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Virological response and safety of 24-week telaprevir alone in Japanese patients infected with hepatitis C virus subtype 1b

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SUMMARY. Hepatitis C virus (HCV) subtype 1b, which infects approximately 70% of Japanese carriers, is likely to be more eradicable by a telaprevir regimen than subtype 1a because of the higher genetic barrier of Val^{36} and Arg^{155} substitutions. The aims of this exploratory study were to evaluate the virological response and safety of 24-week oral administration of telaprevir alone in chronic HCV subtype 1b infection. Fifteen treatment-naïve patients were treated with telaprevir 750 mg every 8 h for 24 weeks. All patients were Japanese whose median age was 58.0 years (range: 45–68), and six patients (40%) were men. Median baseline HCV RNA level was 6.80 $\log_{10}$ IU/mL (range: 3.55–7.10). The HCV RNA levels decreased to undetectable in five patients (33%) within 8 weeks. Three patients (20%) with negative HCV RNA by Week 4 achieved end of treatment response. One patient (7%) who achieved sustained virological response had a low baseline viraemia of 3.55 $\log_{10}$ IU/mL. Most of the adverse events including anaemia and skin disorders were mild to moderate. Developed variants were T54A and A156V/T/F/Y with or without secondary substitutions rather than V36M ± R155K. Telaprevir alone for 24 weeks in Japanese patients with HCV subtype 1b resulted in an sustained viral response rate of 7% (1/15) and was well tolerated for 24 weeks. These results will support the implementation of further studies on oral combination of telaprevir with other direct-acting antiviral agents in patients infected with HCV subtype 1b.

Keywords: hepatitis C virus, monotherapy, subtype 1b, telaprevir.

INTRODUCTION

The World Health Organization (WHO) estimates that approximately 170 million people are infected with hepatitis C virus (HCV) [1]. In Japan, it is estimated that more than 1.5 million people are chronically infected with hepatitis C.

Telaprevir is a novel peptidemimetic HCV NS3-4A protease inhibitor. The mechanism of inhibition involves the formation of a stable, reversible, covalent bond between the ketocarbonyl of telaprevir and the active site serine of NS3 protease. Recently, telaprevir was approved for patients with HCV genotype 1 infection in the United States (US), Canada, European Union (EU) and Japan. The Phase 3 studies showed that patients who received telaprevir in combination with pegylated interferon (PEG-IFN) and ribavirin (RBV) achieved significantly higher rates of sustained viral response (SVR) compared to those who received PEG-IFN and RBV alone, regardless of their prior treatment experience [2–4]. The Japanese Phase 3 studies of the telaprevir-based triple regimen also showed high SVR rates [5,6]. The most common side effects in the telaprevir-based triple regimen were anaemia, rash and IFN-induced systemic symptoms.

The epidemiology of HCV in Japan takes on a different aspect from US and EU; that is, the majority of patients are aged more than 55 years [7]. Accordingly, the RBV dose reduction rate and the frequency of discontinuation of telaprevir treatment in Japan are higher than those in US and EU [2–6]. Taking such problems with telaprevir in combination with PEG-IFN and RBV into consideration, IFN-free

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DAA, direct-acting antiviral agent; EU, European Union; HCV, Hepatitis C virus; LDL, low-density lipoprotein; LOQ, lower limit of quantification; PEG-IFN, pegylated interferon; RBV, ribavirin; SVR, sustained viral response; T-bil, total bilirubin.

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regimens may become very useful options and satisfy important unmet medical needs especially for intolerant patients with IFN-based regimens. Clinical trials of IFN-free therapy for patients with chronic hepatitis C would provide us with meaningful knowledge for the future development of HCV therapy. Interestingly, HCV subtype 1b, which infects approximately 70% of Japanese HCV carriers [8], is likely to be more eradicable by telaprevir regimens than subtype 1a because of the higher genetic barrier of Val^{36} and Arg^{155} substitutions [9,10]. When treating with direct-acting antiviral agent (DAA), HCV subtypes of genotype 1 are now an important factor that affects treatment response. The main aim of this exploratory study is to evaluate the virological response and safety of telaprevir as monotherapy for 24 weeks in Japanese patients infected with HCV subtype 1b.

PATIENTS AND METHODS

Study design and organization

This Phase 2, single-arm, open-label study was conducted from January 2008 to February 2009 at Sapporo Kosei General Hospital, Musashino Red Cross Hospital, Toranomon Hospital and Hiroshima University Hospital. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practices. Before starting the study, the protocol and informed consent forms were reviewed and approved by the institutional review board in each site. All patients provided written informed consent following sufficient explanation before participating in the study. All the patients received 750 mg telaprevir orally every 8 h (q8h) (2250 mg/day) after a meal for 24 weeks. Telaprevir was given as a 250-mg tablet. This study is registered in ClinicalTrials.gov NCT 00621296.

Patients

Participants enrolled in this study were treatment-naïve, male or female chronic hepatitis C patients with the characteristics shown in Table 1 who met the inclusion criteria and did not conflict the exclusion criteria described previously [11], except the age and HCV RNA levels at the time of enrolment; age from 20 to 70 years and HCV RNA levels were not defined.

Virological responses

Virological response to telaprevir was evaluated based on the HCV RNA kinetics in patients. Serum HCV RNA levels were measured using the COBAS TaqMan HCV test (Roche Diagnostics Co., Ltd., Tokyo, Japan). The linear dynamic range was 1.2–7.8 $\log_{10}$ IU/mL. A qualitative result below the lower limit of quantification (LOQ) was also determined as positive (1.0) and negative (0.5). Measurements were obtained on Week 4 before the first dose, Days 1 (prior to the first dosing) and 3, Weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 of the treatment period, and Weeks 2, 4, 8, 12, 16, 20, and 24 of the follow-up period. Day 1 was defined as the date of starting telaprevir treatment.

Table 1 Patient characteristics, treatment duration and viral response

	Sex	Age	BMI (kg/m^2)	Baseline HCV RNA ($\log_{10}$ IU/mL)	Treatment duration (day)	HCV RNA Nadir ($\log_{10}$ IU/mL)	Virological response
1	M	67	25.2	5.85	169 (complete)	Undetectable	Relapse
2	M	59	24.5	3.55	169 (complete)	Undetectable	SVR
3	F	45	18.7	6.80	44*	2.8	Breakthrough
4	F	68	20.9	7.05	43†	<1.2 detectable	Partial responder
5	F	48	21.5	6.45	169 (complete)	Undetectable	Breakthrough
6	F	57	20.9	4.75	43*	1.8	Breakthrough
7	F	51	19.9	5.95	170 (complete)	Undetectable	Partial responder
8	F	58	19.2	6.85	105*	1.5	Breakthrough
9	M	62	20.4	6.25	14†	1.4	Partial responder
10	M	58	24.5	7.10	39*	3.1	Breakthrough
11	M	63	16.2	7.00	74*	<1.2 detectable	Breakthrough
12	F	53	25.0	7.10	169 (complete)	Undetectable	Relapse
13	F	60	19.7	5.00	10‡	<1.2 detectable	Breakthrough
14	F	55	23.8	6.95	78*	<1.2 detectable	Breakthrough
15	M	50	27.5	6.90	26‡	1.3	Partial responder

HCV, Hepatitis C virus; SVR, sustained viral response. Subjects discontinued telaprevir because of *viral breakthrough, †AE and ‡other reasons.

Sustained viral response was defined as an undetectable HCV RNA level at 24 weeks after the end of treatment. Relapse was defined as the reappearance of serum HCV RNA during the follow-up period from the state of undetectable serum HCV RNA at the end of treatment. Breakthrough was defined as the state when the viral level increased by 2 $\log_{10}$ IU/mL from nadir or a level of more than 3 $\log_{10}$ IU/mL after reaching undetectable levels during treatment. Partial responders were subjects whose HCV RNA level dropped by at least 2 $\log_{10}$ IU/mL during treatment but was still detected at the end of treatment.

Sequence analysis at HCV NS3 protease domain

HCV RNA was isolated from serum samples collected on the same day for the measurement of HCV RNA levels. A DNA fragment of 543 bases long (181 amino acids) from the NS3 protease domain was amplified by nested RT-PCR and cloned. At least 39 clones per specimen were sequenced bi-directionally. The limit of detection for the sequencing analysis was 3.0 $\log_{10}$ IU/mL.

Safety assessments

Safety of telaprevir was assessed by clinical laboratory tests, vital signs, abdominal ultrasonography and AEs. Twelve-lead electrocardiogram (ECG) examinations were performed once during the screening period. These safety parameters were reported at regular intervals from 4 weeks before the first dosing to the end of the follow-up period.

Statistical analysis

Statistical analyses were performed using the statistical software SAS Version 9.1.3 (SAS Institute Inc., Cary, NC, USA). Reported AEs were classified according to MedDRA/J version 12.0 (MedDRA Japanese Maintenance Organization, Tokyo, Japan).

RESULTS

Baseline characteristics

Fifteen treatment-naïve patients infected with HCV subtype 1b were enrolled in this study. Baseline characteristics of patients are shown in Table 1. All patients were Japanese whose median age was 58.0 years (range: 45–68); 6 (40.0%) patients were men. Patients over 54 years of age accounted for 66.7% (10 of 15). Median baseline HCV RNA level was 6.80 $\log_{10}$ IU/mL (range: 3.55–7.10). The median BMI was 20.9 kg/m^2 (range: 16.2–27.5).

Virological response

Telaprevir alone caused a rapid decrease in HCV RNA levels after the initiation of treatment in all patients. The average changes were −3.24 $\log_{10}$ IU/mL on Day 3 and −4.24 $\log_{10}$ IU/mL on Week 1 (Fig. 1). The average of maximum reduction in each patient was 5.01 $\log_{10}$ IU/mL. The HCV RNA levels became undetectable in 1, 3, 3 and 5 patients at Weeks 1, 4, 6 and 8, respectively. Three patients with negative HCV RNA after 4 weeks achieved end of treatment response (ETR), of whom one patient achieved a SVR. The patient who achieved SVR had the lowest baseline viral load (3.55 $\log_{10}$ IU/mL) among all the patients.

Ten of 15 patients discontinued the telaprevir treatment because of the following reasons: six patients because of viral breakthrough, two patients because of AEs, one patient because of own drug discontinuation and one patient who met the exclusion criteria after administration.

Safety

AEs observed in two or more patients in this study are shown in Table 2. During the study, 14 of 15 patients experienced 80 AEs in total and 62 events were judged as adverse drug reactions. The common AEs that occurred in

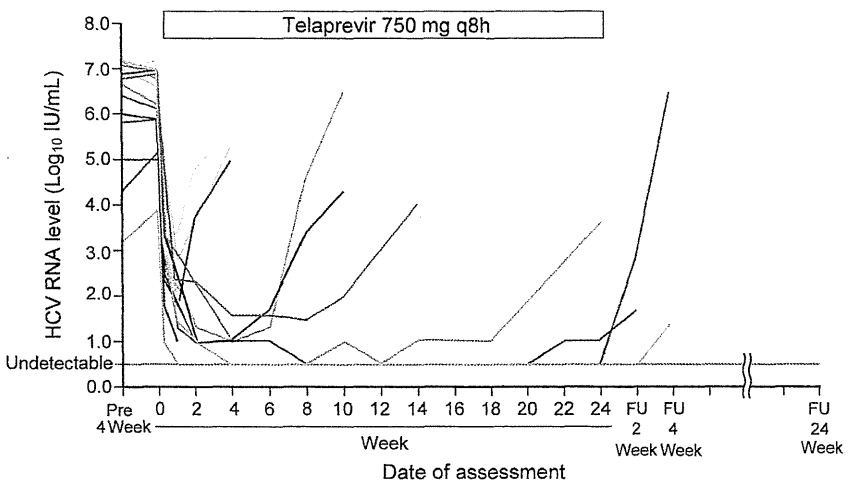

Fig. 1 HCV RNA kinetics during and after treatment with telaprevir monotherapy.

Table 2 Incidence of adverse events that occurred in two or more patients

	N = 15			
	Mild n (%)	Moderate n (%)	Severe n (%)	Total n (%)
Rash	5 (33.3)	3 (20.0)	0 (0.0)	8 (53.3)
Anaemia	7 (46.7)	0 (0.0)	0 (0.0)	7 (46.7)
Low-density lipoprotein increased	6 (40.0)	0 (0.0)	0 (0.0)	6 (40.0)
Blood uric acid increased	4 (26.7)	0 (0.0)	0 (0.0)	4 (26.7)
Pruritus	3 (20.0)	1 (6.7)	0 (0.0)	4 (26.7)
Anorexia	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Dysgeusia	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Headache	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Diarrhoea	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Pyrexia	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Thirst	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Nasopharyngitis	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Blood creatinine increased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Blood triglycerides increased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Platelet count decreased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Dizziness	1 (6.7)	1 (6.7)	0 (0.0)	2 (13.3)

MedDRA (Ver.12.0).

more than 25% of patients were rash (53.5%), anaemia (46.7%), low-density lipoprotein (LDL) increases (40.0%), blood uric acid increase (26.7%) and pruritus (26.7%). Two patients discontinued telaprevir treatment because of AEs (herpes zoster or rash pruritic). Except for the herpes zoster whose severity was judged as severe and serious, all the events were mild to moderate. Fifty of the 80 AEs were observed within the first 4 weeks.

In relation to skin AEs, rash, pruritus and rash pruritic were observed in 8, 4 and 1 patients, respectively. The onset day of these events is described in Fig. 2. The range of the onset day was Day 1 to Day 113, and the median was Day 15. Rash in three patients, pruritus in one patient and rash pruritic in one patient were moderate, and the others were mild. One patient discontinued telaprevir at Week 6 because of moderate rash pruritic. Most of the skin AEs were treated with oral antihistamines or topical steroids.

A decrease in haemoglobin levels was observed in all patients (Fig. 3a). Seven of 15 patients developed anaemia during and after the treatment. All anaemia events were mild and no patient needed discontinuation of telaprevir. Uric acid and LDL cholesterol increased during the treatment (Fig. 3b,c), but these changes were mild and no patient needed any medication for these AEs. There were no substantial increases in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (T-bil).

Sequence analysis at HCV NS3 protease domain

Amino acid substitutions in the NS3 protease domain were examined in 39 clones or more in each sample. Before Week 8, V36A/G, T54A and A156T/V as single substitutions, and T54A + R155K and A156T/V + V158I as multiple substitutions were observed. Among two patients who discontinued telaprevir within 2 weeks, all clones but three in one patient were wild-type variants after withdrawal of telaprevir. In three patients who discontinued at Weeks 5–7 because of viral breakthrough, predominant clones possessed A156V/T substitutions after the nadir of viral load. Predominant variants observed during and after telaprevir monotherapy in the eight patients who received telaprevir beyond 8 weeks are shown in Fig. 4 together with HCV RNA levels. In the two patients who showed the lowest HCV RNA level of on Week 4, the predominant clones detected after

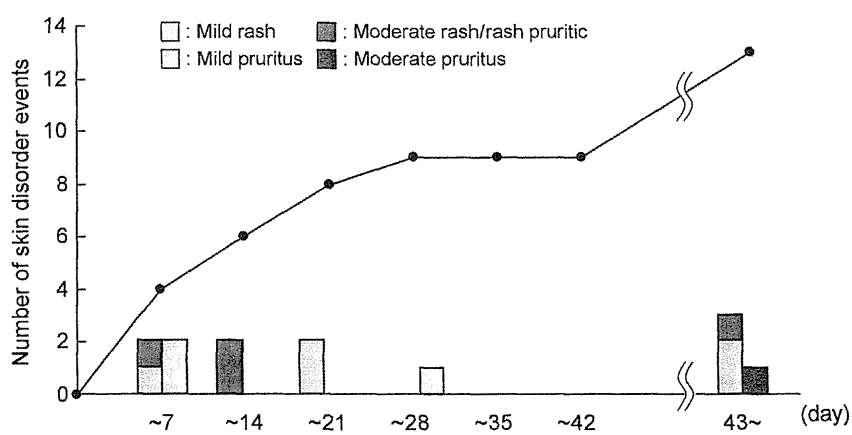

Fig. 2 Rash and pruritus occurrence.

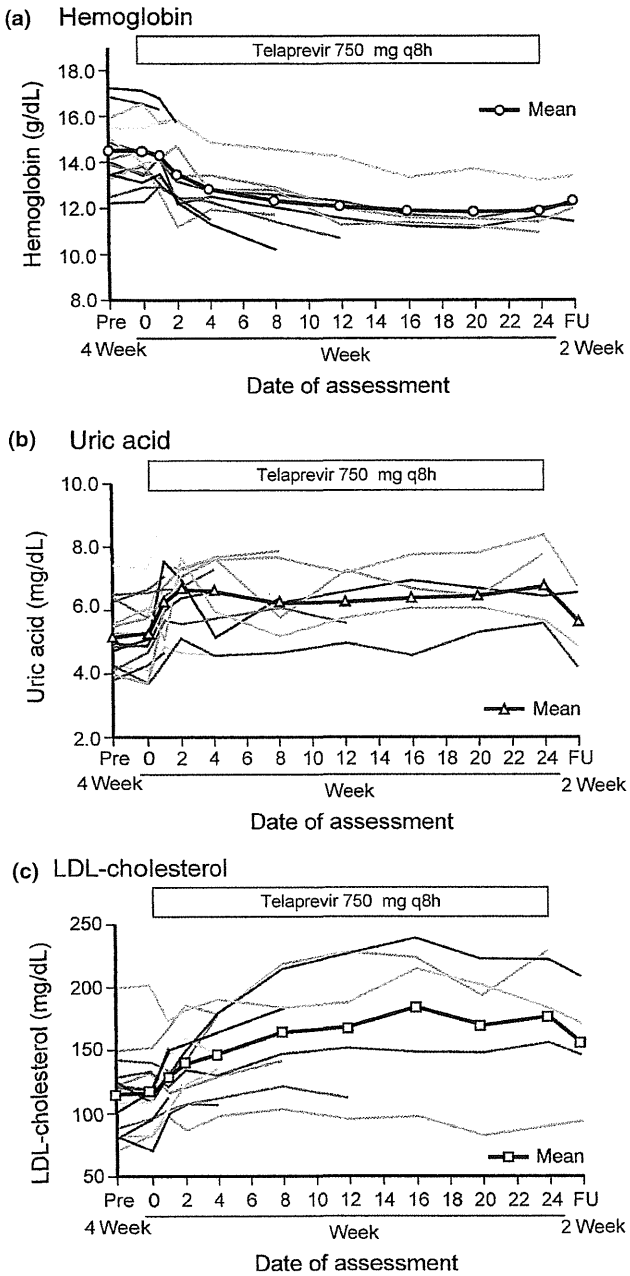

Fig. 3 Changes in (a) hemoglobin, (b) uric acid, (c) LDL-cholesterol.

viral breakthrough were A156F and T54A. One other patient with nadir HCV RNA level on Week 8 had a predominant clone of T54A + I132L after viral breakthrough. Among the five patients who completed the telaprevir treatment for 24 weeks as scheduled, two patients were HCV RNA positive at the end of treatment. One of these two patients had an A156F substitution at the end of treatment, and a A156Y substitution was also detected on Week 1 of the follow-up period. In the two patients who relapsed during the follow-up period, the predominant clone was T54A which shifted to the wild-type variant in one patient.

DISCUSSION

Although higher SVR rates and shorter duration of treatment were achieved by telaprevir in combination with PEG-IFN and RBV in US, EU and Japan [2–6], the DAA combination regimens also increased the frequency and severity of side effects usually observed in the PEG-IFN and RBV therapy. As most patients in Japan are aged people, IFN-free regimens are in urgent need because these patients are intolerant to IFN-based therapies [12–14].

In this exploratory study, one of 15 patients on telaprevir monotherapy was able to achieve SVR. A low viral load of <4 $\log_{10}$ IU/mL in this patient probably contributed to the achievement of SVR, and Suzuki *et al.* [15] published this case report in detail. Although the SVR rate obtained in the study was not beneficial enough, the telaprevir monotherapy could decrease HCV RNA levels dramatically in all cases. The severity of skin-related AEs during telaprevir monotherapy was milder than those of cases developing in the co-administration with PEG-IFN and RBV [5,6,16–18]. All the events were mild to moderate and manageable with antihistamines or topical steroids. Similarly to the skin-related events, decreases in haemoglobin levels were mild, and the incidence of anaemia was 46.7%. As all the anaemia events were mild, there was no need for discontinuation of telaprevir or use of any medications. Severe skin rash and anaemia observed in the therapy with telaprevir in combination with PEG-IFN and RBV are probably ascribable to the synergistic effect of these three drugs. Although the mechanism of uric acid and LDL cholesterol elevation during treatment with telaprevir has been established, these changes disappeared at the end of telaprevir dosing. Telaprevir was generally well tolerated in all the patients.

Amino acid substitutions in the HCV NS3 protease domain were monitored during the study. The relationship between these substitutions and resistance to NS3-4A protease inhibitors has been well documented by *in vitro, in vivo* and clinical studies [19–22]. In the eight patients who received the telaprevir monotherapy beyond 8 weeks, the predominant breakthrough variants were T54A and A156F, which were not observed at the earlier time points (Fig. 4). Furthermore, in the clones accounting for more than 10% of each specimen, the secondary substitution of V158I and I132L was identified along with the primary resistant-associated substitution of A156T/V and T54A, respectively, and a novel substitution of A156Y was also observed. This study confirms the higher genetic barrier of HCV subtype 1b against the V36M ± R155K substitutions. Our results clearly indicate that the prolonged telaprevir monotherapy leads to the development of various variants. As the replication fitness of drug-resistant variants tends to be lower than that of wild type, the former are likely to be overtaken by the wild-type virus under drug-free conditions within 3–7 months [11,23,24]. As Ozeki *et al.* [25] reported that four patients with favourable IL28B SNP who failed to eradicate HCV with telaprevir monotherapy were

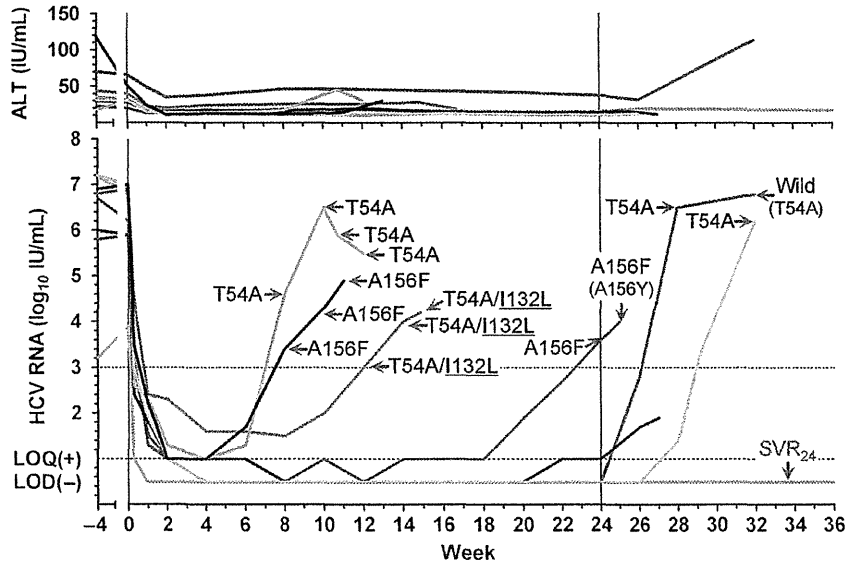

Fig. 4 Viral kinetics and predominant variants during and after telaprevir monotherapy beyond 8 weeks. Besides predominant clones, minority clones which account for 10% and more in a specimen are also summarized by brace notation. Putative secondary resistant-associated mutation is indicated by underline.

responsive to sequential therapy with PEG-IFN and RBV, the substitutions in the NS3 protease domain by the telaprevir treatment are not correlated with resistance to PEG-IFN and/or RBV directly as described previously [23,24]. Sequential therapy with PEG-IFN and RBV after relapse or viral breakthrough on telaprevir monotherapy might be a therapeutic option in some cases, including the case of low haemoglobin. By taking the error-prone nature of HCV replication into account, successful eradication with IFN-free DAA(s) regimens probably depends on how efficiently DAA can suppress various DAA-resistant variants that pre-exist and are selected under DAA pressure. The telaprevir-based combination therapy with other DAA(s) such as NS5A or NS5B polymerase inhibitors may be useful for successful treatment. Using a human chimeric liver mouse model for HCV infection, Ohara *et al.* [26] reported that the combination of telaprevir with a high-dose nucleoside analogue could successfully eradicate HCV infection. Recently, it was reported that the dual therapy with daclatasvir, an NS5A replication complex inhibitor, and asunaprevir, NS3-4A protease inhibitor, had high SVR rates in difficult-to-treat patients with subtype 1b and null responders [27,28]. These successful results are also helpful for us to consider telaprevir-based IFN-free regimens in combination with other DAAs against HCV.

In conclusion, telaprevir monotherapy was well tolerated and provided potent but temporary antiviral activity in Japanese patients with subtype 1b HCV, with an SVR rate of 7%. Most AEs were mild to moderate and much milder than those recorded in patients on combinations with PEG-IFN and RBV. As the essential characteristics of DAAs including telaprevir are substantially masked in the co-administration with other antivirals, the knowledge obtained from the long-term telaprevir monotherapy is most likely to contribute to the future HCV treatment with DAA-based regimens.

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Research Article

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Characterization of virologic escape in hepatitis C virus genotype 1b patients treated with the direct-acting antivirals daclatasvir and asunaprevir

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Background & Aims: Daclatasvir and asunaprevir are NS5A and NS3 protease-targeted antivirals currently under development for treatment of chronic hepatitis C virus infection. Clinical data on baseline and on-treatment correlates of drug resistance and response to these agents are currently limited.

Methods: Hepatitis C virus genotype 1b Japanese patients (prior null responders to PegIFN-α/RBV [n = 21] or PegIFN-α/RBV ineligible or intolerant [n = 22]) were administered daclatasvir/asunaprevir for 24 weeks during a phase 2a open-label study. Genotypic and phenotypic analyses of NS3 and NS5A substitutions were performed at baseline, after virologic failure, and post-treatment through follow-up week 36.

Results: There were three viral breakthroughs and four relapsers. Baseline NS3 polymorphisms (T54S, Q80L, V170M) at amino acid positions previously associated with low-level resistance (<9-fold) to select NS3 protease inhibitors were detected in four null responders and three ineligibles, but were not associated with virologic failure. Baseline NS5A polymorphisms (L28M, L31M, Y93H) associated with daclatasvir resistance (<25-fold) were detected in five null responders and six ineligibles. All three viral breakthroughs and 2/4 relapsers carried a baseline NS5A-Y93H polymorphism. NS3 and NS5A resistance-associated variants were detected together (NS3-D168A/V, NS5A-L31M/V-Y93H) after virologic failure. Generally, daclatasvir-resistant substitutions persisted through 48 weeks post-treatment, whereas asunaprevir-resistant substitutions were no longer detectable. Overall, 5/10 patients with baseline NS5A-Y93H experienced virologic failure, while 5/10 achieved a sustained virologic response.

Conclusions: The potential association of a pre-existing NS5A-Y93H polymorphism with virologic failure on daclatasvir/asunaprevir combination treatment will be examined in larger studies. The persistence of treatment-emergent daclatasvir- and asunaprevir-resistant substitutions will require assessment in longer-term follow-up studies.

Introduction

The introduction of direct-acting antivirals (DAAs) targeting hepatitis C virus (HCV) NS3 protease activity has substantially increased sustained virologic response (SVR) in chronic HCV genotype 1 (GT1) infection. In combination with peginterferon-alfa and ribavirin (PegIFN-α/RBV), treatment with the recently approved protease inhibitors boceprevir or telaprevir results in SVR rates of around 70–75% in treatment-naive patients [1,2]. Despite these improvements, SVR rates vary by genotype and remain suboptimal in some patients, such as null responders to PegIFN-α/RBV [3], and patients for whom PegIFN-α/RBV is poorly tolerated or medically contraindicated. Furthermore, PegIFN-α/RBV is associated with frequent side effects [3], and the addition of these DAAs results in elevated rates of anemia and additional events such as dysgeusia (boceprevir), or rash, pruritus, and nausea (telaprevir) [4,5].

Daclatasvir (DCV) and asunaprevir (ASV) are currently undergoing clinical development for HCV infection. DCV (BMS-790052) is a first-in-class, highly selective NS5A replication complex inhibitor with picomolar potency and broad HCV genotypic coverage [6] that has demonstrated antiviral efficacy and good tolerability in combination with PegIFN-α/RBV [7]. ASV (BMS-650032) is a selective inhibitor of NS3 protease with antiviral activity *in vitro* against GT1 and GT4 [8]; it has also been shown to be

Keywords: Asunaprevir; Daclatasvir; Drug resistance; Direct-acting antivirals; Hepatitis C; Peginterferon-sparing.
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Abbreviations: DAA, direct-acting antiviral; HCV, hepatitis C virus; SVR, sustained virologic response; GT, genotype; PegIFN-α/RBV, peginterferon alfa and ribavirin; DCV, daclatasvir; ASV, asunaprevir; LLOQ, lower limit of quantitation; PCR, polymerase chain reaction; FU, follow-up; RAV, resistance-associated variant; BL, baseline; VBT, viral breakthrough; SD, standard deviation.

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Table 1. Baseline viral and host characteristics among genotype-1b null responders and their virologic outcome.

Patient	*IL28B* GT	HCV RNA, $\log_{10}$ IU/ml	NS5A polymorphism(s)[a]	NS3 polymorphism(s)[a]	Virologic outcome
P-1	CT	7.2	Q54H/Q-Q62Q/E-Y93H/Y	T54S-Q80L	SVR
P-2	CT	7.0		Q80L-V170I/M	SVR
P-3	CT	7.4	Q54H		SVR
P-4	CT	6.7	R30Q		SVR
P-5	CT	7.0	L31L/M-P58P/S		SVR
P-6	CC	5.3	P58P/T-Q62E		D/C at Wk2 due to SAE[b]
P-7	CC	7.2		S122S/G	SVR
P-8	CT	7.0	Q54H	Q80L	SVR
P-9	CT	7.1	Q54H-Y93H/Y	S122N	SVR
P-10	CT	6.4	L28M-R30Q		SVR
P-11	CT	6.8			D/C at Wk12 due to AE; SVR
P-12	CT	6.4	Q54H-P58S-Q62E		SVR
P-13	CT	7.4	Q54H		D/C at Wk6; PDR not achieved[c]
P-14	CT	6.5			SVR
P-15	CT	6.3	R30Q/R-Q62Q/R		SVR
P-16	CT	6.6	Q54H		SVR
P-17	CT	6.6	Q54H-Q62E		SVR
P-18	CT	6.9	Q54Y	Q80L	SVR
P-19	CT	6.6	Q54H-Y93H	N77A	SVR
P-20	CT	7.0	R30Q	S122G	SVR
P-21	CC	6.6	Q54L		SVR

[a]All NS3 and NS5A amino acids were examined with focus on polymorphisms at positions known to be associated with resistance to NS3 protease inhibitors (36, 43, 54, 55, 77, 78, 79, 80, 122, 123, 138, 155, 156, 158, 168, 170, 175) and NS5A inhibitors (21, 23, 24, 28, 30, 31, 32, 54, 58, 62, 92, 93). When a mixture of substitutions is indicated, the most predominant is identified first.
[b]HCV RNA undetectable at post-treatment week 24.
[c]PegIFN-α/RBV added; HCV RNA undetectable at post-treatment week 24 following 52 weeks of therapy.
AE, adverse event; D/C, discontinued; GT, genotype; HCV, hepatitis C virus; PDR, protocol-defined response; SAE, serious adverse event; SVR, sustained virologic response; Wk, week.

with NS3-S122G (P-20, no fold-change to either DCV/ASV), or NS5A-Q54H (P-13, no fold-change to DCV). P-13 was the only patient with HCV RNA <15 IU/ml (target detectable) at week 6 and was, therefore, considered a treatment failure. Treatment-emergent resistance at week 1 in the five patients could not be determined because of PCR failure. A comparison of initial virologic response *vs.* dose and polymorphisms associated with resistance revealed no differences. Among null responders who received ASV 600 mg, mean HCV RNA declines at week 1 for those with *vs.* without RAVs were −4.6 *vs.* −4.3 $\log_{10}$ IU/ml, which were similar to the week 1 declines among those who received ASV 200 mg (−4.5 $\log_{10}$ IU/ml with RAVs [one patient] *vs.* −4.3 $\log_{10}$).

Baseline HCV RNA levels did not have an impact on response to treatment; patients with high baseline viral load still experienced rapid and robust responses to therapy (Fig. 1; Table 1).

Ineligible/intolerant patients

Virologic response.

Virologic response at week 4 was greater in PegIFN-α/RBV ineligible patients than in null responders. Undetectable HCV RNA at week 4 was observed in 86% of the ineligible group *vs.* 52% of null responders. However, by week 12, undetectable HCV RNA was similar in both groups. Early HCV RNA declines appeared unaffected by *IL28B* genotype, the presence of baseline polymorphisms associated with resistance, or virologic outcome (Fig. 3). Adherence to therapy, assessed through pill counts, was found to be high in six of the seven patients experiencing virologic failure. However, DCV/ASV exposures were high in the one non-compliant patient (P-31) who subsequently experienced relapse.

Baseline analysis.

Baseline *IL28B* genotype, polymorphisms associated with resistance, and virologic outcome are shown in Table 2 and Fig. 2B. Three patients presented with DCV resistance at baseline: one (P-25) with an NS5A-L31M-Y93H combination (7105-fold DCV resistance [13]) and two with an NS5A-Q54Y-Y93H (58-fold resistance). All three subsequently experienced viral breakthrough at week 10 or 16.

Other patients had baseline polymorphisms conferring minimal or low-level resistance to DCV and/or protease inhibitors; NS5A-Y93H (n = 4), NS5A-L28M-R30L (n = 1), NS3-T54S (n = 1), and NS3-Q80L (n = 5). Variable responses were observed among these patients (Fig. 2B); the majority responded, but two patients with baseline NS5A-Y93H experienced post-treatment relapse. One patient (P-24) with baseline NS5A-L28M-R30L-Q54H-A92T and NS3-Q80L-S122G had a slower response to treatment at week 1 when compared with mean HCV RNA reductions (SD) for ineligible/intolerant patients on the study (−3.4 *vs.* −4.74 [0.58] $\log_{10}$ IU/ml), but subsequently achieved SVR with only 16 weeks of treatment. Neither NS3-Q80L-S122G nor NS5A-L28M-R30L-Q54H-A92T conferred resistance to ASV or DCV, respectively.

Baseline viral load did not appear to affect response; mean HCV RNA levels (SD) were 6.4 (0.7) $\log_{10}$ IU/ml among patients

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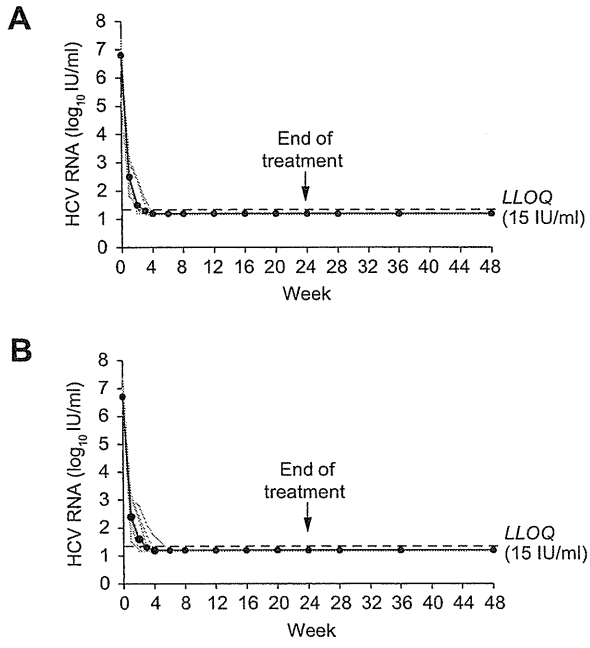

Fig. 1. HCV RNA levels among genotype-1b null responders. Treatment was initiated with (A) asunaprevir 600 mg BID or (B) asunaprevir 200 mg BID, in combination with daclatasvir 60 mg QD. Individual patient HCV RNA levels are shown in grey. Mean HCV RNA levels are shown in black. BID, twice daily; HCV, hepatitis C virus; LLOQ, lower limit of quantitation; QD, once daily.

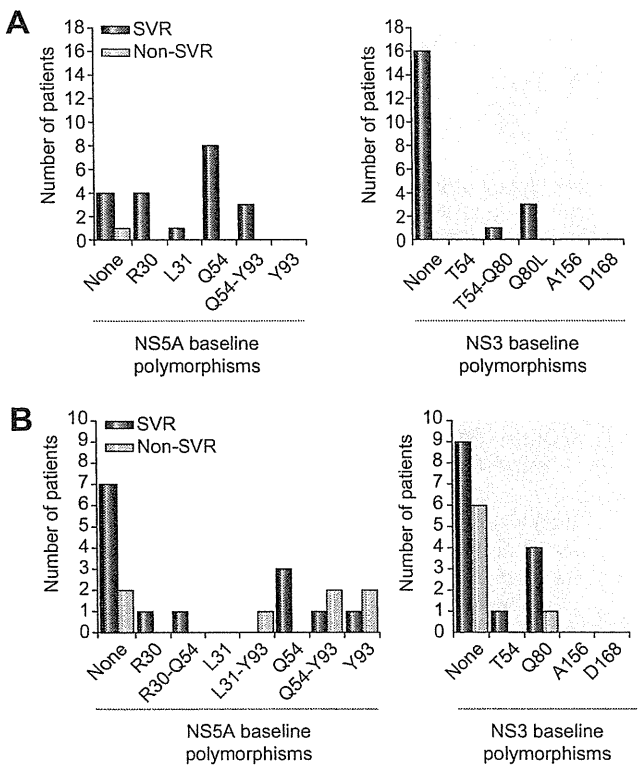

Fig. 2. Impact of baseline polymorphisms associated with resistance on virologic outcome among genotype-1b (A) null responders or (B) ineligible/intolerant patients. The ineligible/intolerant analysis excludes one patient (P-40) who discontinued therapy and was subsequently lost to follow-up. SVR, sustained virologic response.

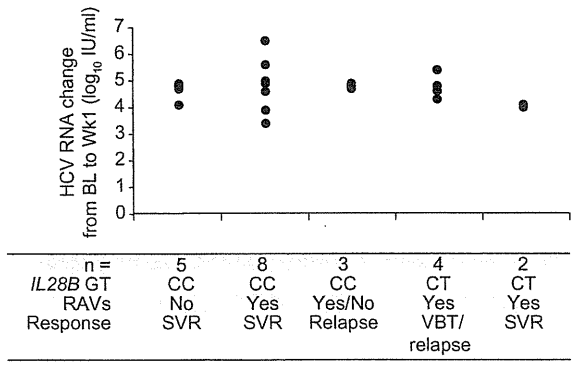

Fig. 3. Early (week 1) declines in HCV RNA were similar among PegIFN-α/RBV ineligible or intolerant patients with and without baseline polymorphisms associated with resistance, virologic failure, and *IL28B* CT genotype. BL, baseline; GT, genotype; HCV, hepatitis C virus; RAV, resistance-associated variant; SVR, sustained virologic response; VBT, viral breakthrough.

Patient P-36 relapsed with an NS5A-L31V/M-Y93H genotype (L31V-Y93H: 14,789-fold DCV resistance *vs.* L31M-Y93H: 7105-fold) [13] and NS3-D168V. The remaining two patients had detectable NS5A-Y93H at baseline (24-fold DCV resistance) and additional substitutions at NS5A-L31 and NS3-D168 were detected after relapse. Patient P-31 displayed NS5A-L31M-Y93H (7105-fold DCV resistance) [13] and NS3-D168A (~120-fold ASV resistance); patient P-37 relapsed with the same NS5A-L31V/M-Y93H and NS3-D168V, as described for patient P-36.

Baseline HCV RNA and *IL28B* genotype did not appear to influence relapse; three of four relapse patients were *IL28B* CC genotype, and baseline HCV RNA was not appreciably higher than for those with SVR (mean HCV RNA [SD]: 6.8 [0.4] *vs.* 6.4 [0.7] $\log_{10}$ IU/ml, respectively).

Changes in the DCV resistance pattern present at relapse through follow-up week 48 were seen in three of four relapsers, with Y93H changing to wild type (100% of 68 clones) in patient P-32. Clonal analysis of the baseline sequence revealed the presence of Y93H as a minor species (~2%; 1/61 clones). Genotypic changes resulting in a lower level of phenotypic resistance (L31V-Y93H to L31M-Y93H) were detected in patients P-36 and P-37. NS3 substitutions observed at relapse were not detectable by population sequencing by follow-up week 36. The D168V substitution detected in patient P-37 was replaced by D168E (78-fold ASV resistance [19]) at follow-up weeks 36 and 48. As with the patients who experienced virologic breakthrough, ASV and DCV trough values in the three drug-compliant patients who relapsed were less than the observed EC_{90} values for the respective RAVs.

Discussion

This study assessed resistance and virologic failure in a difficult-to-treat population of null responders and PegIFN-α/RBV ineligible/intolerant patients treated with the dual oral combination of DCV and ASV. Overall, 77% achieved an SVR [11], with all viral breakthroughs and post-treatment relapses occurring in the ineligible/intolerant subpopulation. It is possible that pharmacokinetics may have played a role in these failures, since patients experiencing failure had DCV and/or ASV trough values below median or documented non-compliance [11]. However, since

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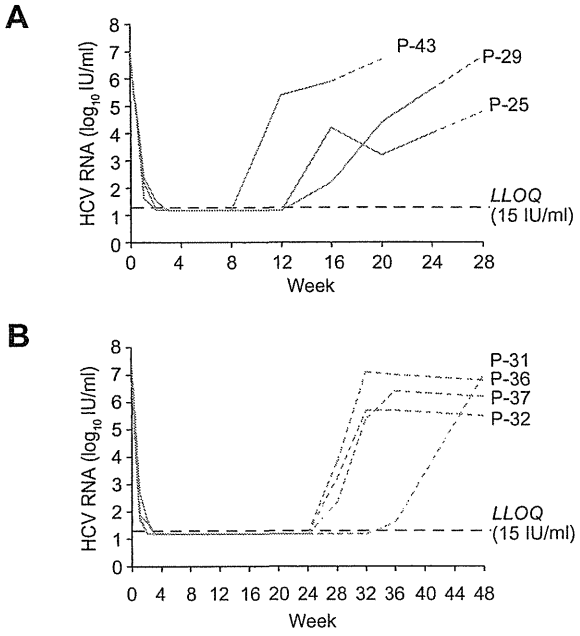

Fig. 4. HCV RNA levels on-treatment and during post-treatment follow-up for genotype-1b ineligible/intolerant patients experiencing (A) viral breakthrough or (B) relapse. Solid lines indicate on-treatment period. Dashed lines indicate post-treatment follow-up. HCV, hepatitis C virus; LLOQ, lower limit of quantitation.

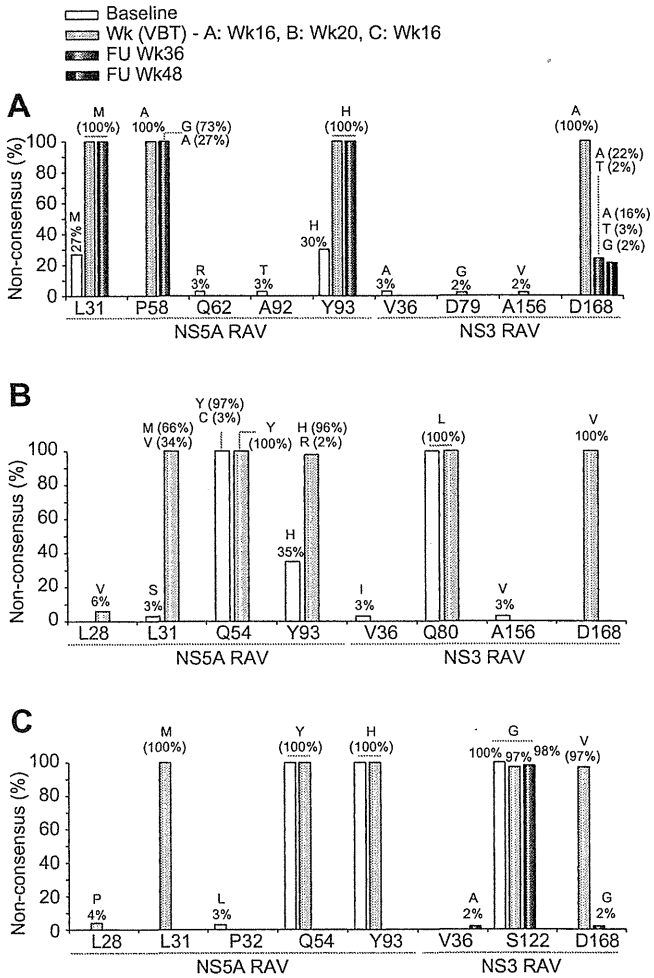

Fig. 5. Clonal analysis of NS3 protease and NS5A resistance-associated variants in patients experiencing virologic breakthrough. (A) Patient P-25. NS5A RAV: baseline 30 clones; Wk16 39 clones; FU Wk36 33 clones; FU Wk48 not performed (no change from FU Wk36 by population sequencing). NS3 RAV: baseline 32 clones; Wk16 41 clones; FU Wk36 56 clones; FU Wk48 63 clones. (B) Patient P-29. NS5A RAV: baseline 37 clones; Wk20 50 clones; FU Wk36/48 analyses not performed (no change from VBT by population sequencing). NS3 RAV: baseline 34 clones; Wk20 47 clones; FU Wk36/48 analyses not performed (no change from VBT by population sequencing). (C) Patient P-43. NS5A RAV: baseline 32 clones; Wk10 47 clones; FU Wk36/48 analyses not performed (no change from VBT by population sequencing). NS3 RAV: baseline 31 clones; Wk10 32 clones; FU Wk36 103 clones; FU Wk48 60 clones. FU, follow-up; VBT, viral breakthrough; RAV, resistance-associated variant.

DCV inhibition were minimal (Y93H EC_{50} = 49 pM [6]) compared with C_{trough} values that ranged from 75 to 620 nM. The global prevalence of NS5A-Y93H is approximately 4%, based on data from the Los Alamos database [20] and unpublished data from nine DCV studies, and is approximately 11% in other recent Japanese DCV studies [21], which is considerably lower than the 23% (10/43) prevalence observed in this study. Further analysis of DCV study data indicates that Y93H pre-exists at higher levels in patients infected with GT1b (10%) than GT1a (1%); however, the link with *IL28B* is not so clear given that most failures to date with DCV have been observed in GT1a patients with no baseline Y93H. Other polymorphisms observed at a higher frequency among this GT1b population included NS3-Q80L (~19%, 8/43) *vs.* Q80K, which has been observed more frequently in GT1a populations [18,19].

Baseline HCV RNA did not appear to influence virologic response in either population, and response was too rapid to allow successful genomic sequencing after 1 week of treatment. ASV dose (600 mg or 200 mg twice daily) did not impact the initial decline in HCV RNA in null responders, and the *IL28B* CT allele, present in 86% (18/21) of null responders, did not prevent patients from achieving a very high (90%) SVR. By contrast, although only 27% (6/22) of ineligible/intolerant patients were *IL28B* CT, this genotype was present in all three viral breakthroughs and one of four relapses. While *IL28B* genotype is known to influence response to PegIFN-α/RBV, its apparent impact on virologic suppression in alfa-sparing regimens is unexpected. However, given the small number of patients, any such correlation will require evaluation in a larger dataset.

The emergent RAVs at viral breakthrough or relapse (signature NS5A-L31 and -Y93 substitutions for DCV and NS3-D168 substitutions for ASV) were similar to observations from other clinical studies of DCV, and from *in vitro* GT1b replicon resistance studies with ASV [19], although this study represents the first demonstration of emergent clinical ASV resistance. It is possible that signature resistance variants to both DCV and ASV pre-existed as minor species, and subsequently enriched by selective pressure, as predicted by viral kinetic modeling [22]. Although a combination of these NS3 and NS5A variants was not detected by clonal sequencing at baseline, their low-level pre-existence cannot be ruled out. However, assessment of minor NS3 plus NS5A variants from the same RNA sequence is currently not feasible using available deep-sequencing technologies. Nevertheless, additional studies to assess the presence and dynamics of minority baseline variants under drug selection are indicated.

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日消誌　2013；110：44―55

原　著

B型慢性肝疾患における核酸アナログ多剤耐性例の検討

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要旨：核酸アナログを投与したB型慢性肝疾患547例中18例（観察期間中央値53カ月）に多剤耐性を検出した．耐性出現を契機に肝不全に陥った症例を認めなかった．多剤耐性をきたした治療はラミブジン（LAM）耐性例に対するエンテカビル（ETV）投与，LAM耐性例に対するアデホビル（ADV）併用，LAMからETVへの切り替え，ETV初回投与で，それぞれ，7例，8例，2例，1例であった．多剤耐性例に対する治療の反応性はLAMとETV耐性例に比べてLAMとADV耐性例で良好であった．18例中1例ではADVとETV併用療法にも不応であり，テノホビルの投与が望まれた．

索引用語：B型肝炎ウイルス，核酸アナログ，多剤耐性

はじめに

B型肝炎ウイルス（hepatitis B virus；HBV）は約3200塩基対の不完全二本鎖DNAウイルスで，複製の際にRNA中間体から逆転写の過程を要する[1)]ため，他のDNAウイルスに比較して遺伝子変異を生じやすい．核酸アナログは逆転写の際のウイルス複製を競合阻害するため，特にこの逆転写酵素をコードするHBVポリメラーゼ遺伝子に変異が生じると薬剤耐性を獲得する可能性を有する薬剤である．

本邦では，現在ラミブジン（lamivudine；LAM），アデホビル（adefovir dipivoxil；ADV），エンテカビル（entecavir；ETV）の3種類の核酸アナログが使用可能である．LAMは本邦で最初に認可された薬剤で，2000年に薬価収載された．LAMは強力な抗ウイルス作用を有する薬剤であるが，長期投与によりポリメラーゼ領域のBドメインのrtL180M，CドメインのrtM204V/Iの変異をおこし高率に薬剤耐性をきたした[2)3)]．このLAM耐性に対してはLAMをADVに切り替えると高率にADV耐性が出現する[4)]ため，現在ではLAMにADVを併用投与する方法が推奨されている[5)6)]．なお，LAMとの大規模比較試験にて明らかに抗ウイルス効果が良好で，かつ，耐性出現率が低率であることが示されたETV[7)8)]は，本邦では2006年に承認され，以後，LAMに代わる第一選択薬となった．ETVの耐性はLAM耐性のrtL180M，rtM204Vに加えて，rtT184G，rtS202I，rtM250Vの3カ所のうち1つ以上の耐性を獲得することで生じる．これらの変異を同時に獲得することはウイルスにとって容易なことではなく，genetic barrierが高いことがこの薬剤の特徴として挙げられる．しかし，既にLAM耐性を獲得してしまった症例に対するETVの投与はETV耐性出現を助長する[9)10)]ことから推奨されていない．

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Table 1. 当院における核酸アナログ投与例の治療内容別内訳

			症例数	観察期間中央値(月)
LAM N=293	LAM耐性出現 N=141	ETV切り替え	12	93
		ADV併用	129	84
	LAM耐性なし N=152	ETV切り替え	107	64
		LAM継続	45	28
ETV			254	34
		合計	547	53

LAM；lamivudine, ADV；adefovir dipivoxil, ETV；entecavir.

核酸アナログはHBV pregenomic RNAからDNAへの転写を阻害し，肝細胞から血液中へのDNAの放出を抑制するが，肝細胞内に入り込んだ完全2本鎖DNA（covalently closed circular DNA；cccDNA）を排除できない[11)~13)]．したがって，投与を中止するとcccDNAを鋳型にウイルスの複製が再開され，ウイルス増殖に引き続き肝炎の再燃をきたす．短期投与ではHBs抗原が消失するような症例はまれであり[14)15)]，長期にわたる肝病変のコントロールのためにも核酸アナログは原則的には長期投与が必要となる[16)17)]．一方で，長期投与には耐性変異出現の危険性を増加させるというジレンマが生じる．

本邦における核酸アナログによる治療法としては，主に，LAM単独療法，LAMとADV併用療法，ETV単独療法が挙げられる．これらの治療による多剤耐性出現率，多剤耐性出現時のウイルス動態や肝炎の程度は明らかではない．また，多剤耐性例に対する治療法は確立されていないのが現状である．

今回，われわれは，当院において経験した多剤耐性例について，耐性をきたした治療内容別に，核酸アナログ初回投与時の患者背景因子・耐性部位の詳細，ALTとHBV DNA量を記し，多剤耐性例に対して行った治療法とその効果を検討したので報告する．

I 対象と方法

当院において1年以上核酸アナログの投与を行ったB型慢性肝疾患547例（観察期間中央値53カ月）を対象とし，投与薬剤別に多剤耐性例の検討を行った．複数の薬剤投与に対して生じた耐性を多剤耐性と称するが，本検討ではETV単独投与で生じた耐性も多剤耐性に含めて検討した．各製剤に対する耐性については，LAMではrtL180M, rtM204V/I, ADVではrtA181G/S/T/V, rtN236T, ETVではrtT184A/C/F/G/I/L/M/S, rtS202C/G/I, rtM250I/L/Vについて耐性の有無を測定した．

対象患者は，2000年以降に当院において核酸アナログの継続的な投与を受け，2012年1月を最終観察時期とした．547例中293例はLAMにより導入されたが，経過中に141例にLAM耐性が出現した．LAM耐性を認めなかった152例では，107例をETVに切り替え，45例はLAM継続投与とした．LAM耐性検出例では，ETVへの切り替え臨床試験参加の12例を除き，129例にADVを併用投与した（Table 1）．LAMからETVへの切り替え例では，事前にrtL180M, rtM204V/Iの耐性がないことを確認した．LAMとADV併用後のADV耐性検出例には，患者の同意が得られた場合は，ADVとETVの併用療法を原則とした．ETV耐性検出例では，まずLAMとADVの併用療法を行い，HBV DNA 3.0 logcopies/ml（以下単位LC/mlと略す）以上で推移する症例はADVとETVの併用療法に切り替えた．1例のみ，主治医判断でADVとETVの併用療法で開始した．

多剤耐性の測定対象は，治療不応例（HBV DNA≧3.0LC/ml），あるいは，ウイルス学的ブレイクスルー（nadirより1.0LC/ml以上上昇）を

示した例であった．HBV DNAはAmplicor法（Roche Diagnostics, Tokyo, Japan. 定量域2.6～7.6LC/ml）あるいはTaqMan® PCR法（Roche Diagnostics, Tokyo, Japan. 定量域2.1～9.0LC/ml）で測定し，耐性の検出はPCR-Invader®法とINNO-LiPA HBV DR version 2 plus（Innogenetics, Ghent, Belgium）の2つの方法で行った．ノンパラメトリックデータの解析にWilcoxonの符号付き順位検定を用い，$p<0.05$を有意とした．

II 結 果

1. 当院の多剤耐性出現状況

多剤耐性の測定が行われた32例中18例で多剤耐性が検出された．18例の内訳は，3例がウイルス学的ブレイクスルー，15例が治療不応例であった．

2. 核酸アナログの治療内容と多剤耐性の出現

1）LAM耐性に対するETV単独投与例

この治療は臨床試験として12例（観察期間中央値93カ月）に行われた．7例（58.3%）でLAM耐性に加えてETV耐性が検出された．多剤耐性検出時ALT 100IU/L以上を示した症例は2例（16.7%）で，HBV DNAは，それぞれ，5.4LC/ml，6.6LC/mlであった．多剤耐性検出後，7例中6例ではLAMとADV併用療法を行い，さらに，HBV DNAが2.1LC/ml未満を呈した症例1を除く5例でADVとETV併用療法に移行した．この5例では観察最終時点でHBV DNA量は2.1LC/ml未満が2例，3.0LC/ml未満が4例となった．一方，症例6は，多剤耐性検出後，主治医の判断でADVとETV併用療法を行い，最終時点でHBV DNA量は陰性となった（Table 2i）．

2）LAM耐性に対するADV併用投与例

129例のLAM耐性例に対してADV 10mg/日の追加併用（観察期間中央値84カ月）を行ったところ，8例（6.2%）でLAM，ADVに対する多剤耐性を検出した．ADVの耐性はrtA181Tが3例，rtA181Vが1例，rtN236Tが1例，rtA181VとrtN236Tが1例であった．症例13，14，15はETVの投与歴のない段階で，ETVの耐性が検出された．症例13，14の2例ではLAM耐性出現時に遡って当時の保存血清を検索したところ，多剤耐性検出時と同様のETV耐性を検出した．耐性検出時ALT 100IU/L以上を示した症例は3剤耐性を呈した1例（12.5%）のみで，HBV DNAの中央値は4.2（3.4～6.6）LC/mlであった．多剤耐性検出後もLAMとADV併用療法を継続した症例は2例，ADVとETV併用に移行した症例は6例で，後者の治療では6例中4例がHBV DNA 2.1LC/ml未満，5例が3.0LC/ml未満を呈した（Table 2ii）．

症例9，10，11，12の4例ではLAM耐性検出時の保存血清を用いてBドメインコドン181の変異の検出を試みた．症例9，10，11ではコドン181の耐性は認めなかったが，症例12ではLAM耐性出現時に同時にrtA181Tが検出された．この症例は2003年7月にrtM204Iの変異を認め，breakthrough hepatitisをおこしLAMとADV併用療法を行った．2007年5月よりHBV DNAの上昇を認め，rtN236Tの耐性を検出していた．その後はADVとETV併用療法を行い2009年5月にはHBV DNAは1.8LC/ml未満となったが，同年6月には肝癌の進行で永眠された（Figure 1）．

3剤耐性の1例（症例15）は2006年に悪性リンパ腫の治療と同時に核酸アナログを開始した症例で，LAMとADV併用療法中にbreakthrough hepatitisをきたし2010年9月当院紹介となった．なお，悪性リンパ腫は2008年以降完全寛解となり，抗癌剤投与は受けていなかった．当院受診後，薬剤耐性の検索を行い，LAM耐性に加えてrtA181V，rtN236TのADV耐性，rtS202GのETV耐性を認めた．ADVとETV併用療法の同意が得られず，LAMとADV併用療法を継続している（Figure 2）．

3）LAMからETVへの切り替え例

この治療は107例（観察期間中央値64カ月）で行われ，2例（1.9%）でLAMとETVの多剤耐性を検出した．LAMからETV切り替え時のHBV DNA量別に多剤耐性例の出現頻度を検討すると，HBV DNAが2.6LC/ml未満，2.6～4.0LC/mlの症例では多剤耐性例は出現していないが，4.0LC/ml以上の13例中の2例（症例16，17）に多剤耐性を認めた．

Table 2. 多剤耐性検出例における耐性株とその後の治療経過

(i) LAM 耐性に対する ETV 投与例

LAM 開始時背景因子					多剤耐性時の変異検出部位							多剤耐性検出時		多剤耐性検出後の治療					
					LAM 耐性		ADV 耐性		ETV 耐性					1次治療			2次治療		
年齢(歳)/性/肝病変	e 抗原	遺伝子型	投与期間(月) LAM	ETV	rt 180	rt 204	rt 181	rt 236	rt 184	rt 202	rt 250	ALT (IU/L)	HBV DNA (LC/ml)		期間(月)	HBV DNA (LC/ml)		期間(月)	HBV DNA (LC/ml)
1. 41/男/CH	陽性	C	19	28	●	●			●	●		131	5.4	L+A	70	<2.1			
2. 67/男/LC	陰性	C	28	42	●	●			●			150	6.6	L+A	28	3.1	A+E	34	<2.1
3. 59/男/CH	陽性	C	51	53	●	●			●	●		73	6.8	L+A	18	4.0	A+E	33	2.4
4. 60/男/CH	陽性	C	31	56	●	●			●	●		49	7.6	L+A	13	4.9	A+E	34	3.1
5. 45/男/CH	陰性	B	29	37	●	●			●			14	6.2	L+A	35	3.6	A+E	31	<2.1
6. 64/男/CH	陰性	C	14	72	●	●				●		30	3.6	A+E	28	(−)			
7. 80/女/CH	陽性	C	77	8	●	●				●		32	6.1	L+A	17	5.0	A+E	35	2.8

(ii) LAM 耐性に対する ADV 併用投与例

LAM 開始時背景因子					多剤耐性時の変異検出部位							多剤耐性検出時		多剤耐性検出後の治療			
					LAM 耐性		ADV 耐性		ETV 耐性								
年齢(歳)/性/肝病変	e 抗原	遺伝子型	投与期間(月) LAM	L+A	rt 180	rt 204	rt 181	rt 236	rt 184	rt 202	rt 250	ALT (IU/L)	HBV DNA (LC/ml)		期間(月)	HBV DNA (LC/ml)	
8. 43/男/CH	陽性	C	15	48	●	●	●					22	3.5	L+A 継続	30	2.7	
9. 53/男/CH	陽性	C	110	19	●	●	●					12	6.6	A+E	33	<2.1	
10. 68/男/LC	陽性	C	13	27		●	●					27	3.0	A+E	34	<2.1	
11. 64/男/LC	陰性	C	18	28	●	●	●					28	3.1	A+E	34	<2.1	
12. 61/男/LC	陽性	C	14	54	●			●				91	5.9	A+E	19	<2.1	
13. 63/女/LC	陽性	C	40	49	●	●				●		28	4.9	A+E	34	3.0	
14. 72/女/CH	陽性	C	46	34	●	●			●			13	3.4	A+E	34	2.2	
15. 61/女/CH	陽性	C	5	61	●	●	●	●			●	497	6.3	L+A 継続	16	4.4	

(iii) LAM から ETV への切り替え例

LAM 開始時背景因子					多剤耐性時の変異検出部位							多剤耐性検出時		多剤耐性検出後の治療					
					LAM 耐性		ADV 耐性		ETV 耐性					1次治療			2次治療		
年齢(歳)/性/肝病変	e 抗原	遺伝子型	投与期間(月) LAM	ETV	rt 180	rt 204	rt 181	rt 236	rt 184	rt 202	rt 250	ALT (IU/L)	HBV DNA (LC/ml)		期間(月)	HBV DNA (LC/ml)		期間(月)	HBV DNA (LC/ml)
16. 48/男/CH	陰性	C	25	45	●	●			●	●		66	3.6	L+A	45	(−)			
17. 61/女/CH	陽性	C	41	14	●	●			●			27	4.2	L+A	14	4.2	A+E	35	2.3

(iv) ETV 初回投与例

ETV 開始時背景因子				多剤耐性時の変異検出部位							多剤耐性検出時		多剤耐性検出後の治療					
				LAM 耐性		ADV 耐性		ETV 耐性					1次治療			2次治療		
年齢(歳)/性/肝病変	e 抗原	遺伝子型	投与期間(月) ETV	rt 180	rt 204	rt 181	rt 236	rt 184	rt 202	rt 250	ALT (IU/L)	HBV DNA (LC/ml)		期間(月)	HBV DNA (LC/ml)		期間(月)	HBV DNA (LC/ml)
18. 44/男/CH	陽性	C	24	●	●				●		53	7.7	L+A	11	6.2	A+E	7	5.3

CH；chronic hepatitis, LC；liver cirrhosis, L+A；LAM+ADV 療法, A+E；ADV+ETV 療法.

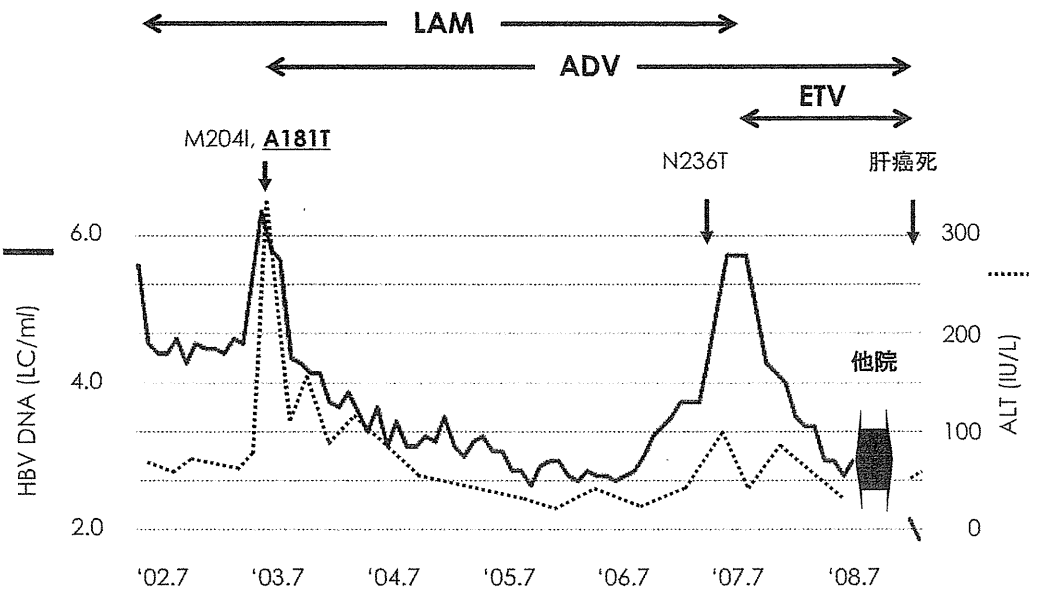

Figure 1. LAM 耐性出現時 ADV 併用前に rtA181T を認めた1例—61歳，男性，e抗原陽性，遺伝子型C型，HCC 合併例—：2003年7月に LAM 耐性（rtM204I）にて LAM と ADV 併用療法を開始した．2007年5月には HBV DNA の上昇をきたし rtN236T が検出され，ADV と ETV 併用療法に移行した．2009年5月には HBV DNA 1.8LC/ml 未満まで低下したが，同年6月には肝細胞癌のため永眠された．2003年7月の保存血清を用いて多剤耐性の検出を行ったところ，rtA181T の耐性が認められた．

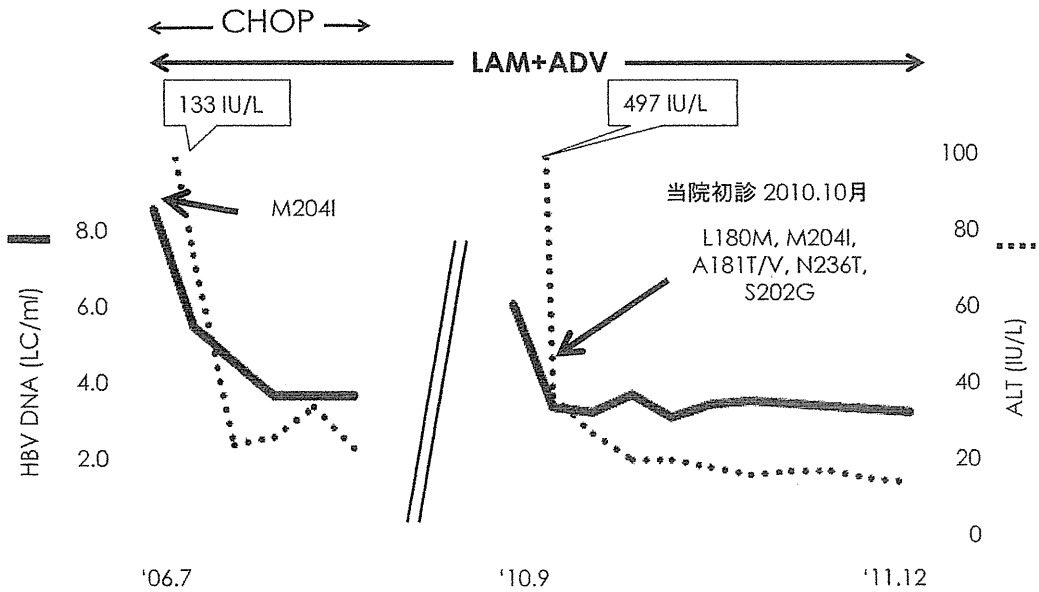

Figure 2. LAM と ADV 併用療法中に3剤耐性を認めた1例—61歳，女性，e抗原陽性，遺伝子型C型，悪性リンパ腫治療後寛解例—：LAM と ADV 併用療法後に HBV DNA と ALT 上昇を認め2010年9月当院紹介．悪性リンパ腫については2007年まで cyclophosphamide, hydroxydaunorubicin, oncovin, prednisolone を用いた CHOP 療法を行い，以後完全寛解となった．耐性検査では rtL180M，rtM204I，rtA181T/V，rtN236T，rtS202G の3剤耐性を認めた．現在まで LAM と ADV 併用療法を継続している．

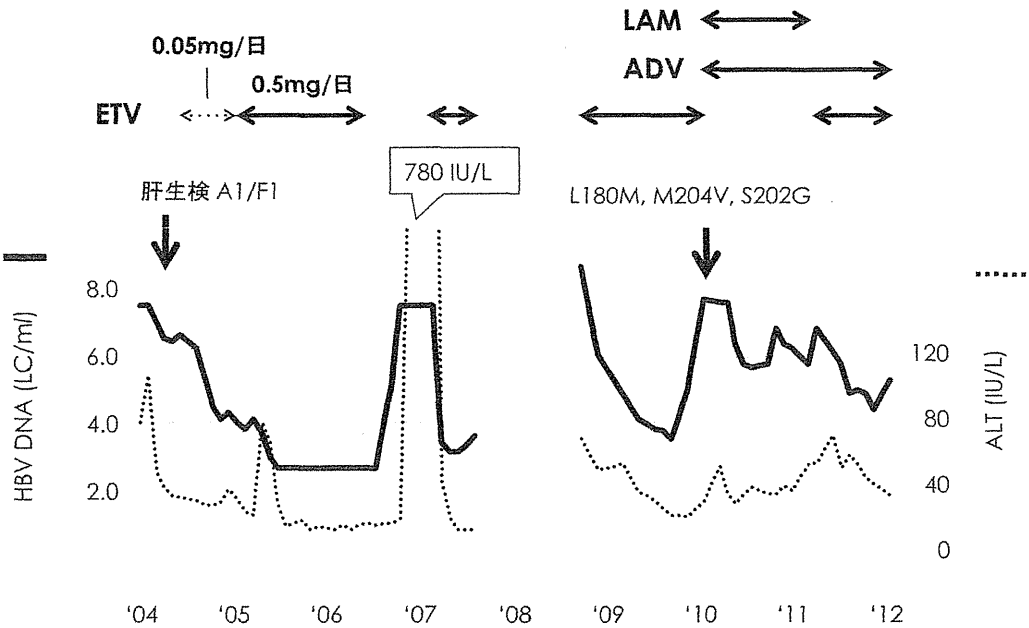

Figure 3. ETV初回投与例で多剤耐性を獲得した1例―44歳，男性，e抗原陽性，遺伝子型C型―：2004年6月からETV用量比較試験に参加し，当初は0.05mg/日，同年11月より0.5mg/日の投与を受けた．経過中，2回にわたるETVの投与中断の経緯を有した．2008年10月よりETV再々投与を行ったが，2010年3月HBV DNAの上昇を認め，rtL180M，rtM204Vの変異に加えてrtS202GのETV耐性が検出された．LAMとADV併用療法にてHBV DNAの低下が得られず，2011年5月よりADVとETV併用療法に切り替えたが，十分量のウイルス低下は得られていない．

この2例では耐性検出時のALTはいずれも100IU/L未満であった．症例16ではLAMとADV併用療法でHBV DNAの陰性化が得られたが，症例17はLAMとADV併用療法でHBV DNAが4.0LC/ml以上で推移するため，ADVとETV併用療法に移行し，観察最終時点で2.3LC/mlまで低下した（Table 2iii）．

4）LAM単独投与例

LAM投与例は死亡や転院にて治療が中止された症例が多く，現在までLAM単独療法が行われている症例は当院では8例に過ぎない．観察期間中央値は28カ月と短く，この群における多剤耐性例は認めていない．

5）ETV初回投与例

この治療は254例（観察期間中央値34カ月）で行われた．1例（0.4%）でLAMとETVの多剤耐性を検出した（Table 2iv）．この症例は44歳，男性で2004年6月よりETV用量比較試験に参加し，当初，0.05mg/日，同年11月より0.5mg/日の投与を受けた．2006年9月試験終了にともない一時ETVの投与を中止したが肝炎の再燃を認め，2007年4月にETVを再開した．その後，自己判断にて内服を中止し，2008年10月，当院再診により再びETVの投与を受けた．2010年3月ウイルス学的ブレイクスルーを認め，rtL180M，rtM204Vの変異に加えてrtS202Gの耐性を認めた．同年4月よりLAMとADV併用療法を行ったが，HBV DNAは6.0LC/ml前後で推移するため，2011年5月よりADVとETV併用療法に切り替えた（Figure 3）．

3．多剤耐性に対する治療

多剤耐性出現18例で，観察期間最終時点における治療内容とHBV DNA量を比較検討した（Figure 4）．LAMとADV併用療法を行った4例では，3例（75.0%）が2.1LC/ml未満，1例（25.0%）が3.5LC/mlとなった．ADVとETV

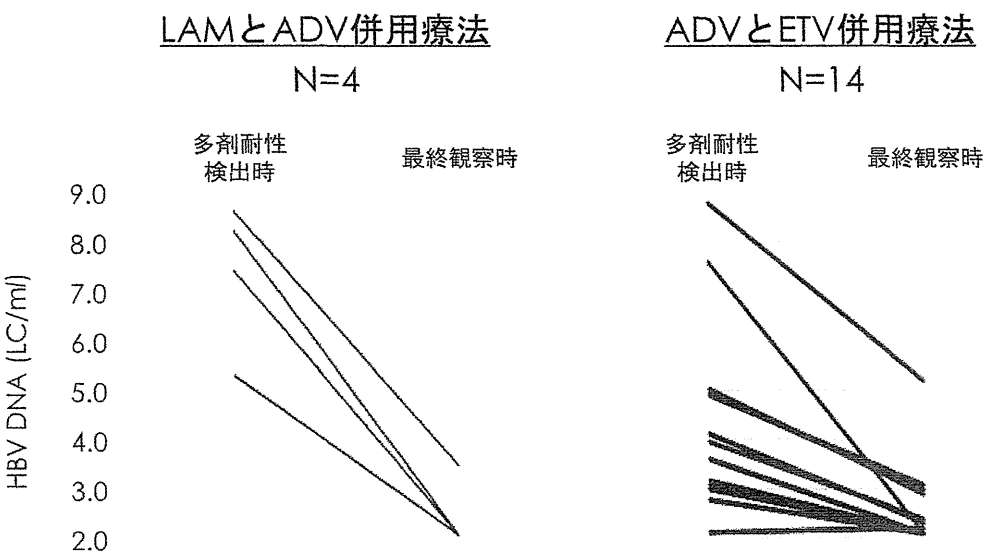

Figure 4. 多剤耐性例に対するウイルス学的効果：観察期間最終時点でHBV DNAが2.1LC/ml未満となった症例の頻度は，LAMとADV併用療法で3例（75.0%），ADVとETV併用療法で12例（85.7%）であった．

併用療法を行った14例では7例（50.0%）が2.1LC/ml未満，12例（85.7%）が3.0LC/ml未満となった．

LAMとETVの多剤耐性出現後の初回治療として，LAMとADVの併用療法を9例に行った．観察期間中央値18（11～70）カ月の経過でHBV DNA中央値は6.4LC/mlから4.1LC/mlと有意に低下（p＝0.002）したが，7例（77.8%）ではHBV DNAが3.0LC/ml以上で推移するため，ADVとETV併用療法に移行した．ADVとETV併用療法34（7～15）カ月の経過でHBV DNA中央値は2.4LC/mlへ低下（p＝0.011）し，HBV DNAは7例中5例（71.4%）が3.0LC/ml未満へ低下した．（Figure 5）．

III 考 案

米国肝臓病学会のガイドラインでは，24週投与でHBV DNAが2.0LC/ml以上低下しない例を核酸アナログ不応と定義している．しかし，海外で行われたLAMとtelbivudine（本邦未承認）の大規模比較試験では，投与2年でHBV DNAが3.0LC/ml以上の症例で高い頻度で薬剤耐性が生じており[18][19]，また，ADVを4年投与した臨床試験においても治療開始から48週の時点でHBV DNAが3.0LC/ml以上の症例ではADV耐性が高頻度に認められた[20]．ETVやテノホビル（tenofovir disoproxil fumarate；TDF）では投与1年でほとんどの症例でHBV DNAが陰性となり，3.0LC/ml以上の症例は少ない[7][8][21]．このように耐性変異を早期の段階で積極的に検出するために，われわれは治療不応の定義を治療1年以上経過してもHBV DNA 3.0LC/ml以上で推移する症例とした．

今回のわれわれの検討では1年以上核酸アナログが投与された547例中，53カ月の観察期間内において，32例で治療不応あるいはウイルス学的ブレイクスルーをきたし，18例で多剤耐性を検出した．14例では多剤耐性が検出されていないが，ほとんどの症例がLAMとADV併用療法での不応例であった．他の要因としては，薬剤のアドヒアランス低下，PCR-Invader法，INNO-LiPA法では検出できない部位の変異などが挙げられる．多剤耐性を示した18例では，ウイルス学的ブレイクスルー3例に対し，治療不応例が15例と多数を占めた．耐性検出時にALTが100 IU/L以上を示した症例は18例中3例（16.7%）と低頻度であり，3剤耐性をきたした1例でALT値は497IU/Lまで上昇したものの，これらの症

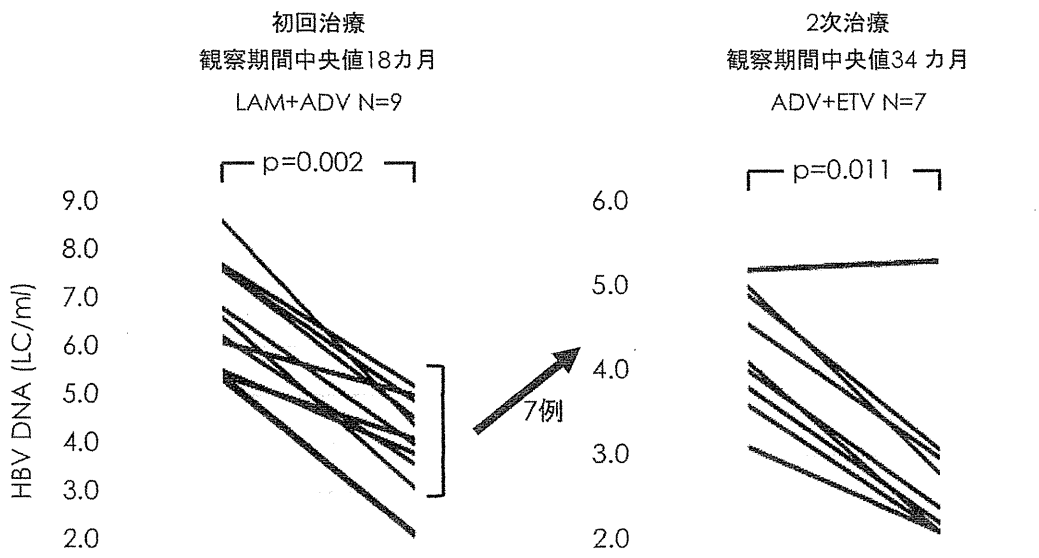

Figure 5. LAMとETV耐性例に対するLAMとADV併用・ADVとETV併用療法の抗ウイルス効果：LAMとADV併用療法を中央値で18カ月行った9例のHBV DNAは，2例(22.2%)が3.0LC/ml未満となった．その時点で3.0LC/ml以上で推移した7例が，ADVとETV併用療法に移行し，34カ月の経過でHBV DNAは7例中5例（71.4%）が3.0LC/ml未満へ低下した．

例では，その後の経過で肝不全をきたすことなく肝炎は鎮静化を認めた．ADV耐性，ETV耐性例ではLAM耐性出現時のような重症肝障害をきたす症例の頻度は低率であると報告されており[21)22)]，当院の症例においても同様の傾向が示された．

薬剤耐性は，INNO-LiPA法およびPCR-Invader法で検出した．INNO-LiPA法は，リバースハイブリダイゼーション法に基づきラインプローブアッセイにより耐性ウイルスを検出する方法で，version 2ではコドン80，173，180，181，204，236の変異を，version 2 plusでは，version 2の項目に加えてコドン184，194，202，250の変異が検出可能であり，LAM，ADV，ETV，TDFの耐性に対応して検出可能である．INNO-LiPA法は既知の耐性部位の検出に限られるが，direct sequencing法と比較して，感度，特異性が優れている[23)24)]とされる．一方，direct sequencing法より感度が優れ，INNO-LiPA法より検査法が簡便で，短時間で検査が可能な方法としてPCR-Invader法[25)]が登場したが，ADVのコドン236の薬剤耐性の検出に対応していない．今回の検討ではPCR-Invader法とINNO-LiPA法の両者を用いることで，既知のADV耐性，ETV耐性の検出を確実なものとした．

LAM耐性を認めずにETVに切り替えた症例とETV初回投与例では多剤耐性出現例はまれであった．LAMからETVに切り替えた症例では，切り替え時のHBV DNA量が4.0LC/ml以上であった2例で耐性が検出された．この2例は，LAMによるウイルス学的ブレイクスルーをおこした時期の検査系ではLAM耐性は検出されていないが，既にLAM耐性が獲得されていた可能性は否定できない．特に切り替え時のHBV DNA量が多い症例では，LAM耐性を有する症例が潜んでいる可能性があり，注意が必要である．

ETVによる耐性出現は核酸アナログ初回投与例ではまれと報告されている[26)]．今回の検討においてもETV耐性の出現頻度は，投与1年後は254例中0例（0%），投与2年後190例中1例（0.5%），投与3年後139例中0例（0%），投与4年後71例中0例（0%），投与5年後23例中1例（4.3%）と低率であった．ETV耐性を認めた症例は，2回にわたりETVの投与を中断したとい