each participating institution.

### Measurements of Serum HCV RNA Levels, Amino Acid Substitution at Residue 70 in the HCV Core, Amino Acid Sequence of ISDR, and Genetic Polymorphism Near the *IL28B* Gene

After each patient gave informed consent, serum samples were obtained during the patient's regular hospital visits just prior to beginning treatment, every 4 weeks during the treatment period, and during the 24-week follow-up period after treatment. Serum samples were stored at  $-80^{\circ}\text{C}$  until they were analyzed. HCV RNA levels were measured using a quantitative

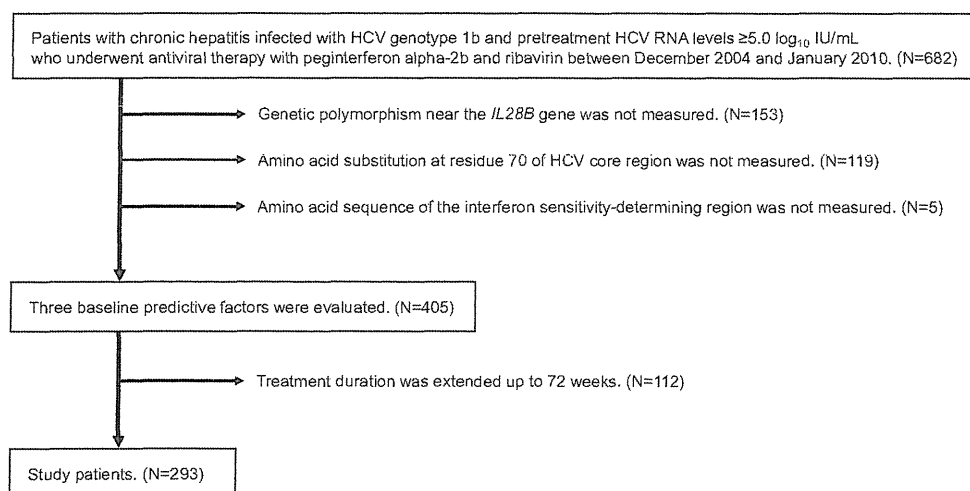


Fig. 1. Schematic representation of the study patients.

real-time PCR-based method (COBAS AmpliPrep/COBAS TaqMan HCV Test). The reductions in HCV RNA 4 and 12 weeks after starting therapy were calculated based on the pretreatment HCV RNA levels. When calculating the reduction in HCV RNA levels, HCV RNA concentration was defined as zero when HCV RNA was undetectable (i.e., rapid virologic response at week 4 and complete early virologic response at week 12).

Amino acid 70 of the HCV core region and the amino acid sequence of the ISDR were analyzed by direct nucleotide sequencing of each region as described previously [Enomoto et al., 1996; Akuta et al., 2007b]. The following PCR primer pairs were used for direct sequencing of the HCV core region:

5'-GCCATAGTGGTCTGCGGAAC-3' (outer, sense primer), 5'-GGAGCAGTCCTTCGTGACATG-3' (outer, antisense primer), 5'-GCTAGCCGAGTAGTGTT-3' (inner, sense primer), and 5'-GGAGCAGTCCTTCGTGACATG-3' (inner, antisense primer). The following PCR primers were used for direct sequencing of ISDR: 5'-TTCCACTACGTGACGGGCAT-3' (outer, sense primer), 5'-CCCGTCCATGTGTAGGACAT-3' (outer, antisense primer), 5'-GGGTCACAGCTCCCTGTGAGCC-3' (inner, sense primer), and 5'-GAGGGTTGTAATCCGGCGTGC-3' (inner, antisense primer). When evaluating the ISDR, HCV was defined as wild-type when there were zero or one amino acid substitutions in residues 2,209–2,248 as compared with the HCV-J strain [Kato et al., 1990], and as non-wild-type when there was more than one substitution.

Genotyping of rs8099917 polymorphisms near the *IL28B* gene was performed using the TaqMan SNP assay (Applied Biosystems, Carlsbad, CA) according to the manufacturer's guidelines. A pre-designed and functionally tested probe was used for rs8099917 (C\_11710096\_10, Applied Biosystems). Genetic polymorphism of rs8099917 reportedly corresponds to

rs12979860 in more than 99% of individuals of Japanese ethnicity [Tanaka et al., 2010]. The TT genotype of rs8099917 corresponds to the CC genotype of rs12979860, the GG genotype of rs8099917 corresponds to the TT genotype of rs12979860, and the TG heterozygous genotype of rs8099917 corresponds to the CT of rs12979860.

## RESULTS

### Patients Characteristics and Baseline Variables

Table I summarizes patient characteristics. The polymorphism of rs8099917 was TT genotype in 204 patients (69.6%). Amino acid substitution at residue 70 was arginine in 200 patients (68.3%). HCV-ISDR was non-wild-type in 78 patients (26.6%). All these variables (TT genotype of rs8099917, arginine at residue 70, and non-wild-type ISDR) were reportedly associated with favorable response to therapy.

As a final outcome, 113 patients (38.6%) achieved sustained virologic response. Sensitivity, specificity, PPV, NPV, and accuracy were 97%, 48%, 54%, 97%, and 67%, respectively, according to genotypes of rs8099917 near the *IL28B* gene. They were 85%, 42%, 48%, 82%, and 59%, respectively, according to amino acid substitutions at residue 70 in the HCV core region, and 43%, 84%, 63%, 70%, and 78%, respectively, according to ISDR of HCV NS5A region.

### Association Between Week 4 Viral Reduction and Treatment Outcome Based on Baseline Predictive Factors

Table II shows the predictive value of a reduction in serum HCV RNA levels at week 4 of therapy in all patients and based on each baseline predictive variable. Week 4 viral reduction demonstrates a high predictive ability for a sustained virologic response with



TABLE I. Characteristics of Study Patients

Age (years), median (range)	60 (20–80)
Sex (male/female) (%)	150 (51.2)/143 (48.8)
BMI, median (range)	22.6 (15.8–33.3)
Prior treatment for HCV (no/yes) (%)	201 (68.6)/92 (31.4)
Initial dose of PEG-IFN ( $\mu\text{g}$ ), median (range)	80.0 (40.0–150.0)
Initial dose of ribavirin (mg), median (range)	600 (200–1,000)
Pretreatment HCV RNA levels ( $\log_{10}$ IU/ml), median (range)	6.1 (5.0–7.4)
Platelet count ( $\times 10^3/\mu\text{l}$ )	159 (43–373)
Hemoglobin (g/dl)	13.9 (8.6–18.1)
Neutrophil count ( $\mu\text{l}^{-1}$ )	2,430 (4,670–7,480)
Alanine aminotransferase (IU/L)	49 (10–485)
Genetic polymorphisms of rs8099917 (TT/TG or GG) (%)	204 (69.6)/89 (30.4)
Amino acid at residue 70 of HCV core (arginine/glutamine or histidine) (%)	200 (68.3)/93 (31.7)
Amino acid sequence of ISDR (non-wild-type/wild-type) (%)	78 (26.6)/215 (73.4)

(N = 293).

BMI, body mass index; HCV, hepatitis C virus; PEG-IFN, peginterferon; ISDR, interferon sensitivity-determining region.

a high AUROC in all patients, in which sensitivity, specificity, PPV, NPV, and accuracy were more than 80%. The best cut-off for the prediction was 3.1- $\log_{10}$  reduction. When patients were stratified according to baseline predictive factors, AUROC remained above 0.85, indicating retention of high predictive ability. However, the best cut-off levels differ depending on baseline factors, and they were lower in patients with unfavorable baseline predictors (TG/GG genotype of rs8099917 near the *IL28B* gene, glutamine/histidine at residue 70 of the HCV core region, and wild-type of ISDR). Especially, when patients had the TG/GG rs8099917 genotype, the calculated best cut-off level was markedly lower than that of patients with the TT genotype. Sensitivity, specificity, PPV, NPV, and accuracy were more than 70% in all patient subgroups, except for patients with the TG/GG genotype in whom PPV was only 10%.

#### Association Between Week 12 Viral Reduction and Treatment Outcome Based on Baseline Predictive Factors

Table III shows the predictive value of a reduction in serum HCV RNA levels at week 12 of therapy in all patients and based on each baseline predictive variable. The predictive ability of week 12 viral reduction

for sustained virologic response was decreased in comparison to that of week 4 with a low AUROC in all patients. The specificity, PPV, and accuracy of the prediction at week 12 were also lower than those at week 4. The best cut-off levels increased to 5.0- $\log_{10}$  reduction. When patients were stratified according to the genetic polymorphisms of rs8099917 near the *IL28B* gene and according to amino acid substitutions at residue 70 of the HCV core region, the differences of the best cut-off levels based on these baseline factors were less marked than those at week 4, although the best cut-off levels remained lower in patients with unfavorable baseline predictors. The difference of best cut-off levels between patients with TT genotype and with TG/GG genotype of rs8099917 also decreased, but PPV in patients with TG/GG genotype remained low (21%). In contrast, the difference in the best cut-off levels increased when patients were stratified according to amino acid sequences in ISDR. The best cut-off level of the reduction in HCV RNA levels at week 12 for predicting sustained virologic response was higher in patients with HCV of wild-type ISDR, an unfavorable baseline variable, than in patients with HCV of favorable non-wild-type ISDR, which was inverse to the evaluation with week 4 viral reduction in which the cut-off level was higher in patients with HCV of non-wild-type ISDR.

TABLE II. AUROC, Best Cut-Off Level, Sensitivity, Specificity, PPV, NPV, and Accuracy of the Reduction in Serum HCV RNA Levels 4 Weeks After Starting PEG-IFN and Ribavirin Combination Therapy From Pretreatment Levels for Predicting Sustained Virologic Response

	N	AUROC	Best cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Overall	293	0.92746	3.1 $\log_{10}$	88	87	81	92	87
<i>IL28B</i> -TT	204	0.88353	3.2 $\log_{10}$	87	78	82	84	83
<i>IL28B</i> -TG or GG	89	0.84302	1.1 $\log_{10}$	100	69	10	100	70
Core 70-R	200	0.91023	3.2 $\log_{10}$	86	83	82	87	85
Core 70-Q or H	93	0.94350	2.8 $\log_{10}$	88	93	75	97	92
ISDR-non-wild type	78	0.93455	3.0 $\log_{10}$	90	90	94	84	90
ISDR-wild type	215	0.92654	2.9 $\log_{10}$	92	84	71	96	87

AUROC, area under the receiver-operating characteristics curve; PPV, positive predictive value; NPV, negative predictive value; HCV, hepatitis C virus; PEG-IFN, peginterferon; R, arginine; Q, glutamine; H, histidine; ISDR, interferon sensitivity-determining region.

TABLE III. AUROC, Best Cut-Off Level, Sensitivity, Specificity, PPV, NPV, and Accuracy of the Reduction in Serum HCV RNA Levels 12 Weeks After Starting PEG-IFN and Ribavirin Combination Therapy From Pretreatment Levels for Predicting Sustained Virologic Response

	N	AUROC	Best cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Overall	293	0.86907	5.0 log <sub>10</sub>	88	73	67	91	79
<i>IL28B</i> -TT	204	0.79216	5.11 log <sub>10</sub>	81	61	70	73	71
<i>IL28B</i> -TG or GG	89	0.92829	4.6 log <sub>10</sub>	100	87	21	100	88
Core 70-R	200	0.81791	5.0 log <sub>10</sub>	88	63	69	86	75
Core 70-Q or H	93	0.94272	4.9 log <sub>10</sub>	100	84	59	100	87
ISDR-non-wild type	78	0.87298	5.0 log <sub>10</sub>	88	79	88	79	85
ISDR-wild type	215	0.89572	5.4 log <sub>10</sub>	84	79	63	92	81

AUROC, area under the receiver-operating characteristics curve; PPV, positive predictive value; NPV, negative predictive value; HCV, hepatitis C virus; PEG-IFN, peginterferon; R, arginine; Q, glutamine; H, histidine; ISDR, interferon sensitivity-determining region.

## DISCUSSION

This study was conducted to confirm the predictive value of week 4 viral dynamics of HCV for predicting sustained virologic response to the combination therapy with PEG-IFN and ribavirin in patients infected with HCV genotype 1 and with pretreatment HCV RNA levels of  $\geq 5.0$  log<sub>10</sub> IU/ml in a large multicenter study of Japan. The comparison of the predictability for sustained virologic response between week 4 and week 12 viral reductions revealed the higher predictive ability of week 4 viral response. In a recent study, Marcellin et al., [2012] suggested that a  $\geq 3$  log<sub>10</sub> reduction in HCV RNA levels at week 4 of PEG-IFN and ribavirin combination therapy is a reliable factor for predicting sustained virologic response in patients with HCV genotype 1. Our current results are consistent with their analysis for patients with HCV genotype 1b and those with pretreatment HCV RNA levels  $\geq 5.0$  log<sub>10</sub> IU/ml overall. The reduction in HCV RNA levels at week 4 appears to be a good and reliable predictor for a sustained virologic response. Although week 12 viral response (i.e., early virologic response) has been used as a pivotal decision criterion to extend treatment duration or to discontinue treatment, the predictive value is lower when the reduction in HCV RNA levels is compared to week 4 viral response.

When patients were stratified based on baseline predictive factors, however, the best cut-off levels for sustained virologic response were not constant. The cut-off levels decreased in patients with unfavorable baseline factors, that is, TG/GG genotype of rs8099917, glutamine/histidine at residue 70 of the HCV core region, and wild-type sequence of ISDR, indicating that the reduction in HCV RNA occurs slowly in patients with these unfavorable baseline variables. Conversely and paradoxically, the results may indicate that one can expect sustained virologic response in patients with a smaller reduction in HCV RNA levels at week 4 if they have unfavorable baseline variables.

When predictive value was evaluated using week 12 viral reduction, the best cut-off levels remained lower in patients with unfavorable TG/GG rs8099917 genotype and patients with HCV of unfavorable

glutamine/histidine at residue 70 of the HCV core region. In contrast, the best cut-off level was higher in patients with HCV of unfavorable wild-type ISDR. Previous studies reported the association between the genetic polymorphisms near the *IL28B* gene (rs12979860 and rs8099917) and amino acid substitution at residue 70 of HCV core region [Abe et al., 2010; Kobayashi et al., 2010], whereas no associations were reported between these two variables and ISDR mutation. This might explain the difference in the relationship of early viral response during therapy between with two baseline predictive factors, *IL28B* genetic polymorphisms and amino acid substitution of HCV core region and with ISDR mutation.

The calculated PPV was markedly low in patients with the unfavorable TG/GG genotype of rs8099917 (CT/TT genotype of rs12979860) both by the evaluations at weeks 4 and 12 viral responses. Therefore, it appears to be difficult to identify patients in this subgroup who are likely to achieve a sustained virologic response by their week 4 viral response, although week 4 viral response can be a factor used to identify patients with a high likelihood of achieving sustained virologic response in other subgroups.

In conclusion, week 4 viral response can be a predictor of sustained virologic response in patients with HCV genotype 1. However, the cut-off levels should be modified based on baseline host and viral predictive variables. In addition, week 4 viral response is not predictive in patients with unfavorable genotype of genetic polymorphism near the *IL28B* gene.

## REFERENCES

- Abe H, Ochi H, Maekawa T, Hayes CN, Tsuge M, Miki D, Mitsui F, Hiraga N, Imamura M, Takahashi S, Ohishi W, Arihiro K, Kubo M, Nakamura Y, Chayama K. 2010. Common variation of *IL28* affects gamma-GTP levels and inflammation of the liver in chronically infected hepatitis C virus patients. *J Hepatol* 53: 439–443.
- Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2005. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 48:372–380.
- Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K,

- Kumada H. 2007a. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: Amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 46:403–410.
- Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Miyakawa Y, Kumada H. 2007b. Prediction of response to pegylated interferon and ribavirin in hepatitis C by polymorphisms in the viral core protein and very early dynamics of viremia. *Intervirology* 50:361–368.
- Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, Buggisch P, Goeser T, Rasenack J, Pape GR, Schmidt WE, Kallinowski B, Klinker H, Spengler U, Martus P, Alshuth U, Zeuzem S. 2006. Extended treatment duration for hepatitis C virus type 1: Comparing 48 versus 72 weeks of peginterferon- $\alpha$ -2a plus ribavirin. *Gastroenterology* 130:1086–1097.
- Buti M, Sanchez-Avila F, Lurie Y, Stalgis C, Valdes A, Martell M, Esteban R. 2002. Viral kinetics in genotype 1 chronic hepatitis C patients during therapy with 2 different doses of peginterferon  $\alpha$ -2b plus ribavirin. *Hepatology* 35:930–936.
- Colucci G, Ferguson J, Harkleroad C, Lee S, Romo D, Soviero S, Thompson J, Velez M, Wang A, Miyahara Y, Young S, Sarrazin C. 2007. Improved COBAS TaqMan hepatitis C virus test (version 2.0) for use with the High Pure system: Enhanced genotype inclusivity and performance characteristics in a multisite study. *J Clin Microbiol* 45:3595–3600.
- de Segadas-Soares JA, Villela-Nogueira CA, Perez RM, Nabuco LC, Brandao-Mello CE, Coelho HSM. 2009. Is the rapid virologic response a positive predictive factor of sustained virologic response in all pretreatment status genotype 1 hepatitis C patients treated with peginterferon- $\alpha$ 2b and ribavirin? *J Clin Gastroenterol* 43:362–366.
- Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, Belle SH, Di Bisceglie AM, Aurora R, Tavis JE, Virahep-C Study Group. 2007. Pretreatment sequence diversity differences in the full-length hepatitis C virus open reading frame correlate with early response to therapy. *J Virol* 2007; 81:8211–8224.
- Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. 1996. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 334:77–81.
- 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.
- Hayes NC, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, Miki D, Imamura M, Ochi H, Kamatani N, Nakamura Y, Chayama K. 2011. HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy. *Gut* 60:261–267.
- Huang CF, Yang JF, Huang JF, Dai CY, Chiu CF, Hou NJ, Hsieh MY, Lin ZY, Chen SC, Hsieh MY, Wang LY, Chang WY, Chuang WL, Yu ML. 2010. Early identification of achieving a sustained virological response in chronic hepatitis C patients without a rapid virological response. *J Gastroenterol Hepatol* 25:758–765.
- Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K. 1990. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. *Proc Natl Acad Sci USA* 87:9524–9528.
- Kobayashi M, Suzuki F, Akuta N, Suzuki Y, Sezaki H, Yatsuji H, Hosaka T, Kobayashi M, Kawamura Y, Hirakawa M, Arase Y, Ikeda K, Mineta R, Iwasaki S, Watahiki S, Nakamura Y, Chayama K, Kumada H. 2010. Relationship between SNPs in the IL28B region and amino acid substitutions in HCV core region in Japanese patients with chronic hepatitis C. *Kanzo* 51:322–323; [in Japanese with English abstract].
- Maekawa S, Enomoto N. 2009. Viral factors influencing the response to the combination therapy of peginterferon plus ribavirin in chronic hepatitis C. *J Gastroenterol* 44:1009–1015.
- Marcellin P, Reau N, Ferenci P, Hadziyannis S, Messinger D, Tatsch F, Jensen D. 2012. Refined prediction of week 12 response and SVR based on week 4 response in HCV genotype 1 patients treated with peginterferon  $\alpha$ -2a (40KD) and ribavirin. *J Hepatol* 56:1276–1282.
- Martinez-Bauer E, Crespo J, Romero-Gomez M, Moreno-Otero R, Sola R, Tesei N, Pons F, Forns X, Sanchez-Tapias JM. 2006. Development and validation of two models for early prediction of response to therapy in genotype 1 chronic hepatitis C. *Hepatology* 43:72–80.
- Martinot-Peignoux M, Maylin S, Moucari R, Ripault M-P, Boyer N, Cardoso A-C, Giuily M, Castelnau C, Pouteau M, Stern C, Auperin A, Bedossa P, Asselah T, Marcellin P. 2009. Virological response at 4 weeks to predict outcome of hepatitis C treatment with pegylated interferon and ribavirin. *Antivir Ther* 14:501–511.
- McCarthy JJ, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, Tillman HL, Muir AJ, McHutchison JG. 2010. Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. *Gastroenterology* 138:2307–2314.
- Pittaluga F, Alice T, Abate ML, Ciancio A, Cerutti F, Varetto S, Colucci G, Smedile A, Ghisetti V. 2008. Clinical evaluation of the COBAS Ampliprep/COBAS TaqMan for HCV RNA quantitation in comparison with the branched-DNA assay. *J Med Virol* 80:254–260.
- Poordad F, Reddy KR, Martin P. 2008. Rapid virologic response: A new milestone in the management of chronic hepatitis C. *Clin Infect Dis* 46:78–84.
- 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, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY, Swiss Hepatitis C Cohort Study; Swiss HIV Cohort Study. 2010. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: A genome-wide association study. *Gastroenterology* 138:1338–1345.
- 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, Müller 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, Nishida N, Sugiyama M, Tokunaga K, Mizokami M. 2010.  $\lambda$ -interferons and the single nucleotide polymorphisms: A milestone to tailor-made therapy for chronic hepatitis C. *Hepatology* 40:449–460.
- Toyoda H, Kumada T, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Tada T, Arakawa T, Fujimori M, Niinomi T, Ando N, Yasuda S, Sakai K, Kimura J. 2011. High ability to predict the treatment outcome of peginterferon and ribavirin combination therapy based on the reduction in HCV RNA levels at 4 weeks after starting therapy and amino acid substitutions in hepatitis C virus in patients infected with HCV genotype 1b. *J Gastroenterol* 46:501–509.
- Yu JW, Wang GQ, Sun LJ, Li XG, Li SC. 2007. Predictive value of rapid virological response and early virological response on sustained virological response in HCV patients treated with pegylated interferon  $\alpha$ -2a and ribavirin. *J Gastroenterol Hepatol* 22:832–836.
- Zeuzem S, Herrmann E, Lee JH, Fricke J, Neumann AU, Modi M, Colucci G, Roth WK. 2001. Viral kinetics in patients with chronic hepatitis C treated with standard or peginterferon  $\alpha$ 2a. *Gastroenterology* 120:1438–1447.

# Predictive Value of Early Viral Dynamics During Peginterferon and Ribavirin Combination Therapy Based on Genetic Polymorphisms Near the *IL28B* Gene in Patients Infected With HCV Genotype 1b

Hidenori Toyoda,<sup>1\*</sup> Takashi Kumada,<sup>1</sup> Toshifumi Tada,<sup>1</sup> Kazuhiko Hayashi,<sup>2</sup> Takashi Honda,<sup>2</sup> Yoshiaki Katano,<sup>2</sup> Hidemi Goto,<sup>2</sup> Takahisa Kawaguchi,<sup>3</sup> Yoshiki Murakami,<sup>3</sup> and Fumihiko Matsuda<sup>3</sup>

<sup>1</sup>Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan

<sup>2</sup>Department of Gastroenterology, Nagoya University Graduate School of Medicine, Nagoya, Japan

<sup>3</sup>Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

A study was carried out to determine whether early viral dynamics retain prediction of the outcome of peginterferon (PEG-IFN) and ribavirin combination therapy based on different genetic polymorphisms near the *IL28B* gene, the strongest baseline predictor of response to this therapy. A total of 272 patients infected with hepatitis C virus (HCV) genotype 1b were grouped according to genetic polymorphisms near the *IL28B* gene (rs8099917). The ability of reduced HCV RNA levels at 4 and 12 weeks after starting therapy to predict a sustained virologic response was evaluated based on these genotypes. Among patients with the TT genotype for rs8099917 (associated with a favorable response), the rates of sustained virologic response were higher in patients with a  $\geq 3 \log_{10}$  reduction in serum HCV RNA levels at 4 weeks after starting therapy ( $P < 0.0001$ ). In contrast, among patients with the TG/GG genotype (associated with an unfavorable response), there were no differences in this rate based on the reduction in HCV RNA levels at 4 weeks. Early viral dynamics at 4 weeks after starting therapy retains its predictive value for sustained virologic response in patients with the TT genotype for rs8099917, but not in patients with the TG/GG genotype. Patients who are likely to achieve sustained virologic response despite unfavorable TG/GG genotype cannot be identified based on early viral dynamics during therapy. In contrast, lack of early virologic response at 12 weeks retains a strong predictive value for the failure of sustained virologic response regardless of *IL28B* polymorphisms, which remains useful as a factor to stop therapy. **J. Med. Virol.** 84:61–70, 2012.

© 2011 Wiley Periodicals, Inc.

**KEY WORDS:** chronic hepatitis C; early viral dynamics; genetic polymorphisms near the *IL28B* gene; peginterferon; response-guided therapy; ribavirin

## INTRODUCTION

The current standard antiviral therapy for patients with chronic hepatitis C is combination therapy with peginterferon (PEG-IFN) and ribavirin [Ghany et al., 2009]. Although this treatment regimen has increased markedly the number of patients with a sustained virologic response, i.e., the eradication of hepatitis C virus (HCV), only 50% of patients infected with HCV genotype 1 achieved a sustained virologic response approximately.

Many investigators have examined factors that predict the treatment outcome of PEG-IFN and ribavirin combination therapy in patients infected with HCV genotype 1. In addition to the baseline factors, the response of HCV during combination therapy, i.e., the changes in serum HCV RNA levels after starting therapy, has been shown to be an important predictor of the treatment outcome [Zeuzem et al., 2001; Buti

Conflict of interest: None.

\*Correspondence to: Hidenori Toyoda, MD, PhD, Department of Gastroenterology, Ogaki Municipal Hospital 4-86, Minaminokawa, Ogaki, Gifu, 503-8502, Japan.

E-mail: hmtoyoda@spice.ocn.ne.jp

Accepted 28 September 2011

DOI 10.1002/jmv.22272

Published online in Wiley Online Library

(wileyonlinelibrary.com).