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APPLICATION NUMBER:

201917Orig1s000

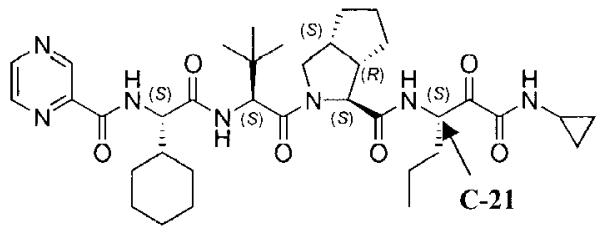
MICROBIOLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW (ADDENDUM)
NDA: 201917 **SN**: 000 **DATE REVIEWED:** 05/20/11
Virology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 201917 **Serial #:** 000
Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Sponsor's Name and Address:
Vertex Pharmaceuticals, Inc.
130 Waverly Street
Cambridge, MA 02139-4242

Initial Submission Dates:
Correspondence Date: 11/19/10
CDER Receipt Date: 11/23/10
Assigned Date: 11/23/10
Review Complete Date: 04/18/11 (Addendum 5/20/11)
PDUFA Date: 5/23/11
Amendments: none
Related/Supporting Documents: IND71832
Product Name(s)
Proprietary: INCIVEK
Non-Proprietary/USAN: telaprevir
Code Name/Number: VX-950
Chemical Name: (1S, 3aR, 6aS)-2-((S)-2- {(S)-2-Cyclohcxyl-2-[(pyrazine-2-carbonyl)amino]-acetylamino}-3,3-dimethyl-butyryl)octahydrocyclopenta[c]pyrrole-1-carboxylic acid ((5)-1-cyclopropylaminooxalylbutyl)amide (IUPAC)
Molecular Weight: 679.85
Molecular Formula: $C_{36}H_{53}N_7O_6$
Structural Formula:



VX-950 (telaprevir, INCIVEK)

Dosage Form(s): 750 mg q8h
Route(s) of Administration: Oral
Indication(s): Treatment of chronic hepatitis C virus infection
Dispensed: Rx
Abbreviations:
CC, cytotoxic concentration; CI, combination index; DAA, direct acting antiviral; DS, delayed start; EC, effective concentration; HCV, hepatitis C virus; EVR, early virologic response; GT, genotype; IC, inhibitory concentration; IFU, infectious units; LOD, limit of detection; LLOQ, lower limit of quantitation; NNPI, non-nucleoside polymerase inhibitor; NS3, nonstructural protein 3; NS4A, nonstructural protein 4A; PEG-IFN-α, pegylated interferon alpha; RBV, PR, pegylated interferon and ribavirin; RBV, ribavirin; RT-PCR, reverse transcriptase-polymerase chain reaction; RVR, rapid virologic response; eRVR, extended rapid virologic response; SEAP, secreted placental alkaline phosphatase; SVR, sustained virologic response; T/PR, telaprevir in combination with pegylated interferon and ribavirin; TVR, telaprevir; WT, wild type;

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ADDENDUM

This review is an addendum to the Clinical Virology review of the Original NDA for telaprevir (INCIVEK). The purpose of this addendum is to document the following information:

- Virology-related post-marketing requirements (PMRs) agreed to by the sponsor.
- Final version of Virology-related sections of the INCIVEK label.
- Results from additional exploratory analyses to assess the clinical relevance of HCV RNA results that are detectable but below the lower limit of assay quantification.
- SVR rates of previous null-responders by Week 4 response in lead arm of Study 216.

VIROLOGY-RELATED PMRs

1. Conduct a study to assess the impact of the following telaprevir treatment emergent amino acid substitutions on phenotypic susceptibility of telaprevir in the HCV replicon system.
    - I132V (genotype 1a and 1b replicon)
    - K244R (genotype 1a and 1b replicon)
    - K360R (genotype 1a and 1b replicon)
    - R155K ± NS4A_A36V (genotype 1a)
    - NS4A_E53K (genotype 1a and 1b replicon)

2. Conduct a study to analyze a representative subset of samples from subjects who experienced virologic failure in the Phase 3 studies, but for whom no clear resistance-associated substitutions in NS3/4A were detected, for the presence of substitutions in NS3/4A protease cleavage sites.

FINAL VERSION OF INCIVEK LABEL (Virology-related sections)
INDICATIONS AND USAGE

1 **INDICATIONS AND USAGE**
1.1 **Chronic Hepatitis C**

INCIVEK™ (telaprevir), in combination with peginterferon alfa and ribavirin, is indicated for the treatment of genotype 1 chronic hepatitis C in adult patients with compensated liver disease, including cirrhosis, who are treatment naïve or who have previously been treated with interferon-based treatment, including prior null responders, partial responders, and relapsers [see Clinical Studies (14.2 and 14.3), including definitions of these terms].

The following points should be considered when initiating treatment with INCIVEK:

- INCIVEK must not be administered as monotherapy and must only be prescribed with both peginterferon alfa and ribavirin [see Warnings and Precautions (5.6)].
- A high proportion of previous null responders (particularly those with cirrhosis) did not achieve a Sustained Virologic Response (SVR) and had telaprevir resistance-associated substitutions emerge on treatment with INCIVEK combination treatment [see Microbiology (12.4) and Clinical Studies (14.3)].

- (b) (4)
- INCIVEK efficacy has not been established for patients who have previously failed therapy with a treatment regimen that includes INCIVEK or other HCV NS3-4A protease inhibitors [see Microbiology (12.4)].

2. Dosage and Administration
2.3 Discontinuation of Dosing
Patients with inadequate viral response are unlikely to achieve SVR, and may develop treatment-emergent resistance substitutions [see *Microbiology (12.4)*]. Discontinuation of therapy is recommended in all patients with (1) HCV-RNA levels of greater than or equal to 1000 IU/mL at Treatment Week 4 or 12; or (2) confirmed detectable HCV-RNA levels at Treatment Week 24 (see Table 2).

5.5 Laboratory Tests
HCV-RNA levels should be monitored at weeks 4 and 12 and as clinically indicated. Use of a sensitive real-time RT-PCR assay for monitoring HCV-RNA levels during treatment is recommended. The assay should have a lower limit of HCV-RNA quantification equal to or less than 25 IU/mL and a limit of HCV-RNA detection of approximately 10-15 IU/mL. For the purpose of assessing response-guided therapy eligibility, an "undetectable" HCV-RNA result is required; a confirmed "detectable but below limit of quantification" HCV-RNA result should not be considered equivalent to an "undetectable" HCV-RNA result.

12.1 Mechanism of Action
Telaprevir is a direct-acting antiviral agent (DAA) against the hepatitis C virus [see Microbiology (12.4)].

12.4 Microbiology
Mechanism of Action
Telaprevir is an inhibitor of the HCV NS3/4A serine protease that is necessary for the proteolytic cleavage of the HCV encoded polyprotein into mature forms of the NS4A, NS4B, NS5A and NS5B proteins and essential for viral replication. In a biochemical assay, telaprevir inhibited the proteolytic activity of the recombinant HCV NS3 protease domain with an IC_{50} value of 10 nM.

Antiviral Activity in Cell Culture
In an HCV genotype 1b replicon assay, the telaprevir EC_{50} value against wild-type HCV was 354 nM in a 2-day cell culture assay, and in a genotype 1a infectious virus assay, the EC_{50} value was 280 nM in a 5-day cell culture assay. In biochemical enzymatic assays, the median IC_{50} values of telaprevir against genotype 2, 3a, and 4a were 16 nM (range 6-32 nM; n=5), 40 nM (range 39-88 nM; n=5), and 130 nM (n=1), respectively, compared to a median IC_{50} values of 20 nM (range 16-23; n=2) for genotype 1a and 20 nM for genotype 1b (range 13-33; n=4). The presence of 40% human serum reduced the anti-HCV activity of telaprevir by approximately 10-fold. Evaluation of telaprevir in combination with interferon alfa or ribavirin showed no evidence of antagonism in reducing HCV RNA levels in HCV replicon cells.

Resistance

In Cell Culture

HCV genotype 1b replicons with reduced susceptibility to telaprevir have been selected in cell culture and characterized for telaprevir genotypic and phenotypic resistance. Additionally, resistance to telaprevir was evaluated in both biochemical and HCV genotype 1b replicon assays using both site-directed mutants and recombinant NS3/4A (b) (4). Variants V36A/M, T54A/S, R155K/T, A156S, R155T+D168N, and V36A+T54A conferred 3- to 25-fold reduced susceptibility to telaprevir; A156V/T variants and the V36M/A+R155K/T and T54S/A+A156S/T double variants conferred >62-fold reduced susceptibility to telaprevir. No amino acid substitutions were observed at the proteolytic cleavage sites.

In Clinical Studies

In a pooled analysis of subjects who did not achieve SVR (on-treatment virologic failure or relapse) from the controlled Phase 3 clinical trials, NS3 amino acid substitutions V36M/A/L, T54A/S, R155K/T, and A156S/T were determined to emerge frequently on INCIVEK treatment (Table 8). Nearly all of these substitutions have been shown to reduce telaprevir anti-HCV activity in cell culture and/or biochemical assays. No clear evidence of treatment-emergent substitutions in the NS3 helicase domain or NS4A coding regions of the HCV genome was observed among INCIVEK-treated subjects who did not achieve SVR.

Telaprevir treatment-emergent resistance substitutions emerged in the majority of isolates from subjects who did not achieve SVR (Table 8): in almost 100% of subjects who failed during 12 weeks of T/PR and in the majority of subjects who failed on PR after Week 12 or who relapsed. HCV genotype 1 subtype-associated patterns of INCIVEK treatment-emergent amino acid substitutions were observed. Subjects with HCV genotype 1a predominately had V36M and R155K substitutions or the combination of these variants, while subjects with HCV genotype 1b predominately had V36A, T54A/S, and A156S/T variants (Table 8). Among subjects treated with telaprevir, on-treatment virologic failure was more frequent in subjects with genotype 1a than with genotype 1b and more frequent in prior null responders [see Clinical Studies (14)].

Table 8. Treatment Emergent Substitutions in Pooled Phase 3 Studies: Subjects Who Did Not Achieve SVR24 in INCIVEK Combination Treatment arms

Emerging Substitutions[1] in NS3	Percent of No SVR Subjects (n) N=525	Percent Subtype 1a No SVR Subjects (n) N=356	Percent Subtype 1b No SVR Subjects (n) N=169
Any substitution at V36, T54, R155, V156 or D168	62% (323)	69% (247)	45% (76)
R155K or T	38% (201)	56% (200)	0.6% (1)
V36M	33% (178)	49% (173)	3% (5)
V36M + R155K[2]	27% (142)	40% (142)	0% (0)
T54A or S	13% (68)	9% (31)	22% (37)
V36A or L	12% (65)	10% (37)	17% (28)
A156S or T	9% (48)	8% (28)	12% (20)
V36G/I, I132V, A156V/F/N, R155M/G or D168N	Less than 2%	Less than 2%	Less than 2%

[1]Alone or in combination with other substitutions (includes mixtures)
[2]Subjects with this combination are also encompassed in two V36M and R155K rows above.

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Persistence of Resistance-Associated Substitutions

Persistance of telaprevir-resistant [redacted] (b) (4) has been observed following treatment failure. Of a combined 255 treatment-naïve and previously treated subjects from Studies 108, 111, and C216 in whom telaprevir resistant variants had emerged during treatment, 103 (40%) had detectable resistant variants by population nucleotide sequencing (present at >25% of the viral population) at the end of study (follow-up range 2-70 weeks median 45 weeks) and results for loss of variants were similar across the three studies. In the combined studies, 46% of the telaprevir resistant substitutions in genotype 1a and 16% of the substitutions in genotype 1b were still detected by the end of study: 29% of V36, 16% of T54, 38% of R155, 14% of A156, and 44% of V36M+NS3-R155K variants were detected at the end of study.

In a 3-year follow-up study of 56 treatment-naïve and prior treatment-failure subjects who did not achieve SVR with a telaprevir regimen in a Phase 2 study and had telaprevir-resistant variants after treatment failure, variants were detected by population sequencing in 11% (6/56) of subjects (median follow up of 25 months). Telaprevir-resistant variants V36L/M, T54S, and R155K were detectable (present at greater than 25% of the viral population) in some subjects at 24 months. [redacted] (b) (4)

The lack of detection of a substitution based on a population-based assay does not necessarily indicate the substitution has declined to the pre-treatment level. The long-term clinical impact of the emergence or persistence of detectable INCIVEK resistance-associated substitutions is unknown. No data are available regarding INCIVEK efficacy among subjects who were previously exposed to INCIVEK, or who previously failed treatment with an INCIVEK-containing regimen.

Effect of Baseline HCV Substitutions/Polymorphisms on Treatment Response

A pooled analysis was conducted to explore the association between the detection (population-based assay) of baseline NS3/4A amino acid substitutions/polymorphisms and treatment outcome in [redacted] (b) (4) 108, 111, and C216. Baseline polymorphisms at NS3 position Q80 (Q80K, Q80L, Q80R), which are frequently observed in HCV genotype 1a-infected [redacted] (b) (4) and have been reported to reduce the activity of some HCV NS3/4A protease inhibitors [redacted] (b) (4), were not associated with reduced INCIVEK efficacy.

Telaprevir-associated resistance substitutions (substitutions at positions V36, T54, R155, or D168) were present at baseline in 5% (117/2217) of the available subject samples in the combined clinical trials. Given the small number of subjects with baseline telaprevir resistance substitutions, conclusions about their effect on response outcomes when these substitutions are present at baseline cannot be determined.

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Cross-resistance

Treatment-emergent NS3 amino acid substitutions detected in INCIVEK-treated subjects who did not achieve SVR in the clinical trials (substitutions at positions V36, T54, R155, A156 or D168) have been demonstrated to reduce the anti-HCV activity of boceprevir and other HCV NS3/4A protease inhibitors (b) (4). The impact of prior INCIVEK exposure or treatment failure on the efficacy of boceprevir or other HCV NS3/4A protease inhibitors has not been studied. INCIVEK efficacy has not been established for patients with a history of exposure to NS3/4A protease inhibitors.

Cross-resistance is not expected between INCIVEK and interferons, or INCIVEK and ribavirin. HCV replicons expressing telaprevir-associated resistance substitutions remained fully sensitive to interferon-alfa and ribavirin, as well as other direct-acting antivirals with different mechanisms of action, such as NS5B polymerase inhibitors.

(b) (4)

ADDITIONAL EXPLORATORY ANALYSES OF HCV RNA LOD/BLOQ

Response guided therapy in the Phase 3 clinical trials of telaprevir and boceprevir was based on different definitions of rapid virologic response and limits of detection (10 IU/mL for telaprevir and 9.3 IU/mL for boceprevir). The Roche COBAS TaqMan assay, which was used in clinical trials for both telaprevir and boceprevir, has a BLOQ of 25 IU/mL. It is not known yet whether decisions made on Roche COBAS TaqMan assay measurements "below the level of quantification (<25 IU/mL) but detectable" and "undetectable" measurements will have different clinical outcomes such as SVR rates. (Reference: Jean-Michel Pawlotsky, Gastroenterology 2011; 140:746-760.)

We performed exploratory analyses on SVR outcomes in Studies 216 (treatment-experienced) and Study 108 (treatment-naïve) based on BLOQ and LOD at different timepoints on treatment in the clinical trials to determine if the different thresholds of the assay have an affect on clinical outcomes (See Figures and Tables below).

In Study 216, (b) (4) was used as the vendor for HCV viral load. The data show clear differences in the SVR rates using LOD vs. BLOQ measurements on treatment (See Figures and Tables below). This observation was also seen with the boceprevir results in Study P05216 (See Pat Harrington's NDA-202258 review and addendum review). The results from boceprevir and telaprevir clinical trials together support that making decisions based on detectable/BLOQ and LOD measurements is clinically relevant.

In Study 108, (b) (4) was used as the vendor for HCV viral load. Results from Study 108 supported the conclusions made in Studies P05216 and 216, although the correlation in Study 108 was weaker. Further analyses shown below revealed a high false positive rate in this study probably as a result of the different vendor.

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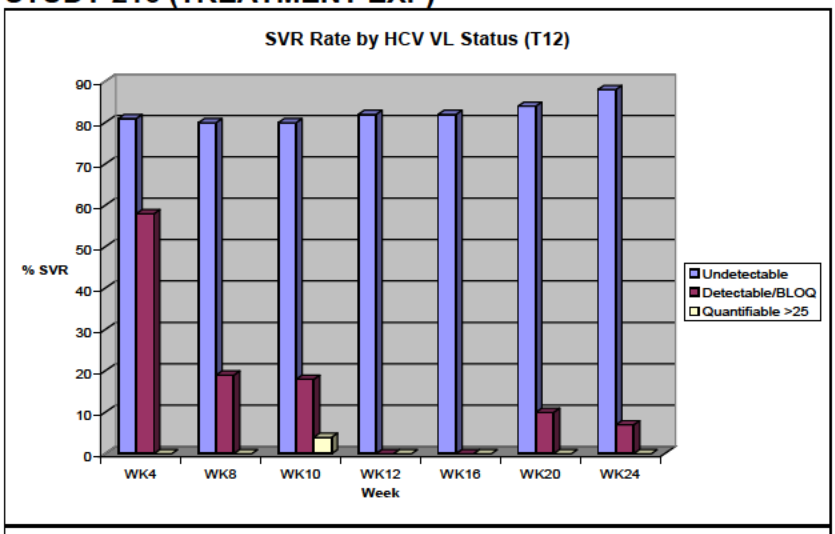
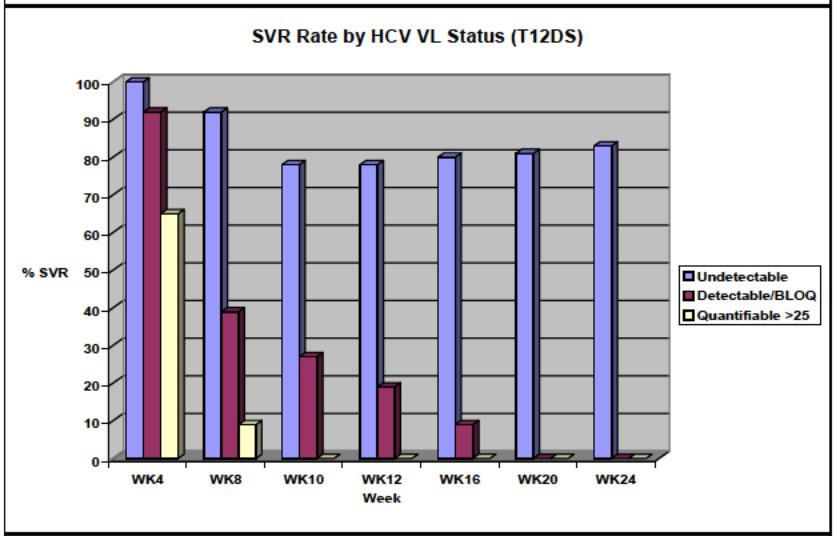
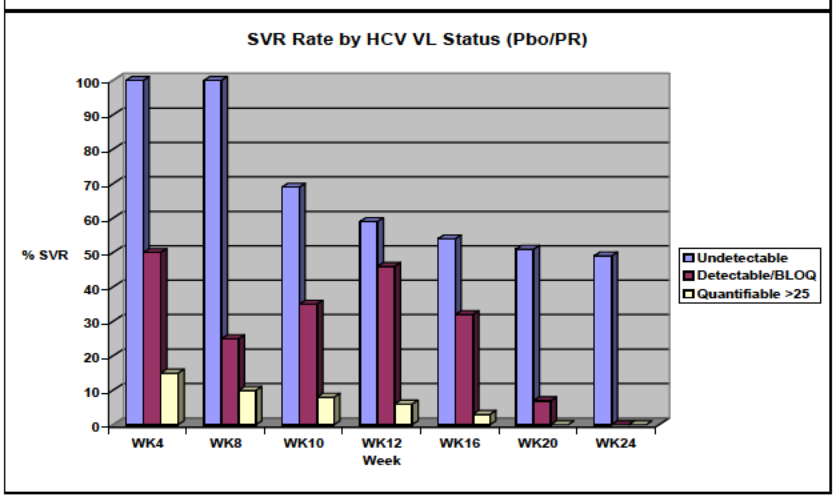
STUDY 216 (TREATMENT EXP)







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STUDY 216 (n=652)

	HCV VL Status On-Treatment (All Arms)		
Timepoint	Undetectable	Detectable/BLOQ	Quantifiable (≥25)
WK 4	154/599 (26%)	85/599 (14%)	360/599 (60%)
WK 8	397/622 (64%)	74/622 (12%)	151/622 (24%)
WK 10	431/616 (70%)	50/616 (8%)	135/616 (22%)
WK 12	437/609 (72%)	40/609 (7%)	132/609 (22%)
WK 16	444/552 (80%)	37/552 (7%)	71/552 (13%)
WK 20	447/532 (84%)	33/532 (6%)	52/532 (10%)
WK 24	439/519 (85%)	38/519 (7%)	42/519 (8%)

	SVR Status On-Treatment (T12)		
Timepoint	Undetectable	Detectable/BLOQ	Quantifiable (≥25)
WK 4	81% (119/147)	58% (41/71)	0% (0/25)
WK 8	80% (163/204)	19% (3/16)	0% (0/26)
WK 10	80% (163/204)	18% (2/11)	4% (1/24)
WK 12	82% (166/202)	0% (0/11)	0% (0/24)
WK 16	82% (167/204)	0% (0/7)	0% (0/11)
WK 20	84% (166/197)	10% (1/10)	0% (0/10)
WK 24	88% (165/188)	7% (1/15)	0% (0/7)

	SVR Status On-Treatment (T12DS)		
Timepoint	Undetectable	Detectable/BLOQ	Quantifiable (≥25)
WK 4	4/4	92% (11/12)	65% (144/222)
WK 8	82% (152/185)	39% (18/46)	9% (2/23)
WK 10	78% (167/214)	27% (6/22)	0% (0/19)
WK 12	78% (170/218)	19% (3/16)	0% (0/18)
WK 16	80% (169/212)	9% (1/11)	0% (0/20)
WK 20	81% (170/209)	0% (0/8)	0% (0/13)
WK 24	83% (170/206)	0% (0/6)	0% (0/14)

	SVR Status On-Treatment (Pbo)		
Timepoint	Undetectable	Detectable/BLOQ	Quantifiable (≥25)
WK 4	3/3	1/2	15% (17/113)
WK 8	8/8	25% (3/12)	10% (10/102)
WK 10	69% (9/13)	35% (6/17)	8% (7/92)
WK 12	59% (10/17)	46% (6/13)	6% (5/90)
WK 16	54% (15/28)	32% (6/19)	3% (1/40)
WK 20	51% (21/41)	7% (1/15)	0% (0/29)
WK 24	49% (22/45)	0% (0/17)	0% (0/21)

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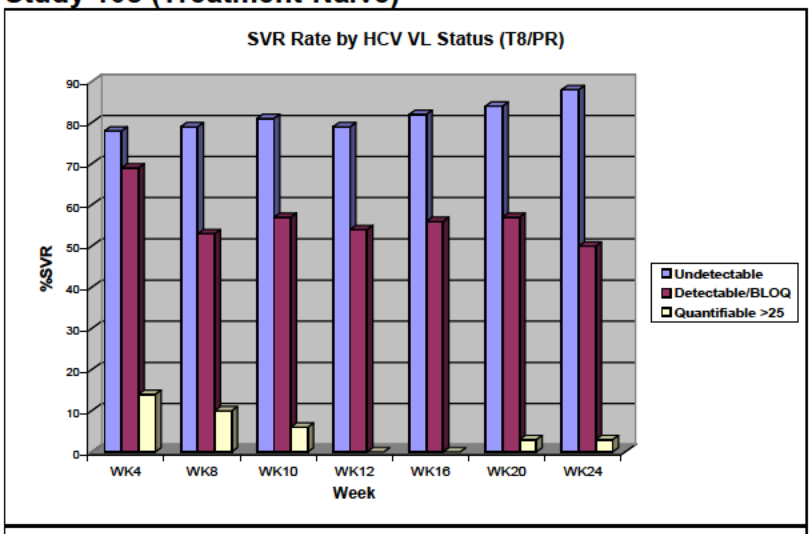
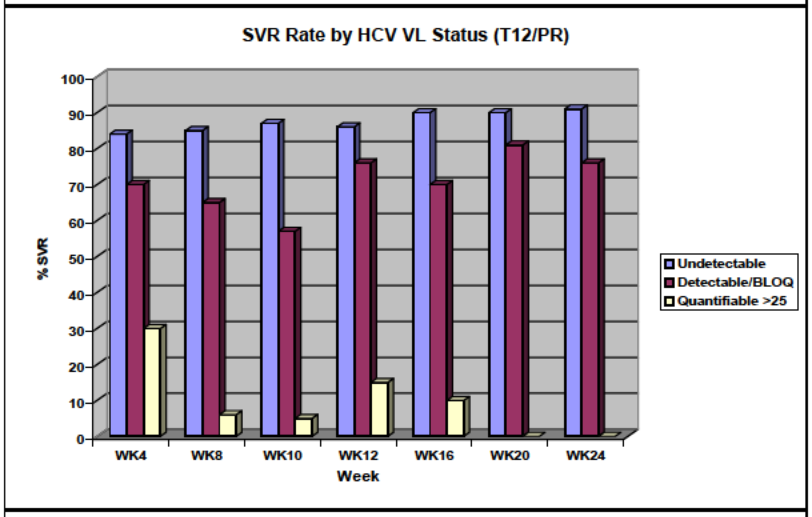
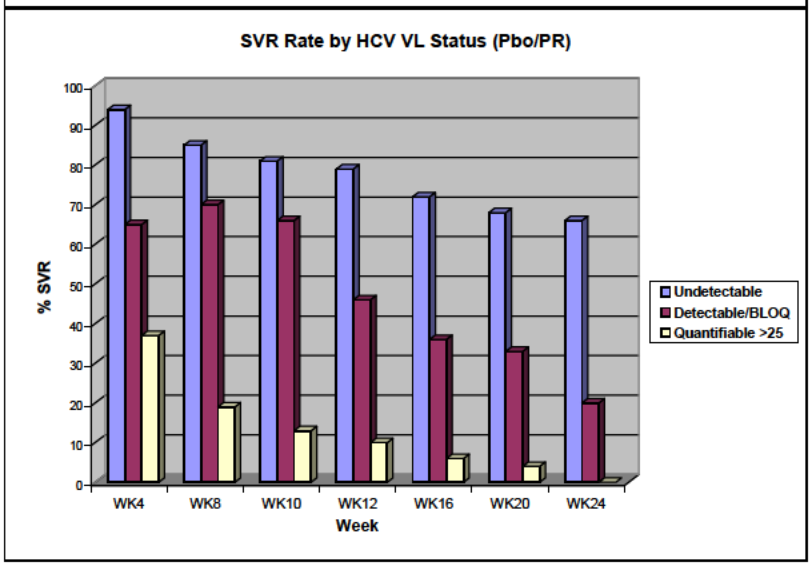
Study 108 (Treatment-Naïve)







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STUDY 108

	HCV VL Status On-Treatment (All Arms)		
Timepoint	Undetectable	Detectable/BLOQ	Quantifiable (≥25)
WK 4	49% (503/1025)	19% (193/1025)	32% (329/1025)
WK 8	64% (645/1002)	13% (126/1002)	23% (231/1002)
WK 10	67% (670/995)	12% (123/995)	20% (202/995)
WK 12	68% (688/1010)	14% (139/1010)	18% (183/1010)
WK 16	79% (771/970)	13% (128/970)	14% (131/970)
WK 20	75% (736/980)	11% (104/980)	14% (140/980)
WK 24	75% (731/973)	11% (111/973)	13% (131/973)

	SVR Status On-Treatment (Pbo)		
Timepoint	Undetectable	Detectable/BLOQ	Quantifiable (≥25)
WK 4	94% (29/31)	65% (17/26)	36% (104/285)
WK 8	85% (78/92)	69% (37/54)	19% (36/194)
WK 10	80% (90/112)	66% (40/61)	13% (22/166)
WK 12	79% (111/141)	45% (30/66)	10% (13/135)
WK 16	72% (129/180)	35% (20/57)	6% (5/85)
WK 20	67% (134/199)	30% (16/53)	4% (3/84)
WK 24	66% (146/222)	18% (7/40)	0% (0/72)

	SVR Status On-Treatment (T12)		
Timepoint	Undetectable	Detectable/BLOQ	Quantifiable (≥25)
WK 4	84% (201/238)	70% (56/80)	32% (7/22)
WK 8	84% (233/276)	67% (24/36)	6% (1/16)
WK 10	87% (246/284)	59% (16/27)	6% (1/18)
WK 12	86% (236/276)	76% (25/33)	17% (4/24)
WK 16	90% (234/260)	70% (26/37)	11% (3/27)
WK 20	90% (243/271)	79% (19/24)	0% (0/27)
WK 24	91% (229/251)	74% (31/42)	0% (0/26)

	SVR Status On-Treatment (T8)		
Timepoint	Undetectable	Detectable/BLOQ	Quantifiable (≥25)
WK 4	77% (181/234)	68% (59/87)	14% (3/22)
WK 8	79% (218/277)	53% (19/36)	10% (2/21)
WK 10	80% (220/274)	54% (19/35)	6% (1/18)
WK 12	82% (222/271)	53% (21/40)	0% (0/24)
WK 16	82% (222/271)	56% (19/34)	0% (0/19)
WK 20	84% (223/266)	56% (15/27)	3% (1/29)
WK 24	88% (226/258)	48% (14/29)	3% (1/33)

How often are measurements <25 IU/mL sample detectable vs. undetectable?

7-19% of all measurements on-treatment in Studies 108 and 216 are detectable but <25 IU/mL, so these measurements are not infrequent and thus may have consequences on on-treatment decision points (See Table below).

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SUMMARY

- In Study 216 (which used (b) (4)), the trend of higher SVR rates for "Undetectable" HCV viral load status compared to "Detectable/BLOQ" and the lowest SVR rates for Quantifiable >25 viral load was similar to the data from boceprevir P05216 trial.
- This same trend of higher SVR rates for "undetectable" measurements vs. "detectable/BLOQ" was first determined in the boceprevir trial P05216 (Pat Harrington).
- Even though SVR rates were higher for the "Detectable/BLOQ" group in Study 108 (likely because of the higher false positive HCV RNA detection rate [see below]), there was still a trend of a reduced SVR rate for subjects with "Detectable/BLOQ" HCV RNA results versus those with "Undetectable" HCV RNA results at the same on-treatment timepoint.
- "Detectable/BLOQ" and "Undetectable" during treatment are different HCV RNA results. During treatment, "Undetectable" measurements are indicative of having a better SVR rate compared to "Detectable/BLOQ" measurements.
- "Detectable/BLOQ" should not substitute for "Undetectable" in response-guided therapy decisions

FALSE-POSITIVE DETECTION RATE DIFFERENT IN TELAPREVIR PHASE 3 TRIALS

(Assay false-positive rate according to COBAS TaqMan label: 1.3%)

Telaprevir Tx-naïve trial (Study 108-<u>all arms</u>):
For samples in dataset

- 11930 follow-up samples from 707 subjects who achieved SVR (based on <25 IU/mL cutoff)
- 783 samples (7%) from 206 subjects with detectable HCV RNA
- Estimates a 7% false-positive detection rate
- VL assessments conducted by (b) (4)

For only "Actual SVR24 analysis"
N=4632 Follow up and Post SVR follow-up samples
276 (6%) samples from 205 subjects with detectable HCV RNA

N=2456 Follow-up samples from 730 subjects
221 (9%) samples from 172 subjects with detectable HCV RNA

Telaprevir Tx-exp trial (Study 216-<u>all arms</u>):

- 1957 follow-up samples from 362 subjects who achieved SVR (based on <25 IU/mL cutoff)
- 5 samples (0.3%) with detectable HCV RNA
- Estimates a 0.3% false-positive detection rate
- VL assessments conducted by (b) (4)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW (ADDENDUM)
NDA: 201917 **SN**: 000 **DATE REVIEWED:** 05/20/11
Virology Reviewer: Lisa K. Naeger, Ph.D.

SUMMARY POINTS

- In the telaprevir Study 216 (which used [redacted] (b) (4) for HCV viral load testing) there was a 0.3% rate of detectable HCV RNA during follow-up for subjects who apparently achieved SVR based on a <25 IU/mL cutoff.
- In Study 108, [redacted] (b) (4) was used to conduct HCV viral load testing. In this trial, there was a 6-9% rate (~20-fold higher than in 216) of detectable HCV RNA during follow-up for subjects who apparently achieved SVR based on a <25 IU/mL cutoff.
- As shown in my review, the majority (60%) of HCV RNA samples that were <25 IU/mL detected when analyzed at [redacted] (b) (4) were not detected when reanalyzed at [redacted] (b) (4), indicating that most fluctuations in Study 108 are due to higher assay variability at [redacted] (b) (4).

Additional information from Dr. Pat Harrington's analysis of the boceprevir NDA (please see Dr. Pat Harrington's review and addendum of these data for more details)

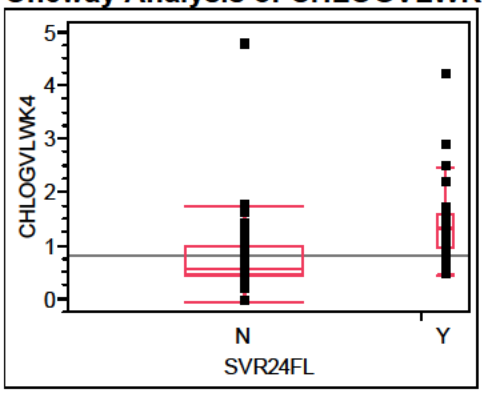
- "The 0.5% rate of detectable HCV RNA during follow-up for Study P05216 subjects who apparently achieved SVR may represent the overall false-positive rate of detectable HCV RNA during the conduct of the trial (assays conducted by [redacted] (b) (4)).
- The reported false-positive rate of the Roche COBAS TaqMan assay in the assay label is 1.3%.

SVR RATES BY LEAD-IN WEEK 4 RESPONSE IN PREVIOUS NULL RESPONDERS

Data from the lead-in phase in the T12DS/PR arm of Study 216 were examined to determine if viral load decline at Week 4 on PR treatment alone in previous null-responders is predictive of likelihood to achieve an SVR on triple therapy following the lead-in.

N= 75 prior null responders in T12DS/PR Arm of Study 216
Overall SVR rate of previous null-responders = 25/75 (33%)

SVR Rate by Change in VL from BL at Week 4
Oneway Analysis of CHLOGVLWK4 By SVR24FL



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Quantiles

Level	Minimum	10%	25%	Median	75%	90%	Maximum
N	-0.06244	0.316476	0.44147	0.55042	0.975026	1.207754	4.746412
Y	0.442385	0.602806	0.940747	1.319585	1.586504	2.583584	4.194052

SVR rate by Week 4 Response
≤1 log = 6/41 (15%)

>1 log = 15/28 (53%)

6 subjects had no Week 4 VL (4 of which had SVR)

USUBJID	VLBL	LOGVLBL	HCVVLW2	SVR24
VX-950-C216-0046	8520000	6.93043959	4000000	Y
VX-950-C216-0057	8620000	6.93550727	5120000	Y
VX-950-C216-0097	9010000	6.95472479	1710000	Y
VX-950-C216-0123	19000000	7.2787536	4900000	Y
VX-950-C216-0334	3745000	6.57345182	2750000	N
VX-950-C216-0347	2440000	6.38738983	.	N

Summary
Previous Null-Responders who had a >1 log decline from baseline at Week 4 during the lead-in phase had higher SVR rates than those than had ≤1 log decline at Week 4.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LISA K NAEGER
05/20/2011

JULIAN J O'REAR
05/22/2011

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 201917 **SN**: 000 **DATE REVIEWED:** 4/15/11
Virology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 201917 **Serial #:** 000
Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Sponsor's Name and Address:
Vertex Pharmaceuticals, Inc.
130 Waverly Street
Cambridge, MA 02139-4242

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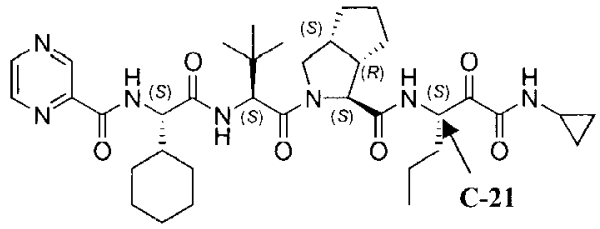
Related/Supporting Documents: IND71832
Product Name(s)
Proprietary: not finalized as of 4/21/11
Non-Proprietary/USAN: telaprevir
Code Name/Number: VX-950
Chemical Name: (1S, 3aR, 6aS)-2-((S)-2- {(S)-2-Cyclohcxyl-2-[(pyrazine-2-carbonyl)amino]-acetylamino}-3,3-dimethyl-butyryl)octahydrocyclopenta[c]pyrrole-1-carboxylic acid ((5)-1-cyclopropylaminooxalylbutyl)amide (IUPAC)
Molecular Weight: 679.85
Molecular Formula: $C_{36}H_{53}N_7O_6$
Structural Formula:



VX-950

Dosage Form(s): 750 mg q8h
Route(s) of Administration: Oral
Indication(s): Treatment of chronic hepatitis C virus infection
Dispensed: Rx
Abbreviations:
CC, cytotoxic concentration; CI, combination index; DAA, direct acting antiviral; DS, delayed start; EC, effective concentration; HCV, hepatitis C virus; EVR, early virologic response; GT, genotype; IC, inhibitory concentration; IFU, infectious units; LOD, limit of detection; LLOQ, lower limit of quantitation; NNPI, non-nucleoside polymerase inhibitor; NS3, nonstructural protein 3; NS4A, nonstructural protein 4A; PEG-IFN-α, pegylated interferon alpha; RBV, PR, pegylated interferon and ribavirin; RBV, ribavirin; RT-PCR, reverse transcriptase-polymerase chain reaction; RVR, rapid virologic response; eRVR, extended rapid virologic response; SEAP, secreted placental alkaline phosphatase; SVR, sustained virologic response; T/PR, telaprevir in combination with pegylated interferon and ribavirin; TVR, telaprevir; WT, wild type;

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EXECUTIVE SUMMARY

Telaprevir is an inhibitor of the HCV NS3/4A protease necessary for proteolytic cleavage of the viral nonstructural polyprotein developed for the treatment of chronic hepatitis C virus infection. The EC_{50} value of telaprevir against wild-type HCV in a 2-day HCV subtype 1b replicon assay was 354 nM. In a subtype 1a infectious virus assay, the EC_{50} value was 280 nM in a 5-day cell culture assay. Telaprevir also demonstrated activity against genotypes 2, 3a, and 4a in biochemical enzymatic assays. The presence of 40% human serum reduced the anti-HCV activity of telaprevir by approximately 10-fold. The average CC_{50} value was 83 μM, resulting in a selective index of 230. Evaluation of telaprevir in combination with interferon alfa or ribavirin showed no evidence of antagonism in reducing HCV RNA levels in HCV replicon cells.

Clinical Virology of Phase 3 Studies 108, 111 and 216
For the clinical virology analysis, the applicant submitted extensive genotypic data of the entire NS3-4A coding region and response outcome data from 2,260 baseline subject isolates and comprehensive post-baseline and follow-up samples from 628 subjects who did not achieve SVR in the Phase 3 Studies: treatment-naïve Studies 108 and 111 and treatment-experienced Study 216.

Telaprevir Treatment-Emergent Substitutions
In a pooled analysis of subjects who did not achieve SVR from the Phase 3 studies, NS3 amino acid substitutions V36M, A or L, T54A or S, R155K or T, A156S, T or V and D168N were determined to emerge frequently on telaprevir treatment. Variants at position D168, known to confer decreased susceptibility to the macrocyclic NS3/4A protease inhibitors, had not previously been reported to be associated with telaprevir resistance. In replicon-based and enzymatic phenotypic assays using site-directed mutant NS3, the V36M/A, T54A/S, R155K/T, A156S and R155T+D168N amino acid substitutions have been shown to confer 4- to 25-fold reduced susceptiblity to telaprevir and substitutions V36M+R155K, A156T, or A156V have been shown to confer >62-fold reduced susceptiblity to telaprevir. Telaprevir susceptibility changes for post-baseline Phase 2 clinical recombinant isolates containing telaprevir resistant NS3 substitutions correlated with the degree of susceptibility changes observed with the site-directed mutants.

Telaprevir-associated resistance substitutions (substitutions at positions V36, T54, R155, A156 or D168) were present at baseline in 5% (117/2217) of the available subject samples in the combined Phase 3 Studies. Given the small number of subjects whose HCV had telaprevir resistance substitutions at baseline, it is difficult to make conclusions on response outcomes when these substitutions are present at baseline.

STUDY 108: Treatment-Naïve T8/PR vs. T12/PR
In subjects who did not achieve SVR24, the most frequently observed outcome was discontinuation due to virologic stopping rules. The percentage of subjects who did not achieve SVR was higher in the T8 arm than the T12 arm. The proportion of subjects who failed on 12 weeks of T/PR as well as the proportion of subjects who relapsed were

similar between the two arms; however, the proportion of subjects who had virologic failure after Week 12 on PR treatment was higher in the T8 arm.

Overall, the proportion of telaprevir resistance substitutions that emerged on treatment was comparable between the T8/PR and T12/PR arms with more substitutions emerging in subtype 1a than 1b treatment failures. Almost all of the treatment failures who failed on T/PR ≤Week 12 had treatment-emergent substitutions in their HCV and 60% of isolates from subjects who failed after Week 12 on PR or who relapsed had treatment-emergent substitutions. The substitutions V36M and R155K and combination of both emerged most frequently in subtype 1a failures and V36A, T54A or S and A156T emerged most frequently in subtype 1b failures.

STUDY 111: Treatment-Naïve T12/PR24 eRVR+ vs. T12/PR48 eRVR+ vs. T12/PR48 eRVR-
In Study 111, a high percentage of telaprevir treatment failure isolates had treatment-emergent substitutions. Of the treatment failures who failed after Week 12 on PR or relapsed on T12-containing regimens, 90% (46/51) had virus with treatment-emergent substitutions. As in Study 108, V36M and R155K and the combination of both emerged most frequently in subtype 1a failure isolates. In the few subtype 1b failures, T54A was the only substitution that emerged in the T12-containing arms.

STUDY 216: Treatment-Experienced T12/PR48 vs. T12(DS)/PR48
Overall, the number of treatment failures who did not achieve SVR was similar in the T12/PR48 (36%) and lead-in arm T12(DS)/PR48 (34%). Overall, 70% of No SVR failures had treatment-emergent substitutions when they experienced failure on treatment or relapsed. The proportion of treatment-emergent substitutions was also similar between the two arms. Over half the treatment failure subjects in Study 216 were prior null responders. Consistent with these data, the prior null responders also had the most treatment-emergent substitutions in their failure isolates. The V36M and R155K substitutions and the combination of both emerged most frequently in subtype 1a treatment failures. The V36A, T54S or A and A156T, S or V emerged most frequently in subtype 1b failures.

Persistence of Telaprevir Resistant Variants/Follow-up Analysis
In Study 112, changes in telaprevir resistance-associated HCV variants over time were evaluated in subjects who did not achieve an SVR 24. Study 112 was a 3-year, virology follow-up study in subjects previously treated with telaprevir from Phase 2 Studies 104, 104EU, 106, and 107. Follow-up periods in Study 112 ranged from 5 - 40 months with a median of 25 months. A total of 56 subjects were used for the analysis of persistance of resistant variants V36A/M/L, T54S/A, R155T/K/I, A156S/T in the absence of telaprevir selection. V36M, T54A and S, R155K, and A156S or T or N were detectable by population nucleotide sequencing (present at >25% of the viral population) at 6, 18, and 24 months. By 36 months, V36M, T54S or A, and A156S/T/N variants had fallen below the level of detection in all subjects. Three percent of the subject isolates that had the R155K variant still had detectable R155K variants by population sequencing at 36 months. The lack of detection of a substitution based on a population-based assay does not necessarily indicate that viral populations carrying that substitution have declined to a background level that may have existed prior to treatment.

In addition, the viral populations of subjects failing a telaprevir-containing regimen in Studies 108, 111, and 216 were assessed at multiple time points after treatment-failure by population nucleotide sequencing to determine if the telaprevir resistant variants initially present at the post-nadir visit were detectable in the viral population by the end of study (EOS) visit. Of the combined subjects from Phase 3 studies with a total of 443 resistant variants, 176 (40%) had detectable resistant variants by population sequencing by EOS (follow-up range 5-71 weeks median 45 weeks) and results for loss of variants were similar across the three studies. In the combined studies, 50% of these substitutions in subtype 1a and 20% of the substitutions in subtype 1b were still detected by the EOS.

Summary of Telaprevir Treatment-Emergent Substitutions

- The majority of isolates from subjects who did not achieve SVR had telaprevir resistance-associated treatment-emergent substitutions.
- More treatment failures were subtype 1a than subtype 1b.
- Most prior null-responders did not achieve SVR on telaprevir and of these, 80% had treatment-emergent telaprevir substitutions.
- There are divergent resistance pathways for subtype 1a and 1b
  - The most frequent emergent substitutions in subtype 1a failures were V36M and R155K and the combination of both of these.
  - The most frequent emergent substitutions in subtype 1b failures were T54A or S, V36A, and A156T, S or V.
- Variants expressing telaprevir resistance-associated substitutions can persist at >25% of the virus population out to at least 3 years after the end of treatment

1. RECOMMENDATIONS

1.1. Recommendation and Conclusion on Approvability

This supplemental NDA for telaprevir is approvable with respect to virology for the treatment of chronic Hepatitis C virus (HCV) infection.

- Indicated for use in combination with peginterferon alfa and ribavirin, for the treatment of genotype 1 chronic hepatitis C in adult patients with compensated liver disease, including cirrhosis, who are treatment naïve or who have been previously treated, including prior null responders, partial responders, and relapsers.
  - Consideration should be taken when treating previous Null responders with T/PR: A high proportion of previous null responders did not achieve SVR and had telaprevir resistance-associated substitutions emerge on treatment with a T/PR regimen (See Microbiology 12.4 and Clinical Studies 14.1)
  - The long term clinical impact of the emergence and persistence of dectable telaprevir resistance-associated substitutions is unknown (See Microbiology 12.4).

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable.

1. Conduct a study to assess the impact of the following telaprevir treatment emergent amino acid substitutions on phenotypic susceptibility of telaprevir in the HCV replicon system.

   - I132V (genotype 1a and 1b replicon)
   - K244R (genotype 1a and 1b replicon)
   - K360R (genotype 1a and 1b replicon)
   - R155K ± NS4A_A36V (genotype 1a)
   - NS4A_E53K (genotype 1a and 1b replicon)

2. Conduct a study using the HCV replicon system to assess phenotypic susceptibility of baseline and treatment-failure isolates from a subset of telaprevir-treated subjects in Phase 3 studies who did not achieve SVR with representative genotypic resistance patterns. Isolates from some telaprevir-treated subjects without known telaprevir substitutions and baseline samples from subjects who achieved SVR should also be included in these assessments for comparison.

3. Conduct a study to analyze a representative subset of samples from subjects who experienced virologic failure in the Phase 3 studies, but for whom no clear resistance-associated substitutions in NS3/4A were detected, for the presence of substitutions in NS3/4A protease cleavage sites.

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Non-Clinical Virology

Telaprevir is a peptidomimetic ketoamide inhibitor of the HCV NS3•4A serine protease that is necessary for the proteolytic cleavage of the HCV encoded polyprotein into mature forms of the NS4A, NS4B, NS5A and NS5B proteins and essential for viral replication. It was designed from the [redacted](b) (4). In a standard peptide cleavage assay for HCV NS3, telaprevir inhibited the proteolytic activity of the recombinant HCV NS3 protease domain with an apparent K_i value of 44 nM and IC_{50} value of 10 nM. The steady state K_i in a continuous assay was 7 nM. Biochemical data indicate that telaprevir has slow-binding inhibition and forms a tightly bound enzyme/inhibitor complex [redacted](b) (4) and $t_{1/2}$ life of 58 minutes. Telaprevir is selective for the HCV NS3 protease and did not interfere with other serine proteases (kallikrein, thrombin, plasmin, and Factor Xa) at physiologically relevant concentrations.

Two assay systems, primary human hepatocytes infections with patient-derived infectious virus and the HCV replicon assay in Huh7 cells, were used to evaluate anti-HCV activities of telaprevir. In an HCV subtype 1b replicon assay, the telaprevir EC_{50} value against wild-type HCV was 354 nM in a 2-day cell culture assay, and in a subtype 1a infectious virus assay, the EC_{50} value was 280 nM in a 5-day cell culture assay. In

biochemical enzymatic assays, the median IC_{50} values of telaprevir against genotype 2, 3a, and 4a were 16 nM (range 6-32 nM; n=5), 40 nM (range 39-88 nM; n=5), and 130 nM (n=1), respectively, compared to a median IC_{50} value of 20 nM (range 16-23; n=2) for genotype 1a and 20 nM for genotype 1b (range 13-33; n=4). The presence of 40% human serum reduced the anti-HCV activity of telaprevir by approximately 10-fold. At 100% human serum, the extrapolated EC_{50} value of telaprevir would be 8 μM. The average CC_{50} value of 83 μM, resulting in a selective index of 230. The CC_{50} value of telaprevir in PBMC was >30 μM.

Evaluation of telaprevir in combination with interferon alfa or ribavirin showed no evidence of antagonism in reducing HCV RNA levels in HCV replicon cells.

In serially passaged selection experiments in the presence of increasing telaprevir concentrations in the replicon system, substitutions at A156 in the protease domain were observed at days 21 and 56. In replicon cells, which had been cultured in the presence of 28 μM telaprevir for 63 days, 79% (60/76) of clones had an A156S substitution. No substitution was found at any of the four proteolytic sites in the HCV nonstructural protein region that are cleaved by the NS3•4A serine protease.

The EC_{50} value of telaprevir against the A156S and A156T or V replicon cells was 10-fold and >75-fold higher than that against the wild-type replicon cells, respectively. There was no decrease in telaprevir susceptibility against the D168V or D168A mutant replicons compared with the wild-type replicon cells, but D168N was not tested.

2.2 Clinical Virology

STUDY 108: Treatment-Naïve T8/PR vs. T12/PR
In subjects who did not achieve SVR24, the most frequently observed outcome was discontinuation due to virologic stopping rules. The percentage of subjects who did not achieve SVR was higher in the T8 arm than the T12 arm. The proportion of subjects who failed during 12 weeks of T/PR and the proportion of subjects who relapsed were similar between the two arms; however, the proportion of subjects who had virologic failure after Week 12 on PR treatment was higher in the T8 arm.

Overall, the proportion of telaprevir resistance substitutions that emerged on treatment was comparable between the T8/PR and T12/PR arms with more substitutions emerging in subtype 1a than 1b treatment failures. Almost all of the treatment failures who failed on T/PR ≤Week 12 had treatment-emergent substitutions in their virus and 60% of isolates from subjects who failed after Week 12 on PR or who relapsed had treatment-emergent substitutions. The substitutions V36M and R155K and combination of both emerged most frequently in subtype 1a failure isolates and V36A, T54A or S and A156T emerged most frequently in subtype 1b failure isolates.

STUDY 111: Treatment-Naïve T12/PR24 eRVR+ vs. T12/PR48 eRVR+ vs. T12/PR48 eRVR-
In Study 111, a high percentage of telaprevir treatment failure isolates had treatment-emergent substitutions. Of the treatment failures who failed after Week 12 on PR or relapsed on T12-containing regimens, 90% (46/51) had treatment-emergent

substitutions in their HCV. As in Study 108, V36M and R155K and the combination of both substitutions emerged most frequently in 50-60% of Subtype 1a failures. In the subtype 1b failures, T54A emerged most frequently.

STUDY 216: Treatment-Experienced T12/PR48 vs. T12(DS)/PR48
Overall, the number of treatment failures who did not achieve SVR was similar in the T12/PR48 (36%) and lead-in arm T12(DS)/PR48 (34%). Overall, 70% of No SVR failure isolates had treatment-emergent substitutions when they experienced failure on treatment or relapsed. The proportion of treatment-emergent substitutions was also similar between the two arms. Over half the treatment failure subjects in Study 216 were prior null responders. Consistent with these data, isolates from the prior null responders also had the most treatment-emergent substitutions. The V36M and R155K substitutions and the combination of both emerged most frequently in subtype 1a treatment failures. The V36A, T54S/A and A156T/S/V substitutions emerged most frequently in subtype 1b failures.

Telaprevir Treatment-Emergent Substitutions
In a pooled analysis of subjects who did not achieve SVR from the Phase 3 studies, NS3 amino acid substitutions V36M, A or L, T54A or S, R155K or T, A156S, T or V and D168N were determined to emerge frequently often in combination on telaprevir treatment. Variants at position D168, known to confer decreased susceptibility to the macrocyclic NS3/4A protease inhibitors, had not previously been reported to be associated with telaprevir resistance.

Telaprevir-associated resistance substitutions (substitutions at positions V36, T54, R155, A156 or D168) were present at baseline in 5% (117/2217) of the available subject samples in the combined Phase 3 Studies. Conclusions on response outcomes cannot be made given the small number of subjects with baseline telaprevir resistance substitutions.

Telaprevir Phenotypic Studies
Phenotypic characterization of site-directed mutants and recombinant HCV NS3 of patient isolates from Phase 2 clinical trials of telaprevir, conducted in both replicon and enzymatic assays, showed a 3- to 25-fold decrease in telaprevir susceptibility for variants V36M/A, T54A/S, R155K/T, A156S and R155T+D168N and >62-fold decrease in susceptibility for variants A156T/V, V36M+R155K and most double variants. A range in telaprevir susceptibilities for post-baseline isolates with the same substitutions indicates that genetic variation of HCV clinical isolates may also contribute to differences in telaprevir susceptibility. Post-baseline clinical isolates containing V36A/M, T54A/S, R155K/T, A156T or V36M+R155K exhibited susceptibility levels to telaprevir consistent with those obtained in the site-directed mutant evaluations. For both the site-directed mutants and recombinant clinical isolates, the phenotypic results obtained with the replicon assays were consistent with the phenotypic results from enzymatic assays.

Baseline isolates with V36M, R155K or R155K+T54S substitutions showed less susceptibility to telaprevir compared to baseline isolates without telaprevir-resistant variants. Post-baseline isolates lacking telaprevir-resistant mutations showed susceptibilities comparable to those seen with the corresponding baseline isolates.

Persistence of Telaprevir Resistant Variants/Follow-up Analysis
In Study 112, changes in telaprevir resistance-associated HCV variants over time were evaluated in subjects who did not achieve an SVR24. Study 112 was a 3-year, virology follow-up study in subjects previously treated with telaprevir from Phase 2 Studies 104, 104EU, 106, and 107. Follow-up periods in Study 112 ranged from 5 - 40 months with a median of 25 months. A total of 56 subjects were used for the analysis of persistance of resistant variants V36A/M/L, T54S/A, R155T/K/I, A156S/T in the absence of telaprevir selection. All variants were detectable (present at >25% of the viral population) in some subjects at 24 months. By 36 months, V36M, T54S or A, and A156S/T/N variants had fallen below the level of detection in all subjects. Three percent of the subject isolates that had the R155K variant still had detectable R155K variants by population sequencing at 36 months. The lack of detection of a substitution based on a population-based assay does not necessarily indicate that viral populations carrying that substitution have declined to a background level that may have existed prior to treatment.

In addition, the viral populations of subjects failing a telaprevir-containing regimen in Studies 108, 111, and 216 were assessed at multiple time points after treatment-failure by population nucleotide sequencing to determine if the telaprevir resistant variants initially present at the post-nadir visit were detectable in the viral population by the end of study (EOS) visit. Of the combined subjects from Phase 3 studies with a total of 443 resistant variants, 176 (40%) had detectable resistant variants by population sequencing by EOS (follow-up range 5-71 weeks median 45 weeks) and results for loss of variants were similar across the three studies. In the combined studies, 50% of these substitutions in subtype 1a and 20% of the substitutions in subtype 1b were still detected by the EOS.

3. ADMINISTRATIVE

3.1. Reviewer's Signature(s)

Lisa K. Naeger
[Lisa K. Naeger, Ph.D.]
Sr. Microbiologist, HFD-530

3.2. Concurrence

HFD-530/Micro TL _____Date _____

4. VIROLOGY REVIEW

4.1 Important Milestones in Product Develo10pment

OFF-TREATMENT CUTOFF FOR SVR24 DETERMINATION

The primary endpoint for efficacy in the telaprevir studies and for previously approved products is defined as SVR24 (sustained virologic response at Week 24) using BLOD. However, BLOD can be a different numerical value depending on which assay is used.

The telaprevir studies used the COBAS® TaqMan HCV Test, v2.0 (for use with The High Pure System), which was approved in 2010. In the telaprevir viral load results from Studies 108 and 111, we have seen much fluctuation or "blipping" (jumping around of results from limit of detection [LOD was <10 IU/mL as an investigational assay but was approved at 15 IU/mL for GT 1] and below the lower limit of quantification [BLOQ was <25 IU/mL as an investigational assay but approved at 23 IU/mL]. This viral load fluctuation is not necessarily unexpected for patients currently on anti-HCV therapy at the time of measurement when the viral load is near the LOD and LLOQ. However, what is unexpected, in this case, is that the fluctuations are frequently observed in subjects who have been off anti-HCV therapy and are currently several weeks into their treatment-free follow-up phase. The HCV viral load fluctuations do not occur as frequently in the third study, Study 216. The vendor for Studies 108 and 111 was (b) (4) laboratories and the vendor for Study 216 was (b) (4) laboratories.

Data (see below), supplied from Vertex upon our request, indicate that the viral load data from the vendor used for viral load assessments in Studies 108 and 111 did have significantly more viral load fluctuations after EOT off-treament than the viral load data from the other vendor used in Study 216. These differential viral load fluctuations raised the issue as to whether "undetectable" or BLOD (<10 IU/mL) is the appropriate measure for determining the SVR24 primary endpoint.

We requested the following information from the applicant, Vertex. Each FDA comment is shown in italics followed by Vertex's response.

1. Please determine the number of fluctuations and rate of fluctuation (percentage of subjects with fluctuation) from 5 IU/mL (<10 BLOD) to 17.5 IU/mL (<25 BLOQ but detectable) back to 5 IU/mL after treatment during follow-up in Study 108, Study 111 and Study 216.

The number of fluctuations and rate of fluctuations after treatment during follow-up are provided in Table 1 (Study 108 and Study 111) and Table 2 (Study C216). HCV RNA samples for Study 108 and Study 111 were analyzed by the (b) (4) HCV RNA samples for Study C216 were analyzed by the (b) (4). Since two (b) (4) laboratory sites were used in Study 108 and Study 111, Table 1 provides the data by the laboratory sites individually and in total from both laboratory sites.

Table 1. Number and Percentage of Subjects with Fluctuations by Central Lab Site and by Study for Phase 3 Studies 108 and 111

Study	Treatment	Fluctuation (b) (4)	Fluctuation All	No Fluctuation (b) (4)	No Fluctuation All
108	T8/PR (N=364)	29 (12.4)	48 (36.6)	77 (21.2)	204 (87.6)	83 (63.4)	287 (78.8)
	T12/PR (N=363)	23 (10.6)	52 (35.6)	75 (20.7)	194 (89.4)	94 (64.4)	288 (79.3)
	Pbo/PR48 (N=361)	6 (2.7)	31 (22.3)	37 (10.2)	216 (97.3)	108 (77.7)	324 (89.8)
	All (N=1088)	58 (8.6)	131 (31.5)	189 (17.4)	614 (91.4)	285 (68.5)	899 (82.6)
111	T12/PR24/eRVR+ (N=162)	13 (8.4)	3 (37.5)	16 (9.9)	141 (91.6)	5 (62.5)	146 (90.1)
	T12/PR48/eRVR+ (N=160)	11 (7.3)	3 (33.3)	14 (8.8)	140 (92.7)	6 (66.7)	146 (91.3)
	T12/PR48/eRVR- (N=118)	2 (1.9)	1 (8.3)	3 (2.5)	104 (98.1)	11 (91.7)	115 (97.5)
	Other (N=100)	5 (5.1)	-	5 (5.0)	93 (94.9)	2 (100.0)	95 (95.0)
	All (N=540)	31 (6.1)	7 (22.6)	38 (7.0)	478 (93.9)	24 (77.4)	502 (93.0)

Table 2. Number and Percentage of Subjects with Fluctuations for Phase 3 Study C216

Study	Treatment	Fluctuation (b) (4)	No Fluctuation (b) (4)
C216	T12/PR48 (N=266)	2 (0.8)	264 (99.2)
	T12(DS)/PR48 (N=264)	-	264 (100.0)
	Pbo/PR48 (N=132)	1 (0.8)	131 (99.2)
	All (N=662)	3 (0.5)	659 (99.5)

2. We have noted that different vendors were used for HCV viral load analysis in the Studies 108/111 and Study 216. Please provide an explanation for the variability in viral load fluctuation from BLOD and BLOQ following treatment in the different studies with a report from (b) (4) laboratories on possible reasons for the viral load fluctuations between BLOD and BLOQ in Studies 108 and 111.

Variability in results for HCV RNA samples with concentrations below the limit of quantitation has been reported for highly sensitive, PCR-based assays such as the COBAS Taqman assay used in these studies [Sarrazin C et al., 2008].

The number of fluctuations was greater in Studies 108 and 111 than in Study C216 (see Table 1 and Table 2). In addition, for Studies 108 and 111, the number of fluctuations was greater for subjects who had their HCV RNA samples analyzed at the (b) (4) central laboratories in (b) (4) than for subjects who had their HCV RNA samples analyzed at the (b) (4) central laboratories in (b) (4). In response to the noted fluctuations in Study 108, Vertex has conducted a reanalysis on selected HCV RNA samples at (b) (4) laboratories.

A report from (b) (4) Laboratories (*See Appendix A*) on possible reasons for the viral load fluctuations between BLOD and BLOQ was provided. The (b) (4) report concluded:

- the reproducibility of measurements decrease at <25 IU/mL,
- samples repeated twice might be BLOD one time and BLOQ the next,

- contamination of the samples during the run is unlikely because fluctuations would also blip to >25 IU/mL

Given the issue with the fluctuations after EOT, that the package insert for the COBAS assay indicates the lower limit of quantification is 23 IU/mL and the fact that prior products for treatment of chronic HCV were approved using <50 IU/mL, we wanted to determine if it was appropriate to use <25 IU/mL or <50 IU/ml instead of LOD for follow-up off-treatment samples to determine SVR24 primary efficacy analysis.

We sent a consult to CDRH regarding this issue. They state that current recommendations from the AASLD recognize <50 IU/mL as "undetectable" and sufficient for defining SVR. They recommended <50 IU/mL (*See CDRH consult in Appendix B*).

Literature Review
Subsequently, I contacted Dr. Leonard Seeff, senior author on the AASLD Guideline on Treatment of HCV, for his perspective on which cutoff should be used. Discussions with him and a review of the literature [Bortoletto, G. et al., 2010; deLeuw, P. et al., 2011; Fytili, P., et al., 2007; Kadam, J.S., et al., 2007; Lange, C.M., et al., 2010; Morishima, C., et al., 2006; Morishima, C., et al., 2008; Sarrazin, C., et al., 2008; Sarrazin, C., et al., 2010; Schlosser, B. et al., 2011; Toyoda, H., et al., 2010] suggested <25 IU/mL would be appropriate.

The papers above compared TMA (trans-mediated amplification) (Bayer VERSANT) 5-10 IU/mL LOD to conventional PCR (<50-100 IU/mL LOD). All the data was on-treatment and this reviewer could not find data off-treatment. The data showed that one detectable viral load on-treatment reading might be spurious and not accurate. In total, the data also showed that on-treatment, the most sensitive detection of HCV RNA is better at predicting SVR. However, a number of current clinical trials are testing whether BLOQ is appropriate for RVR determination. This reviewer's conclusions from studying the literature are that for decisions on-treatment, the LOD is the most accurate and no changes in the cutoff for the on-treatment decision points or end-of-treatment (EOT) are necessary for our approach to analyzing the data in the telaprevir Phase 3 studies. However, for analysis of SVR24 during follow-up, a viral load cutoff of <25 IU/mL is reasonable and appropriate.

Request to Vertex for SVR24 Analysis Using <25 IU/mL Cutoff in Extend Study 3-Year Follow-up Data
For additional support to use <25 IU/mL rather than below level of detection as the cutoff for the SVR24 primary efficacy analysis for follow-up off-treatment samples, we requested the following from the applicant:

1. *Please provide a comparison of the durability of SVR rates in the subjects who achieved an SVR using BLOD (<10 IU/mL) and BLOQ (<25 IU/mL) cutoffs from EXTEND Study 112. From the study report, 122 (99.2%) of the 123 subjects who had achieved SVR following treatment with a telaprevir-based regimen maintained their SVR status out to 3 years. This SVR assessment used undetectable as cutoff. Please provide the number of subjects who maintained*

their SVR status if the <25 IU/mL cutoff was used to determine SVR. Please submit this information by Feb. 11, 2011.

Vertex Response: As of Interim Analysis 2 (cut-off date 09/01/2010), there were no subjects enrolled in Study 112 who would have been re-classified as SVR if the <25 IU/mL cutoff during follow-up was used to determine SVR. However, there was one subject (Subject 108-211009) enrolled in Study 112 who was classified as non-SVR from Study 108 because of a <25 detectable HCV RNA value at the end of treatment (Week 24), but who had undetectable HCV RNA at the Week 24 follow-up timepoint. Additionally, there is a second subject (Subject 111-125006) enrolled in Study 112 who was undetectable throughout follow-up period of Study 111 through Week 24 of follow-up (SVR_{actual}), but who was classified as non-SVR because the subject was lost-to-follow-up for $SVR_{planned}$. Both of these subjects remain undetectable in Study 112, and would have maintained their SVR status.

2. *Please submit the data and a report on the reanalysis of selected HCV RNA samples from Study 108 by* (b) (4) *Laboratories.*

Reanalysis of Selected HCV RNA Samples from Study 108 by (b) (4) **Laboratories**

During review of the HCV RNA data for Studies 108 and 111, fluctuations of transient detectable HCV RNA values, defined as detectable HCV RNA values below the limit of quantitation (LOQ; 25 IU/mL) preceded and followed by undetectable HCV RNA values (<10 IU/mL) were observed. The HCV RNA samples in Studies 108 and 111 were analyzed by (b) (4) Laboratories and Study 216 was analyzed by (b) (4) Laboratories. To further understand whether transient detectable HCV RNA values reflect actual detectable HCV RNA levels, HCV RNA samples were randomly selected from a subset of subjects in Study 108 who had HCV RNA values of <25 IU/mL detected at (b) (4) Laboratories. These samples were reanalyzed at (b) (4) Laboratories.

For the original and reanalysis results, HCV RNA levels in Study 108 were analyzed using the COBAS TaqMan HCV assay, Version 2.0. This version of the assay has a linear range from 25 to 300,000,000 IU/mL, with a LOQ of 25 IU/mL. Samples with detectable HCV RNA above the LOQ were reported as “detected.” Samples with detectable HCV RNA below the LOQ were reported as "<25 IU/mL detected.” Samples with no detectable HCV RNA were reported as “not detected.”

Analyses were conducted on a subgroup of the full analysis set (n=270), including subjects from each treatment group. Each of the 270 subjects met at least 1 of the following criteria:

- Subjects who had $SVR24_{planned}$ with HCV RNA values of <25 IU/mL detected preceded and followed by undetectable HCV RNA values
- Subjects who had HCV RNA values of <25 IU/mL detected at Week 4
- Subjects who had HCV RNA values of <25 IU/mL detected at Week 12
- Control-matching cases: subjects who had extended rapid viral response (eRVR; undetectable HCV RNA at Weeks 4 and 12)

From the subjects in the subgroups above, 759 HCV RNA samples were selected using computer generated random listing from the subset of 270 subjects.

Of the 307 samples that were <25 IU/mL detected when analyzed at (b) (4) Laboratories, 101 (33%) samples remained <25 IU/mL detected, while 185 (60%) samples were not detected and 21 (7%) samples were detected when reanalyzed at (b) (4) Laboratories (Table 3). Most (92%) HCV RNA samples that were not detected when analyzed at (b) (4) Laboratories were also not detected at (b) (4) Laboratories; 32 (8%) samples were <25 IU/mL detected and 2 (0.5%) samples were detected when reanalyzed at (b) (4) Laboratories. Of 36 HCV RNA samples that were detected when analyzed at (b) (4) Laboratories, only 1 sample had a different result (not detected) when analyzed at (b) (4) Laboratories.

Table 3 Reanalysis of HCV RNA Results from (b) (4) Labs Using (b) (4) Labs

HCV RNA Original Result (b) (4)	HCV RNA Reanalysis Result (b) (4)			
	Not detected	<25 IU/mL detected	Detected[a]	Total
Not detected	382	32	2	416
<25 IU/mL detected	185	101	21	307
Detected	1	0	35	36

[a] Detected: HCV RNA >25 IU/mL

When looking at the subjects who had SVR24 with transient values of <25 IU/mL detected, 92% of samples were undetected at (b) (4) Laboratories and only 3 (6%) of the 52 reanalyzed samples remained <25 IU/mL detected (Table 4).

For subjects who had HCV RNA samples that were <25 IU/mL detected at Week 4 at (b) (4) Laboratories, 40% of HCV RNA samples were undetected at (b) (4) Laboratories and 51% of the 137 reanalyzed HCV RNA samples remained <25 IU/mL detected. For subjects who had HCV RNA samples that were <25 IU/mL detected at Week 12 at (b) (4) Laboratories, 70% of the samples were undetected at (b) (4) Laboratories and 24% of the 118 reanalyzed samples remained <25 IU/mL detected. For subjects who had eRVR (undetectable HCV RNA at Weeks 4 and 12) at (b) (4) Laboratories, 94% of the Week 4 or Week 12 HCV RNA samples reanalyzed at (b) (4) Laboratories remained undetected.

The majority (60%) of HCV RNA samples that were <25 IU/mL detected when analyzed at (b) (4) Laboratories were not detected when reanalyzed at (b) (4) Laboratories, suggesting that most fluctuations in Study 108 are due to higher assay variability at (b) (4) Laboratories. For subjects who had $SVR24_{planned}$ with transient detectable HCV RNA values, 92% of HCV RNA samples with <25 IU/mL detected at (b) (4) Laboratories were not detected when reanalyzed at (b) (4) Laboratories. These results indicate that most of the fluctuations during the follow-up period post-treatment were likely due to assay variability at (b) (4) Laboratories. In contrast, approximately half of the HCV RNA samples at Week 4 that were <25 IU/mL detected at (b) (4) Laboratories were confirmed to have low levels of detectable HCV RNA.

Table 4. Reanalysis by Timepoint of HCV RNA Results from (b) (4) Labs Using (b) (4) Labs

HCV RNA Original Result (b) (4)	HCV RNA Reanalysis Result (b) (4)			
	Not detected	<25 IU/mL detected	Detected[a]	Total
Samples for Subjects Who Had HCV RNA <25 IU/mL detected at Week 4[b], n				
Not detected	67	15	0	82
< 25 IU/mL detected	55	70	12	137
Detected	1	0	19	20
Total	123	85	31	239
Samples for Subjects Who Had HCV RNA <25 IU/mL detected at Week 12[b], n				
Not detected	106	9	1	116
< 25 IU/mL detected	82	28	8	118
Detected	0	0	16	16
Total	188	37	25	250
Samples for Subjects Who Had eRVR (undetectable HCV RNA at Weeks 4 and 12)[c], n				
Not detected	143	8	1	152
< 25 IU/mL detected	NA	NA	NA	NA
Detected	NA	NA	NA	NA
Total	143	8	1	152
Samples for Subjects Who Had $SVR24_{Planned}$ With <25 IU/mL Detected HCV RNA Values[d], n				
Not detected	66	0	0	66
< 25 IU/mL detected	48	3	1	52
Detected	0	0	0	0
Total	114	3	1	118

NA: not applicable
[a] Detected: HCV RNA >25 IU/mL
[b] For subjects who had HCV RNA samples that were <25 IU/mL detected at Week 4 or Week 12, HCV RNA samples were reanalyzed for Week 4 or Week 12 as well as available HCV RNA samples that preceded and followed the Week 4 or Week 12 HCV RNA sample.
[c] For subjects who had eRVR (HCV RNA not detected at Week 4 and Week 12), HCV RNA samples were reanalyzed for Week 4 and/or Week 12.
[d] For subjects who had $SVR24_{planned}$ with transient values of <25 IU/mL detected, HCV RNA samples that were <25 IU/mL detected were reanalyzed as well as available HCV RNA samples that preceded and followed <25 IU/mL detected sample.

Reviewer's Conclusions:
Clinically, an off-treatment cutoff of <10 IU/mL or <25 IU/mL for after EOT is unlikely to be relevant, because there is little monitoring of viral load from EOT to the 6 month SVR24 timepoint and even if the value is >25 IU/mL, there will be no change in treatment decisions because treatment is over. For analysis of this NDA, during follow-up, a viral load cutoff of <25 IU/mL for determination of SVR24 is acceptable and appropriate. This is differentiated from EOT and on-treatment decision timepoints in the Phase 3 studies where <10 IU/mL (LOD) was still used.

4.2 Methodology

Subjects with on-treatment virologic failure (met stopping rule or had detectable HCV RNA at the end of assigned treatment) continued all scheduled visits through Week 72.

If the HCV RNA became detectable, including subjects who prematurely discontinued treatment for non-virologic reasons, samples were collected at 4 and 24 weeks after HCV RNA became detectable. Subjects with relapse (undetectable HCV RNA at the end of treatment, but became detectable later) were followed 4 and 24 weeks after relapse. For each subject with on-treatment virologic failure or relapse, the viral sequence at the post-nadir visit was determined and any non-WT variants at the 4 positions canonically associated with telaprevir-treatment failure (NS3_V36, T54, R155 and A156) were indexed. The fraction of subjects who no longer had observable resistant variants during the observation period was tabulated by both position and individual resistance profile. Subjects were excluded from these tabulations if the variant present at the post-nadir visit was also present at baseline, given that expectations of variant loss would be different for these subjects due to the variant being part of the natural dominant quasispecies prior to treatment.

HCV RNA VIRAL LOAD

Study 108 and 111: Central Clinical Laboratory (b) (4)

Study 216: Central Clinical Laboratory (b) (4)

All plasma HCV RNA levels were assessed using the Roche COBAS TaqMan® HCV/HPS assay (Version 2.0, lower limit of quantification [LLOQ] of 25 IU/mL). In the clinical study protocol, all plasma HCV RNA concentrations levels were planned to be assessed as HCV RNA values ≤10 IU/mL were considered undetectable and HCV RNA values >10 IU/mL were considered detectable. After study initiation, it was determined that the LLOQ of this assay should be reported as 25 IU/mL. Only HCV RNA values ≥25 IU/mL could be reliably quantitated. Therefore, if HCV RNA values were <25 IU/mL, they were reported as <25 IU/mL, detected or <25 IU/mL, undetected. Note: The Roche COBAS TaqMan® HCV/HPS assay (Version 2.0) was approved by FDA (CDRH) in late 2010 with a reported LOD of 15 IU/mL for genotype 1 and BLOQ of 23 IU/mL.

GENOTYPIC METHODS

HCV genotyping (1a/1b) was performed for randomization stratification using commercially available assays, which are based on analysis of the HCV 5' noncoding region (5' NC method). HCV genotype for virology analyses was based on sequence analysis of the HCV NS3-4A region (NS3 method). A comparison of the NS5B, NS3 and 5'NC assays was done (see below).

HCV Subtyping in Studies 108 and 111

Genotyping at screening was performed by (b) (4) using the VERSANT HCV genotype 2.0 (5 NC InnoLipa [5 NC-I]) assay. This line probe assay analyzes variations in the 5 NC region of the HCV to determine the genotype and subtype. Additional genotyping was performed by Vertex (NS3 assay) and was done by

amplification and nucleotide sequencing of the HCV NS3•4A region. The NS3 assay is the primary genotyping method used for the virology analysis and subjects with genotyping discrepancies with the 5 NC-I assay are noted in the tables and dataset.

HCV Subtyping in Study 216

Genotyping to determine study eligibility was performed by the central laboratory based on the 5'NC genotyping method (Trugene). Additional subtype determination was based on NS3 genotyping.

Concordance between Screening Subtype and NS3 Subtype Determination

A comparison between the NS5B, 5'NC, and NS3 sequencing methods for genotype 1 HCV subtype determination was performed using 158 samples from Study VX-950-TiDP24-C208 in subjects with genotype 1 chronic HCV infection. NS5B- and NS3-based subtyping was performed using phylogenetic analysis or BLAST. When comparing the 5'NC and NS5B sequencing methods, concordant results were found in 124/158 (78.5%) samples and 13/158 (8.2%) samples gave discordance. There was a 97.5% (154/158 samples) agreement for HCV subtyping between the NS5B and NS3 methods.

Table 5. Overview of Discordant Samples Comparing NS5B-Based, NS3-Based and 5'NC-Based HCV Subtyping Methods

Subject CRF ID	NS5B Genotyping[b]	NS3 Genotyping[c]	5'NC Genotyping (Trugene)
0005	1b	1b	1a
0052	1[d]	1a	1a
0056	1b	1b	1a
0058	1b	1b	1a
0070	1b	1a	1b
0079	1a	1b	1b
0095	1a	1a	1b
0098	1b	1b	1a
0107	1a	1a	1b
0125	1b	1b	1a
0151	1a	1a	1b
0158	1b	1b	1a
0160	1g[e]	1[f]	1c
0162	1a	1a	1b
0178	1a	1a	1b

[a] 20 samples that could not be subtyped by the 5'NC method are not included in this table
[b] Followed by phylogenetic analysis (subtype reference set 2008)
[c] Subtype was defined as 1a, 1b, 1c or 1 (untyped) by NS3 genotyping followed by phylogenetic analysis
[d] Subtype 1c based on phylogenetic analysis using the subtype reference set 2007
[e] Subtype 1e based on phylogenetic analysis using the subtype reference set 2007
[f] No subtype could be assigned by phylogenetic analysis as the subtype reference set did not contain subtype 1g references

The discordant samples with the NS5B-based, NS3-based and 5'NC-based HCV subtyping methods are shown in Table 5. Two samples gave discordant results between the NS5B and NS3 genotyping methods: 1 genotype 1a sample and 1 genotype 1b sample by the NS5B method resulted in genotype 1b and genotype 1a,

respectively, by the NS3 method (Table 5). One untyped genotype 1 sample by the NS5B method resulted in genotype 1a by the NS3 method. As the concordance between NS5B and NS3-based genotyping for subtype determination within genotype 1 was high, the NS3 method was used for subtype determination in the telaprevir phase 3 studies.

Nucleotide Sequence Analysis

Population nucleotide sequencing of the NS3-4A region was performed in subjects who did not achieve an SVR in a telaprevir-treatment group at time points with HCV RNA levels greater than 1,000 IU/mL. (b) (4)

Purified DNA was then sent to for sequencing of the NS3 and NS4A regions. The limit of detection (LOD) for the sequencing assay was 1,000 IU/mL of HCV RNA.

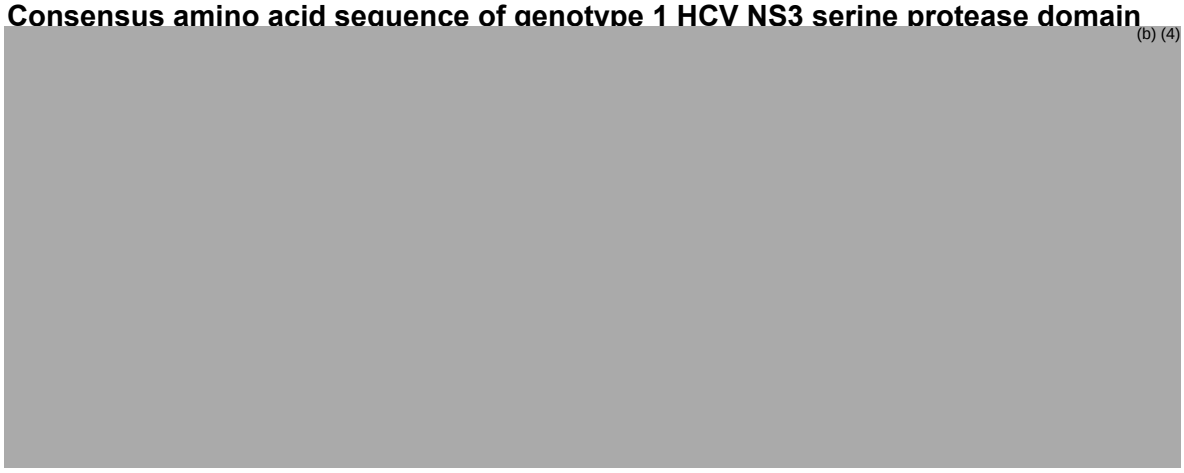
Sequence traces were aligned and interpreted using the software Mutational Surveyor (SoftGenetics, State College, PA). Amino acid substitutions were detected by comparing sequences to the subject-specific baseline sequence. Potential resistance substitutions were identified using a Poisson distribution.

Consensus of NS3 Serine Protease Domain in Genotype 1a/1b

The NS3 protease sequences of over 500 genotype 1a or 1b HCV isolates were found in GenBank™ as of May 2006. Alignment of these amino acid sequences resulted in the identification of the following consensus sequences for the genotype 1 (1a/1b) HCV NS3 serine protease domain.

Consensus amino acid sequence of genotype 1 HCV NS3 serine protease domain

(b) (4)



PHENOTYPIC METHODS

Phenotypic analysis (replicon and enzymatic) of the telaprevir-resistant variants was performed. See "Phenotypic Analysis" in Clinical Virology Section of this review.

4.3 Prior FDA Virological Reviews

IND-71832 reviews were done by Lisa K. Naeger, Ph.D., Sr. Virology Reviewer

4.4 State of Antivirals Used for the indication (s) Sought*:*

Globally, 170 million people are estimated to be infected with HCV, which induces liver necrosis and inflammation and increases the risk of progressive liver failure and liver cancer (WHO, 2010). The prevalence of chronic HCV infection in the United States (3.9 million infected) is approximately 4 times that of HIV or HBV. An estimated 75% of chronically HCV-infected individuals remain undiagnosed compared with individuals infected with HIV (21%) or HBV (65%). HCV accounts for about 15% of acute viral hepatitis, 60-70% of chronic hepatitis and up to 50% of cirrhosis, end-stage liver disease and liver cancer.

HCV is a small, enveloped, single-stranded RNA virus of the Flaviviridae family. The virus contains a single, 10 kb, positive-sense RNA genome which encodes both structural proteins necessary for virus particle formation and nonstructural proteins necessary for replication. Viral RNA encodes a single, long open reading frame producing a polyprotein. Structural proteins are cleaved by cellular proteases and the nonstructural proteins are cleaved by the viral encoded NS2 and NS3/4A proteases.

There are 6 major HCV genotypes with different geographic distributions. Genotypes 1a and 1b are most common in the US representing about 75% of the infected population with genotype 1a predominating. Genotypes 2 and 3 are present in only 10-20% of US patients, and genotype 4 is found in about 7% of US patients. Unlike HBV and HIV, which currently require long-term therapy to maintain viral suppression, clearance of HCV is possible with therapy, because of a presumed lack of an archival form of the HCV RNA genome. A durable sustained virologic response (SVR) has been consistently observed in long-term studies following HCV treatment. However, the current treatments for chronic HCV infection are limited in efficacy and have significant toxicities. The current standard-of-care for adults with genotype 1 chronic hepatitis C (CHC) virus infection is 48 weeks of pegylated recombinant human interferon α (Peg-IFN) combined with ribavirin (RBV) and results in sustained clearance of HCV RNA in just over half of all US patients.

This combination is more effective for genotypes 2 and 3 than genotype 1. The efficacy (sustained virologic response) for genotype 1 is 40-45% in contrast to 80-90% with genotypes 2 and 3. Subjects with chronic genotype 1 HCV infection usually require a full 48 weeks of therapy to maximize the likelihood of achieving SVR, although subjects who achieve a rapid virologic response (RVR) after 4 weeks of treatment may benefit from a shorter duration of treatment. There are limitations in Peg/RBV treatment for some groups of patients. Patients with high viral loads, cirrhosis, homozygous or heterozygous "T" allele in the polymorphic IL28B gene, and African Americans are reported to have substantially lower rates of SVR with standard-of-care. In addition, Peg/RBV treatment is not tolerable in all chronic HCV patients, because of the significant adverse events associated with interferon and ribavirin. Furthermore, subjects who have previously failed to respond to Peg/RBV therapy have poor treatment response (6-11%) after retreatment with Peg/RBV. Therefore, there is an unmet need for effective and safe treatment options in certain patient populations and shorter treatment options for most subjects with genotype 1 HCV.

Direct-acting antivirals (DAAs) specifically target the HCV proteins involved in the HCV life cycle. The HCV NS3•4A protease is essential for viral replication [redacted] (b) (4) Telaprevir (VX-950) is part of this new class of DAAs targeting the NS3-4A protease in HCV. It is a specific, reversible, covalent NS3-4A inhibitor that was derived through structure-based drug design. Currently, no DAAs are marketed.

Variants resistant to DAAs likely pre-exist in all patients, because of the high HCV replication rate and error rate. HCV has a high rate of replication (up to 1 $x10^{12}$ virions produced each day) (Neumann et al., 1998), which is error-prone because the HCV RNA-dependent RNA polymerase lacks a proofreading function. The error rate of the polymerase is approximately 10^{-4}/base/generation for a single mutation, which results in one mutation being introduced into every genome that is copied. New variants are constantly being generated, and it has been estimated that every possible point mutation along the HCV genome occurs at least once and probably many times each day (Kieffer et al., 2010). Treatment with a DAA monotherapy can rapidly select for resistant variants in some patients, as has been demonstrated by the selection of variants with resistance to a number of DAAs, including NS3-4A protease inhibitors and polymerase

inhibitors (Sarrazin and Zeuzem, 2010). Therefore, telaprevir is being studied in chronic HCV patients in combination with Peg/RBV to reduce the selection of resistant variants, improve SVR rates over treatment with Peg/RBV alone, and potentially shorten treatment duration.

4.5 NON-CLINICAL VIROLOGY

MECHANISM OF ACTION

VX-950 is an active site directed peptidomimetic ketoamide inhibitor of HCV NS3 protease designed from the (b) (4). A standard peptide cleavage assay for HCV NS3 proteas (b) (4) was used to evaluate the activity of VX-950. (b) (4) Under these conditions, the following apparent K_i values for VX-950 and isomeric forms VRT-108720 and VRT-127394 were 44 nM, 78 nM and 1200 nM, respectively.

To evaluate the time dependence of inhibition by these peptidomimetic ketoamides, a continuous assay was used, (b) (4) for the measurement of HCV NS3 protease activity. Activity for VX-950 is achieved within the first 20 minutes. The IC_{50} values determined after a 4 hour pre-incubation were 10 nM, 18 nM and 60 nM for VX-950, VRT-108720, and VRT-127394, respectively. The progress curves of VX-950-treated HCV NS3 protease activity are biphasic, which is indicative of slow-binding inhibition. The K_i values, determined from steady state velocities for VX-950, VRT-108720 and VRT-127394 were 7 nM, 15 nM and 58 nM, respectively.

Off-rates (k_{off}) for the tightly bound enzyme/inhibitor complex were measured directly by (b) (4) The half-life of the complexes was calculated from k_{off} values (Table 6).

Table 6. Measured Off-Rates from Enzyme Inhibitor Complexes

	VX-950	VRT-108720	VRT-127394
k_{off} (sec-1)	(b) (4)		
$t_{1/2}$ (min)	58	(b) (4)	

The selectivity of VX-950, VRT-108720, and VRT-127394 were assessed against a representative panel of serine proteases: kallikrein, thrombin, plasmin, and Factor Xa. At a test concentration of 10 µM, VX-950 showed no inhibition of any of these proteases (Table 7). The other two isomeric substances showed 7 percent or less inhibition against this same panel. These data indicate that VX-950 is highly selective for the HCV NS3 protease and should not interfere with other serine proteases at physiologically relevant concentrations.

Table 7. Selectivity of Ketoamide Inhibitors against Selected Serine Proteases

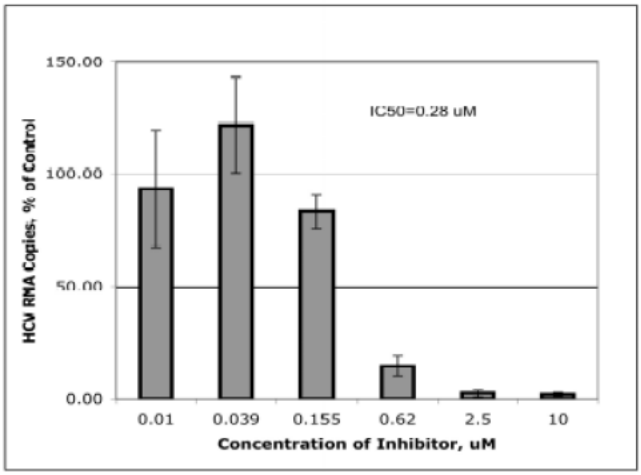
Percent Inhibition Tested at 10 μM	VX-950	VRT-108720	VRT-127394
Kallikrein	0	7	1
Thrombin	0	0	7
Plasmin	0	0	0
Factor Xa	0	0	0

ANTIVIRAL ACTIVITY IN CELL CULTURE

Two assay systems were used to evaluate anti-HCV activities of VX-950: infection of primary human hepatocytes with virus derived from the serum of HCV infected patients and the HCV replicon, a non-infectious system that utilizes a sub-genomic clone of HCV viral RNA, which stably replicates in Huh7 cells.

Vertex developed (b) (4) VX-950 demonstrated a concentration-dependent inhibition of HCV replication in this culture system. When inhibition of HCV infection was analyzed after 5-day VX-950 exposure, an EC_{50} value of 0.28 μM for VX-950 was obtained (Figure 1) and no difference in cell density or morphology between VX-950-treated and control medium-treated cultures could be observed by microscopic observation.

Figure 1. Effect of VX-950 on HCV Replication in Cell Culture



In the replicon assay, cells were treated with VX-950 for 1, 2, 3 and 5 days, a time and concentration dependent decrease in HCV RNA levels was observed. Consistent with the decreased HCV RNA levels, Western blot analysis showed that HCV NS3 and NS5A proteins diminished over 4 days of treatment with VX-950. Loss of NS3 protein in HCV replicons cells treated with VX-950 was also demonstrated using immunofluorescence.

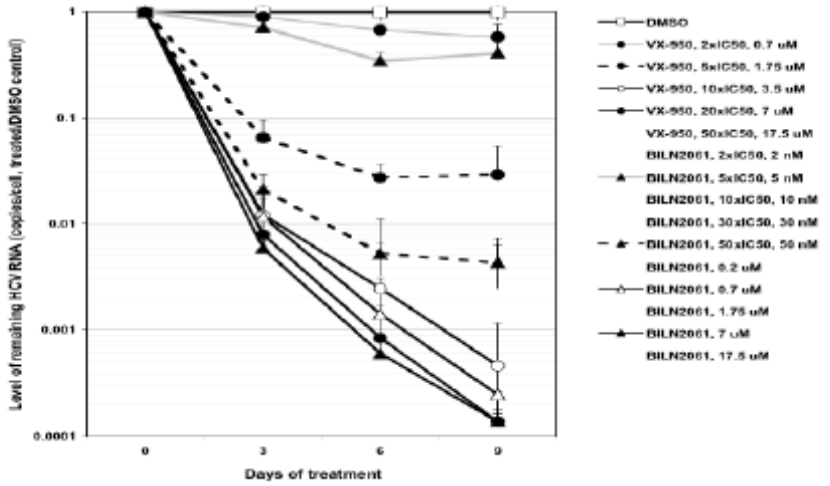
Anti-HCV activity and cytotoxicity of VX-950 were evaluated in a standard 2-day HCV replicon assay in three independent assays (Table 8). The average EC_{50} value of VX-950 following 2-day treatment was 0.354 µM with an average CC_{50} value of 83 µM, resulting in a selective index of 230.

Table 8. EC_{50} and CC_{50} Values of VX-950 in 2-Day HCV Replicon Assay

Experiment	IC_{50} (µM)	IC_{90} (µM)	CC_{50} (µM)	% Cytotoxicity at 100 µM	S.I. (CC_{50}/IC_{50})
12/05/00	0.319	0.636	51.9	57.4	163
12/12/00	0.354	1.015	95.8	60.1	271
02/13/01	0.389	0.835	100	50.0	257
Average	0.354	0.830	83	55.8	230

Replicon cells were incubated with various concentrations of VX-950 or BILN 2061 for 3, 6, or 9 days to determine whether VX-950 can induce a multi-log reduction of HCV RNA. The number of cells was determined in the MTS-based cell viability assay and the level of HCV RNA in the cells was determined by the quantitative RT-PCR (Taqman) assay. The copy number of HCV replicon RNA molecules per cell was calculated for cells treated with compound and compared to that of control cells treated with 0.2% DMSO in media. After 9-day incubation, 3.5 µM VX-950 resulted in a greater than 3.5 log_{10} reduction in HCV replicon RNA (Fig. 2). To determine whether VX-950 could clear all the HCV RNA from replicon cells, cells were incubated with compound in the absence of G418 for 27 days.

Figure 2. Comparison of VX-950 and BILN 2061 in 9-Day HCV Replicon Assay



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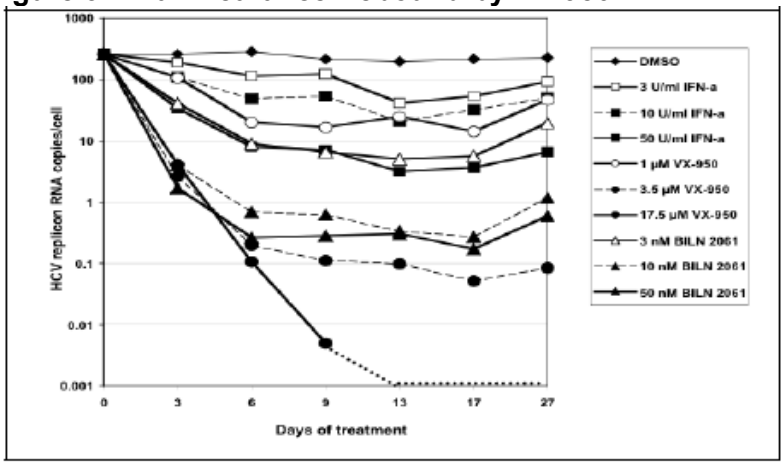
To confirm that viable HCV replicon had been cleared from these cells, a rebound experiment was performed in which the inhibitors were withdrawn from the culture media on day 13. Unlike a viral infection system, the HCV replicon is a stable cell line maintained under G418 selection, which enriches the population of replicon-positive cells over replicon-negative cells. Therefore, when the inhibitors were withdrawn, 250 µg/mL G418 was added back to the culture media in order to expand any cell in which

viable HCV replicon had not been completely cleared. The cells were cultured for two additional weeks in the presence of G418. Cells that had completely lost the HCV replicon died between 10-14 days in the presence of 250 µg/mL G418. The levels of HCV replicon RNA in cells incubated with 3 different concentrations of IFN α, BILN 2061, or 1 and 3.5 µM VX-950 rebounded to the same level of control cells within two weeks after the withdrawal of inhibitors. In contrast, no HCV replicon RNA was recovered from cultures treated with 17.5 µM VX-950, indicating that all the viable HCV replicon had been completely cleared from these cells (Fig. 3).

Figure 3. Viral Clearance/Rebound by VX-950



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Antiviral Activity against Genotypes 2, 3, and 4

Enzymatic studies were designed to compare the inhibition of telaprevir (VX-950) against the genotype 2, 3, or 4 HCV NS3 serine protease domain proteins derived from patient isolates in complex with the corresponding NS4A cofactor, vs. genotypes 1 HCV enzymes.

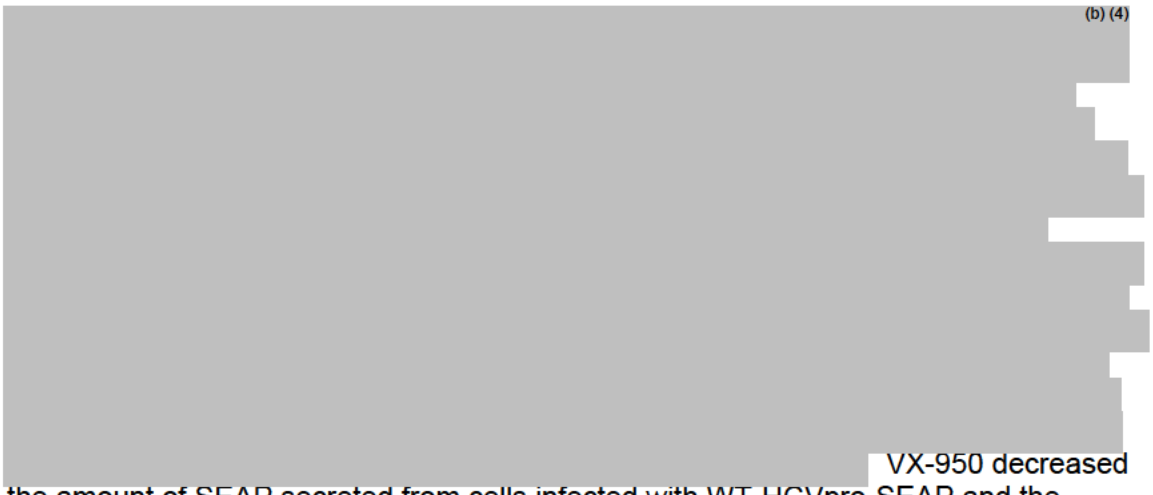
The mean IC_{50}(1h) values of telaprevir against 5 genotype 2 HCV NS3•4A proteases were 16, 32, 6.4, 6.8 and 18 nM, respectively, resulting in a mean value of 16 ± 10 nM (median 16 nM), which is similar to the mean value for the genotype 1 enzymes of 21 ± 7 nM (median 20 nM; median 20 nM (range 16-23; n=2) for genotype 1a and 20 nM for genotype 1b (range 13-33; n=4)). The mean IC_{50}(1h) values of telaprevir against 5 genotype 3a HCV NS3•4A proteases were 77, 88, 40, 39, and 40 nM (median 40 nM), respectively, resulting an mean value of 57 ± 24 nM. The mean IC_{50}(1h) values of telaprevir against one genotype 4a HCV NS3•4A protease was 130 nM.

Antiviral Activity of Metabolite VRT-842291

In a peptide cleavage assay using a truncated form of HCV NS3 protease, the K_i for the metabolite VRT-842291 was 1.65 µM, which is an ~38-fold reduction in activity compared to VX-950. Additionally, in a 2-day HCV replicon assay, VRT-842291 showed an ~15-fold reduced activity in comparison to VX-950. The level of remaining HCV RNA

in cells incubated with 17.5 µM of VX-950 after 13 days was below the detection limit of the quantitative RT-PCR assay.

ANTIVIRAL ACTIVITY IN VIVO



VX-950 decreased the amount of SEAP secreted from cells infected with WT-HCVpro-SEAP and the amount of inhibition was dependent on the concentration of VX-950 and was significantly higher than that observed in cells that received Ad-MT-HCVpro-SEAP or cells infected with Ad-SEAP alone.

To determine the kinetics of expression, $5x10^9$ and 10^{10} IFU of Ad-WT-HCVpro-SEAP or Ad-MT-HCVpro-SEAP was injected into the tail vein of severe combined immunodeficiency (SCID) mice (N=6). At 1, 2, 4, and 15 days post injection, serum was harvested from the infected mice by retro-orbital bleeding and the concentration of SEAP in the serum was determined. At 24 hours post infection, there was a 50- to 60-fold increase in SEAP levels in the serum of mice infected with Ad-WT-HCVpro-SEAP compared to the level obtained in mice infected with the Ad-MT-HCVpro-SEAP. The amount of SEAP in the serum peaked at 48 hours post infection followed by persistent, measurable levels up to 15 days. SEAP levels in the serum of mice infected with 10^{10} IFU of Ad-WT-HCVpro-SEAP were approximately 10-fold higher than the level in mice infected with 5×10^9 IFU/mouse of Ad-WT-HCVpro-SEAP. To determine the localization and distribution of HCV NS3 protein in mice infected with Ad-WT-HCVpro-SEAP, lysates of heart, lungs, spleen, kidney and liver tissue from 3 infected mice were prepared and subjected to immunoblotting. The results showed that >95% of adenovirus intravenously administered to a mouse via tail vein injection ends up in the liver and that HCV protease expression was mostly detected in the liver of the Ad-WT-HCVpro-SEAP infected mice. Expression of HCV protease in the liver of infected mice was analyzed by immunohistochemistry of paraffin embedded liver sections. HCV protease expression was observed mainly in the cytoplasm as granular staining with a reticular mesh pattern and more than 50 to 60% of the hepatocytes were expressing HCV protease, which was readily detectable up to 15 days after infection.

SCID mice were dosed 2 hours before infection and 10 hours post infection with 0, 0.3, 1, 3, 10, 30 mg/kg of VX-950 and 2 hours later, infected with Ad-WT-HCVpro-SEAP. At

24 hours post infection, they were sacrificed and SEAP levels in the circulation were measured. Inhibition of HCV protease dependent SEAP release was observed. Mice dosed with 0.3 mg/kg of VX-950 had more than 50% inhibition of SEAP release compared to the vehicle alone dosed animals. These results indicate that the 50% effective dose (ED_{50}) value of VX-950 in the HCV protease animal model is <0.3 mg/kg. The concentration of VX-950 in the liver was 6- to 16-fold higher than the concentration in the plasma, supporting that active VX-950 targets the liver. Another HCV protease, BILN 2061 inhibitor, showed similar activity in the HCV protease animal model.

ANTIVIRAL ACTIVITY IN THE PRESENCE OF HUMAN SERUM PROTEINS

The potential serum-binding effect on antiviral activity of VX-950 was evaluated by performing the two-day HCV replicon assay in the presence of various percentages of human serum (b) (4) The EC_{50}, EC_{90}, and CC_{50} values of VX-950 in the presence of 10%, 20%, or 40% of human serum and the fold of increase in EC_{50} or EC_{90} values over those in the absence of human serum were calculated (Table 9). The EC_{50} value increased by ~10-fold and the EC_{90} value increased by 6-fold with 40% human serum. At 100% human serum, the extrapolated EC_{50} and EC_{90} values of VX-950 would be 8 μM and 10.78 μM, respectively.

Table 9. Effect of Serum on VX-950 in the Standard 2-day HCV Replicon Assay

Human Serum	IC_{50} (μM)	Fold Increase	IC_{90} (μM)	Fold Increase	CC_{50} (μM)
0%	0.33	—	0.79	—	>30
10%	0.88	2.7	1.37	1.7	>30
20%	1.40	4.2	2.52	3.2	>30
40%	3.45	10.4	4.76	6.0	>100

CYTOTOXICITY

To evaluate cyctotoxicity in resting PBMC, PBMC were prepared from fresh blood donated by healthy volunteers, and then the cells were incubated with various concentrations of VX-950 in the absence of bovine or human serum, and the cell viability was determined by the MTS-based assay after 48 h of incubation. The CC_{50} value of VX-950 in PBMCs was >30 μM (Table 9).

ANTIVIRAL ACTIVITY IN COMBINATION WITH OTHER ANTIVIRALS

Combination with IFN α
The HCV replicon cells were treated with VX-950 and interferon α, either alone or in combination. HCV replicon cells were treated with various concentrations of VX-950 and IFN α for two days. Total cellular RNA was extracted and the level of HCV RNA remaining in replicon cells was determined by quantitative RT-PCR. The data were further analyzed using MacSynergy™ and CalcuSyn™ to determine whether the effect of drug-drug combination was antagonistic, additive, or synergistic (Fig. 4 and Table 10). Combination of VX-950 and IFN α was additive to moderately synergistic in reducing HCV RNA levels in the replicon cells. The cell viability was determined by an MTS-

based cell viability assay. The combination of VX-950 and IFN α did not cause a significant increase in cytotoxicity.

Figure 4. MacSynergy Analysis of Combination of VX-950 with IFN α

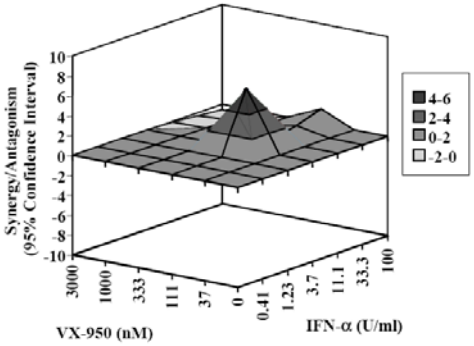


Table 10. Analysis of the Combination of VX-950 and IFN α at Various Ratios by CalcuSyn™

Ratio (VX-950 : IFN-α)[a]	Combination Index (CI) [b]		
	at IC_{50}	at IC_{75}	at IC_{90}
1 : 100	1.24	0.73	0.56
3 : 100	0.74	0.53	0.53
9 : 100	0.77	0.58	0.61
27 : 100	0.67	0.61	0.68

Combination Index (CI) value of <1 indicates synergy.

Replicon cells were treated with VX-950 and IFN α alone or in combination for 3, 6, or 9 consecutive days. The number of cells was determined in the MTS-based cell viability assay using an established standard curve. The level of HCV RNA in the cells was determined by the quantitative RT-PCR (Taqman). The copy number of HCV replicon RNA molecules per cell was calculated for cells treated with compound and compared to that of control cells treated with 0.2% DMSO in media. After 9-day treatment, combination of 2 μM VX-950 and 50 U/mL IFN α resulted in a greater than 3 $\log_{10}$ reduction in HCV replicon RNA level which can only be achieved with higher concentrations of either agent alone. VX-950 and IFN α combined resulted in a multi-log reduction of HCV RNA following a 9-day treatment of the replicon cells.

Combination with Ribavirin

Replicon experiments were performed to evaluate the effect of combinations of VX-950 and ribavirin on HCV RNA replication using the standard 48-hour culture method. Concentrations tested included a wide range spanning the inhibition range. The decrease in HCV RNA in the treated cultures was measured and compared to the untreated control cultures using the bDNA method. The combination index values for the VX-950 and ribavirin combinations using the Loewe additivity (or median-effect) method were 1.04, 0.99, and 0.94, at the 50% effect, 75% effect and 90% effect, respectively. Combination index values greater than 1.2 are considered to indicate

antagonism, values less than 0.85 are considered to indicate synergy, and values near 1.0 indicate additivity. Therefore, these combination index values are indicative of additivity. The same data was evaluated in an isobologram plot and the values from the combinations fell very close to the isobol lines at the 50% effect, 75% effect, and 90% effect levels, again suggesting additivity.

Viability measurements, employing cellular ATP content at 48 hours as readout, indicated that VX-950 did not have major effects on the viability of cells under these conditions. High concentrations of ribavirin showed modest effects on cell viability both in the presence and absence of VX-950. The concentrations of ribavirin at which viability effects were observed were higher than the 10 µM plasma concentration typically observed in standard HCV therapy options.

Interaction with HIV-1 Protease Inhibitors
Vertex has not conducted a study looking at the potential for antagonism of anti-HIV-1 protease inhibitors by telaprevir in cell culture. However it has been demonstrated that telaprevir is not a selective inhibitor against HIV-1 in cell culture. In addition, the ability of telaprevir to interact with a panel of HIV-1 protease inhibitors has been studied in healthy volunteers (See Clinical Pharmacology review of Dr. Shirley Seo). Additionally, in the synopsis of Study VX09-950-110 provided in the NDA, the applicant states that no unexpected changes in HIV-1 viral load were seen (presented at CROI 2011).

RESISTANCE DEVELOPMENT IN CELL CULTURE SELECTION EXPERIMENTS

HCV sub-genomic replicon cells were serially passaged in the presence of increasing concentrations of VX-950, BILN 2061, or both, to select resistant replicon cells. The concentrations of VX-950 ranged from 3.5 µM (or 10X EC_{50} value) to 28 µM (80X EC_{50} value). For BILN 2061, the starting concentration was 80 nM (80X EC_{50} value), and the final concentration was 12.5 µM (12,500 EC_{50} value). The purified 1.7-kb RT-PCR products of PI-treated replicons from several different culture time points were sequenced. Emergent PI resistance-associated substitutions in the HCV NS3 protease domain were identified by comparison of the HCV RNA sequences in the resistant replicon cells versus the naive replicon cells. Each of the individual substitutions was then subcloned into appropriate plasmids for generation of HCV replicon cell lines. The resistance phenotype of these mutations was subsequently confirmed in enzymatic and virological assays. After 10 days, the replicon cells grew significantly slower and a significant amount of cell death was observed between day 10 and day 17. Normal growth did not resume until day 21. The EC_{50} value of VX-950 against the resistant replicon cells at day 56 was 12.1 µM, which is 34-fold higher than the EC_{50} value (354 nM) against wild-type replicon cells. At days 21 and 56, substitutions at A156 in the protease domain were observed. In replicon cells, which had been cultured in the presence of 28 µM VX-950 for 63 days, 79% (60/76) of clones had an alanine to serine substitution at residue 156 (A156S). No substitution was found at any of the four proteolytic sites in the HCV nonstructural protein region that are cleaved by the NS3•4A serine protease.

HCV replicon cells resistant to BILN 2061 were selected in a similar manner as for VX-950. Wild-type Con1 sub-genomic HCV replicon cells were serially passed in the

presence of 0.25 mg/mL G418 and slowly increasing concentration of BILN 2061. BILN 2061 had an EC_{50} value of 1.0 to 1.8 µM against the replicon cells at day 59, which is 250- to 450-fold higher than the EC_{50} value (4 nM) against wild-type replicon cells. By day 24, a variety of substitutions were observed at amino acid 168 of the NS3 protein. Individual clones from the HCV serine protease of the replicon at day 98 which was cultured in the presence of 3.2 µM BILN 2061, were sequenced. Sixty out of 94 clones or 64% had a D168V substitution, and 23 clones or 24% had a D168A substitution.

CROSS-RESISTANCE

See also "Phenotypic Analysis" in this review on page 61. Two HCV sub-genomic replicon stable cell lines (the wild-type (wt) and the A156S mutant), were generated using (b) (4) The EC_{50} value of VX-950 against the A156S replicon cells was 3.98 µM, which is 10 times higher than that against the wild-type replicon cells (0.40 µM). The EC_{50} values of BILN 2061 against the A156S (7 nM) and the wild-type replicon (4 nM) cells were similar (Table 11).

Table 11. Resistance to VX-950 and BILN2061 in the HCV Replicon 2-Day Assay (µM)

Mutant	VX-950	BILN 2061
wt	0.402	0.004
A156S	3.98	0.007
A156T	>30	1.09
A156V	>30	5.76
D168V	0.163	4.27
D168A	0.193	0.88

The EC_{50} values were determined against the three HCV replicon cell lines in the standard 48-hour assay, and the average of two independent assays is shown.

The D168V or D168A substitution was also introduced into the wild-type HCV replicon by site-directed mutagenesis and a stable replicon cell line carrying either substitution was generated. BILN 2061 had an EC_{50} value of 4.27 µM against the D168V replicon cells, which is more than 1,000 times higher than against wild-type replicon cells (4 nM) (Table 11). The EC_{50} value of BILN 2061 was 0.88 µM against the D168A mutant replicon. There was a decrease in the EC_{50} values of VX-950 against the D168V or D168A mutant replicon compared with the wild-type replicon cells, so these substitutions are susceptible to VX-950.

In addition, Con1 sub-genomic replicon cells derived from pBR322-HCV-Neo-mADE were serially passaged in the presence of increasing concentrations of both VX-950 and BILN 2061. The starting concentration of VX-950 was 3.5 µM and the highest concentration was 14 µM. The starting concentration of BILN 2061 was 80 nM and the final concentration was 1.6 µM. In the replicon cells that had been cultured in the presence of 3.5 µM VX-950 and 0.32 µM BILN 2061 for 14 days, 65% (30/46) of the clones had the A156T substitution, while the substitution A156V was found in 35% (16/46) of the clones. For replicon cells that had been cultured in higher concentrations

(14 µM VX-950 and 1.6 µM BILN 2061) for 14 days, 80% (35/44) clones had the A156T substitution, while the A156V substitution was found in 20% or 9 out of 44 clones.

Site-directed mutagenesis was used to replace A156 with either Val or Thr in the wild-type NS3 protease domain and was introduced into a sub-genomic replicon for characterization in the HCV replicon system. No significant reduction of HCV replicon RNA by up to 30 µM VX-950 was observed in either mutant replicon cell line, indicating at least 75-fold decreased susceptibility conferred by either subsitution (Table 12). The EC_{50} values of BILN 2061 against the A156T and A156V replicon cells was 1.09 µM and 5.76 µM, respectively, which is 272-times and >1,400-fold higher, respectively, than that against the wildtype replicon cells (4 nM). The A156V and A156T mutant replicons remain sensitive to IFN-α and ribavirin. The A156V and A156T mutant replicons have diminished replication capacity in supporting HCV RNA replication in the replicons cells.

Table 12. HCV Replicon Mutants Remain Susceptible to IFN α and RBV

Mutant	Replicon IC_{50}	
	IFN-α(U/mL)	ribavirin (µM)
Wild-type	1.01	40.5
A156T	1.03	49.7
A156V	1.20	75.1

4.6 CLINICAL STUDIES

VX07-950-108

Clinical study VX07-950-108 (Study 108) was a Phase 3 trial of telaprevir (VX-950) 750 mg q8h, in combination with pegylated interferon alfa-2a (Peg-IFN-alfa-2a) 180 µg/week and ribavirin (RBV) 1000 or 1200 mg/day in treatment-naive subjects with genotype 1 chronic hepatitis C virus infection. The telaprevir regimens were 24 or 48 weeks in treatment duration, with telaprevir given in combination with Peg-IFN alfa-2a and RBV for either the first 8 weeks (T8/PR group) or the first 12 weeks (T12/PR group). For the control group, the total treatment duration was 48 weeks, with telaprevir-matching placebo given for the first 12 weeks and Peg-IFN alfa-2a and RBV dosed for the entire 48 weeks (Pbo/PR48 group). For subjects who achieved an extended rapid viral response (eRVR, defined as undetectable HCV RNA at Week 4 and Week 12), Peg-IFN alfa-2a and RBV were dosed for a total of 24 weeks. For subjects who did not achieve eRVR, Peg-IFN-alfa-2a and RBV were dosed for a total of 48 weeks. The SVR rates were 68.7% versus 43.8% for T8/PR group versus Pbo/PR48 group (P<0.0001) and 74.7% versus 43.8% for T12/PR group versus Pbo/PR48 (P<0.0001).

VX08-950-111

Clinical Study VX08-950-111 was Phase 3, randomized, open-label, multicenter study conducted in treatment-naïve subjects with genotype 1, chronic hepatitis C virus infection. The study was designed to evaluate the SVR rates in subjects who achieved an eRVR (undetectable HCV RNA levels at Week 4 and Week 12 on treatment) with

telaprevir in combination with Peg-IFN alfa-2a and RBV. The treatment regimens were 24 or 48 weeks in duration, with telaprevir administered in combination with Peg-IFN alfa-2a and RBV for the first 12 weeks (i.e., T12/PR24 arm or T12/PR48 arms, respectively). Subjects who achieved an eRVR and completed the Week 20 visit were randomized in a 1:1 ratio to stop all study treatment at Week 24 (randomized withdrawal; T12/PR24/eRVR+ group) or to continue treatment with Peg-IFN alfa-2a and RBV to Week 48 (T12/PR48/eRVR+ group). Randomization was stratified by genotype (1a, 1b, or unknown) and race (Black or non-Black; self-identified). Randomization occurred after the Week 20 visit, but before the Week 24 visit. Subjects who did not achieve an eRVR were assigned a total treatment with Peg-IFN alfa-2a and RBV for 48 weeks (T12/PR48/eRVR- group). Subjects who received at least 1 dose of study drug, but prematurely discontinued treatment before Week 20, were not randomized or assigned to a treatment regimen. These subjects were included in the group designated 'Other'. The total RVR rate for all subjects in the study was 72.0%; the total eRVR rate for the study was 65.2%. The total SVR24 planned rate for the study was 72% (Table 13).

Table 13. SVR Rate in Study 111

$SVR24_{planned}$ Rates, Full Analysis Set				
	Randomized (eRVR+)		Assigned (eRVR-)	
Variable	T12/PR24 N = 162 n (%)	T12/PR48 N = 160 n (%)	T12/PR48 N = 118 n (%)	Other N = 100 n (%)
$SVR24_{planned}$	149 (92.0)	140 (87.5)	76 (64.4)	23 (23.0)
SVR Week 72: n (%)	141 (87.0)	140 (87.5)	76 (64.4)	20 (20.0)
$SVR24_{actual}$: n (%)	149 (92.0)	144 (90.0)	78 (66.1)	27 (27.0)

Abbreviations: eRVR: extended rapid viral response; SVR: sustained viral response
Note: Subjects in the Other treatment group prematurely discontinued treatment before Week 20 and were not randomized or assigned to a treatment regimen.
Source: Table 14.2.1a

VX-950-TiDP24-C216

Clinical Study VX08-950-216 was a randomized, double-blind, placebo-controlled Phase 3 study with telaprevir in subjects with genotype 1 chronic HCV infection who failed prior treatment with Peg-IFN (Peg-IFN alfa-2a or Peg-IFN alfa-2b) plus RBV. The study was designed to compare the efficacy, safety, and tolerability of 2 regimens of telaprevir (with and without delayed start of telaprevir) combined with Peg-IFN alfa-2a and RBV versus standard treatment (Peg-IFN alfa-2a and RBV). Subjects were eligible to enroll in the study if they 1) had an undetectable hepatitis C virus (HCV) RNA level at the end of a prior course of Peg-IFN/RBV therapy but did not achieve SVR (prior relapsers), or 2) never had an undetectable HCV RNA level during or at the end of a prior course of Peg-IFN/RBV therapy (prior non-responders = null and partial responders). Approximately 650 subjects (350 prior relapsers and 300 prior null and partial responders) were planned to be randomized in a 2:2:1 ratio to one of 3 treatment groups, all with a planned total treatment duration of 48 weeks. Randomization was stratified based on screening HCV RNA value (<800,000 IU/mL or ≥800,000 IU/mL) and on type of prior response (prior relapser or prior null and partial responder). Furthermore, for the stratum of prior null and partial responders, an additional stratification was for prior null-

responsers or prior partial responders, defined as subjects with <2-log_{10} drop in HCV RNA at Week 12 of prior therapy (null-responders) or subjects with ≥2-log_{10} drop in HCV RNA at Week 12 of prior therapy but who never achieved undetectable HCV RNA levels while on treatment (partial responders). Enrollment was limited such that neither of these strata would represent more than 55% of the non-responder subpopulation.

For the prior relapser population, SVR24 rates were 83.4% and 87.9% for the T12/PR48 and T12(DS)/PR48 groups compared to 23.5% for the Pbo/PR48 group (Table 14). For the prior non-responder population, SVR24 rates were 41.3% and 41.5% for the T12/PR48 and T12(DS)/PR48 groups compared to 9.4% for the Pbo/PR48 group. For the prior null-responder population, SVR24 rates were 29.2% and 33.3% for the T12/PR48 and T12(DS)/PR48 groups compared to 5.4% for the Pbo/PR48 group. For the prior partial responder population, SVR24 rates were 59.2% and 54.2% for the T12/PR48 and T12(DS)/PR48 groups compared to 14.8% for the Pbo/PR48 group.

Table 14. SVR24 by Prior Response in Study 216

	Overall population					
	T12/PR48		T12(DS)/PR48		Pbo/PR48	
$SVR24_{planned}$, n (%)	N	n (%)	N	n (%)	N	n (%)
Prior relapsers	145	121 (83.4)	145	126 (86.9)	71	17 (23.9)
Prior non-responders	121	50 (41.3)	119	49 (41.2)	61	5 (8.2)
Prior null-responders	68	22 (32.4)	67	21 (31.3)	35	1 (2.9)
Prior partial responders	53	28 (52.8)	52	28 (53.8)	26	4 (15.4)

N: number of subjects with data; n: number of subjects with SVR

4.7 CLINICAL VIROLOGY IN PHASE III STUDIES

STUDY VX07-950-108: TREATMENT NAIVES

TREATMENT OUTCOMES IN STUDY 108

Of the 356 subjects in the T8 group, 244 achieved an SVR (69%), and of the 353 subjects in the T12 group, 264 achieved an SVR (75%). Subjects who did not achieve SVR after treatment with a telaprevir-based regimen can be subdivided into the following outcomes

- on-treatment virologic failure (stopping rules) (Table 15)
- relapse
- detectable HCV RNA after premature discontinuation from assigned treatment (for reasons other than the virologic stopping rules)
- undetectable HCV RNA at the end of treatment and discontinued study before SVR

Table 15. Virologic Stopping Rules

Week	Subjects	HCV RNA Criteria	Treatment and Procedural Modification
Week 4	T8/PR and T12/PR subjects	≤1000 IU/mL	At Week 6, subjects continued study drug dosing as planned.
		>1000 IU/mL (Virologic Failure)	At Week 6, subjects were discontinued from telaprevir, but continued Peg-IFN-alfa-2a and RBV dosing and continued on study until Week 72.
Week 12 (EVR Assessment)	All subjects	≥2-$\log_{10}$ decrease in HCV RNA at Week 12 compared to baseline[a] (EVR)	At Week 14, subjects in Pbo/PR48 group continued study drug dosing as planned. Subjects in T8/PR and T12/PR group underwent the Week 12 Virologic Failure Assessment (see below).
		<2-$\log_{10}$ decrease in HCV RNA at Week 12 compared to baseline[a]	At Week 14, subjects discontinued all study drugs, but continued on study until Week 72 and participated in all required follow-up procedures.[a]
Week 12 (Virologic Failure Assessment)	T8/PR and T12/PR subjects with HCV RNA ≤1000 IU/mL at Week 4	HCV RNA ≤1000 IU/mL	At Week 14, subjects continued study drug dosing as planned.
		HCV RNA >1000 IU/mL (Virologic Failure)	At Week 14, subjects continued Peg-IFN-alfa-2a and RBV dosing and continued on study until Week 72.[b]
Week 24	T8/PR and T12/PR subjects who achieved eRVR	Undetectable at Week 4 and Week 12 (eRVR)	Between Weeks 23 and 26, investigator was informed that planned treatment duration was 24 weeks. Subjects were discontinued from all study drugs, but continued on study until Week 72 and participated in a Safety Follow-up Visit.[a]
	All other subjects	Undetectable HCV RNA	After Week 24 (by Week 26), investigator was informed that planned treatment duration was 48 weeks and subjects continued on study drug dosing.
		Detectable (HCV RNA >10 IU/mL, Virologic Failure)	After Week 24 (by Week 26), investigator was informed that planned treatment duration was 48 weeks, subjects were discontinued from all study drugs, but continued on study until Week 72 and participated in all required follow-up procedures.[a]
Weeks 28, 36 and 40	All subjects still receiving treatment	Undetectable HCV RNA	Subjects continued study drug dosing as planned.
		Detectable (HCV RNA >25 IU/mL, Virologic Failure)	As soon as HCV RNA results were available, subjects discontinued all study drugs, but continued on study until Week 72 and participated in all required follow-up procedures.[a,c]

EVR: early viral response.
Note: EVR:is defined as ≥2--$\log_{10}$ decrease in HCV RNA at Week 12 compared to baseline. Virologic failure was defined as meeting virologic stopping rules or having detectable HCV RNA at the end of treatment.

[a] Follow-up procedures included a Safety Follow-up visit (including HCV RNA and viral sequencing samples) 4 weeks after last actual dose of study drug and testing of HCV RNA and collecting of viral sequencing samples at 4 and 24 weeks after becoming detectable.

[b] For subjects who had virologic failure at Week 12, there was no treatment modification. This classification was used in the analysis.

[c] If HCV RNA was detectable, but <1000 IU/mL, the investigator could have repeated the HCV RNA assessment to confirm the value before making changes to treatment.

Of the 709 subjects in the telaprevir treatment groups, 201 (28%) did not achieve an SVR (112 of 356 in the T8/PR group and 89 of 353 in the T12/PR group) (Table 16). Among subjects who did not have SVR24, the most frequently observed outcome was discontinuation due to virologic stopping rules (Table 17). Overall, the proportion of subjects with on-treatment virologic failure (No SVR) was higher in the T8/PR group than in the T12/PR group. This difference can be attributed to higher on-treatment virologic failure during the PR treatment phase. During the telaprevir treatment phase, the virologic failure (subjects who met the Week 4 or the Week 12 stopping rules) was similar in the T8/PR and T12/PR group: 2.8% in the T8/PR group and 3.4% in the T12/PR group (Table 16). The proportion of subjects with on-treatment virologic failure after Week 12 during the PR treatment phase (subjects who met the Week 24 or 36 stopping rules or had detectable HCV RNA at EOT) was higher in the T8/PR group than in the T12/PR group: 16% versus 10% (Table 16 and 17). Relapse rates were similar between the T8/PR and T12/PR arms (8% vs. 7%).

Table 16. Summary of No SVR Failures: Study 108 (% of all subjects/arm)

	T8/PR n=356	**T12/PR n=353**	**PR n=355**
No SVR24	112 (31%)	89 (25%)	201 (57%)
Failure during T/PR	10 (2.8%)	12 (3.4%)	43 (12%)
Failure during PR	58 (16%)	37 (10%)	88 (25%)
Relapse	28 (8%)	25 (7%)	63 (18%)
Relapse with eRVR	18 (5%)	14 (4%)	0
Relapse No eRVR	10 (3%)	11 (3%)	63 (18%)

Response rates and time of failure (e.g. virologic stopping rules) are analyzed by subtype and treatment arms in Table 17 and summarized in Table 18. The rates of No SVR were higher in subjects with subtype 1a viruses than subtype 1b viruses across treatment arms (Tables 17 and 18). This increase occurs from higher rates of failure on T/PR before Week 12 in subtype 1a viruses. In the T8/PR arm, there were higher rates of failure for genotype 1a subjects No SVR failures, compared to genotype 1b No SVR failures, both on T/PR and PR after Week 12. However, relapse rates were higher for subtype 1b across the treatment arms. In addition, relapse rates were similar in subjects in T/PR arms who achieved an eRVR and completed a 24-week treatment regimen to subjects who did not achieve an eRVR and completed a 48-week treatment regimen (Table 18).

Table 17. Treatment Outcomes in Study 108

	T8/PR		**T12/PR**		**PR**	
	GT 1a N=212	**GT 1b N=144**	**GT 1a n= 215**	**GT 1b N=138**	**GT 1a n=208**	**GT 1b N=147**
SVR24	139 (66%)	105 (73%)	157 (73%)	109 (79%)	85 (41%)	69 (47%)
RVR	133 (63%)	103 (72%)	141 (66%)	97 (70%)	22 (10%)	10 (6%)

eRVR	120 (57%)	83 (58%)	126 (59%)	80 (58%)	20 (10%)	8 (5%)
No SVR (n=400)	73 (34%)	39 (27%)	59 (27%)	30 (22%)	123 (59%)	78 (53%)
WK 4 VF	6 (8%)	0	5 (9%)	1	-	-
WK 12 VF	2 (3%)	2 (5%)	4 (7%)	2 (7%)	30 (24%)	13 (17%)
WK 24 VF	17 (23%)	5 (13%)	10 (17%)	3 (10%)	32 (26%)	24 (31%)
WK 28 VF			1		1	1
WK 36 VF	2 (3%)					1
EOT	22 (30%)	13 (33%)	14 (24%)	9 (30%)	20 (16%)	9 (12%)
RELAPSE	16 (22%)	12 (31%)	15 (25%)	10 (33%)	36 (29%)	27 (35%)
OTHER	8	7	10	5	4	3

Table 18. Summary of No SVR Failures by Time of Failure and Subtype: Study 108

	T8/PR		**T12/PR**		**PR**	
	GT 1a N=212	**GT 1b N=144**	**GT 1a n= 215**	**GT 1b N=138**	**GT 1a n=208**	**GT 1b N=147**
No SVR (n=400)	73 (34%)	39 (27%)	59 (27%)	30 (22%)	123 (59%)	78 (53%)
Failure **≤WK12** T/PR	8 (11%)	2 (5%)	9 (15%)	3 (10%)	30 (24%)	13 (17%)
Failure **>WK12 PR**	41 (56%)	17 (44%)	25 (42%)	12 (40%)	53 (43%)	35 (45%)
Relapse	16 (22%)	12 (31%)	15 (25%)	10 (33%)	36 (29%)	27 (35%)
Relapse with eRVR	9 (12%)	9 (23%)	7 (12%)	7 (23%)	0	0
Relapse No eRVR	7 (10%)	3 (8%)	8 (14%)	3 (10%)	36 (29%)	27 (35%)

In Study 108, of those who achieved RVR, approximately 77-79% achieved SVR24 in the T8/PR arm compared to 84-86% in the T12/PR arm (Table 19). Of those who did not achieve RVR, approximately 50% of subtype 1a and 60% of subtype 1b achieved SVR24 with slightly higher SVR24 proportions in the T12/PR arm than in the T8/PR arm.

Table 19. Analysis of RVR and SVR24 Status in Study 108

Subtype		**T8/PR**	**T12/PR**	**PR**
1a	**RVR and SVR24**	102/133 (77%)	118/141 (84%)	20/22 (91%)
	RVR no SVR24	31/133 (23%)	23/141 (16%)	2/22 (9%)
	No RVR but SVR24	37/79 (47%)	39/74 (53%)	65/186 (35%)
	No RVR or SVR24	42/79 (53%)	35/74 (47%)	121/186 (65%)
	eRVR and SVR24	96/212 (45%)	111/215 (52%)	19/208 (9%)
	SVR24 if SVR12 (% of SVR24)	139/141 (98%)	157/161 (98%)	85/87 (98%)
	SVR12 but no SVR24	2	4	2
1b	**RVR and SVR24**	81/103 (79%)	83/97 (86%)	10/10 (100%)
	RVR no SVR24	22/103 (21%)	14/97 (14%)	

	No RVR but SVR24	24/41 (59%)	26/41 (63%)	59/137 (43%)
	No RVR or SVR24	17/41 (41%)	15/41 (37%)	78/137 (57%)
	eRVR and SVR24	71/144 (49%)	71/138 (51%)	8/147 (5%)
	SVR24 if SVR12 (% of SVR24)	105/108 (97%)	108/110 (98%)	69/70 (99%)
	SVR12 but no SVR24	3	2	1
Overall	**SVR24 if SVR12 (% of SVR24)**	244/249 (98%)	265/271 (98%)	154/157 (98%)

Overall concordance between SVR12 and SVR24 is 98%.

STUDY 108 GENOTYPIC ANALYSIS

Population nucleotide sequence analysis of the HCV NS3•4A region was performed from subjects who did not achieve a SVR (on-treatment virologic failure, relapse, or detectable HCV RNA at time of early discontinuation of treatment) to investigate the emergence of viral variants with decreased susceptibility to telaprevir during dosing withT/PR in Study 108.

In the sponsor's analysis, for each subject who failed to achieve SVR in a telaprevir-containing regimen, a single time point to define the sequence that could be considered as representative of the viral population present at the time of failure (termed the 'post-nadir visit') was derived based the following algorithm (Source ADSQLST):

- if the latest nadir time point occurred during telaprevir treatment, the post-nadir sequence data that was latest in time during telaprevir treatment was used,
- if the latest nadir time point occurred after the end of telaprevir treatment, the first post-nadir sequence data was used.

This algorithm results in the post-nadir time point being the first time point with an HCV RNA level ≥1,000 IU/mL for subjects who had relapse or had viral breakthrough during Peg-IFN alfa-2a and RBV treatment (after the end of telaprevir dosing) and the last time point on telaprevir after nadir with an HCV RNA level ≥1,000 IU/mL for subjects who had viral breakthrough during telaprevir treatment, met a stopping rule, or had detectable HCV RNA at the end-of-treatment.

STUDY 108 BASELINE GENOTYPIC ANALYSES

The majority of subjects (94%; 1004/1064) had WT virus at baseline (i.e., without substitutions at V36, T54, R155, A156 or D168). A listing of the subjects with virus having these baseline substitutions is included in Appendix B. Five subjects' isolates had either a V36M substitution (119002 and 405012) or a R155K substitution (169017, 173004 and 179004) present by population nucleotide sequencing at baseline. These variants both confer phenotypic resistance to telaprevir in cell culture. The low prevalence (99.5%; 1075/1080) of these two telaprevir-resistant variants at baseline in Study 108 was similar to that previously observed in Phase 2 Studies. Telaprevir-resistant variants have rarely been observed (<1%) in the combined public Genbank and Vertex baseline HCV sequence databases (Table 20).

Table 20. Frequency of V36M and R155K HCV Genotype 1 in Genbank and Vertex Sequence Databases (as of December 2008)

HCV NS3 amino acid position	36		155	
Subtype	1a (n=2311)	1b (n=2935)	1a (n=2401)	1b (n=2976)
Wild-type	V 98. 44%	V 98.29%	R 98. 95%	R 99.86%
Resistant variant	M 0.41 %	M 0%	K 0.83%	K 0%

All 3 subjects with R155K variants at baseline had a continuous decline of HCV RNA and 2 subjects (169017 and 179001) completed T12/PR48 treatment and achieved an SVR. The other subject (173004) withdrew consent at Week 5 and discontinued treatment with a detected HCV RNA level of <25 IU/mL. Both of the subjects with V36M at baseline had a continuous decline of HCV RNA. Subject 405012 was in the T12/PR24 treatment group and achieved an SVR. Subject 119002 had a significant decline in HCV RNA and reached undetectable HCV RNA at Week 8, but had detectable HCV RNA and met a stopping rule at Week 24. The viral quasispecies remained unchanged from baseline and contained V36M with no additional telaprevir-resistant variants. These limited data are insufficient to determine if the presence of telaprevir-resistance associated substitutions V36M or R155K at baseline has an effect on virologic response, because response in these individuals may be driven by the PR background.

Response rates were examined in subjects with any baseline substitution at NS3 positions V36, T54, R155, A156 or D168 (Table 21). Interestingly, response rates for subjects with V36L, T54S or R155K substitutions at baseline were lower in the T8/PR arm than the T12/PR arm. Although these numbers are small and interpretations should be made cautiously, these data indicate that if baseline telaprevir substitutions are present, 8 weeks of telaprevir treatment may not be a sufficient duration to prevent subsequent failure.

Table 21. Response Rates by Baseline Substitution in Study 108

	T8/PR	T12/PR	PR
V36L	0/4	63% (5/8)	20% (1/5)
V36M		50% (1/2)	
R155K	0/1	2/2	
T54S	57% (8/14)	77% (10/13)	63% (5/8)
D168E	2/2	0/1	0/1
V55A	56% (5/9)	64% (7/11)	17% (1/6)
V55I	71% (5/7)	78% (7/9)	67% (4/6)
I170V (GT 1a)	70% (7/10)	67% (4/6)	50% (6/12)
I170T (GT 1a)	1/2		
A150V/I/L	47% (7/15)	83% (10/12)	62% (8/13)
V151A	25% (3/12)	75% (6/8)	70% (7/10)
Overall	69%	75%	43%

STUDY 108 TREATMENT EMERGENT SUBSTITUTIONS IN SUBJECTS WHO DID NOT ACHIEVE AN SVR

NS3 amino acid substitutions V36M, A or L, T54A or S, R155K or T, A156S, T or V and D168E emerged on telaprevir regimens most frequently in a pooled analysis of the Phase 3 studies (See "TREATMENT EMERGENT SUBSTITUTIONS IN SUBJECTS WHO DID NOT ACHIEVE AN SVR FROM POOLED PHASE 3 STUDIES" in this review). These amino acid substitutions have been shown to confer 4- to 20-fold decreased susceptibility to telaprevir for V36M/A, T54A or S, R155K or T, A156S and 60-fold decreased susceptibility for V36M+R155K and A156T or V (See "PHENOTYPIC ANALYSIS" in this review). Thus, we focused our genotypic resistance analysis on these treatment-emergent substitutions in the subjects who did not achieve SVR24.

The number of treatment-emergent substitutions in subtype 1a and 1b were determined at failure before or after Week 12 and relapse. Overall, the proportion of substitutions that emerged on treatment was comparable between the T8/PR and T12/PR arms with more substitutions emerging in subtype 1a than 1b. Substitutions emerged on treatment in over 50% of the failures with GT 1a and about 40% of GT 1b. Most of the treatment-emergent substitutions emerged during breakthrough on PR after Week 12, which correlates with the higher proportion of failures during breakthrough on PR after WK 12 (Table 22).

Table 22. Treatment-Emergent Substitutions by Subtype and Time of Failure: Study 108

	# with Treatment Emergent Substitutions					
	T8/PR N=356			T12/PR N=353		
	GT 1a N=212	GT 1b N=144	All N=365	GT 1a N=215	GT 1b N=138	All N=353
No SVR Total (n=201)	73 (34%)	39 (27%)	112 (31%)	59 (27%)	30 (22%)	89 (25%)
Treatment Emergent Substitutions	41 (56%)	17 (44%)	58 (52%)	34 (59%)	11 (38%)	45 (51%)
Breakthrough on T/PR	**8 (20%)**	**1 (6%)**	**9 (16%)**	**9 (26%)**	**2 (18%)**	**11 (24%)**
Breakthrough on PR	**24 (59%)**	**9 (53%)**	**33 (57%)**	**15 (44%)**	**4 (36%)**	**19 (42%)**
Relapse	**9 (22%)**	**7 (41%)**	**16 (28%)**	**10 (29%)**	**5 (45%)**	**15 (33%)**

Treatment-emergent substitutions were also analyzed by proportion of failures at each failure timepoint group - before or after Week 12 or relapse. Interestingly, almost all of the ≤WK12 breakthrough failure isolates had treatment-emergent substitutions (Table 23); most of the 1a subtypes had a combination of V36M/R155K and the 1b subtypes had V36A or M, V36A+T54S/T or T54S+A156S. In the failures who had failure during PR treatment after Week 12, approximately 60% of the failure isolates had treatment-emergent substitutions (Table 23) with a combination of V36M/R155K in most of the 1a

subtype failures and combinations of V36A or L, T54A or S and A156S or T substitutions in the 1b subtype failures.

The majority of failure isolates in subjects who relapsed had treatment-emergent substitutions (Table 23). Most of the relapse subtype 1a variants had the R155K substitution often in combination with V36M or T54A or S while most relapse subtype 1b variants had T54A.

Table 23. Proportion of Treatment-Emergent Substitutions at Each Failure Timepoint: Study 108

	T8/PR N=356		T12/PR N=353	
	GT 1a N=212	GT 1b N=144	GT 1a N=215	GT 1b N=138
% Failure at:				
≤WK12 on treatment	8/8 (100%)	1/2 (50%)	9/9 (100%)	2/2 (100%)
>WK12 PR treatment	24/41 (59%)	9/17 (53%)	15/24 (63%)	4/12 (33%)
Relapse	9/16 (56%)	7/12 (58%)	10/15 (67%)	5/10 (50%)

The proportion of treatment-emergent substitutions in subjects who had RVR was similar between the T8 and T12 arms 40% vs. 38% (Table 24). There were more treatment-emergent substitutions in subjects who did not have RVR (60% and 62% in T8 and T12 arm, respectively).

Table 24. Treatment-Emergent Substitutions by RVR

	# with Treatment-Emergent Substitutions in NO SVR Subset					
	T8/PR N=356			T12/PR N=353		
	GT 1a N=212	GT 1b N=144	All N=356	GT 1a N=215	GT 1b N=138	All N=353
No SVR Total (n=201)	73 (34%)	39 (27%)	112 (31%)	59 (27%)	30 (22%)	89 (25%)
Treatment Emergent Substitutions	41 (56%)	17 (44%)	58 (52%)	34 (59%)	11 (38%)	45 (51%)
RVR	13	10	23 (40%)	14	3	17 (38%)
No RVR	28	7	35 (60%)	20	9	29 (62%)

The V36M and R155K substitutions emerged most frequently in subtype 1a failures often in combination with each other (Table 25). The R155K substitution only emerged in the subtype 1a failures. The V36M+R155K substitutions emerged in a higher proportion of No SVR failures in the T12/PR arm than in the T8/PR arm. The V36A and T54A substitutions emerged most frequently in the subtype 1b failures. The V36A substitution exists in combination with T54S or A and the T54A substitution only

emerged in subtype 1b failures. The D168D/N substitution mixture emerged in combination with V36L+T54S in one subject subtype 1a in the T8 arm.

In the relapsers, 45% (30/67) had wildype virus; 32% (10/31) of the relapsers with substitutions had the combinations of V36M+R155K, V36M+T54S, or T54S+R155K and the remaining had emergent substitutions R155K, V36M/A/L or A156S. Most subtype 1b relapsers had emergent V36A/L or T54A substitutions.

Table 25. Specific Treatment-Emergent Substitutions in Study 108

	# with Treatment-Emergent Substitutions					
	T8/PR N=356			T12/PR N=353		
	GT 1a N=212	GT 1b N=144	All N=356	GT 1a N=215	GT 1b N=138	All N=353
No SVR Total (n=201)	73 (34%)	39 (27%)	112 (31%)	59 (27%)	30 (22%)	89 (25%)
V36M	24 (33%)	3 (8%)	27 (24%)	28 (48%)	0	28 (31%)
V36L	5 (7%)	0	5 (4%)	0	3 (10%)	3 (3%)
V36A	5 (7%)	6 (15%)	11 (10%)	3 (5%)	4 (14%)	7 (8%)
T54S	3 (4%)	3 (8%)	6 (5%)	6 (10%)	2 (7%)	8 (9%)
T54A	0	8 (21%)	8 (7%)	0	5 (17%)	5 (6%)
R155K	35 (48%)	0	35 (31%)	30 (52%)	0	30 (34%)
R155T	4 (5%)	0	4 (4%)	1 (2%)	0	1 (1%)
A156T	1 (1%)	3 (8%)	4 (4%)	2 (3%)	2 (7%)	4 (5%)
A156V	0	1 (3%)	1 (1%)	0	1 (3%)	1 (1%)
A156S	1 (1%)	2 (5%)	3 (3%)	1 (2%)	1 (3%)	2 (2%)
D168N/D*	1 (1%)	0	1 (1%)	0	0	0
V36M + R155K	23 (32%)	0	23 (21%)	24 (41%)	0	24 (27%)
V36L + R155K	2 (3%)	0	0	0	0	0
Two	28 (38%)	2 (5%)	30 (27%)	29 (50%)	3 (10%)	32 (36%)
Three	2 (3%)	0	2 (2%)	3 (5%)	0	3 (3%)
Total Emergent Substitutions	41 (56%)	17 (44%)	58 (52%)	34 (59%)	11 (38%)	45 (51%)

*with V36L + T54S
V36A exists with T54S or A in geno 1b

STUDY VX08-950-111: TREATMENT-NAIVES

TREATMENT OUTCOMES IN STUDY 111

Of the 534 subjects in the Study 111 dataset, 317 achieved an eRVR and were randomized into the T12/PR24/eRVR+ (n=159) or T12/PR48/eRVR+ (n=158) group. Of these subjects, 146 (92%) in the T12/PR24/eRVR+ group and 143 (91%) in the T12/PR48/eRVR+ group achieved an SVR. For the 118 subjects who did not have eRVR (eRVR- T12/PR48 group), 78 (66%) achieved an SVR. There were 99 subjects who did not reach the Week 20 randomization timepoint (Other group), and 26 (26%) of these subjects achieved an SVR.

Among subjects who did not have SVR24, the most commonly observed outcomes were discontinuation due to virologic stopping rules and discontinuation with undetectable HCV RNA at EOT without SVR. Subjects who did not achieve SVR are subdivided into the following outcomes:

- on-treatment virologic failure (including stopping rules described in Table 26) and viral breakthrough,
- detectable HCV RNA at the end of treatment without viral breakthrough;
- relapse;
- undetectable HCV RNA at end of treatment and discontinued study before SVR.

Stopping Rules

The Study 111 protocol included several stopping rules designed to prevent continuation of treatment in subjects who did not meet certain HCV RNA response criteria. At Week 4, subjects who had HCV RNA >1,000 IU/mL discontinued telaprevir, and were eligible to receive a total of 48 weeks of Peg-IFN alfa-2a and RBV. Subjects who did not have an early viral response (EVR) (a 2-$\log_{10}$ decrease from baseline in HCV RNA level at Week 12) discontinued treatment. All subjects who received treatment beyond Week 24 were assessed for viral response at the Week 24, 28, or 36 visits. Subjects who had detectable HCV RNA at any of these times discontinued treatment, consistent in agreement with standard treatment practices for Peg-IFN alfa-2a and RBV. Although viral breakthrough was not included in the predefined stopping rules and was not monitored during this study as it was previously in Phase 2 studies, subjects who had viral breakthrough (≥1 $\log_{10}$ increase in HCV RNA from nadir or >100 IU/mL if previously undetectable: <25 IU/mL (HCV RNA not detected) were noted in the virologic analysis to investigate the pattern of HCV RNA response in subjects who met virologic stopping rules.

Table 26. Stopping Rules in Study 111

Study Week	HCV RNA Criteria	Actions to be Taken: Treatment and Procedural Modification
Week 4 Virologic Failure (blinded to investigator)	≤ 1000 IU/mL	Subject continues study drug dosing as planned.
	> 1000 IU/mL (Virologic Failure)	Subjects discontinued telaprevir, but continued Peg-IFN-alfa-2a and RBV dosing
Week 12 Virologic Failure[a] **EVR Assessment** (blinded to investigator)	< 2-$\log_{10}$ decrease in HCV RNA at Week 12 compared to baseline[a]	Subjects discontinued all study drugs and were required to complete a Treatment Discontinuation Study Visit and Post-Treatment Follow-Up visits.
	≥ 2-$\log_{10}$ decrease in HCV RNA at Week 12 compared to baseline[a] (EVR) or Undetectable HCV RNA (HCV RNA <10 IU/mL)	Subjects continued Peg-IFN-alfa-2a and RBV dosing as planned
Week 24 through Week 36 Virologic Failure (unblinded to investigator)	Undetectable HCV RNA (HCV RNA <10 IU/mL)	Subject continues study drug dosing as planned.
	Detectable (HCV RNA >10 IU/mL, Virologic Failure)[b]	Subjects discontinued all study drugs and were required to complete a Treatment Discontinuation Study Visit and Post-Treatment Follow-Up visits

[a] Baseline was calculated as the median of predose values
[b] If HCV RNA was detectable, but <1000 IU/mL, the investigator should have repeated the HCV RNA assessment and obtained a viral sequencing sample to confirm the value before making changes to treatment
Source: clinical study protocol (Appendix 16.1.1).

In Study 111, SVR24 rates were very high in subjects who achieved eRVR, approximately 90%. Likewise, the rate of on-treatment virologic failures was low in these randomized groups with eRVR: 2% (3/159) in the T12/PR24/eRVR+ group and 4% (6/158) in the T12/PR48/eRVR+ group. The percentage of subjects with on-treatment virologic failure was highest in the groups who did not achieve eRVR: 18% (21/118) in the T12/PR48 eRVR- arm and 54% (53/99) in the Other arm. For subjects who did not achieve an eRVR, most failed by breakthrough >WK12 on PR (Table 27). Relapse rates were also higher in the no eRVR groups with 8% for the T12/PR48 eRVR- arm and 13% for the Other group compared to 6% (9/158) for the T12/PR24 eRVR+ arm and 2% (3/158) for the T12/PR48 eRVR+ arm.

Interestingly, relapse rates were higher for subtype 1a failures in the T12/PR24 eRVR+ arm compared to T12/PR48 eRVR+ arm; in subtype 1a failures, the relapse rates were 7% (8/114) in the T12/PR24 eRVR+group and 0.8% (1/116) in the T12/PR48 eRVR+ group (Table 28). However, the No SVR failure numbers were small, so it is hard to make definitive conclusions. The applicant notes that, because of the study design and the Week 20 randomization, there was a 48 week period during which subjects could have relapse in the randomized T12/PR24/eRVR+ group (i.e. from >24 wk until the final 72 Week visit), but only 4 weeks in which they could have virologic failure. In contrast, in the randomized T12/PR48/eRVR+ group, virologic failure could occur during the 28 weeks of treatment, while relapse could occur only during the 24 weeks off treatment prior to the Week 72 visit. These differences in duration of treatment may have contributed to fact that there were numerically more relapses in the T12/PR24/eRVR+ group and numerically more virologic failures in the T12/PR48/eRVR+ group.

Table 27. Treatment Outcomes in Study 111 N=534

	T12/PR24 eRVR+ N=159		**T12/PR48 eRVR+ N=158**		**T12/PR48 eRVR- N=118**		**Other N=99**	
	GT 1a N=114	**GT 1b N=45**	**GT 1a n=116**	**GT 1b N=42**	**GT 1a n=85**	**GT 1b N=33**	**GT 1a n=72**	**GT 1b N=27**
SVR24 n=393	102 (89%)	44 (98%)	105 (91%)	38 (90%)	51 (60%)	27 (82%)	21 (29%)	5 (19%)
RVR	114	45	115	42	11	4	37 (51%)	15 (56%)
eRVR	114	45	115	42	0	0	22 (31%)	8 (30%)
No SVR (n=141)	12 (11%)	1 (2%)	11 (9%)	4 (10%)	34 (40%)	6 (18%)	51 (71%)	22 (81%)
WK 4 VF							7	1
WK 12 VF							3	1
WK 24 VF			2 (2%)	1 (2%)	16 (19%)			
WK 28 VF			1			1		
WK 36 VF					1			
DET EOT	3 (3%)		2 (2%)		2 (2%)	1 (3%)	20 (28%)	9 (33%)

RELAPSE	8 (7%)	1 (2%)	1 (0.8%)	2 (5%)	9 (11%)	1 (3%)	8 (11%)	5 (19%)
OTHER	1		5	1	6	3	13	6

Table 28. Summary of No SVR Subjects by Time of Failure and Subtype: Study 111

	T12/PR24 eRVR+ N=159		**T12/PR48 eRVR+ N=158**		**T12/PR48 eRVR- N=118**		**Other N=99**	
	GT 1a N=114	**GT 1b N=45**	**GT 1a n=116**	**GT 1b N=42**	**GT 1a n=85**	**GT 1b N=33**	**GT 1a n=72**	**GT 1b N=27**
No SVR (n=141)	12 (11%)	1 (2%)	11 (9%)	4 (10%)	34 (40%)	6 (18%)	51 (71%)	22 (81%)
≤WK12 on treatment							10 (14%)	2 (7%)
>WK12 PR treatment	3 (3%)		5 (4%)	1 (2%)	19 (22%)	2 (6%)	30 (42%)	11 (41%)
Relapse	8 (7%)	1 (2%)	1 (0.8%)	2 (5%)	9 (11%)	1 (3%)	8 (11%)	5 (19%)

In Study 111, the concordance between SVR12 and SVR24 in the eRVR+ randomized groups was 99% (Table 29). In the T12/PR48 eRVR- and Other arms the concordance was 96% and 90%, respectively.

Table 29. Analysis of SVR24 Status in Study 111

Subtype		**T12/PR24 eRVR+ N=159**	**T12/PR48 eRVR+ N=158**	**T12/PR48 eRVR- N=118**	**Other N=99**
1a	**SVR24 if SVR12 (% of SVR24)**	98% (102/104)	98% (105/107)	96% (51/53)	95% (21/22)
	SVR12 but no SVR24	2	2	2	1
1b	**SVR24 if SVR12 (% of SVR24)**	100% (44/44)	100% (38/38)	96% (27/28)	71% (5/7)
	SVR12 but no SVR24			1	2
Overall	**SVR24 if SVR12 (% of SVR24)**	99% (146/148)	99% (143/145)	96% (78/81)	90% (26/29)

Overall concordance in all three phase 3 trials between SVR12 and SVR24 is 98%.

STUDY 111 GENOTYPIC ANALYSIS

Population nucleotide sequence analysis of the HCV NS3•4A region was performed from subjects who did not achieve a SVR (on-treatment virologic failure, relapse, or detectable HCV RNA at time of early discontinuation of treatment) to investigate the emergence of viral variants with decreased susceptibility to telaprevir during dosing withT/PR in Study 111.

STUDY 111 BASELINE GENOTYPIC ANALYSIS

The majority of subjects (95%; 496/523) had WT virus at baseline (i.e., without amino acid substitutions at V36, T54, R155, A156 or D168). The substitutions T54S (n=12) and V36L (n=9) were present at baseline most frequently. Four subjects with the V36L at baseline did not achieve SVR; two relapsed and two were detectable at EOT (Table 30). None of these 4 subjects had eRVR. One subject with T54S at baseline was detectable at EOT and was in the Other group. However, most subjects in Study 111 with these substitutions at baseline were able to achieve SVR with a T/PR regimen especially those who achieved an eRVR (Table 31). One subject (156004) had a V36M substitution and 3 subjects (128002, 157004, and 165001) had a R155K substitution present by population sequencing at baseline. All 4 of these subjects achieved an SVR after completing a telaprevir containing regimen with either 24- or 48-week of PR (Table 31).

Table 30. Baseline Substitutions of Subjects with NO SVR in Study 111

PID	ARM	Baseline Substitution	OUTCOME	Subtype
111-105-105004	T12/PR48/eRVR-	V36L	relapse	1a
111-152-152011	T12/PR48/eRVR-	V36L	DET EOT	1a
111-164-164009	T12/PR48/eRVR-	V36L	Relapse	1a
111-120-120011	Other	V36I	relapse	1a
111-139-139005	Other	V36L	DET EOT	1a
111-120-120017	Other	T54S	DET EOT	1a

Table 31. Response by Baseline Substitution in Study 111 (n=523)

	T12/PR24 eRVR+	T12/PR48 eRVR+	T12/PR48 eRVR-	Other
V36L	2/2	1/1	40% (2/5)	0/1
V36M		1/1		
V36I				0/1
R155K	1/1	1/1	1/1	
T54S	1/1	4/4	4/4	67% (2/3)
D168E	1/1		1/1	
V55A	4/4	3/3	2/2	75% (3/4)
V55I		1/1	3/3	1/1
I170V (GT 1a)	67% (2/3)	89% (8/9)	50% (2/4)	67% (2/3)
I170T (GT 1a)			1/1	
A150V/I/L	80% (4/5)	83% (5/6)	5/5	1/1
V151A	3/3	75% (3/4)	67% (2/3)	
V151T	0/1			

STUDY 111 TREATMENT EMERGENT SUBSTITUTIONS IN SUBJECTS WHO DID NOT ACHIEVE AN SVR

Nucleotide sequence analysis was performed on the subset of subjects who did not achieve SVR after treatment with a telaprevir-based regimen. The sequence of the full-length NS3 and 4A regions was determined in these subjects for samples with sufficient levels of HCV RNA (LOD of the sequencing assay was ~1,000 IU/mL HCV RNA). All the individual subjects in Study 111 whose virus had emerging telaprevir resistance-associated substitutions are listed in Appendix D.

The number of treatment-emergent substitutions at positions V36, T54, R155, A156 and D168 in subtype 1a and 1b were determined at breakthrough, before or after Week 12, and relapse (Table 32). In Study 111, a high percentage of No SVR failures had treatment-emergent substitutions but conclusions are difficult to make about differences between the arms because the numbers of No SVR failures are small.

Table 32. Treatment-Emergent Substitutions by Subtype and Failure: Study C111

	# with Treatment-Emergent Substitutions							
	T12/PR24 eRVR+ N=159		T12/PR48 eRVR+ N=158		T12/PR48 eRVR- N=118		Other N=99	
	GT 1a N=114	GT 1b N=45	GT 1a n=116	GT 1b N=42	GT 1a n=85	GT 1b N=33	GT 1a n=72	GT 1b N=27
No SVR Total (n=141)	12	1	11	4	34	6	51	22
Treatment Emergent Substitutions	**11 (92%)**	**1/1**	**5 (45%)**	**2 (50%)**	**27 (79%)**	**0**	**29 (57%)**	**9 (41%)**
Breakthrough on T/PR	**0**	**0**					**11 (38%)**	**1 (11%)**
Breakthrough on PR	**3 (27%)**	**0**	**4 (80%)**		**19 (70%)**		**12 (41%)**	**3 (33%)**
Relapse	**8 (73%)**	**1**	**1 (20%)**	**2**	**8 (30%)**		**6 (21%)**	**5 (56%)**

Treatment-emergent substitutions were also analyzed by proportion of failures at each failure timepoint group. Of the failures who had breakthrough on PR or relapsed on T12-containing regimens, 90% (46/51) had treatment-emergent substitutions. In the Other group, 92% (12/13) of the failures who discontinued telaprevir before Week 12 had breakthrough emergent substitutions in their isolates (Table 33).

Table 33. Proportion of Treatment-Emergent Subst at Each Failure Timepoint: Study 111

	# with Treatment-Emergent Substitutions							
	T12/PR24 eRVR+ N=159		T12/PR48 eRVR+ N=158		T12/PR48 eRVR- N=118		Other N=99	
	GT 1a N=114	GT 1b N=45	GT 1a n=116	GT 1b N=42	GT 1a n=85	GT 1b N=33	GT 1a n=72	GT 1b N=27

No SVR Total (n=141)	12	1	11	4	34	6	51	22
≤WK12 on treatment							11/11	1/2
>WK12 PR treatment	3/3		4/5		19/19	0/2	12/30	3/11
Relapse	8/8	1/1	1/1	2/2	8/9	0/1	6/8	5/5

As in Study 108, V36M and R155K and the combination of both emerged most frequently in 50-60% of Subtype 1a failures (Table 34). The D168N emerged in 2 subjects with the R155T or G in subtype 1a. In the subtype 1b failures, T54A emerged most frequently.

Treatment-emergent substitutions were also analyzed by proportion of failures at each failure timepoint group – failure after Week 12 or relapse. In the failures who failed during PR after Week 12, most subtype 1a failures had a V36M or R155K or combination of V36M/R155K and subtype 1b failures had combinations of V36A and T54A. Most of the relapse subtype 1a variants had a combination with V36M and R155K while relapse subtype 1b variants had substitutions V36A, T54A or A156S. In the T12/PR24/eRVR+ group, 9 subjects had telaprevir-resistant variants (V36A/M, n=3; T54A, n=1; R155G/K/T, n=4) and 1 subject had the combination V36M+R155K. In the T12/PR48/eRVR+ group, one subtype 1a variant had substitutions V36M+R155K and 2 subtype 1b variants had the substitution T54A. In the T12/PR48/eRVR- group, 2 relapse subject variants had the V36M+R155K combination and the other 6 relapse variants had single V36L, V36M or R155K or A156T substitutions.

Variants with A156A/T mixtures emerged early with or without V36 and R155 substitutions (often as mixtures) and then were overgrown by variants with V36M and R155K substitutions; A156T usually was not present with substitutions at V36 and R155. T54S usually emerged later in treatment after V36M and R155K.

Table 34. Specific Treatment-Emergent Substitutions in Study 111

	# with Treatment-Emergent Substitutions							
	T12/PR24 eRVR+ N=159		**T12/PR48 eRVR+ N=158**		**T12/PR48 eRVR- N=118**		**Other N=99**	
	GT 1a N=114	**GT 1b N=45**	**GT 1a n=116**	**GT 1b N=42**	**GT 1a n=85**	**GT 1b N=33**	**GT 1a n=72**	**GT 1b N=27**
No SVR (n=141)	12 (11%)	1 (2%)	11 (9%)	4 (10%)	34 (40%)	6 (18%)	51 (71%)	22 (81%)
V36M	4 (33%)	0	2 (18%)	0	21 (62%)	0	23 (45%)	1 (5%)
V36L	0	0	0	0	3 (9%)	0	1 (2%)	0
V36A	1 (8%)	0	1 (9%)	0	0	0	0	3 (14%)
T54S	0	0	0	0	0	0	4 (8%)	0
T54A	1 (8%)	1 (1/1)	0	2 (50%)	0	0	0	3 (14%)
R155K	5 (42%)	0	5 (45%)	0	19 (56%)	0	24 (47%)	0
R155T	1 (8%)	0	0	0	1 (3%)	0	1 (2%)	0

A156T	0	0	1 (9%)	0	4 (12%)	0	6 (12%)	0
A156V	0	0	0	0	0	0	0	0
A156S	0	0	0	0	0	0	0	4 (18%)
D168N*	1 (8%)	0	0	0	1 (3%)	0	0	0
V36M + R155K	4 (33%)	0	2 (18%)	0	20 (59%)	0	21 (41%)	1 (5%)
V36L + R155K	0	0	0	0	1 (3%)	0	1 (2%)	0
Two	2 (17%)	0	3 (27%)	0	18 (53%)	0	22 (43%)	2 (9%)
Three	0	0	0	0	0	0	8 (16%)	0
Total Emergent Substitutions	11 (92%)	1/1	5 (45%)	2 (50%)	27 (79%)	0	29 (57%)	9 (41%)

*with R155T or R155G

STUDY VX-950-C216: TREATMENT-EXPERIENCED TRIAL

VX-950-C216 was a randomized, double-blind, placebo-controlled Phase 3 study in subjects with genotype 1 chronic HCV infection who failed prior treatment with Peg-IFN (Peg-IFN alfa-2a or Peg-IFN alfa-2b) plus RBV. The trial consisted of a screening period of approximately 4 weeks, a 48-week treatment period, and a 24-week follow-up period.

Viral response during prior treatment was categorized as follows:

- **Prior Non-Responder**: never reached an undetectable HCV RNA level during or at the end of a prior course of Peg-IFN/RBV therapy
  - **Prior null-responder**: had <2 $\log_{10}$ drop in HCV RNA at Week 12 of previous therapy and never achieved undetectable HCV RNA levels while on treatment
  - **Prior partial-responder**: had ≥2 $\log_{10}$ drop in HCV RNA at Week 12 of previous therapy, but never achieved undetectable HCV RNA levels while on treatment
- **Prior Relapser**: had undetectable HCV RNA at the end of treatment but reverted to detectable levels of HCV RNA after stopping treatment in parent study

In Study C216, 662 subjects (308 prior null and partial responders and 354 prior relapsers) were randomized in a 2:2:1 ratio and treated in one of 3 treatment groups: 2 telaprevir regimens (T12/PR48, without delayed start (DS) of telaprevir, and T12(DS)/PR48, with DS of telaprevir, i.e. 4 weeks of Peg-IFN/RBV prior to start telaprevir) and one control group (Pbo/PR48).

TREATMENT OUTCOMES IN STUDY 216

Of the 262 subjects in the T12/PR48 arm in the Study 216 virology dataset, 168 (64%) achieved an SVR, and of the 262 subjects in the T12(DS)/PR48 arm, 173 (66%) achieved an SVR. Both telaprevir containing arms achieved a higher SVR rate in the treatment-experienced subjects than the control PR48, which had an SVR rate of 17%.

The SVR rate in the telaprevir treatment groups was higher in subjects with genotype 1b (72%; 172/239) than genotype 1a (59%; 169/285).

In the T12/PR48, 36% (94/262) of subjects did not achieve SVR comparable to the 34% (89/262) of subjects in the lead-in T12 (DS)/PR48 arm. Most of the on-treatment virologic failures met a virologic stopping rule (Table 35).

Table 35. C216 Virology Stopping Rules

Week	Subjects	HCV RNA Criterion	Treatment and Procedural Modification
4, 6 and 8 weeks after start of telaprevir (telaprevir arms)			
Week 4, 6, and 8 Week 8, 10 and 12	T12/PR48 T12(DS)/PR48	>100 IU/mL HCV RNA	Subjects were discontinued from telaprevir but continued Peg-IFN and RBV dosing and continued on study until Week 72
12 Weeks after start of telaprevir (telaprevir arms) or placebo (control group)			
Week 12 Week 16	T12/PR48 and Pbo/PR48 T12(DS)/PR48	<2-log decrease in HCV RNA compared to baseline	Subjects were discontinued from all study drugs but continued on study until Week 72 and participated in all required follow-up procedures[a]
Week 24	All subjects	Confirmed detectable HCV RNA (>25 IU/mL or <25 IU/mL detectable)[b,c]	Subjects were discontinued from all study drugs but continued on study until Week 72 and participated in all required follow-up procedures[a]
Week 36	All subjects	Confirmed detectable HCV RNA (>25 or <25 IU/mL detectable) [b,c]	Subjects were discontinued from all study drugs but continued on study until Week 72 and participated in all required follow-up procedures[a]

[a] Follow-up procedures included a Safety Follow-up visit (including HCV RNA and viral sequencing sampling) 4 weeks after last dose of study medication and HCV RNA testing and collecting of viral sequencing samples 24 weeks after the last dose of study medication and at SVR assessment time point (i.e., Week 72)
[b] If HCV RNA was detectable after previously being undetectable the HCV RNA assessment must be repeated within 4 weeks to confirm detectability before making changes to the treatment
[c] Note that in the protocol a different terminology was used: <10 IU/mL HCV RNA instead of <25 IU/mL undetectable HCV RNA

Response rates and time of failure (e.g. virologic stopping rules) are analyzed by subtype and treatment arms in Table 36 and summarized in Table 37. The No SVR failure rate was 44% in the no lead-in arm compared to 38% in the lead-in arm for genotype 1a failures. Interestingly, rates of failure before Week 12 were slightly higher in GT 1a subjects in the no lead-in arm: 15% vs. 9%. However, rates of failure after Week 12 on PR and relapse rates were similar between the two telaprevir arms and also between subtypes.

Table 36. Treatment Outcomes in Study 216 (N=652)

	T12/PR48 N=262		**T12(DS)PR48 N=262**		**PR48 N=128**	
	GT 1a N=136	**GT 1b N=126**	**GT 1a n=149**	**GT 1b N=113**	**GT 1a n=67**	**GT 1b N=61**
SVR24 n=363	76 (56%)	92 (73%)	93 (62%)	80 (71%)	14 (21%)	8 (13%)
RVR	65 (48%)	82 (65%)	3*	1*	2	1
eRVR	61 (45%)	79 (63%)	102 (68%)	76 (67%)	2 (3%)	1 (2%)
No SVR (n=289)	60 (44%)	34 (27%)	56 (38%)	33 (29%)	53 (79%)	53 (87%)
WK 4 VF	14	2	11	3		
WK 6 VF	3	2	2			

WK 8 VF	1	1				
WK 12 VF	3		1		17	18
WK 24 VF	10	5	10	4	18	10
WK 28 VF						
WK 36 VF	1		3	1		1
DET EOT	12	8	10	9	8	6
RELAPSE	13	13	15	12	10	18
OTHER	3	3	4	4		

***Small number with RVR because of 4 week PR Lead-In**

Table 37. Summary of No SVR Subjects by Time of Failure and Subtype: Study 216

	T12/PR48 N=262		**T12(DS)PR48 N=262**		**PR48 N=128**	
	GT 1a N=136	**GT 1b N=126**	**GT 1a n=149**	**GT 1b N=113**	**GT 1a n=67**	**GT 1b N=61**
No SVR (n=289)	60 (44%)	34 (27%)	56 (38%)	33 (29%)	53 (79%)	53 (87%)
Failure≤WK12 on treatment	21 (15%)	5 (4%)	14 (9%)	3 (3%)	17 (25%)	18 (30%)
Failure>WK12 PR treatment	23 (17%)	13 (10%)	23 (15%)	14 (12%)	26 (39%)	17 (28%)
Relapse	13 (10%)	13 (10%)	15 (10%)	12 (11%)	10 (15%)	18 (30%)

In Study 216, of those who achieved RVR in the T12/PR48, 77% of subtype 1a subjects and 84% of subtype 1b subjects achieved SVR24 (Table 38). Of those who did not achieve RVR in this arm, 37% of subtype 1a and 52% of subtype 1b achieved SVR24. This analysis could not be compared to the T12(DS)/PR48 arm because the RVR timepoint was not applicable with a 4 week lead-in phase.

Table 38. Analysis of RVR and SVR24 Status in Study 216

Subtype		**T12/PR48 N=262**	**T12(DS)/PR48 N=262**	**PR48 N=128**
1a	**RVR and SVR24**	50 (77%)	3	2
	RVR no SVR24	15 (23%)		0
	No RVR but SVR24	26 (37%)	90	12
	No RVR or SVR24	45 (63%)	56	53
	eRVR and SVR24	51 (84%)	87 (85%)	2
	SVR24 if SVR12 (% of SVR24)	99% (76/77)	97% (93/96)	14/14 (100%)
	SVR12 but no SVR24	1	3	0
1b	**RVR and SVR24**	69 (84%)	1	1
	RVR no SVR24	13 (16%)	-	-
	No RVR but SVR24	23 (52%)	79	7
	No RVR or SVR24	21 (48%)	33	53
	eRVR and SVR24	72 (91%)	65 (86%)	1
	SVR24 if SVR12 (% of SVR24)	97% (92/95)	99 % (80/81)	8/8 (100%)
	SVR12 but no SVR24	3	1	0

Overall	**SVR24 if SVR12 (% of SVR24)**	98% (168/172)	98% (173/177)	100% (22/22)

The reason for prior failure (i.e., null responder, partial responder or relapser) was an important factor in outcomes in Study 216. Over half the No SVR failure subjects in Study 216 were prior null responders with the remaining failure subjects divided approximately half between prior partial responders and relapsers (Table 39).

Table 39. Distribution of No SVR Failures by Prior Response

	T12/PR48			**T12(DS)PR48**		
Prior Response	**GT 1a N=136**	**GT 1b N=126**	**All N=262**	**GT 1a n=149**	**GT 1b N=113**	**All N=262**
No SVR	N=60 (44%)	N=34 (27%)	N=94 (36%)	N=56 (50%)	N=33 (29%)	N=89 (34%)
NULL	34 (57%)	17 (50%)	51 (54%)	30 (54%)	20 (61%)	50 (56%)
PARTIAL	13 (22%)	7 (21%)	20 (21%)	16 (29%)	6 (18%)	22 (25%)
RELAPSE	13 (22%)	10 (29%)	23 (24%)	10 (18%)	7 (21%)	17 (19%)

Table 40. Total Subjects in Each Prior Responder Group (n)

Prior Response	T12/PR48	T12(DS)/PR48	Pbo/PR48
NULL	72	75	37
PARTIAL	48	47	26
RELAPSE	142	140	65

The number of subjects in each prior responder group are listed in Table 40. In the T12/PR48 arm, 71% (51/72) of prior Null-responders, 42% (20/48) of prior partial responders and 16% (23/142) of prior relapsers did not achieve SVR (Table 41). In the T12(DS)/PR48 arm, 67% (50/75) of prior Null-responders, 47% (22/47) of prior partial responders and 12% (17/140) of prior relapsers did not achieve SVR.

Table 41. Proportion of No SVR Failures by Prior Response

Prior Response	**T12/PR48 N=262**	**T12(DS)/PR48 N=262**
NULL	**71% (51/72)**	**67% (50/75)**
PARTIAL	**42% (20/48)**	**47% (22/47)**
RELAPSE	**16% (23/142)**	**12% (17/140)**

STUDY 216 GENOTYPIC ANALYSIS

Population-based sequencing of the NS3•4A protease domain was performed to determine the amino acid composition of the predominant HCV quasispecies present in all subjects at baseline and at subsequent time points in those who did not achieve SVR after treatment with a telaprevir-based regimen. Sequence analysis of the full-length NS3•4A region was performed for samples with sufficient levels of HCV RNA (LOD of the sequencing assay ~ 1,000 IU/mL HCV RNA).

STUDY 216 BASELINE GENOTYPIC ANALYSIS

The majority of subjects (95%; 622/652) had WT virus at baseline (i.e., without substitutions at positions V36, T54, R155, A156 or D168). Twelve subjects had a baseline substitution at V36, 12 subjects had a T54S substitution at baseline, 4 subjects had an R155K, and 3 subjects had a D168E substitution at baseline.

Response rates were examined in subjects with any baseline substitution at NS3 positions V36, T54, R155, A156 or D168 (Table 42). The subjects who did not reach SVR and had baseline substitutions are listed in Table 43. Two subjects with a baseline V36M substitution (C216-0684 and C216-0393), both in the T12(DS)/PR48 arm, did not achieve SVR. Only 1 of 4 subjects (C216-0734, C216-0372, C216-0260, C216-0191) with a baseline R155K substitution achieved an SVR and that subject was in the T12(DS)/PR48 arm, while the other 3 were in the T12/PR48 arm (Table 42 and 43). Interestingly, all 3 of the subjects in the T12(DS)/PR48 arm with a V36L baseline substitution attained SVR, while 40% (2/5) of the subjects with baseline V36L in the T12/PR48 arm reached SVR. Of the 12 subjects with baseline T54S, 8 (67%) reached SVR and there did not appear to be a difference between arms (Table 42). All 3 subjects with the D168E substitution at baseline reached SVR and were in the T12/PR48 arm. Interestingly, response rates for subjects with V36L or R155K substitutions at baseline were lower in the T12/PR48 arm than the lead-in T12 (DS)/PR48 arm. However, these numbers are small and interpretations should be made cautiously.

Table 42. Response by Baseline Substitution in Study 216 (n=652)

	T12/PR48 N=262	**T12(DS)/PR48 N=262**	**PR48 N=128**
Any Major (V36, T54, R155, A156, D168)	**6/12 (50%)**	**8/13 (62%)**	2/3 (67%)
V36L	2/5 (40%)	3/3	0/1
V36M		0/2	
V36I			1/1
R155K	0/3	1/1	
T54S	2/3 (67%)	4/7 (57%)	1/1
D168E	3/3		
V55A	2/4 (50%)	2/3 (67%)	
V55I	1/1	1/4 (25%)	0/1
I170V (GT 1a)	2/4 (50%)	4/6 (67%)	0/3
A150V/I/L	8/10 (80%)	8/10 (80%)	3/11 (27%)
V151A	4/6 (67%)	0/3	0/3
V151T	-	-	-
Q80K	26/42 (62%)	18/37 (49%)	4/21 (19%)
Q80L	6/14 (43%)	4/6 (67%)	1/3 (33%)
Q80K/L/R	32/56 (57%)	23/44 (52%)	5/25 (20%)
Q41H	2/3	0/1	
Overall	162/254 (64%)	167/254 (66%)	21/122 (17%)

Table 43. Baseline Substitutions of Subjects with NO SVR in Study 216

PID	ARM	PRIOR RESPONSE	Baseline Substitution	OUTCOME	Subtype
C216-0052	T12 (DS)	PARTIAL	I170V	DET EOT	1a
C216-0083	T12 (DS)	NULL	I170V	DET EOT	1a
C216-0094	PBO	NULL	I170V	WK12	1a
C216-0125	Pbo/PR48	RELAPSER	V36L	WK 24	1a
C216-0191	T12	PARTIAL	R155K	WK4	1a
C216-0224	T12 (DS)	NULL	T54S	WK4	1a
C216-0260	T12	NULL	V36L R155K I170V	WK4	1a
C216-0273	T12	NULL	V36L	WK4	1a
C216-0322	PBO	NULL	1170V	WK12	1a
C216-0334	T12 (DS)	NULL	T54S	WK4	1a
C216-0372	T12	NULL	R155K/R	WK4	1a
C216-0393	T12 (DS)	RELAPSE	V36M	DET EOT	1a
C216-0439	T12	RELAPSE	V36L	RELAPSE	1b
C216-0442	T12 (DS)	NULL	T54S	WK4	1a
C216-0684	T12 (DS)	RELAPSE	V36M	LOST TO FU	1a
C216-0753	T12	PARTIAL	I170V/I	WK24	1a
C216-0771	PBO	NULL	I170V	WK12	1a
C216-0797	T12	RELAPSE	T54S	LOST FU	1b

We performed an analysis to try to determine the effect of the background PR therapy on telaprevir resistance-associated baseline substitutions using the Week 4 response in the lead-in arm (Table 44). Making interpretations from this analysis are difficult because the numbers of subjects with baseline substitutions in this arm are very small. In subjects with a poor early response to PR, the SVR rates are comparable between subjects with baseline telaprevir substitutions and subjects without baseline substitutions. In subjects with a good early response to PR with ≥2 $\log_{10}$ decrease from baseline at Week 4, SVR rates were lower (70%) for subjects with baseline substitutions compared to 93% for subjects without baseline substitutions. These limited data indicate that the presence of baseline substitutions does not appear to be affected by the Week 4 response to background PR therapy, but may decrease response to telaprevir-containing regimens.

Table 44. The Effect of the Background PR Therapy on Telaprevir Resistance-Associated Baseline Substitutions Using the Week 4 Response in the Lead-In Arm in Study 216

Subject Population Analyzed	SVR Rate According to HCV RNA Decline through Treatment Week 4		
	<1 $\log_{10}$ IU/mL	<2 $\log_{10}$ IU/mL	≥2 $\log_{10}$ IU/mL
Telaprevir-Treated Subjects **with** Baseline Resistance Substitution(s) n=13	1/3 (33%)		7/10 (70%)
Telaprevir-Treated Subjects **without** Baseline Resistance Substitution(s)	22/68 (32%)	49/72 (68%)	78/84 (93%)
Control Arm Pbo/PR48	0/37 (0%)	6/40 (15%)	15/41 (37%)

STUDY 216 TREATMENT-EMERGENT SUBSTITUTIONS IN SUBJECTS WHO DID NOT ACHIEVE AN SVR

Nucleotide sequence analysis was performed on samples from subjects who did not achieve SVR after treatment with a telaprevir-based regimen. The number of treatment-emergent substitutions in subtype 1a and 1b were determined at failure before or after Week 12 and relapse (Table 45). Subjects in Study 216 with emerging substitutions in their HCV are listed in Appendix E. The proportion of treatment-emergent substitutions was similar between the T12 and T12 lead-in arm. There were more treatment-emergent substitutions in subtype 1a failures than 1b.

Overall, 70% of No SVR failures had treatment-emergent substitutions when they experienced failure on T/PR treatment or relapsed (Table 46), and the proportion of substitutions that emerged on telaprevir treatment was comparable between arms. There were more treatment-emergent substitutions before Week 12 in the subtype 1a failures of the T12/PR48 arm and more treatment-emergent substitutions after Week 12 in the subtype 1a failures of the T12(DS)/PR48 arm. This result is most likely due to the different timing of telaprevir dosing in each arm because of the PR lead-in phase. The proportion of substitutions emerging during relapse was also generally comparable between the two arms.

Table 45. Treatment-Emergent Substitutions by Subtype and Failure: Study C216

	# with Treatment-Emergent Substitutions					
	T12/PR48 N=262			T12(DS)PR48 N=262		
	GT 1a	GT 1b	All	GT 1a	GT 1b	All
No SVR Total (n=289)	60	34	94 (36%)	56	33	89 (34%)
Treatment-Emergent Substitutions	47 (78%)	19 (56%)	66 (70%)	47 (84%)	15 (45%)	62 (70%)
Failure on T/PR≤WK12	21 (45%)	5 (26%)	26 (39%)	14 (30%)	3 (20%)	17 (27%)
Failure on PR>WK12	16 (34%)	7 (37%)	23 (35%)	21 (45%)	5 (33%)	26 (42%)
Relapse	10 (21%)	7 (37%)	17 (26%)	12 (26%)	7 (47%)	19 (31%)

Table 46. Proportion of Treatment-Emergent Substitutions at Each Failure Timepoint: Study 216

	# with Treatment-Emergent Substitutions			
	T12/PR48 N=262		T12(DS)PR48 N=262	
	GT 1a	GT 1b	GT 1a	GT 1b
No SVR Total (n=289)	60	34	56	33
≤WK12 on treatment	21/21 (100%)	5/5 (100%)	14/14 (100%)	3/3 (100%)

>WK12 PR treatment	16/23(70%)	7/13 (54%)	21/23 (91%)	5/14 (36%)
Relapse	10/13 (77%)	7/13 (54%)	12/15 (80%)	7/12 (58%)

Consistent with the data that most of the No SVR failures (50-60%) were previous Null responders (Table 47), the previous null responders also had the most treatment-emergent substitutions (Table 48).

Table 47. Treatment-Emergent Substitutions by Prior Treatment: Study C216

	# with Treatment-Emergent Substitutions					
	T12/PR48 N=262			T12(DS)PR48 N=262		
Prior Response	**GT 1a N=47**	**GT 1b N=19**	**All N=66**	**GT 1a N=47**	**GT 1b N=15**	**All N=62**
NULL	30 (64%)	11 (58%)	41 (62%)	28 (60%)	12 (80%)	40 (65%)
PARTIAL	11 (23%)	1 (5%)	12 (18%)	14 (30%)	1 (7%)	15 (24%)
RELAPSE	6 (13%)	7 (37%)*	13 (20%)	5 (11%)	2 (13%)	7 (11%)

*V36L (n=2) or T54A (n=4) or A156N (n=1)

Table 48. Proportion of Treatment-Emergent Substitutions by Prior Treatment: Study C216

	# with Treatment-Emergent Substitutions					
	T12/PR48 N=262			T12(DS)PR48 N=262		
Prior Response	**GT 1a N=47**	**GT 1b N=19**	**All N=66**	**GT 1a N=47**	**GT 1b N=15**	**All N=62**
NULL	**30/34 (88%)**	**11/17 (65%)**	**41/51 (80%)**	**28/30 (93%)**	**12/20 (60%)**	**40/50 (80%)**
PARTIAL	**11/12 (92%)**	**1/7 (14%)**	**12/19 (63%)**	**14/16 (88%)**	**1/6 (17%)**	**15/22 (68%)**
RELAPSE	**6/13 (46%)**	**7/10 (70%)**	**13/23 (57%)**	**5/10 (50%)**	**2/7 (29%)**	**7/17 (41%)**

V36M and R155K and the combination of both emerged most frequently in GT 1a failures (Table 49). The V36A, T54S or A, and A156T emerged most frequently in GT 1b failures. The majority of subjects failing on telaprevir treatment had the combination of V36M+R155K in subtype 1a or A156T in subtype 1b; this is especially true of failures at the Week 4 and 8 stopping rules.

In the relapsers, 45% (30/67) had wildype virus; 20% (7/35) of the subtype 1a failures had the combination of V36M+R155K and the remaining 35% had emergent substitutions R155K, V36M/A/L or A156T/S/V. Most subtype 1b relapsers had emergent V36A/L or T54A substitutions. The D168N/D substitution emerged with substitutions V36M/A + R155R/T in two subtype 1a subjects in the T12/PR48 arm.

Table 49. Specific Treatment-Emergent Substitutions in Study 216

	# with Treatment Emergent Substitutions					
	T12/PR48 N=262			T12(DS)PR48 N=262		
	GT 1a	GT 1b	All	GT 1a	GT 1b	All
No SVR Total (n=289)	60	34	94 (36%)	56	33	89 (34%)
V36M	34 (57%)	0	34 (36%)	33 (59%)	1 (3%)	34 (38%)
V36L	6 (10%)	1 (3%)	7 (7%)	0	0	0
V36A	8 (13%)	5 (15%)	13 (14%)	2 (4%)	6 (18%)	8 (9%)
T54S	3 (5%)	4 (12%)	7 (7%)	8 (14%)	3 (9%)	11 (12%)
T54A	2 (3%)	5 (15%)	7 (7%)	2 (4%)	2 (6%)	4 (4%)
R155K	36 (60%)	1 (3%)	37 (39%)	40 (71%)	0	40 (45%)
R155T	5 (8%)	0	5 (5%)	3 (5%)	0	3 (3%)
A156T	3 (5%)	4 (12%)	7 (7%)	5 (9%)	4 (12%)	9 (10%)
A156V	1 (2%)	1 (3%)	2 (2%)	1 (2%)	2 (6%)	3 (3%)
A156S	0	1 (3%)	1 (1%)	4 (7%)	3 (9%)	7 (8%)
D168N/D*	2 (3%)	0	2 (2%)	0	0	0
V36M + R155K	27 (45%)	0	27 (29%)	29 (52%)	0	29 (33%)
V36L + R155K	5 (8%)		5 (5%)			
Two	32 (53%)	3 (9%)	35 (37%)	30 (54%)	3 (9%)	33 (37%)
Three	7 (12%)	1 (3%)	8 (9%)	10 (18%)		10 (11%)
Total Emergent Substitutions	47 (78%)	19 (56%)	66 (70%)	47 (84%)	15 (45%)	62 (70%)

*with V36M/A + R155R/T

GENOTYPIC ANALYSIS OF POOLED PHASE 3 STUDIES

The genotypic data from all 3 Phase 3 studies were pooled to examine:

i) whether telaprevir resistance-associated baseline substitutions affected SVR rates on telaprevir-containing regimens and
ii) which treatment emergent substitutions emerged most frequently on telaprevir-containing regimens.

GENOTYPIC BASELINE ANALYSIS FROM COMBINED PHASE 3 STUDIES

A pooled analysis was conducted to explore the association between the presence of baseline NS3/4A amino acid substitutions or polymorphisms detected by population sequencing and treatment outcome in the the 3 Phase 3 studies 108, 111 and 216. Baseline polymorphisms at NS3 positions Q41R, Q80K, L, or R, and V170T, which have been observed in HCV genotype 1a-infected patients and have been reported to reduce the activity of some HCV NS3/4A protease inhibitors in development, did not appear to be associated with reduced efficacy to telaprevir. At baseline, telaprevir-associated resistance substitutions (substitutions at positions V36, T54, R155, A156 or D168) were

present in 5% (117/2239) of the subjects in the combined Phase 3 studies. Given the small number of baseline telaprevir resistance substitutions, it is difficult to make conclusions on response outcomes when these substitutions are present at baseline (Table 50).

Table 50. Response by Baseline Substitution in Phase 3 Studies 108, 111, and 216 (without Lead-in DS arm from Study 216)

	T8	T12	PR
Overall	69% (244/356)	76% (787/1033)	37% (175/477)
V36L	0/4	63% (15/24)	17% (1/5)
V36I		1/1	1/1
V36M		40% (2/5)	
Q41R	67% (2/3)	70% (7/10)	0/1
F43S	0	0	0
R155K	0/1	67% (6/9)	
T54S	57% (8/14)	78% (25/32)	67% (6/9)
D168E	2/2	80% (4/5)	0/1
V55A	56% (5/9)	74% (20/27)	17% (1/6)
V55I	71% (5/7)	72% (13/18)	57% (4/7)
Q80K	65% (58/89)	77% (203/264)	37% (37/100)
Q80L	75% (6/8)	54% (13/24)	38% (3/8)
Q80R	67% (2/3)	78% (7/9)	(1/3)
P96S	1/1	1/1	0/2
V107I	0	50% (2/4)	67% (2/3)
A150V/I/L	47% (7/15)	83% (40/48)	46% (11/24)
V151A	25% (3/12)	64% (18/28)	54% (7/13)
I170V (GT 1a)	70% (7/10)	69% (22/32)	40% (6/15)
I170T (GT 1a)	1/2	1/1	

TREATMENT-EMERGENT SUBSTITUTIONS IN SUBJECTS WHO DID NOT ACHIEVE AN SVR FROM POOLED PHASE 3 STUDIES

The majority of isolates from subjects who did not achieve SVR had telaprevir resistance-associated treatment-emergent substitutions. In total, 62% of the No SVR T/PR subjects' isolates had an emerging substitution at positions V36, T54, R155, V156 or D168 (Table 51). More treatment failures were subtype 1a than 1b. The most frequent emergent substitution in subtype 1a failures were V36M and R155K emerging in 48% and 54% of No SVR T/PR subjects, respectively (Tables 51 and 52). The combination of V36M + R155K emerged together in 40% of subtype 1a telaprevir failures. The most frequent emergent substitutions in subtype 1b failures were T54A or S, V36A, and A156T or S emerging in 6 to 15% of the No SVR T/PR subjects, (Tables 51 and 52).

Table 51. Treatment-Emergent Substitutions in Pooled Phase 3 Studies (n=2266 Total Subjects; Subjects Who Did Not Achieve SVR24 in T/PR arms (n=525))

Emerging Substitutions in NS3	% of No SVR T/PR Subjects n=525	% GT 1a No SVR Subjects N=356	% GT 1b No SVR Subjects N=169	PIDs of Key Isolates
Any substitution at V36, T54, R155, V156 or D168	324 (62%)	247 (69%)	77 (46%)	
Frequently Emergent Telaprevir Resistance Substitutions				
R155K	193 (37%)	192 (54%)	1 (0.6%)	GT 1b: VX-950-C216-0074
V36M	175 (33%)	170 (48%)	5 (3%)	
V36M + R155K	142 (27%)	142 (40%)		
V36A	40 (8%)	17 (5%)	23 (14%)	
V36L	4 (0.8%)	4 (1%)		
V36G/I	3 (0.6%)		3 (2%)	
T54S	25 (5%)	14 (4%)	11 (7%)	
T54A	30 (6%)	5 (1%)	25 (15%)	
R155T	16 (3%)	16 (4%)		All GT 1a
R155M/G	2 (0.4%)	2 (0.6%)		
A156T	37 (7%)	23 (6%)	14 (8%)	GT 1a: n=23 and GT 1b: n=14
A156S	16 (3%)	6 (2%)	10 (6%)	GT 1a: n=6 and GT 1b: n=10
A156V/F/N	8 (2%)	1 (0.3%)	7 (4%)	GT 1a: n=1 and GT 1b: n=7
D168N	5 (1%)	5 (1%)		All GT 1a; 4/5 with R155T or G
R155T D168N	3 (0.6%)	3 (0.8%)		
Other Emerging Substitutions				
Q41R	1 (0.2%)	1 (0.3%)		VX-950-C216-0510
F43C	1 (0.2%)		1 (0.6%)	VX-950-C216-0429
P96L/S/A	6 (1%)	3 (0.8%)	3 (2%)	
V107I	2 (0.4%)	2 (0.6%)		VX08-950-111-104-104002; VX07-950-108-166-166007
G120S	2 (0.4%)	1	1	VX08-950-111-104-104007; VX07-950-108-403-403010
I132V	5 (1%)	5 (1%)		VX07-950-108-151-151003; VX07-950-108-301-301002; VX07-950-108-

				508-508004; VX07-950-108-705-705005; VX-950-C216-0207
V151A	3 (0.6%)	2 (0.6%)	1 (0.6%)	VX07-950-108-106-106006; VX07-950-108-311-311005; VX07-950-108-214-214002
I170V	9 (2%)	9 (3%)		
I170T	0			
I170A	0			
S181A	1 (0.2%)	1 (0.3%)		
M242I or V	2 (0.4%)	2 (0.6%)		VX07-950-108-162-162005; VX08-950-111-103-103002
K244R or E	12 (2%)	8 (2%)	4 (2%)	N=54 responders
P250S	1 (0.2%)	1 (0.3%)		VX07-950-108-119-119008
T305S/T	2 (0.4%)		2 (1%)	VX07-950-108-110-110006;VX-950-C216-0585
K360R	10 (2%)	5 (1%)	5 (3%)	N=41 responders
G362R/G	3 (0.6%)	3 (0.8%)		VX07-950-108-160-160008; VX-950-C216-0160; VX-950-C216-0386
S439T	1 (0.2%)	1 (0.3%)		VX07-950-108-709-709003; S439G n=3 responders
T449I	4 (0.8%)	3 (0.8%)	1 (0.6%)	VX-950-C216-0213; VX-950-C216-0330; VX-950-C216-0501; VX07-950-108-140-140008
T449A	2 (0.4%)		2 (1%)	VX-950-C216-0002; VX-950-C216-0821;
T505M	1 (0.2%)	1 (0.3%)		VX07-950-108-162-162005
P574L/S/Q	8 (2%)	6 (2%)	2 (1%)	N=44 responders
NS4A Emergent Substitutions				
A36V or A/V	7 (1%)	6 (2%)	1 (0.6%)	
E53E/K E53G E53E/V	9 (2%)	9 (3%)		

D168N Substitution Summary

Study 108: D168D/N emerged after Week 48 in 1 subtype 1a subject in the T8 arm (failed at Week 4 with baseline V36L and T54S).
Study 111: The D168N emerged in 2 subtype 1a subjects at Week 20 and Week 48 (1 each in T12/PR24 eRVR+ and T12/PR48 eRVR-) with the R155T or G in subtype 1a.
Study 216: The D168N/D substitution emerged with substitutions V36M/A and R155R/T during follow-up in two subtype 1a subjects (1 previous null at Wk 36 stopping rule; 1 previous partial responder who relapsed) in the T12/PR48 arm.

Table 52. Treatment-Emergent Substitutions in Pooled Phase 3 Studies: Subjects Who Did Not Achieve SVR24 in T/PR arms (n=525)

Emerging Substitutions in NS3	% of No SVR T/PR Subjects n=525	% GT 1A No SVR Subjects N=356	% GT 1B No SVR Subjects N=169
Any substitution at V36, T54, R155, V156 or D168	324 (62%)	247 (69%)	77 (46%)
R155K	193 (37%)	192 (54%)	1 (0.6%)
V36M	175 (33%)	170 (48%)	5 (3%)
V36M + R155K	142 (27%)	142 (40%)	
V36A	40 (8%)	17 (5%)	23 (14%)
T54A	30 (6%)	5 (1%)	25 (15%)
V36L/G/I, T54S, I132V R155T/M/G, A156T/S/V/F/N, D168N, I170V	1% to <10%	1% to <10%	1% to <10%

PHENOTYPIC ANALYSIS

Enzymatic and replicon-based phenotypic assays were performed to characterize substitutions identified in the HCV NS3 protease domain that were observed after treatment failure in clinical studies of telaprevir. The recombinant NS3 protease domain used in enzymatic assays was derived from a genotype 1a subject and the HCV replicon was in a genotype 1b background. The susceptibility to telaprevir of these protease variant enzyme and replicons was tested. In the HCV replicon-based phenotypic assay, the reduction in susceptibility to telaprevir conferred by a variant was defined by the increase in the EC_{50} value from wild-type and a less than 3-fold change from wild-type was not considered significant by the applicant because this was within the variability of the assay. FDA considers results of phenotypic assays to contribute to confirmation of a resistance association, but negative phenotypic results do not exclude a resistance association.

Generally, a good agreement was observed between the enzyme and replicon-based phenotypic assays for the NS3 protease variants that were tested in both assays using site-directed mutations. The mean fold increases in EC_{50} values for telaprevir to NS3 variants in the replicon cells are shown in Table 53. Variants V36M/A, T54A/S, R155K/T, and A156S conferred 3- to 25-fold decreases in telaprevir susceptibility, while the A156T/V and V36M+R155K variants conferred higher levels (>62-fold) of resistance to telaprevir in the replicon assay (Tables 53 and Appendix H). The V36L variant

conferred a 2.2-fold reduction in susceptibility to telaprevir. Multiple double variants with various combinations of a V36, T54, R155 or A156 substitutions [V36A+R155K/T, V36M+R155T, V36M/A+A156T, T54S+A156S/T, and T54A+A156S] were tested and all had >62-fold changes in susceptibility to telaprevir (Appendix H). The triple variant V36M+T54S+R155K also conferred a >62-fold decrease in telaprevir susceptibility.

The substitution D168N was detected often in combination with the R155T substitution in the Phase 3 clinical trial subtype 1a subjects who did not achieve SVR. The D168N variant was also tested for phenotypic changes alone and in combination with R155T in the replicon. The D168N variant alone did not confer decreased telaprevir susceptibility; however, the D168N+R155K conferred a 24-fold decrease in telaprevir susceptibility (Table 53). The two variants, I132V and V151A, observed at a higher frequency in the treatment failures in the Phase 3 trials than expected, are currently being assessed phenotypically.

Additionally, NS3 variants (Q41R, V55A, Q80R, R109K, and V170A) that have been reported to confer resistance to other protease inhibitors were tested for telaprevir susceptibility in replicon cells. Variants with R109K have been identified during cell culture resistance selection with SCH6. V55A and V170A have been observed in cell culture selection experiments and in clinical trials with boceprevir. NS3 protease variants Q41R, Q80R and D168A/V/N have been selected using macrocyclic protease inhibitors such as MK-7009, BILN-2061, ITMN-191and TMC-435350 in cell culture and in clinical trials. Less than 3-fold reductions in telaprevir susceptibility were detected for each of these single variants (Table 53).

Table 53. Phenotypic Susceptibility (EC_{50} fold change) of HCV Protease Variants in Replicons

	Telaprevir	Boceprevir	BILN-2061	ITMN-191	TMC-435350	MK-7009
V36M	7.0	3.2	1.0	1.8	2.1	1.8
V36A	7.4	3.8	1.2	1.8		
V36L	2.2	1.3	1.2	1.3		
T54A	6.3	3.8	0.7	1.1	1.0	1.1
R155K	7.4	7.3	250	82	18	116
R155T	20	12	456	10		
R155S	4.1	2.1	418	7.9		
R155I	24	7.7	26	1.3		
A156S	10	37	1.4		0.35	2.9
A156T	>62	46	222	5.3	33	60
A156V	>62	40	2041	6.1		
V36M+R155K	64	13	559	259	60	376
V36A+T54A	20	5.3	0.5	0.5		
V36M+A156T	>62	>55	>1600	12.1		
D168N	0.6					
D168V	0.3		1039	13		
D168A	0.4		380	34		
R155T+D168N	24					

Q41R	1.5					
V55A	2.1					
Q80R	0.5					
R109K	0.8	0.9	0.9	0.8		
V170A	2.6	3.9	1.4	0.8		

Cross-Resistance

The susceptibility of these protease variant enzyme and/or replicons to telaprevir and to 5 other HCV NS3•4A protease inhibitors [boceprevir, ciluprevir (BILN 2061), danoprevir (ITMN-191, RG-7227), TMC-435350 and vaniprevir (MK-7009)] was determined. Substitutions at HCV NS3 protease residue V36 (V36A/M/G/L), T54 (T54A), or both (V36A+T54A) conferred no significant reduction in susceptibility to BILN-2061 and ITMN-191. Similarly no significant reduction in susceptibility was observed for TMC-435350 and MK-7009 against a V36M and a T54A variant.

Substitutions at R155 conferred decreased susceptibility to BILN-2061, TMC-435350 and MK-7009 (Table 53). Substitutions at A156 and double variant with substitutions at V36 and R155 conferred high level resistance to all the HCV protease inhibitors tested (BILN-2061, ITMN-191, TMC-435350, and MK-7009).

Phenotypic characterization of NS3 variants observed with other classes of HCV protease inhibitors determined that Q41R, V55A, Q80R, R109K, D168A/V/N and V170A had <3-fold change in EC_{50} value.

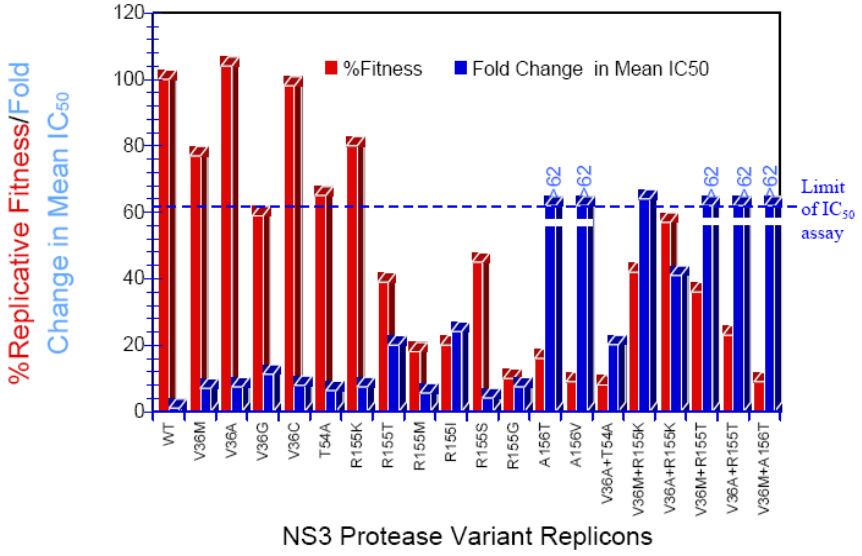
All variants (single or double) tested remained fully sensitive to nucleoside inhibitors PSI-6130 (RG-7128) and NM-107 and non-nucleoside inhibitors VX-222 (NS5B thumb domain), VRT-830353 (NS5B Palm 1 domain) HCV-796 (NS5B Palm 2 domain), and VRT-832554 (NS5B Finger Loop domain) in HCV replicon cells (Table 54).

In addition, the sensitivity of replicon variants to interferon alpha (IFN α) and RBV and to representative NS5B polymerase nucleoside [RG-7128, NM-107] and non-nucleoside (VX-222, HCV-796, VRT-832554 and VRT-830353) inhibitors was determined. The EC_{50} values of IFN α and ribavirin were determined against the HCV replicon cell lines in the standard 48-hr assay. The EC_{50} values of IFN α and RBV did not vary between WT and HCV replicon cells containing either single variants (V36M, V36A, T54A, R155K, R155T or R155M) or double variants (V36M+R155K, V36M+R155T, V36A+R155K, or V36A+R155T) [Range 0.3 – 1.3]. Thus, telaprevir resistant variants remain fully sensitive to IFN α and RBV. Furthermore, testing of NS3 protease variants against other NS5B inhibitors that include several NS5B nucleoside (PSI-6130 and NM-107) and non-nucleoside polymerase inhibitors (VX-222, HCV-796, VRT-830353 and VRT-832554) showed no cross-resistance with the telaprevir resistant variants (Table 54). In replicon assays, NS3 variants observed with other HCV protease inhibitors Q41R, V55A, Q80R, R109K, D168A/V/N and V170A had <3-fold change in telaprevir EC_{50} values.

Table 54. Replicon Fold Change in EC_{50} Values for NS5B Inhibitors against NS3 Variants

	IFN-α	RBV	PSI-6130	VCH-222	HCV-796	NM-107	VRT-832554	VRT-830353
Target			NS5B nuc	NS5B NNPI	NS5B NNPI	NS5B nuc	NS5B NNPI	NS5B NNPI
Site			Active site	Thumb	Palm 2		Finger loop	Palm 1
V36M	1.0	0.6	0.9	1.0	1.2	1.2	0.7	1.0
V36A	0.9	0.8				1.6	1.2	1.6
T54A	0.3	0.4	0.9	1.1	1.2	1.5	1.1	1.6
R155K	1.3	0.6	0.7	0.9	0.7	1.8	1.2	1.3
R155T	0.4	0.6						
R155M	0.4	0.7						
A156S			0.4	0.8	0.9			
A156T			0.5	0.8	0.8	0.9	1.0	0.9
A156V						1.0	0.9	0.8
V36M+R155K	0.9	0.7	1.1	1.1	1.0	2.0	1.3	1.7
V36M+R155T	0.3	0.6						
V36A+R155K	0.6	0.6						
V36A+R155T	0.3	0.7						

Figure 5. Comparison of Replicative Fitness of NS3 Protease Variant Replicons with Fold Change in Replicon EC_{50} Values of Telaprevir



The replication capacity of these variants was determined in Huh-7.5 cells transiently transfected with replicon RNA. The replication capacity of all telaprevir-resistant variants was lower than that of WT in replicon cells. Generally, there appears to be an inverse correlation between replicon replication capacity and resistance levels for these variants. Resistance variants that confer >25-fold decreases in telaprevir susceptibility in replicon

or enzyme susceptibility relative to wild type) such as A156T/V and V36M+A156T, generally tend to have the lowest replicon replicative capacity, whereas mutants with lower decreases in telaprevir susceptibility, such as V36M/A, T54A and R155K, tend to be more fit (Figure 5).

Phenotypic Studies from Patient Isolates in Phase 2 Clinical Trials of Telaprevir

Sixty-five clinical isolates (41 genotype 1a and 24 genotype 1b) were used for this analysis. Paired baseline and post-baseline (on-treatment and post-treatment/follow-up) isolates were available for 30 subjects and for 5 subjects with only baseline samples. Samples were obtained from treatment-naïve or treatment-experienced subjects enrolled in the T12/PR12, T12/PR24, T12/PR48 or T24/PR48 treatment arms of Study 104 (VX05-950-104), Study 104EU (VX05-950-104EU), Study 106 (VX06-950-106), and VX-950-C208 clinical trials of telaprevir. Reasons for treatment failure of these isolates included breakthrough on T/PR, breakthrough on PR, Week 4 stopping rule, relapse, and baseline samples with telaprevir-resistant variants. The NS3 gene (Tyr6–Pro191) from clinical isolates was reverse transcribed, amplified and cloned into a shuttle replicon, and replicon clones were pooled to mimic the intrinsic genetic heterogeneity of the viral quasispecies in infected patients. The drug susceptibility (EC_{50} values) was determined after transfection of the transcribed RNA pools into Huh7-lunet cells. A subset of the 65 isolates was also tested in a NS3 enzymatic assay for comparison with the replicon data.

The range of mean EC_{50} values for the 26 subjects with WT baseline isolates was from 0.034-0.32 μM, with a grand mean (±SE) of 0.12 μM (±0.014). One baseline isolate containing V36L (subject 129006) and one baseline isolate (subject 40007) bearing V170V/I had mean EC_{50} values of 0.23 μM and 0.51 μM, respectively.

Six baseline isolates contained V36M (n=3), R155K (n=2) or R155K+T54S (n=1). The mean EC_{50} values of isolates containing V36M (0.76, 0.76 and 3.3 μM), R155K (1.1 and 1.5 μM) and R155K+T54S (5.2 μM) were higher than those of the mean baseline WT EC_{50} value (0.12 μM), indicating subjects with these variants detectable at baseline may have reduced response to TVR treatment compared to subjects with WT virus at baseline. Of the 5 subjects who had available day 4 viral loads, 4 subjects with V36M R155K or R155K+T54S had a 1.1, 0.78, 2.6 or 1.4 $\log_{10}$ decrease in HCV RNA from baseline while subjects with WT baseline had a 3 to 5 $\log_{10}$ drop.

The EC_{50} values of post-baseline samples with substitutions of V36M, V36A, T54A, T54S, R155K, R155T, A156S, A156T or V36M+R155K were significantly higher than that of the corresponding baseline for each subject (Table 55). The telaprevir susceptibility changes for the post-baseline isolates with telaprevir resistance-associated substitutions were consistent with the telaprevir susceptibility changes seen with the corresponding site-direct variants. Interestingly, the emergence of V170A, a boceprevir-resistant substitution, in the post-baseline isolates of subject 40007 and 129006 was associated with a 9.5- and 3.6-fold of increase in EC_{50} values from baseline, respectively, which are higher than the 2.6-fold change in susceptibility obtained with site-directed mutant repicons. Clinical isolates with the same telaprevir-resistant

substitutions had different ranges in telaprevir susceptibilities, which may be due to genetic differences of the HCV isolates.

Table 55. Fold Change in Replicon EC_{50} values for Baseline and Post-Baseline Clinical Isolates

Subject ID	Genotype	Substitution Post Baseline	Mean EC_{50} Value (μM)	Fold Change from Baseline	Fold Change from WT*
122002	1a	V36M	0.39	4	3.3
126005	1a	V36M	0.87	6.6	7.3
108001	1b	V36A	0.72	5.6	6
18001	1b	T54A	1.1	6.8	9
126010	1b	T54A	1.0	8.9	8
138003	1b	T54A	0.37	3.1	3
121010	1b	T54S	3.0	9.3	25
127008	1a	R155K	4.6	16	38
140011	1a	R155K	0.59	18	5
303009	1a	R155T	3.5	32	29
115007	1a	A156S	2.9	47	24
4008	1b	A156T	6.0	45	50
205012	1b	A156T	>25	>116	>208
8002	1a	V36M+R155K	8.4	83	70
102006	1a	V36M+R155K	1.7	43	14
104007	1a	V36M+R155K	12	110	100
106007	1a	V36M+R155K	4.3	72	36
109007	1a	V36M+R155K	12	95	100
306007	1a	V36M+R155K	9.9	249	83
40007	1b	V170A	4.9	9.5	41
129006	1b	V36L[$]+V170A	0.82	3.6	6.8

*Mean WT value from Clinical Baseline samples = 0.12 μM
[$]Present at Baseline

Seven subjects in this study had baseline and post-baseline isolates that did not contain canonical TVR-resistant substitutions and had breakthrough or relapsed with WT virus. The fold change of EC_{50} values of their post-baseline samples relative to their corresponding baseline isolates ranged from 1.0 to 2.2, indicating that the susceptibility to telaprevir of these post-baseline samples was similar to those of their baseline isolates.

The NS3 protease domain (Ala1-Ser181) of baseline and post-baseline isolates of 13 subjects was cloned, expressed, purified, and tested for sensitivity to TVR in a NS3 enzymatic assay. The sequences of the cloned NS3 protease at positions associated with resistance to TVR were confirmed identical to plasma and replicon sequences obtained through population sequencing. The fold change in IC_{50} values between baseline and post-baseline isolates was calculated and compared with the replicon data (Table 56). In general, a good correlation was seen (r=0.77) between the fold change values obtained in the enzymatic and replicon assays for the clinical isolates tested.

Table 56. Comparison of Fold Change of IC_{50} Values in Replicon Assay and NS3 Enzymatic Assay

Subject ID	GT	PI mutation		FC in IC_{50} compared to BL (for replicon and enzyme) or WT (for site-directed mutants)		
		Baseline	Post-Baseline	Replicon	Enzyme	Site-directed mutants
122002	1a	WT	V36M	4.0	2.2	
126005	1a	WT	V36M	6.6	5.6	7.0
108001	1b	WT	V36A	5.6	3.7	7.4
126010	1b	WT	T54A	8.9	7.9	
138003	1b	WT	T54A	3.1	4.6	6.3
140011	1a	WT	R155K	18	17	7.4
115007	1a	WT	A156S	47	49	
212008	1b	WT	A156S	-	14	9.6
4008	1b	WT	A156T	45	188	
205012	1b	WT	A156T	>116	192	>62
104007	1a	WT	V36M+R155K	110	82	
306007	1a	WT	V36M+R155K	249	54	64
40007	1b	WT(V170V/I)	WT(V170A)	9.5	0.48	2.6

LATE RELAPSE RATES: Study VX08-950-112 EXTEND

Cohort A subjects (99.2%; 122/123) had a durable response and maintained their undetectable HCV RNA and SVR status. The duration of follow-up was up to 35 months after SVR was achieved and the median follow-up time was 22 months. The one instance of late relapse occurred at 5.3 months during a previous study (104EU) and was previously reported. This subject was in the T12/PR24 group of Study 104EU and had undetectable HCV RNA at the time of premature discontinuation of study drug dosing (Day 66) through the follow-up Week 36. The late relapse was observed at the follow-up Week 48 visit with a HCV RNA level of 12,700 IU/mL. The subject's virus had the substitution NS3_T54S at baseline, throughout treatment and at follow-ups. At the time of late relapse, the subject's virus had a V170V/A subsitution. Four weeks after the late relapse time point, the V170V/A polymorphism had returned to WT.

PERSISTENCE OF RESISTANCE-ASSOCIATED SUBSTITUTIONS

STUDY 112

Study 112 was a 3-year, virology follow-up study in subjects previously treated with telaprevir from Studies 104, 104EU, 106, and 107. Subjects who did and did not achieve a SVR following therapy were included in this study. In subjects who achieved an SVR following a telaprevir-based treatment, the durability of the response and late relapse was assessed. In subjects who did not achieve an SVR, the changes in HCV variants over time were evaluated (Table 57). Follow-up periods in Study 112 ranged from 5 - 40 months with a median of 25 months. A total of 56 subjects had both post-nadir data from their original studies and a Day 1 time point from Study 112 and were used for the analysis of evolution of resistant variants V36A/M/L, T54S/A, R155T/K/I, A156S/T and combinations of these. Eighty-nine percent (50/56) of all analyzed subjects were WT by population sequencing at Day 1 of Study 112. The majority of

subjects enrolled in Cohort B are infected with genotype-subtype 1a. Of the 44 subjects infected with subtype 1a that were not WT after the previous study, the viral populations in 38 (86%) subjects changed to WT by population sequencing. The viral populations in the 12 subjects (100%) infected with subtype 1b changed to WT by population sequencing.

The proportions of subjects who had detectable (present at >25% of the viral population) variants (V36A/M/L, T54S/A, R155T/K/I, A156S/T) in available samples at 6, 12, 24 and 36 months were evaluated (Tables 58 and 59; Fig. 6). At 6 months, a high proportion of these substitutions persisted. All variants were still detectable (present at >25% of the viral population) in some subjects at 24 months. By 36 months, V36M, T54S or A, and A156S/T/N variants had fallen below the level of detection by population sequencing in these subjects. However, the R155K variant was still detectable by population sequencing at 36 months in 3% of subject isolates. The lack of detection of a substitution based on a population-based assay does not necessarily indicate that viral populations carrying that substitution have declined to a background level that may have existed prior to treatment.

Clonal sequence analysis was performed on a subset of samples (n=20 subjects; 10 subjects per subtype) that were considered wild-type by population sequencing, with 96 clones picked for each sample to determine if resistant variants could be detected by clonal analysis for samples considered wild-type by population sequence analysis. Results were tabulated by counts of resistant and wild-type variants at each of four resistance-associated positions in NS3. Of the 35 variants that were part of the post-nadir resistance profile but were determined to be undetectable by population sequencing of the Study 112 sample, 25 (71%) remained undetectable by clonal sequence analysis (Table 57). The variant T54A was detected in 1 of 85 clones (1.1%) in Subject 106-110005, 1 of 77 clones in Subject 106-134004, 1 of 85 clones in Subject 106-134007 and 1 of 160 clones (0.6%) in Subject 106-131002 at approximately 28 months. The variant V36M was detected in 4 of 128 (3%) in Subject 106-134003, 1 of 85 clones (1%) of Subject 106-134007, and 1 of 158 clones (0.6%) of Subject 107-3303302 at 21-28 months. The A156 variants were detected in 1 of 85 clones (1%) in Subject 106-110005, 1 of 160 clones (0.6%) in Subject 106-131002, and 2 of 158 clones (1.3%) in Subject 107-3303302. The R155K substitution in Subject 106-116008 was still present at and after 24 months by population sequencing and 62/62 clones detected the R155K substitution by clonal sequencing.

Overall, the frequency of resistant clones was 0.9% at baseline (14 of 1567 clones) and 0.9% in Study 112 follow-up (16 of 1769 clones). Thus, in general, the clonal sequencing data indicate that the resistant viral populations from subjects who failed on telaprevir treatment return to pre-treatment levels after 2 years.

There was no evidence that previous study failure type (viral breakthrough, relapse), previous study treatment arm, or duration of telaprevir dosing in the previous study affected the virus population change back to WT, although the sample size is too small to draw firm conclusions.

Table 57. Study 112 Subjects with Resistance-Associated Substitutions and Time to WT

PID	Variant	Subtype	Present (post nadir + #months)	WT months	Post Nadir visit (week)	# Clones at FU	Duration of T/ TRT (Weeks)
104-002002	V36M		6	36			
	R155K		6	36			
104-002008	V36M			24			
104-022004	V36M		6	36			
	R155K		6	36			
104-033007	V36M		6	36			
104-040001	V36M			24			
	R155K			24			
104E-102001	V36M			12			
	R155K		12				
104E-102006	V36M			36			
	R155K			36			
104E-104013	R155K			36			
104E-112010	T54A	1b		36	142	0/80	12
104E-204013	V36M		6	36			
	R155K		36+				
104E-204017	V36M			36			
	R155K			36			
104E-205009	T54S		6	36			
	A156N		6	36			
104E-211003	A156S			36			
104E-211006	T54A/T			36			
	A156S/A			36			
106-101002	V36M	1a		6	90	0/70	16
	R155K		6	18		0/70	
106-101004	R155K	1a		18	81	0/54	24
106-108008	V36M			24			

	R155K			24			
106-110005	T54A	1b	6	24	118	1/85	24
	A156					1/85	
106-111005	V36M		6	24			
	R155K		6	24			
106-111006	V36M	1a	6	36	136	0/72	6.3
	R155K		6	36		0/72	
106-111009	V36M		6	36			
	T54S/T		6	36			
	R155K/R		6	36			
106-112003	R155I/T/R			6			
106-112007	T54T/A	1b		6	126	0/45	6
106-113002	V36V/M		24+				
106-113003	R155K		6	24			
106-113006	V36V/M		6	24			
	A156A/S		6	24			
106-115001	R155K/R			6			
106-116001	V36M			18			
	R155K			18			
106-116008	V36M	1a	6	24	105	0/62	20
	R155K		24+			62/62	
106-118008	V36M	1a	6	18	91	0/78	24
	R155K		6	18		0/78	
106-122002	V36M			6			
106-122003	V36M/V		6	24			
	T54T/A			6			
	R155K		6	24			
106-124002	V36V/A	1b	6	24	117	0/61	24
	T54T/A			6		0/61	
106-124005	V36M	1a	6	36	134	0/37	11
	R155K		6	36		0/36	
106-	R155T		6	24			

126001							
106-126002	V36M		6	36			
	R155K		6	36			
106-126005	V36M		24+				
106-126008	R155K		6	24			
106-126010	T54A	1b		6	107	0/251	16
106-129006	V36L		24+				
106-129007	V36M		6	24			
	R155K		6	24			
106-131002	T54A/T	1b	6	24	110	1/160	24
	A156					1/160	
106-131007	V36M		6	24			
	R155K		6	24			
106-134003	V36M	1a	6	24	105	**4/128**	14
	R155K		6	24		0/128	
106-134004	V36M	1a	6	24	101	0/77	16
	R155K		6	24		0/77	
	T54A					1/77	
106-134007	V36M	1a		24	112	**1/85**	6
	R155K			24		0/85	
	T54A					1/85	
106-301004	V36M			36			
	R155K			36			
106-306003	V36M		6	36			
	R155K		6	36			
106-306006	V36A/M		6	24			
	R155K		24+				
106-401012	V36M		6				
	R155K		6				
106-402001	T54A	1b		24	101	0/73	24
106-403001	V36M		6	36			
	T54S/T		6	36			
	R155K/R		6	36			

106-403003	T54A/T	1b	6	24	116	0/23	16
106-403007	T54A	1b	6	24	106	0/82	24
106-403008	A156A/T	1b		24	100	0/89	16
107-3108313	V36V/M			18			
107-3115103	V36M		12+				
	R155K		12+				
107-3115305	V36A			6			
107-3303302	V36M	1a	6	18	85	1/158	24
	T54S/T		6	18		0/158	
	R155K		6	18		0/158	
	A156					2/158	

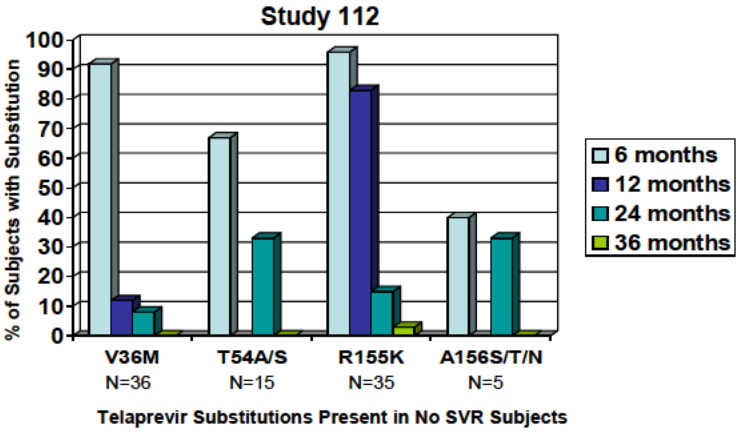
Table 58. Proportion of Persistence Resistance-Associated Substitutions (% of Subjects with Substitution and Available Data at Timepoint)

Variant	n	% Mutant 6 mo	% Mutant 12 mo	% Mutant 24 mo	% WT 6 mo	% WT 1.5 yr	% WT 2 yr	% WT 3 yr
V36M	36	24/26 (92%)	3/26 (12%)	2/26 (8%)	2/36 (6%)	7/34 (21%)	19/34 (56%)	32/32 (100%)
V36L	1	1/1	1/1	1/1				
V36A	3	2/3			1/3	1/3	3/3	
T54A	11	4/8 (50%)			4/8 (50%)		9/11 (82%)	11/11
T54S	4	4/4			0/4	1/4	1/4	4/4
R155K	35	25/26 (96%)	5/6 (83%)	3/20 (15%)	1/26 (4%)	6/9 (67%)	17/19 (89%)	29/30 (97%)
R155T	2	1/2			1/2		2/2	
A156S	3	1/3			0/1		1/1	3/3
A156N/T	2	1/2					1/2	2/2

Table 59. Study 112 Summary of Persistence of Telaprevir Resistance Substitutions (% of Subjects with Substitution and Available Data at Timepoint)

Variant	n	% Mutant 6 mo	% Mutant 12 mo	% Mutant 24 mo	% Mutant 36 mo
V36M/L/A	40	90% (27/30)	15% (4/27)	11% (3/27)	
T54A/S	15	67% (8/12)		33% (5/15)	0% (0/15)
R155K	35	96% (25/26)	83% (5/6)	15% (3/20)	3%
A156S/T	5	40% (2/5)		33% (1/3)	0% (0/5)

Figure 6. Persistence of Telaprevir Resistance-Associated Substitutions in Study 112



STUDY 108 FOLLOW-UP

The viral populations of subjects failing a telaprevir-containing regimen with non-WT variants observed at NS3-36, 54, 155, and/or 156 (n=91) in Study 108 were assessed at multiple time points after treatment failure. By protocol, the viral populations of subjects experiencing on-treatment virologic failure were assessed at all regularly scheduled visits after enforcement of the stopping rule and subjects with relapse were assessed 4 and 24 weeks after relapse. Viral populations were assessed by population sequencing to determine if the variants initially present at the post-nadir visit were retained in the viral population or replaced by WT virus. Three subjects had no follow-up visits after their post-nadir visit, but the remaining 88 subjects had at least 1 followup visit, with the majority of subjects (~89%) having 2 or more post-nadir observations. The median time between the post-nadir visit and the End of Study (EOS) visit was 45 weeks

Of, the follow-up samples in Study 108, 55/91 (60%) were wildtype by End of Study: 52% of the genotype 1a population (median follow-up time 34 weeks) and 86% of the genotype 1b population (median follow-up time 12 weeks) changed completely to WT. The proportion of subjects with variants that changed to WT after the post-nadir visit during the study period is shown by each subtype in Table 60 and 61 (Report vx07-950-108-vsr-g141; p. 48).

The fraction of NS3-V36 variants in both genotype 1a and genotype 1b that changed to WT during the study period were comparable between the subtypes, with overall 65% (37/57) of post-nadir V36 variants no longer detectable by EOS (median time to apparent loss of variant, 36 weeks).

The T54 variants were observed primarily in genotype 1b and only rarely in genotype 1a. All but 1 genotype 1a subject and 1 genotype 1b subject changed to WT, for 86% (12/14) total change to WT (median time to apparent loss of variant, 13 weeks).

Variants at R155 were observed exclusively in genotype 1a, with the majority of genotype 1a viral populations in treatment-failure subjects developing an R155 variant.

Of the 61 subjects with an R155 variant at the post-nadir visit, 36 (59%) changed to WT by EOS (median time to apparent loss of variant, 44 weeks).

Variants at A156 occurred rarely with 2 genotype 1a subjects and 4 genotype 1b subjects having variants at the post-nadir visit. Overall, 67% (4/6) of A156 variants (1/2 genotype 1a and 3/4 genotype 1b) changed to WT by EOS (median time to apparent loss of variant, 24 weeks).

Substitutions at positions V36 and R155 occurred together frequently (~61%) in subtype 1a but were not observed in subtype 1b. Overall, 52% (22/42) of subjects whose virus possessed the double variants V36 and R155K had virus that changed to WT at both positions by EOS (median time to apparent loss of variant, 46 weeks).

Table 60. Follow-up in Study 108: Change to Wild-type by Position in Genotype 1a

		Time (weeks; post-nadir visit to End of Study)		WT by EOS	
Resistance profile	N	Median	Range	N	%
V36A	1	35	N.A.	1	100
V36V/M	1	40	N.A.	1	100
V36M	5	41	34, 67	2	40
R155T	2	49	45, 52	2	100
R155R/K	1	66	N.A.	1	100
R155K	14	43	4, 57	6	43
A156S	1	8	N.A.	0	0
V36A/M+R155R/K	1	50	N.A.	0	0
V36V/M+R155R/K	3	44	35, 45	2	67
V36V/M+R155K	5	50	26,68	3	60
V36M+R155K	32	52	0, 70	16	50
T54T/S+R155R/K	1	64	N.A.	1	100
T54S+R155K	1	55	N.A.	0	0
V36M+R155K+A156A/S	1	46	N.A.	1	100
Any mutation profile	69	45	0, 70	36	52

Source: ADSQFU

Table 61. Follow-up in Study 108: Change to Wild-type by Position in Genotype 1b

		Time (weeks; post-nadir visit to EOS)		WT by EOS	
Resistance profile	N	Median	Range	N	%
V36A	2	17	0, 33	1	50
V36V/A	3	56	12, 60	3	100
V36M	1	59	N.A.	1	100
T54T/A	4	49	2, 57	4	100
T54A	7	44	12, 52	6	86
A156A/T	1	69	N.A.	1	100
A156S/T/V	1	65	N.A.	1	100
A156S	1	44	N.A.	1	100
V36V/A+T54T/A	1	73	N.A.	1	100
V36V/M+A156A/S	1	0	N.A.	0	0
Any mutation profile	22	49	0, 73	19	86

Source: ADSQFU

The data indicate that substitutions in subtype 1b variants return to WT in higher proportions during the 1 year study observation period than GT1a variants. Furthermore, at 1 year, approximately half of the substitutions in subtype 1a variants are still present.

Table 62. Non-Parametric Estimation of Median Times to Change to WT

Position	N		Time to loss of detectable variant (weeks)			
	Changed	Failed to change	Median	95% CI[a]	1st and 3rd Quartiles[a]	Worst-case Median[b]
NS3-36	37	20	35.9	28,44	22,51	42.0
NS3-54	12	2	12.7	4,28	8,32	12.7
NS3-155	36	25	44.0	36,50	24,56	50.7
NS3-156	4	2	24.0	2,24	18,33	28.6
NS3-36+NS3-155	22	20	46.1	36,56	29,70	57.4

[a] 95% CI and Quartiles are formatted with a comma separating the lower and upper ends of each range
[b] Derived from KM estimate based on replacing all censored times with times greater than the largest observation time
Source: ADSQFU

Non-parametric estimation was utilized to determine the median time to change to WT for each position. These estimates are based on 89 event observations (with the event being a change to WT) and 49 censored observations. A subject was considered censored for the event if the change to WT had not yet occurred by the time of the subject's last sequencing assessment. Generally, T54 variants changed to WT more rapidly than all other variants and A156 variants changed to WT more rapidly than did V36 and R155 variants. Based on these data, the median time to reversion for T54 variants was ~13 weeks, followed by 24 weeks in the case of A156 variants, and 36 and 44 weeks for V36 and R155 variants, respectively (Table 62; Report vx07-950-108-vsr-g141; p. 52)). Although the sample size is small and should be interpreted cautiously, there is no evidence from the data that the median time to loss of detectable resistant variants varies between the single (V36M or R155K) or double variants.

STUDY 111 FOLLOW-UP

The viral populations of subjects who failed telaprevir-containing regimens were followed at multiple timepoints after treatment-failure in the absence of drug. Change at positions NS3_36, 54, 155, and 156 were assessed by population sequencing and the association between time to change to wild-type was determined. The median time between the post-nadir visit and the last sequencing assessment visit of the 71 subjects with at least 1 follow-up visit was 41 weeks (range 14-44 weeks).

In Study 111, 46% of the GT 1a population (median follow-up time 39 weeks) and 75% of the GT 1b population (median follow-up time 46 weeks) changed completely to wild-type by end of study (Table 63 and 64; Clinical Virology Report vx08-950-111; p. 35). Overall, 50% (40/80) changed to wild-type by the end of the study.

Table 63. Follow-up in Study 111: Change to Wild-type by Position in Genotype 1a

		Time (weeks; post-nadir visit to last assessment)		WT by End of Study	
Resistance profile	N	Median	Range	N	%
V36A	1	39	NA	1	100
V36V/M	1	49	NA	1	100
V36M	8	24	0,46	3	38
T54T/A	1	44	NA	1	100
R155G	1	25	NA	0	0
R155R/T	1	0	NA	0	0
R155T	2	31	13,50	1	50
R155R/K	1	12	NA	0	0
R155K	13	36	0,55	4	31
V36V/A+R155R/K	1	20	NA	0	0
V36A/M+R155R/K	1	10	NA	0	0
V36V/M+R155R/K	8	19	0,64	3	38
V36V/M+R155K	3	44	41,54	3	100
V36M+R155K	26	54	0,70	14	54
Any mutation profile	68	39	0,70	31	46

Source: ADSQFU

Table 64. Follow-up in Study 111: Change to Wild-type by Position in Genotype 1b

		Time (weeks; post-nadir visit to last assessment)		WT by End of Study	
Resistance profile	N	Median	Range	N	%
V36A	1	54	NA	1	100
V36M	1	0	NA	0	0
T54A	4	33	12,46	3	75
A156A/S	1	56	NA	1	100
A156S	3	48	45,48	2	66
V36V/A+T54T/A	2	30	8,51	2	100
Any mutation profile	12	46	0.51	9	75

Source: ADSQFU

Variants at NS3_36 were observed in both genotype 1a (n=49) and genotype 1b (n=4). In genotype 1a subjects the variant identified was primarily V36M, which was found in combination with R155K in ~80% (38 of 47) of these subjects. The fraction of V36 variants that changed to wild-type during the study period was comparable between the subtypes, with 70% (37/53) no longer detectable by EOS (Kaplan-Meier estimate of median time to apparent loss of variant, 31 weeks). Variants at NS3_T54 (T54A) were observed primarily in genotype 1b (n=6) and only rarely in genotype 1a (n=1). All but 1 genotype 1b subject changed to wild-type by EOS, for 86% (6/7) total change to wild-type (Kaplan-Meier estimate of median time to apparent loss of variant, 15 weeks). NS3_R155 was observed exclusively in genotype 1a, with the majority of genotype 1a treatment-failures developing an R155K variant. Of the 57 subjects with an R155 variant at the post-nadir visit, 30 (53%) changed to wild-type by EOS (Kaplan-Meier estimate of median time to apparent loss of variant, 41 weeks). Overall, 75% (3/4) of post-nadir

A156 variants were no longer detectable by EOS (Kaplan-Meier estimate of median time to apparent loss of variant, 44 weeks). By EOS, 53% (20/38) of subjects possessing the combination of NS3_V36 and NS3_R155 variants post-nadir no longer had detectable resistant variants at both positions (Kaplan-Meier estimate of median time to apparent loss of variant, 48 weeks).

STUDY 216 FOLLOW-UP

The viral populations of subjects failing a telaprevir-containing regimen in Study 216 were assessed at multiple time points after treatment-failure by population sequencing to determine if the variants initially present at the post-nadir visit were present in the viral population by the EOS visit. Only subjects with telaprevir-resistant variants observed at positions NS3_V36, T54, R155, and/or A156 were included in this analysis. Subjects who had baseline telaprevir-resistant variants were excluded from the analysis. The majority of genotype 1a subjects (51%, median follow-up time 47.7 weeks) and of genotype 1b subjects (80%, median follow-up time 24.4 weeks) no longer had detectable telaprevir-resistant variants by the EOS visit (Tables 65 and 66). Overall, 60 of 104 (58%) subjects had no detectable telaprevir-resistant variants by EOS (median follow-up time 46.4 weeks).

V36A/M variants were observed in both genotype 1a (n=13) and genotype 1b (n=11) subjects with V36M primarily found in genotype 1a subjects and V36A in genotype 1b subjects. The fraction of these variants that became undetectable during the study period was comparable between the genotypes, with 62% and 64% of subjects who no longer had detectable V36A/M variants in genotypes 1a and 1b, respectively. T54A/S variants were observed in both genotype 1a (n=2) and genotype 1b (n=9). Variant T54S was only identified in genotype 1a subjects and T54A was primarily identified in genotype 1b subjects. All but one genotype 1b subjects and 1 of 2 genotype 1a subjects no longer had detectable resistant variants at EOS. R155 variants, primarily R155K, were exclusively observed in genotype 1a. Of the 64 subjects with a R155K/M/T variant at the post-nadir visit, 35 (55%) no longer had detectable R155K/M/T variants at EOS. A156S/T/V variants were observed in both genotype 1a (n=6) and genotype 1b (n=7) with primarily A156T in genotype 1b subjects. All of these variants were no longer detectable within the follow-up period. Substitutions V36M+R155K occurred together frequently in genotype 1a (n=48) but not in genotype 1b. Overall, 50% (24/48) of subjects that possessed V36M+R155K variants at the post-nadir visit no longer had detectable resistant variants at these positions by EOS. Of subjects with V36M+R155K at the post-nadir visit, 23% (11/48) lost only one of the variants – 13% (6/48) lost only the V36M variant and 10% (5/48) lost only the R155K variant by EOS.

Table 65. Follow-up in Study 216: Change to Wild-type by Position in Genotype 1a

Mutation profile	N	WT[a] by EOS N	WT[a] by EOS %	Follow-up Time Median (Weeks)	Follow-up Time Range (Weeks)
V36A	2	1	50	35.6	19;52
V36A/M	1	0	0	23.9	24;24
V36M	4	3	75	46.6	5;56
V36M/V	3	2	67	21.4	13;40
R155K	13	5	38	36.3	3;68
R155K/R	1	0	0	6.9	7;7
R155M	1	0	0	4.1	4;4
R155T	1	1	100	12.0	12;12
A156S	1	1	100	11.7	12;12
A156T	1	1	100	12.0	12;12
V36M+T54S	1	0	0	7.6	8;8
V36L/M+R155K	1	0	0	47.7	48;48
V36M+R155K	37	19	51	60.0	8;71
V36M/V+R155K	3	1	33	45.4	36;62
V36M/V+R155K/R	4	2	50	44.4	30;65
V36M+A156S	1	1	100	56;0	56;56
V36M/V+A156A/T	1	1	100	49.7	50;50
V36M/V+T54S/T+R155K	1	1	100	57.7	58;58
V36M/V+R155K/R+A156A/S	1	1	100	57.0	57;57
V36M/V+R155K/R+A156T/V	1	0	0	32.3	32;32
Any Mutation Profile	79	40	51	47.7	3;71

[a] WT = no telaprevir-resistant variants
Source: Refer to Module 5.3.5.1/VX-950-C216-Anal-EFF/Display ADD.11 and Listing VIR.2

Table 66. Follow-up in Study 216: Change to Wild-type by Position in Genotype 1b

Mutation profile	N	WT[a] by EOS N	WT[a] by EOS %	Follow-up Time Median (Weeks)	Follow-up Time Range (Weeks)
V36A	7	4	57	16.0	12;20
V36M	1	0	0	26.1	26;26
V36A/V	2	2	100	31.8	13;51
T54A	3	2	67	45.7	13;47
T54A/S	1	1	100	52.9	53;53
T54S	1	1	100	52.0	52;52
T54A/T	2	2	100	20.6	19;22
A156A/S	1	1	100	19.9	20;20
A156T	5	5	100	63.1	24;66
V36A/V+T54A/T	1	1	100	55.1	55;55
T54S+A156T	1	1	100	31.0	31;31
Any Mutation Profile	25	20	80	24.4	12;66

[a] WT = no telaprevir-resisant variants
Source: Refer to Module 5.3.5.1/VX-950-C216-Anal-EFF/Display ADD.11 and Listing VIR.2

Of the combined subjects from Phase 3 studies (108, 111, and 216) with a total of 443 resistant variants, 176 (40%) had detectable resistant variants by population sequencing by the end of study (median follow-up 45 weeks) and results for loss of variants by EOS

were similar across studies (Table 67). Overall, 50% of substitutions (V36M/A or R155K) persisted to EOS in subtype 1a subjects and 20% of substitutions (T54A) persisted in subjects with subtype 1b (Table 68 and Fig. 7). At EOS, 63% of the R155K variants (all genotype 1a) and 15% T54A (all genotype 1b) were still detectable. Of the V36 variants, 48% of V36M variants (45% genotype 1a; 67% genotype 1b) and 31% of V36A variants (25% genotype 1a; 33% genotype 1b) were still detectable at EOS (Table 68). Half of the V36M+R155K variants were still detectable, but no A156T subsitutions were detectable at EOS by population sequencing.

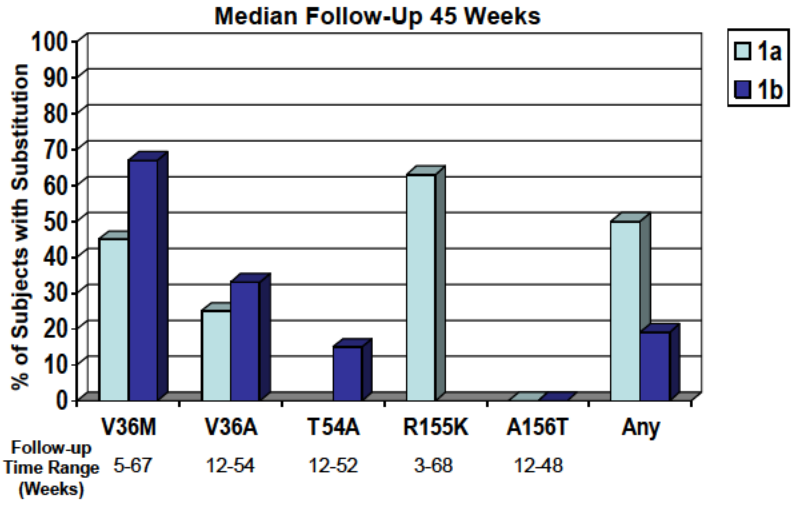
Table 67. Proportion of Subjects with Variants that Changed to WT by Study

	N (with any teleprevir-resistant variant)	Loss of Variant by ESO (N)	Loss of Variant (%)
Study 108	180	111	62%
Study 111	159	96	60%
Study 216	104	60	58%
Total	443	267	60%

Table 68. Summary of Persistence Off-Treatment of Telaprevir Resistant Variants at End of Study in Studies 108, 111, and 216 (% of Subjects with Substitution)

	Genotype 1a	Genotype 1b	Range in Follow-up (Weeks)
V36M	10/22 (45%)	2/3 (67%)	5-67
V36A	1/4 (25%)	4/12 (33%)	12-54
T54A		3/20 (15%)	12-52
R155K	27/43 (63%)		3-68
A156S	0/2 (0%)	1/6 (17%)	12-48
A156T	0/1 (0%)	0/7 (0%)	12-69
V36M+R155K	62/125 (50%)		8-71
Any	109/216 (50%)	11/59 (19%)	0-73

Figure 7. Summary of Persistence of Telaprevir Substitutions at EOS in Phase 3 Studies



Kaplan-Meier estimates determined by the applicant for the median time to change to WT showed that substitutions at A156 and T54 generally changed to WT the fastest followed by V36 and then R155 substitutions (Table 69). The applicant presented data from Studies 108, 111, and C216 that indicate that the rates of loss of V36M or R155K variants are similar whether each variant is present alone or in combination.

Table 69. Kaplan-Meier Estimation of Median Time to Change to WT

	Study 108	Study 111	Study 216
V36	36	31	47
T54	13	15	12
R155	44	41	58
A156	24	44	15

5. CONCLUSIONS

- The majority of isolates from subjects who did not achieve SVR had telaprevir resistance-associated treatment-emergent substitutions.
- More subjects with subtype 1a failed treatment with telaprevir than subjects with subtype 1b.
- Most prior null-responders did not achieve SVR on telaprevir and of these, 80% had treatment-emergent telaprevir substitutions.
- There are divergent resistance pathways for subtype 1a and 1b
  - The most frequent emergent substitutions in subtype 1a failures were V36M and R155K and the combination of both of these.
  - The most frequent emergent substitutions in subtype 1b failures were T54A or S, V36A, and A156T, S or V.
- Variants expressing telaprevir resistance-associated substitutions can persist at >25% of the virus population out to at least 3 years after the end of treatment

This supplemental NDA for telaprevir is approvable with respect to virology for the treatment of chronic Hepatitis C (HCV) virus infection.

- Indicated for use in combination with peginterferon alfa and ribavirin, for the treatment of genotype 1 chronic hepatitis C in adult patients with compensated liver disease, including cirrhosis, who are treatment naïve or who have been previously treated, including prior null responders, partial responders, and relapsers.
  - Consideration should be taken when treating previous Null responders with T/PR: A high proportion of previous null responders did not achieve SVR and had telaprevir resistance-associated substitutions emerge on treatment with a T/PR regimen (See Microbiology 12.4 and Clinical Studies 14.1)
  - The long term clinical impact of the emergence and persistence of dectable telaprevir resistance-associated substitutions is unknown (See Microbiology 12.4).

6. PACKAGE INSERT

Applicant Proposed Package Insert

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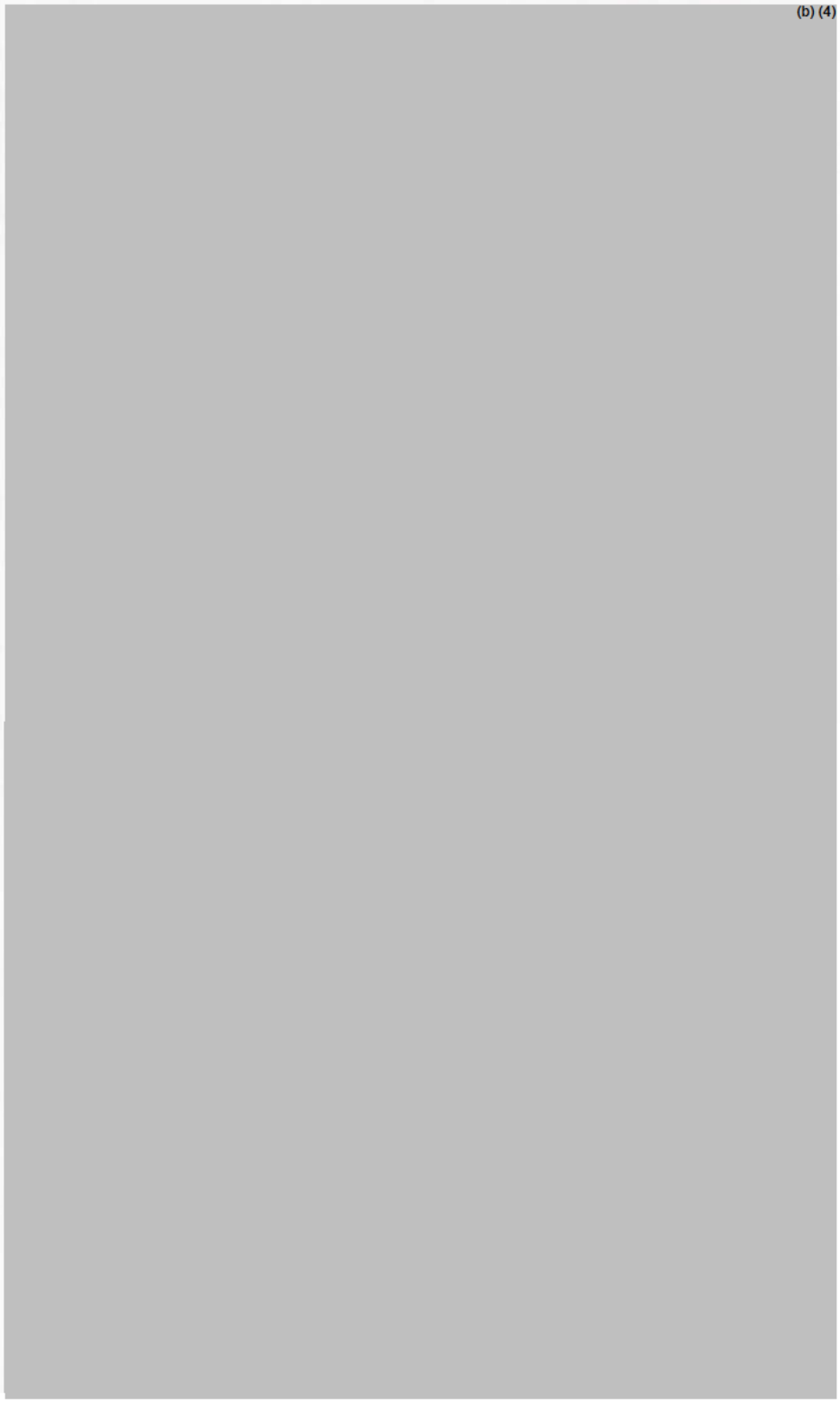
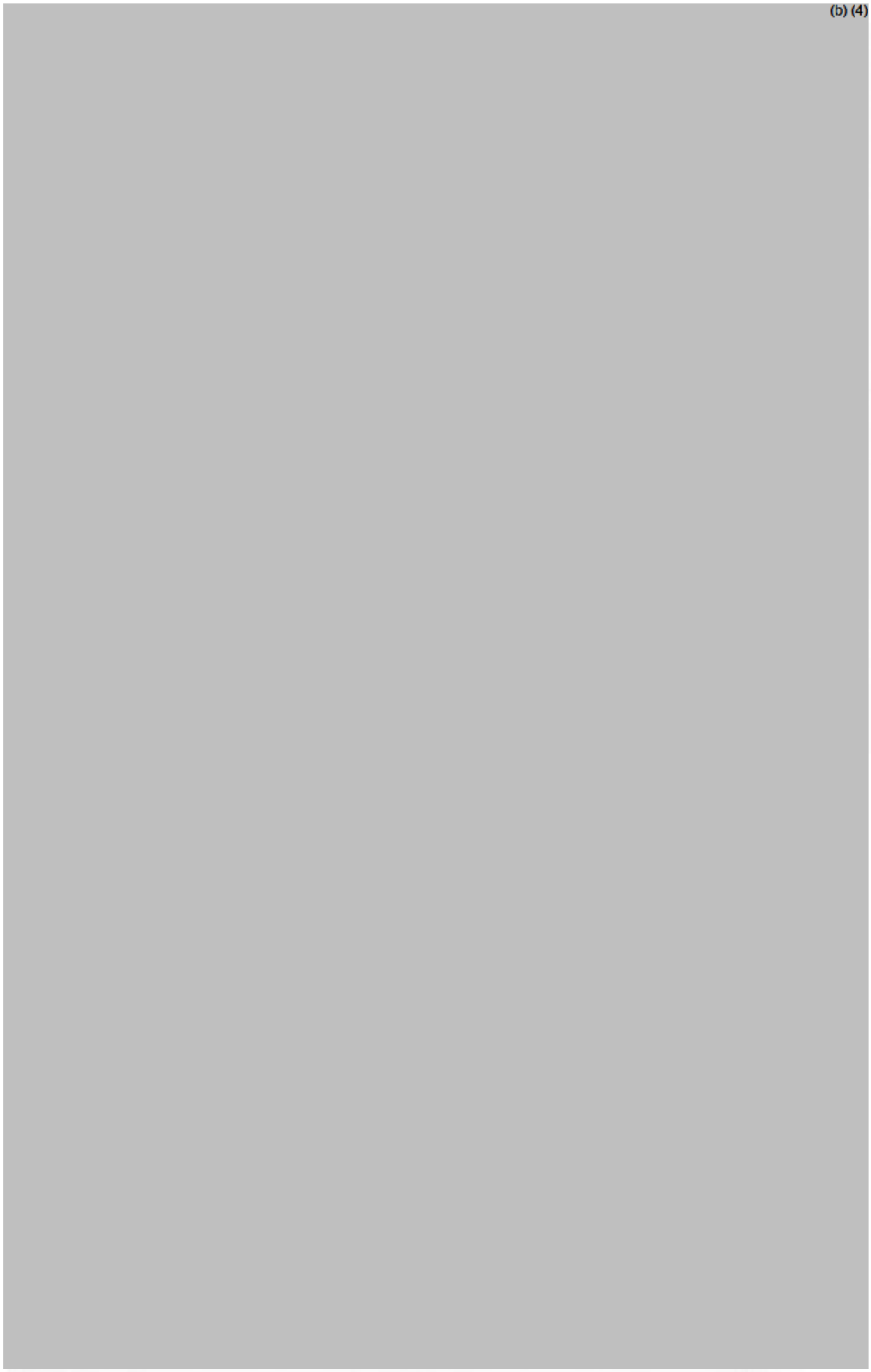
APPENDICES

APPENDIX A.



DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 201917 **SN**: 000 **DATE REVIEWED:** 4/15/11
Virology Reviewer: Lisa K. Naeger, Ph.D.

(b) (4)



APPENDIX B. CDRH CONSULT

CDER CONSULT QUESTIONS : *CDRH/OIVD Response***:**

1. Do you agree with changing the criteria for the primary endpoint efficacy analysis from <10 IU/mL BLOD to <25 IU/mL BLOQ?

Current recommendations from the AASLD recognize below 50 IU/ml as "undetectable" and sufficient for defining SVR. The assay has only been approved for an LoQ of 25 IU/ml. Below this is an unquantifiable measurement and should not be used for determining SVR. Samples will be positive at varying rates below this measurement. A truly negative sample will be below the LoD.

We have done analyses for several studies using less than 50 IU/ml or less than 25 IU/ml (no numerical assignments) to define SVR and have seen no difference in the percentage of patients assigned an SVR status.

Please see the description below on the relationship between the limit of blank, the LoB, the LoD, and the LoQ. The LoQ represents the lowest limit of the accurate measuring range. Truly negative samples should not be above the LoD however you can see that a sample with LoD has a bell shaped distribution (see EP-17P for more information on this figure).

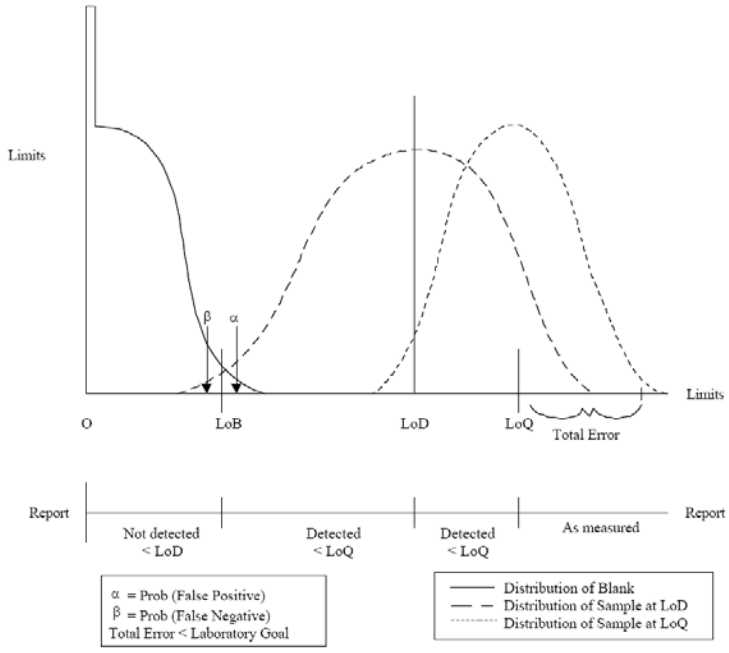


Figure 5. Distribution of Results for Blank, Low Positive at LoD, and Low Positive at LoQ. Report recommendations are shown for results at various points relative to Limits.

2. We have two NDA applications and studies within an application with different variability in the viral load results in the <25 IU/mL range over time, despite the same assay being used. We are not yet sure what if anything this means clinically. Having reviewed the assay, how would you interpret results at the lower end of quantification <25 IU/mL –

specifically viral load results that bounce from <10 IU/mL to <25 IU/mL back to <10 IU/mL?

As noted above, clinically any result below 50 IU/ml is used to define SVR. If a sample falls between the LoD and the LoQ one would expect some variability in the absolute value of that sample. The sample is positive (above the LoD) but not quantifiable (below the LoQ). See the figure above.

Is this a true measure of detectable HCV, an artifact of the assay, operator or run variability?

There is an inherent variability in this portion of the assay (10 – 25 IU/ml) and thus results in this area of the assay are not reliable for determining SVR.

How often would you expect to obtain a result of HCV RNA detectable but BLOQ in a panel of plasma samples from a patient population with no history of HCV infection?

In our specificity studies using patients with signs and symptoms similar to those of viral hepatitis we have not seen any samples with RNA detectable but below the LoQ. Please refer back to the graph in response to question #1. A truly negative HCV sample should not test above the LoD. If truly negative samples are testing positive, there is a problem with assay performance due to operator error, machine calibration, or contaminated assay reagents.

3. In the COBAS label, Section E, Tables 5-8, the component of Variance %CV results seem to indicate that reproducibility is variable on the lower end of viral load 23-50 IU/mL. Is this a correct interpretation?

*NO, %CV = (SD/Mean)*100. Since the mean is much lower at the lower concentrations, the CV is higher even though the SD is also lower.*

If so, should caution be used in interpreting test results <50 IU/mL?

AASLD considers values below 50 IU/ml to be sufficient for determining SVR. Samples falling between the LoD and LoQ are only positive, not a specific viral load, but they are positive. Samples testing with values below the LoD are negative.

4. Does the fact that different vendors performed the HCV viral load assays in the different studies seem a plausible explanation for the difference in “blipping” from BLOD and BLOQ in the different studies and applications?

Yes. Again, it could be machine build, machine calibration, or operator associated. Refer to the figure above and note the variability of results with a sample at LoD.

If so, what are the potential factors (e.g., differences in assay setup-sample handling, improper cleaning, improper workflow, differences in data analysis-no, contamination-yes, etc.) that might explain why two different sites using the same standardized assay have different frequencies of these observations?

There are many factors that can contribute to the perceived differences. These include but are not limited to sample handling, improper cleaning, and improper workflow. Data analysis should not affect the results unless machine settings have been changed. Proper controls should be in place to detect contamination (i.e. negative controls run on each plate, changes in standard curve values).

APPENDIX C.
Table. Baseline Substitutions of Subjects in Study 108

PID	ARM	Baseline Substitution	OUTCOME	Subtype
108-149-149005	T8PR	V36L	Week 24 VF	1a
108-173-173009	T8PR	V36L	Week 24 VF	1a
108-173-173004	T8PR	R155K	DC	1A
108-311-311005	T8PR	V36L	relapse	1a
108-709-709003	T8PR	V36L T54S	Week 4 VF	1a
108-184-184003	T8PR	D168E	SVR24	1B
108-211-211001	T8PR	D168E	SVR24	1B
108-121-121008	T8PR	T54S	DET DC	1A
108-129-129002	T8PR	T54S	SVR24	1A
108-142-142010	T8PR	T54S	SVR24	1A
108-151-151017	T8PR	T54S/T	DET EOT	1A
108-171-171003	T8PR	T54S	SVR24	1A
108-181-181003	T8PR	T54S	SVR24	1A
108-206-206007	T8PR	T54S	RELAPSE	1A
108-507-507002	T8PR	T54S	SVR24	1A
108-702-702001	T8PR	T54S	WK36	1A
108-709-709003	T8PR	T54S	WK4	1A
108-106-106001	T8PR	T54S	SVR24	1B
108-179-179005	T8PR	T54S/T	SVR24	1B
108-508-508008	T8PR	T54S	DET DC	1B
108-605-605009	T8PR	T54S	SVR24	1B
108-101-101005	T12PR	V36L	SVR24	1a
108-115-115004	T12PR	V36L	SVR24	1a
108-119-	T12PR	V36M	WK24VF	1a

119002				
108-207-207004	T12PR	V36L	SVR24	1a
108-210-210004	T12PR	V36L	SVR24	1a
108-158-158010	T12PR	V36I/L	LOST TO FU for SVR24	1b
108-305-305006	T12PR	V36L	DET EOT	1b
108-405-405009	T12PR	V36I	SVR24	1b
108-405-405012	T12PR	V36M	SVR24	1b
108-504-504003	T12PR	V36V/L	RELAPSE	1b
108-508-508007	T12PR	V36L	SVR24	1b
108-169-169017	T12PR	R155K	SVR24	1A
108-173-173004	T12PR	R155K	DET EOT	1A
108-179-179001	T12PR	R155K	SVR24	1B
108-134-134001	T12PR	D168E	AE	1B
108-119-119008	T12PR	T54S	RELAPSE	1A
108-141-141004	T12PR	T54S	SVR24	1A
108-143-143006	T12PR	T54S	RELAPSE	1A
108-151-151001	T12PR	T54S	SVR24	1A
108-167-167003	T12PR	T54S	SVR24	1A
108-179-179006	T12PR	T54S	SVR24	1A
108-305-305002	T12PR	T54S	SVR24	1A
108-309-309004	T12PR	T54S	SVR24	1A
108-602-602005	T12PR	T54S	SVR24	1A
108-705-705005	T12PR	T54S	RELAPSE	1A
108-149-149009	T12PR	T54S	SVR24	1B
108-405-405005	T12PR	T54S	SVR24	1B
108-405-405009	T12PR	T54S	SVR24	1B

108-110-110002	PR	V36L	SVR24	1a
108-130-130005	PR	V36L	EOT	1A
108-133-133006	PR	V36L	WK12 VF	1A
108-202-202003	PR	V36L	LOST TO FU for SVR	1A
108-802-802001	PR	V36L	WK24 VF	1A
108-112-112005	PR	T54S/T	RELAPSE	1A
108-132-132009	PR	T54S	SVR24	1A
108-133-133012	PR	T54S	SVR24	1A
108-133-133013	PR	T54S/T	DET EOT	1A
108-169-169023	PR	T54S/T	RELAPSE	1A
108-137-137008	PR	T54S	SVR24	1B
108-205-205010	PR	T54S	SVR24	1B
108-208-208010	PR	T54S	SVR24	1B
108-605-605002	PR	D168E/D	RELAPSE	1B

APPENDIX D.

Table. Study 108: No SVR with Substitutions Emerging

PID	Arm	Outcome	Reason	Subtype	RVR4	Baseline Subst	Emerging Substitutions
108-125-125009	T8	Week 4 VF	**Lost to FU**	**1a**	N		**V36M R155K**
108-128-128008	T8	Week 4 VF		**1a**	N		**V36M R155K** I586I/T V609V/I
108-130-130003	T8	Week 4 VF		**1a**	N		**V36V/M R155K** G534G/D; *I29I/V*
108-143-143003	T8	Week 4 VF		**1a**	N		**V36M R155K** H201H/Y
108-169-169016	T8	Week 4 VF		**1a**	N		**V36M R155K/R** A200A/T V329V/I D375D/N A383A/G L384L/M V399V/I Y618F
108-709-709003	T8	Week 4 VF		**1a**	N	**V36L T54S**	**D168D/N** M179M/L A192A/D V248V/I I288I/M I329I/V S439T M485M/L A515A/T V630V/A
108-124-124014	T8	Week 12 VF	Lost to FU	1a	N		**V36M** S122S/G **R155K** T591T/S
108-211-211008	T8	Week 12 VF		1a	N		**V36M R155K**
108-128-128004	T8	Week 12 VF	Withdrew consent	1b	N		4A: *I37I/V*
108-207-207003	T8	Week 12 VF		1b	N		V36A/M T54T/S
108-108-108008	T8	Week 24 VF		1a	N		V36M R155K
108-110-110007	T8	Week 24 VF		1a	Y		**V36M R155K** G237A H593Q
108-	T8	Week 24		1a	N		V36M R155K

113-113015		VF					
108-114-114008	**T8**	Week 24 VF		1A	N	S189T	**V36A/M R155R/K**
108-120-120004	**T8**	Week 24 VF	NOT BLOD LOST FU	1A	N	A379A/T	**V36M R155K**
108-126-126002	**T8**	Week 24 VF		1A	N		V33V/I **V36M R155K** I615M
108-140-140008	**T8**	Week 24 VF		1a	N		R155K I170V
108-149-149005	T8	Week 24 VF		1A	N	V55A	**V36L**
108-149-149011	T8	Week 24 VF		1A	N		**V36V/M R155R/K**
108-150-150001	T8	Week 24 VF		1A	Y		**V36M/V R155K I170I/V**
108-151-151003	T8	Week 24 VF		1A	N		I132I/V **R155K:** *E53E/K*
108-165-165001	T8	Week 24 VF		1A	N	I170V/I A189S/T	**V36V/M R155K**
108-169-169007	T8	Week 24 VF		1A	N	A379S	**V36M R155K**
108-173-173009	T8	Week 24 VF		1A	N		**V36L R155K**
108-303-303003	T8	Week 24 VF		1A	N		**V36M R155K**
108-173-173003	T8	Week 24 VF		1B	N		**V36V/M/A**
108-156-156005	T8	Week 24 VF		1B	Y		**T54A**
108-508-508006	T8	Week 24 VF		1B	N		L13V S61S/T **T54A/T A156T** I586V
108-118-118004	T8	Week 36 VF		1A	**Y**		I18I/V **R155K:** *A36V*

108-702-702001	**T8**	Week 36 VF		1A	**N**		V55I **R155K** A515A/S: *A36A/V*
108-140-140004	T8	EOT		**1A**	**N**		**V36M R155K**
108-151-151017	T8	EOT		**1A**	**Y**	**T54S/T**	**V36M/A/V T54S/T R155R/K**
108-180-180002	T8	EOT		**1A**	**Y**		**V36M R155K**
108-128-128010	T8	EOT		**1B**	**Y**		**T54T/A**
108-169-169024	T8	EOT		**1B**	**N**		**T54T/S** G124G/R **A156T and V** I347I/V P595P/S; *V6V/I I37I/V M51V/M A263A/T*
108-104-104005	T8	EOT		**1A**	**N**		**R155R/K** T402T/S
108-181-181006	T8	EOT		**1A**	**Y**		A477A/T S553G A573A/T P574S
108-118-118007	**T8**	**DC**		**1A**	**N**		**V36M R155K** V358V/A
108-157-157005	**T8**	**DC**		**1A**	**Y**		**V36M/V R155R/K**
108-312-312001	**T8**	**DC**		**1B**	**N**		**V36M/V A156A/S**
108-402-402006	**T8**	**DC**		**1B**	**Y**		**T54T/A**
108-508-508008	**T8**	**DC**		**1B**	**N**	**T54S**	
108-166-166007	**T8**	**DC Wk2**		**1A**	**N**		V107V/I **R155R/K A156T** P264/S V358V/A
108-129-129012	**T8**	**DC Wk2**		**1b**	**N**		**A156T** V535V/I

108-137-137004	**T8**	***Relapse but det at Wk 16***		**1a**	**n**		**A156S**
108-117-117005	**T8**	***Relapse DC det a Wk 20***	**AE**	**1B**	**Y**		**V36A**
108-311-311005	**T8**	**Relapse**		**1A**	**Y**	**V36L**	I3V V151A D249E S263N V609I
108-205-205009	T8	**Relapse**		**1A**	**Y**		**V36V/M R155K**
108-704-704004	T8	**Relapse**		**1A**	**Y**		**V36M**
108-181-181004	T8	**Relapse**		**1B**	**Y**		**V36V/A**
108-313-313001	T8	**Relapse**		**1B**	**Y**		**V36V/A**
108-504-504004	T8	**Relapse**		**1B**	**Y**		**T54A**
108-605-605001	T8	**Relapse**		**1B**	**Y**		**T54T/A**
108-113-113002	T8	**Relapse**		**1A**	**N**		**R155K**
108-119-119005	T8	**Relapse**		**1A**	**Y**		**R155K**
108-144-144006	T8	**Relapse**		**1A**	**Y**		**R155T/K**
108-162-162005	T8	**Relapse**		**1A**	**Y**		**R155K** T505M
108-151-151006	T8	**Relapse**		**1B**	**Y**		**A156S**

PID	Arm	Outcome	Reason	Subtype	RVR4	Baseline Substitutions	Emerging Substitutions
108-106-106006	T12 PR	WK4 VF		**1A**	**N**		**V36M** V151V/A **R155K A156A/T**
108-121-	T12 PR	WK4 VF		**1A**	**N**		**V36M** (V36A first) **R155K**

121004							S189S/T V339A V358V/A I359I/V I386I/V K469K/R
108-133-133003	T12 PR	WK4 VF		**1A**	**N**		**V36M R155K A156T**
108-166-166006	T12 PR	WK4 VF		**1A**	**N**		**V36M R155K A156T/A**
108-305-305008	T12 PR	WK4 VF		**1A**	**N**		**V36M R155K**
108-401-401011	T12 PR	WK4 VF		**1B**	**N**		**T54S A156V (»T/V/S »S)**
108-122-122001	T12 PR	WK12 VF		**1A**	**Y**		**V36M R155K**
108-129-129004	T12 PR	WK12 VF		**1A**	**N**		**V36M R155K**
108-164-164003	T12 PR	WK12 VF		**1A**	**N**		**V36M R155K**
108-804-804003	T12 PR	WK12 VF		**1A**	**Y**		**V36M R155K**
108-201-201008	T12 PR	WK12 VF		**1B**	**N**		**V36A A45A/V**
108-119-119002	T12 PR	WK24 VF		**1A**	**N**	**V36M**	V71I
108-111-111002	T12 PR	WK24 VF		**1A**	**N**		**V36M T54S/T R155K**
108-140-140006	T12 PR	WK24 VF		**1A**	**N**		**V36M R155K**
108-145-145010	T12 PR	WK24 VF		**1A**	**Y**		**V36M R155K**
108-160-160008	T12 PR	WK24 VF		**1A**	**N**		**V36V/M R155K**
108-161-161005	T12 PR	WK24 VF		**1A**	**N**		**V36V/M/A R155K/R**

108-205-205007	T12 PR	WK24 VF		**1A**	**N**		**V36M R155K**
108-307-307006	T12 PR	WK24 VF		**1A**	**Y**		**V36M R155K**
108-508-508004	T12 PR	WK24 VF		**1A**	**N**		**V36M R155K**
108-149-149015	T12 PR	WK24 VF		**1B**	**N**		**V36A/V A156A/T**
108-407-407006	T12 PR	WK24 VF		**1B**	**N**		**T54A**
108-702-702006	T12 PR	WK24 VF		**1A**	**N**		**R155K**
108-202-202006	T12 PR	WK28 VF		**1A**	**Y**		**V36M R155K**
108-119-119009	T12 PR	EOT		**1A**	**Y**		**V36M R155K A156A/S**
108-152-152004	T12 PR	DC WK24		**1A**	**N**		**V36M R155K**
108-313-313009	T12 PR	DC WK10		**1A**	**N**		**V36M**
108-214-214002	T12 PR	EOT		**1B**	**N**		**V36V/A T54T/A T402T/S**
108-305-305006	T12 PR	DC WK8		**1B**	**N**	**V36L**	**V36L**
108-160-160003	T12 PR	EOT		**1A**	**Y**		**R155T**
108-105-105006	T12 PR	EOT		**1A**	**N**		L356I T402S
108-142-142005	T12 PR	EOT		**1A**	**N**		T402S
108-130-130004	T12 PR	Relapse DC		**1a**	**y**		**V36M** T95A **R155K**
108-129-129006	T12 PR	Relapse		**1A**	**N**		**V36M R155K**

108-143-143006	T12 PR	Relapse		**1A**	**N**		**V36M T54S**
108-144-144008	T12 PR	Relapse		**1A**	**Y**		**V36M R155R/K**
108-704-704003	T12 PR	Relapse		**1A**	**Y**		**V36M R155K**
108-705-705005	T12 PR	Relapse		**1A**	**Y**		**V36M T54S**
108-139-139005	T12 PR	Relapse		**1B**	**N**		**V36V/A**
108-504-504003	T12 PR	Relapse		**1B**	**N**		**V36L/V**
108-158-158010	T12 PR	LOST TO FU		**1B**	**N**	**V36I/L**	**V36L**

APPENDIX E.

Table. Study 111: Treatment Emergent Substitutions in Subjects with No SVR

PID	Arm	Outcome	Subtype	Baseline Substitutions	Emerging Substitutions
111-113-113012	**T12/ PR24**	**DET at EOT**	1a		**R155K** I472T I615I/V
111-166-166002	**T12/ PR24**	**DET at EOT**	1a		**T54A/T** I170V E357Q
111-172-171004	**T12/ PR24**	**DET at EOT**	1a		**V36M (wk1) R155K (wk28)**
111-102-102004	**T12/ PR24**	**RELAPSE**	1a		**R155K** F557L
111-105-105006	**T12/ PR24**	**RELAPSE**	1a		**R155G D168N** A315V; K34R
111-129-129003	**T12/ PR24**	**RELAPSE**	1a		**V36A**
111-134-134004	**T12/ PR24**	**RELAPSE**	1a		I18V **V36M** V113I K244E S280L T402S D405N
111-145-145012	**T12/ PR24**	**RELAPSE**	1a		**R155K**
111-147-147007	**T12/ PR24**	**RELAPSE**	1A		**V36V/M R155K** N174S
111-166-166003	**T12/ PR24**	**RELAPSE**	1A		**V36M**
111-201-201005	**T12/ PR24**	**RELAPSE**	1B	A150V V151T	**T54A**
111-120-120014	**T12/ PR24**	**RELAPSE**	1A		P96L **R155R/T**
111-123-123009	**T12/ PR48+**	**WK24**	1A		**V36M/V R155K A156T (wk1)** [S66T A234A/D wk1]
111-162-162006	**T12/ PR48+**	**WK24**	1A		**R155K**
111-	**T12/ PR48+**	**WK28**	1A		**V36V/A R155K**

202-202003					I72I/V
111-111-111005	**T12/ PR48+**	**DET at EOT**	1A		**R155K**
111-141-141006	**T12/ PR48+**	**RELAPSE**	1B		**T54A** I347V
111-148-148001	**T12/ PR48+**	**RELAPSE**	1A		**V36M R155K**
111-154-154010	**T12/ PR48+**	**RELAPSE**	1B		**T54A**
111-101-101007	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K: A156T(not with 36 155)** I64I/L Q526Q/L; A36V
111-111-111006	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K** S122G P264S
111-114-114003	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K** I18V F557L; R28R/K
111-120-120002	**T12/ PR48-**	**WK24**			**V36M/V R155K** P67P/S T98T/A
111-120-120008	**T12/ PR48-**	**WK24**	**1A**		**V36V/M R155K/R;** D40D/E
111-140-140007	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K** I64L E357D P574L; V30I
111-142-142005	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K** V33I/V
111-143-143016	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K** I18V
111-147-147005	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K: A156T** (not in combination with 36 or 155)
111-147-147009	**T12/ PR48-**	**WK24**	**1A**		**R155K**
111-152-	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K**

152005					
111-154-154008	**T12/ PR48-**	**WK24**	**1A**		**R155T D168N;** G8S
111-162-162001	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K: A156A/T (with V36/V)**
111-162-162004	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K** I64L A383A/G
111-165-165002	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K** I586T
111-201-201001	**T12/ PR48-**	**WK24**	**1A**		**V36M R155K** K360K/R I615V; Q46R
111-104-104007	**T12/ PR48-**	**WK36**	**1A**		**V36M**
111-152-152011	**T12/ PR48-**	**DET EOT**	**1A**	**V36L**	**R155K**
111-103-103002	**T12/ PR48-**	**RELAPSE**	**1A**		M242V
111-105-105004	**T12/ PR48-**	**RELAPSE**	**1A**	**V36L**	
111-120-120009	**T12/ PR48-**	**RELAPSE**	**1A**		**V36M** I265I/V
111-132-132006	**T12/ PR48-**	**RELAPSE**	**1A**		**V36M** T40A
111-138-138007	**T12/ PR48-**	**RELAPSE**	**1A**		**A156T** D249E V329I
111-143-143010	**T12/ PR48-**	**RELAPSE**	**1A**		**R155K**
111-146-146001	**T12/ PR48-**	**RELAPSE**	**1A**		**V36M R155K**
111-164-164009	**T12/ PR48-**	**RELAPSE**	**1A**	**V36L**	P67S A71V/A
111-	**T12/ PR48-**	**RELAPSE**	**1A**		**V36M R155K** A383G

202-202002					
111-146-146002	**T12/ PR48-**	**DC**	**1A**		**V36M**
111-155-155004	**T12/ PR48-**	**RELAPSE**	**1B**		G383S A410S I615V
111-105-105005	**Other**	**WK4**	**1A**		**V36M/V R155K A156A/T**
111-120-120018	**Other**	**WK4**	**1A**		**V36M T54S/T R155K A156A/T**
111-127-127012	**Other**	**WK4**	**1A**		**V36M R155K A156A/T**
111-136-136002	**Other**	**WK4**	**1A**		**V36M T54T/S R155K**
111-166-166009	**Other**	**WK4**	**1A**		**V36M R155K A156A/T; A156T (WK 1,3)**
111-166-166012	**Other**	**WK4**	**1A**		**V36M R155K A156T (WK1-3)**
111-168-168003	**Other**	**WK4**	**1A**		**V36M R155K**
111-114-114004	**Other**	**WK12**	**1A**		**V36M T54S/T R155K**
111-117-117001	**Other**	**WK12**	**1A**		**V36M R155K**
111-120-120001	**Other**	**WK12**	**1A**		**V36M/V R155K/R**
111-201-201002	**Other**	**WK12**	**1B**		**V36M**
111-102-102002	**Other**	**DET EOT**	**1A**		**R155K**
111-104-104002	**Other**	**DET EOT**	**1A**		**V36M/V R155K**

111-104-104015	**Other**	**DET EOT**	**1B**		**V36A/V T54A/T**
111-106-106001	**Other**	**DET EOT**	**1A**		**V36M/V R155K/R**
111-120-120006	**Other**	**DET EOT**	**1A**		**V36M R155K/R**
111-120-120017	**Other**	**DET EOT**	**1A**		**T54S A155K**
111-132-132011	**Other**	**DET EOT**	**1B**		**V36A/V T54A/T**
111-139-139005	**Other**	**DET EOT**	**1A**	**V36L**	**R155K**
111-141-141003	**Other**	**DET EOT**	**1B**		**T54A**
111-146-146003	**Other**	**DET EOT**	**1A**		**R155K**
111-147-147008	**Other**	**DET EOT**	**1A**		**V36M/V R155K/R**
111-150-150009	**Other**	**DET EOT**	**1A**		**V36M R155K**
111-154-154013	**Other**	**DET EOT**	**1A**		**V36M R155K**
111-167-167003	**Other**	**DET EOT**	**1A**		**V36M R155K A156T/A (WK1-2)**
111-168-168004	**Other**	**DET EOT**	**1A**		**V36M/V**
111-107-107002	**Other**	**RELAPSE**	**1B**		**V36A**
111-120-120011	**Other**	**RELAPSE**	**1A**	**V36I**	**V36I/V R155K/R**
111-123-	**Other**	**RELAPSE**	**1A**		**V36M/V R155K/R**

123006					
111-131-131011	**Other**	**RELAPSE**	**1A**		**V36M**
111-135-135001	**Other**	**RELAPSE**	**1A**		**V36M/V**
111-143-143009	**Other**	**RELAPSE**	**1B**		**A156S WK20 (A156T/V WK1)**
111-152-152010	**Other**	**RELAPSE**	**1A**		**R155T**
111-153-153001	**Other**	**RELAPSE**	**1A**		**V36M/A R155K**
111-154-154009	**Other**	**RELAPSE**	**1B**		**A156S**
111-167-167005	**Other**	**RELAPSE**	**1B**		**A156S/A**
111-170-170004	**Other**	**RELAPSE**	**1B**		**A156S**
111-157-157009	**Other**	**DC**	**1A**		**V36M** I170V

APPENDIX F.

Table. Study 216: No SVR with Substitutions Emerging

PID	Arm	Previous TRT	Outcome	Subtype	RVR4	Baseline Substitutions	Emerging Substitutions
C216-0004	T12 (DS)	NULL	RELAPSE	1A	N		**R155K**
C216-0007	T12	NULL	DET EOT	1B	Y		T54T/A/S
C216-0009	T12	NULL	WK 4	1A	N		**V36M R155K**
C216-0010	T12 (DS)	NULL	WK 4	1A	N		**V36M T54S R155K**
C216-0017	T12 (DS)	NULL	DET EOT	1A	N		**V36M R155K**
C216-0020	T12 (DS)	NULL	DET EOT	1A	N		**V36M R155K**
C216-0021	T12	NULL	WK 24	1A	N		**V36L R155K**
C216-0022	T12 (DS)	NULL	DET EOT	1A	N		**V36M R155K**
C216-0023	T12 (DS)	RELAPSE	RELAPSE	1A	N		**R155T**
C216-0027	T12 (DS)	NULL	WK4	1A	N		**V36M R155K**
C216-0030	T12	RELAPSE	**WK4**	1A	N		**V36M R155K**
C216-0032	T12	PARTIAL	**WK12**	1A	N		**A156A/T**
C216-0033	T12	PARTIAL	**DET EOT**	1A	Y		**V36M R155K**
C216-0035	T12	NULL	**DET EOT**	1B	Y		**V36A**
C216-0052	T12 (DS)	PARTIAL	**DET EOT**	1A	N	I170V	**V36M**
C216-0054	T12	PARTIAL	**DET EOT**	1A	Y		**R155K**
C216-0061	T12 (DS)	NULL	**WK4**	1B	N		**T54T/S A156T**
C216-0062	T12 (DS)	RELAPSE	**RELAPSE**	1A	N		**V36V/M R155R/K A156A/S**
C216-0068	T12	NULL	**WK4**	1A	N		**V36M R155K R155(T)**
C216-0073	T12	NULL	**RELAPSE**	1A	N		**V36V/M R155K A156A/V**
C216-0074	T12	NULL	**WK4**	1B	N		**T54S/T R155K**
C216-0076	T12	RELAPSE	**RELAPSE**	1A	N		**V36M**

C216-0083	T12 (DS)	NULL	**DET EOT**	1A	N	**I170V**	**A156T**
C216-0089	T12 (DS)	NULL	**WK24**	1A	N		**V36M R155K**
C216-0090	T12 (DS)	NULL	**WK6**	1A			**V36M T54T/A R155K**
C216-0096	T12	NULL	**WK4**	1A			**V36L/A/M R155K**
C216-0103	T12 (DS)	NULL	**WK4**	1A			**V36M T54S (early) R155K A156T/V/S**
C216-0111	T12	NULL	**WK8**	1A			**V36L R155K**
C216-0114	T12	NULL	**WK6**	1B			**A156T then A156F**
C216-0122	T12	NULL	**WK24**	1B			**V36V/A**
C216-0124	T12	NULL	**WK24**	1A			**V36M R155K**
C216-0132	T12 (DS)	NULL	**WK4**	1A			**V36M R155K**
C216-0133	T12 (DS)	PARTIAL	**DET EOT**	1A			**R155K**
C216-0138	T12	NULL	**WK6**	1A			**V36M T54S/T R155K**
C216-0148	T12 (DS)	NULL	**DET EOT**	1B			**T54T/A**
C216-0153	T12	NULL	**WK4**	1B			**A156T**
C216-0156	T12 (DS)	NULL	**WK24**	1A			**V36M R155K**
C216-0158	T12	RELAPSE	**RELAPSE**	1B			**T54A**
C216-0160	T12	NULL	**WK24**	1A			**V36M/V**
C216-0161	T12 (DS)	NULL	**WK4**	1A			**V36M T54S/T R155K**
C216-0167	T12	NULL	**WK4**	1A			**V36M R155K**
C216-0168	T12 (DS)	PARTIAL	**RELAPSE**	1A			**V36A/V**
C216-0170	T12 (DS)	NULL	**WK4**	1A			**V36M R155K A156A/T**
C216-0186	T12	NULL	**WK4**	1A			**V36M T54T/S R155K**
C216-0187	T12	RELAPSE	**RELAPSE**	1A			**V36A**
C216-0189	T12 (DS)	PARTIAL	**WK4**	1A			**V36M T54T/A R155K A156A/T**

C216-0191	T12	PARTIAL	**WK4**	1A		**R155K**	**V36A then V36V/M T54A/T R155K**
C216-0200	T12	NULL	**DET EOT**	1A			**V36M R155K**
C216-0207	T12 (DS)	PARTIAL	**WK24**	1A			**V36M R155K**
C216-0213	T12	NULL	**WK 6**	1A			**V36M R155K**
C216-0224	T12 (DS)	NULL	**WK4**	1A		**T54S**	**V36V/M R155K**
C216-0227	T12	RELAPSE	**RELAPSE**	1A			**V36A**
C216-0232	T12 (DS)	NULL	**WK24**	1A			**V36M R155K**
C216-0246	T12 (DS)	NULL	**WK4**	1A			**V36M R155K**
C216-0257	T12	NULL	**WK24**	1A			**V36M R155K**
C216-0260	T12	NULL	**WK4**	1A		**V36L R155K I170V**	**T54T/A**
C216-0270	T12	RELAPSE	**RELAPSE**	1B			**A156N**
C216-0273	T12	NULL	**WK4**	1A		V36L	**R155K**
C216-0276	T12	NULL	**WK8**	1B			**A156A/S**
C216-0279	T12	RELAPSE	**WK12**	1A			**V36L R155K**
C216-0285	T12	NULL	**WK12**	1A			**V36M R155K I170I/V**
C216-0286	T12	NULL	**WK6**	1A			**V36M R155K**
C216-0288	T12	NULL	**RELAPSE**	1B			**V36G then A**
C216-0298	T12	NULL	**WK4**	1A			**V36M R155K**
C216-0301	T12	NULL	**RELAPSE**	1A			**V36M/V R155K/R**
C216-0308	T12 (DS)	NULL	**RELAPSE**	1A			**A156S**
C216-0317	T12	NULL	**DET EOT**	1A			**V36M R155K**
C216-0321	T12	NULL	**WK36**	1A			**V36V/A/M R155K/R [FU >24: V36V/A R155T/R D168D/N]**

C216-0327	T12 (DS)	NULL	**WK24**	1B			**A156B**
C216-0329	T12 (DS)	NULL	**RELAPSE**	1A			**R155K**
C216-0330	T12	NULL	**RELAPSE**	1A			**A156T**
C216-0334	T12 (DS)	NULL	**WK4**	1A		T54S	**V36V/M R155K**
C216-0353	T12	NULL	**DET EOT**	1B			**T54S/T A156T/A**
C216-0355	T12 (DS)	NULL	**WK6**	1A			**V36M R155K**
C216-0361	T12 (DS)	NULL	**DET EOT**	1B			**V36M**
C216-0362	T12 (DS)	RELAPSE	**RELAPSE**	1B			**V36A**
C216-0368	T12 (DS)	NULL	**WK36**	1A			**V36M R155K**
C216-0369	T12	NULL	**WK4**	1A			**V36M R155K**
C216-0370	T12 (DS)	NULL	**RELAPSE**	1B			**V36A/V**
C216-0372	T12	NULL	**WK4**	1A		**R155K/R**	**V36M R155K/R**
C216-0377	T12 (DS)	NULL	**WK24**	1A			**V36M R155K**
C216-0379	T12 (DS)	NULL	**WK 12**	1A			**V36M/V T54S R155K**
C216-0381	T12 (DS)	NULL	**WK4**	1B			**A156T and A156S/A/T**
C216-0386	T12	NULL	**WK4**	1A			**V36M R155K**
-C216-0389	T12 (DS)	NULL	**RELASPE**	1B			**V36V/A T54T/A**
C216-0400	T12	PARTIAL	**DET EOT**	1A			**V36M/A**
C216-0414	T12	NULL	**WK24**	1B			**T54A**
C216-0417	T12	NULL	**WK 4**	1A			**V36M R155K**
C216-0425	T12 (DS)	PARTIAL	**DET EOT**	1A			**R155K**
C216-0427	T12 (DS)	NULL	**WK36**	1A			**V36M R155K and R155T (day 31)**
C216-0429	T12 (DS)	NULL	**WK24**	1B			**T54S**
C216-0439	T12	RELAPSE	**RELAPSE**	1B		**V36L**	**V36L**

C216-0442	T12 (DS)	NULL	**WK4**	1A		**T54S**	**R155K**
C216-0446	T12 (DS)	NULL	**RELAPSE**	1B			**V36A**
C216-0448	T12	NULL	**WK6**	1B			**V36V/A T54S A156T and A156V (day 3)**
C216-0449	T12 (DS)	NULL	**WK24**	1B			**V36G**
C216-0461	T12 (DS)	NULL	**WK24**	1A			**V36M R155R/K A156S I170V**
C216-0466	T12	NULL	**WK24**	1A			**V36A R155K**
C216-0473	T12 (DS)	RELAPSE	**RELAPSE**	1A			**V36V/M A156A/T I170V**
C216-0476	T12	NULL	**WK24**	1A			**V36M R155K**
C216-0497	T12	RELAPSE	**DET EOT**	1B			**V36V/I**
C216-0499	T12 (DS)	NULL	**WK36**	1A			**V36A and V36V/M**
C216-0501	T12 (DS)	NULL	**WK4**	1B			**T54T/S A156T**
C216-0507	T12 (DS)	PARTIAL	**RELAPSE**	1B			**V36A**
C216-0510	T12	PARTIAL	**DET EOT**	1A			**V36M T54S**
C216-0511	T12 (DS)	PARTIAL	**RELAPSE**	1A			**R155K**
C216-0515	T12 (DS)	NULL	**RELAPSE**	1B			**V36A**
C216-0526	T12	PARTIAL	**RELAPSE**	1B			**V36A**
C216-0558	T12 (DS)	**RELAPSE**	**RELAPSE**	1B			**A156T**
C216-0577	T12 (DS)	**RELAPSE**	**RELAPSE**	1A			**R155K**
C216-0578	T12	**RELAPSE**	**RELAPSE**	1A			**R155T (day 3) V36V/M R155R/K**
C216-0580	T12 (DS)	PARTIAL	**WK24**	1A			**V36M R155K**
C216-0587	T12	**PARTIAL**	**RELAPSE**	1A			**R155K**
0609	T12	NULL	**WK24**	1A			**V36M**
C216-0610	T12 (DS)	NULL	**WK24**	1A			**V36M T54S/T R155K**
C216-	T12	**RELAPSE**	**RELAPSE**	1A			**R155K**

0638	(DS)						
C216-0643	T12 (DS)	PARTIAL	**RELAPSE**	1A			**R155K**
C216-0660	T12	RELAPSE	**DET EOT**	1B			**T54A/T**
C216-0663	T12	PARTIAL	**RELAPSE**	1A			**V36M R155K [FU24:V36V/M R155R/T D168D/N]**
C216-0693	T12 (DS)	PARTIAL	**RELAPSE**	1A			**V36M R155K**
C216-0711	T12	**RELAPSE**	**RELAPSE**	1B			**T54A**
C216-0724	T12 (DS)	PARTIAL	**DET EOT**	1A			**R155K/R**
C216-0742	T12 (DS)	PARTIAL	**WK24**	1A			**R155K**
C216-0750	T12 (DS)	PARTIAL	**RELAPSE**	1A			**V36M R155K**
C216-0752	T12	PARTIAL	**RELAPSE**	1A			**V36V/M**
C216-0753	T12	PARTIAL	**WK24**	1A		**I170V/I**	**V36V/M I170V**
C216-0796	T12 (DS)	PARTIAL	**WK24**	1A			**V36M R155K**
C216-0798	T12	**RELAPSE**	**RELAPSE**	1B			**T54A**
C216-0834	T12	PARTIAL	**WK24**	1A			**R155K**

APPENDIX G. TREATMENT EMERGENT SUBSTITUTIONS BY STOPPING RULES/FAILURE TIMEPOINT IN EACH STUDY

Table A. Emergent Substitutions at V36, T54, R155, A156, D168 by Stopping Rule/Failure Timepoint: Study 108

	T8/PR		T12/PR		PR
	Number (%)	**# with Treatment Emergent Substitutions**	**Number (%)**	**# with Treatment Emergent Substitutions**	
No SVR (n=199)	**112**	**54 (48%)**	**87**	**40 (46%)**	**201**
Week 4 Virologic Failure	**6**	6 (11%)	**6**	6 (15%)	**-**
Week 12 Virologic Failure	**4**	3 (5%)	**6**	5 (13%)	**43**
Week 24 Virologic Failure	**22**	18 (%)	**13**	11 (%)	**56**
Week 28 Virologic Failure	**0**		**1**	**1**	**2**
Week 36 Virologic Failure	**2**	**2**	**0**		**1**
Detectable at EOT	**14**	6 (%)	**11**	5 (%)	**29**
Detectable at DC	**22**	6 (%)	**12**	3 (%)	
Relapse	**27**	13 (%)	**25**	8 (%)	**63**
NO DC	**22**	11	**23**	7	
DC	***5***	*2*	**2**	1	
Lost to FU/Withdrew for SVR	**7**	0	**6**	1	
Other (censored)	**8**		**10**		**7**

Table B. Treatment Emergent Substitutions at V36, T54, R155, A156, D168 in Study 111

	# with Treatment Emergent Substitutions			
	T12/PR24 eRVR+ N=159	**T12/PR48 eRVR+ N=158**	**T12/PR48 eRVR- N=118**	**Other N=99**
No SVR	**13**	**15**	**40**	**73**

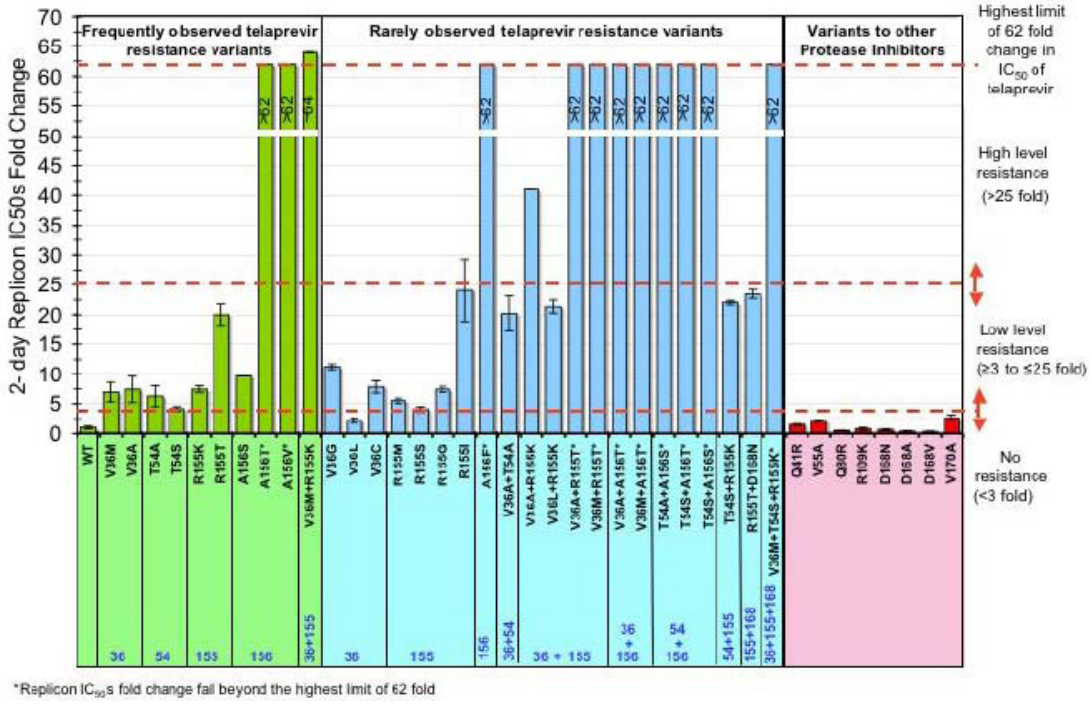
(n=141)				
Total Treatment Emergent Substitutions	**12 (92%)**	**7 (47%)**	**25 (63%)**	**38 (52%)**
Week 4 Virologic Failure				7/8
Week 12 Virologic Failure				4/4
Week 24 Virologic Failure		2/3	16/16	
Week 28 Virologic Failure		1/1	0/1	
Week 36 Virologic Failure			1/1	
Detectable at EOT	3/3	1/2	1/3	15/29
Detectable at DC			1	1
Relapse	9/9	3/3	6/10	11/13
NO DC	9	3	10	13
DC		*5*	5	8
Lost to FU/Withdrew for SVR	1		4	6
Other (censored)		1		5

Table C. Treatment Emergent Substitutions at V36, T54, R155, A156, D168, I170 in NO SVR Subset of Study 216 (N=289)

	# with Treatment Emergent Substitutions		
	T12/PR48 N=262	**T12(DS)PR48 N=262**	**PR48 N=128**
No SVR (n=289)	**94 (36%)**	**89 (34%)**	**106 (83%)**
Total Treatment Emergent Substitutions	**66 (70%)**	**62 (70%)**	**0**
Week 4 Virologic Failure	16/16	14/14	

Week 6 Virologic Failure	5/5	2/2	
Week 8 Virologic Failure	2/2		
Week 12 Virologic Failure	3/3	1/1	
Week 24 Virologic Failure	11/15 (73%)	13/14 (93%)	
Week 36 Virologic Failure	1/1	3/4	
Detectable at EOT	11/20 (55%)	10/19 (53%)	
Detectable at DC			
Relapse	17/26 (65%)	19/27 (70%)	
NO DC			
DC			

APPENDIX H. Telaprevir replicon EC_{50} fold-change for WT and NS3 variants (Report C128, page 19)



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/s/

LISA K NAEGER
04/22/2011

JULIAN J O'REAR
04/22/2011

MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 201,917 **Applicant: Vertex** **Stamp Date: Nov. 23, 2010**

Drug Name: Telaprevir **NDA Type: Original**

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comments
1	Is the virology information (nonclinical and clinical) provided and described in different sections of the NDA organized in a manner to allow substantive review to begin?	X		
2	Is the virology information (nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	X		
3	Is the virology information (nonclinical and clinical) legible so that substantive review can begin?	X		
4	On its face, has the applicant submitted cell culture data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	X		
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?	X		Studies not required.
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	X		
7	Has the applicant submitted the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?	X		
8	Has the applicant used standardized or nonstandardized methods for virologic outcome measures? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	X		
9	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		
10	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	X		
11	Have all the study reports, published articles, and other references been included and cross-referenced in the	X		

MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
	annotated draft labeling or summary section of the submission?			
12	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		X	

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE? ***Yes***__

If the NDA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Please determine the number of fluctuations and rate of fluctuation (percentage of subjects with fluctuation) from 5 IU/mL (<10 BLOD) to 17.5 IU/mL (<25 BLOQ but detectable) back to 5 IU/mL after treatment during follow-up in Study 108, Study 111 and Study 216.

We have noted that different vendors were used for HCV viral load analysis in the Studies 108/111 and Study 216. Please provide an explanation for the variability in viral load fluctuation from BLOD and BLOQ following treatment in the different studies with a report from (b) (4) **on possible reasons for the viral load fluctuations between BLOD and BLOQ in Studies 108 and 111.**

Lisa K. Naeger	12/22/10
Reviewing Microbiologist	Date
Jules O'Rear	12/22/10
Microbiology Team Leader	Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LISA K NAEGER
12/22/2010