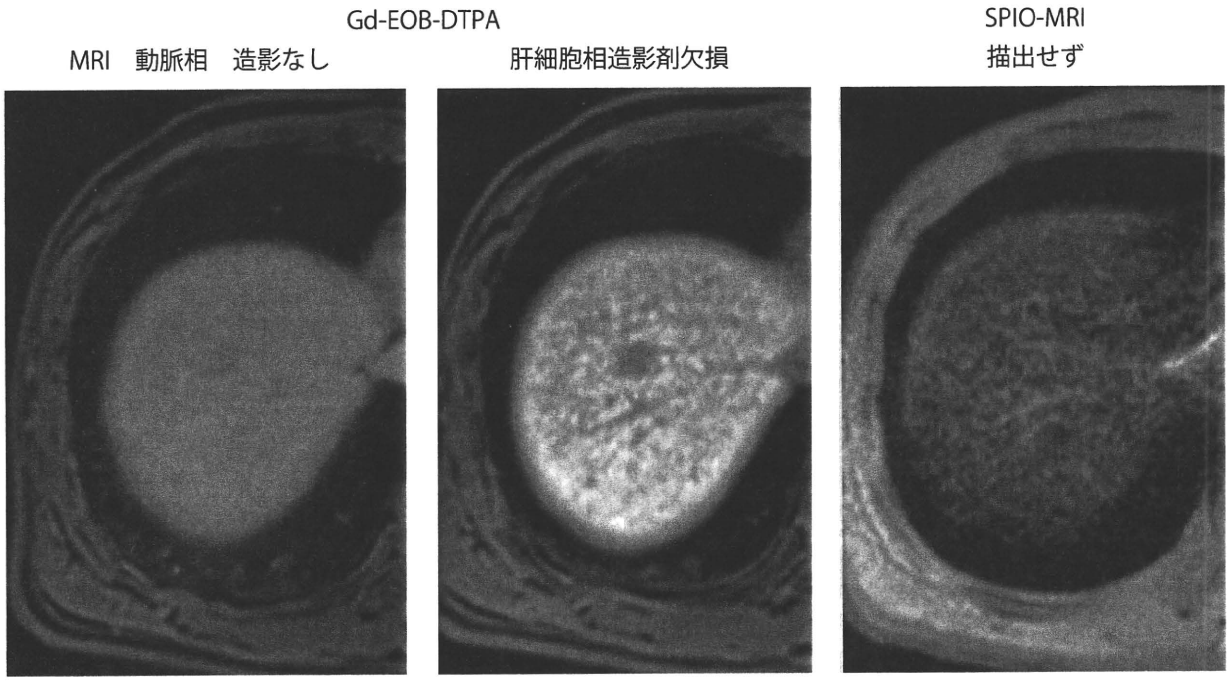



**図2 MRIによるスクリーニングで検出された高分化型肝癌**

Gd-EOB-DTPAによる造影の肝細胞相で造影剤欠損所見を示し，臨床経過から新規に出現した高分化型肝癌と診断された．

（**図2**）．EOB造影MRI検査で発見される小型の早期肝癌は腹部超音波検査での描出が困難であるばかりでなく，治療の必要性に関しても今後の議論が必要である．

B型慢性肝炎や，線維化がF1段階程度のC型慢性肝炎，さらにHBV・HCVのキャリア（ALT正常者）では，肝硬変症例に比し，肝癌発癌率は低い．とりわけ，HBVDNA低値（$10^{3.7}$ copy/m*l*未満）のB型慢性肝炎，トランスアミナーゼ正常のC型慢性肝炎での発癌率は低い．このことから，漠然とこれらの軽度の慢性肝疾患では画像スクリーニングの間隔を6カ月～1年と延ばす意見も出されている．しかし，肝硬変症例よりも発癌率は低いとはいえ，健常人よりははるかに高い発癌リスクを有しているのであり，直径3 cm以下の小型肝癌で発見するためには，やはり2～6カ月ごとに超音波検査などを励行せねばならないことに変わりはない．すなわち，これは供給できる医療環境に依存するものであって，軽度の慢性肝炎では1年に1回の画像スクリーニングが適切であるというものではない．

## 肝癌の生物学的悪性度診断

門脈などの脈管に浸潤する肝癌，他臓器への転移を伴う肝癌は，治療困難で予後不良である．これらは生物学的悪性度が高いという評価がされるよりも，一般的には高度に進行した肝癌であると表現される．「生物学的悪性度」は，一般的に，「現在の診断方法では癌進行度分類が同様となるにもかかわらず，転移・再発・生存のいずれかの性質が平均的な腫瘍より明らかに不良であるもの」と理解されることが多い（**図3**）．

このような観点から，性質の悪い肝癌をできる限り見極めて「より根治的な」治療を行おうとする努力は画像診断やさまざまなバイオマーカーで行われている．

画像診断では，いくつかの「悪性所見」が示されている．ダイナミックCTでみられる辺縁のリング状造影を示す結節型肝癌で，内部が広く低吸収値となる腫瘍は小型でも低分化型のことが多い（**図4**）．リング状の辺縁部濃染ではなく厚みのある染影であれば，内部に壊死を伴う通常の中分化

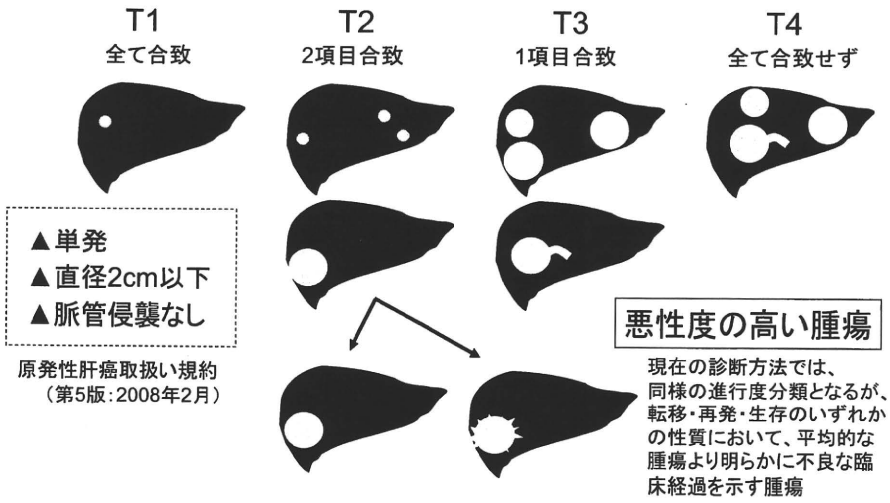



**図3　肝細胞癌の病期分類と生物学的悪性度**

T3・T4のような「進行した」肝癌を意味するのではなく，転移・再発・生存などの点で不良な経過を示す腫瘍を指す概念.

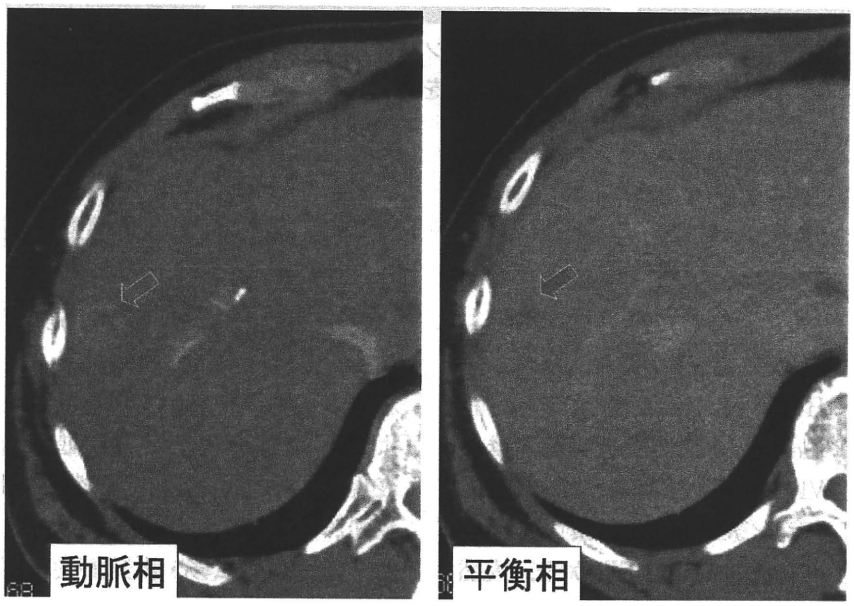



**図4　高い悪性度が疑われて外科的肝切除を行った小型肝癌**

術前診断　Stage Ⅰ（T1N0M0），肝障害度　A

直径2 cm弱であるが，リング状の濃染と内部は低吸収値となるダイナミックCT所見で，切除組織は低分化型肝癌であった.

型肝癌の可能性も多い．また，肝癌取扱い規約上の単純結節周囲増殖型・多結節癒合型は，単純結節型より予後が悪いことが知られており，この画像上の確認が治療選択・予後推定に重要である（**図5**）．これらの症例では，積極的に根治的外科切除（可能なら系統的切除）を行うべきである．単純結節周囲増殖型・多結節癒合型の病理学的検索では，顕微鏡的肝内転移（im），顕微鏡的門脈浸潤（vp）が高率であるほか，低分化型肝癌もしばしばみられることが，再発・生存予後に影響すると考えられている．

腫瘍マーカーのうち，PIVKA-IIは腫瘍の生物学的悪性度と相関するとする報告が増えている．当院でRFAまたは肝切除にて「根治治療」した症例

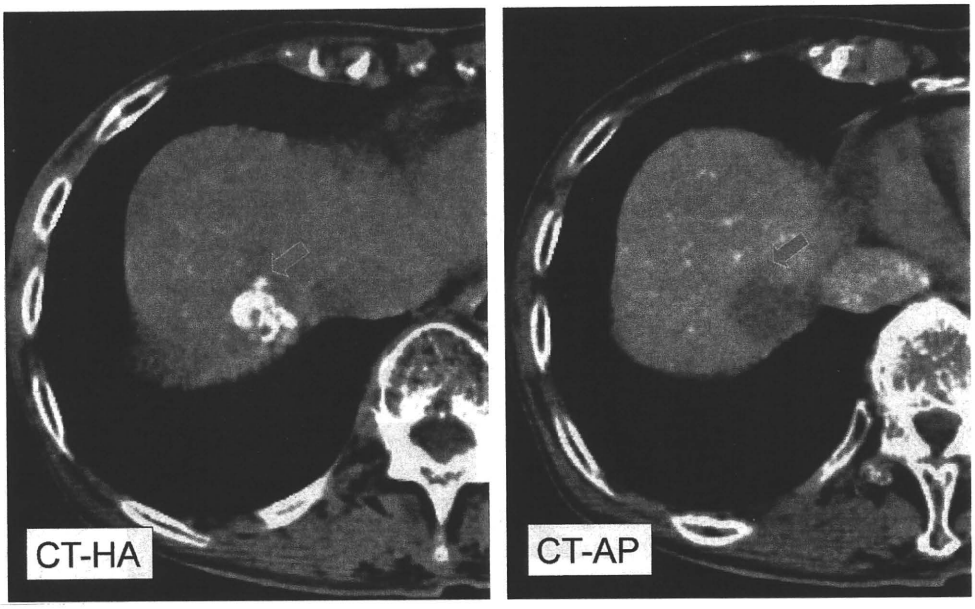



**図5　単純結節周囲増殖型を示す小型肝癌の画像診断**

CT-HAでは腫瘍の前方に突出する結節部分を示し，CT-APでは腫瘍前方に突出する造影欠損像を示している．

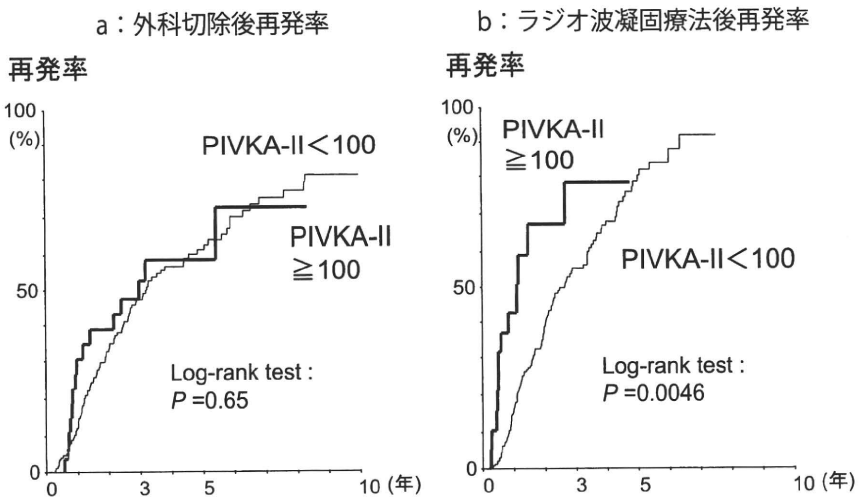
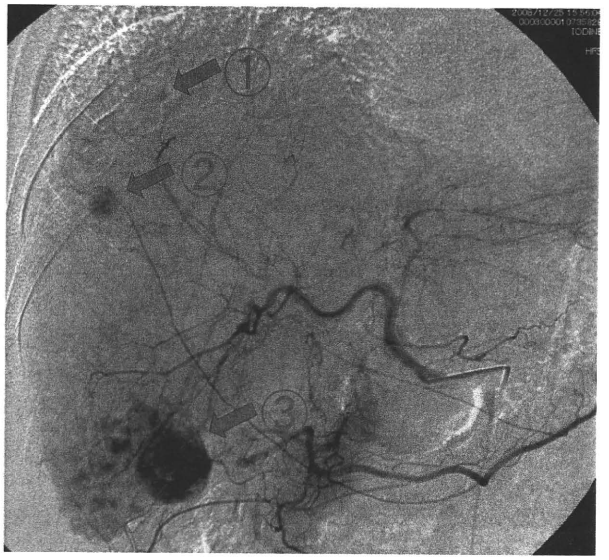


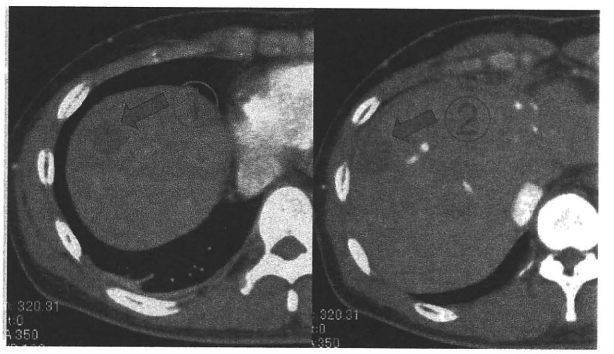

**図6　悪性度を示すPIVKA-II値と治療法による治療後再発率の比較**

a：外科切除後再発率，b：ラジオ波凝固療法後再発率．外科治療ではPIVKA-IIの値による再発率の差はみられないが，ラジオ波凝固療法ではPIVKA-IIが100 AU/L以上の症例で再発率が有意に高くなる．

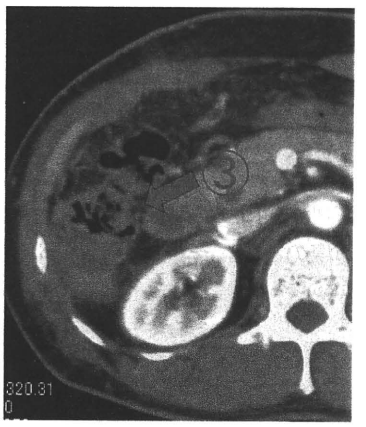
の再発率を検討すると，PIVKA-IIが100 AU/L以上の症例での再発率が高い傾向にあった．これを治療法別にみると，肝切除施行例ではPIVKA-IIの高低により再発率は同様であった(**図6-a**)が，PIVKA-IIが100以上の症例ではRFAを行った後の再発率が有意に高率であった(**図6-b**)．ラジオ波凝固療法は外科的肝切除に比べると，局所再発率や同亜区域内の再発がやや高いのは事実であり，PIVKA-II値が100 AU/L以上で「悪性度が高い」と推定される症例では，経皮的局所治療よりも，外科的切除(可能なら系統的切除)を行うことを考慮すべきである[6]．




a：治療前血管造影



b：治療後CT（病変①）　c：治療後CT（病変②）




c：治療後CT
（病変③）

**図7　右葉に多発する肝癌を集学的に「根治治療」した症例**

a：血管造影では，S8に1 cm以下の小型の肝細胞癌結節が2カ所みられ，S6には直径28 mmの結節とその周囲に散布する10数個の転移結節が認められる．
b：S8の2結節は経皮的ラジオ波凝固療法を施行．
c：S6の多発病変に対しては肝動脈化学塞栓の後に肝切除術を施行．

## 経皮的ラジオ波凝固療法の現状

内科的局所治療は将来の新規の肝癌発癌に備えて，より肝機能の温存を図ろうとして発達してきた．1980年代のエタノール局注療法の時代にはともすると，肝機能温存を重視するあまり肝癌根治性の点では肝切除より劣る場面もみられた．しかし，マイクロ波凝固療法を経てラジオ波凝固療法が加わった現在，直径25～30 mmまでの肝癌であれば内科的局所療法で肝癌根治性と肝機能温存の両面で満足のいく治療ができる時代となった．

RFAが治療手段の一つに入ることにより，従来は肝癌が多発であるからという理由で，TAE・肝動脈抗癌剤動注などのinterventional radiologyにとどまっていた症例の一部は，より根治的な治療効果を目指す段階に引き上げることができた．またRFAの導入は，従来は多発結節で困難としていた肝切除の適応の場面で，大型の肝癌のみを外科切除し他はRFA治療を行う（**図7**）というように，外科医の考え方を柔軟なものに変化させつつある．ウイルス性慢性肝疾患，特に肝硬変を基礎疾患とした肝細胞癌症例では，根治的に肝癌治療を行っても，再発をきたすことが多く，異時性多発が避けられない．このため，異時的に種々の治療を組み合わせて行う症例は今後もますます増えていくと考えられる．

最近の肝癌局所治療症例の約75％は，再発肝癌に対する治療である．すなわち，腹部超音波検査で観察すると，既治療病変と未治療病変とが同時に肝内に存在する状況である．再発新規病変が

既治療病変の近傍に出現したり，腹部超音波検査所見が似通っていると，経皮的局所治療での正確なターゲッティングが困難となる．再発病変・描出困難病変・CT/MRIでのみ検出できる病変に対して，現在，①ソナゾイド造影超音波検査，②Real time virtual sonography（RVS），③血管造影下での炭酸ガス動注エコー検査などが行われ，それぞれの長所・短所のもとに使い分けられている．最近では，EOB造影MRI検査のみで検出された小型肝癌の治療の場合には，④CT下またはMRI下穿刺によるRFA治療も行われている．

RFA治療を行う症例は，高齢化・全身合併症併存・反復治療・ターゲッティング困難など，さまざまの社会的・医学的背景の変化を示しており，10年前にRFAが導入された時期より明らかに治療困難症例を扱う方向となっている．

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# ミリプラチン

池 田 健 次*

索引用語：Miriplatin（ミリプラチン），Hepatocellular carcinoma（肝細胞癌），Lipiodol（リピオドール），Lipiodolization（リピオドリゼーション），TACE（肝動脈化学塞栓療法）

## 1 はじめに

肝細胞癌は小型であれば肝切除やラジオ波凝固療法などの根治的治療が可能であるが，大型肝癌・多発肝癌などでは内科的・経カテーテル治療が行われる．従来の経カテーテル治療は，アントラサイクリン系薬剤や白金製剤を動注し，そのあと多孔性ゼラチン粒で塞栓する肝動脈化学塞栓療法(TACE)として行われている．脂溶性造影剤ヨード化ケシ油脂肪酸エチルエステル(以下，リピオドール™)を動注すると，リピオドールが肝癌組織に特異的に停滞する性質を利用して，TACEの際にはこれら水溶性抗癌剤と用手的に混和し，リピオドール混和液として動注使用されることが一般的であった．

2010年より第三世代白金製剤としてリピオドールに親和性のミリプラチンが認可され，従来の「水と油」を強制的に混和するのではなく，「親油性抗癌剤と油性造影剤」を自然に懸濁することが可能となった．このことにより，肝細胞癌組織に，リピオドールに伴われた白金性抗癌剤が選択的かつ長期停滞することとなり，強い抗癌剤効果が期待できる薬剤として登場した．

## 2 薬剤の特徴

肝細胞癌の動注化学療法を目的として開発された，DACH構造を有する白金製剤であり，側鎖にミリスチン酸をもつことで親油性を向上し，リピオドールへの懸濁性を良好にしたものである(図1)．

ミリプラチンのリピオドールから腫瘍細胞内への移行と活性化は図2のように考えられている．肝動注を行うと，親油性のミリプラチンはリピオドール内では安定で，均一に分布している．リピオドールとともに腫瘍局所に長時間滞留するが，ミリプラチン分子の長い脂肪鎖が切れてリピオドール滴から徐々に遊離するとともに，外れた脱離基の代わりに生体成分中の塩素イオンが配位し，活性体DPCとなる．腫瘍細胞内に入った活性化

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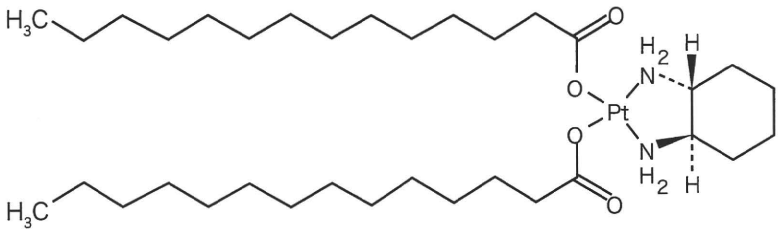


図1　ミリプラチンの分子構造

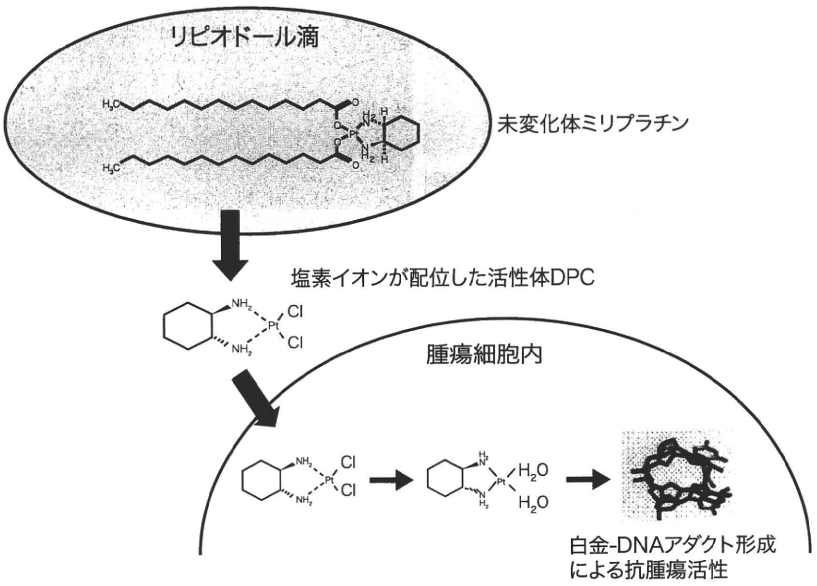



図2　ミリプラチンの腫瘍細胞への移行と生体内での活性化機序

DPCは，白金-DNA架橋(アダクト)を形成し，腫瘍増殖抑制効果を発揮する．

## 3　適応・禁忌

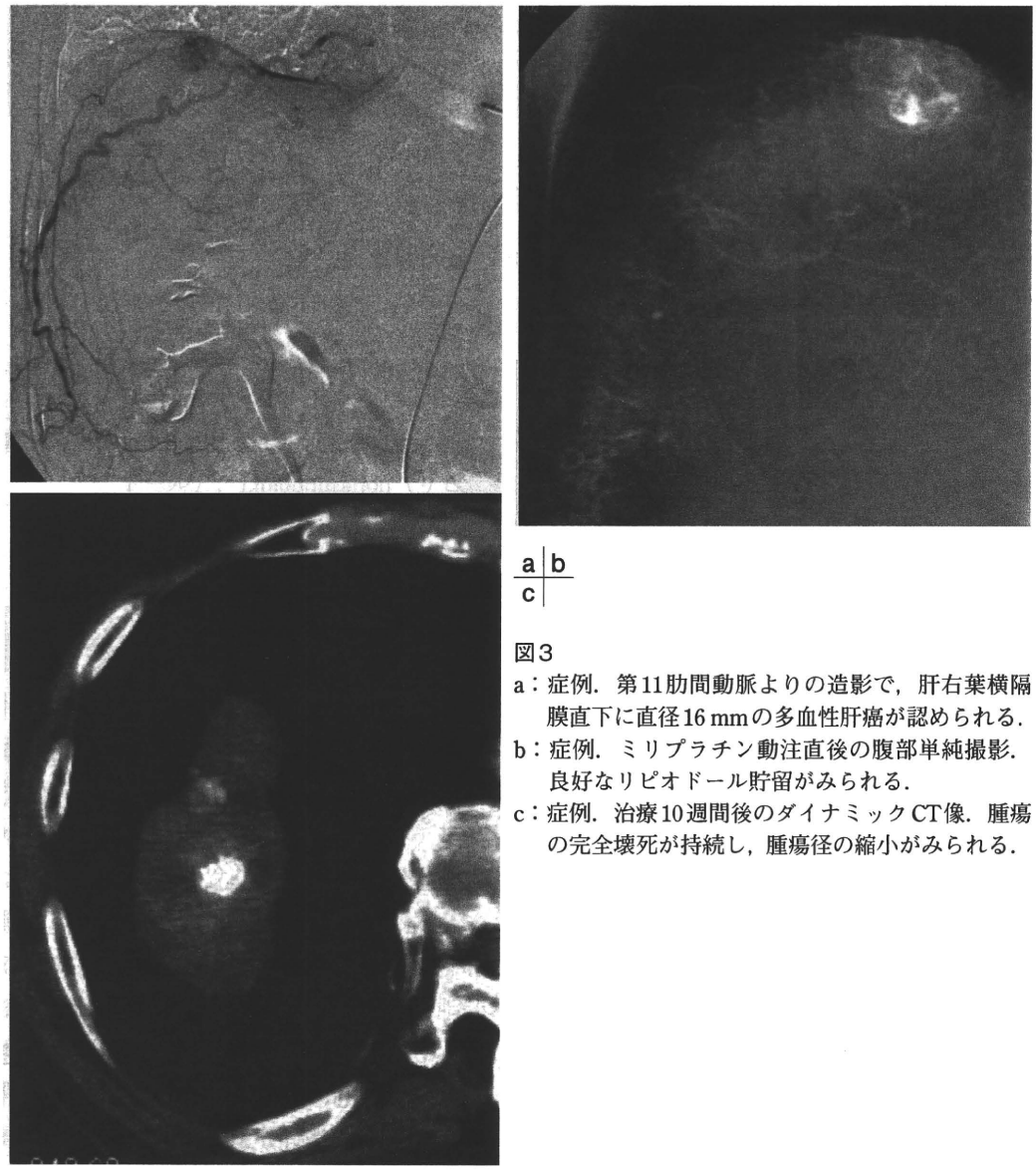
根治的切除・経皮的穿刺治療が行えない肝細胞癌がミリプラチンの適応で，これらの治療が可能であれば，根治的な外科的・内科的治療を優先する．

禁忌は通常の薬剤同様，本剤・他の白金製剤・ヨード系薬剤に対して過敏症のある患者，ヨード化合物であるリピオドールを使用するため重篤な甲状腺疾患患者，妊婦・妊娠している可能性のある婦人も禁忌である．肝機能上，ビリルビン3 mg/dl以上の顕性黄疸患者や肝障害度Cの高度肝機能不良例では，肝不全を起こす可能性があるため，投与しないことを原則とする．

## 4　用量・用法

肝細胞癌におけるリピオドリゼーション(リピオドールと懸濁して行う動注化学療法)として使用する．

ミリプラチン70 mgを本剤懸濁用液(リピオドール) 3.5 mLに懸濁し，1日1回肝動脈内に挿入されたカテーテルより投与する．本剤の投与は，腫瘍血管に懸濁液が充満した時



a b
c

図3
a：症例．第11肋間動脈よりの造影で，肝右葉横隔膜直下に直径16 mmの多血性肝癌が認められる．
b：症例．ミリプラチン動注直後の腹部単純撮影．良好なリピオドール貯留がみられる．
c：症例．治療10週間後のダイナミックCT像．腫瘍の完全壊死が持続し，腫瘍径の縮小がみられる．

点で終了する．ただし，上限を1回6 mL（ミリプラチンとして120 mg)とする．また，繰り返し投与する場合には，4週間以上の観察期間をおく．

2010年9月現在，ミリプラチンはリピオドールに懸濁して使用することのみが認可されており，多孔性ゼラチン粒併用によるTACEの施行に関しては，安全性・有効性が確認されていない．

## 5 使用上の注意・コツ

肝細胞癌結節の大きさに応じて，リピオドール(すなわちミリプラチン)の投与量を決定する．肝機能不良例，肝動脈-門脈シャント例，肝動脈-肝静脈シャント例，門脈血流が著しく不良な例，門脈腫瘍栓合併例では，リピオドリゼーションに伴う合併症の危険があるため，超選択的投与を行ったり投与量を

減らしたりするなどの必要がある.

## 6 有効性

認可に使用された後期第Ⅱ相試験では,ミリプラチン最終投与3カ月後の画像診断による抗腫瘍効果では,TEⅤ(壊死効果100%または腫瘍縮小率100%)が83例中22例(26.5%),TEⅣ(壊死効果50%以上100%未満または腫瘍縮小率50%以上100%未満)が21例(25.3%)で,両者をあわせた奏効率は51.8%であった.多孔性ゼラチン粒を使用しない(TACEを行わない)抗がん剤の治療成績としては,白金製剤やアントラサイクリン系薬剤より奏効率が高い結果であった.

## 7 治療の実例

70歳代男性.7年前よりC型肝硬変・肝細胞癌として治療を行っており,これまでに,肝切除1回,ラジオ波凝固療法10回,エタノール局注療法3回,TACE5回が行われており,TACEの際にはすでにエピルビシン・シスプラチンの使用歴がある.今回,ダイナミックCTにてS8横隔膜直下に直径16 mmの多血性結節が認められ,肝癌再発として精査入院となった.

血管造影では,反復してTACEを行っている経緯があり,S8に新規に出現した肝癌結節は,固有肝動脈・右横隔動脈・肋下動脈・第10肋間動脈などからは栄養されず,唯一,右第11肋間動脈より栄養されていた(図3a).これに対し右第11肋間動脈よりミリプラチン60 mg+リピオドール3.0 mlを動注した.多孔性ゼラチン粒による塞栓は行わなかった.注入直後の腹部単純撮影ではリピオドールは腫瘍に良好に貯留している像が見られた(図3b).

治療翌日・1週間後・10週間後のダイナミックCTでもリピオドールは腫瘍内に100%の貯留を持続し,腫瘍径は10 mmにまで縮小した(図3c).

## 8 本薬剤の将来性

リピオドリゼーションのみで約半数の症例に奏効が得られることより,従来の抗癌剤より高い成績がみられる.このことは,肝機能不良例などTACEが困難な症例では第一に選択できる治療であるといえる.また,肝動注を行うことで血管閉塞・動脈-門脈シャント・肝葉萎縮などの血管障害の副作用が少なく,安全な反復治療に適している.

執筆時点では,ミリプラチン動注に多孔性ゼラチン粒を併用すること(TACE)が認可されていないが,肝動脈塞栓を併用することでさらに抗腫瘍効果が高まることが期待されている.

ミリプラチンの活性体DPCは,各種のシスプラチン耐性癌細胞株に対して弱い交叉耐性示すのみで,シスプラチン無効肝癌症例など,「従来のTACEに抵抗性」の肝癌症例に対しても治療効果が期待される.

今後は,従来のアントラサイクリン系抗癌剤,他の白金製剤との適切な比較試験を通じて抗癌剤作用の「実力」を確認するとともに,リピオドールとの配合比などより最適な投与方法についての検討が必要である.また,長期的には「無進行再発率」や生存率に及ぼす影響についても早期に明らかにされていくことも求められている.

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# Influence of Amino-Acid Polymorphism in the Core Protein on Progression of Liver Disease in Patients Infected With Hepatitis C Virus Genotype 1b

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The substitution of amino acid (aa) 70 of arginine for glutamine and/or that of aa91 of leucine for methionine in the core protein in patients infected with hepatitis C virus (HCV) genotype 1b is associated with a poor response to pegylated interferon and ribavirin. Factors influencing these substitutions were sought in 1,097 patients infected with HCV-1b who had not received antiviral treatment. HCV variants with Arg70 and Leu91 (wild-type) decreased, while those with Gln70 and/or Met91 (mutant types) increased with age ($P < 0.001$). Of the 1,097 patients, 464 (42.3%) were infected with the Gln70 variant and the remaining 633 patients with the Arg70 variant. The proportion of patients with the Gln70 variant increased with the severity of liver disease ($P < 0.001$), elevated γ-glutamyl transpeptidase (γ-GTP) levels ($P < 0.001$) and a decrease in platelet count ($P = 0.008$). In univariate analysis patients with hepatocellular carcinoma, elevated aspartate aminotransferase (AST ≥ 58 IU/L) and γ-GTP (≥61 IU/L), and decreased albumin levels (<3.9 g/dl) were more frequent in the patients with the Gln70 variant than the Arg70 variant ($P = 0.003$, 0.005, <0.001, and 0.031, respectively). In multivariate analysis HCC (odds ratio 1.829 [95% confidence interval 1.147–2.917]) and γ-GTP ≥61 IU/L (1.647 [1.268–2.139]) increased the risk for the Gln70 variant. In conclusion, the substitution of amino aa70 of Arg for Gln in patients infected with HCV-1b increases with age, and it is associated with severe liver disease accompanied by elevated AST and γ-GTP levels, as well as the development of hepatocellular carinoma. ***J. Med. Virol. 82:41–48, 2010.*** © 2009 Wiley-Liss, Inc.

**KEY WORDS:** cirrhosis; core protein; hepatitis C; hepatocellular carcinoma; interferon; ribavirin



## INTRODUCTION

Worldwide, an estimated 170 million people are infected with hepatitis C virus (HCV) persistently [Cohen, 1999]. Decompensated cirrhosis and hepatocellular carcinoma (HCC) can develop in about 30% of patients infected with HCV [Alberti et al., 1999; Seeff, 2002]. HCV has six major genotypes and dozens of subgenotypes, and they have distinct geographic distributions and are associated with the progression of liver disease [Simmonds, 1995]. Host and virological factors can influence the severity of liver disease and the response to antiviral treatment. HCV infection in the childhood and women runs a milder course than that in adulthood and men, and the intake of alcohol accelerates the progression of liver disease [Poynard et al., 1997; Kenny-Walsh, 1999; Vogt et al., 1999; Wiese et al., 2000]. Genotypes 1 and 4 aggravate liver disease and decrease the response to antiviral treatment, in comparison with genotypes 2, 3, and 6 [Tsubota et al., 1994; Hui et al., 2003; Hadziyannis et al., 2004; Legrand-Abravanel et al., 2005; Yuen and Lai, 2006]. High levels of HCV RNA in the serum can induce severe liver disease and decrease treatment response [Tsubota et al., 1994].

In Japan, genotype 1b in a high viral load (>100 KIU/ml) accounts for >70% of HCV infection, and decreases the treatment response in patients with chronic hepatitis C [Kumada et al., 2006]. Even with pegylated interferon (PEG-IFN) combined with ribavirin, the sustained virological response for longer than 24 weeks after the withdrawal of treatment is achieved merely in

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50% of the patients with HCV-1b in high levels [Manns et al., 2001; Fried et al., 2002]. It is necessary to predict the response to PEG-IFN/ribavirin before the start of antiviral therapy, to avoid severe side-effects in the patients who will barely gain sustained virological response.

The core protein of HCV is coded for by the C gene, and consists of 191 amino acids (aa) [Rosenberg, 2001]. Although the core protein is conserved better than the other structural and non-structural proteins of HCV, polymorphisms of core protein are known, and they influence the response to antiviral treatment. In patients infected with HCV-1b, for example, the substitution of arginine at position 70 (Arg70) for glutamine (Gln70) and that of leucine at position 91 (Leu91) for methionine (Met70) decrease sustained virological response in the patients with chronic hepatitis C who are treated with PEG-IFN/ribavirin and increase the development of HCC [Akuta et al., 2007a,b,d, 2008].

In the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo, the amino-acid sequence of the core-protein was determined in 1,079 patients infected with HCV-1b who had not received antiviral treatment. The substitution of Arg70 for Gln70 and that of Leu91 or Met 91 were correlated with the age at presentation, liver function tests and the severity of liver disease. Based on the results obtained, Gln70 would contribute to the progression of chronic hepatitis C.

## MATERIALS AND METHODS

### Patients

During 1966–2008, 1,097 patients infected with HCV-1b visited the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo. They were: (1) negative for hepatitis B surface antigen by radioimmunoassay (Dainabot, Tokyo, Japan) or antibody to human immunodeficiency virus type-1; (2) positive for anti-HCV by a third-generation enzyme immunoassay (Chiron Corp., Emeryville, CA) and HCV RNA by the polymerase chain reaction (PCR) (Cobas Amplicor HCV Monitor ver.2.0, Roche Diagnostics, Tokyo, Japan); (3) infected with HCV genotype 1b but not with other genotypes; (4) without previous antiviral treatment; (5) without other forms of hepatitis, including hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic liver disease and autoimmune liver disease; and (6) had serum samples stored at −80°C. Of the 1,097 patients, 778 (70.9%) had chronic hepatitis, 221 (20.1%) cirrhosis, and 98 (8.9%) HCC. Amino acids in the core protein at positions 70 and 91 were determined, and were correlated with liver disease and biochemical and virological markers. Informed consent was obtained from each patient in this study, and the protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by approval by Ethic Committee of the institution.

### Histopathological Diagnoses of Liver Disease

Liver biopsy was performed under laparoscopy by a modified Vim Silverman needle (Tohoku University style, Kakinuma Factory, Tokyo). The sample was fixed in 10% formalin, and was stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff. It contained at least six portal areas. The pathological diagnosis was made by one of the authors (H.K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on the scoring system of Desmet et al. [1994]. Cirrhosis was diagnosed by imaging on ultrasonography (US), computed tomography (CT), or magnetic resonance imaging (MRI). HCC was diagnosed by US and/or CT. Angiography was performed when HCC was strongly suspected by US, CT, MRI, or liver biopsy. An increasing trend of tumor markers was taken into consideration for the diagnosis of HCC.

### Determination of Amino-Acid Substitutions in the Core Protein

Amino acid (aa) at position 70 of Arg or Gln and aa91 of Leu or Met were determined by PCR with primers specific for each of them [Okamoto et al., 2007]. It is highly reproducible, and has a sensitivity of 94.4% in the determination of aa70 or aa91 in samples with HCV RNA titers >10 KIU/ml. The concordance of the results of this method with those of direct sequencing reached 97.1%. Amino acids at positions 70 and 91 were confirmed by direct sequencing of most samples [Akuta et al., 2005].

### Statistical Analysis

Changes of Arg70/Leu91 (wild-type) and Gln70 and/or Met91 (mutant types) with age were analyzed by the Cochran–Armitage trend test (SAS version 9.1.3; SAS Institute, Inc., Cary, NC). Frequencies were compared between groups by the Kruskal–Wallis test and Fisher's exact test. Univariate and multivariate logistic regression analyses were used for the evaluation of factors independently associated with the substitution of aa70. They included the following ten variables: age, sex, liver disease, platelet count, hemoglobin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), and substitution of aa at position 91 in the core protein. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. Variables that achieved statistical significance on univariate analysis were tested by the multivariate Cox proportional hazard model to identify independent factors. Statistical comparisons were performed using SPSS ver.11.0 (SPSS, Inc., Chicago, IL). A $P$-value $<0.05$ by the two-tailed test was considered significant.

## RESULTS

### Clinical and Virological Characteristics of the 1,097 Patients Who Were Infected With HCV-1b

Table I lists the baseline characteristics of the 1,097 patients who were infected with HCV-1b and had not received antiviral treatment. They had the median age of 60 years and included 590 (53.8%) men. The median transaminase levels were elevated, and alpha-fetoprotein was within the normal limit (<10 μg/L). The majority of the patients (70.9%) had chronic hepatitis, while HCC had developed in 8.9% of the patients. Amino acids at positions 70 and 91 in the core protein were both the wild-type (Arg70 and Leu91) in 37.6% of them, and both mutant types (Gln70 and Met91) in 16.4%. The Gln70 variant was detected in 464 of the 1,097 (42.3%) patients.

### The Prevalence of Amino-Acid Substitutions Stratified by Age and Sex

The 1,097 patients infected with HCV-1b were classified into three age groups, and the prevalence of Arg70/Leu91 (wild-type) and that of Gln70 and/or Met91 (mutant types) were compared (Fig. 1). Ag70/Leu91 decreased with age by trend analysis, from 63.6% in the patients aged ≤30 years to 36.6% in those ≥41 years ($P < 0.001$ by the Cochran–Armitage trend test). Table II lists the prevalence of the Gln70 variant in men and women stratified by the age. There were no sex differences in the prevalence of the Gln70 variant.

### The Prevalence of the Gln70 Variant in Patients With Different Liver Diseases

Figure 2 compares the prevalence of the Gln70 variant among patients infected with HCV-1b who presented with different liver diseases at the baseline. The prevalence of the Gln70 variant increased with the progression of liver disease from chronic hepatitis (32.6%) to cirrhosis (43.0%) and HCC (53.1%) ($P < 0.001$ by the Kruskal–Wallis test). In patients with cirrhosis, the 126 patients with the Arg70 variant were aged with the mean of 62 years (range: 32–78 years) in comparison with the 95 patients with the Gln70 variant who were aged 59 years (25–80). In patients with HCC, the 47 patients with the Arg70 variant were aged with the mean of 66 years (range: 37–81 years) in comparison with the 51 patients with the Gln70 variant who were aged 66 years (46–78).

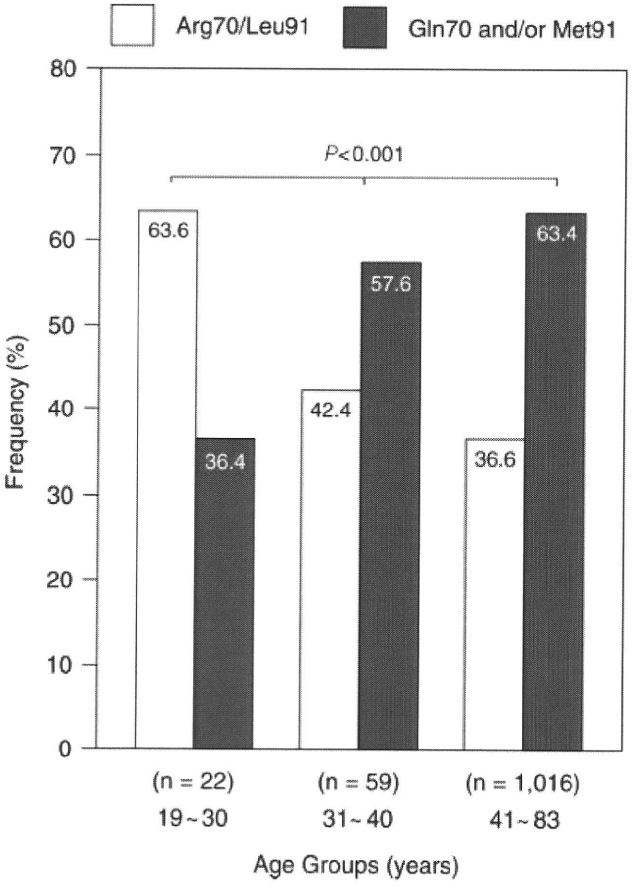


Fig. 1. The age-specific prevalence of Gln70 in treatment-naive patients infected with HCV-1b.

TABLE I. Clinical and Virological Characteristics of the 1,097 Patients Who Were Infected With Hepatitis C Virus of Genotype 1b

| | |
|---|---|
| Age (years) | 60 (19–83) |
| Men | 590 (53.8%) |
| Follow-up period (years) | 8 (3–28) |
| Hemoglobin (g/dl) | 14.0 (4.5–26.8) |
| Platelets ($\times 10^3/mm^3$) | 15.4 (2.0–34.1) |
| Aspartate aminotransferase (IU/L) | 58 (8–617) |
| Alanine aminotransferase (IU/L) | 69 (6–776) |
| Alpha-fetoprotein (μg/L) | 6 (2–65,700) |
| Liver disease | |
| Chronic hepatitis | 778 (70.9%) |
| Cirrhosis | 221 (20.1%) |
| Hepatocellular carcinoma | 98 (8.9%) |
| Amino acids in the core protein | |
| Arg70/Leu91 (double wild-type) | 412 (37.6%) |
| Gln70/Leu91 (mutant type) | 284 (25.9%) |
| Arg70/Met91 (mutant type) | 221 (20.1%) |
| Gln70/Met91 (double mutant type) | 180 (16.4%) |

Values are the median with range in parentheses or the number with percentage in parentheses.

TABLE II. Frequency of Gln70 in the Core Protein in Patients Infected With HCV-1b Stratified by Age and Sex

| Age (years) | Men | Women | Differences |
|---|---|---|---|
| 19–30 | 23.5% (4/17) | 20% (1/5) | 1.0 |
| 31–40 | 34.1% (14/41) | 38.9% (7/18) | 0.773 |
| 41–50 | 37.2% (45/121) | 40% (14/35) | 0.763 |
| 51–60 | 39.1% (72/184) | 40.1% (63/157) | 0.912 |
| 61–70 | 36.0% (62/172) | 30.1% (74/246) | 0.205 |
| 70–83 | 45.5% (25/55) | 43.5% (20/46) | 0.842 |
| Total | 37.6% (222/590) | 35.3% (179/507) | 0.451 |

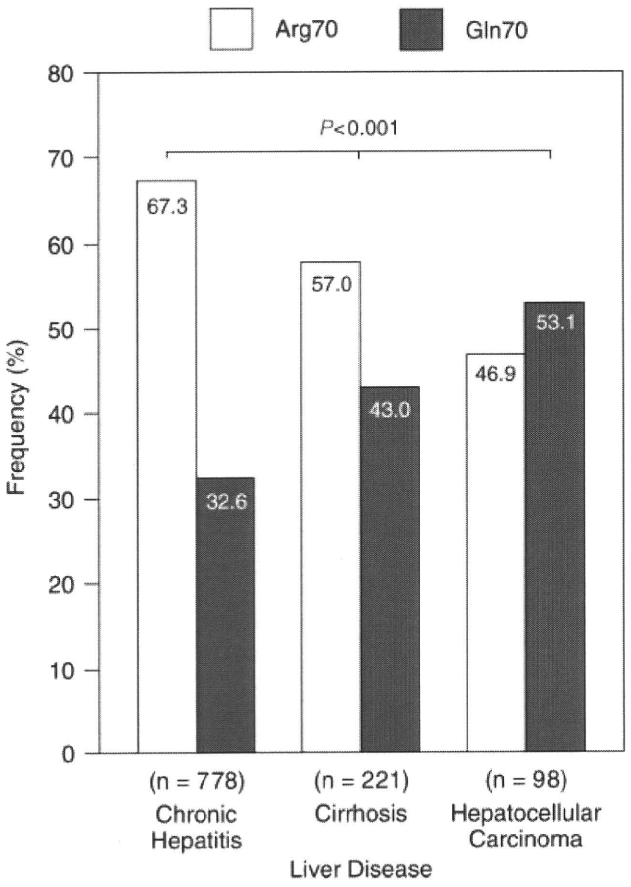


Fig. 2. The prevalence of the Gln70 variant among patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma.

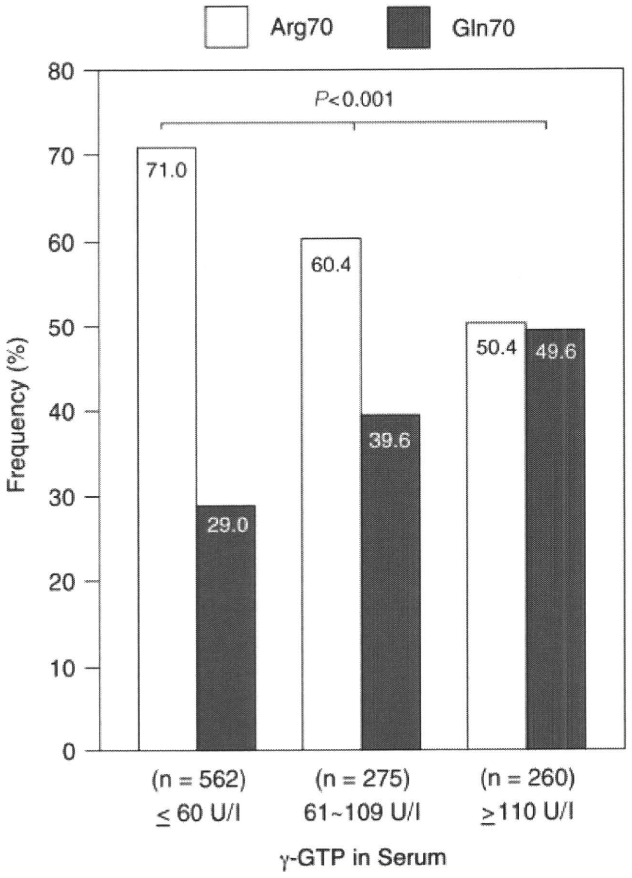


Fig. 3. The prevalence of the Gln70 variant among patients with different γ-GTP levels.

### The Influence of γ-GTP Levels on the Prevalence of the Gln70 Variant

The prevalence of Gln70 was compared among patients with different γ-GTP levels at the baseline (Fig. 3). The prevalence of the Gln70 variant increased in parallel with the γ-GTP levels from 29.0% to 49.6% ($P < 0.001$ by the Kruskal–Wallis test).

### The Influence of Platelet Count on the Prevalence of the Gln70 Variant

The prevalence of the Gln70 variant was compared among three groups of patients with various platelet counts at the baseline (Fig. 4). The prevalence of the Gln70 variant increased as the platelet count decreased ($P = 0.008$ by the Kruskal–Wallis test).

### Factors Associated With the Gln70 Variant in Patients Infected with HCV-1b

Since the Gln70 variant, in comparison with the Arg70 variant, aggravated liver disease in patients infected with HCV-1b (Figs. 2–4), ten factors were evaluated for the association with the Gln70 variant by the univariate analysis (Table III); the cut-off value was set at the median of studied patients. Among them, HCC, elevated levels of AST (≥58 IU/L) and γ-GTP (>61 U/L), as well as decreased albumin concentration (<3.9 g/dl), were associated with the Gln70 variant ($P = 0.003$, 0.005, <0.001, and 0.031, respectively). A similar analysis was performed for the substitution of Leu91 for Met91 (Table IV). Except for the association with the substitution of Arg70 for Gln70, the Met91 variant had no influence on any variable examined.

Two factors associated independently with the Gln70 variant were identified by the multivariate analysis (Table V). The risk for the Gln70 variant was increased by HCC (odds ratio 1.829 [95% confidence interval 1.147–2.917], $P = 0.011$) and γ-GTP ≥61 IU/L (1.647 [1.268–2.139], $P < 0.001$).

## DISCUSSION

The response to PEG-IFN and ribavirin is influenced by genotypes and viral load, and is poorest in patients with HCV-1b in high HCV RNA levels [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004]. The prediction of sustained virological response would circumvent side-effects and costs in non-responders. Amino-acid substitutions in the core protein are useful

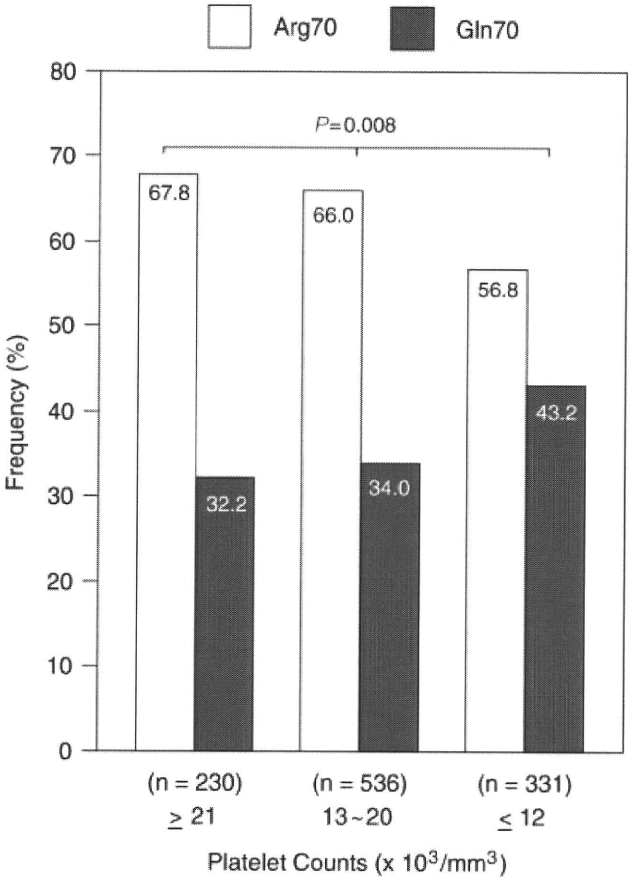


Fig. 4. The prevalence of the Gln70 among three groups of patients with different platelet counts.

for predicting the non-response in patients infected with HCV-1b. The substitution of Arg70 for Gln70 in the prototype sequence of HCV-1b [Kato et al., 1990] and/or that of Leu91 for Met91 can predict the non-response to IFN-based treatment [Akuta et al., 2005, 2006, 2007c,d]. It has been beyond the scope of previous studies, however, whether or not these amino-acid substitutions influence the progression of hepatitis C in the patients who have not received antiviral treatment. The availability of pre-treatment sera from many patients with chronic hepatitis C permitted the evaluation of the influence of aa substitutions in the core protein on the progression of liver disease without therapeutic intervention.

First, the prevalence of the Gln70 variant increased with the age of patients until they had reached 50 years (Fig. 1). It is not certain if HCV-1b with Arg70 underwent a point mutation for Gln70 (G-to-A at nucleotide 209), or these amino-acid residues were present in HCV-1b strains prevalent at the time of infection. Follow-up of patients for aa substitutions will resolve this issue. Another possibility for this difference would be a selection bias. If the patients with the Arg90 variant fare better than those with the Gln70 variant, they would not develop liver disease severe enough to visit hospital.

Secondly, the patients infected with HCV-1b with Gln70 increased in parallel with γ-GTP levels and the severity of liver disease from chronic hepatitis to cirrhosis and HCC, as well as with a decrease in platelet count (Figs. 2–4). Since the Met91 variant did not make such difference, the aggravation of liver disease would have been due to the Gln70 variant, but not to the Met91 variant. Increases in the γ-GTP level may have been related to the development of HCC; γ-GTP has been proposed as a sensitive marker of cirrhosis and HCC [Penn and Worthington, 1983]. Decreased platelet counts have been associated with HCC [Ikeda et al., 2001; Lu et al., 2006; Kumada et al., 2009]. Although the proportion of the Gln70 variant increases with the severity of liver disease (Fig. 2), the median age of patients with cirrhosis or HCC did not differ between the patients with the Arg70 variant and Gln70 variant who

TABLE III. Factors Associated With the Substitution of aa70 of Arginine for Glutamine in the Core Protein in 1,097 Patients Infected With HCV Genotype1b by Univariate Analysis

| Factor | Category | Gln70 | *P*-value |
|---|---|---|---|
| Sex | 1: Male | 38.6% (228/590) | 0.663 |
| | 2: Female | 37.3% (189/507) | |
| Age (years) | 1: <60 | 40.6% (219/540) | 0.093 |
| | 2: ≥60 | 35.5% (198/557) | |
| AST (IU/L) | 1: <58 | 33.9% (184/543) | 0.005 |
| | 2: ≥58 | 42.2% (234/554) | |
| ALT (IU/L) | 1: <75 | 36.9% (213/578) | 0.376 |
| | 2: ≥75 | 39.3% (204/519) | |
| Albumin (g/dl) | 1: <3.9 | 42.5% (194/457) | 0.031 |
| | 2: ≥3.9 | 35.8% (229/640) | |
| γ-GTP (IU/L) | 1: <61 | 29.0% (163/562) | <0.001 |
| | 2: ≥ 61 | 44.4% (238/535) | |
| Hemoglobin (g/dl) | 1: <14 | 35.1% (176/501) | 0.083 |
| | 2: ≥14 | 40.4% (241/596) | |
| Platelet count ($\times 10^3/mm^3$) | 1: <150 | 39.9% (207/519) | 0.253 |
| | 2: ≥150 | 36.3% (210/578) | |
| Hepatocellular carcinoma | 1: No | 36.6% (366/999) | 0.003 |
| | 2: Yes | 53.1% (52/ 98) | |
| Substitutions of core aa91 | 1: Leucine | 35.6% (227/638) | 0.051 |
| | 2: Methionine | 41.4% (190/459) | |

TABLE IV. Factors Associated With the Substitution of aa91 of Leucine for Methionine in the Core Protein in 1,097 Patients Infected With HCV Genotype1b by Univariate Analysis

| Factor | Category | Met91 | *P*-value |
|---|---|---|---|
| Sex | 1: Male | 40.8% (241/590) | 0.500 |
| | 2: Female | 43.0% (218/507) | |
| Age (years) | 1: <60 | 43.5% (235/540) | 0.271 |
| | 2: ≥60 | 40.2% (220/517) | |
| AST (IU/L) | 1: <58 | 43.6% (234/537) | 0.196 |
| | 2: ≥58 | 39.7% (217/547) | |
| ALT (IU/L) | 1: <75 | 42.4% (238/561) | 0.618 |
| | 2: ≥75 | 40.8% (205/502) | |
| Albumin (g/dl) | 1: <3.9 | 42.0% (177/421) | 0.797 |
| | 2: ≥3.9 | 41.2% (249/604) | |
| γ-GTP (IU/L) | 1: <61 | 40.4% (237/586) | 0.327 |
| | 2: ≥61 | 43.4% (222/511) | |
| Hemoglobin (g/dl) | 1: <14 | 40.8% (193/473) | 0.658 |
| | 2: ≥14 | 42.3% (240/567) | |
| Platelet count ($\times 10^3/mm^3$) | 1: <150 | 40.5% (202/499) | 0.454 |
| | 2: ≥150 | 42.9% (240/559) | |
| Hepatocellular carcinoma | 1: No | 42.3% (423/999) | 0.334 |
| | 2: Yes | 36.7% (36/ 98) | |
| Substitutions of core aa71 | 1: Arginine | 49.0% (269/680) | 0.051 |
| | 2: Glutamine | 45.6% (190/417) | |

had cirrhosis (62 years vs. 59 years) of HCC (66 years vs. 66 years). This would indicate a possibility that the Gln70 variant would be a factor for the aggravation of liver disease that might be independent of age.

The distinct capacity of Gln70 and Met91 for decreasing the response to combined treatment in patients infected with HCV-1b was proposed in a recent study [Okanoue et al., 2008]. The Gln70 variant decreased sustained virological response, while the Met91 variant did not, although the Met91 variant reduced the rate of rapid virological response within 4 weeks after the start of therapy. The role of the Gln70 variant greater than that of the Met91 variant in the progression of liver disease has been confirmed in this study (Tables III and IV). In the multivariate analysis, the risk for Gln70 was increased by HCC (odds ratio 1.829 [95% confidence interval 1.147–2.917]) and γ-GTP ≥61 U/L (1.647 [1.268–2.139]). The Gln70 variant would aggravate liver disease toward the development of HCC in patients infected with HCV-1b who have not received antiviral treatment.

It would be a matter of conjecture how the Gln70 variant influences the severity of liver disease. Previous suggestions for a reduced response of patients with the Gln70 variant were confined to interaction of the core protein with IFN receptors and IFN-signaling pathways [Alexander, 2002; Blindenbacher et al., 2003; Bode et al., 2003]; these studies were restricted to patients receiving IFN-based treatments [Akuta et al., 2007a,b,d, 2008]. The ability of the Gln70 variant for accelerating the progression of liver disease, in the absence of exogenous IFN, has changed this issue into a wider perspective. There still remains a possibility, however, that the Gln70 variant would interact with the endogenous IFN induced by HCV infection, and aggravate liver disease.

Another possibility may be the cytotoxic T-cell (CTL) response, as has been demonstrated for the pathogenesis of chronic hepatitis B [Chisari and Ferrari, 1995]. Since both hepatitis B virus (HBV) and HCV do not have a cytopathic capacity, hepatitis B and C would be mediated by immune responses directed at viral proteins. Amino-acid sequences bearing a CTL epitope restricted by the MHC class-I are demonstrated in the HBV core protein [Bertoletti et al., 1993; Bertoletti and Gehring, 2006], and are implicated in liver disease in the patients with the HLA-2 phenotype [Penna et al., 1991; Bertoletti et al., 1994]. It is tempting to speculate that the substitution of Arg70 for Gln70 might generate a CTL epitope and stimulate cytotoxic lymphocytes toward inflammation of the liver [Kita et al., 1993; Jackson et al., 1999].

In conclusion, amino-acid substitutions in the core protein influence the progression of liver disease, and the Gln70 variant aggravates hepatic inflammation and increases the risk for HCC in the patients who have not received antiviral treatment. The ability of the Gln70

TABLE V. Factors Associated with the Substitution of aa70 of Arginine for Glutamine in the Core Protein in 1,097 Patients Infected with HCV Genotype1b by Multivariate Analysis

| Factor | Category | Odds ratio (95%CI) | *P*-value |
|---|---|---|---|
| Hepatocellular carcinoma | 1: No | 1 | 0.011 |
| | 2: Yes | 1.829 (1.147–2.917) | |
| γ-GTP (IU/L) | 1: <61 | 1 | <0.001 |
| | 2: ≥61 | 1.647 (1.268–2.139) | |

variant to aggravate liver disease, in the absence of exogenous IFN, would lend further support on its capacity of predicting sustained virological response before the start of therapy. It is possible that mechanisms other than the resistance to IFN, such as cytotoxic T-cell responses, might be involved in an increased pathogenetic potential of HCV-1b with Gln70.

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***Original Article***

# Development of HCC in patients receiving adefovir dipivoxil for lamivudine-resistant hepatitis B virus mutants

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*Aim:* To identify factors for the development of hepatocellular carcinoma (HCC) in the patients who receive adefovir add-on lamivudine for treatment of lamivudine-resistant hepatitis B virus (HBV) mutants.

*Methods:* A total of 247 patients who developed lamivudine-resistant HBV mutants, with an increase of HBV DNA ≥ 1 log copies/mL, received adefovir dipivoxil 10 mg add-on lamivudine 100 mg daily during a median of 115 weeks (range: 25–282 weeks). They were followed for the development of HCC by imaging modalities every 3–6 months.

*Results:* HCC developed in 18 of the 247 (7.3%) patients. Eight factors were in significant association with the development of HCC by the univariate analysis. They included age, cirrhosis, platelet counts, levels of bilirubin, aspartate aminotransferase (AST), alanine aminotransferase and α-fetoprotein, as well as YMDD mutants at the start of adefovir dipivoxil. By the multivariate analysis, AST levels, YIDD mutants, cirrhosis and age were independent factors for the development of HCC. By the Kaplan-Meier analysis, AST levels ≥ 70 IU/L, YIDD mutants, cirrhosis and age ≥ 50 years increased the risk of HCC ($P = 0.018$, $P = 0.035$, $P = 0.002$ and $P = 0.014$, respectively). HCC developed more frequently in the patients with than without cirrhosis at the start of adefovir (10/59 [16.9%] vs. 8/188 [4.3%], $P = 0.002$).

*Conclusion:* HCC can develop in cirrhotic patients receiving adefovir add-on lamivudine. Hence, the patients with baseline AST ≥ 70 IU/L and YIDD mutants would need to be monitored closely for HCC.

**Key words:** adefovir dipivoxil, chronic hepatitis B, hepatitis B virus, hepatocellular carcinoma, lamivudine, rescue therapy

## INTRODUCTION

WORLDWIDE, AN ESTIMATED 400 million people are infected with hepatitis B virus (HBV) persistently, and one million die of decompensated cirrhosis and/or hepatocellular carcinoma (HCC) annually.[1,2] Interferon (IFN) was introduced for treatment of chronic hepatitis B, and it has been replaced for pegylated-IFN.[3] Due to substantial side-effects and requirement for injection, however, IFN-based therapies are not favored.

In 1998, lamivudine was approved as the first nucleot(s)ide analogue for treatment of chronic hepatitis B,[4] and then adefovir in 2002.[5] Due to its lower costs and safety records, lamivdine has gained a wide popularity for treatment of chronic hepatitis B. However, drug-resistant mutants arise in parallel with the duration of lamivudine, in 12.5% after 1 year, in 43.8% after 3 years, and 62.5–70.2% after 5 years.[6,7] For preventing breakthrough hepatitis induced by lamivudine-resistant HBV mutants, additional adefovir dipivoxil 10 mg daily has been recommended;[8,9] it is more effective than switching to adefovir monotherapy and has fewer chances of developing drug-resistant mutants.[10,11]

Since 1995, 930 patients with chronic hepatitis have been treated with lamivudine in the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo.[12] HBV mutants with mutations in the thyrosine-methionine-aspartic acid-aspartic acid (YMDD) motif elicited in the 247 (26.5%) patients, and they started to receive additional adefovir since December, 2002.[13,14] However, HCC developed in 18 (7.3%) of them during the combination therapy for 25–282 weeks; HCC has

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not been reported in any of the patients who have received adefovir add-on lamivudine for 5 years.[15–17] Hence, factors for the development of HCC in the patients receiving adefovir add-on lamivudine were sought for in a retrospective study.

## METHODS

### Patients

OVER A PERIOD of 13 years, from September 1995 to September 2007, 930 patients with chronic hepatitis B received long-term lamivudine treatment at the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo. Drug-resistant YMDD mutants developed in 247 (26.5%) of them, accompanied by an increase in HBV DNA ≥ 1 log copies/mL, and they received adefovir 10 mg in addition to lamivudine 100 mg daily during the median of 115 weeks (range: 25–282 weeks). They have been followed for liver function and virological markers of HBV infection monthly, as well as blood counts and tumor makers including alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II). Cirrhosis was diagnosed by laparoscopy or liver biopsy, and in the patients who had not received them, by clinical data, imaging modalities and portal hypertension. HCC was diagnosed by hypervascularity on angiography and/or histological examination, characteristic features of computed tomography, magnetic resonance imaging and ultrasonography. An informed consent was obtained from each patient in this study, and the protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a *priori* approval by the institution's human research committee.

### Markers of HBV infection

Hepatitis B e antigen (HBeAg) was determined by enzyme-linked immunosorbent assay (ELISA) with commercial kits (HBeAg EIA, Institute of Immunology, Tokyo). HBV DNA was quantitated by the Amplicor monitor assay (Roche Diagnostics, Tokyo) with a dynamic range over 2.6–7.6 log copies/mL. Genotypes of HBV were determined serologically by the combination of epitopes expressed on the pre-S2 region product, which is specific for each of the seven major genotypes (A–G),[18,19] with use of commercial kits (HBV Genotype EIA, Institute of Immunology).

### Detection of YMDD mutants

YMDD mutants were determined by polymerase chain reaction (PCR)-based enzyme-linked mini-sequence assay (PCR-ELIMA) with commercial kits (Genome Science Laboratories, Tokyo).

### Statistical analyses

Categorial variables were compared between groups by the $\chi^2$ test, and non-categorical variables by the Mann–Whitney *U*-test. A *P*-value < 0.05 was considered significant. Factors associated with HCC by univariate analysis were evaluated by the multivariate analysis by the stepwise Cox proportional hazard model. Development of HCC with time was analyzed by the Kaplan–Meier method, and differences were evaluated by the log-rank test. Data were analyzed by the SPSS software, version 11.0 (Chicago, IL).

## RESULTS

### Baseline characteristics of the patients who did and who did not develop hepatocellular carcinoma during adefovir add-on lamivudine treatment

TABLE 1 COMPARES characteristics at the start of adefovir between the 18 patients who developed HCC and the 229 who did not. Eight factors were associated with the development of HCC by the univariate analysis. They included age, cirrhosis, platelet counts, bilirubin, AST, alanine aminotransferease (ALT) and α-fetoprotein (AFP) levels, as well as YMDD mutants. HCC developed more frequently in the patients with than without cirrhosis at the start of adefovir (10/59 [16.9%] vs. 8/188 [4.3%], $P = 0.002$). There were 61 (26.6%) patients who had cirrhosis at the start of adefovir. Of them, one of the 18 (2.2%) with HCC and 18 of the 229 (2.2%) without HCC presented with decompensation; no patients developed decompensation after the start of adefovir.

Rates of HBV DNA disappearance from serum (< 2.6 log copies/mL) were: 55% (113/207) at 1 year, 71% (119/168) at 2 years, 77% (78/101) at 3 years and 85% (35/41) at 4 years. Rates of AST nomarlization (< 38 IU/L) were: 87% (179/207) at 1 year, 90% (151/168) at 2 years, 92% (93/101) at 3 years and 95% (39/41) at 4 years; and those of ALT normalization (< 50 IU/L) were: 88% (183/207) at 1 year, 91% (153/168) at 2 years, 93% (94/101) at 3 years and 98% (40/41) at 4 years. There were no differences in the rate of HBV DNA disappearance from serum between the patients with and without HCC: 57% (8/14) vs. 54% (105/193) at 1 year ($P = 1.0$); 86% (12/14) vs. 70% (107/154) at 2 years ($P = 0.229$); and 89% (8/9) vs.

Table 1 Characteristics of patients who did and did not develop hepatocellular carcinoma (HCC) at the start of adefovir†

| | HCC developed ($n = 18$) | HCC did not develop ($n = 229$) | Differences *P*-value |
|---|---|---|---|
| Duration of lamivudine before the start of adefovir | 128 (31–346) | 144 (13–617) | 0.321 |
| Age (years) | 52 (35–75) | 45 (26–75) | 0.008 |
| Men | 15 (83%) | 183 (80%) | 1.000 |
| Cirrhosis | 10 (56%) | 51 (22%) | 0.004 |
| Platelets ($\times 10^3/mm^3$) | 12.0 (4.6–19.7) | 16.3 (3.1–31.9) | 0.001 |
| Albumin (g/dL) | 3.6 (2.3–4.7) | 3.9 (2.8–4.7) | 0.073 |
| Bilirubin (mg/dL) | 0.8 (0.5–15.5) | 0.7 (0.2–6.0) | 0.046 |
| Creatinine (mg/dL) | 0.8 (0.5–1.0) | 0.8 (0.4–1.6) | 0.950 |
| AST (IU/L) | 119 (55–248) | 66 (14–1413) | 0.003 |
| ALT (IU/L) | 151 (61–576) | 104 (13–1563) | 0.035 |
| AFP (ng/dL) | 8 (2–130) | 4 (1–282) | 0.026 |
| HBV genotypes | | | 0.228 |
| C | 18 (100%) | 189 (87%) | |
| Others | 0 | 27 (13%) | |
| HBeAg | 8 (44%) | 132 (58%) | 0.323 |
| HBV DNA (log copies/mL) | 7.1 (4.4->7.6) | 7.1 (<2.6->7.6) | 0.623 |
| YMDD mutants | | | 0.041 |
| YIDD | 13 (72%) | 109 (45%) | |
| YVDD | 5 (28%) | 62 (25%) | |
| YI/VDD | 0 | 56 (23%) | |

†Values are the median with the range in parentheses or *n* with percent in parentheses.
AFP, alpha-fetoprotein; ALT, alaine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

92% (85/92) at 3 years ($P = 0.555$). Rates of normalized AST levels in the patients with and without HCC were: 50% (7/14) vs. 90% (173/193) at 1 year ($P < 0.001$); 79% (11/14) vs. 91% (140/154) at 2 year ($P = 0.166$); and 67% (6/9) vs. 95% (87/92) at 3 year ($P = 0.037$). Rates of ALT normalization in the patients with and without HCC were: 71% (10/14) vs. 90% (174/193) at 1 year ($P = 0.037$); 79% (11/14) vs. 90% (139/154) at 2 year ($P = 0.189$); and 56% (5/9) vs. 92% (85/92) at 3 year ($P = 0.015$). Thus, normalization of AST and ALT was less frequent in the patients with than without HCC.

Characteristics of the 18 patients who developed HCC are compared between the baseline and at the development of HCC (Table 2). At the start of adefovir, 10 (56%) of them had developed cirrhosis and 16 (89%) had AST levels ≥ 70 IU/L. HBV DNA was not detectable in 10 (56%) of them at the development of HCC. Of the eight patients with detectable HBV DNA levels (≥ 2.6 log copies/mL), five (63%) developed HCC within 1 year after the start of adefovir. AST was elevated (> 38 IU/L) in eight patients, including four (50%) without detectable HBV DNA levels.

## Factors independently associated with the development of hepatocellular carcinoma

Eight factors associated with the development of HCC by the univariate analysis, including age, cirrhosis, platelet counts, bilirubin, AST, ALT and AFP levels, as well as YMDD mutants (Table 1), were evaluated by the multivariate analysis. AST ≥ 70 IU/L, YIDD mutants, age ≥ 50 years and cirrhosis at the baseline were independent risk factors for the development of HCC (Table 3). There were no differences in the distribution of YIDD, YVDD and the mixture thereof among the patients with distinct AST, ALT or HBV DNA levels or between those with and without cirrhosis at the start of adefovir. HBV mutants with mutations resistant to adefovir (rtA181T/S, rtN236T) occurred in two of the 247 (0.8%) patients; none of them developed HCC.

The median time between the elevation of HBV DNA > 5.0 log copies/mL and the administration of adefovir was 124 (range: 0–815) days for the 13 patients who developed HCC and 147 (0–3268) days for the 166 patients who did not ($P = 0.605$). The median time between the elevation of ALT > 43 IU/L and the start of