

# 厚生労働科学研究費補助金(肝炎等克服緊急対策研究事業)

分担研究報告書

## 高齢者に対する IFN 治療効果と発癌抑制について

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### 研究要旨:

急速に高齢化する我が国の情勢から考えると C 型慢性肝疾患に罹患している高齢者に対する治療法を確立していくことは急務である。抗ウイルス療法によるウイルスの排除が予後の改善に最も寄与しており、治療の最終目標であることは紛れも無い事実であるが、高齢者においては全ての症例に完全著効を期待して最強の治療を行えるわけではない。今年度は、全体研究としてデータマイニング解析を行っているため、我々は高齢者の症例を個別研究として取り上げた。最近増加傾向にある高齢者症例に対する治療の実態はいかなるものか、その効果はどのようになっているか？また、どのような治療法が有効かつ安全であり、著効がえられなかった原因としての副作用や合併症の問題をいかにして解決するかにつき検討した。さらには、この三年間にわたり検討してきたインターフェロンの発癌抑制効果についても論文発表しているものにつき報告する。

### A. 研究目的

C 型慢性肝炎に対するインターフェロン(IFN)治療の目的は、ウイルス血症を改善し肝臓の組織学的進展を改善し、患者の予後の向上を図ることである。当院の検討でも IFN により完全著効がえられた症例では、無治療例や IFN 無効症例に比して著明に発癌率は低下している。一方高齢化する我が国において近年問題となっているのは高齢者の慢性肝炎症例からの発癌である。このために我々は、治療効果の向上のための新たな方法や、効果的に予後を予測する方法を検討し報告してきた。今年度は高齢者に対する IFN 治療の実態とその結果につき明らかにするとともに、従来著効率の低いといわれてきた高齢女性の著効率をいかにして向上させるかという取り組みを取り上げていく。その上で今後の治療の選択肢の幅を広げることで更なる治療効果の向上を目指すことが研究の目的である。また、高率な治療効果が期待しうるのであればいかに安全に治療が行えるかについても検討し、各個人にあった治療法を見出すことを目的とした。

### B. 研究方法

対象は、2010 年 12 月現在までに IFN 治療を施行し効果判定可能な高齢者症例(IFN 導入時満 65 歳以上で、65 歳から 69 歳と 70 歳以上の二群に分けた。) 596 例である。導入した IFN 別の治療法と

その効果につき検討を行った。

これまでに副反応で中止になった症例を治療別、年齢別に検討し、中止になった理由や要因を解析し、その上で、いかにして IFN 治療の再投与を行い治療効果を上げるかにつき、個々の症例について詳細な検討を行うことで新たな治療法を模索してきた。また、高齢者やうつ病などの合併症症例を対象としどのような工夫をすることで IFN 治療が可能となり、治療効果の向上が期待しうることについても検討しこの二年間報告してきた。対象症例は全例において、HCV-RNA 量、genotype を測定し、治療効果判定は厚生労働省の治療効果判定基準に準じて行った。

(倫理面への配慮)

臨床試験の目的・方法、治療の副作用、患者に関する個人情報の守秘義務、患者の権利保護等について十分な説明を行い、患者が熟考するに十分な時間と理解の後に書面による同意を得た上で臨床試験を遂行した(新 GCP に遵守)。既に医療保険が認められている治療法においても上記に準じて書面の同意書を得ている。本研究の遂行にあたっては、必要な申請を行い当院の倫理規定を遵守した。

### C. 研究結果

これまでに当施設で IFN 治療を行ってきた症例は 4895 例ありこのうち高齢者症例は 1b 型 503 例

(10.3%)、2a型 442例(9%)、2b型 75例(1.5%)存在した。今年度はこれらのうち治療目的に IFN 治療を行い解析可能であった症例を 1b型高ウイルス例 292例とその他の症例(いわゆる others 症例)304例に分けて報告する。図1に示すように60歳代の高齢者ではPEG+Ribaを選択している頻度が41.5%と高率なのに対し70歳以上では23.5%と低下し、 $\alpha$  IFNのみの投与が61.8%と高率であった。Others症例においては図2のように $\beta$  IFNの投与症例が多く特に70歳代では41.3%と最も多数であった。これは、対治療効果を勘案した場合、より安全な $\beta$  IFNによる治療が選択されていることを示している。一方SVRは新規治療であるtelaprevir(TVR)併用療法が症例数は少ないものの50%であり、65歳までの今後の治療はTVRを含めた三剤併用が主流となる可能性が示された(図3)。また、 $\beta$  IFN治療においては二群間のSVR率が乖離しており、症例数が少ないこともあり今後の検討を待つこととなる。others症例においては、いずれもSVR率は高いが、リバビリン併用が不可能な症例においても工夫をすることで著効率を向上させることが可能であることが示された(図4)。

最後にこの三年間に報告した発癌抑制に関する論文報告をまとめておく。AraseらがJ Med Virolに報告した結果を図5に示すが、60歳以上の検討ではあるものの、IFNの発癌抑制効果は著明であり、ALT、AFPとも有意差を持って低下することが明らかとなった。図6-7では、前治療が無効であった症例においてもIFNの長期療法を行うことで肝癌の発生を抑え生存率を向上させることが示され1b型に限ってもこの傾向ははっきりとしている。

#### D. 考察

IFN治療の目的は完全著効をえることであり、その完全著効率はこの15年で著明に向上した。しかしながら、対象患者の高齢化が明らかとなった現在、全ての症例に対してペグインターフェロンとリバビリン併用療法を行うことは副作用中止を考えると不可能と言わざるをえない。そこで今回対象としたような高齢者症例のように別の選択肢も可能な集団の治療成績を明らかにするとともに新たなオプション治療を工夫することでより安全な方法を見いだしていきたいと考えている。その上で増加の一途をたどる高齢者発癌を抑止するためにはIFN無効症例に対してもQOLを考慮しつつ長期のIFN治療を行っていくことが発癌抑制、ひいては生存率の向上にもつながることが示された。今後は新規の治療も導入予定であることより、対象を決めた上でprospectiveに新たな治療を試み、また症例数を積むことでevidenceに基づいた成績を出していきたいと考えている。

#### E. 結論

高齢者症例に対する治療成績を示したが、高齢者や合併症を持つ症例においては、治療法を工夫することで効果向上が期待しうることが示された。今後は、その治療反応性に関わる要因をさらに解析していくことが肝要である。

#### F. 健康危険情報

なし

#### G. 研究発表

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#### H. 知的財産権の出願・登録状況

(※予定を含む)

1.特許取得:なし

2. 実用新案登録:なし

3. その他:なし

## 書籍

著者氏名	論文タイトル名	書籍全体の 編集者名	書 籍 名	出版社 名	出版地	出版年	ページ

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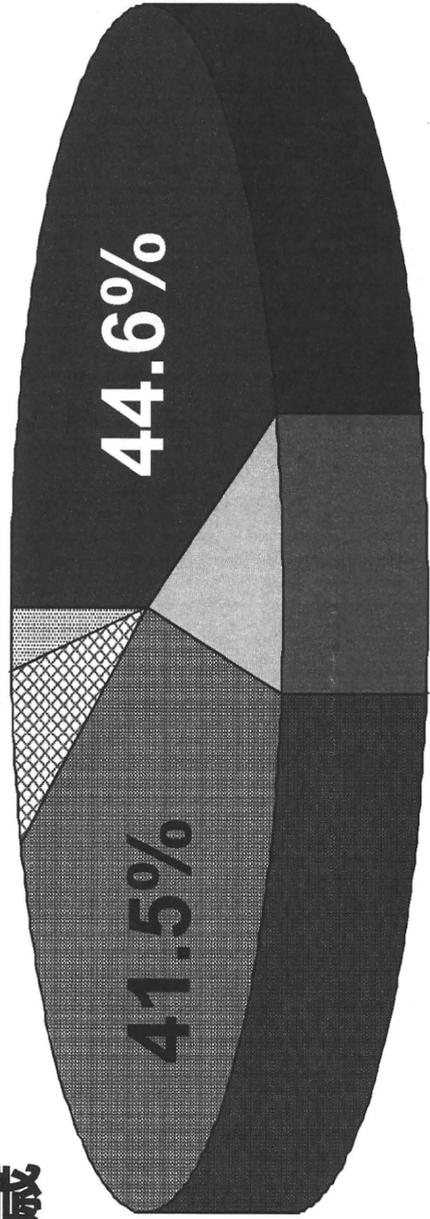
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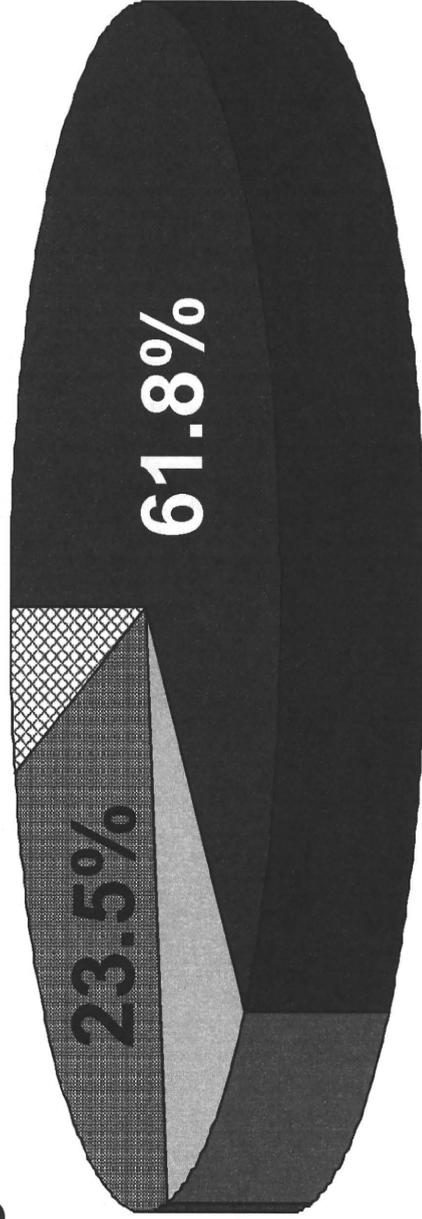
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65-69歳  
n=224



- α-IFN
- β-IFN
- PEG-R
- β-R
- P-R-TVR

70歳~  
n=68



- α-IFN
- β-IFN
- PEG-R
- β-R

図1.高齢者のIb high症例に対するIFN投与の内訳

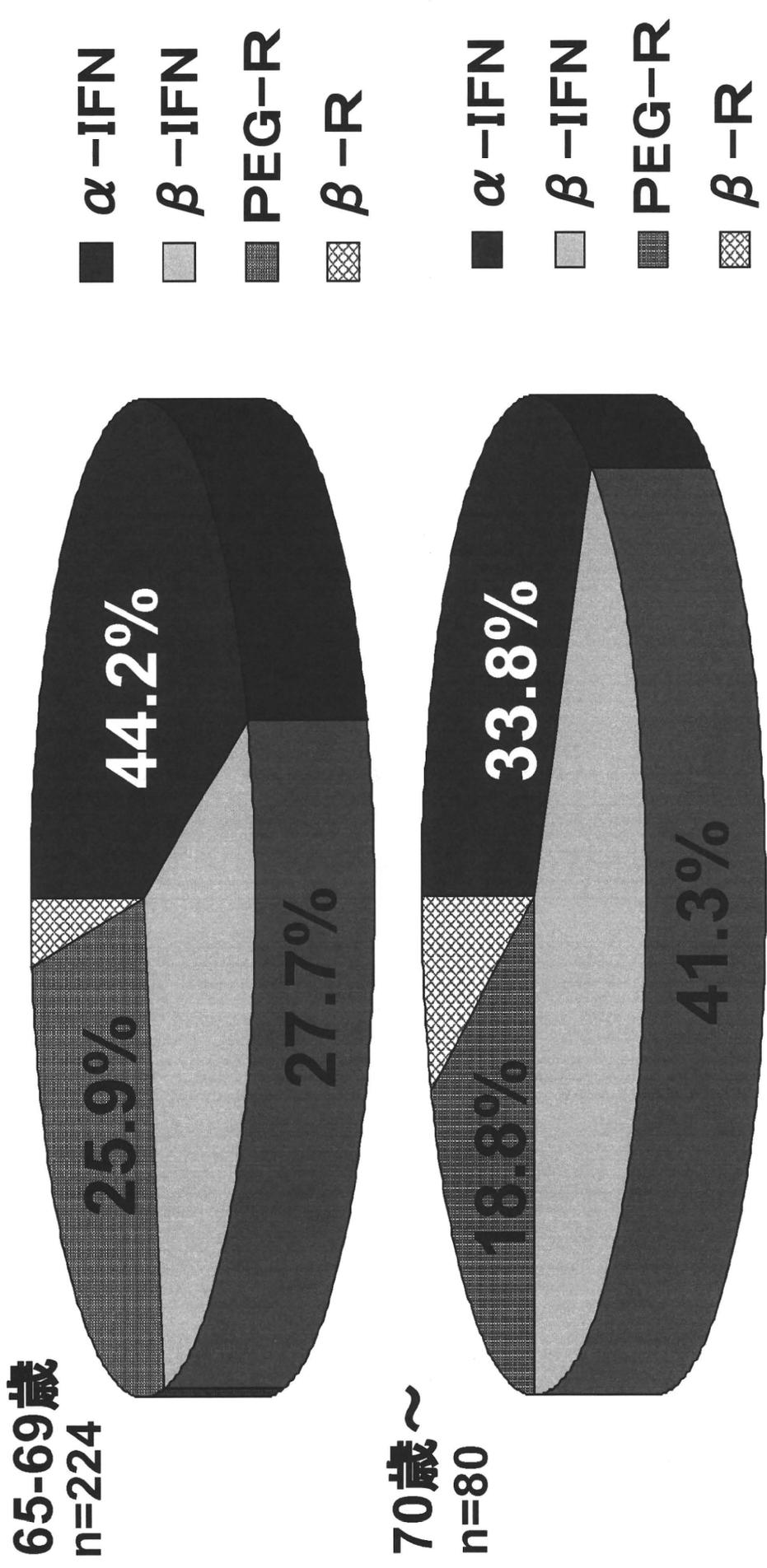


図2. 高齢者のothers症例に対するIFN治療の内訳

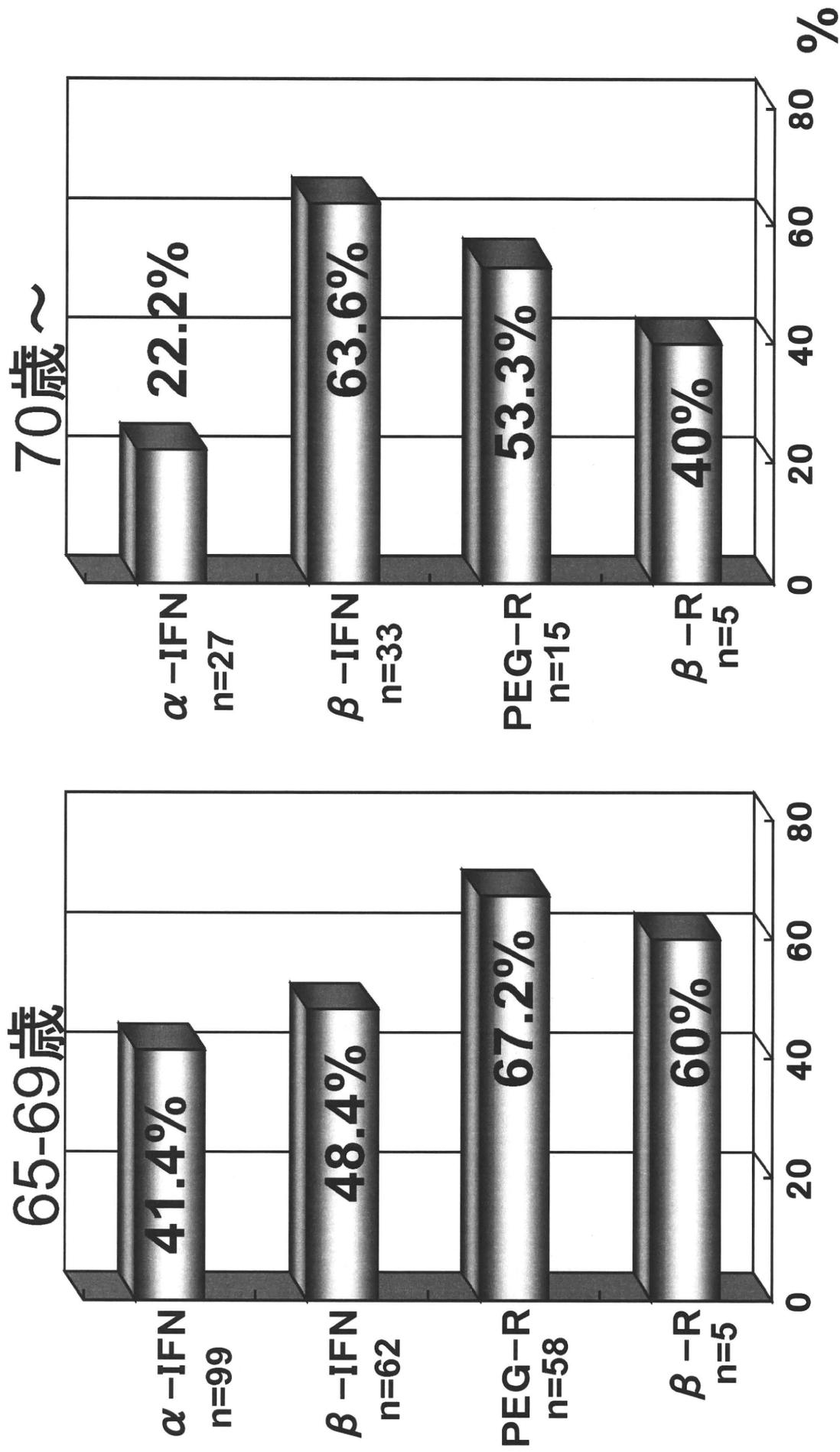


図4. 高齢者のothers症例に対するIFN治療の著効率

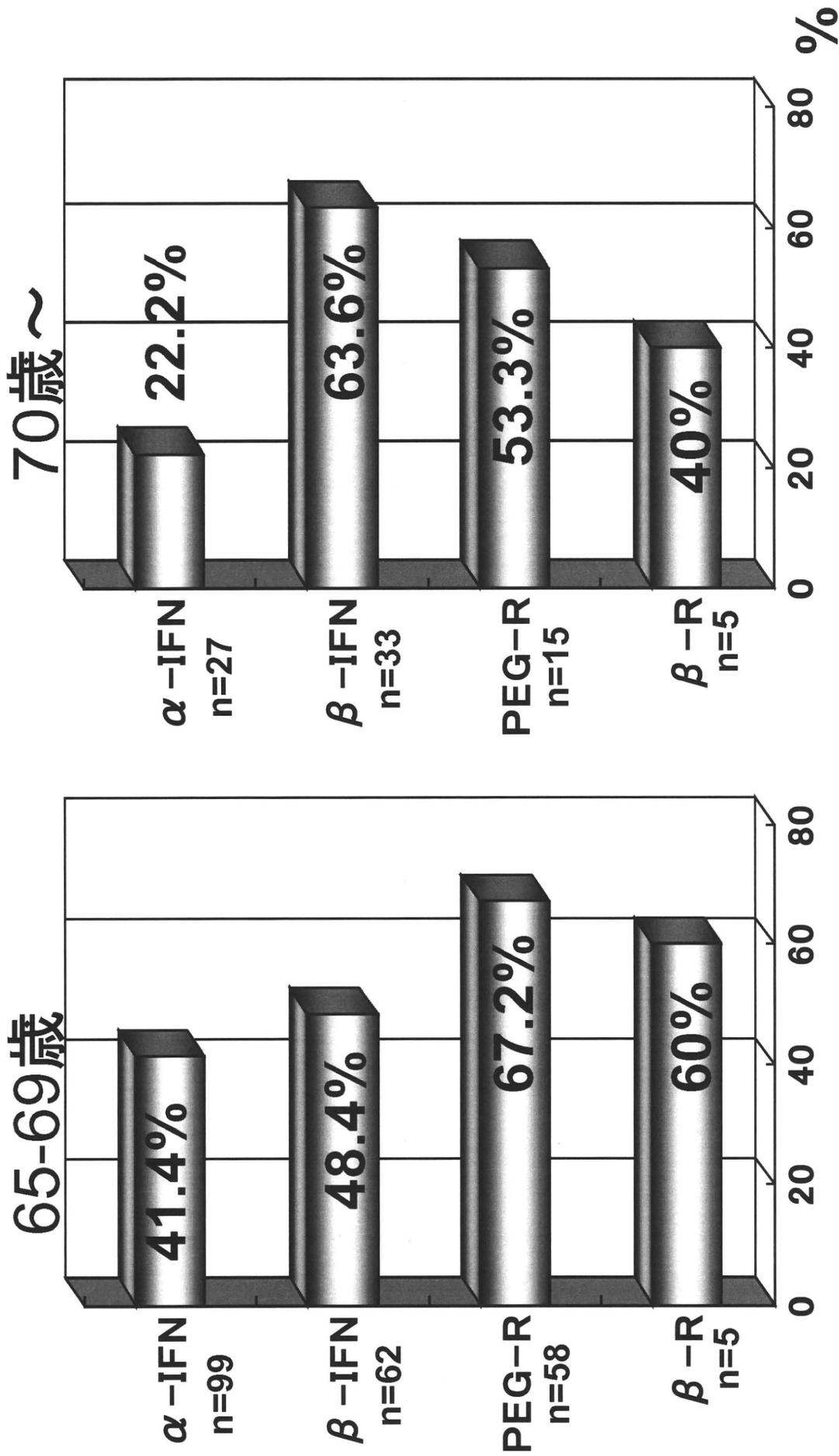
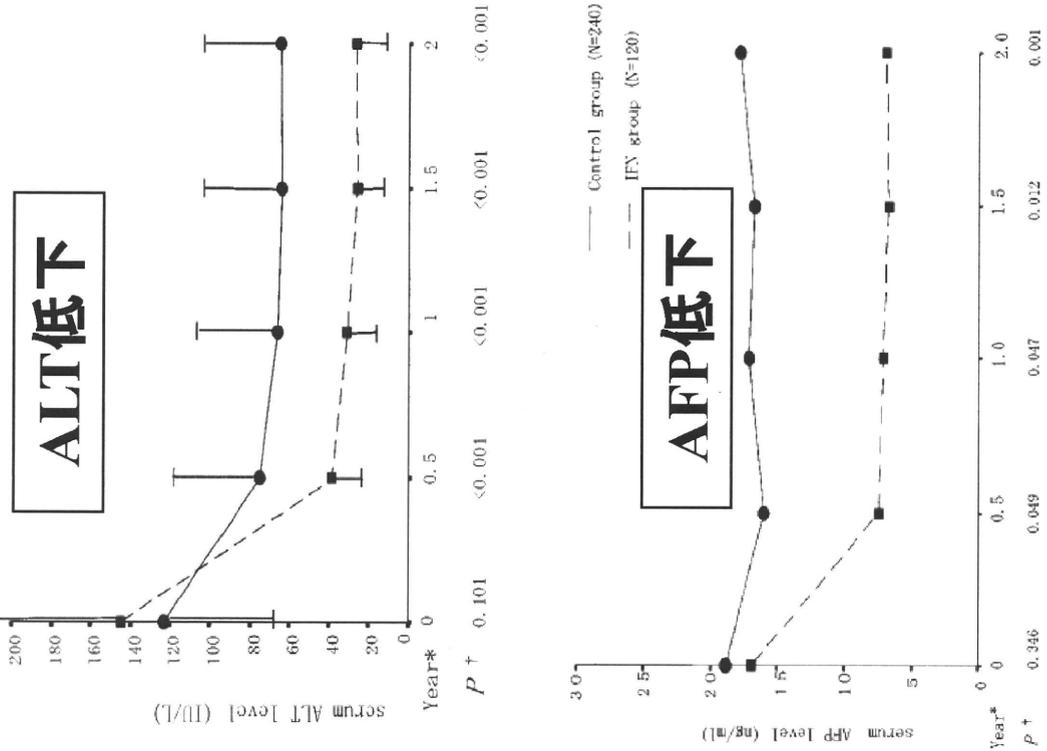
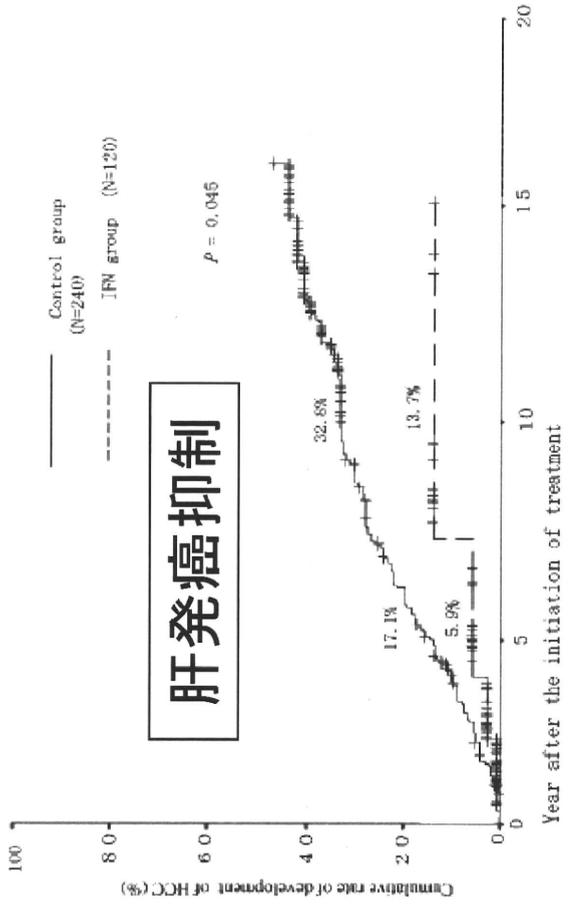


図4.高齢者のothers症例に対するIFN治療の著効率

# 図5. IFN-少量間歇投与の肝発癌抑制効果



60歳以上にSumiferon 300万単位を  
週2~3回(平均2.5年間)



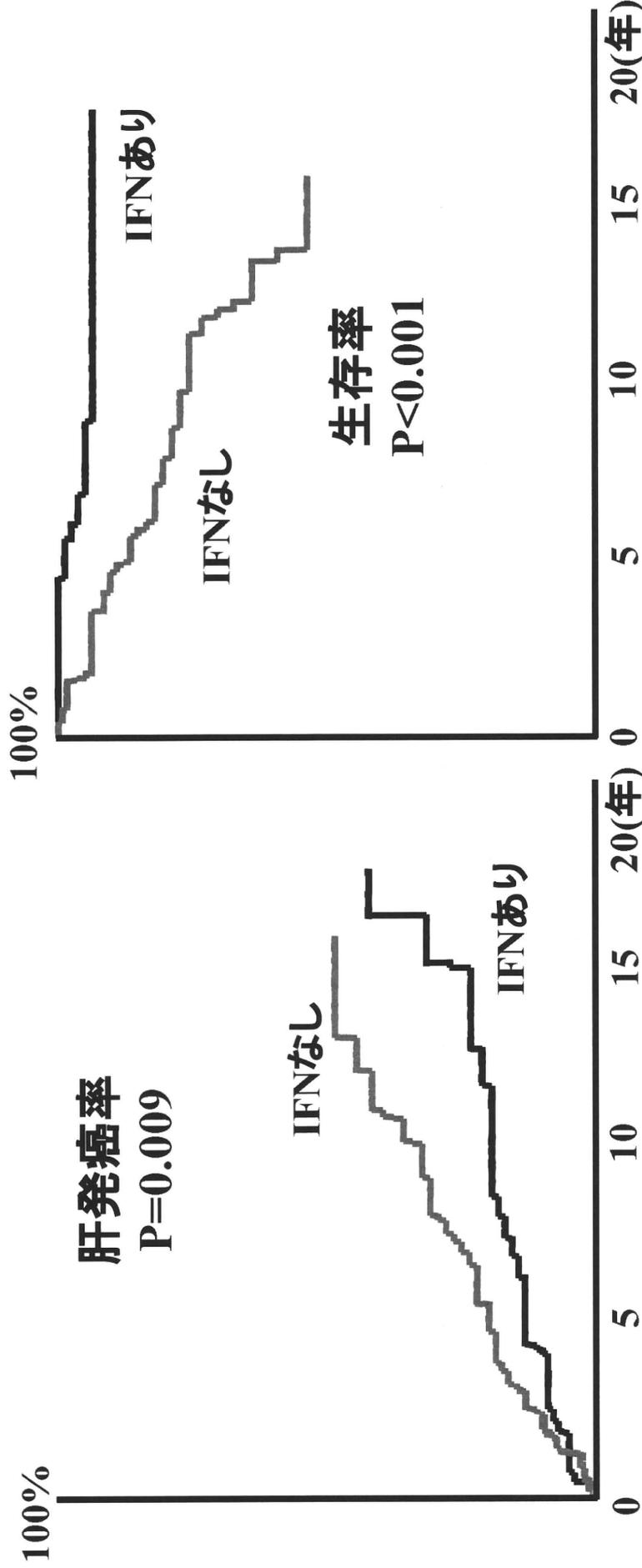


図7.HCV-1b症例に対する初回IFN単独療法NVRに  
IFNを投与した症例の肝発癌率・生存率

# Amino Acid Substitution in Hepatitis C Virus Core Region and Genetic Variation Near the Interleukin 28B Gene Predict Viral Response to Telaprevir with Peginterferon and Ribavirin

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Genetic variation near the IL28B gene and substitution of amino acid (aa) 70 and 91 in the core region of hepatitis C virus (HCV) genotype 1b can predict the response to pegylated interferon (PEG-IFN)/ribavirin combination therapy, but its impact on triple therapy of telaprevir/PEG-IFN/ribavirin is not clear. The aims of this study were to investigate the predictive factors of sustained virological response to a 12-week or 24-week regimen of triple therapy in 72 of 81 Japanese adults infected with HCV genotype 1. Overall, sustained virological response and end-of-treatment response were achieved by 61% and 89%, respectively. Especially, the sustained virological response was achieved by 45% and 67% in the 12- and 24-week regimens, respectively. Multivariate analysis identified rs8099917 near the IL28B gene (genotype TT) and substitution at aa 70 (Arg70) as significant determinants of sustained virological response. Prediction of response to therapy based on a combination of these factors had high sensitivity, specificity, and positive and negative predictive values. The efficacy of triple therapy was high in the patients with genotype TT, who accomplished sustained virological response (84%), irrespective of substitution of core aa 70. In the patients having genotype non-TT, those of Arg70 gained high sustained virological response (50%), and sustained virological response (12%) was the worst in patients who possessed both genotype non-TT and Gln70(His70). **Conclusion:** This study identified genetic variation near the IL28B gene and aa substitution of the core region as predictors of sustained virological response to a triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV genotype 1b. (HEPATOLOGY 2010;52:421-429)

Abbreviations: aa, amino acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase;  $\gamma$ GTP, gamma-glutamyl transpeptidase; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; NPV, negative predictive value; PEG-IFN, pegylated interferon; PPV, positive predictive value

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Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).<sup>1,2</sup> At present, treatments based on interferon (IFN), in combination with ribavirin, are the mainstay for combating HCV infection. In Japan, HCV genotype 1b (HCV-1b) in high viral loads (>100 KIU/mL) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C.<sup>3</sup> Such background calls for efficient treatments of Japanese patients with chronic HCV infection.

Even with pegylated IFN (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b and high viral loads.<sup>4,5</sup> Recently, a new strategy was introduced in the treatment of chronic HCV infection by

means of inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection.<sup>6</sup> Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, gains a robust antiviral activity.<sup>7,8</sup> Specifically, HCV RNA is suppressed below the limits of detection in the blood in almost all patients infected with HCV-1 during triple therapy of telaprevir with PEG-IFN and ribavirin.<sup>9</sup> However, treatment-resistant patients who do not achieve sustained virological response by the triple therapy have been reported.<sup>9-11</sup> The underlying mechanism of the response to the treatment is still not clear.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with HCV-1b and high viral loads are pretreatment predictors of poor virological response to PEG-IFN plus ribavirin combination therapy,<sup>12-14</sup> and also affect clinical outcome, including hepatocarcinogenesis.<sup>15,16</sup> Furthermore, a recent report showed that aa substitutions in the core region can also be used before therapy to predict very early dynamics (within 48 hours) after the start of triple therapy of telaprevir with PEG-IFN and ribavirin.<sup>17</sup> However, it is not clear at this stage whether aa substitutions in the core region can be used before therapy to predict sustained virological response to triple therapy.

Recent reports showed that genetic variations near the IL28B gene (rs8099917, rs12979860) on chromosome 19 is a host-related factor, which encodes IFN- $\lambda$ -3, are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1,<sup>18,21</sup> and also affect clinical outcome, including spontaneous clearance of HCV.<sup>22</sup> However, it is not clear at this stage whether genetic variation near the IL28B gene can be used before therapy to predict sustained virological response to triple therapy.

The present study included 81 patients with HCV-1b and high viral loads who received the triple therapy of telaprevir with PEG-IFN plus ribavirin. The aims of the study were to identify the pretreatment factors that could predict sustained virological response, including viral- (aa substitutions in the HCV core and NS5A regions) and host-related factors (genetic variation near the IL28B gene).

## Patients and Methods

**Study Population.** Between May 2008 and September 2009, 81 patients infected with HCV were

recruited for this study at the Department of Hepatology in Toranomon Hospital in Metropolitan Tokyo. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki and was approved by the Institutional Review Board. Each patient gave informed consent before participating in this trial. Patients were divided into two groups: 20 (25%) patients were allocated to a 12-week regimen of triple therapy (telaprevir [MP-424], PEG-IFN, and ribavirin) (the T12PR12 group), and 61 patients (75%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks (the T12PR24 group).

All of 81 patients met the following inclusion and exclusion criteria: (1) diagnosis of chronic hepatitis C. (2) HCV-1 confirmed by sequence analysis. (3) HCV RNA levels of  $\geq 5.0$  log IU/mL determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (4) Japanese (Mongoloid) ethnicity. (5) Age at study entry of 20-65 years. (6) Body weight  $\geq 35$  kg and  $\leq 120$  kg at the time of registration. (7) Lack of decompensated liver cirrhosis. (8) Negativity for hepatitis B surface antigen (HBsAg) in serum. (9) Negative history of HCC. (10) No previous treatment for malignancy. (11) Negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C. (12) Negative history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of  $\leq 50$  mL/minute at baseline, diabetes requiring treatment or fasting glucose level of  $\geq 110$  mg/dL, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication or anaphylaxis at baseline. (13) Hemoglobin level of  $\geq 12$  g/dL, neutrophil count  $\geq 1500/\text{mm}^3$ , and platelet count of  $\geq 100,000/\text{mm}^3$  at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study. Furthermore, 72 of 81 patients were followed for at least 24 weeks after the completion of triple therapy. The treatment efficacy was evaluated by HCV-RNA negative at the end of treatment (end-of-treatment response) and 24 weeks after the completion of therapy (sustained virological response), based on the COBAS TaqMan HCV test (Roche Diagnostics).

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at 750 mg or 500 mg

**Table 1. Profile and Laboratory Data at Commencement of Telaprevir, Peginterferon and Ribavirin Triple Therapy in Japanese Patients Infected with HCV Genotype 1**

Demographic data	
Number of patients	81
Sex (M/F)	44 / 37
Age (years)*	55 (23-65)
History of blood transfusion	24 (29.6%)
Family history of liver disease	13 (16.0%)
Body mass index (kg/m <sup>2</sup> )*	22.5 (13.2-32.4)
Laboratory data*	
HCV genotype (1a/ 1b)	1/80
Level of viremia (log IU/mL)	6.7 (5.1-7.6)
Serum aspartate aminotransferase (IU/L)	34 (15-137)
Serum alanine aminotransferase (IU/L)	42 (12-175)
Serum albumin (g/dL)	3.9 (3.2-4.6)
Gamma-glutamyl transpeptidase (IU/L)	36 (9-229)
Leukocyte count (/mm <sup>3</sup> )	4,800 (2,800-8,100)
Hemoglobin (g/dL)	14.3 (11.7-16.8)
Platelet count ( $\times 10^3$ /mm <sup>3</sup> )	17.1 (9.1-33.8)
Alpha-fetoprotein ( $\mu$ g/L)	4 (2-39)
Total cholesterol (mg/dL)	180 (110-276)
Fasting plasma glucose (mg/dL)	92 (64-125)
Treatment	
PEG-IFN $\alpha$ -2b dose ( $\mu$ g/kg)*	1.5 (1.3-2.0)
Ribavirin dose (mg/kg)*	11.7 (7.2-18.4)
Telaprevir dose (1,500 / 2,250 mg/day)	10/71
Treatment regimen (T12PR12 group / T12PR24 group)	20/61
Amino acid substitutions in the HCV genotype 1b	
Core aa 70 (arginine / glutamine [histidine] /ND)	47/33/1
Core aa 91 (leucine / methionine / ND)	43/37/1
ISDR of NS5A (wild-type / non wild-type / ND)	76/4/1
Genetic variation near IL28B gene	
rs8099917 genotype (TT / TG / GG / ND)	42/30/2/7
rs 12979860 genotype (CC / CT / TT / ND)	42/32/2/5
Past history of IFN therapy	
Treatment-naïve / Relapsers to previous treatment / nonresponders to previous treatment	27/33/21

Data are number and percentages of patients, except those denoted by asterisk (\*), which represent the median (range) values. ND, not determined.

three times a day at an 8-hour (q8) interval after the meal. PEG-IFN $\alpha$ -2b (PEG-Intron; Schering Plough, Kenilworth, NJ) was injected subcutaneously at a median dose 1.5  $\mu$ g/kg (range: 1.3-2.0  $\mu$ g/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200-600 mg twice a day after breakfast and dinner (daily dose: 600-1000 mg).

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1500/mm<sup>3</sup>, neutrophil count below 750/mm<sup>3</sup>, or platelet count below 80,000/mm<sup>3</sup>; PEG-IFN was discontinued when these counts decreased below 1000/mm<sup>3</sup>, 500/mm<sup>3</sup> or 50,000/mm<sup>3</sup>, respectively. When hemoglobin decreased to <10 g/dL, the daily dose of ribavirin was reduced from 600 to 400 mg, from 800 to 600 mg

and 1000 mg to 600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dL. However, the dose of telaprevir (MP-424) remained the same, and its administration was stopped when the discontinuation was appropriate for the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFN $\alpha$ -2b and ribavirin was also terminated.

Table 1 summarizes the profiles and laboratory data of the 81 patients at the commencement of treatment. They included 44 males and 37 females, ages 23 to 65 years (median, 55 years).

**Measurement of HCV RNA.** The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at least once every month before, during, and after therapy. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2-7.8 log IU/mL, and the undetectable samples were defined as negative.

**Detection of Amino Acid Substitutions in Core and NS5A Regions of HCV-1b.** In the present study, aa substitutions of the core region and NS5A-ISDR (IFN-sensitivity determining region) of HCV-1b were analyzed by direct sequencing. HCV RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo). Nucleic acids were amplified by polymerase chain reaction (PCR) using the following primers: (1) Nucleotide sequences of the core region: The first-round PCR was performed with CE1 (sense, 5'-GTC TGC GGA ACC GGT GAG TA-3', nucleotides: 134-153) and CE2 (antisense, 5'-GAC GTG GCG TCG TAT TGT CG-3', nucleotides: 1096-1115) primers, and the second-round PCR with CC9 (sense, 5'-ACT GCT AGC CGA GTA GTG TT-3', nucleotides: 234-253) and CE6 (antisense, 5'-GGA GCA GTC GTT CGT GAC AT-3', nucleotides: 934-953) primers. (2) Nucleotide sequences of NS5A-ISDR: The first-round PCR was performed with ISDR1 (sense, 5'-ATG CCC ATG CCA GGT TCC AG-3', nucleotides: 6662-6681) and ISDR2 (antisense, 5'-AGC TCC GCC AAG GCA GAA GA-3', nucleotides: 7350-7369) primers, and the second-round PCR with ISDR3 (sense, 5'-ACC GGA TGT GGC AGT GCT CA-3', nucleotides: 6824-6843) and ISDR4 (antisense, 5'-GTA ATC CGG GCG TGC CCA TA-3', nucleotides: 7189-7208) primers. ([1,2]; nested PCR.) All samples were initially denatured at 95°C for 2 minutes. The 35 cycles of

amplification were set as follows: denaturation for 30 seconds at 95°C, annealing of primers for 30 seconds at 55°C, and extension for 1 minute at 72°C with an additional 7 minutes for extension. Then 1  $\mu$ L of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (PerkinElmer, Tokyo).

With the use of HCV-J (Access. No. D90208) as a reference,<sup>23</sup> the sequence of 1-191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on 81 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91).<sup>12</sup> The sequence of 2209-2248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al.<sup>24</sup> was determined and the numbers of aa substitutions in ISDR were defined as wildtype (0, 1) or nonwildtype ( $\geq 2$ ).

**Genetic Variation Near the IL28B Gene.** Samples for genome-wide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of the Invader assay, TaqMan assay, or direct sequencing as described.<sup>25,26</sup>

In this study, genetic variations near the IL28B gene (rs8099917, rs12979860), reported as the pretreatment predictors of treatment efficacy and clinical outcome,<sup>18-22</sup> were investigated.

**Statistical Analysis.** Nonparametric tests (chi-squared test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to sustained virological response. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P* values less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance (*P* < 0.05) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. The

potential pretreatment factors associated with sustained virological response included the following variables: sex, age, history of blood transfusion, family history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase ( $\gamma$ -GTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, alpha-fetoprotein, total cholesterol, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, treatment regimen of triple therapy, past history of IFN therapy, genetic variation near the IL28B gene, and aa substitution in the core region, and NS5A-ISDR. Statistical analyses were performed using SPSS (Chicago, IL). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated to determine the reliability of predictors of the response to therapy.

## Results

**Virological Response to Therapy.** Sustained virological response was achieved by 44 of 72 (61.1%) patients. In all, 64 of 72 (88.9%) patients were considered end-of-treatment response. According to treatment regimen, sustained virological response were achieved by 45.0% (9 of 20 patients) and 67.3% (35 of 52 patients), in the T12PR12 group and the T12PR24 group, respectively. Of eight patients who could not achieve end-of-treatment response, six (75.0%) patients resulted in reevaluation of viral loads regardless of HCV-RNA temporary negative, and the other two patients (25.0%) did not achieve HCV-RNA negative during treatment.

Especially in the T12PR24 group, according to the past history of treatment, sustained virological response were achieved by 76.4% (13 of 17 patients), 86.4% (19 of 22 patients), and 23.1% (3 of 13 patients), in treatment-naïve, relapsers to previous treatment, and nonresponders to previous treatment, respectively.

**Sustained Virological Response According to Amino Acid Substitutions in Core and NS5A Regions.** According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions (74.4%) showed sustained virological response than that of patients who showed Gln70(His70) (41.4%) (Fig. 1, *P* = 0.007). In contrast, according to the substitution of core aa 91, the sustained virological response rate was not significantly different between Leu91 (65.0%) and Met91 (56.3%) (Fig. 1). Likewise, according to the numbers of aa substitutions in ISDR, the sustained virological response rate was not significantly different between wildtype

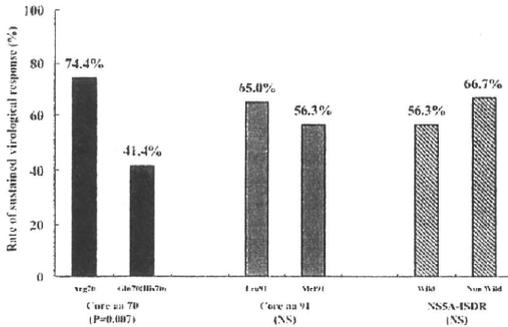


Fig. 1. According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions showed sustained virological response than that of patients who showed Gln70(His70) ( $P = 0.007$ ). In contrast, according to the substitution of core aa 91, the sustained virological response rate was not significantly different between Leu91 and Met91. Likewise, according to the numbers of aa substitutions in ISDR, the sustained virological response rate was not significantly different between wildtype and nonwildtype.

(56.3%) and nonwildtype (66.7%) (Fig. 1). Thus, sustained virological response was influenced by the substitution of core aa 70.

**Sustained Virological Response According to Genetic Variation Near the IL28B Gene.** According to the genetic variation in rs8099917, sustained virological response was achieved by 83.8% (31 of 37 patients), 29.6% (8 of 27 patients), and 0% (0 of 2 patients) in patients with genotype TT, TG, and GG, respectively. Thus, a significantly higher proportion of patients with genotype TT (83.8%) showed sustained virological response than that of patients who showed genotype non-TT (27.6%) (Fig. 2,  $P < 0.001$ ) (Table 2).

According to the genetic variation in rs12979860, sustained virological response was achieved by 83.8% (31 of 37 patients), 34.5% (10 of 29 patients), and 0% (0 of 2 patients), in patients with genotype CC, CT, and TT, respectively. Thus, a significantly higher proportion of patients with genotype CC (83.8%) showed sustained virological response than that of patients who showed genotype non-CC (32.3%) (Fig. 2,  $P < 0.001$ ) (Table 2).

**Predictive Factors Associated with Sustained Virological Response.** Univariate analysis identified three parameters that correlated with sustained virological response significantly: substitution of aa 70 (Arg70; OR 4.12,  $P = 0.007$ ), genetic variation in rs8099917 (genotype TT; OR 13.6,  $P < 0.001$ ), and rs12979860 (genotype CC; OR 10.8,  $P < 0.001$ ). Two factors were identified by multivariate analysis as independent

parameters that significantly influenced sustained virological response (rs8099917 genotype TT; OR 10.6,  $P < 0.001$ ; and Arg70; OR 3.69,  $P = 0.040$ ) (Table 3).

**Assessment of Amino Acid Substitutions in Core Region and Genetic Variation Near the IL28B Gene as Predictors of Sustained Virological Response.** The ability to predict sustained virological response by substitution of core aa 70 and rs8099917 genotype near the IL28B gene was evaluated. The sustained virological response rates of patients with a combination of Arg70 or rs8099917 genotype TT were defined as PPV (prediction of sustained virological response). The nonsustained virological response rates of patients with a combination of Gln70(His70) or rs8099917 genotype non-TT were defined as NPV (prediction of nonsustained virological response).

In patients with rs8099917 genotype TT, the sensitivity, specificity, PPV, and NPV for sustained virological response were 79.5, 77.8, 83.8, and 72.4%, respectively. Thus, genotype TT has high sensitivity, specificity, and PPV for prediction of sustained virological response. In patients with Arg70 the sensitivity, specificity, PPV, and NPV were 76.9, 63.0, 75.0, and 65.4%, respectively. Thus, Arg70 has high sensitivity and PPV in predicting sustained virological response. Furthermore, when both predictors were used the sensitivity, specificity, PPV, and NPV were 61.5, 85.2, 85.7, and 60.5%, respectively. When one or more of the two predictors were used the sensitivity, specificity, PPV, and NPV were 94.9, 55.6, 75.5, and 88.2%, respectively. These results indicate that the use of the combination of the above two predictors has high sensitivity, specificity, PPV, and NPV for prediction of sustained virological response (Table 4).

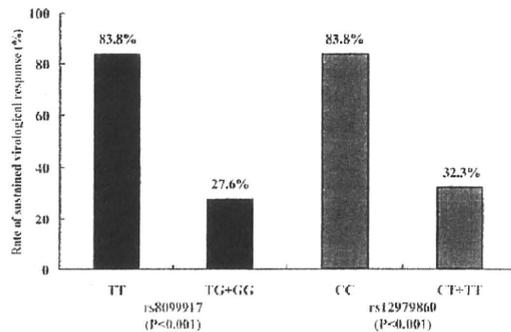


Fig. 2. According to the genetic variation in rs8099917 or rs12979860 near the IL28B gene, a significantly higher proportion of patients with genotype TT or CC showed sustained virological response than that of patients who showed genotype non-TT or non-CC, respectively ( $P < 0.001$  or  $P < 0.001$ , respectively).

**Table 2. According to Genetic Variation Near the IL28B Gene, Background at Commencement of Triple Therapy and Treatment Efficacy**

	rs8099917 genotype			rs12979860 genotype		
	TT (n = 42)	TG+GG (n = 32)	TT vs. TG+GG P	CC (n = 42)	CT+TT (n = 34)	CC vs. CT+TT P
<b>Demographic data</b>						
Sex (M/F)	22 / 20	18 / 14	NS	22 / 20	19 / 15	NS
Age (years)*	54 (23-65)	56 (36-65)		54 (23-65)	55 (36-65)	NS
History of blood transfusion	15 (35.7%)	9 (28.3%)	NS	15 (35.7%)	9 (26.5%)	NS
Family history of liver disease	6 (14.3%)	6 (18.8%)	NS	6 (14.3%)	6 (17.6%)	NS
Body mass index (kg/m <sup>2</sup> )*	22.1 (13.2-32.4)	22.4 (18.7-26.5)	NS	22.1 (13.2-32.4)	22.3 (18.7-26.5)	NS
<b>Laboratory data*</b>						
HCV genotype (1a / 1b)	0 / 42	1 / 31	NS	0 / 42	1 / 33	NS
Level of viremia (log IU/mL)	6.9 (5.4-7.5)	6.6 (5.1-7.4)	NS	6.9 (5.4-7.5)	6.5 (5.1-7.4)	NS
Serum aspartate aminotransferase (IU/L)	38 (15-118)	31 (20-137)	0.036	38 (15-118)	31 (20-137)	0.031
Serum alanine aminotransferase (IU/L)	50 (12-175)	36 (17-136)	0.029	50 (12-175)	35 (17-136)	0.014
Serum albumin (g/dL)	3.9 (3.3-4.6)	3.9 (3.2-4.6)	NS	3.9 (3.3-4.6)	3.9 (3.2-4.6)	NS
Gamma-glutamyl transpeptidase (IU/L)	29 (9-194)	53 (9-154)	0.008	29 (9-194)	53 (9-229)	0.004
Leukocyte count (/mm <sup>3</sup> )	4,800 (2,800-8,100)	4,800 (3,000-7,800)	NS	4,800 (2,800-8,100)	4,800 (3,000-7,800)	NS
Hemoglobin (g/dL)	14.3 (12.3-16.5)	14.3 (11.7-16.8)	NS	14.3 (12.3-16.5)	14.3 (11.7-16.8)	NS
Platelet count ( $\times 10^4$ /mm <sup>3</sup> )	16.8 (9.9-33.8)	17.1 (9.1-24.8)	NS	16.8 (9.9-33.8)	17.8 (9.1-28.8)	NS
Alpha-fetoprotein ( $\mu$ g/L)	4 (2-39)	5 (2-38)	NS	4 (2-39)	5 (2-38)	NS
Total cholesterol (mg/dL)	184 (112-276)	178 (110-263)	NS	184 (112-276)	178 (110-263)	NS
Fasting plasma glucose (mg/dL)	97 (80-125)	90 (66-111)	0.038	97 (80-125)	91 (66-111)	0.030
<b>Treatment regimen</b>						
T12PR12 group / T12PR24 group	12 / 30	7 / 25	NS	12 / 30	7 / 27	NS
<b>Amino acid substitutions in the HCV genotype 1b</b>						
Core aa 70 (arginine / glutamine [histidine])	30 / 12	13 / 18	0.016	30 / 12	13 / 20	0.009
Core aa 91 (leucine / methionine)	25 / 17	13 / 18	NS	25 / 17	14 / 19	NS
ISDR of NS5A (wild-type / non wild-type)	39 / 3	30 / 1	NS	39 / 3	32 / 1	NS
<b>Past history of IFN therapy</b>						
Treatment-naive / Relapsers to previous treatment / Nonresponders to previous treatment	16 / 24 / 2	7 / 6 / 19	<0.001	16 / 24 / 2	8 / 7 / 19	<0.001
<b>Treatment efficacy**</b>						
End-of-treatment response (%)	35 (94.6%)	23 (79.3%)	NS	35 (94.6%)	25 (80.6%)	NS
Sustained virological response (%)	31 (83.8%)	8 (27.6%)	<0.001	31 (83.8%)	10 (32.3%)	<0.001

Data are number and percentages of patients, except those denoted by asterisk (\*), which represent the median (range) values.

\*\*Treatment efficacy according to rs8099917 genotype was evaluated in 66 patients, and that according to rs12979860 genotype was evaluated in 68 patients.

**Predicting Sustained Virological Response by Amino Acid Substitutions in Core Region in Combination with Genetic Variation Near the IL28B Gene.** Sustained virological response by core aa 70 in combination with rs8099917 genotype is shown in Fig. 3. In patients with rs8099917 genotype TT, sustained virological response was not different between Arg70 (85.7%) and Gln70(His70) (77.8%). In contrast, in patients with rs8099917 genotype TG and GG, a significantly higher proportion of patients with Arg70 (50.0%) showed sustained virological response than that of patients with Gln70(His70) (11.8%) ( $P = 0.038$ ).

Based on a strong power of substitution of core aa 70 and rs8099917 genotype in predicting sustained virological response (Table 3), how they increase the predictive value when they were combined was evaluated. The results are schematically depicted in Fig. 3.

Together they demonstrate three points: (1) the efficacy of triple therapy was high in patients with genotype TT who accomplished sustained virological response at 83.8%, irrespective of substitution of core aa 70; (2) in patients having genotype TG and GG, those of Arg70 gained high sustained virological response (50.0%); and (3) sustained virological response (11.8%) was the worst in patients who possessed both of genotype TG and GG, and Gln70(His70).

## Discussion

Two previous studies (PROVE1 in the US, and PROVE2 in Europe) showed that the T12PR12 and T12PR24 group of telaprevir, PEG-IFN, and ribavirin could achieve sustained virological response rates of 35%-60% and 61%-69%, respectively.<sup>10,11</sup> In the