Table 1. Virological outcomes

	Treatment-naive				Non-responders			
Virological response	DCV 10 mg Study 021 (n=9)	DCV 10 mg Study 022 (n=9)	DCV 60 mg Study 021 (n=10)	DCV 60 mg Study 022 (n=8)	DCV 10 mg Study 021 (n=9)	DCV 10 mg Study 022 (n=8)	DCV 60 mg Study 021 (n=9)	DCV 60 mg Study 022 (n=9)
HCV RNA undetectable at end of treatment, n (%)	7 (78)	8 (89)	10 (100)	8 (100)	5 (56)	7 (88)	5 (56)	8 (89)
SVR_{24}, n (%)	6 (67)	8 (89)	9 (90)	8 (100)	2 (22)	4 (50)	3 (33)	7 (78)
All virological failures, n	3	1	1	0	7[a]	4[b]	6[c]	2[d]
No EVR, n	1	–	–	–	–	–	–	–
Virological breakthrough, n	1	1	–	–	4	1	4	1
Post-treatment relapse, n	1	–	1	–	3	3	2	1

All patients also received ribavirin and peginterferon alfa-2b (Study 021) or peginterferon alfa-2a (Study 022). [a]4/4 prior null responders and 3/5 prior partial responders. [b]4/5 prior null responders and 0/3 prior partial responders. [c]4/5 prior null responders and 2/4 prior partial responders. [d]2/8 prior null responders and 0/1 prior partial responders. DCV, daclatasvir; EVR, early virological response (2 log_{10} decrease in HCV RNA from baseline at week 12 of treatment); SVR_{24}, sustained virological response 24 weeks post-treatment.

Table 2. Baseline and on-treatment DCV resistance-associated substitutions among treatment-naive patients with virological failure

PID	VR	Tx, weeks	Visit, week	L28	R30	L31	Q54	P58	Q62	A92	Y93	DCV EC_{50}, nM (SD)[a]
DCV 10 mg												
TN04	Relapse	24	0	–	–	–	–	–	–	–	–	NA
			FU4, FU12, FU24	–	–	V	–	–	–	–	H	101 (20)
TN05	No EVR	13	0	–	–	–	H	–	Q/E	–	–	≤0.003
			2, 12, FU4	–	–	V	H	–	–	–	H	26 (7)
TN06	VBT	24	0	–	–	–	Q/H	–	–	–	–	0.003
			FU4	–	–	M	–	–	–	–	H	48 (16)
			FU4	–	–	V	–	–	–	–	H	101 (20)
TN17	VBT	28	0	–	–	–	H/N	S	–	–	–	ND
			28	–	–	V	–	–	–	–	H	101 (20)
			FU4	–	–	M	H	S	–	–	H	53 (20)
DCV 60 mg												
TN20	Relapse	24	0	–	–	–	–	–	–	–	H/Y	0.062
			FU4, FU12, FU16	–	–	V	–	–	–	–	H	101 (20)

[a]Daclatasvir (DCV) 50% effective concentration (EC_{50}) in a transient HCV replicon luciferase reporter assay for HCV replicons (GT1b [Con1]) with substitutions inserted by site-directed mutagenesis. Results represent the mean ±sd of ≥ two independent experiments, with each experiment representing the mean of ≥ two determinations. EVR, early virological response; FU, follow-up; NA, not applicable; ND, not determined; PID, patient identification; Tx, treatment duration; VBT, virological breakthrough; VR, virological response.

for NS5A resistance-associated polymorphisms (Additional file 2 and Additional file 4). Baseline HCV RNA was 6.1–7.6 (median 6.7) log_{10} IU/ml among patients with virological failure and 5.5–7.4 (median 6.7) log_{10} IU/ml among patients with SVR_{24}. Baseline polymorphisms at amino acid positions associated with DCV resistance were observed in 28/35 patients (Additional file 4). These included 14/16 patients who achieved SVR_{24} (L31M and Y93H each detected in one 60 mg recipient, respectively) and 14/19 patients with virological failure. Among virological failures, one DCV 10 mg recipient had baseline Y93H (patient NR09) and two DCV 60 mg recipients had baseline L31 polymorphisms (patient NR25 L31L/M; patient NR22 L31V).

The majority of non-responders (91%; 32/35 patients) had non-CC IL28B genotypes with only one patient in the alfa-2b study having CC genotype and two patients (one each in the alfa-2a and alfa-2b studies) had no valid IL28B genotype determination (Additional file 4). Of the 32 non-CC patients, 17 were treatment failures (6 receiving alfa-2a, 11 receiving alfa-2b). Only two had signature NS5A resistance-associated polymorphisms (patient NR25, L31M; patient NR09, Y93H). The one patient with IL28B genotype CC (NR22) was infected with an HCV NS5A-L31V variant associated with a 24-fold reduction in DCV susceptibility. HCV RNA decline was relatively slow in this patient and, although HCV RNA was undetectable by week 12 and

maintained through week 48, the patient relapsed at four weeks post-treatment. In general, pharmacokinetic parameters indicated no obvious deficiency in DCV exposure in most non-responders with virological failure (Additional file 3).

Variants emerging on treatment
DCV-resistant variants emerged during treatment in all 19 non-responders with virological failure (13 receiving alfa-2b, 6 receiving alfa-2a; Table 3). In all cases, $\geq$ two DCV-resistant variants were detected in the on-treatment and post-treatment failures. Patterns of emerging variants were generally similar to those observed in treatment-naive patients, with the principal combinations being L31V plus Y93H (nine patients) and L31M plus Y93H (six patients). Of previously reported NS5A resistance-associated variants, only L31V+Y93H was detected in six patients (EC_{50}=101 nM; patients NR06, NR04, NR07, NR17, NR26 and NR22), with the addition of Q54H in two patients (EC_{50}=26 nM; NR15 and NR37) and multiple additional substitutions in one patient (NR21, described below). L31M plus Y93H was always associated with other substitutions at positions Q54H with or without P58S and Q62E. Generally, these combinations were associated with a >8,000-fold decrease in DCV susceptibility *in vitro* (EC_{50}>25 nM) compared with the reference replicon (EC_{50}=0.003 nM). It should be noted that combining DCV signature resistance substitutions with Q54H generally enhanced drug susceptibility (Tables 2, 3 and 4). In almost all cases, DCV-resistant variants detected during virological breakthrough persisted post-treatment and DCV-resistant variants detected during early relapse persisted at post-treatment week 24.

One relapser (NR22) had HCV RNA>1,000 IU/ml over the first few weeks of treatment. At week 1 NS5A-Y93H emerged, which when combined with a baseline L31V variant, conferred significant loss in DCV potency *in vitro*. This patient subsequently achieved undetectable HCV RNA by week 12 and received 48 weeks of treatment before relapsing. The NS5A sequence at week 1 was similar to that at relapse. In 5 of 19 patients experiencing virological failure, DCV-resistant variants other than L31V/M/I-Y93H were detected. In two patients (NR02 and NR24) the codon encoding NS5A amino acid 32 was deleted, in two others (NR20 and NR21), A92K was detected among the emerging substitutions and in one patient (NR12), R30H plus L31V emerged (Table 3).

Patient NR02 had high baseline HCV RNA (7.6 $\log_{10}$ IU/ml); this patient received DCV 10 mg plus alfa-2b/RBV, which reduced HCV RNA to 2.5 $\log_{10}$ IU/ml by week 2. Virological breakthrough occurred between weeks 2 and 12, leading to cessation of treatment

due to lack of efficacy. At baseline, the NS5A-Q54H variant was detected. At week 8, deletion of P32 was detected, which alone confers a DCV EC_{50}>5,000 nM with a replication capacity of 29% versus wild type (Table 4). Interestingly, this variant was transient and not detected at weeks 12 or 24, or at week 4 of follow-up. Instead, a variant carrying substitutions L31M plus Y93H in combination with the baseline polymorphism Q54H (EC_{50}=13 nM in a transient assay) became the dominant viral population. Phenotypic assessment using the patient-derived full-length NS5A sequence from week 12 (L31M-Q54H-Y93H) yielded a comparable EC_{50} value (19 nM) to the reference GT1b (Con1) carrying these substitutions in the transient assay (Table 4).

Patient NR24 had baseline HCV RNA of 6.7 $\log_{10}$ IU/ml. Treatment with DCV 60 mg plus alfa-2b/RBV reduced HCV RNA to below detection by week 4. At week 8, the patient experienced virological breakthrough and treatment was eventually stopped at week 40 because of lack of efficacy. At baseline, Q62R and A92A/T were detected and at week 24, L31F and a P32 deletion emerged. Unlike patient NR02, ΔP32 combined with L31F was also detected at follow-up week 4. The replication capacity of replicons carrying L31F plus ΔP32 was poor (12%) compared with the reference GT1b (Con1) replicon; however, L31F plus ΔP32 plus Q62R did not replicate in the transient assay and required testing in a stable cell line to obtain an EC_{50} (>5,000 nM).

Patient NR21 had baseline HCV RNA of 7.0 $\log_{10}$ IU/ml. Treatment with DCV 60 mg plus alfa-2b/RBV reduced HCV RNA to a nadir of 2.3 $\log_{10}$ IU/ml by week 4 followed by an increase to 5.8 $\log_{10}$ IU/ml by week 12. Treatment was stopped at week 13 for lack of efficacy. At baseline, NS5A polymorphisms L28M plus R30Q were detected but did not reduce DCV susceptibility *in vitro* when introduced into the GT1b (Con1) replicon reference. However, retention of these two substitutions and addition of L31V plus Y93H substitutions at week 8 resulted in a DCV EC_{50}=1,672 nM in a stable cell line. Population sequencing following amplification of the NS5A region using two different primer pairs revealed a mixture of DCV-resistant variants in this patient at different times after virological failure. Clonal analysis at week 12 showed several distinct linkages: L28M+R30Q+A92K (26% clones), L28M+R30Q+Y93H (approximately 11% clones), L28M+R30H+L31V+A92K (21% clones), L28M+R30Q+L31V+Y93H (16% clones), and L28M+R30Q+L31V+Y93H (11% clones); EC_{50}s of >3,000 nM were observed for each substitution combination (Table 4). Thus, the loss in DCV potency with any of these combinations was sufficient to explain virological failure.

Table 3. Baseline and on-treatment DCV resistance associated substitutions among non-responders with virological failure

PID	VR	Tx, weeks	Visit, week	DCV resistance-associated substitutions									DCV EC$_{50}$, nM (sd)[a]
---	---	---	---	L28	R30	L31	P32	Q54	P58	Q62	A92	Y93	
DCV 10 mg													
NR02	VBT	36	0	–	–	–	–	H	–	–	–	–	0.003
			8	–	–	–	DEL	H	–	–	–	–	ND
			12, 24 and FU4	–	–	M	–	H	–	–	–	H	13 (6)
NR03	VBT	41	0	–	–	–	–	H	–	–	–	–	0.003
			24 and FU4	–	–	M	–	H	–	–	–	H	13 (6)
NR04	Relapse	24	0	–	–	–	–	–	–	–	–	–	ND
			FU4, FU24	–	–	V	–	–	–	–	–	H	101 (20)
NR06	VBT	14	0	–	–	–	–	–	–	–	–	–	0.003
			FU4	–	–	V/I	–	–	–	–	–	H	101 (20)
NR07	Relapse	24	0	–	–	–	–	–	–	–	–	–	ND
			12 and FU24	–	–	V	–	–	–	–	–	H	101 (20)
NR08	Relapse	24	0	–	–	–	–	–	–	–	–	–	ND
			FU12	–	–	I	–	–	–	–	–	H	5.2 (2)
NR09	VBT	45	0	–	–	–	–	–	–	–	–	H	0.062
			4	–	–	M	–	–	–	–	–	H	48 (16)
			FU4	–	–	V	–	–	–	–	–	H	101 (20)
NR10	Relapse	23	0	–	–	–	–	H	–	E	–	–	0.001
			FU4, FU24	–	–	M	–	H	–	E	–	H	28 (4)
NR12	VBT	34	0	M	Q	–	–	H	–	–	T	–	0.002
			24 and FU4	M	H	V	–	H	–	–	T	–	59 (20)
NR15	Relapse	24	0	–	–	–	–	H	–	–	–	–	0.003
			FU4, FU12, FU24	–	–	V	–	H	–	–	–	H	26 (7)
NR17	Relapse	24	0	–	–	–	–	–	–	–	–	–	ND
			FU12	M	–	–	–	–	–	–	–	H	2.8 (1)
			FU24	–	–	I	–	–	–	–	–	H	5.2 (2)
			FU24	–	–	V	–	–	–	–	–	H	101 (20)
DCV 60 mg													
NR20	Relapse	24	0	–	–	–	–	H	–	–	–	–	0.003
			FU4, FU24	–	Q	–	–	H	–	–	K	–	26 (18)
NR21	VBT	13	0	M	Q	–	–	–	–	–	–	–	0.003
			8	M	Q	V	–	–	–	–	–	H	1,672[b]
			12	M	Q	V/L	–	–	–	–	A/E/K/T	Y/H	–[c]
			12	M	Q	–	–	–	–	–	K	–	3,932 (684)
			FU4	M	H/Q	L/V	–	–	–	–	A/E/K/P/S/T	Y/H	ND
			FU4	M	Q	V	–	–	–	–	–	H	ND
NR22	Relapse	48	0	–	–	V	–	–	–	–	–	–	0.072
			1 and FU4, 24	–	–	V	–	–	–	–	–	H	101 (20)
NR24	VBT	40	0	–	–	–	–	–	–	R	A/T	–	ND
			24 and FU4	–	–	F	DEL	–	–	R	–	–	>5,000[b]
NR25	VBT	13	0	–	–	L/M	–	H	–	–	–	–	0.004
			1, 12 and FU4	–	–	M	–	H	–	–	–	H	13 (6)
NR26	VBT	13	0	–	–	–	–	H	–	–	–	–	ND
			8, 12 and FU4	–	–	V	–	–	–	–	–	H	101 (20)
NR32	Relapse	14	0	–	–	–	–	H	–	E	–	–	0.001
			FU12, FU24	–	–	M	–	H	–	E	–	H	28 (4)
NR37	VBT	25	0	–	–	–	–	Y/H	–	–	–	–	0.003
			FU4	–	–	V	–	H	–	–	–	H	26 (7)

[a]Daclatasvir (DCV) 50% effective concentration (EC$_{50}$) in a transient HCV replicon luciferase reporter assay for HCV replicons (GT1b [Con1]) with substitutions inserted by site-directed mutagenesis. Results represent the mean ±sd of ≥ two independent experiments, with each experiment representing the mean of ≥ two determinations. [b]This variant did not replicate in the transient assay. Result shown is from a cell line assay. [c]See Table 4. DEL, deletion; FU, follow-up; ND, not determined; PID, patient identification; Tx, treatment; VBT, virological breakthrough; VR, virological response.

Table 4. DCV susceptibility and replicative capacity of HCV (Con1) replicons with NS5A substitutions detected in patients

Patient	NS5A DCV resistance substitutions	DCV EC$_{50}$, nM (SD)	DCV EC$_{90}$, nM (SD)	Percentage replication versus GT1b (Con1) (SD)[a]
Deletion observed in patients NR02 and NR24	P32 deletion	>5,000 (NA)	>5,000 (NA)	29 (3)
Patient NR20				
Consensus sequence	R30Q Q54H T56I T64A A92K	95 (22)	423 (181)	47 (22)
Variants constructed to	R30Q	0.002 (0.0003)	0.007 (0.003)	91 (1)
assess the role of individual	Q54H	0.003 (0.0005)	0.011 (0.003)	83 (18)
mutations	T56I	0.002 (0.0008)	0.004 (0.002)	39 (10)
	A92K	33 (0.85)	40 (1.1)	1 (0)
	R30Q A92K	231 (67)	631 (311)	49 (4)
	R30Q Q54H A92K	26 (18)	213 (88)	36 (14)
	R30Q Q54H T56I A92K	102 (26)	373 (143)	100 (17)
	R30Q Q54H T64A A92K	129 (31)	446 (89)	76 (30)
Patient NR21				
Variants detected by	L28M R30Q Y93H (2 clones)	>5,000 (NA)	>5,000 (NA)	5 (1)
clonal analysis (total 19	L28M R30Q A92K (5 clones)	3,932 (684)	>5,000 (NA)	49 (23)
clones, variants observed in	L28M R30H L31V A92K (4 clones)	>5,000	>5,000	36 (32)
>1 clone tested)	L28M R30H L31V Y93H (2 clones)	>5,000 (NA)	>5,000 (NA)	21 (2)
	L28M R30Q L31V Y93H (3 clones)[b]	1,672 (598)	3,637 (627)	ND

Results are from transient assays unless otherwise indicated and represent the mean ±SD of ≥ two independent experiments with each experiment representing the mean of ≥ two determinations. [a]Replication capacity of variant replicons was compared against the reference GT1b (Con1) replicon. [b]This variant did not replicate in the transient assay; result shown is from a cell line assay. DCV, daclatasvir; EC$_{50}$, 50% effective concentration; EC$_{90}$, 90% effective concentration; NA, not applicable; ND, not determined.

Impact of specific variants

In patient NR20, who experienced post-treatment relapse, NS5A substitutions at positions 30 (R30Q), 56 (T56I), 64 (T64A) and 92 (A92K) were detected at failure and Q54H, which was also present at baseline, was retained. Among individual substitutions, A92K was the only one to significantly impact DCV susceptibility in the transient replicon assay (EC$_{50}$=33 nM); however, this substitution also reduced replicative capacity to only 1% of the wild-type GT1b (Con1) reference replicon (Table 4). Addition of R30Q both restored replicative capacity and further reduced susceptibility to DCV inhibition. However, combination with Q54H countered this effect by increasing DCV susceptibility by approximately 10-fold. Subsequent addition of T56I or T64A restored the reduction in DCV susceptibility four- to fivefold and also modulated replicative capacity. Phenotypic assessment using the patient-derived full-length NS5A sequence from follow-up week 4 resulted in DCV EC$_{50}$=369 nM (Additional file 5).

Discussion

In Japanese patients with chronic GT1b infection, triple therapy comprising DCV (10 mg or 60 mg once daily) combined with alfa-2a/RBV or alfa-2b/RBV resulted in a robust response with few virological failures among previously treatment-naive patients. In prior non-responders to alfa/RBV, partial responders (approximately 62%) were more likely to achieve SVR$_{24}$ than null responders (approximately 36%), with more responders among recipients of DCV 60 mg than 10 mg. SVR was more robust in non-responders receiving alfa-2a (50% and 78% achieving SVR$_{24}$ in DCV 10 mg and 60 mg groups, respectively) than alfa-2b (22% and 33% achieving SVR$_{24}$ in DCV 10 mg and 60 mg once-daily groups, respectively).

Overall, the majority of treatment failures carried non-CC IL28B genotypes. Among treatment-naive patients, most patients had IL28B genotype CC and achieved SVR$_{24}$. Among 11 patients with non-CC IL28B genotypes, 5 experienced virological failure (4/5 in the alfa-2b study). This finding is consistent with the known influence of host genotype on response to interferon-based therapy [20]. Low DCV plasma levels may have contributed to poor treatment response or virological breakthrough in three of the treatment-naive patients receiving DCV 10 mg; however, there was no clear pharmacokinetic evidence of a deficiency in DCV exposure in most patients with virological failure.

Virological response rates did not appear to be influenced by baseline NS5A resistance-associated polymorphisms or baseline HCV RNA levels. NS5A polymorphisms reported as associated with DCV resistance were detected at baseline in most patients regardless of virological response, but most did not

reduce DCV potency when tested alone in an *in vitro* HCV replicon assay. Of the 24 failures, 18 had baseline NS5A polymorphisms at sites associated with DCV resistance (L28M, L31M/V, Q54H, Q62E, A92T and Y93H) and 14/18 were still detected at failure. The combination of some baseline polymorphisms with the emergent signature resistance variants *in vitro* modulated loss in potency. GT1b DCV signature substitutions shown to exhibit slight reduction in DCV susceptibility *in vitro* were detected in some baseline samples (three patients with L31M or L31V, seven with Y93H). Only three prior non-responders receiving DCV 60 mg with alfa-2b/RBV had L31 polymorphisms and one responded to treatment. Most (5/7, 71%) patients with pre-existing Y93H responded to treatment, including a GT1a patient who received DCV 10 mg with alfa-2a/RBV. Thus, pre-existence of NS5A-Y93H by itself did not appear to significantly impact risk of failure even though the DCV resistance barrier was effectively lower in the GT1b patients and non-existent in the GT1a patient.

Four of the five patients with baseline NS5A-Y93H who achieved SVR also had IL28B CC genotype, whereas the two failures had non-CC genotypes (both alfa-2b recipients). Therefore, a patient's IL28B genotype may contribute more than baseline NS5A-Y93H to virological outcome. Of the three prior non-responders with baseline NS5A-L31 variants, two experienced virological failure and had high DCV exposure, although only one had non-CC IL28B genotype (both alfa-2b recipients). The one patient with L31M who achieved SVR had a non-CC IL28B genotype. Of the ten patients with pre-existing signature DCV-resistant variants, all four who failed received alfa-2b. The combination of high baseline viral load, non-CC IL28B genotype and pre-existing low-level NS5A resistance-associated polymorphisms may potentially increase risk of failure in prior non-responders treated with DCV plus alfa-2b/RBV. However, because of the low numbers of patients, larger studies are required to confirm possible correlations.

In these studies, all DCV recipients with virological failure had DCV resistance-associated variants at the time of failure. Predominant NS5A variants were the combination of L31V/M/I and Y93H, previously described as the major signature variants in Japanese patients with GT1b [19]. Differences in DCV dose and timing of virological failure did not seem to affect the pathway to resistance. Assessment of different patient groups revealed that only L31 plus Y93 variants emerged in treatment-naive patients, whereas resistance variants in prior non-responders were more varied with novel combinations such as L31F plus ΔP32 being detected. The difference in resistance pathways in prior non-responders (NR12 in the alfa-2a study; NR02, NR20, NR21 and NR24 in

the alfa-2b study) may have been a consequence of the respective baseline NS5A sequences and/or a lower barrier to resistance in this population, since antiviral effects exerted by alfa may have been suboptimal. In general, patterns of emergent DCV-resistant substitutions were similar in patients receiving alfa-2a and alfa-2b.

Previous studies have shown that the DCV resistance barrier in HCV GT1b is higher than in GT1a, with multiple substitutions required for significant reduction of drug susceptibility [14,15]. Findings from this study were consistent; significant loss in DCV antiviral activity was generally only seen against NS5A variants with $\geq$ two resistance-associated substitutions. These emergent substitutions persisted several weeks off-treatment, with HCV RNA levels similar to pre-treatment levels, suggesting that the replicative capacity of the DCV-resistant virus was not significantly compromised. Longer-term follow-up studies are required to fully assess the fitness of NS5A resistance-associated variants.

Of interest was the emergence of an NS5A P32 deletion in two non-responders that was sufficient to confer high-level DCV resistance in HCV GT1b; however, its transient appearance in patient NR02 suggests that it may also reduce replication capacity. Patient NR02 was treated with DCV 10 mg and the level of selective pressure may have been insufficient to provide an advantage to the ΔP32 variant without sufficient compensatory mutations. Conversely, in the patient receiving DCV 60 mg, ΔP32 persisted to follow-up week 4, along with substitutions L31F and Q62R, which in the case of L31F may play a compensatory role in addition to conferring resistance. Selection of a deletion at NS5A codon 30 *in vitro* with GT1a replicons has been described previously; however, GT1b replicons carrying the Δ30 substitution did not replicate *in vitro* [14]. Further studies are needed to clarify the role of these deletions and possible compensatory mutations.

In conclusion, DCV in combination with alfa-2a or alfa-2b and RBV resulted in high response rates in treatment-naive Japanese patients chronically infected with HCV GT1b, particularly at the 60 mg dose. Among previous non-responders to alfa/RBV, virological failure was more frequent, suggesting that alternative combinations with a higher barrier to resistance will be required for this population. NS5A variants emerging at the time of virological failure were generally consistent with those previously reported. The presence of baseline DCV resistance-associated polymorphisms appeared to be less associated with virological failure than IL28B genotype and the type of interferon; combination with alfa-2a/RBV was potentially more efficacious than combination with alfa-2b/RBV. Larger studies may clarify possible correlations

between baseline NS5A polymorphisms and response to DCV-based therapy. Finally, all reported emergent DCV resistant variants persisted with no outgrowth by consensus baseline viral sequences. The fitness of these NS5A resistance variants will require assessment in longer-term follow-up studies.

Acknowledgements

Disclosure statement

Additional files

Additional file 1: Supplementary Table 1 can be found at http://www.intmedpress.com/uploads/documents/3012_McPhee_Additional_file1.pdf

Additional file 2: Supplementary Table 2 can be found at http://www.intmedpress.com/uploads/documents/3012_McPhee_Additional_file2.pdf

Additional file 3: Supplementary Figure 1 can be found at http://www.intmedpress.com/uploads/documents/3012_McPhee_Additional_file3.pdf

Additional file 4: Supplementary Table 3 can be found at http://www.intmedpress.com/uploads/documents/3012_McPhee_Additional_file4.pdf

Additional file 5: Supplementary Table 4 can be found at http://www.intmedpress.com/uploads/documents/3012_McPhee_Additional_file5.pdf

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Relevance of the Core 70 and IL-28B polymorphism and response-guided therapy of peginterferon alfa-2a ± ribavirin for chronic hepatitis C of Genotype 1b: a multicenter randomized trial, ReGIT-J study

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Abstract

Background We conducted a multicenter randomized clinical trial to determine the optimal treatment strategy against chronic hepatitis C virus (HCV) with genotype 1b and a high viral load (G1b/high).

Methods The study subjects included 153 patients with G1b/high. Patients were initially treated with PEG-IFNα-2a alone and then randomly assigned to receive different treatment regimens. Ribavirin (RBV) was administered to all patients with HCV RNA at week 4. Patients negative for HCV RNA at week 4 were randomly assigned to receive PEG-IFNα-2a (group A) or PEG-IFNα-2a/RBV (group B). Patients who showed HCV RNA at week 4 but were negative at week 12 were randomly assigned to receive weekly PEG-IFNα-2a (group C) or biweekly therapy (group D). Patients who showed HCV RNA at week 12 but were negative at week 24 were randomly assigned to receive PEG-IFNα-2a/RBV (group E) or PEG-IFNα-2a/RBV/fluvastatin (group F).

Results Overall, the rate of sustained virological response (SVR) was 46 % (70/153). The total SVR rate in the group (A, D, and F) of response-guided therapy was significantly higher than that in the group (B, C, and E) of conventional therapy [70 % (38/54) versus 52 % (32/61), $p = 0.049$]. Although IL28-B polymorphism and Core 70 mutation were significantly associated with efficacy, patients with rapid virological response (RVR) and complete early virological response (cEVR) achieved high SVR rates regardless of their status of IL-28B polymorphism and Core 70 mutation.

Conclusion In addition to knowing the IL-28B polymorphism and Core 70 mutation status, understanding the likelihood of virological response during treatment is critical in determining the appropriate treatment strategy.

Keywords Chronic hepatitis C · IL-28B · Peginterferon alfa-2a · Ribavirin · Response-guided therapy

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Introduction

The introduction of combined treatment with peginterferon (PEG-IFN) and ribavirin (RBV) has dramatically increased the rate of sustained virological response (SVR) in patients with genotype 1 high virus titer chronic hepatitis C (HCV RNA titer $\geq$ 5 Log IU/mL), a disease generally considered intractable, to approximately 50 % [1–4]. Currently, a protease inhibitor, telaprevir, can be used for the treatment of chronic hepatitis C, further increasing the SVR rate to approximately 70 % after initial treatment; however, adverse events such as severe anemia, dermatopathy, and renal dysfunction due to increased creatinine level have been reported [5, 6].

RBV is also associated with adverse events, such as anemia, dermatopathy and taste disturbance, and these events can be accentuated in elderly patients or patients with renal dysfunction or anemia. In Japan, there are many elderly patients with chronic hepatitis C and they often cannot tolerate a treatment combination involving RBV [7]. For such patients, PEG-IFN monotherapy could be a treatment option. It has been reported that patients with genotype 1 high virus titer chronic hepatitis C are more likely to achieve SVR if their HCV RNA becomes negative within 4 weeks after initiation of PEG-IFN monotherapy (Rapid Virological Response: RVR) [8].

Patients receiving the PEG-IFNα-2a/RBV combination therapy can also achieve an excellent SVR rate if their HCV RNA becomes negative within 12 weeks after initiation of treatment, whereas the rate is known to decrease with a delay in the timing of HCV RNA-negative conversion [3]. Based on these findings, we propose the use of "response-guided therapy", in which a treatment regimen is modified according to viral kinetics. For the treatment of genotype 1 chronic hepatitis C, proposed treatment strategies include shortening of treatment period in patients with RVR and extension of treatment period in patients with a delayed response to the initial treatment as judged at week 12 [9–17]. For the treatment of genotype 1 high virus titer chronic hepatitis C, shortening of the treatment period may not be recommended even if RVR is achieved because of a possible reduction in the SVR rate, whereas extension of the treatment period to 72 weeks has been reported to increase the SVR rate in patients showing a delayed response to the initial treatment [12, 14–18]. In addition, combined use of HMG-CoA reductase inhibitors and IFN has been shown to enhance the antiviral effects in a synergistic manner [19]. Addition of fluvastatin (FLV), an HMG-CoA reductase inhibitor reported to exhibit the highest antiproliferative activity against hepatitis C virus, to PEG-IFNα-2a/RBV combination therapy has improved the SVR rate [20–22].

Factors affecting the efficacy of PEG-IFN/RBV combination therapy can be divided into viral and host factors. The viral factors include virus titer, genotype, amino acid substitution at position 70 of the core protein (Core 70) and mutations in the interferon sensitivity-determining region (ISDR) in the HCV NS5A region [23–27]. The host factors include age, sex, the degree of liver fibrosis, and a single nucleotide polymorphism (SNP) close to the IL-28B gene [28–33].

We therefore conducted a randomized trial to explore the optimal treatment strategy for patients with genotype 1 high virus titer chronic hepatitis C by comparing several treatment regimens modified according to the concept of "response-guided therapy" in consideration of tolerability (PEG-IFNα-2a monotherapy, PEG-IFNα-2a weekly or biweekly/RBV combination, and PEG-IFNα-2a/RBV/FLV combination therapy). We also evaluated the relations of IL-28B polymorphism and Core 70 mutation to the rate of HCV-RNA-negative conversion and SVR.

Patients and methods

Patients

The study subjects included 153 patients with genotype 1b high virus titer chronic hepatitis C (HCV RNA $\geq$ 5 Log IU/mL) who visited 17 institutions from April 2007 to December 2010 and met the following inclusion criteria: laboratory data before study treatment of white blood cell count $\geq$ 3,000/mm^3, neutrophil count $\geq$ 1,500/mm^3, platelet count $\geq$ 90,000/mm^3, and hemoglobin $\geq$ 12 g/dL. Before the study treatments were carried out, all patients gave written informed consent after receiving a sufficient explanation of the therapy. All patients had genotype 1b chronic hepatitis C with a mean HCV RNA titer of 6.4 Log IU/mL. There were 63 male and 90 female patients with a mean age of 56.5 years. Sixty patients had received prior treatment with IFN, though it was ineffective in 30 of these patients (Table 1).

Treatment protocol

The study design is shown in Fig. 1. After a lead-in therapy with PEG-IFNα-2a 180 µg/week alone (for 4 weeks), RBV was added to the treatment for patients without HCV RNA-negative conversion (according to their weight; $\leq$ 60 kg, 600 mg/day; 60–80 kg, 800 mg/day; and >80 kg, 1,000 mg/day). Patients with negative HCV RNA (Taq-Man < 1.2 Log IU/mL) at week 4 (rapid virological response, RVR) were randomly assigned to receive PEG-IFNα-2a alone (group A) or PEG-IFNα-2a/RBV combination (group B). Patients with negative HCV RNA

Table 1 Baseline characteristics of patients (n = 153)

Age (years)	56.5 ± 11.1
Gender (male/female)	63/90
HCV RNA (Log IU/mL)	6.4 ± 0.7
BMI (kg/m^2)	22.8 ± 3.3
ALT (IU/L)	60.5 ± 41.3
AST (IU/L)	51.7 ± 31.5
Previous IFN (no/yes)	93/60 (non-responder for 30)
Fibrosis (F0-2/F3-4)	72/32 (unknown for 49)
Activity (A0-1/A2-3)	49/56 (unknown for 48)
Core 70 (wild/mutant)	54/38 (unknown for 61)
IL-28B, rs8099917 (TT/non-TT)	43/26 (unknown for 84)

Values are mean ± standard deviation (SD)

BMI body mass index, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase
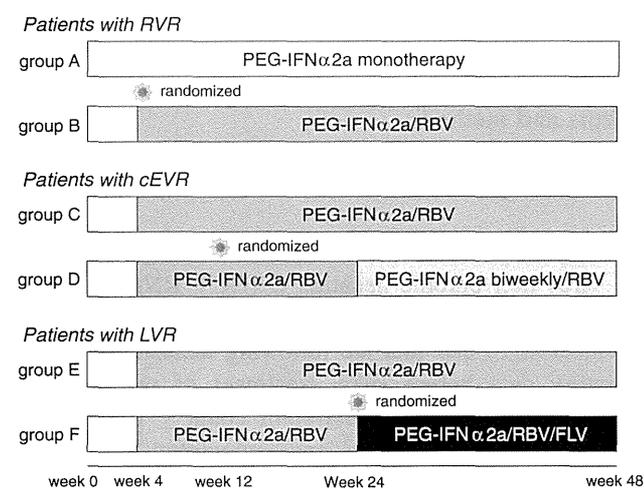

Fig. 1 Study design. After a lead-in therapy with PEG-IFNα-2a for 4 weeks, patients with negative HCV RNA at week 4 (RVR) were randomly assigned to receive PEG-IFNα-2a alone (group A) or PEG-IFNα-2a/RBV combination (group B). Patients with negative HCV RNA at week 12 (cEVR) were randomly assigned to receive weekly PEG-IFNα-2a/RBV combination (group C) or biweekly PEG-IFNα-2a/RBV combination (group D). Patients with negative HCV RNA at week 24 (LVR) were randomly assigned to receive PEG-IFNα-2a/RBV combination (group E) or PEG-IFNα-2a/RBV/fluvastatin (FLV) combination (group F)

at week 12 (complete early virological response, cEVR) were randomly assigned to receive weekly PEG-IFNα-2a/RBV combination (group C) or biweekly PEG-IFNα-2a/RBV combination (group D). Patients with negative HCV RNA at week 24 (late virological response, LVR) were randomly assigned to receive PEG-IFNα-2a/RBV combination (group E) or PEG-IFNα-2a/RBV/fluvastatin (FLV) combination (group F). For assignment, we used Microsoft Access to generate random numbers.

Cases with RVR: evaluation of necessity of RBV (PEG-IFNα-2a monotherapy versus PEG-IFNα-2a/RBV combination therapy)

Patients with negative HCV RNA at week 4 after the introduction of lead-in therapy with PEG-IFNα-2a alone (RVR) were randomly assigned to receive PEG-IFNα-2a alone (group A) or PEG-IFNα-2a/RBV combination (group B) to compare the efficacy and safety between the treatment groups and to evaluate the significance of addition of RBV in RVR cases.

Cases with cEVR: evaluation of dosage interval of PEG-IFNα-2a (weekly versus biweekly PEG-IFNα-2a in combination of RBV)

Patients positive for HCV RNA at week 4 but negative at week 12 (cEVR) were randomly assigned to receive weekly PEG-IFNα-2a/RBV combination (group C) or biweekly PEG-IFNα-2a/RBV combination (group D) after week 24, to compare the efficacy and safety between the treatment groups and to evaluate the dosage interval of PEG-IFNα-2a.

Cases with LVR: evaluation of clinical significance of addition of fluvastatin (PEG-IFNα-2a/RBV combination therapy versus PEG-IFNα-2a/RBV/FLV combination therapy)

Patients with positive HCV RNA at week 4 and 12 but negative HCV RNA at week 24 (LVR) were randomly assigned to a treatment group of PEG-IFNα-2a/RBV (group E) or PEG-IFNα-2a/RBV/FLV (group F) to compare the efficacy and safety between the treatment groups and to evaluate the significance of adding FLV. The dosage of FLV was set to 20 mg/day.

The primary efficacy endpoint was SVR. We also investigated correlations of IL-28B polymorphism (rs8099917) and Core 70 mutation with the rate of HCV RNA-negative conversion and SVR. The IL-28B polymorphism and Core 70 mutation were measured only in patients who wished to have this done. The genetic testing (IL-28B) was performed only in patients who gave written informed consent after obtaining the approval from the ethical committee. This study was a multicenter trial, and the numbers of patients with available HCV-RNA data were different for the week-4, -12, and -24 responses, because not all of the participating institutions completed all of these time points. Therefore, the numbers of patients with regard to IL28B and Core 70 mutation did not completely match at each time point.

If a decrease in the neutrophil count, platelet count, or Hb level reached a critical level or other adverse events