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RESEARCH**

APPLICATION NUMBER:

205123Orig1s000

MICROBIOLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)**VIROLOGY REVIEW****NDA:** 205123 **DATE REVIEWED:** 11/20/2013**Virology Reviewer:** Damon J. Deming, Ph.D.**Applicant Name and Address:**

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Initial Submission Dates:**Correspondence Date:** March 28, 2013**Filing Date:** May 27, 2013**Review Complete Date:** October 28, 2013**PDUFA Date:** November 28, 2013**Electronic Document Room Link:** [\CDSESUB1\EVSPROD\NDA205123](#)**Additional Submissions Reviewed**SDN 035 (eCTD [0031](#))SDN 037 (eCTD [0033](#))SDN 042 (eCTD [0037](#))SDN 045 (eCTD [0039](#))SDN 049 (eCTD [0042](#))SDN 051 (eCTD [0044](#))**Received**

September 4, 2013

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September 5, 2013

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October 18, 2013

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November 19, 2013

Related Supporting Documents: IND 075391**Product Names:**

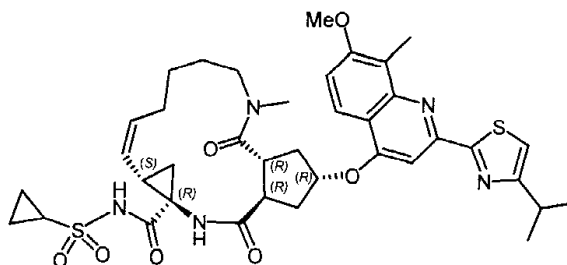
Proprietary: Olysio

Non-Proprietary/USAN: Simeprevir

Code Name/Number: TMC435; TMC435350; R494617; JNJ-38733214-AAA

Chemical Names:

(2*R*,3*aR*,10*Z*,11*aS*,12*aR*,14*aR*)-*N*-(cyclopropylsulfonyl)-2-[[2-(4-isopropyl-1,3-thiazol-2-yl)-7-methoxy-8-methyl-4-quinolinyloxy]-5-methyl-4,14-dioxo-2,3,3*a*,4,5,6,7,8,9,11*a*,12,13,14,14*a*-tetradecahydrocyclopenta[*c*]cyclopropa[*g*][1,6]diazacyclotetradecine-12*a*(1*H*)-carboxamide

Structure:**TMC435 (simeprevir)****Molecular formula:** C₃₈H₄₇N₅O₇S₂**Molecular weight:** 749.94**Drug category:** Antiviral

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 205123 **DATE REVIEWED:** 11/20/2013

Virology Reviewer: Damon J. Deming, Ph.D.

Indication: Treatment of chronic HCV infection

Dosage Form/Route of administration: 150 mg capsule/Oral

Abbreviations:

EOT, end of treatment;; HCV, hepatitis C virus; IFN α , interferon alpha; ITT, intent-to-treat; LLOQ, lower limit of quantification; LOD, limit of detection; NAPI, NS5B nucleoside analog polymerase inhibitor; NNAPI, non-nucleoside analog polymerase inhibitor; PegIFN α -2a, pegylated interferon α -2a; PI, protease inhibitor; q.d. "quaque die" once daily; P/R, PegIFN α plus ribavirin; RBV, ribavirin (Copegus[®]); RGT, response-guided treatment; RVR, rapid virologic response (i.e., HCV RNA <25 IU/mL TND at Week 4); SVR, sustained virologic response; SVR12, HCV RNA not detected at end of treatment and 12 weeks after the end of treatment; TD, HCV RNA target detected (i.e., HCV RNA <LLOQ but detected); TND, HCV RNA target not detected

Dispensed: Rx X OTC

Amendment to the Clinical Virology Review of NDA 205123

This amendment includes:

- I. Corrections to the Clinical Virology Review
- II. Section 12.4 of the agreed upon label (as of November 19, 2013)

I. Corrections

Changed text is shown red, with deletions indicated by strikethrough and insertions by underline.

(b) (4)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 205123 DATE REVIEWED: 11/20/2013

Virology Reviewer: Damon J. Deming, Ph.D.

(b) (4)

II. Proposed Package Insert (November 19, 2013)

The sponsor proposed no additional changes to the Microbiology section of the label other than correction of 2 typographical errors (not shown).

12.4 Microbiology

Mechanism of Action

Simeprevir is an inhibitor of the HCV NS3/4A protease which is essential for viral replication. In a biochemical assay simeprevir inhibited the proteolytic activity of recombinant genotype 1a and 1b HCV NS3/4A proteases, with median K_i values of 0.5 nM and 1.4 nM, respectively.

Antiviral Activity

The median simeprevir EC_{50} and EC_{90} values against a HCV genotype 1b replicon were 9.4 nM (7.05 ng/mL) and 19 nM (14.25 ng/mL), respectively. Activity of simeprevir against a selection of genotype 1a (N=78) and genotype 1b (N=59) chimeric replicons carrying NS3 sequences derived from HCV NS3/4A protease-inhibitor-naïve subjects resulted in median fold change (FC) in EC_{50} values of 1.4 (interquartile range, IQR: 0.8 to 11) and 0.4 (IQR: 0.3 to 0.7) compared to reference genotype 1b replicon, respectively. Genotype 1a (N=33) and 1b (N=2) isolates with a baseline Q80K polymorphism resulted in median FC in simeprevir EC_{50} value of 11 (IQR: 7.4 to 13) and 8.4, respectively. The presence of 50% human serum reduced simeprevir replicon activity by 2.4-fold. Combination of simeprevir with interferon, ribavirin, NS5A inhibitors, NS5B nucleoside analog polymerase inhibitors or NS5B non-nucleoside analog polymerase inhibitors, including NS5B thumb 1-, thumb 2-, and palm-domain targeting drugs, was not antagonistic.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)**VIROLOGY REVIEW****NDA: 205123 DATE REVIEWED: 11/20/2013****Virology Reviewer: Damon J. Deming, Ph.D.****Resistance in Cell Culture**

Resistance to simeprevir was characterized in HCV genotype 1a and 1b replicon-containing cells. Ninety-six percent of simeprevir-selected genotype 1 replicons carried one or multiple amino acid substitutions at NS3 protease positions F43, Q80, R155, A156, and/or D168, with substitutions at NS3 position D168 being most frequently observed (78%). Additionally, resistance to simeprevir was evaluated in HCV genotype 1a and 1b replicon assays using site-directed mutants and chimeric replicons carrying NS3 sequences derived from clinical isolates. Amino acid substitutions at NS3 positions F43, Q80, S122, R155, A156, and D168 reduced susceptibility to simeprevir. Replicons with D168V or A, and R155K substitutions displayed large reductions in susceptibility to simeprevir (FC in EC₅₀ value greater than 50), whereas other substitutions such as Q80K or R, S122R, and D168E displayed lower reductions in susceptibility (FC in EC₅₀ value between 2 and 50). Other substitutions such as Q80G or L, S122G, N or T did not reduce susceptibility to simeprevir in the replicon assay (FC in EC₅₀ value lower than 2). Amino acid substitutions at NS3 positions Q80, S122, R155, and/or D168 that were associated with lower reductions in susceptibility to simeprevir when occurring alone, reduced susceptibility to simeprevir by more than 50-fold when present in combination.

Resistance in Clinical Studies

In a pooled analysis of subjects treated with 150 mg OLYSIO in combination with peginterferon alfa and ribavirin who did not achieve SVR in the controlled Phase 2b and Phase 3 clinical trials, emerging amino acid substitutions at NS3 positions Q80, S122, R155 and/or D168 were observed in 180 out of 197 (91%) subjects. Substitutions D168V and R155K alone or in combination with other substitutions at these positions emerged most frequently (Table 8). Most of these emerging substitutions have been shown to reduce susceptibility to simeprevir in cell culture replicon assays.

HCV genotype 1 subtype-specific patterns of simeprevir treatment-emergent amino acid substitutions were observed in subjects not achieving SVR. Subjects with HCV genotype 1a predominately had emerging R155K alone or in combination with amino acid substitutions at NS3 positions Q80, S122 and/or D168, while subjects with HCV genotype 1b had most often an emerging D168V substitution (Table 8). In subjects with HCV genotype 1a with a baseline Q80K amino acid substitution an emerging R155K substitution was observed most frequently at failure.

Table 8: Treatment-Emergent Amino Acid Substitutions in Pooled Phase 2b and Phase 3 Trials: Subjects Who Did Not Achieve SVR with 150 mg OLYSIO in Combination with Peginterferon Alfa and Ribavirin

Emerging Amino Acid Substitutions in NS3	Genotype 1a[*] N=116 % (n)	Genotype 1b N=81 % (n)
Any substitution at NS3 position F43, Q80, S122, R155, A156, or D168 [†]	95% (110)	86% (70)
D168E	15% (17)	17% (14)
D168V	10% (12)	61% (49)
Q80R [‡]	4% (5)	12% (10)
R155K	77% (89)	0% (0)
Q80X+D168X [#]	4% (5)	14% (11)
R155X+D168X [#]	13% (15)	4% (3)

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Q80K [‡] , S122A/G/I/T [‡] , S122R, R155Q, D168A, D168F [‡] , D168H, D168T, I170T	Less than 10%	Less than 10%
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* May include few subjects infected with HCV genotype 1 viruses of non-1a/1b subtypes.

† Alone or in combination with other substitutions (includes mixtures).

‡ Substitutions only observed in combinations with other emerging substitutions at one or more of the NS3 positions Q80, S122, R155 and/or D168.

Subjects with these combinations are also included in other rows describing the individual substitutions. X represents multiple amino acids. Other double or triple substitutions were observed with lower frequencies.

Note: substitutions at NS3 position F43 and A156 were selected in cell culture and associated with reduced simeprevir activity in the replicon assay but were not observed at time of failure.

Persistence of Resistance–Associated Substitutions

The persistence of simeprevir-resistant NS3 amino acid substitutions was assessed following treatment failure in the pooled analysis of subjects receiving 150 mg OLYSIO in combination with peginterferon alfa and ribavirin in the Phase 2b and Phase 3 trials. The proportion of subjects with detectable levels of treatment-emergent, resistance-associated variants was followed post treatment for a median time of 28 weeks (range 0 to 70 weeks). Resistant variants remained at detectable levels in 32 out of 66 subjects (48%) with single emerging R155K and in 16 out of 48 subjects (33%) with single emerging D168V.

The lack of detection of virus containing a resistance-associated substitution does not necessarily indicate that the resistant virus is no longer present at clinically significant levels. The long-term clinical impact of the emergence or persistence of virus containing OLYSIO-resistance-associated substitutions is unknown.

Effect of Baseline HCV Polymorphisms on Treatment Response

Analyses were conducted to explore the association between naturally-occurring baseline NS3/4A amino acid substitutions (polymorphisms) and treatment outcome. In the pooled analysis of the Phase 3 trials QUEST 1 and QUEST 2, and in the PROMISE trial, the efficacy of OLYSIO in combination with peginterferon alfa and ribavirin was substantially reduced in subjects infected with HCV genotype 1a virus with the NS3 Q80K polymorphism at baseline [see *Pharmacogenomics* (12.5) and *Clinical Studies* (14)].

The observed prevalence of NS3 Q80K polymorphic variants at baseline in the overall population of the Phase 2b and Phase 3 trials was 14%; while the observed prevalence of the Q80K polymorphism was 30% in subjects infected with HCV genotype 1a and 0.5% in subjects infected with HCV genotype 1b. The observed prevalence of Q80K polymorphic variants at baseline in the U.S. population of the Phase 2b and Phase 3 trials was 35% overall, 48% in subjects infected with HCV genotype 1a and 0% in subjects infected with HCV genotype 1b. With the exception of the NS3 Q80K substitution, baseline polymorphic variants at NS3 positions F43, Q80, S122, R155, A156, and/or D168, which were associated with reduced simeprevir activity in replicon assays, were generally uncommon (1.3%) in subjects with HCV genotype 1 infection in the Phase 2b and Phase 3 trials (n=2007).

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Cross-Resistance

Cross-resistance is expected among NS3/4A protease inhibitors. Some of the treatment-emergent NS3 amino acid substitutions detected in OLYSIO-treated subjects who did not achieve SVR in clinical trials, including R155K, which emerged frequently, and I170T, which emerged infrequently, have been shown to reduce the anti-HCV activity of the NS3/4A protease inhibitors, boceprevir and/or telaprevir.

The most frequently occurring boceprevir or telaprevir treatment-emergent NS3 amino acid substitutions that are expected to impact subsequent treatment with OLYSIO include R155K and A156T or V. The NS3 amino substitutions V36A or G and I170A or T, which displayed slight shifts in susceptibility to simeprevir in replicon cultures, may emerge in patients who do not achieve SVR with boceprevir or telaprevir, and may therefore also impact subsequent treatment with OLYSIO.

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/s/

DAMON J DEMING
11/21/2013

JULIAN J O REAR
11/21/2013

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 205123 DATE REVIEWED: 08/28/2013

Virology Reviewer: Damon J. Deming, Ph.D.

Applicant Name and Address:

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1125 Trenton Harbourton RD
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Initial Submission Dates:

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Additional Submissions Reviewed

SDN 006 (eCTD [0003](#))

SDN 007 (eCTD [0004](#))

SDN 026 (eCTD [0022](#))

Received

April 23, 2013

April 29, 2013

August 14, 2013

Related Supporting Documents: IND 075391

Product Names:

Proprietary:

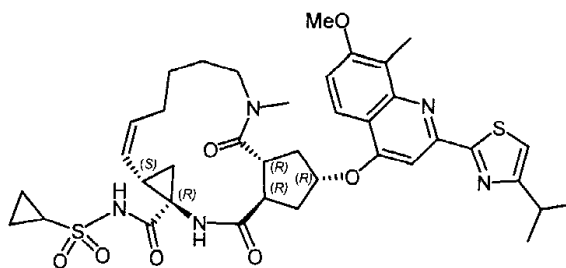
Non-Proprietary/USAN: Simeprevir

Code Name/Number: TMC435; TMC435350; R494617; JNJ-38733214-AAA

Chemical Names:

(2*R*,3*aR*,10*Z*,11*aS*,12*aR*,14*aR*)-*N*-(cyclopropylsulfonyl)-2-[[2-(4-isopropyl-1,3-thiazol-2-yl)-7-methoxy-8-methyl-4-quinolinyl]oxy]-5-methyl-4,14-dioxo-2,3,3*a*,4,5,6,7,8,9,11*a*,12,13,14,14*a*-tetradecahydrocyclopenta[*c*]cyclopropa[*g*][1,6]diazacyclotetradecine-12*a*(1*H*)-carboxamide

Structure:



TMC435 (simeprevir)

Molecular formula: C₃₈H₄₇N₅O₇S₂

Molecular weight: 749.94

Drug category: Antiviral

Indication: Treatment of chronic HCV infection

Dosage Form/Route of administration: 150 mg capsule/Oral

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VIROLOGY REVIEW

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Abbreviations:

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Dispensed: Rx X **OTC**

APPEARS THIS WAY ON ORIGINAL

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 205123 DATE REVIEWED: 08/28/2013

Virology Reviewer: Damon J. Deming, Ph.D.

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DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 205123 **DATE REVIEWED:** 08/28/2013

Virology Reviewer: Damon J. Deming, Ph.D.

Executive Summary

1 Recommendations

1.1 Recommendation and Conclusion on Approvability

This Original NDA is approvable from a Virology perspective for the treatment of chronic HCV genotype 1 infected patients who are either naïve to prior anti-HCV therapy or who relapsed with prior therapy. However, subjects infected with genotype 1a NS3_Q80K variants, a very common polymorphism in the U.S., experienced substantially reduced SVR rates relative to those infected with other polymorphic variants. Those genotype 1a Q80K-infected subjects who received TMC435 and failed to achieve SVR frequently developed NS3_R155K-expressing virus, which is expected to be cross-resistant to other NS3/4A inhibitors, including the approved boceprevir and telaprevir, and to persist, possibly for years. We therefore recommend that genotype 1a Q80K-infected subjects do not use TMC435 in combination with PegIFN α /RBV (P/R).

The review team is currently engaged in discussions regarding the inclusion of prior partial-responders and prior null-responders in the indication. While the limited phase 2b data indicate that TMC435 treatment was generally associated with improved SVR rates, there were too few Q80K variant-infected subjects to determine the magnitude of the impact that the polymorphic variant might have on the TMC435 + P/R regimen. It is the current opinion of this reviewer that the indication should only be extended to include partial- and null-responders if screening for the genotype 1a Q80K variant is conducted and Q80K-infected subjects excluded.

1.2 Recommendation on Phase 4 Commitments

Determine the phenotypic susceptibility of TMC435 against:

- L356F or V106I expressed in genotype 1a replicon cultures, individually and in combination with Q80K
- R24W, K213R, T358F, or T610I expressed in genotype 1b replicon cultures

2 Summary of Virology Assessments

2.1 Nonclinical Virology

TMC435 is a small molecule inhibitor of the hepatitis C virus (HCV) NS3/4A serine protease. Inhibition of the NS3/4A protease prevents the proteolytic processing of the HCV nonstructural polyprotein, which is required for HCV replication. TMC435 inhibited the replication of HCV subgenomic genotype 1b (strain Con1b) replicons in Huh7 cells with median EC₅₀ and EC₉₀ values of 9.4 nM and 19 nM, respectively. The antiviral activity of TMC435 was confirmed against a panel of genotype 1a and genotype 1b replicons expressing the NS3 genes of clinical isolates. The median fold-change in susceptibility of the genotype 1a chimeric replicons relative to the H77 genotype 1a control replicon was 1.4 (interquartile range, IQR: 0.8 to 11.3), while the median fold-change in susceptibility of the genotype 1b chimeric replicons relative to the genotype 1b control replicon was 0.4 (IQR: 0.3 to 0.7). TMC435 was not antagonistic with PegIFN α , RBV, or class-representative direct acting antiviral drugs targeting HCV NS5A or NS5B. TMC435's antiviral activity on replicons was reduced 2.4-fold in the presence of 50% human serum.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

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HCV genotype 1a and genotype 1b replicons with reduced susceptibility to TMC435 were selected and characterized in a series of nonclinical studies. Reduced susceptibility to TMC435 was frequently associated with substitutions in the NS3 protease at amino acids F43, Q80, R155, A156, and/or D168, and less frequently with substitutions at amino acids Q41, Q89, N174, and N176. Phenotypic analysis of treatment-emergent isolates of early clinical studies indicated that R155K and a series of substitutions at D168 represent the primary pathways to resistance.

Cross-resistance between TMC435 and other NS3/4A protease inhibitors (PIs) is expected, and patients who fail to achieve SVR after receiving TMC435 may lose the benefit of other NS3/4A PI-containing regimens.

2.2 Clinical Virology

TMC435 was studied in HCV genotype 1 infected populations in several phase 1, 2, and 3 trials. Data from two pivotal trials in treatment-naïve subjects, TMC435-TiDP16-C208 (C208) and TMC435-TiDP16-C216 (C216), and one pivotal trial in prior P/R-relapsers, TMC435HPC3007 (HPC3007), were included in this NDA submission. Subjects in all three trials received 150 mg of TMC435 q.d. in combination with P/R, followed by either 12 or 36 additional weeks of P/R. The duration of the P/R was determined by the following response-guided treatment (RGT) algorithm: subjects who achieved HCV RNA levels of <25 IU/mL by Week 4 received a total of 24 weeks of P/R, while those with HCV RNA levels ≥25 IU/mL at Week 4 received a total of 48 weeks of P/R.

The most frequent treatment-emergent, resistance-associated substitutions identified during the clinical trials were NS3_R155K, D168E, and D168V for genotype 1a viruses and Q80R, D168E, and D168V for genotype 1b viruses. NS3_R155K and multiple substitutions at NS3_D168 are associated with resistance to NS3/4A protease inhibitors in general, so the majority of subjects who fail TMC435-containing regimens may have limited re-treatment options available until their resistant virus has fully reverted, which may take years, or new classes of anti-HCV drugs become approved.

TMC435 treatment was consistently associated with improved SVR responses in the phase 3 trials. However, analysis of the impact of HCV polymorphic variants on efficacy revealed that the SVR rates of subjects infected with genotype 1a NS3_Q80K at baseline were not significantly improved relative to those who received placebo, despite evidence of antiviral activity (i.e., improvements in early virologic responses). In lieu of screening for the presence of genotype 1a Q80K virus in prospective patients, who could then be excluded from treatment, the sponsor provided an alternative (b) (4) algorithm (b) (4)

(b) (4)

(b) (4) The sponsor's proposed (b) (4) algorithm is therefore an inadequate alternative to screening.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 205123 **DATE REVIEWED:** 08/28/2013

Virology Reviewer: Damon J. Deming, Ph.D.

3 Administrative Signatures

3.1 *Reviewer's Signature*

Damon J. Deming, Ph.D.

Virology Reviewer, Division of Antiviral Products

3.2 *Concurrence*

Julian J. O'Rear, Ph.D.

Virology Team Leader, Division of Antiviral Products

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 205123 DATE REVIEWED: 08/28/2013

Virology Reviewer: Damon J. Deming, Ph.D.

OND Virology Review

1 Introduction and Background

1.1 Important Milestones in Product Development

Simeprevir (TMC435) is a hepatitis C virus (HCV) direct-acting antiviral agent (DAA) that has been developed for the treatment of chronic HCV infection. The Original IND 075391 was received on April 30, 2008. Several non-clinical and clinical studies have been conducted under the IND. Clinical study reports and electronic datasets for three supportive Phase 3 trials, C208, C216, and HPC3007 were included in this submission. The protocols for C208, C216, and HPC3007 were submitted on December 3, 2010 (SDN 236).

1.2 Methodology

HCV genotype determination

HCV genotyping in the phase 2b and 3 studies used two commercially available investigational assays, the Trugene™ assay and the Versant® HCV Genotype 2.0 (LiPA) assay. Subsequently, Janssen Diagnostics repeated the genotyping of Baseline isolates using NS5B nucleotide sequence analysis of a 329 bp region of the viral gene and phylogenetic analysis. Efficacy subgroup analyses were based on the genotyping results of the NS5B nucleotide sequence-based assay, when available. NS5B nucleotide sequence analysis was used to genotype 97.2% (1,970/2,026) of subject isolates, while the LiPA assay was used to genotype 45 of the subjects who were not analyzed by the NS5B assay, and the remaining 11 subjects were genotyped using the Trugene™ assay.

Isolates from 865 subjects were genotyped using both the NS5B assay and the Trugene™ assay, and the two assays were concordant for 77.8% (673/865) of the isolates. A majority of the discordant results (65.6% [126/192]) were related to the lack of a subtype determination by the Trugene™ assay, with another 32.8% (63/192) due to differing subtyping results between assays, and one case of incorrect genotyping (1b reported by Trugene, 6e by NS5B nucleotide sequence analysis). There were two cases where NS5B nucleotide sequence analysis did not successfully identify the isolates' subtypes.

Isolates from 1,105 subjects were genotyped by both the NS5B nucleotide sequence assay and the LiPA assay. The NS5B and LiPA assays were 99.5% (1,100/1,105) concordant, with 4 of the 5 discordant cases due to disagreement between subtype determinations, and one case was due to the LiPA reporting the presence of a mixed genotype while the NS5B assay reported genotype 1a.

Only two isolates were genotyped using both the Trugene™ and LiPA assays.

HCV viral load assessments

Plasma HCV RNA levels were determined using the Roche COBAS® TaqMan® HCV/HPS v2.0 assay, which has a quantitative range of 25 to 300,000,000 IU/mL and a limit of detection (LOD) of 15 IU/mL for HCV genotype 1 and 12 IU/mL for HCV genotype 4. The LOD is defined as the HCV RNA concentration at which the viral RNA is detected 95% of the time.

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Resistance-related Assessments

Population-based Sanger nucleotide sequencing of the HCV NS3/4A region was performed in all subjects at baseline, and at predefined time points to monitor for emerging HCV variants. In subjects with HCV genotype 1 infection, substitutions were defined as changes from reference strain H77 (AF009606) for genotype 1a isolates and from strain Con1 (AJ238799) for genotype 1b isolates.

1.3 Prior FDA Virology Reviews

The original IND submission was reviewed by Jules O'Rear, Ph.D., while IND submissions SDN 006 through SDN 174 were reviewed by Patrick Harrington, Ph.D. The IND submissions after SDN 190 were reviewed by Damon Deming, Ph.D.

1.4 Major Virology Issues that Arose during Product Development

HCV NS3_Q80K is an HCV genotype 1a polymorphic variant that is known to confer reduced-susceptibility to some NS3/4A protease inhibitors ([Bae et al., 2010](#); [Lenz et al., 2010](#)). The Q80K polymorphism is very common among genotype 1a viruses, with an estimated frequency of approximately 30-50% ([Bae et al., 2010](#); [Bartels et al., 2013](#); [Cento et al., 2012](#); DAVP review data). [Bae et al., 2010](#) reported the identification of Q80K variants among 121/268 (45.1%) genotype 1a isolates within their combined HCV Los Alamos and Gilead Science databases, while [Bartels et al., 2013](#) reported that Q80K variants were detected among 37.6% (793/2,111) of the Baseline genotype 1a isolates from treatment-naïve subjects who participated in telaprevir's phase 2 and 3 clinical trials. [Cento et al., 2012](#) identified Q80K variants at a frequency of 41.5% (258/621) among genotype 1a viruses included in their analyses. During the DAVP review of boceprevir (NDA 202285; Virology review by Patrick Harrington, Ph.D.), Q80K variants were detected at a frequency of 40.7% (319/784) among genotype 1a isolates of subjects who participated in that drug's phase 3 trials.

During TMC435's phase 2b and 3 trials, Janssen encountered Q80K variant frequencies among genotype 1a viruses that varied from 7% to 48%, depending on study region. Specifically, Q80K variant frequencies were 7.1% (9/127) in Asia-Pacific sites, 19.2% (71/369) in European sites, 48.3% (185/383) in North American sites, and 9.1% (2/22) in South-American sites. Q80K variant frequencies in the United States sites were 48.0% (143/298), indicating that the proportion of subjects infected with HCV genotype 1 genotype 1a Q80K-variants (34.5% [143/415]) was higher than that of genotype 1b-infected individuals (27.5% [114/415]). Notably, Q80K was very rare in genotype 1b viruses, with an overall frequency of 0.5% (5/1096).

In replicon culture studies, the presence of Q80K was associated with an approximately 10-fold reduction in susceptibility (see Section 2.3). Results from the TMC435 phase 2b studies, C205 and C206, which were conducted in treatment-naïve and treatment-experienced subjects, respectively, indicated that the 150 mg q.d. TMC435 in combination with P/R (TMC435 + P/R) would likely maintain activity against Q80K variants (see Section 4.7). However, data from the phase 3 trials showed that TMC435 + P/R had significantly reduced efficacy against genotype 1a Q80K variants (see Section 4.1). Instead of screening for the presence of the genotype 1a Q80K variant in HCV-infected subjects before administering TMC435 + P/R, the sponsor proposed an alternative (b) (4) algorithm (b) (4)

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(b) (4)

1.5 *State of Antivirals Used for the Indication Sought*

The current standard-of-care therapies for HCV genotype 1 infection include the approved NS3/4a inhibitors, boceprevir (NDA 202258; approved May 13, 2011) and telaprevir (NDA 201917; approved May 23, 2011), in combination with recombinant pegylated interferon alpha and ribavirin (P/R). Boceprevir, telaprevir, and TMC435—along with several other anti-HCV drugs in development—target the NS3/4A protease and share overlapping resistance profiles. Although data comparisons between trials must be interpreted with caution, the treatment-related increases in SVR rates for TMC435, boceprevir, and telaprevir in combination with P/R appear to be similar when compared to the SVR rates of their placebo + P/R controls (~30% increases in treatment naïve subjects). However, analysis of the phase 3 data included in this NDA showed that TMC435 + P/R is less efficacious against HCV genotype 1a NS3_Q80K-expressing variants than against other HCV genotype 1a polymorphic variants in treatment-naïve and prior P/R-relapsers. In contrast, similar efficacy reductions for boceprevir or telaprevir in combination with P/R against the genotype 1a Q80K variant were not observed during the pivotal trials of those drugs.

2 *Nonclinical Virology*

2.1 *Mechanism of Action*

TMC435 is a macrocyclic, noncovalent, peptidomimetic inhibitor of the hepatitis C virus (HCV) NS3/4A serine protease. Inhibition of the NS3/4A protease prevents the proteolytic processing of the HCV nonstructural polyprotein, which is necessary for HCV replication. The sponsor presented biochemical, structural, replicon culture antiviral activity, and genotypic and phenotypic replicon culture resistance study data to support TMC435's mechanism of action.

2.2 *Biochemical Assays*

TMC435 inhibited the enzymatic activity of recombinant HCV genotype 1a (H77) and genotype 1b (Con1) NS3/4A proteases in a fluorescence resonance energy transfer (FRET)-based biochemical assay with a median IC₅₀ value of 2.3 nM (interquartile range [IQR]: 1.8 to 2.3 nM) against HCV genotype 1a and 5.2 nM (IQR: 5.1 to 5.3 nM) against genotype 1b.

The inhibitory activity of TMC435 against HCV genotype 1b (Con1) NS3/4A proteases bearing individual amino acid substitutions known to reduce TMC435's activity in replicon assays—including Q80K, R155K, A156V, D168E, and D168V—were determined using the same biochemical assay. As expected, TMC435 had reduced activity against the mutant recombinant proteases, with median IC₅₀ values of 14 nM (IQR: 13 to 14 nM), 86 nM (IQR: 76 to 120 nM), 98 nM (IQR: 98 to 130 nM), 61 nM (IQR: 49 to 76 nM), and 1200 nM (n=2, no IQR determined), respectively.

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The inhibitory activity of TMC435 against a panel of recombinant NS3/4A proteases derived from 20 clinical isolates representing HCV genotypes 1 through 6 was also determined using the FRET-assay. The range of median IC₅₀ values (where more than one isolate was available) for genotypes 1a, 1b, 2b, 3a, 4a, 4d, 5a, and 6a were <0.2 to 2.0 nM (n=4), 5.7 to 13 nM (n=3), 3.2 nM (n=1), 43 to 767 nM (n=2), 2.0 to 26 nM (n=3), 2.1 to 4.1 nM (n=3), 370 nM (n=1), and 0.6 to 4.3 nM (n=3), respectively. The reduced activity against the genotype 3a isolates, which had 8.3- and 148-fold higher IC₅₀ values than the genotype 1b reference were likely due to presence of a D168Q polymorphism in each isolate. TMC435's inhibitory activity against the genotype 5a isolate was 71-fold higher than against the genotype 1b reference, which was likely due to Q80K and D168E polymorphisms in the isolate.

The inhibitory activity of TMC435 against HCV genotype 1a, 1b, 2a, and 3a recombinant NS5B polymerases was determined using a primer-dependent transcription assay. The median IC₅₀ values of TMC435 were 8.02 µM (IQR: 5.83 to 20.50 µM), 7.07 µM (IQR: 5.99 to 17.30 µM), 13.40 µM (IQR: 8.15 to 18.80 µM), and 6.04 µM (IQR: 5.24 to 11.30 µM), respectively. These data indicate that TMC435 does not have significant inhibitory activity against the HCV NS5B polymerase.

The inhibitory activity of TMC435 against a panel of 20 cellular proteases, including serine proteases (i.e., chymase, chymotrypsin, factor VIIa, factor Xa, proteinase 3, streptokinase, tissue plasminogen activator, tryptase, urokinase, leukocyte-elastase, plasmin, thrombin, trypsin, and cathepsin G), cysteine proteases (cathepsin S, cathepsin L, and cathepsin B), and aspartic acid proteases (cathepsin D and cathepsin E) was determined to assess specificity. TMC435 inhibited the proteolytic activity of 6 out of the 20 cellular enzymes with IC₅₀ values <10 µM, including cathepsin S, leukocyte-elastase, cathepsin G, thrombin, trypsin, and plasmin with IC₅₀ values of 0.8 µM, 1.5 µM, 3.8 µM, 5.6 µM, 5.7 µM, and 5.8 µM, respectively. The remaining cellular proteases had IC₅₀ values >10 µM. The selectivity indices of TMC435 for HCV genotype 1b NS3 against these 6 cellular proteases therefore ranged from 154 (cathepsin S) to 1,115 (plasmin). TMC435's activity against cathepsin S and thrombin were further assessed in secondary cellular assays, an invariant chain degradation assay and spiked human coagulation assay, respectively, and no inhibitory activity was detected at the highest tested concentrations (10 µM and 300 µM, respectively).

2.3 Cell Culture Studies

TMC435's inhibitory activity and cellular cytotoxicity against HCV replicon cultures was evaluated in five HCV genotype 1a and 1b replicon cell culture systems (i.e., Huh7-FL1a, Huh7-SG1a, Huh-21-5, Huh7-Luc, and Huh7-SGCon1b) using either luciferase reporter gene- or quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)-based assays for quantifying replication.

TMC435 inhibited HCV genotype 1b replicon in Huh7-Luc cultures with a median EC₅₀ value of 9.4 nM (IQR: 7.3 to 12 nM) by luciferase assay read-out. TMC435 was cytotoxic in a control cell line with HCV-independent transcription of luciferase, with a CC₅₀ value of 33.7 µM by luciferase assay read-out, yielding a therapeutic index of 3,600.

TMC435 inhibited the replication of two HCV genotype 1a replicon cultures, Huh7-FL1a and Huh7-SG1a, with median EC₅₀ values of 23 nM (IQR: 10 to 28 nM) and 28 nM (IQR: 19 to 40 nM), respectively, by qRT-PCR read-out. TMC435 was cytotoxic on both cultures with median CC₅₀ values of >32 µM by qRT-PCR read-out of a cellular RNA control, yielding therapeutic indices of >1,400 and >1,100, respectively.

TMC435 inhibited the replication of three HCV genotype 1b replicon cultures, Huh-21-5, Huh7-Luc, and Huh7-SGCon1b, with median EC₅₀ values of 58 nM (n=2, no IQR determinations), 29 nM (IQR: 27 to 41 nM), and 115 nM (IQR: 94 to 123 nM), respectively, by qRT-PCR read-out. TMC435 was cytotoxic with

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median CC₅₀ values of >10 µM, 86.7 µM, and >32 µM, respectively, yielding therapeutic indices of >170; 3,000; and >280, respectively.

The impact of human plasma proteins on TMC435's antiviral activity was determined on Huh7-Luc replicon cultures using a luciferase assay-based read-out. The EC₅₀ value of TMC435 was determined in the presence of 1 mg/mL α-1 acid glycoprotein (AAG), 40 mg/mL human serum albumin (HSA), the combination of AAG with HSA, or up to 50% human serum (HS). The largest EC₅₀ value shift was a 2.4-fold increase in the presence of 50% HS. The extrapolated EC₅₀ value shift in 100% HS is approximately 4.1.

TMC435 showed limited antiviral activity against a panel of 11 viruses, including bovine diarrhea virus (BVDV), Coxsackie virus, hepatitis B virus (HBV), herpes simplex virus type-2 (HSV-2), human immunodeficiency virus type 1 (HIV-1), influenza A virus, respiratory syncytial virus (RSV), Sindbis virus (SinV), vesicular stomatitis virus (VSV), West Nile virus (WNV), and yellow fever virus (YFV) with EC₅₀ values greater than the highest concentration tested (i.e., EC₅₀ values ranging from >25 to >100 µM). BVDV, WNV, and YFV are distantly related flaviviruses. In addition, TMC435 inhibited HIV-1 protease activity with an IC₅₀ value >200 µM in an enzymatic assay. Collectively, these data indicate that TMC435's antiviral activity is specific for HCV.

2.4 *Animal Studies*

No virology-related animal studies were reported.

2.5 *Resistance Studies*

Fourteen independent HCV genotype 1 TMC435 reduced-susceptibility selection experiments were performed, including six experiments in genotype 1a (Huh7-SG1a) and eight experiments in genotype 1b (Huh7-Luc and Huh7-con1b) replicon cultures (these data have been published in [Lenz et al., 2010](#)). Replicon variants with reduced susceptibility to TMC435 were selected while grown in either a constant concentration (i.e., 10- or 50-times the EC₅₀ value) of TMC435 or under increasing concentrations of TMC435 with starting concentrations at 1-, 10-, or 20-times the EC₅₀ value. In total, 46 genotype 1a and 63 genotype 1b replicon colonies or cell pools grown in the presence of TMC435 were analyzed for genotypic resistance. In addition, 21 genotype 1a and 91 genotype 1b replicon colonies or pools grown in the absence of TMC435 were genotypically assessed to identify polymorphic sites.

Ninety-six percent (105/109) of the replicons selected under TMC435 expressed NS3 substitutions at F43, Q80, R155, A156, and/or D168, while none of the replicons grown in the absence of TMC435 had variants expressing substitutions at any of those amino acid positions. The selection of NS3_D168 variants, including (in order of decreasing frequency) D168V, A, E, H, G, N, or Y, was observed in 78% (85/109) of the replicons, with the D168A variant most frequently observed in genotype 1a replicons (35% [16/46]) and the D168V variant most frequently observed in genotype 1b replicons (54% [34/63]) (Table 1, Virology Reviewer's analysis of data in nonclinical study reports TMC435-IVS-AVMR and TMC435-SDM-AVMR). Selection of Q80 variants, including Q80R, K, or H, was observed in 13% (14/109) of the replicons, with the Q80R variant most frequently observed in genotype 1a (13% [6/46]) and genotype 1b (4.8% [3/63]) replicons. Selection of Q80K was only observed in genotype 1a replicon cultures. NS3_A156 variants were selected in 9.2% (10/109) of the replicon cultures and included A156V, G, or T-expressing variants. The emergence of NS3_R155K variants was observed in 6.4% (7/109) of the replicon cultures overall, but was limited to genotype 1a replicons. A156V was the most

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frequently observed variant (5.5% [6/109]) in replicon cultures overall, but was limited to genotype 1b replicons. NS3_F43S-expressing variants were selected in 3.7% (4/109) of replicon cultures, but were limited to genotype 1b replicons and were only detected in combination with Q80R or D168E.

Four percent (4/109) of the replicons selected under TMC435 did not express F43, Q80, R155, A156, or D168 variants. Instead, these 4 replicon pools—which were all genotype 1a—harbored NS3_Q41R, Q41R+N174K, Q41R+E176K, or Q89R+N174K expressing variants, respectively. In total, Q41-expressing variants were selected in 18% (20/109) replicon cultures and included Q41R (n=19) or Q41P (n=1) variants. In contrast, only one Q41 variant (0.89% [1/112]) was detected in control cultures. Q41R variants were present in combination with Q80, R155, and/or D168 variants in 16/19 cases, and were primarily detected in genotype 1a replicons. The three remaining Q41R variants were detected as a mixture with wild-type (Q41Q/R), or in combination with N174K or E176K. The Q41P variant was detected in combination with A156G in a genotype 1a replicon.

Table 1. Frequency of replicon variants selected in the presence of TMC435 and TMC435 susceptibility against site-directed mutant replicons

NS3 Substitution ¹	Frequency of Observation ²		Fold Change in EC ₅₀ value ³	
	GT 1a	GT 1b	GT 1a	GT 1b
Q41R	1/46 (2.2%)		5.4	1.8
Q41R+N174K	1/46 (2.2%)			
Q41R+E176K	1/46 (2.2%)			
F43S+Q80R		1/63 (1.6%)		286
F43S+D168E		3/63 (4.8%)		694
Q80H+D168E	1/46 (2.2%)			163
Q80K	2/46 (4.3%)		9.3	7.7
Q80K+R155K	1/46 (2.2%)		1850	364
Q80K+R155K+D168A	1/46 (2.2%)			
Q80R	3/46 (6.5%)	1/63 (1.6%)	13	6.9
Q80R+R155K	2/46 (4.3%)			270
Q80R+D168A	1/46 (2.2%)			2655
Q80R+D168E		1/63 (1.6%)	>1320	
Q89R+N174K	1/46 (2.2%)			
R155K	3/46 (6.5%)		88	33
A156G	1/46 (2.2%)	1/63 (1.6%)		16
A156T		1/63 (1.6%)		44
A156V		4/63 (6.3%)		181
A156T+D168V	1/46 (2.2%)			
A156V+D168A		1/63 (1.6%)		
A156V+D168V		1/63 (1.6%)		
D168A	16/46 (34.8%)	13/63 (20.6%)		948
D168E	2/46 (4.3%)	1/63 (1.6%)	26	43
D168G	1/46 (2.2%)			4.3
D168H	2/46 (4.3%)	2/63 (3.2%)		368
D168I		2/63 (3.2%)		1800
D168N	1/46 (2.2%)			6.6
D168T		2/63 (3.2%)		308
D168V	8/46 (17.4%)	34/63 (54%)	>1610	2830
D168Y		1/63 (1.6%)		651

¹ Q41, Q89, N174K, and E176K variants selected in combination with F43, Q80, R155, A156, or D168 substitutions are not shown.

² Frequency of observed replicon variants selected under TMC435

³ Susceptibility data derived in a separate experiment using site-directed mutant replicons, not from the replicons grown under TMC435 selection

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NS3_Q89 variants were selected in 4.6% (5/109) of the replicon cultures but were only detected in 0.9% (1/112) of controls. NS3_Q89R (genotype 1a) or P89L (genotype 1b) were detected in combination with a Q80, A156, and/or D168 substitution in 4 of the 5 of the cultures, while Q89R was present in combination with N174K in the remaining culture.

NS3_N174K was selected in combination with Q41R, Q89R, or R155K in 6.5% (3/46) of the genotype 1a replicon cultures.

NS3_E176 variants were selected in 28% (13/46) of the genotype 1a replicon cultures. The sponsor pointed out that amino acid substitutions at this position are known to be selected in cell culture ([Krieger et al., 2001](#)), and did not include E176-expressing replicon variants in their phenotypic analyses. However, it should be noted that the sponsor did not report the emergence of any E176 variants in genotype 1a cultures included in the control experiment, so it remains possible that E176 variants emerged specifically in genotype 1a replicons grown under selective pressure of TMC435.

The anti-HCV activity of TMC435 was assessed in a transient replicon assay using HCV genotype 1a (H77) and/or HCV genotype 1b (Con1) replicon cultures. The median fold-change in EC₅₀ value for a subset of site-directed mutant replicons representing variants identified during the replicon selection experiments are summarized in Table 1. The fold-changes (FC) in susceptibility ranged from 5.5 to 2,830 for each single-or double-amino acid variant when tested on the appropriate background.

Activity of TMC435 against Chimeric Replicons

The anti-HCV activity of TMC435 against chimeric replicons expressing the NS3 gene from 78 genotype 1a and 59 genotype 1b clinical isolates obtained from subjects who participated in TMC435's phase 1 and 2 trials was determined by transient replicon assay. The EC₅₀ values of the genotype 1a chimeric replicons ranged from 0.4- to 100-fold different from that of the H77 control replicon, with a median fold-difference of 1.4 (IQR: 0.8 to 11.3), while those of the genotype 1b chimeric replicons differed from the Con1 control from 0.1 to 26-fold, with median differences of 0.4 (IQR: 0.3 to 0.7).

Thirty-six percent (28/78) of the genotype 1a isolates did not harbor variants expressing substitutions at any of the primary sites of interest identified in the replicon selection experiments (i.e., F43, Q80, R155, A156, or D168) and were fully susceptible to TMC435, with a fold-change (FC) in susceptibility ranging from 0.4 to 2.0 and a median of 0.9 (IQR: 0.7 to 1.1). The most common baseline polymorphisms in the genotype 1a clinical isolates that were expected to impact TMC435's antiviral activity included those at Q80 (47% [37/78]), with Q80K being the most common (42% [33/78]), and R155K (5.1% [4/78]). The FC value of TMC435 susceptibility for isolates bearing Q80K ranged from 3.6 to 27, with a median of 11 (IQR: 7.4 to 13). The FC value in TMC435 susceptibility of R155K-expressing isolates ranged from 26 to 100, with a median FC value of 95 (IQR: 43 to 99).

Sixty-three percent (37/59) of the genotype 1b isolates did not harbor variants expressing substitutions at the primary sites of interest and were fully susceptible to TMC435, with FC values ranging from 0.1 to 1.5 and with a median FC value of 0.3 (IQR: 0.3 to 0.4). The most common baseline polymorphisms in the genotype 1b clinical isolates that were expected to impact TMC435's antiviral activity included those at Q80 (19% [11/59]) and D168 (1.7% [1/59]). Two of the genotype 1b clinical isolates expressed Q80K; one had an FC value of 15 relative to the control replicon while the second, which harbored the combination of Q80K+S122G, had an FC value of 1.8 relative to the control. A Q80L polymorphism was present in 14% (8/59) of the isolates and was associated with a median FC value of 0.7. One isolate expressed Q80R and had an FC value of 4.1. The isolate expressing D168E had an FC value of 20.

One genotype 1b-infected subject with a baseline variant expressing S122T had an FC value of 26, although five other subjects with S122T variants were fully susceptible with FC values ranging from 0.2

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to 0.5, indicating that the loss in susceptibility may not have been due to the presence of S122T, but to other polymorphic substitution(s) at positions other than F43, Q80, R155, A156, or D168. Notably, there were 12 genotype 1a isolates bearing S122 polymorphisms, and those were also fully susceptible to TMC435, with a median FC value of 0.5 (IQR: 0.4 to 1.0). However, in a follow up study designed to confirm that TMC435 susceptibility differences observed for some of the baseline clinical isolates could be attributed to the presence of individual substitutions (i.e., they were engineered into wild-type replicon backbone by site-directed mutagenesis), S122R was determined to confer an FC value of 20 when expressed from a genotype 1b replicon. S122K was also later reported to confer a 29-fold reduction in susceptibility in a follow-up study in replicon cultures, although that variant was not observed in cell culture selection studies, as a baseline polymorphism, or among treatment-failure isolates. In contrast, S122G, S122N, and S122T did not confer more than a 1.1 shift in susceptibility in that study.

Cross-Resistance

The impact of TMC435 resistance-associated substitutions on anti-HCV direct acting antiviral drugs, including other NS3/4A protease inhibitors (e.g., boceprevir and telaprevir), and inhibitors of other drug classes in development, including NS5A inhibitors, NS5B nucleoside analog polymerase inhibitors (NAPI), and non-nucleoside analog polymerase inhibitors (NNAPI), was evaluated. The impact of common resistance-associated substitutions of these other drug classes on TMC435 was also conducted.

Boceprevir and telaprevir resistance-associated substitutions typically involve amino acid changes at NS3_V36, T54, V55, V107, R155K, A156, V158, D168, V170, and M175. TMC435's susceptibility to V36A/G/I/L/M, T54A/C/G/S, V55A/C/I, V107I, V158I, V170A/F/I/T, and M175L—those substitutions at positions other than R155, A156, and D168 which are known to reduce susceptibility to TMC435—was determined. Substitutions that conferred a ≥ 2 -fold reduction in TMC435 susceptibility in genotype 1b replicon culture included V36A (2.8-fold), V36G (3.6-fold), V170A (4.7-fold), and V170T (5.4-fold), indicating that these substitutions could contribute to TMC435 resistance. Note that lack of a detectable shift in susceptibility in the replicon assay does not lead to a conclusion that a substitution is not resistance-associated.

TMC435 had ≤ 2 -fold reductions in susceptibility against genotype 1b replicons expressing NS5A inhibitor resistance-associated substitutions, L31F/V or Y93C/H; replicons expressing the NAPI resistance-associated substitution, S96T; and replicons expressing the NNAPI resistance-associated substitutions, C316N/Y, S365T, M414I/L/Q/T, M423T, or P495A.

An NS5A inhibitor, 479103882-AAA, maintained activity (i.e., EC_{50} values < 0.02 nM) against replicons bearing NS3_Q80K, A156V, or D168V. Two NS5B NAPIs, MK-0608 and TMC619688, maintained activity (i.e., < 3 -fold shifts in susceptibility) against replicons expressing NS3_Q80K/L/R, S122K/R, R155K, A156T/V, or D168A/E/V. TMC647055 and SHIRE-2, two NS5B NNAPIs, maintained activity against replicons expressing NS3_Q80K/L/R, S122K/R, R155K/Q, A156S/T/V, or D168A/E/H/V.

In conclusion, TMC435 is expected to have an overlapping resistance profile with the approved NS3/4A inhibitors, boceprevir and telaprevir. Specifically, cross-resistance between TMC435, boceprevir, and telaprevir is expected to be conferred primarily by R155K. Cross-resistance between TMC435 and other classes of HCV DAAs is not anticipated.

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3 Relevant Findings from Other Disciplines

3.1 Summary of Efficacy in Phase 3 Trials

Please see the reviews of the Statistical reviewer, Yanming Yin, Ph.D., and the Biometrics reviewer, Jiang Liu, Ph.D., for detailed primary efficacy analyses.

3.2 Summary of Safety in Phase 3 Trials

Please see the review of the Clinical Reviewer, Adam Sherwat, M.D., for detailed safety analyses.

4 Clinical Virology

4.1 Overview of the Pivotal Phase 3 Trials C208, C216, and HPC3007

Data from three pivotal phase 3 trials in HCV genotype 1-infected subjects were submitted in support of this NDA, including two phase 3 trials in recombinant pegylated interferon alpha/ribavirin (P/R) naïve subjects, C208 and C216, and one phase 3 trial in prior P/R relapsers, HPC3007. Subjects in all three trials received 12 weeks of TMC435 (150 mg q.d.) or placebo (PBO) in combination with P/R. All 3 trials used a response-guided treatment (RGT) algorithm to determine if the additional P/R treatment would extend through Week 24 or Week 48. According to the RGT, P/R treatment could be completed at Week 24 in subjects achieving HCV RNA <25 IU/mL target detected (TD) or target not detected (TND) (i.e., those whose plasma HCV RNA loads were below the lower limit of quantification [<LLOQ]) at Week 4 and HCV RNA <25 IU/mL TND (i.e., HCV RNA was not detected) at Week 12. Subjects who achieved HCV RNA ≤1,000 IU/mL and ≥25 IU/mL at Week 4 and HCV RNA <25 IU/mL TND at Week 12 received P/R for a total of 48 weeks.

All study drugs were halted for subjects who failed to achieve a ≥2 log₁₀ IU/mL reduction in HCV RNA from Baseline levels by Week 12 or had HCV RNA detected at Weeks 24 or 36. TMC435 or PBO were discontinued for all subjects with HCV RNA >1,000 IU/mL at Week 4, although P/R treatment continued through 48 weeks, assuming no other stopping rules or virologic failure conditions were met.

Stratification factors included *IL28B* genotype (CC, CT, or TT) and HCV GT1 subtype. In addition, a subpopulation in study C216 in selected countries was also randomized by use of PegIFNα-2a/RBV (Pegasys® and Copegus®) or PegIFNα-2b/RBV (PegIntron® and RebetoI®).

For the efficacy summaries presented in this review, treatment failures were defined as subjects who did not achieve a sustained virologic response (SVR), defined as HCV RNA <25 IU/mL, either target not detected (TND) or target detected (TD), at follow-up (FU) week 12 (i.e., SVR12) or later, when those data were available (Table 2, Virology Reviewer's summary).

Table 2. Number (%) of subjects with efficacy data available at follow-up (FU) Weeks

Trial	FU Wk 12	FU Wk 24	FU Wk 36	FU Wk 48	FU Wk 60
C208	394/394 (100)	300/394 (76.1)	251/394 (63.7)	67/394 (17)	3/394 (0.8)
C216	391/391 (100)	321/391 (82.1)	258/391 (66)	110/391 (28.1)	2/391 (0.5)
HPC3007	393/393 (100)	322/393 (81.9)	256/393 (65.1)	129/393 (32.8)	1/393 (0.3)

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All intent-to-treat (ITT) subjects (C208: N=394; C216: N=391; HPC3007: N=393) were included in the SVR12 evaluation; subjects who were lost to follow-up prior to reaching FU Week 12 were considered SVR12 failures. The majority of subjects had also met their FU Week 24 time point evaluation at the time of the data cut-off prior to the NDA submission, including 76.1% (300/394) of subjects in C208, 82.1% (321/391) subjects in C216, and 81.9% (322/393) of subjects in HPC3007.

Impact of Baseline HCV Genotype 1a and 1b Polymorphisms on Efficacy

The impact of HCV NS3/4A polymorphisms identified in baseline isolates by Sanger sequencing was evaluated using the pooled data of C208 (n=391), C216 (n=381), and HPC3007 (n=390). First, the number of baseline variants bearing each polymorphism was determined separately for genotype 1a (n=538) and genotype 1b (n=624) isolates. Next, potential associations between each of the selected baseline polymorphisms and SVR outcomes were probed by comparing the proportions of all genotype 1a- or 1b-infected subjects who received TMC435 + P/R with those of the subjects harboring each polymorphic variant using a one-tailed Fisher Exact Test. Polymorphic variants that appeared to be associated with significantly lower responses to TMC435 relative to the overall population were excluded if there were fewer than 10 subjects infected with that polymorphic variant in order to exclude rare polymorphic variants and increase the reliability of analysis. In addition, the proportion of SVR responders of potential polymorphic variants of interest were compared to those treated with PBO in order to determine if subjects receiving TMC435 experienced statistically significant response rates than those treated with PBO (two-tailed Fisher Exact Test), or if there was a TMC435-independent difference in response rates. In total, there were 538 genotype 1a and 675 genotype 1b individual polymorphic variant substitutions reported among the baseline isolates. The results of the analysis are summarized in Table 3 (Virology Reviewer's analysis).

Table 3. Baseline polymorphisms with the potential for efficacy impact

GT	Variant	SVR _{TMC} n/N (%)	SVR _{PBO} n/N (%)	p value (TMC _{subtype} vs. TMC _{variant})	p value (TMC _{variant} vs. PBO _{variant})
All genotype 1a		258/357 (72.3)	75/181 (41.4)	0.53	<0.0001
1a	NS3_Q80K	62/114 (54.4)	30/64 (46.9)	0.00035	0.35
1a	NS3_S91T	19/35 (54.3)	8/20 (40)	0.024	0.4
1a	NS3_I252V	6/13 (46.2)	3/7 (42.9)	0.046	1
1a	NS3_A383G	6/16 (37.5)	2/3 (66.7)	0.0049	0.55
1a	NS3_T402A	4/10 (40)	2/5 (40)	0.036	1
1a	NS3_I586T	25/43 (58.1)	6/25 (24)	0.043	0.011
1a	NS3_I586V	4/11 (36.4)	4/7 (57.1)	0.016	0.63
1a	NS4A_I29V	47/78 (60.3)	18/44 (40.9)	0.026	0.058
All genotype 1b		349/414 (84.3)	99/210 (47.1)	0.54	<0.0001
1b	NS3_S122T	21/31 (67.7)	7/19 (36.8)	0.022	0.043
1b	NS3_K213R	23/33 (69.7)	3/5 (60)	0.034	0.64
1b	NS3_T344I	55/83 (66.3)	10/39 (25.6)	0.00023	<0.0001
1b	NS3_T610I	38/56 (67.9)	17/34 (50)	0.0036	0.12

Consistent with the nonclinical data, the genotype 1a Q80K variant was identified as a potential polymorphism of interest. Other potential polymorphic variants of interest included genotype 1a NS3_S91T, I252V, A383G, T402A, I586T, I586V, and NS4A_I29V, polymorphisms that were prevalent at frequencies of 10.2% (55/538), 3.7% (20/538), 3.5% (19/538), 2.8% (15/538), 12.6% (68/538), 3.3% (18/538), and 22.7% (122/538), respectively. With the exception of the I586T variant, SVR rates

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differed from that of the overall genotype 1a population, but not from those of subjects infected by the same variant who received PBO, as would be expected in cases where TMC435 was less efficacious.

In order to determine if the potential efficacy reduction could be attributed to the co-expression of Q80K within the isolate, the distribution of Q80K among these subjects was also evaluated (Table 4, Virology Reviewer's analysis). Interestingly, several of the SVR failures among the subjects infected by the S91T, I252V, A383G, T402A, I586T, I586V, and NS4A_I29V-expressing variants were infected by virus that co-expressed Q80K, and the significance of these polymorphisms on efficacy was not maintained when subjects infected with Q80K co-expressing variants were excluded. In conclusion, infection by genotype 1a viruses expressing NS3_S91T, I252V, A383G, T402A, I586T, I586V, or NS4A_I29V could not be associated with reduced efficacy of TMC435 in combination with P/R.

Table 4. NS3 Q80K co-expression among genotype 1a polymorphic variants of interest

HCV GT	Polymorphism	SVR _{TMC} n/N (%)	SVR _{PBO} n/N (%)	p value (TMC _{subtype} vs. TMC _{variant})	p value (TMC _{variant} vs. PBO _{variant})
1a	NS3_S91T	19/35 (54.3)	8/20 (40)	0.024	0.4
	No-Q80K	6/8 (75)	2/4 (50)	0.61	0.55
	Q80K	13/27 (48.1)	6/16 (37.5)	0.0094	0.54
1a	NS3_I252V	6/13 (46.2)	3/7 (42.9)	0.046	1
	No-Q80K	1/2 (50)	1/4 (25)	0.48	1
	Q80K	5/11 (45.5)	2/3 (66.7)	0.06	1
1a	NS3_A383G	6/16 (37.5)	2/3 (66.7)	0.0049	0.55
	No-Q80K	6/11 (54.5)	2/3 (66.7)	0.17	1
	Q80K	0/5 (0)	0/0	0.0018	
1a	NS3_T402A	4/10 (40)	2/5 (40)	0.036	1
	No-Q80K	4/7 (57.1)	1/3 (33.3)	0.31	0.58
	Q80K	0/3 (0)	1/2 (50)	0.022	0.4
1a	NS3_I586T	25/43 (58.1)	6/25 (24)	0.043	0.011
	No-Q80K	14/21 (66.7)	2/14 (14.3)	0.37	0.0048
	Q80K	11/22 (50)	4/11 (36.4)	0.027	0.71
1a	NS3_I586V	4/11 (36.4)	4/7 (57.1)	0.016	0.63
	No-Q80K	3/5 (60)	2/3 (66.7)	0.43	1
	Q80K	1/6 (16.7)	2/4 (50)	0.0083	0.5
1a	NS4A_I29V	47/78 (60.3)	18/44 (40.9)	0.026	0.058
	No-Q80K	18/22 (81.8)	7/20 (35)	0.24	0.004
	Q80K	29/56 (51.8)	11/24 (45.8)	0.0021	0.64

In contrast, there were several baseline genotype 1b polymorphic variants that might be associated with reduced TMC435 efficacy. Subjects infected with NS3_K213R and T610-expressing variants may have experienced reduced SVR rates relative to the overall genotype 1b-infected population (p values of 0.034 and 0.0036, respectively), but not to PBO-treated subjects who were also infected with the same variants (p values of 0.64 and 0.12, respectively). S122T and T344I were associated with reduced susceptibilities to TMC + P/R and PBO + P/R, indicating that this variant may be associated with poorer anti-HCV responses in general, including those to P/R, but not specifically to TMC435. Notably, S122T genotype 1b-expressing replicons did not show reduced susceptibility to TMC435, with a FC value of 0.5.

In summary, genotype 1a NS3_Q80K appears to be associated with reduced efficacy of TMC435 + P/R relative to those treated with PBO + P/R. Genotype 1b NS3_K213R and T610-polymorphic variants may also be associated with reduced efficacy among subjects treated with TMC435 + P/R relative to those treated with PBO + P/R. However, the results of the genotype 1b analysis should be interpreted

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carefully given the smaller sample sizes, lack of analyses to identify potential covariates that may have contributed to the observations of reduced efficacy, and lack of supporting nonclinical data.

Efficacy Analyses of C208, C216, and HPC3007

A summary of the individual efficacy results for trials C208, C216, and HPC3007 are summarized in Table 5 (Virology Reviewer's analysis). Subjects in all three pivotal trials benefited from higher SVR rates when treated with TMC435 in combination with P/R relative to those who received PBO in combination with P/R, with improved SVR rates 28.8% (95% CI: 18.8 to 38.4%; $P < 0.0001$), 30.5% (95% CI: 20.6 to 40%; $P < 0.0001$), and 42.0% (95% CI: 31.9 to 50.9%; $P < 0.0001$) in trials C208, C216, and HPC3007, respectively. Evaluation of the treatment effect by HCV genotype 1 subgroup indicated that both HCV genotype 1a and 1b-infected subjects benefited from treatment with TMC435 + P/R when compared to those who received PBO + P/R, with higher SVR rates of 21.4% (95% CI: 7.9 to 34.4%; $p = 0.0017$) for genotype 1a-infected subjects and 38.0% (95% CI: 23.7 to 51.4%; $P < 0.0001$) for genotype 1b-infected subjects in C208, 35.4% (95% CI: 19.7 to 49.4%; $P < 0.0001$) for genotype 1a-infected and 28.1% (95% CI: 15.1 to 40.4%; $P < 0.0001$) for genotype 1b-infected subjects in C216, and 39.5% (95% CI: 23.5 to 52.5%; $P < 0.0001$) for genotype 1a-infected and 44.2% (95% CI: 31.3 to 55.4%; $P < 0.0001$) for genotype 1b-infected subjects in HPC3007.

Table 5. Efficacy evaluation of TMC435 + P/R for ITT population*

		SVR (n/N) (%)		SVR _{TMC435+P/R} - SVR _{PBO}			
		TMC435+P/R	PBO+P/R	Δ (%)	95% CI	Plot	p value
C208	GT1	210/264 (79.5)	66/130 (50.8)	28.8	(18.8, 38.4)		<0.0001
	1a	105/147 (71.4)	37/74 (50)	21.4	(7.9, 34.4)		0.0017
	non-Q80K	73/86 (84.9)	19/43 (44.2)	40.7	(23.4, 55.7)		<0.0001
	Q80K	31/60 (51.7)	17/30 (56.7)	-5.0	(-25.2, 16.3)		0.65
	1b	105/117 (89.7)	29/56 (51.8)	38.0	(23.7, 51.4)		<0.0001
C216	GT1	205/257 (79.8)	66/134 (49.3)	30.5	(20.6, 40)		<0.0001
	1a	83/105 (79)	24/55 (43.6)	35.4	(19.7, 49.4)		<0.0001
	non-Q80K	64/78 (82.1)	17/40 (42.5)	39.6	(21.3, 55.2)		<0.0001
	Q80K	17/24 (70.8)	7/14 (50)	20.8	(-9.8, 48.1)		0.2
	1b	120/150 (80)	40/77 (51.9)	28.1	(15.1, 40.4)		<0.0001
HPC3007	GT1	201/260 (77.3)	47/133 (35.3)	42.0	(31.9, 50.9)		<0.0001
	1a	74/110 (67.3)	15/54 (27.8)	39.5	(23.5, 52.5)		<0.0001
	non-Q80K	59/79 (74.7)	9/34 (26.5)	48.2	(28.5, 62.7)		<0.0001
	Q80K	14/30 (46.7)	6/20 (30)	16.7	(-10.7, 39.8)		0.24
	1b	127/150 (84.7)	32/79 (40.5)	44.2	(31.3, 55.4)		<0.0001

* Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction ([Newcombe, 1998](#)).

A reduction in the benefit of TMC435 was observed among subjects who were infected with the HCV genotype 1a NS3_Q80K polymorphic variant. In C208, subjects infected with genotype 1a non-Q80K variants who received TMC435 + P/R had an SVR rate that was 40.7% (95% CI: 23.4 to 55.5%; $P < 0.0001$) higher than those who received PBO + P/R. In contrast, subjects infected with the Q80K variant at baseline who received TMC435 + P/R had an SVR rate that was 5.0% (95% CI: -25.2 to

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16.3%; P=0.65) lower than that of those who received PBO + P/R. Similarly, C216 subjects infected with the Q80K variant at Baseline who were treated with TMC435 + P/R had an SVR rate of 20.8% (95% CI: -9.8 to 48.1%; P=0.2), which was not significantly different from that of subjects who received PBO + P/R. Likewise, the prior P/R relapsers in HPC3007 who were infected with the Q80K variant and received TMC435 + P/R also experienced a reduced benefit, with a 16.7% (95% CI: -10.7 to 39.8%; P=0.24) difference in SVR relative to those who received PBO + P/R. Notably, similar efficacy reductions were not observed in the boceprevir and telaprevir trials. For example, a comparison of the efficacy data from treatment-naïve subjects of the pivotal TMC435 (pooled C208 and C216), boceprevir (P05216), and telaprevir (108) trials are summarized in Table 6 (Virology Reviewer's analysis). Unlike TMC435 + P/R, boceprevir + P/R and telaprevir + P/R provided significant efficacy improvements (P<0.0001) in the SVR rates of subjects infected with Q80K variants at Baseline relative to those who received PBO + P/R.

Table 6. Impact of HCV 1a Q80K on efficacy of TMC435 and approved NS3/4A inhibitors*

		SVR (n/N) (%)		SVR _{TMC435} -SVR _{PBO}			
		TMC435+P/R	PBO+P/R	Δ(%)	95% CI	Plot	p value
TMC435	GT1	415/521 (79.7)	132/264 (50)	29.7	(22.6 , 36.5)		<0.0001
	1a	188/252 (74.6)	61/129 (47.3)	27.3	(17 , 37.1)		<0.0001
	non-Q80K	137/164 (83.5)	36/83 (43.4)	40.2	(27.7 , 51.4)		<0.0001
	Q80K	48/84 (57.1)	24/44 (54.5)	2.6	(-14.8 , 20.2)		0.78
	1b	225/267 (84.3)	69/133 (51.9)	32.4	(22.8 , 41.7)		<0.0001
Boceprevir	GT1	468/734 (63.8)	133/363 (36.6)	27.1	(20.9 , 33)		<0.0001
	1a	221/366 (60.4)	59/177 (33.3)	27.0	(18.2 , 35.2)		<0.0001
	non-Q80K	110/193 (57)	39/106 (36.8)	20.2	(8.4 , 31.1)		0.00083
	Q80K	111/173 (64.2)	20/71 (28.2)	36.0	(22.4 , 47.4)		<0.0001
	1b	180/267 (67.4)	50/126 (39.7)	27.7	(17.2 , 37.4)		<0.0001
Telaprevir	GT1	270/359 (75.2)	158/359 (44)	31.2	(24.2 , 37.8)		<0.0001
	1a	158/217 (72.8)	87/210 (41.4)	31.4	(22.2 , 39.8)		<0.0001
	non-Q80K	88/128 (68.8)	53/129 (41.1)	27.7	(15.6 , 38.6)		<0.0001
	Q80K	70/89 (78.7)	34/81 (42)	36.7	(22.2 , 49.1)		<0.0001
	1b	112/142 (78.9)	71/149 (47.7)	31.2	(20.3 , 41.1)		<0.0001

* Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction ([Newcombe, 1998](#)).

Antiviral Activity of TMC435 in Combination with P/R

The on-treatment antiviral activity of TMC435 in combination with P/R was assessed by comparing the proportion of subjects who received TMC435 to achieve HCV RNA loads below the lower limit of quantification (<LLOQ; i.e., <25 IU/mL TD or TND) at Weeks 4, 12, and 24 to that of subjects who received PBO (Table 7; Virology Reviewer's analysis). TMC435 was associated with improved antiviral responses at Weeks 4, 12, and 24 for the treatment-naïve subjects of trials C208 and C216 as well as for the prior relapsers of trial HPC3007.

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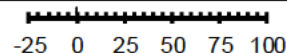
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Among the subjects of trials C208 and C216, 92.6% (474/512) who received TMC435 achieved HCV RNA levels <LLOQ by Week 4 relative to the 20.8% (54/260) of those who received PBO, a difference of 71.8% (95% CI: 65.9 to 76.7%; p value <0.0001). TMC435 treatment was also associated with improved early virologic responses at Weeks 8 and 12, with 39.2% (95% CI: 33.0 to 45.4%) more subjects treated with TMC435 achieving HCV RNA levels <LLOQ at Week 8 relative to those who received PBO, and 24.7% (95% CI: 18.8 to 30.9%) more subjects treated with TMC435 achieving HCV RNA levels <LLOQ at Week 12 relative to those receiving PBO.

Table 7. Impact of TMC435 on early virologic responses

		SVR (n/N) (%)		Response _{TMC435} -Response _{PBO}				
		TMC435+P/R	PBO+P/R	Δ(%)	95% CI	Plot	p value	
C208+ C216	Week 4	GT1	474/512 (92.6)	54/260 (20.8)	71.8	(65.9 , 76.7)		<0.0001
		1a	216/247 (87.4)	24/128 (18.8)	68.7	(59.7 , 75.5)		<0.0001
		non-Q80K	148/162 (91.4)	14/82 (17.1)	74.3	(63.3 , 81.7)		<0.0001
		Q80K	64/81 (79)	10/44 (22.7)	56.3	(38.8 , 68.7)		<0.0001
		1b	256/263 (97.3)	30/130 (23.1)	74.3	(65.9 , 80.8)		<0.0001
	Week 8	GT1	488/507 (96.3)	149/261 (57.1)	39.2	(33 , 45.4)		<0.0001
		1a	226/240 (94.2)	68/127 (53.5)	40.6	(31.4 , 49.6)		<0.0001
		non-Q80K	153/159 (96.2)	41/81 (50.6)	45.6	(34.2 , 56.5)		<0.0001
		Q80K	70/78 (89.7)	26/44 (59.1)	30.7	(14.8 , 46.2)		<0.0001
		1b	260/265 (98.1)	80/132 (60.6)	37.5	(29.2 , 46.1)		<0.0001
	Week 12	GT1	469/501 (93.6)	175/254 (68.9)	24.7	(18.8 , 30.9)		<0.0001
		1a	215/236 (91.1)	80/123 (65)	26.1	(17.1 , 35.3)		<0.0001
		non-Q80K	146/156 (93.6)	48/78 (61.5)	32.1	(20.9 , 43.5)		<0.0001
		Q80K	66/77 (85.7)	31/43 (72.1)	13.6	(-1 , 29.6)		0.069
		1b	252/263 (95.8)	93/129 (72.1)	23.7	(16 , 32.2)		<0.0001
HPC3007	Week 4	GT1	247/260 (95)	15/129 (11.6)	83.4	(75.9 , 88.3)		<0.0001
		1a	103/110 (93.6)	4/51 (7.8)	85.8	(73.5 , 91.5)		<0.0001*
		non-Q80K	77/79 (97.5)	3/34 (8.8)	88.6	(73.2 , 94.7)		<0.0001*
		Q80K	25/30 (83.3)	1/17 (5.9)	77.5	(50.4 , 88)		<0.0001*
		1b	144/150 (96)	11/78 (14.1)	81.9	(71.5 , 88.3)		<0.0001
	Week 8	GT1	250/255 (98)	65/129 (50.4)	47.7	(38.8 , 56.2)		<0.0001
		1a	105/107 (98.1)	28/51 (54.9)	43.2	(29.6 , 56.8)		<0.0001*
		non-Q80K	77/77 (100)	18/34 (52.9)	47.1	(30.7 , 63.3)		<0.0001*
		Q80K	27/29 (93.1)	10/17 (58.8)	34.3	(9.6 , 57.6)		0.0078*
		1b	145/148 (98)	37/78 (47.4)	50.5	(39 , 61.3)		<0.0001*
	Week 12	GT1	249/253 (98.4)	107/126 (84.9)	13.5	(7.8 , 20.8)		<0.0001*
		1a	105/105 (100)	37/49 (75.5)	24.5	(14 , 38.1)		<0.0001*
		non-Q80K	78/78 (100)	27/34 (79.4)	20.6	(9.3 , 36.8)		0.00015*
		Q80K	26/26 (100)	10/15 (66.7)	33.3	(11.1 , 58.3)		0.004*
		1b	144/148 (97.3)	70/77 (90.9)	6.4	(0.3 , 15.1)		0.049*

-25 0 25 50 75 100



* Inadequate data for a valid comparison of the difference between two independent proportions; the difference between proportions and their 95% CI intervals for these groups should be interpreted carefully; p-values were determined using the two-tailed Fisher Exact Test.

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Interestingly, TMC435 treatment was also associated with improved early virologic responses among subjects infected with genotype 1a Q80K variants, although the proportion of subjects achieving HCV RNA <LLOQ was reduced relative to that of subjects infected with genotype 1a non-Q80K variants. At Week 4, 79% (64/81) of the subjects infected with Q80K variants who received TMC435 + P/R achieved HCV RNA <LLOQ, while 91.4% (148/162) of subjects infected with genotype 1a non-Q80K who received TMC435 + P/R achieved HCV RNA <LLOQ, a significant difference (p value of 0.01; two-tailed Fisher Exact Test). While the proportion of subjects infected with genotype 1a Q80K variants who achieved HCV RNA <LLOQ at Weeks 8 and 12 were less than those of subjects infected with non-Q80K variants, the differences did not maintain significance (p values of 0.07 and 0.06 by two-tailed Fisher Exact Tests, respectively).

Comparison of the virologic responses of the prior relapsers in HPC3007 mirrored what was observed for the treatment-naïve subjects of C208 and C216. TMC435 treatment was associated with significantly improved early virologic responses at Weeks 4, 8, and 12. At Week 4, 95.0% (247/260) of the subjects who received TMC435 achieved HCV RNA levels <LLOQ relative to the 11.6% (15/129) of those who received PBO, a difference of 83.4% (95% CI: 75.9 to 88.3%; p value <0.0001). TMC435 treatment was also associated with improved early virologic responses at Weeks 8 and 12, with 47.7% (95% CI: 38.8 to 56.2%; p value <0.0001) more subjects treated with TMC435 achieving HCV RNA levels <LLOQ at Week 8 relative to those who received PBO, and 13.5% (95% CI: 7.8 to 20.8%; p value <0.0001) more subjects treated with TMC435 achieving HCV RNA levels <LLOQ at Week 12 relative to those receiving PBO.

As observed in the treatment-naïve subjects, TMC435 treatment was also associated with improved early virologic responses among subjects infected with genotype 1a Q80K variants, although the proportion of subjects achieving HCV RNA <LLOQ was reduced relative to that of subjects infected with genotype 1a non-Q80K variants. At Week 4, 97.5% (77/79) of subjects infected with genotype 1a non-Q80K who received TMC435 + P/R achieved HCV RNA <LLOQ, while 83.3% (25/30) of the subjects infected with Q80K variants who received TMC435 + P/R achieved HCV RNA <LLOQ, a significant difference (p value of 0.02; two-tailed Fisher Exact Test). While the proportion of subjects infected with genotype 1a Q80K variants who achieved HCV RNA <LLOQ at Weeks 8 and 12 were less than those of subjects infected with non-Q80K variants, the differences did not maintain significance (p values of 0.07 and 1.0 by two-tailed Fisher Exact Test, respectively).

In summary, TMC435 treatment was associated with increased proportions of subjects who achieved HCV RNA levels to <LLOQ at Weeks 4, 8, and 12. Improved early virologic responses were also observed for genotype 1a Q80K infected subjects, although the response rates appeared to be reduced relative to subjects infected with non-Q80K variants.

Virologic Failures

The impact of TMC435 + P/R versus PBO + P/R on SVR failures was evaluated by comparing the proportions of SVR failures in each treatment arm (Table 8; Virology Reviewer's analysis). There were 29.7% (95% CI: 22.6% to 36.5%) and 42.0% (95% CI: 31.9 to 50.9%) fewer SVR failures among subjects who received TMC435 compared to those who received PBO in C208 + C216 and HPC3007, respectively. In C208 and C216, TMC435 was associated with a greater reduction in on-treatment failures than post-treatment failures, with reductions of 23.2% (95% CI: 17.4 to 29.3%) and 6.4% (95% CI: 1 to 12.3%), respectively. Although TMC435 treatment was associated with fewer on- and post-treatment failures in both genotype 1a- and 1b-infected subjects, there was not a significant difference between failure rates of subjects infected with genotype 1a Q80K variants who received TMC435 relative to those who received PBO, with a difference of 8.3% (95% CI: -5.7 to 24.2%) for on-treatment failures and a 5.7% (-19.6 to 10.5%) reduction for post-treatment failures.

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In HPC3007, TMC435 was also associated with a greater reduction in on-treatment failures than post-treatment failures, with reductions of 16.5% (95% CI: 10.2 to 24.1%) and 25.5% (95% CI: 15.7 to 35.0%), respectively. Similar to the results of the analysis for trials C208 and C216, TMC435 treatment was associated with fewer on-treatment failures in both genotype 1a- and 1b-infected subjects, although the benefit of TMC435 treatment extended to subjects infected with genotype 1a Q80K, who experienced a 33.3% (95% CI: 10 to 55%) reduction in on-treatment failures relative to those who received PBO. However, TMC435 treatment was not associated with a significant decrease in post-treatment failures among subjects infected with genotype 1a Q80K, whose failure rate was reduced by 16.7% (-39.8 to 10.7%) relative to subjects who received PBO.

In summary, TMC435's association with increased SVR rates appears to be due to reductions in both on-treatment and post-treatment failure rates relative to those of subjects who received PBO. Subjects infected with genotype 1a Q80K virus may have experienced a small reduction in on-treatment failures, but not post-treatment failures.

Table 8. TMC435 effect on failure events

		Failures (n/N) (%)		Fail _{PBO} -Fail _{TMC}				Plot	p value
		TMC435+P/R	PBO+P/R	Δ (%)	95% CI				
C208+C216	All	GT1	106/521 (20.3)	132/264 (50)	29.7	(22.6, 36.5)			<0.0001
	On-Tx Failures	GT1	35/521 (6.7)	79/264 (29.9)	23.2	(17.4, 29.3)			<0.0001
		1a	25/252 (9.9)	44/129 (34.1)	24.2	(15.4, 33.3)			<0.0001
		non-Q80K	11/164 (6.7)	32/83 (38.6)	31.8	(20.9, 43)			<0.0001
		Q80K	14/84 (16.7)	11/44 (25)	8.3	(-5.7, 24.2)			0.26
		1b	10/267 (3.7)	35/133 (26.3)	22.6	(15.2, 30.8)			<0.0001
	Post-Tx Failures	GT1	71/521 (13.6)	53/264 (20.1)	6.4	(1, 12.3)			0.019
		1a	39/252 (15.5)	24/129 (18.6)	3.1	(-4.5, 11.7)			0.44
		non-Q80K	16/164 (9.8)	15/83 (18.1)	8.3	(-0.4, 18.6)			0.062
		Q80K	22/84 (26.2)	9/44 (20.5)	-5.7	(-19.6, 10.5)			0.47
		1b	32/267 (12)	29/133 (21.8)	9.8	(2.2, 18.3)			0.01
HPC3007	All	GT1	59/260 (22.7)	86/133 (64.7)	42.0	(31.9, 50.9)			<0.0001
	On-Tx Failures	GT1	6/260 (2.3)	25/133 (18.8)	16.5	(10.2, 24.1)			<0.0001
		1a	3/110 (2.7)	14/54 (25.9)	23.2	(12.2, 36.3)			<0.0001*
		non-Q80K	1/79 (1.3)	6/34 (17.6)	16.4	(5.5, 32.3)			0.003*
		Q80K	2/30 (6.7)	8/20 (40)	33.3	(10, 55.2)			0.009*
		1b	3/150 (2)	11/79 (13.9)	11.9	(4.9, 21.3)			0.0007*
	Post-Tx Failures	GT1	53/260 (20.4)	61/133 (45.9)	25.5	(15.7, 35)			<0.0001
		1a	33/110 (30)	25/54 (46.3)	16.3	(0.7, 31.5)			0.04
		non-Q80K	19/79 (24.1)	19/34 (55.9)	31.8	(12.3, 49.1)			0.001
		Q80K	14/30 (46.7)	6/20 (30)	-16.7	(-39.8, 10.7)			0.24
		1b	20/150 (13.3)	36/79 (45.6)	32.2	(19.9, 44.1)			<0.0001

* Inadequate data for a valid comparison of the difference between two independent proportions; the difference between proportions and their 95% CI intervals for these groups should be interpreted carefully; p-values were determined using the two-tailed Fisher Exact Test.

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4.2 *Treatment-Emergent Resistance Analysis*

Genotypic data collected from isolates of subjects who received 150 mg TMC435 and had on- or post-treatment emergent variants were pooled in order to evaluate associations between TMC435 + P/R exposure, SVR failure, and virologic resistance. The data pool included isolates collected from subjects in phase 2 trials, C205 and C206, as well as those from subjects who participated in the pivotal phase 3 trials, C208, C216, and HPC3007.

There were 245 SVR failures among subjects who received 150 mg TMC435, including 144 genotype 1a (76 non-Q80K variants and 65 Q80K variants), 100 genotype 1b, and 1 genotype 1d-infected subjects. Three subjects were missing Baseline data and 31 subjects had only Baseline data, so data from these 34 SVR failures had to be excluded from the analysis because emergent substitutions could not be identified. The remaining eight SVR failures had Baseline and on- or post-treatment data, but no emergent variants were detected, and included two genotype 1a and six genotype 1b infections. Four of the eight subjects discontinued therapy prematurely (i.e., within three weeks), two were relapsers, one subject was lost to follow-up, and one subject experienced virologic breakthrough during the first week of treatment.

Paired baseline, on-treatment, and/or post-failure genotypic data were available for 127 genotype 1a-infected subjects, including those of 68 non-Q80K and 59 Q80K-infected subjects, and 85 genotype 1b-infected subjects. The majority of subjects with treatment-emergent variants did not achieve SVR, including 96.1% (122/127) of the genotype 1a-infected and 95.3% (81/85) of the genotype 1b-infected subjects. Overall, paired genotypic data were available for 86.8% (125/144) of the genotype 1a SVR failures, including 88.2% (67/76) of the non-Q80K and 89.2% (58/65) of the Q80K variants, 86.0% (86/100) of the genotype 1b, and 0% (0/1) of the genotype 1d-infected SVR failures.

In order to identify variants that likely emerged under selective pressure of TMC435 from those that might only represent natural shifts between common variants present within the quasispecies, the proportion of subjects harboring each putative treatment-emergent variant was compared to that of subjects at Baseline. Emergent variants that were identified in a significantly higher proportion of 150 mg TMC435-treated subjects than in those at Baseline (p value ≤ 0.05 by Fisher Exact test) are summarized in Table 9 (Virology Reviewer's analysis).

The most prevalent genotype 1a variants that emerged with TMC435-treatment were R155K, D168E, and D168V-expressing viruses, which were detected in 78.7%, 18.9%, and 11.8% of subjects with treatment-associated emergent genotypic data, respectively. The prevalence of the R155K, D168E, and D168V-variants among the genotype 1a-infected SVR failures was 79.2% (99/125), 19.2% (24/125), and 11.2% (14/125), respectively. Emergent NS3_R155 and/or D168-expressing variants were detected in 93.6% (117/125) of the genotype 1a-infected subjects who failed to achieve SVR, which in combination with the selection studies in replicon culture, provide strong evidence that selection of R155 and D168-expressing variants represent the primary genetic pathways to resistance.

The six genotype 1a-infected subjects who did not achieve SVR and had post-baseline isolates without emergent R155 or D168 variants, C205-0283, C208-0047, C208-0383, 3007-6001, and 3007-6152, were analyzed in further detail.

- Subject C205-0283 was infected with genotype 1a R155K-expressing variant at Baseline, experienced a reduction in HCV RNA levels from 3.10×10^6 IU/mL at Baseline to 7.58×10^3 IU/mL at Week 4, achieved HCV RNA levels < 25 IU/mL TND at Week 12, and stopped therapy at Week 21 due to an adverse event. HCV RNA levels remained undetected from Week 12 through Week 28, and did not otherwise fail due to a protocol-defined virologic failure, indicating

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that Subject C205-0283 likely responded well to the P/R component of the regimen. No post-relapse isolates were collected for genotypic analysis.

Table 9. NS3/4A amino acid substitutions associated with emergent variants

Substitution	Genotype 1a			Genotype 1b		
	Baseline n/901 (%)	Emergent n/127 (%)	p value	Baseline n/1096 (%)	Emergent n/85 (%)	p value
A5V	0 (0)	2 (1.6)	0.015			
A39T				7 (0.6)	6 (7.1)	0.00013
S61L				0 (0)	2 (2.4)	0.0051
Q80K				5 (0.5)	4 (4.7)	0.0024
Q80R	6 (0.7)	7 (5.5)	0.00034	6 (0.5)	16 (18.8)	<0.0001
G120S	0 (0)	2 (1.6)	0.015			
S122C	1 (0.1)	2 (1.6)	0.042			
S122R	0 (0)	10 (7.9)	<0.0001			
R123M	0 (0)	2 (1.6)	0.015			
I132L	1 (0.1)	6 (4.7)	<0.0001			
R155G	0 (0)	2 (1.6)	0.015			
R155K	6 (0.7)	100 (78.7)	<0.0001			
R155Q				0 (0)	4 (4.7)	<0.0001
R155T	0 (0)	2 (1.6)	0.015			
D168A	0 (0)	13 (10.2)	<0.0001	0 (0)	7 (8.2)	<0.0001
D168E	3 (0.3)	24 (18.9)	<0.0001	5 (0.5)	20 (23.5)	<0.0001
D168H				0 (0)	2 (2.4)	0.0051
D168T				0 (0)	3 (3.5)	0.00036
D168V	0 (0)	15 (11.8)	<0.0001	0 (0)	52 (61.2)	<0.0001
I170T	1 (0.1)	6 (4.7)	<0.0001			
N174R	0 (0)	2 (1.6)	0.015			
S174F				4 (0.4)	3 (3.5)	0.01
T178I	3 (0.3)	5 (3.9)	0.0011			
L356F	1 (0.1)	2 (1.6)	0.042			
I426V				2 (0.2)	2 (2.4)	0.028
T448A				2 (0.2)	2 (2.4)	0.028
V511I	0 (0)	2 (1.6)	0.015			
V535A				0 (0)	2 (2.4)	0.0051

- Subject C208-0047 was infected with a genotype 1a non-Q80K expressing variant at Baseline and achieved HCV RNA levels <25 IU/mL TND by Week 4, and remained undetected through Week 24, the End of Treatment timepoint. HCV RNA levels were <25 IU/mL TD at Week 28 and 1.32×10^7 IU/mL by Week 28. The Week 48 isolate was genotypically characterized, at which time emergent NS3_L356F, V358T, I586N, and NS4A V43A were detected (Table 10; Virology Reviewer's analysis). The L356F substitution does not appear to be a common polymorphism, and was observed at a frequency of 0.11% (1/901) in genotype 1a baseline isolates and in no (0/1096) genotype 1b baseline isolates. An L356F-expressing variant was also detected in another genotype 1a infected subject who relapsed, HPC3007-6152 who is included in this analysis (see below), for a total treatment-emergent frequency of 1.6% (2/127). V358T was detected as a genotype 1a polymorphism in 5.8% (52/901) genotype 1a and 0.1% (1/1096) genotype 1b Baseline isolates, and among 3.9% (5/127) of genotype 1a isolates with emergent variants, indicating that it may be an uncommon polymorphic variant. I586N was detected

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among 3.8% (34/901) genotype 1a baseline isolates and 2.4% (3/127) emergent isolates, indicating that it may also be an uncommon polymorphic variant. NS4A V43A variants were detected among 2.4% (22/901) of genotype 1a and 4.8% (53/1096) of genotype 1b Baseline variants, and emerged at a frequency of 1.6% (2/127) in genotype 1a and 1.2% (1/85) in genotype 1b-infected subjects.

The L356F variant, which was very infrequently identified among baseline isolates and was identified in a second TMC435-exposed subject, is the most likely to have been selected by TMC435. However, it is also possible that variants more frequently associated with TMC435 exposure, such as R155K or D168E, were overgrown by other variants by the time the relapse isolates had been collected, which was 24 weeks after the end of treatment. It is unclear if the impact of L356F has been determined in replicon cultures.

Table 10. NS3/4A emergent amino acid substitutions in genotype 1a SVR failures without detected resistance-associated R155 or D168 substitutions

Subject	EOT (Wk)	Isolate (Wk)	NS3							NS4A
			S122	I170	P264	L356	V358	V406	I586	V43
C208-0047	24	48				F	T		N	A
C208-0383	2	2						I		
HPC3007-6001	24	28		T						
HPC3007-6001	24	36		T						
HPC3007-6001	24	48		T						
HPC3007-6001	24	60			S					
HPC3007-6001	24	72			S					
HPC3007-6152	48	52	R			F				
HPC3007-6152	48	60	G			F				

- Subject C208-0383 discontinued at Week 2 with an HCV RNA load of 2.55×10^3 IU/mL. Only a V406I expressing variant was detected at Week 2, the last post-Baseline isolate for which genotypic data are available. V106I-expressing variants were not detected among any Baseline isolates or among any other post-Baseline isolates. While it is possible that the V106I variant had been selected during the two weeks of TMC435 exposure, it is unclear if the impact of V106I on TMC435 susceptibility has been determined in replicon cultures.
- Subject HPC3007-6001 was infected with genotype 1a Q80K-expressing variant at Baseline and achieved HCV RNA levels <25 IU/mL TD by Week 4 and <25 IU/mL TND by Week 8. HCV RNA remained undetected through Week 24, the End of Treatment timepoint. HCV RNA levels were 77 IU/mL by Week 28 and 1.12×10^6 IU/mL 10 days later. I170T variants were selected in the earlier post-relapse isolates, while P264S was detected in the later isolates. I170T was identified in 0.11% (1/901) of genotype 1a baseline isolates and as a treatment-associated substitution in 4.7% (6/127) of failure isolates. Notably, this is the only one of the six I170T-expressing variant that did not also harbor an R155 or D168 substitution. V170T conferred a median 4.7-fold reduction in TMC435 susceptibility in genotype 1b replicon cultures. P264S is a common polymorphism that was detected in 5.9% (53/901) of genotype 1a and 19.6% (215/1096) of genotype 1b Baseline isolates.
- Subject HPC3007-6152 was infected with a genotype 1a Q80K-expressing variant at Baseline and achieved HCV RNA levels <25 IU/mL TD by Week 8 and TND by Week 12. HCV RNA remained undetected through Week 48, the End of Treatment time point. HCV RNA levels were

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305 IU/mL by Week 52 and 9.14×10^5 IU/mL by Week 60. P264S was detected among 5.9% (53/901) of genotype 1a and 19.6% (215/1096) of genotype 1b baseline isolates. The P264S variant was also detected among 3.9% (5/127) of genotype 1a failure isolates. S122R was not detected in any baseline isolates, but was found in 7.1% (9/127) of genotype 1a SVR failures and in a Week 8 isolate of a subject (C208-0361) who achieved SVR. In contrast, S122G was detected in 9.2% (83/901) of genotype 1a and 4.3% (47/1096) genotype 1b Baseline isolates, and also emerged in 2.4% (3/127) genotype 1a post-Baseline isolates. L356F-frequencies among Baseline and treatment-associated isolates were described above for Subject C208-0047. S122R, which was shown to confer a 20-fold reduction in TMC435 susceptibility in genotype 1b replicon cultures, and L356F appear to be TMC435-treatment associated variants.

The most prevalent treatment-associated emergent genotype 1b variants were D168V, D168E, and Q80R, which were detected at frequencies of 61.2%, 23.5%, and 18.8% among subjects with treatment-emergent genotypic data, respectively. The prevalence of D168V, D168E, and Q80R among SVR failures was 60.5% (52/86), 23.3% (20/86), and 18.6% (16/86), respectively. Emergent NS3 D168-expressing variants were detected in 92.6% (75/81) of the genotype 1b-infected subjects with treatment-emergent genotypic data, and in 87.2% (75/86) of the SVR failures with available genotypic data. The six genotype 1b-infected subjects who failed to achieve SVR and lacked treatment-associated emergent D168 are discussed below in further detail (Table 11; Reviewer's analysis).

Table 11. NS3 emergent amino acid substitutions in genotype 1b SVR failures without detected resistance-associated D168 substitutions

Subject	EOT (Wk)	Isolate (Wk)	S7	R24	T46	T95	S138	A156	H246	T269	I288	N335	T358	S403
C206-0569	28	1						V						
C206-0569	28	36											F	
C206-0569	28	48											F	
C206-0569	28	72											F	
C208-0007	10	24		W										
C208-0409	1	4				S			Y					
C208-0409	1	24	A		S									
C208-0409	1	36			S									
C216-3316	24	36									M			
C216-3316	24	42									M			
C216-3316	24	48									M			
C216-3316	24	60									M			
HPC3007-6164	24	36												T
HPC3007-6164	24	48										G		T
HPC3007-6164	24	60					P							T
HPC3007-6164	24	72												T
HPC3007-6166	24	36												
HPC3007-6166	24	42								S				

- Subject C206-0569 achieved an HCV RNA load of <25 IU/mL TD by Week 3 and <25 IU/mL TND at Week 6. HCV RNA levels were either <25 IU/mL TND or TD until Week 24, at which time HCV RNA was quantifiable at 48 IU/mL, and Week 24 virologic failure was noted. All anti-HCV therapies were stopped at Week 28. An A156V-expressing variant was transiently detected at Week 1, and a T358F variant was detected at Weeks 36, 48, and 72. No A156V-expressing variants were detected among genotype 1a or 1b isolates, while one other A156V

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variant was transiently detected at Week 4 in a genotype 1a-infected subject who experienced virologic breakthrough. T358F was detected in one genotype 1b isolate at Baseline, but not in any other treatment-associated emergent variants. A156V was shown to reduce TMC435 susceptibility by 181-fold in genotype 1b replicon cultures, but it is unclear if the impact of T358F was determined.

- Subject C208-0007 achieved <25 IU/mL TD by Week 1 and <25 IU/mL TND by Week 2 and remained TND until Week 16. This subject discontinued therapy at Week 10 and had an HCV RNA level of 1.47×10^6 IU/mL at Week 24. The Week 24 isolate had an emergent R24W, which was not detected in any Baseline or in any other post-Baseline isolates. It is unclear if the impact of R24W on TMC435 susceptibility was determined on replicon cultures, so it is difficult to determine if the variant was selected by the drug. While it is possible that the R24W variant randomly emerged or was the result of an assay artifact, the variant may have been selected by TMC435. It is also possible that the more commonly observed treatment-emergent variants were overgrown by susceptible virus during the 14 week period between the end of treatment and collection of the post-relapse isolate.
- Subject C208-0409 achieved <25 IU/mL TD by Week 1, at which time all therapy was discontinued following an adverse event. A T95S and H246Y-expressing variant was transiently detected at Week 4, which was replaced by an S7A and T46S-expressing variant at Week 24. T95S variants were detected in 0.1% (1/901) of genotype 1a and 0.5% (6/1096) of genotype 1b Baseline isolates, but not among any other treatment associated emergent variants. H246Y was detected in 0.9% (8/901) of genotype 1a and 12.2% (134/1096) of genotype 1b baseline variants, and in 3.5% (3/85) of genotype 1b-infected subjects exposed to TMC435. S7A was detected among 0% of genotype 1a and 43.2% (473/1096) of genotype 1b Baseline isolates, and in 2.4% (2/85) genotype 1b post-treatment variants. T46S was detected in 3.8% (34/901) of genotype 1a and 4.7% (51/1096) of genotype 1b Baseline isolates, and was not observed to emerge in any other isolates. All of the emergent substitutions were polymorphic variants, although the T95S variant was rare. It is possible that the one week exposure was too short to enrich reduced susceptibility variants to a detectable level, or that such a population was overgrown in the three weeks between the end of treatment and collection of the isolate.
- Subject C216-3316 achieved HCV RNA loads <25 IU/mL TD by Week 4 and TND by Week 12, remained TND until Week 28, then relapsed at Week 36. An emergent I288M was detected in the Week 36 isolate and persisted at least through Week 60. I288M was detected in 0.1% (1/901) of genotype 1a and 13.1% (144/1096) of genotype 1b Baseline isolates, but not among any other treatment-emergent substitutions. It is possible that the emergence of this common polymorphic variant represents a TMC435-independent shift in the viral quasispecies, and that variants selected by TMC435 were overgrown during the period between the end of treatment and collection of the post-relapse isolates.
- Subject HPC3007-6164 achieved HCV RNA levels <25 IU/mL TND by Week 4, remained TND through Week 28, then relapsed by Week 36, twelve weeks after the end of treatment. An S403T variant was detected at Week 36 that persisted through Week 72. An N335G variant was transiently detected at Week 48 and an S138P variant at Week 60. S403T was detected in 0.2% (2/901) of genotype 1a and 1.2% (13/1096) of genotype 1b Baseline isolates, but not in any other post-Baseline isolates. N335G was not detected among any baseline isolates or among any other post-Baseline emergent viruses. S138P was detected in 0.1% (1/901) genotype 1a and no genotype 1b Baseline isolates, and not in any other post-Baseline variants. The S403T variant, the only one detected at Week 36, the first post-relapse isolate genotypically characterized, is an uncommon polymorphic variant that may represent a drug-independent shift

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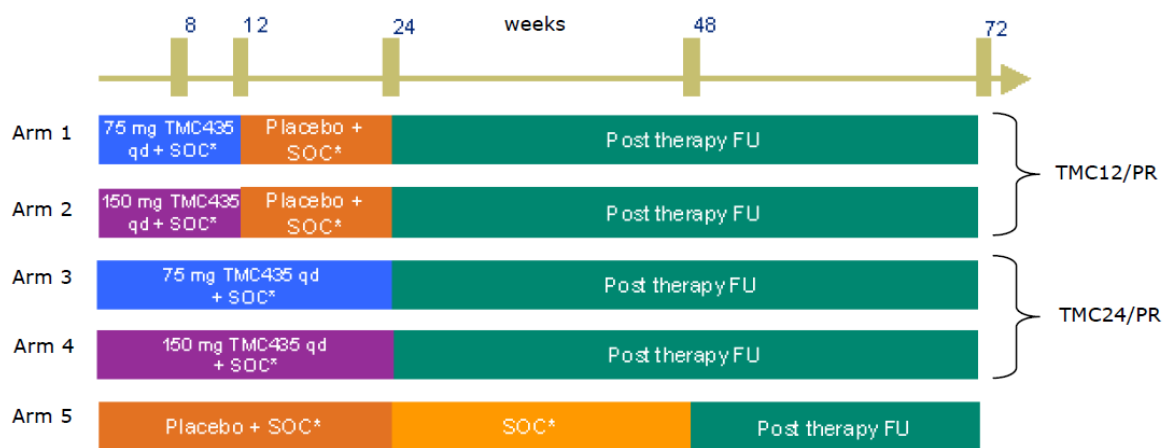
in the virus quasispecies. Although S138P and N335G were not observed as genotype 1b polymorphic variants among Baseline isolates, it is unlikely that they were selected by TMC435 given their absence within the earliest post-relapse isolate. It is more likely that the TMC435-selected variants were overgrown by susceptible variants at the time of the first post-relapse isolate collection.

- Subject HPC3007-6166 achieved HCV RNA <25 IU/mL TD by Week 1, TND by Week 2, remained TND until Week 28, then relapsed at Week 36, 14 weeks after the End of Treatment. An emergent T269S variant was detected at Week 42. T269S was not detected among any Baseline isolates or among any other treatment-associated emergent variants, and would therefore be suspect as a rare treatment-associated variant. However, the absence of the variant at Week 36, which was the first isolate collected post-relapse, casts doubt on its relevance.

4.3 Virology Summaries of other Supportive Trials

4.3.1 TMC435-TiDP16-C205

Trial C205 was a Phase 2 randomized, double blind, five-arm, placebo-controlled study to investigate the efficacy, tolerability, safety, and pharmacokinetics of TMC435 (75 and 150 mg q.d.) in combination with P/R in treatment-naïve HCV genotype-1 infected subjects. The design of C205, which was conducted in Europe, Australia/New Zealand, and North America, is illustrated in Figure 1 (C205 Clinical Study Report, pg. 41).



* Standard of Care (SoC) treatment, i.e., PegIFN α -2a and RBV

PR: standard treatment

Figure 1. Schematic of the C205 study design

Subjects received either 75 mg or 150 mg of TMC435 for 12 or 24 weeks in combination with 24 or 48 weeks of P/R; the duration of P/R was determined by virologic response at Weeks 4 and 12 (i.e., HCV RNA loads <12 IU/mL TND). No significant efficacy differences between the cohorts treated with different TMC435 durations (i.e., 12 or 24 weeks) or doses (i.e., 75 or 150 mg) were observed (Table 12; Virology Reviewer's analysis), and subjects in each TMC435 treatment arm experienced increased SVR rates of 8.4% to 19.8% relative to PBO-treated subjects. Notably, the SVR rates of subjects infected with genotype 1a Q80K variants were lower on average than those infected with other polymorphic variants, although the number of subjects was very small and the study was not sufficiently powered to detect significant differences between subgroups.

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Table 12. Summary of C205 SVR data

		SVR (n/N) (%)		SVR _{TMC435} -SVR _{PBO}			
		TMC435+P/R	PBO+P/R	Δ(%)	95% CI	Plot	p value
TMC435 75 mg 12 WKS	GT1	64/78 (82.1)	51/77 (66.2)	15.8	(2, 28.9)		0.024
	1a	27/37 (73)	21/30 (70)	3.0	(-17.8, 24.3)		0.79
	non-Q80K	20/25 (80)	15/23 (65.2)	14.8	(-10.1, 38)		0.25
	Q80K	7/12 (58.3)	5/6 (83.3)	-25.0	(-54.7, 20.5)		0.6*
	1b	37/41 (90.2)	30/47 (63.8)	26.4	(8.7, 41.9)		0.0052*
TMC435 75 mg 24 WKS	GT1	56/75 (74.7)	51/77 (66.2)	8.4	(-6.1, 22.4)		0.25
	1a	20/34 (58.8)	21/30 (70)	-11.2	(-32.5, 12)		0.35
	non-Q80K	16/26 (61.5)	15/23 (65.2)	-3.7	(-28.5, 22.2)		0.79
	Q80K	4/8 (50)	5/6 (83.3)	-33.3	(-64.9, 15.5)		0.3*
	1b	35/40 (87.5)	30/47 (63.8)	23.7	(5.4, 39.6)		0.011
TMC435 150 mg 12 WKS	GT1	62/77 (80.5)	51/77 (66.2)	14.3	(0.3, 27.6)		0.045
	1a	29/37 (78.4)	21/30 (70)	8.4	(-12.1, 29)		0.43
	non-Q80K	24/31 (77.4)	15/23 (65.2)	12.2	(-11.3, 35.4)		0.32
	Q80K	5/6 (83.3)	5/6 (83.3)	0.0	(-42, 42)		1*
	1b	31/38 (81.6)	30/47 (63.8)	17.7	(-1.6, 34.7)		0.071
TMC435 150 mg 24 WKS	GT1	68/79 (86.1)	51/77 (66.2)	19.8	(6.5, 32.5)		0.0036
	1a	32/37 (86.5)	21/30 (70)	16.5	(-3.2, 35.9)		0.099
	non-Q80K	29/31 (93.5)	15/23 (65.2)	28.33	(6.9, 49.2)		0.012*
	Q80K	3/6 (50)	5/6 (83.3)	-33.3	(-67.4, 17.2)		0.55*
	1b	36/42 (85.7)	30/47 (63.8)	21.88	(3.6, 38.1)		0.019

-75 -50 -25 0 25 50

* Inadequate data for a valid comparison of the difference between two independent proportions; the difference between proportions and their 95% CI intervals for these groups should be interpreted carefully; p-values were determined using the two-tailed Fisher Exact Test.

4.3.2 TMC435-TiDP16-C206

Trial C206 included P/R-experienced subjects and compared the efficacy of TMC435 + P/R to PBO + P/R. Subjects received either 100 mg or 150 mg of TMC435 q.d. for 12, 24, or 48 weeks in combination with 48 weeks of P/R (Figure 2; C206 Clinical Study Report, pg. 41).

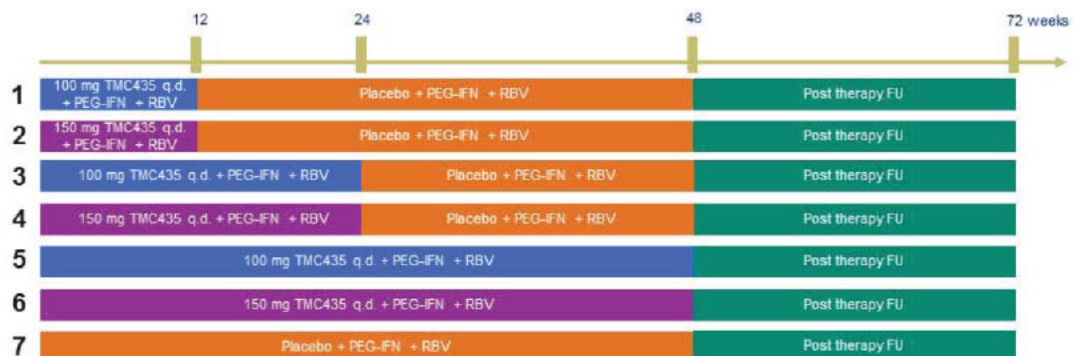


Figure 2. Schematic of the C206 study design

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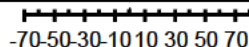
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Note that there was no RGT component to this protocol. No clear SVR differences were observed between the cohorts who received different durations or doses of TMC435 (Table 13; Clinical Virology Reviewer's analysis), with genotype 1 SVR rates ranging from 60.6% to 80%. However, the 150 mg dose did appear to be associated with an improved benefit to genotype 1a Q80K-infected subjects, who experienced a 60.9% (14/23) SVR rate, relative to those who received the 100 mg dose, who experienced an SVR rate of 26.1% (6/23). Note that the 150 mg TMC435-treated cohort only included 8 partial and 4 null-responders who were infected with genotype 1a Q80K, so the impact of the Q80K variant on the treatment of this harder-to-treat population could not be determined.

Table 13. Summary of C206 SVR data

		SVR (n/N) (%)		SVR _{TMC435} -SVR _{PBO}			
		TMC435+P/R	PBO+P/R	Δ(%)	95% CI	Plot	p value
TMC435 100 mg 12 WK	GT1	48/66 (72.7)	15/66 (22.7)	50.0	(33.6, 62.5)		<0.0001
	1a	17/26 (65.4)	5/27 (18.5)	46.9	(20.5, 65.3)		0.00054
	non-Q80K	13/18 (72.2)	4/22 (18.2)	54.0	(23.3, 72.8)		0.0011*
	Q80K	4/8 (50)	1/5 (20)	30.0	(-21.1, 62.9)		0.56*
	1b	30/39 (76.9)	10/39 (25.6)	51.3	(29.6, 66.5)		<0.0001
TMC435 100 mg 24 WK	GT1	43/65 (66.2)	15/66 (22.7)	43.4	(26.8, 56.8)		<0.0001
	1a	17/29 (58.6)	5/27 (18.5)	40.1	(14.6, 59)		0.0021
	non-Q80K	17/25 (68)	4/22 (18.2)	49.8	(21.6, 68.2)		0.001*
	Q80K	0/4 (0)	1/5 (20)	-20.0	(-62.4, 31.7)		1*
	1b	26/35 (74.3)	10/39 (25.6)	48.6	(26.2, 64.6)		<0.0001
TMC435 100 mg 48 WK	GT1	40/66 (60.6)	15/66 (22.7)	37.9	(21.3, 51.7)		<0.0001
	1a	13/25 (52)	5/27 (18.5)	33.5	(7.5, 54.2)		0.011
	non-Q80K	11/14 (78.6)	4/22 (18.2)	60.4	(27.3, 78)		0.00053*
	Q80K	2/11 (18.2)	1/5 (20)	-1.8	(-46.2, 31.9)		1*
	1b	27/40 (67.5)	10/39 (25.6)	41.9	(20, 58.5)		0.00019
TMC435 150 mg 12 WK	GT1	44/66 (66.7)	15/66 (22.7)	43.9	(27.4, 57.2)		<0.0001
	1a	19/30 (63.3)	5/27 (18.5)	44.8	(19.4, 62.9)		0.00062
	non-Q80K	14/22 (63.6)	4/22 (18.2)	45.5	(16.4, 65.3)		0.0051*
	Q80K	5/8 (62.5)	1/5 (20)	42.5	(-10.6, 71.4)		0.27*
	1b	25/36 (69.4)	10/39 (25.6)	43.8	(21.4, 60.5)		0.00015
TMC435 150 mg 24 WK	GT1	49/68 (72.1)	15/66 (22.7)	49.3	(33, 61.9)		<0.0001
	1a	20/30 (66.7)	5/27 (18.5)	48.1	(22.6, 65.6)		0.00025
	non-Q80K	17/24 (70.8)	4/22 (18.2)	52.7	(24.1, 70.6)		0.00044*
	Q80K	3/5 (60)	1/5 (20)	40.0	(-16.3, 72.6)		0.29*
	1b	27/35 (77.1)	10/39 (25.6)	51.5	(29.2, 67)		<0.0001
TMC435 150 mg 48 WK	GT1	52/65 (80)	15/66 (22.7)	57.3	(41.2, 68.8)		<0.0001
	1a	14/24 (58.3)	5/27 (18.5)	39.8	(13.2, 59.9)		0.0033
	non-Q80K	8/13 (61.5)	4/22 (18.2)	43.4	(10.3, 66.8)		0.024*
	Q80K	6/10 (60)	1/5 (20)	40.0	(-11.3, 68.4)		0.28*
	1b	38/41 (92.7)	10/39 (25.6)	67.0	(47.4, 79.1)		<0.0001*



* Inadequate data for a valid comparison of the difference between two independent proportions; the difference between proportions and their 95% CI intervals for these groups should be interpreted carefully; p-values were determined using the two-tailed Fisher Exact Test.

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4.4 Virology Perspective of Proposed (b) (4) Alternative to Q80K Screening

In replicon culture studies, the presence of Q80K was associated with an approximately 10-fold reduction in susceptibility (see Section 2.3). Results from the TMC435 phase 2b studies, C205 and C206, which were conducted in treatment-naïve and treatment-experienced subjects, respectively, indicated that the 150 mg q.d. TMC435 in combination with P/R would likely maintain activity against Q80K variants (see Sections 4.3.2 and 4.3.3). However, data from the phase 3 trials showed that subjects infected with a genotype 1a Q80K variant experienced significantly reduced efficacy (see Section 4.1).

In lieu of screening for the presence of genotype 1a Q80K variant-infected subjects before administering TMC435 + P/R, the sponsor proposed an alternative (b) (4) treatment algorithm (b) (4)

(b) (4)

(b) (4)

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(b) (4)



The sponsor's proposal is reasonable from a virology perspective

(b) (4)



(b) (4)



(b) (4)



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(b) (4)

In conclusion, the proposed (b) (4) algorithm does not appear adequate

(b) (4)

5 Conclusion

This NDA is approvable from a Virology perspective for the treatment of chronic HCV genotype 1a non-Q80K and genotype 1b-infected subjects who are either treatment-naïve or prior P/R-relapsers. We recommend that genotype 1a Q80K-infected subjects not be treated with TMC435 + P/R due to a substantially reduced efficacy benefit and an increased risk of developing virus that is cross-resistant with other NS3/4A PIs, including boceprevir and telaprevir, two approved drugs that otherwise maintain their full activity against the polymorphic variant. There are inadequate data to determine the impact of genotype 1a Q80K in prior partial-responders and prior null-responders, so those subjects should not be treated with TMC435 + P/R until a benefit can be demonstrated in an adequately powered clinical trial.

6 Package Insert

6.1 Proposed Package Insert (with Reviewer-recommended changes)

The following section was copied from the draft label submitted by the sponsor. Changes to the proposed label are shown in red text, with deletions indicated by strikethrough and additions by underline. Note that the numbering convention of the label's tables was preserved.

12.4 Microbiology

Mechanism of Action

Simeprevir is an inhibitor of the HCV NS3/4A protease which is essential for viral replication. In a biochemical assay simeprevir inhibited the proteolytic activity of recombinant genotype 1a and 1b HCV NS3/4A proteases, with median K_i values of 0.5 nM and 1.4 nM, respectively.

(b) (4)

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(b) (4)

Resistance in Cell Culture

Resistance to simeprevir was characterized in HCV genotype 1a and 1b replicon-containing cells. Ninety-six percent of simeprevir-selected genotype 1 replicons carried one or multiple amino acid substitutions at NS3 protease positions F43, Q80, R155, A156, and/or D168, with substitutions at NS3 position D168 being most frequently observed (78%). (b) (4)

Resistance to simeprevir was evaluated in HCV genotype 1a and 1b replicon assays using site-directed mutants and chimeric replicons carrying NS3 sequences derived from clinical isolates. Amino acid substitutions at NS3 positions F43, Q80, S122, R155, A156, and D168 reduced (b) (4) D168V or A, and R155K, substitutions (b) (4) displayed large reductions in susceptibility (b) (4) to simeprevir (FC in EC₅₀ value > 50), whereas other substitutions such as Q80K or R, S122R, and D168E displayed (b) (4) slight reductions in susceptibility (FC in EC₅₀ value between 2 and 50). Other substitutions such as Q80G or L, S122G, N or T did not reduce (b) (4) in the replicon assay (FC in EC₅₀ value (b) (4) 2). Amino acid substitutions at NS3 positions Q80, S122, R155, and/or D168, (b) (4) with lower reductions in susceptibility (b) (4) to simeprevir when occurring alone, reduced (b) (4) by more than 50-fold when present in combination.

Resistance in Clinical Studies

In a pooled analysis of (b) (4) subjects treated with 150 mg TRADENAME in combination with peginterferon alfa and ribavirin who did not achieve SVR in the controlled Phase 2b and Phase 3 clinical (b) (4), emerging amino acid substitutions at NS3 positions Q80, S122, R155 and/or D168 were observed in 180 out of 197 (91%) (b) (4) subjects. Substitutions D168V and R155K alone or in combinations with other (b) (4) substitutions at these positions emerged most frequently (b) (4). Most of these emerging substitutions have been shown to reduce (b) (4) in cell culture replicon assays.

HCV genotype 1 subtype-specific patterns of simeprevir treatment-emergent amino acid substitutions were observed in (b) (4) subjects not achieving SVR. (b) (4) Subjects with HCV genotype 1a predominantly had emerging R155K alone or in combination with amino acid substitutions at NS3 positions Q80, S122 and/or D168, while (b) (4) subjects with HCV genotype 1b had most often an emerging D168V substitution (b) (4). In (b) (4) subjects with HCV genotype 1a with a baseline Q80K amino acid substitution an emerging R155K substitution was observed most frequently at failure.

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Table 7: Treatment-Emergent Amino Acid Substitutions in Pooled Phase 2b and Phase 3 Studies: (b) (4) Subjects Who Did Not Achieve SVR With 150 mg TRADENAME in Combination With Peginterferon Alfa and Ribavirin

Emerging Amino Acid Substitutions in NS3	Genotype 1a N=116 % (n)	Genotype 1b N=81 % (n)
Any substitution at NS3 position <u>F43</u> , <u>Q80</u> , <u>S122</u> , <u>R155</u> , <u>A156</u> , or <u>D168</u> [†] <u>D168A</u> D168E D168V Q80R [†] R155K Q80X+D168X [#] R155X+D168X [#] Q80K [†] , S122A/G/I/T [†] , S122R, R155Q, <u>D168A</u> , D168F [†] , D168H, D168T, <u>I170T</u>		

* May include few (b) (4) subjects infected by HCV genotype 1 viruses of non-1a/1b subtypes.

† Alone or in combination with other substitutions (includes mixtures).

‡ Substitutions only observed in combinations with other emerging substitutions at one or more of the NS3 positions Q80, S122, R155 and/or D168.

(b) (4) Subjects with these combinations are also included in other rows describing the individual substitutions. X represents multiple amino acids. Other double or triple (b) (4) substitutions were observed with lower frequencies.

Note: substitutions at NS3 position F43 and A156 were selected in cell culture and associated with reduced simeprevir activity (b) (4) in the replicon assay but were not observed at time of failure. (b) (4)

Persistence of Resistance–Associated Substitutions

The persistence of simeprevir-resistant NS3 amino acid substitutions was assessed following treatment failure. ~~In the~~ a pooled analysis of (b) (4) subjects receiving 150 mg TRADENAME in combination with peginterferon alfa and ribavirin in the Phase 2b and Phase 3 studies. The proportion of subjects with detectable levels of treatment-emergent, resistance-associated variants was followed for a median time of 28 weeks (range 0 to 70 weeks). Resistant variants remained at detectable levels in 32 of 66 (b) (4) subjects (48%) with single emerging R155K and in 16 of 48 (b) (4) subjects (33%) with single emerging D168V.

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(b) (4)

Effect of Baseline HCV Polymorphisms on Treatment Response

Analyses were conducted to explore the association between naturally-occurring baseline NS3/4A amino acid substitutions (polymorphisms) and treatment outcome. In the pooled analysis of the

Phase 3

(b) (4)

(b) (4)

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(b) (4)

Cross-Resistance

Some of the treatment-emergent NS3 amino acid substitutions detected in TRADENAME-treated (b) (4) subjects who did not achieve SVR in clinical (b) (4) including R155K, substitutions at D168, and I170T) have been shown to reduce the anti-HCV activity of the NS3/4A PIs, boceprevir and/or telaprevir. (b) (4)

(b) (4)

6.2 *Proposed package insert (clean)*

(b) (4)

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7 Recommendations

As part of a post-marketing study, we recommend that the sponsor determine the phenotypic susceptibility of TMC435 against:

- L356F or V106I expressed in genotype 1a replicon cultures, individually and in combination with Q80K
- R24W, K213R, T358F, or T610I expressed in genotype 1b replicon cultures

8 References

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/s/

DAMON J DEMING
08/28/2013

JULIAN J O REAR
08/28/2013

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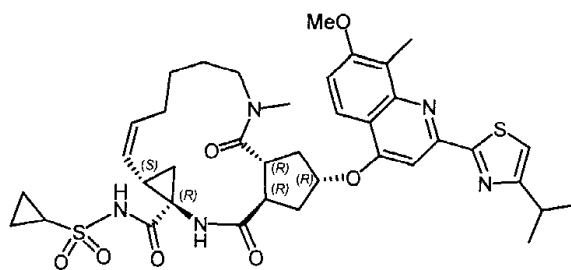
Reviewer: Eric F. Donaldson, Ph.D.
Date Submitted: 3/29/13
Date Received: 3/29/13

Date Assigned: 4/1/13

Sponsor: Janssen Research and Development
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Product Names: Simeprevir; TMC435; TMC435350; R494617; JNJ-38733214-AAA
Chemical Names: (2*R*,3*aR*,10*Z*,11*aS*,12*aR*,14*aR*)-*N*-(cyclopropylsulfonyl)-2-[[2-(4-isopropyl-1,3-thiazol-2-yl)-7-methoxy-8-methyl-4-quinolinyl]oxy]-5-methyl-4,14-dioxo-2,3,3*a*,4,5,6,7,8,9,11*a*,12,13,14,14*a*-tetradecahydrocyclopenta[*c*]cyclopropa[*g*][1,6]diazacyclotetradecine-12*a*(1*H*)-carboxamide

Structure:



TMC435 (simeprevir)

Molecular formula: C₃₈H₄₇N₅O₇S₂
Molecular weight: 749.94

Drug category: Antiviral

Indication: Treatment of chronic HCV infection with GT1a lacking a Q80K polymorphism or with GT1b in combination with pegylated recombinant human interferon α and ribavirin

Dosage Form: 150 mg "solid dose form"
Route of administration: Oral

Supporting documents: IND 075391; Submission IND75391 SDN545 (Analysis of NGS mock data)

Abbreviations: cEVR, complete early virologic response (i.e., HCV RNA not detected at Week 12); EVR, early virologic response (i.e., $\geq 2 \log_{10}$ change of HCV RNA at Week 12); HCV, hepatitis C virus; IFN α , interferon alpha; LPVPS, last planned visit of that previous study; NGS, next generation sequencing; PegIFN α -2a, pegylated interferon α -2a (Pegasys[®]); PPSR, pediatric plan study request; q.d., P/R, PegIFN α plus ribavirin; quaque die (once daily); RBV, ribavirin (Copegus[®]); RVR, rapid virologic response (i.e., HCV RNA undetectable at Week 4); SoC, standard of care (PegIFN α +RBV); SVR, sustained virologic response; SVR4, HCV RNA not detected at the end of treatment (Week 24) and 4 weeks after the end of treatment; SVR12, HCV RNA not detected at end of treatment and 12 weeks after the end of treatment

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DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA# 205123 SDN 003 DATE REVIEWED: 08/10/13

EXECUTIVE SUMMARY

TMC435 or simeprevir is a noncovalent small-molecule inhibitor of the hepatitis C virus (HCV) NS3/4A trypsin-like serine protease, which is essential for processing the polyprotein that forms the viral replication complex. The sponsor submitted a New Drug Application seeking the following indication for TMC435:

1. For the treatment of chronic hepatitis C (CHC) genotype 1 infection, in combination with peginterferon α and ribavirin, in adult patients with compensated liver disease (including cirrhosis) who are treatment naïve or who have failed previous interferon therapy (pegylated or non pegylated) with or without ribavirin
2. One 150 mg capsule taken once daily with food, to be administered with both pegylated-interferon α and ribavirin.
3. The recommended treatment duration for TMC435 with pegylated-interferon α and ribavirin is 12 weeks, followed by either 12 or 36 additional weeks of pegylated-interferon α and ribavirin depending on treatment viral response and prior response status

The sponsor provided next generation nucleotide sequencing data (NGS) used in the resistance analysis of two phase 2 clinical trials, TMC435-C205 and TMC435-C206, which tested TMC435 at doses of 75, 100, and 150 mg and for durations of 12-, 24- and 48-weeks. Given that next generation sequencing is an emerging technology with no current standards for analysis, the division requested raw NGS sequence data in the form of fastq sequences so that an independent analysis could be performed with the NGS data. The Division of Antiviral Products (DAVP) worked with the Office of Computational Science (OCS) within the Center for Drug Evaluation and Research (CDER) to acquire the resources required to analyze NGS data for review purposes. An analysis pipeline was established and used to conduct an independent resistance analysis for these two phase 2 clinical trials using the raw NGS data provided in this submission. The results were compared to those reported by the sponsor.

In general, there was good agreement between the results and conclusions reported by the sponsor and those determined by DAVP; however, there were some notable differences related to the different approaches used by the two parties. The sponsor focused its analyses on two lists of substitutions that were predetermined to be sites of interest based on cell culture selection studies and clinical studies of other protease inhibitors, whereas DAVP performed an unbiased analysis of all substitutions. Moreover, DAVP generated the NGS summary data using two variant detection algorithms and evaluated different filtering strategies to determine which provided the most reliable results.

The predominant treatment emergent resistance-associated substitutions were R155K and Q80K for genotype 1 subtype a (GT1a) and D168V/E for GT1b. Several additional substitutions were identified in two or more subjects, and these sites were reported by the sponsor, with many being tested in phenotypic assays designed to determine if the presence of a given substitution has an impact on the EC₅₀ value of TMC435 in the HCV replicon system. It is important to note that DAVP requests phenotypic data to confirm an association, but not to exclude a resistance association because some known resistance-associated substitutions have shown little or no effect in phenotypic assays.

While in general, the conclusions reached by the sponsor and DAVP were similar, additional potential resistance-associated substitutions were identified. The predominant resistance-associated substitutions observed in the analyses of TMC435-C205 and –C206 have been identified previously:

- GT1a: Q80K, R155K
- GT1b: D168V/E
- Both: S122R/T122S (S122A/G/R reduce susceptibility to TMC435 (0.9-, 0.5- and 22-fold change in EC₅₀ values, respectively).

In addition, 2 potential low frequency resistance-associated substitutions were observed at highly conserved sites: P574A/S (1b) and V629I (GT1a and 1b). Clinical virology recommends that these substitutions be further evaluated in a phenotypic assay as potential resistance-associated substitutions as post-marketing considerations.

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BACKGROUND AND SUMMARY

TMC435 or simeprevir is a noncovalent small-molecule inhibitor of the hepatitis C virus (HCV) NS3/NS4A trypsin-like serine protease, which is essential for processing the polyprotein that forms the viral replication complex.

NDA205123 was submitted on March 29, 2013 and received priority review status. The sponsor provided next generation sequencing data (NGS) used in the resistance analysis of two phase 2 clinical trials, TMC435-C205 and TMC435-C206. The sponsor provided these data on a hard drive, including 1) frequency tables showing amino acid variation that occurred at each position of NS3/4A for each failure sample that was successfully sequenced using Illumina; 2) raw sequence data in fastq format for all samples that were deep sequenced; and 3) summary resistance data for each study.

Given that next generation sequencing is an emerging technology with no current standards for analysis, the Division requested raw data so that an independent analysis could be performed on the NGS data. The sponsor's summary NGS data were compared to the results generated by the Division of Antiviral Products following these criteria:

1. The sponsor's frequency tables were used to generate a summary and do a direct comparison of the results reported by the sponsor;
2. Frequency tables were generated by DAVP using an independent mapping of reads to a reference for each sample and using two different variant detection algorithms and the results were compared with those reported by the sponsor and those generated using the sponsor's frequency table; and
3. The conclusions from the NGS data were compared to the results reported by the sponsor using Sanger population sequence analysis.

Rationale for Requesting and Analyzing NGS data

In general, the FDA does not analyze raw data in conjunction with new drug applications (NDAs); however, when the technology used to generate the data is relatively new, it is necessary to perform independent assessments of the data to confirm that the review division understands how the data are analyzed. Next Generation Sequencing (NGS) is an emerging technology that presents many potential data integrity issues that must be considered upon careful review:

1. There are currently multiple sequencing platforms available for resistance analysis by NGS (454, Illumina, Ion Torrent, PacBio), and these technologies are continuously emerging. Each platform has different error rates and chemistries that contribute to unique types of base calling errors
2. There are currently no standardized analysis pipelines with which to analyze NGS data and more than 200 algorithms can be used to generate an assembly of small reads, with each algorithm employing unique strategies and using unique parameters. Comparison of different platforms and algorithms has shown that often differences in data interpretation are attributed to the bioinformatics analysis and not the sequencing platform
3. To date, each sponsor submitting NGS data has generated data with an unique NGS analysis pipeline that includes internal scripts and programs not available in the public domain

The Division of Antiviral Products (DAVP) reviews Investigational New Drugs (IND) and New Drug Applications (NDA) with indications for the treatment of a variety of viral diseases. In nearly all cases, these drugs select for resistant viruses that escape antiviral therapies. Therefore, viral resistance has important implications for the durability of a new drug and the potential impact on future treatment options for patients infected with the virus. DAVP performs independent analyses of all resistance data associated with antiviral drugs being developed to ensure that the emergence of resistance is carefully characterized and explained in the label of newly approved antiviral drug products. Providing accurate resistance information is imperative for protecting public health to prevent emergence of novel resistant and cross-resistant viral variants that have the potential to infect others and cause major outbreaks of disease that cannot be controlled by approved drugs. In addition, the resistance information provides important guidance for health care professionals who oversee the use of these therapeutics and is included in the drug product information approved by DAVP.

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Until recently, the genotypic resistance data reviewed by DAVP virology reviewers were generated using the Sanger DNA sequencing method. However, NGS is an emerging technology that provides an inexpensive and comprehensive approach for performing high throughput sequencing and is being employed more and more frequently by sponsors when performing resistance analyses. Because it determines the sequence for all RNAs or DNAs in a clinical sample, NGS adds complexity to the resistance analysis process while reducing sequencing costs. In contrast to Sanger sequencing which provides an average sequence of the virus population, NGS provides sequence information for cDNA sequences derived from individual viruses within a virus population, potentially providing millions of short sequences per sample. The complexity of the data makes it challenging for virology reviewers to analyze and validate the sequence information, which is complicated by the fact, as mentioned above, that there are currently no standard bioinformatics analysis approaches for analyzing these large datasets. Moreover, nearly every sponsor performing NGS has developed their own proprietary bioinformatics analysis pipeline. Given that there are over two hundred assembly algorithms alone, it is expected that each pipeline will provide a unique interpretation of the data.

Currently, industry is rapidly adopting the use of NGS technology in support of product development and application submissions. This technology has created unique review challenges for CDER where no NGS data analysis/review capabilities had previously existed. To address this gap in the review process which could have a significant impact on public health, DAVP teamed up with CDER's Computational Science Center to develop an independent NGS analysis pipeline that would allow virology reviewers to perform a robust and independent analysis of NGS resistance datasets submitted in support of antiviral drugs in development.

NGS Data Analysis Pipeline

The Division of Antiviral Products worked with the Office of Scientific Computing within CDER to acquire the resources to analyze NGS data for review purposes. The CLC Genomics Workbench was installed for use on the High Performance Computer at CDRH and was used to establish an analysis pipeline for independently analyzing NGS data. CLC Genomics was used to evaluate each of the sequence runs, trim and filter the sequences prior to mapping, and to map the sequences to HCV GT1a or HCV GT1b reference sequences (H77 or Con1, respectively). Two independent variant detection algorithms were used to call variants from each mapping, and the variant tables were exported from CLC Genomics Workbench and combined to generate frequency tables and resistance summary tables (Figure 1).

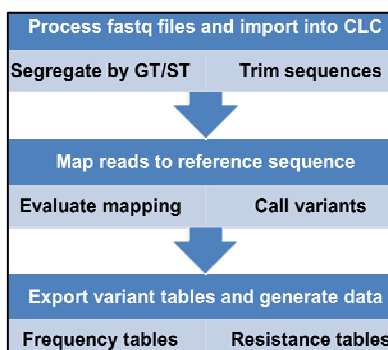


Figure 1. An overview of the NGS Analysis Pipeline using CLC Genomics Workbench.

NGS Analysis Parameters and Overview of Data Analysis

Each step of the analysis process is described in detail below.

- 1. Processing fastq files with CLC Genomics Workbench.** Data were received on a portable hard drive, which included fastq files for each subject and time point that was sequenced using the Illumina platform. The sequences were uploaded via the CLC Genomics interface, using the Illumina specific criteria. Failed reads were removed, read names were discarded, and Quality scores were calculated using the NCBI/Sanger (Illumina Pipeline 1.8) option.

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2. **Segregating sequences by HCV genotype and trimming the sequence reads prior to mapping.** The fastq files were separated by genotype and subtype and the NS3/4A genes for HCV GT1a (H77) and HCV GT1b (Con1) were imported and annotated as coding sequences so that these could be used as the reference sequences. The individual reads from each fastq file were subjected to trimming using the default parameters for CLC Genomics Workbench and these are defined in Figure 2.

The screenshot shows the 'Trim Sequences' dialog box with the following settings:

- Ambiguous trim: ☒
- Ambiguous limit: 2
- Quality trim: ☒
- Quality limit: 0.05
- Use colorspace: ☐
- Also search on reversed sequence: ☒
- Remove 5' terminal nucleotides: ☐
- Number of 5' terminal nucleotides: 1
- Maximum number of nucleotides in reads: 1000
- Minimum number of nucleotides in reads: 15
- Discard short reads: ☐
- Remove 3' terminal nucleotides: ☐
- Number of 3' terminal nucleotides: 1
- Trim adapter list: (empty field)
- Discard long reads: ☐

Figure 2. CLC Genomics Workbench default trimming parameters.

3. **Mapping reads to the appropriate reference sequence for each HCV genotype and subtype.** The reads from each fastq file were aligned to the appropriate reference sequence to generate a mapping for each time point (Figure 3). The mapping contained the target of interest (the NS3/4A gene sequence) and was used to generate a consensus sequence for each sequence run. The consensus sequences were conceptually translated to amino acid sequences to compare changes that occurred at the amino acid level.

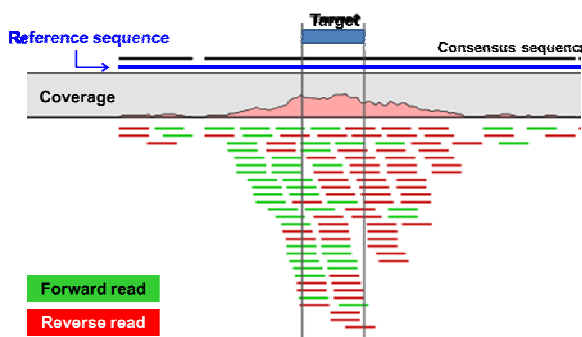


Figure 3. Schematic representation of a sequence mapping.

In general, the mappings were assessed to determine the depth of coverage at each nucleotide position and to evaluate read directionality (ratio of forward to reverse reads) to identify regions of bias. The parameters used for the mapping are shown in Figure 4.

The screenshot shows the 'Map Reads to Reference' dialog box with the following settings:

- References: HCV_GT1a_H77_NS3_4A
- Masking mode: No masking
- Masking track: (empty field)
- Mismatch cost: 2
- Insertion cost: 3
- Deletion cost: 3
- Length fraction: 0.5
- Similarity fraction: 0.8
- Global alignment: ☐
- Color space alignment: ☒
- Color error cost: 3
- Auto-detect paired distances: ☒
- Non-specific match handling: Map randomly

Figure 4. Parameters for mapping sequence reads to the reference sequence.

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4. Generating frequency tables of amino acid substitutions. From the read mappings, two algorithms were used to call variants based on independent criteria, and variant tables were generated for each sequence run and variant detection method. The variant tables included the following column headers: Reference Position, Type, Length Reference, Allele Linkage, Zygosity, Count Coverage, Frequency, Forward/reverse balance, Average quality, Overlapping annotations, Coding region change, and Amino acid change. The two variant detection systems employed different strategies for calling variants, and the variant detection parameters were relaxed from default to maximize the number of variants called, given that true variants would likely be identified in multiple subjects, allowing those that were of low quality or probability to be filtered out at the analysis stage. The two detection methods were:

- 1. Probabilistic Variant detection (PVD75)** – calls variants from a read mapping using a probabilistic model (combines a Bayesian model and a Maximum Likelihood approach to calculate prior and error probabilities)(Figure 5). Parameters are calculated on the mapped reads without considering the reference sequence. The variant probability parameter was reduced from a default value of 90 to 75 to increase the number of variant calls, given that false calls would likely be filtered during data analysis.

The screenshot shows the 'Probabilistic Variant Detection' settings. The parameters are as follows:

Parameter	Value
Ignore non-specific matches	<input checked="" type="checkbox"/>
Ignore broken pairs	<input checked="" type="checkbox"/>
Minimum coverage	500
Variant probability	75.0
Require presence in both forward and reverse reads	<input checked="" type="checkbox"/>
Filter 454/Ion homopolymer indels	<input type="checkbox"/>
Maximum expected variants	2
Genetic code	1 Standard
Discard coalesce SNVs	<input checked="" type="checkbox"/>
Ignore quality scores	<input type="checkbox"/>

Figure 5. Parameter settings used for Probabilistic Variant Detection. The Variant Probability was set to 75% in order to increase the likelihood of a variant call, to provide a comprehensive view of potential resistance-associated substitutions.

- 2. Quality-based variant detection (QbVD)** - based on the Neighborhood Quality Standard algorithm, it uses a combination of quality filters and user-specified thresholds for coverage and frequency to call variants covered by aligned reads. The parameters used for this algorithm are shown in Figure 6.

The screenshot shows the 'Quality-based Variant Detection' settings. The parameters are as follows:

Parameter	Value
Neighborhood radius	5
Maximum gap and mismatch count	2
Minimum neighborhood quality	15
Minimum central quality	20
Ignore non-specific matches	<input checked="" type="checkbox"/>
Ignore broken pairs	<input checked="" type="checkbox"/>
Minimum coverage	500
Minimum variant frequency (%)	1.0
Maximum expected alleles	2
Advanced	<input type="checkbox"/>
Maximum coverage	99999
Sufficient variant count	5
Required variant count	1
Require presence in both forward and reverse reads	<input checked="" type="checkbox"/>
Filter 454/Ion homopolymer indels	<input type="checkbox"/>
Genetic code	1 Standard

Figure 6. Parameter settings used for Quality based Variant Detection. The minimum variant frequency (%) was set at 1% in order to increase the likelihood of a variant call, to provide a comprehensive view of potential resistance-associated substitutions.

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Frequency tables were generated by exporting the variant tables for both variant detection methods (PVD75 and QBVD) for each mapping and then reformatting the data to reflect variation at the amino acid level with these pertinent changes (Figure 7):

1. The variant tables were combined by genotype/subtype and study
2. The variant tables were filtered to remove synonymous substitutions
3. The variant tables were reformatted to be directly comparable to the frequency tables submitted by the sponsor

Reference Position	Type	Length	Reference	Allele	Linkage	Zygosity	Count	Coverage	Frequency	Forward/reverse balance	Average quality	Overlapping annotations	Coding region change	Amino acid change
6	SNV	1	C	T		Heterozygous	315	27155	1.160007365	0.495238095	37.60952381	CDS: NS3/4A	NS3/4A:c.6C>T	
9	SNV	1	C	T		Heterozygous	824	28830	2.858133888	0.422330097	38.1407767	CDS: NS3/4A	NS3/4A:c.9C>T	
19	SNV	1	G	A		Heterozygous	372	35474	1.048655353	0.438172043	38.41397849	CDS: NS3/4A	NS3/4A:c.19G>A	NS3/4A:p.Ala7Thr
27	SNV	1	G	A		Heterozygous	38493	39913	96.44226192	0.434442626	37.88890448	CDS: NS3/4A	NS3/4A:c.27G>A	
30	SNV	1	G	A		Heterozygous	39899	40967	97.39302365	0.435850523	37.58547833	CDS: NS3/4A	NS3/4A:c.30G>A	
33	SNV	1	A	G		Homozygous	43607	43951	99.21731019	0.433577178	36.91932488	CDS: NS3/4A	NS3/4A:c.33A>G	
42	SNV	1	A	G		Heterozygous	14323	47086	30.41890814	0.44225791	37.45081987	CDS: NS3/4A	NS3/4A:c.42A>G	
48	SNV	1	T	C		Homozygous	46283	46506	99.51621226	0.443656276	36.45859171	CDS: NS3/4A	NS3/4A:c.48T>C	
54	SNV	1	C	T		Heterozygous	45538	47038	96.81108891	0.434933462	36.64447275	CDS: NS3/4A	NS3/4A:c.54C>T	
61	SNV	1	C	T		Heterozygous	1656	50736	3.263954588	0.414855072	37.11231884	CDS: NS3/4A	NS3/4A:c.61C>T	
66	SNV	1	T	C		Heterozygous	758	52197	1.452190739	0.420844327	37.95778364	CDS: NS3/4A	NS3/4A:c.66T>C	
75	SNV	1	C	T		Heterozygous	799	52447	1.523442714	0.441802253	37.46933667	CDS: NS3/4A	NS3/4A:c.75C>T	
78	SNV	1	A	G		Homozygous	50303	50568	99.47595317	0.445400871	37.63379918	CDS: NS3/4A	NS3/4A:c.78A>G	
84	SNV	1	A	G		Heterozygous	1575	53794	2.927835818	0.442539683	37.9447619	CDS: NS3/4A	NS3/4A:c.84A>G	
93	SNV	1	T	C		Heterozygous	1755	51227	3.425927733	0.476353276	36.90826211	CDS: NS3/4A	NS3/4A:c.93T>C	
96	SNV	1	G	A		Heterozygous	798	53910	1.480244853	0.439849624	37.32205514	CDS: NS3/4A	NS3/4A:c.96G>A	
105	SNV	1	C	T		Homozygous	52558	53068	99.03896887	0.475569847	37.82362343	CDS: NS3/4A	NS3/4A:c.105C>T	
114	SNV	1	T	C		Heterozygous	50744	52327	96.97479313	0.484668138	37.88684376	CDS: NS3/4A	NS3/4A:c.114T>C	
118	SNV	1	A	G		Homozygous	52699	52807	99.79548106	0.486878309	37.94667831	CDS: NS3/4A	NS3/4A:c.118A>G	NS3/4A:p.Thr40Ala
123	SNV	1	A	G		Heterozygous	1802	55129	3.268897056	0.49445061	37.73140954	CDS: NS3/4A	NS3/4A:c.123A>G	
126	SNV	1	C	T		Heterozygous	49617	53768	92.27979467	0.487111727	37.35344741	CDS: NS3/4A	NS3/4A:c.126C>T	
129	SNV	1	C	T		Heterozygous	1976	56723	1.89693775	0.476765799	37.66263941	CDS: NS3/4A	NS3/4A:c.129C>T	
135	SNV	1	A	G		Heterozygous	677	57024	1.187219416	0.462333826	38.12703102	CDS: NS3/4A	NS3/4A:c.135A>G	
147	SNV	1	T	C		Heterozygous	1267	57134	2.217593727	0.499605367	38.13259668	CDS: NS3/4A	NS3/4A:c.147T>C	

USUBJID	NGSPL	VISIT	NS3/4A AAPOS	TCOV	AAREF	AASUB	VCOV	AAFREQ
TMC435-C205-0015	ILLUMINA GAIIX	SCREENING	7	35474	A	T	372	0.0105
TMC435-C205-0015	ILLUMINA GAIIX	SCREENING	40	52807	T	A	52699	0.998
TMC435-C205-0015	ILLUMINA GAIIX	WEEK48	40	33463	T	A	33396	0.998
TMC435-C205-0015	ILLUMINA GAIIX	SCREENING	80	32472	Q	K	32390	0.9975
TMC435-C205-0015	ILLUMINA GAIIX	WEEK48	80	25183	Q	K	24982	0.992
TMC435-C205-0015	ILLUMINA GAIIX	SCREENING	86	30335	P	A	5462	0.1801
TMC435-C205-0015	ILLUMINA GAIIX	SCREENING	86	30335	P	S	734	0.0242
TMC435-C205-0015	ILLUMINA GAIIX	SCREENING	86	30335	P	T	2186	0.0721
TMC435-C205-0015	ILLUMINA GAIIX	WEEK48	86	25415	P	A	606	0.0238
TMC435-C205-0015	ILLUMINA GAIIX	WEEK48	86	25415	P	T	739	0.0291
TMC435-C205-0015	ILLUMINA GAIIX	SCREENING	119	35747	R	Q	17622	0.493
TMC435-C205-0015	ILLUMINA GAIIX	WEEK48	119	22826	R	Q	2332	0.1022
TMC435-C205-0015	ILLUMINA GAIIX	SCREENING	153	36063	L	I	36040	0.9994
TMC435-C205-0015	ILLUMINA GAIIX	WEEK48	153	24207	L	I	24175	0.9987
TMC435-C205-0015	ILLUMINA GAIIX	WEEK48	155	26156	R	K	296	0.0113
TMC435-C205-0015	ILLUMINA GAIIX	WEEK48	168	28353	D	E	2960	0.1044

Figure 7. Comparing the QbVD table to the Frequency table. The variant detection table for the Quality based Variant Detection (QbVD) method for subject TMC435-C205-0015 of clinical trial TMC435-C205 is shown in the top panel. This table shows information at the nucleotide level, with variation defined as differences from the reference sequence. The frequency table (bottom panel) contains the appropriate information for each subject at the amino acid level. **USUBJID**, Unique Subject ID; **NGSPL**, NGS Sequencing platform; **VISIT**, visit time point; **NS3/4A AAPOS**, gene name and amino acid position; **AAREF**, amino acid present in the reference sequence; **AASUB**, amino acid substitution; **TCOV**, total coverage; **VCOV**, variant coverage (number of reads that contain the nucleotides encoding the substitution); and **AAFREQ**, the frequency of the variant.

5. Generating resistance analysis tables. An ETL/Kettle script was developed to convert the Frequency tables into Resistance Analysis tables, allowing the resistance tables to be populated using different frequency thresholds. For example, the frequency tables generated from CLC Genomics Workbench output or submitted by the sponsor contained all variants with a frequency greater than or equal to 1%, and this tool allowed Resistance Analysis tables to be generated showing variants at different levels of sensitivity (5%, 15%, 25%, etc.) as defined by the user. Figure 8 shows a Frequency table and three Resistance Analysis tables generated from the same frequency table at different levels of sensitivity (0, 0.05, and 0.20).

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USUBJID	NGSPL	VISIT	NS3 AAPOS	TCOV	AAREF	AASUB	VCOV	AAFREQ
TMC435-C205-0197	ILLUMINA GAlx	WEEK 4	167	40439	V	I	467	0.012
TMC435-C205-0197	ILLUMINA GAlx	WEEK 12	167	52461	V	I	2871	0.055
TMC435-C205-0197	ILLUMINA GAlx	WEEK 36	167	46812	V	I	521	0.011
TMC435-C205-0197	ILLUMINA GAlx	WEEK 2	168	54499	D	V	40234	0.738
TMC435-C205-0197	ILLUMINA GAlx	WEEK 2	168	54499	D	A	13539	0.248
TMC435-C205-0197	ILLUMINA GAlx	WEEK 3	168	30625	D	V	24929	0.814
TMC435-C205-0197	ILLUMINA GAlx	WEEK 3	168	30625	D	A	4262	0.139
TMC435-C205-0197	ILLUMINA GAlx	WEEK 3	168	30625	D	E	893	0.029
TMC435-C205-0197	ILLUMINA GAlx	WEEK 3	168	30625	D	T	358	0.012
TMC435-C205-0197	ILLUMINA GAlx	WEEK 4	168	40487	D	V	36392	0.899
TMC435-C205-0197	ILLUMINA GAlx	WEEK 4	168	40487	D	A	2868	0.071
TMC435-C205-0197	ILLUMINA GAlx	WEEK 4	168	40487	D	E	473	0.012
TMC435-C205-0197	ILLUMINA GAlx	WEEK 12	168	27317	D	E	21849	0.8
TMC435-C205-0197	ILLUMINA GAlx	WEEK 12	168	52112	D	E	39049	0.749
TMC435-C205-0197	ILLUMINA GAlx	WEEK 12	168	27317	D	V	4892	0.179
TMC435-C205-0197	ILLUMINA GAlx	WEEK 12	168	52112	D	V	6894	0.132
TMC435-C205-0197	ILLUMINA GAlx	WEEK 12	168	52112	D	T	1358	0.026
TMC435-C205-0197	ILLUMINA GAlx	WEEK 12	168	27317	D	T	333	0.012

USUBJID	VISIT	NGSPL	NS3 150	NS3 151	NS3 152	NS3 153	NS3 155	NS3 159	NS3 160	NS3 167	NS3 168	NS3 170
TMC435-C205-0197	SCREENING	ILLUMINA GAlx										
TMC435-C205-0197	WEEK 12	ILLUMINA GAlx								I	E,V	
TMC435-C205-0197	WEEK 2	ILLUMINA GAlx									AV	
TMC435-C205-0197	WEEK 3	ILLUMINA GAlx									A,E,V	
TMC435-C205-0197	WEEK 36	ILLUMINA GAlx								I		
TMC435-C205-0197	WEEK 4	ILLUMINA GAlx								I	A,E,V	
TMC435-C205-0197	WEEK 48	ILLUMINA GAlx										
TMC435-C205-0197	WEEK 72	ILLUMINA GAlx										

USUBJID	VISIT	NGSPL	NS3 150	NS3 151	NS3 152	NS3 153	NS3 155	NS3 159	NS3 160	NS3 167	NS3 168	NS3 170
TMC435-C205-0197	SCREENING	ILLUMINA GAlx										
TMC435-C205-0197	WEEK 12	ILLUMINA GAlx								I	E,V	
TMC435-C205-0197	WEEK 2	ILLUMINA GAlx									AV	
TMC435-C205-0197	WEEK 3	ILLUMINA GAlx									AV	
TMC435-C205-0197	WEEK 36	ILLUMINA GAlx										
TMC435-C205-0197	WEEK 4	ILLUMINA GAlx									AV	
TMC435-C205-0197	WEEK 48	ILLUMINA GAlx										
TMC435-C205-0197	WEEK 72	ILLUMINA GAlx										

USUBJID	VISIT	NGSPL	NS3 150	NS3 151	NS3 152	NS3 153	NS3 155	NS3 159	NS3 160	NS3 167	NS3 168	NS3 170
TMC435-C205-0197	SCREENING	ILLUMINA GAlx										
TMC435-C205-0197	WEEK 12	ILLUMINA GAlx									E	
TMC435-C205-0197	WEEK 2	ILLUMINA GAlx									AV	
TMC435-C205-0197	WEEK 3	ILLUMINA GAlx									V	
TMC435-C205-0197	WEEK 36	ILLUMINA GAlx										
TMC435-C205-0197	WEEK 4	ILLUMINA GAlx									V	
TMC435-C205-0197	WEEK 48	ILLUMINA GAlx										
TMC435-C205-0197	WEEK 72	ILLUMINA GAlx										

Figure 8. Generating Resistance Analysis tables from Frequency tables at different levels of sensitivity. A Frequency table is shown for subject TMC435-C205-0197 at NS3 amino acid positions 167 and 168 (top panel). Resistance Analysis tables were generated at frequency thresholds of 0, 0.05 and 0.20 (bottom panels). Blank spaces represent amino acid positions that had no variation for a given timepoint. ω_t = frequency threshold.

6. Conducting independent resistance analysis. The frequency tables and resistance analysis tables were then analyzed to identify substitutions that occurred above an arbitrary frequency threshold. Three filtering thresholds were tested, including:

- SUBS20** – Identified all substitutions that were different by a frequency of 0.20 compared to baseline (i.e., 0.10 at baseline to 0.30 at a later timepoint or not detected at baseline but detected at 0.20 or greater at later timepoints or detected at baseline at 0.20 but not detected at later timepoints)
- SUBS75** - Identified all substitutions that were different by a frequency of 0.75 compared to baseline (using the same criteria as for SUBS20)
- SUBS10+-** – Identified all substitutions that were not detected at baseline (<0.01 frequency) but detected at a frequency of 0.10 or greater at later timepoints or detected at baseline at 0.10 and were not detected at later timepoints (Figure 9).

All three filtering thresholds identified the major substitutions associated with resistance and showed good overlap; however, SUBS10+- identified substitutions that were more likely to be associated with resistance and reduced the amount of background noise (substitutions at polymorphic sites, substitutions occurring in only one subject, etc.).

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USUBJID	NGSPL	VISIT	NS3 APOS	TCOV	AAREF	AASUB	VCOV	AAFREQ	Diff	SUBS20	SUBS75	SUBS10+	VARDECT
TMC435-C205-0197	ILLUMINA GA IX	WEEK 12	167	52461	V	I	2871	0.055	0.055				JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 36	167	46812	V	I	521	0.011	0.011				JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 4	167	40439	V	I	487	0.012	0.012				JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 12	168	52112	D	E	39049	0.749	0.749	D168E	D168E	D168E	JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 12	168	27317	D	T	1358	0.012	0.012				JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 12	168	52112	D	T	1358	0.026	0.026				JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 12	168	52112	D	V	6894	0.132	0.132			D168V	JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 12	168	27317	D	V	4892	0.179	0.179			D168V	JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 12	168	27317	D	E	21849	0.8	0.8	D168E	D168E	D168E	JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 2	168	54499	D	A	13539	0.248	0.248	D168A	D168A	D168A	JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 2	168	54499	D	V	40234	0.738	0.738	D168V	D168V	D168V	JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 3	168	30625	D	V	24929	0.814	0.814	D168V	D168V	D168V	JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 3	168	30625	D	T	359	0.012	0.012				JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 3	168	30625	D	E	893	0.029	0.029				JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 3	168	30625	D	A	4262	0.139	0.139			D168A	JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 4	168	40487	D	V	36392	0.899	0.899	D168V	D168V	D168V	JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 4	168	40487	D	E	473	0.012	0.012				JAN
TMC435-C205-0197	ILLUMINA GA IX	WEEK 4	168	40487	D	A	2868	0.071	0.071				JAN

Figure 9. Identifying substitutions in the Frequency tables. For emergence analysis, amino acid substitutions were compared to the values present at baseline and the differences are shown in the Diff column. SUBS20 identified substitutions using a 0.20 threshold, SUBS75 identified substitutions using a 0.75 threshold and SUBS10+ identified substitutions using 0.10 threshold (described in the main text). VARDECT, reports the variant detection method used to identify the substitutions. This is a portion of the Frequency table generated by the sponsor for subject TMC435-C205-0197. Green, frequency that are greater than or equal to 0.10; blue, positions where coverage was below the acceptable limits for calling variants (limits included, <1000 for TCOV and <500 for VCOV).

Specific sites of interest were also plotted to determine how frequency emerged (Figure 10). It should be noted that these frequency changes do not necessarily reflect changes in the absolute levels of the specific HCV RNA expressing the indicated variant, but could change as a result of an increase or decrease in other HCV RNA variants.

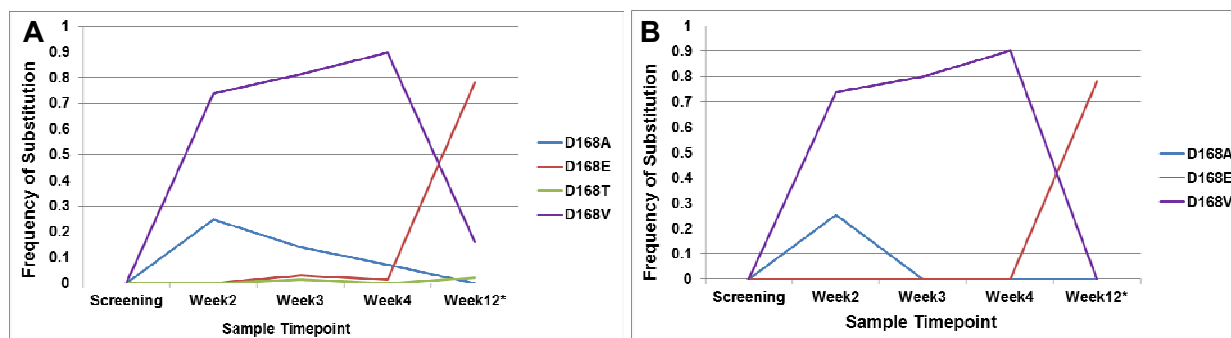


Figure 10. Comparing resistance profiles at NS3 amino acid position 168 for subject TMC435-C205-0197. There was reasonably good agreement between frequencies reported by the sponsor (panel A) and those calculated using the PDV75 variant detection method (panel B). Similar results were obtained with the QbVD method.

7. **Comparing results to those submitted by the sponsor.** The remainder of this review provides details on how the NGS data submitted by the sponsor was independently evaluated using the above described NGS analysis pipeline. In general, the NGS data analysis was performed using data generated in this pipeline and provided by the sponsor, and the results were compared as follows:
 - a. Frequency and Resistance Analysis tables were compared directly and major differences were noted
 - b. Amino acid substitutions were identified by the three algorithms (the sponsor's algorithm (Jan) and QbVD and PVD75 used by DAVP) and major differences between algorithms were reported
 - c. Novel resistance-associated amino acid substitutions reported by different NGS analysis approaches were compared and major differences were reported
 - d. NGS analysis results were compared to results obtained and reported by the sponsor using Sanger population sequencing
 - e. Novel resistance-associated substitutions identified by the independent analysis were noted and discussed with the review team for potential labeling/post-marketing actions

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CLINICAL STUDIES

The sponsor provided NGS data for two phase 2 clinical trials that were used to support the NDA for TMC435 (simeprevir). This portion of the NDA review focuses exclusively on these two trials and the NGS data associated with them. The primary review of the pivotal phase 3 trials and overall conclusions drawn from phase 2 and phase 3 resistance data can be found in the review of Clinical Virology Reviewer Damon Deming, Ph.D.

REVIEW OF TMC435-C205

Summary of TMC435-C205

TMC435-C205 was a Phase IIb, randomized, double-blind, placebo-controlled, 5-arm study designed to compare the efficacy, tolerability and safety of different regimens of TMC435 in combination with peginterferon α -2a and ribavirin (PegIFN/RBV) versus PegIFN/RBV alone in adult treatment-naïve subjects with chronic hepatitis C virus (HCV) genotype 1 infection (Figure 11).

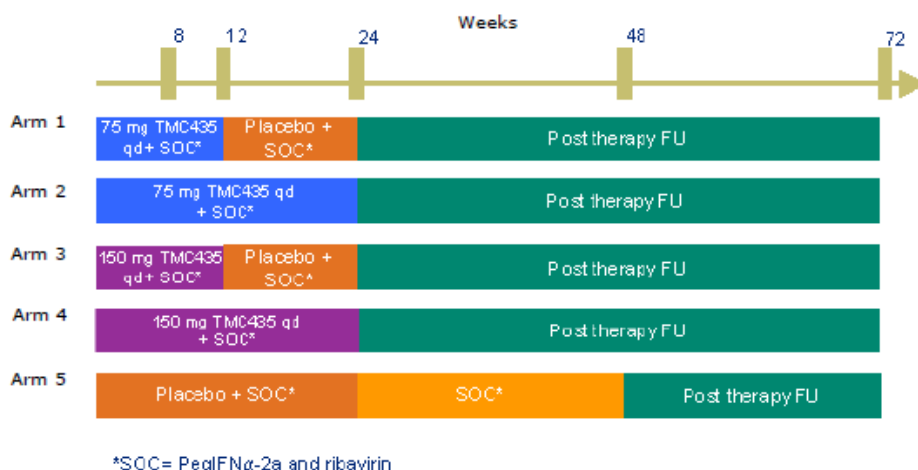


Figure 11. Schematic of TMC435-C205 study design (Figure 1, page 14, TMC435-C205 Antiviral Microbiology Report).

The sponsor reported that treatment with TMC435 in combination with PegIFN/RBV resulted in higher SVR24 rates (74.7%-86.1%) with no substantial differences between the 75 mg and 150 mg doses of TMC435 or 12 week or 24 week lengths of TMC435 therapy in the overall population compared to the placebo group (64.9%). However, the sponsor also reported that some subgroups, including subjects infected with HCV genotype 1a and subjects with Metavir Scores of F3, showed a trend for higher virologic response in the 150 mg dose compared to the 75 mg dose of TMC435. This observation was part of the justification for going with the 150 mg dose in the pivotal phase 3 clinical trials.

The virology datasets based on Sanger population sequencing submitted by the sponsor for TMC435-C205 were analyzed to determine which subjects would be appropriate for resistance analysis. Data were submitted for 386 subjects, but 7 subjects were removed due to inadequate information for assessing resistance (no baseline sequences were available; the subject was lost to follow up while suppressed, etc.). A total of 379 subjects were included in the resistance analysis, including 170 subjects (44.9%) infected with HCV GT1a and 206 subjects (54.4%) infected with HCV GT1b (Table 1, DAVP analysis). Three additional subjects were included who were infected with a non-specified HCV GT1 (n=1), GT1e (n=1) or GT6e (n=1) determined by sequence analysis (Table1).

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Table 1. TMC435-C205 censored population.

ARM	No. Subjects	GT1a	GT1b	GT1	GT1e	GT6e
PLACEBO	76	29	47	0	0	0
TMC435 150MG/12WKS	75	35	38	1	1	0
TMC435 150MG/24WKS	77	35	42	0	0	0
TMC435 75MG/12WKS	76	37	39	0	0	0
TMC435 75MG/24WKS	75	34	40	0	0	1
Totals	379	170	206	1	1	1

SVR24 rates in the censored population ranged from 74.7% to 88.3% compared to 63.1% in the placebo group (Table 2, DAVP analysis). This range was very similar to the range reported by the sponsor of 74.7%-86.1% in the treatment groups and 64.9% in the placebo group.

Table 2. SVR12 and SVR24 rates in the censored population of TMC435-C205.

ARM	SVR12	Rate	SVR24	Rate
PLACEBO	49	64.47%	48	63.16%
TMC435 150MG/12WKS	60	80.00%	60	80.00%
TMC435 150MG/24WKS	68	88.31%	68	88.31%
TMC435 75MG/12WKS	63	82.89%	62	81.58%
TMC435 75MG/24WKS	57	76.00%	56	74.67%
Totals	297	78.36%	294	77.57%

The 150 mg dose for 24 weeks provided the best treatment outcome in TMC435-C205 with an SVR24 rate of 88.3%, which was the same SVR12 rate for this treatment arm, indicating that there were no post-SVR12 relapsers (Figure 12). Of note, the 75 mg dose for 24 weeks had the worse outcome (SVR12/SVR24 rates ≤76% versus ≥80% for all other treatment arms, including 75 mg for 12 weeks) (Figure 12, Table 2).

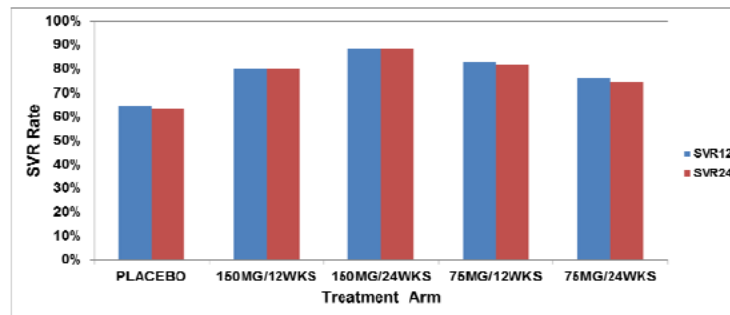


Figure 12. SVR12 and SVR24 rates in the censored TMC435-C205 population. More late relapsers were observed in the 75 mg dose groups after SVR12 was detected.

In addition to determining SVR rates for the censored population, SVR12 rates were assessed to determine if substitutions at NS3 amino acid position Q80 reduced these rates. A total of 330 subjects (87.1%) had wild type Q80 at baseline, including 131 subjects (77.1%) infected with HCV GT1a and 196 (95.2%) subjects infected with HCV GT1b. Among subjects infected with HCV GT1a, 35 (20.6%) had a Q80K substitution at baseline compared to 2 subjects (~1%) infected with HCV GT1b (Table 3).

Table 3. Frequency of substitutions at NS3 position 80 in the baseline censored population. Data not shown for subjects who were infected with non-GT1a or GT1b HCV.

Baseline Population	Q80	Q80K	Q80L	Q90L/Q	Q80R	Q80K/Q	Totals
All	330	37	8	2	1	1	379
Percent	87.07%	9.76%	2.11%	0.53%	0.26%	0.26%	
GT1a	131	35	2	1	0	1	170
Percent	77.06%	20.59%	1.18%	0.59%	0.00%	0.59%	
GT1b	196	2	6	1	1	0	206
Percent	95.15%	0.97%	2.91%	0.49%	0.49%	0.00%	

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The combined SVR12 rate in the censored population across all treatment arms for GT1a subjects with Q80 at baseline was ~77% (101/131) compared to 81.6% (160/196) for subjects infected with HCV GT1b. However, for subjects in the censored population who were infected with HCV GT1a bearing a substitution at NS3 position 80 (Table 3, 39 subjects total), the SVR12 rate dropped to ~62% (24/39). A SVR12 rate of 100% (10/10) was observed for subjects infected with HCV GT1b bearing a substitution at this position (Figure 13).

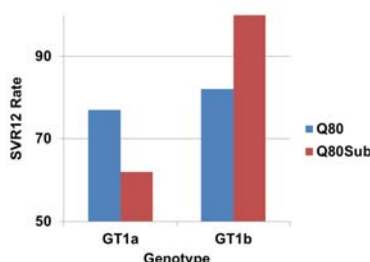


Figure 13. Comparison of SVR12 rates among subjects in the censored population infected with HCV GT1a or GT1b with or without a substitution at position 80. Q80, wildtype; Q80Sub, a substitution at position 80 (Table 3).

In addition to the impact of Q80K and other substitutions at this position at baseline on the SVR12 rate in subjects infected with HCV GT1a, among treatment failures infected with HCV GT1a, 42% had a substitution at NS3 position Q80 at baseline (13/39), compared to 0% of treatment failures infected with HCV GT1b (n=17; Figure 14). Q80K in a GT1a background appears to confer resistance to TMC435, but the number of subjects in this study was too small to draw a firm conclusion.

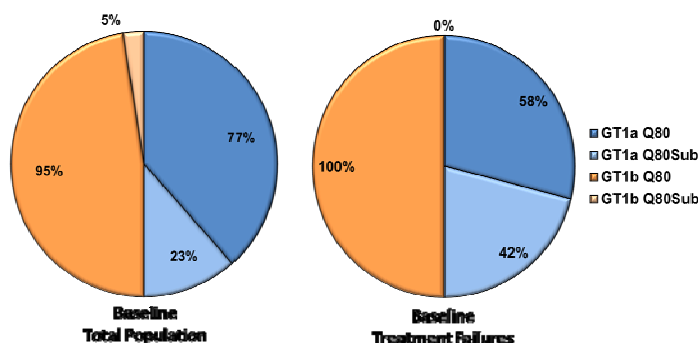


Figure 14. Comparison of frequencies of substitutions at NS3 position 80 in the censored population versus treatment failures.

In addition to any impact at baseline, substitutions at NS3 position 80 were also associated with emerging resistance with substitutions at this position increasing in isolates from 6% of virologic failures infected with HCV GT1b and 10% of virologic failures infected with HCV GT1a (Figure 15). The emergence of Q80K substitutions and impact at baseline indicates that Q80K is a TMC435 resistance substitution.

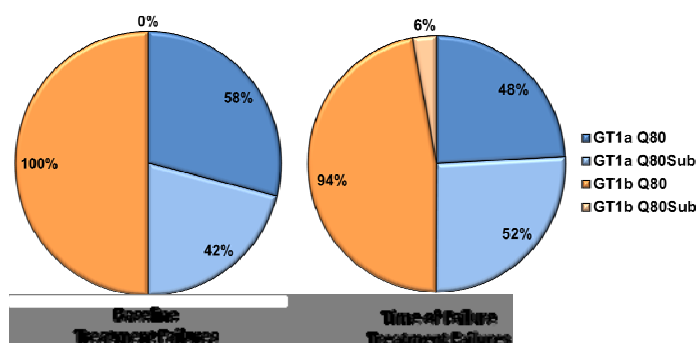


Figure 15. Comparison of frequencies of substitutions at NS3 position 80 in the censored population treatment failures at baseline versus time point closest to failure.

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A comprehensive analysis of Q80K can be found in the [Additional Studies](#) section below. In this section, (b) (4) structures are compared between HCV GT1a and GT1b to show that Q80K has a profound impact on the NS3/4a binding pocket, which likely reduces binding of TMC435. In addition, the frequency of Q80K at baseline and emerging Q80sub are analyzed across both studies.

TMC435-C205 Resistance Analysis

The sponsor provided resistance analysis datasets that were derived from both population sequencing using the Sanger sequencing method and from Next Generation Sequencing using the Illumina platform.

The sponsor compiled two lists with NS3 positions of interest (NS3 protease domain 1-181) that were defined to facilitate the analyses of baseline polymorphisms and emerging substitutions. The “short” list was comprised of six amino acid positions in NS3 that were specific to TMC435, including positions 43, 80, 122, 155, 156 and 168. Specific amino acid changes at one or more of these positions were known to confer reduced susceptibility to TMC435 in cell culture using the HCV replicon system to assess the impact of a given substitution at a specific position on the EC₅₀ value of TMC435. In addition, other substitutions were observed during cell culture selection experiments.

The “long” list was comprised of NS3 amino acid positions that have been associated with resistance to other HCV NS3/4A protease inhibitors or were considered of interest based on observations in TMC435 in cell culture or clinical studies. This list had 18 NS3 positions, including 36, 41, 43, 54, 55, 80, 107, 122, 132, 138, 155, 156, 158, 168, 169, 170, 174, and 175 (Table 4).

Table 4. Background information for NS3 Amino Acid Positions of Interest (Table 4, page 22, TMC435-C205 Antiviral Microbiology Report).

NS3 Position of Interest	Background Information
36	Described in telaprevir (TVR) and boceprevir (BOC) USPI ^{15,16} and EU SmPC ^{17,18}
41	Observed in vitro in selection experiments with TMC435 ¹³
43	Observed in vitro in selection experiments with TMC435 ¹³
54	Described in TVR and BOC USPI ^{15,16} and EU SmPC ^{17,18}
55	Described in BOC USPI ¹⁶ and EU SmPC ¹⁸
80	Observed in vitro in selection experiments with TMC435 ¹³
107	Described in BOC USPI ¹⁶
122	Effect of polymorphisms at position 122 on HCV NS3/4A protease inhibitor activity has been described. ¹⁹ Selected amino acid changes at position 122 reduce susceptibility to TMC435. ¹²
132	Described in TVR USPI ¹⁵
138	Described for HCV NS3/4A protease inhibitor ITMN-191 ²⁰
155	Described in TVR and BOC USPI ^{15,16} and EU SmPC ^{17,18} and observed in vitro in selection experiments with TMC435 ¹³
156	Described in TVR and BOC USPI ^{15,16} and EU SmPC ^{17,18} and observed in vitro in selection experiments with TMC435 ¹³
158	Described in BOC USPI ¹⁶
168	Described in TVR and BOC USPI ^{15,16} and observed in vitro in selection experiments with TMC435 ¹³
169	Observed in clinical studies TMC435-C201 ²¹ (Cohort 5) and TMC435-C202 ²²
170	Described in BOC USPI ¹⁶ and EU SmPC ¹⁸
174	Observed in vitro in selection experiments with TMC435 ¹³
175	Described in BOC USPI ¹⁶

USPI: United States Package Insert; SmPC: Summary of Product Characteristics

Note: Most of these amino acid positions have been described in association with other HCV NS3/4A protease inhibitors as well. Not all references to other HCV NS3/4A protease inhibitors are listed.

TMC435-C205 Sanger Population Sequencing analysis

The sponsor focused on the sites listed in Table 4 for conducting resistance analysis, first assessing the presence of substitutions at these sites at baseline and then to see if emerging substitutions appeared at these sites at or near the time of breakthrough or relapse. In addition, the sponsor focused on the first 180 amino acids of NS3/4A. The independent analysis performed by DAVP did not limit the analyses to the specific positions identified by the sponsor, but instead analyzed all substitutions that were observed in the entire

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NS3/4A sequence. DAVP does not use preselected lists to identify resistance-associated substitutions but considers all amino acid positions. The sponsor's results for baseline resistance analysis are shown in Table 5. There was good agreement between the results reported by the sponsor and the independent assessment of baseline substitutions performed by DAVP (Figure 16).

Table 5. Number of subjects with baseline substitutions at 18 NS3 positions of interest (Table 8, page 28, TMC435-C205 Antiviral Microbiology Report).

	TMC435 75mg 12 Wks	TMC435 75mg 24 Wks	TMC435 150mg 12 Wks	TMC435 150mg 24 Wks	Placebo	Total
Analysis set: ITT	78	75	77	79	77	386
All HCV geno/subtypes	78	75	77	79	77	386
Subjects with sequencing data	78	75	77	79	76	385
No polymorphism at positions of interest	21 (26.9%)	19 (25.3%)	21 (27.3%)	27 (34.2%)	26 (34.2%)	114 (29.6%)
Polymorphism at positions of interest ^a	57 (73.1%)	56 (74.7%)	56 (72.7%)	52 (65.8%)	50 (65.8%)	271 (70.4%)
036	2 (2.6%)	1 (1.3%)	1 (1.3%)	1 (1.3%)	6 (7.9%)	11 (2.9%)
041	0	1 (1.3%)	0	2 (2.5%)	2 (2.6%)	5 (1.3%)
043	0	0	0	0	0	0
054	1 (1.3%)	1 (1.3%)	5 (6.5%)	2 (2.5%)	3 (3.9%)	12 (3.1%)
055	2 (2.6%)	3 (4.0%)	4 (5.2%)	1 (1.3%)	2 (2.6%)	12 (3.1%)
080	16 (20.5%)	10 (13.3%)	7 (9.1%)	9 (11.4%)	9 (11.8%)	51 (13.2%)
107	0	1 (1.3%)	1 (1.3%)	0	0	2 (0.5%)
122	8 (10.3%)	14 (18.7%)	7 (9.1%)	8 (10.1%)	12 (15.8%)	49 (12.7%)
132	16 (20.5%)	14 (18.7%)	11 (14.3%)	10 (12.7%)	10 (13.2%)	61 (15.8%)
138	0	0	0	0	0	0
155	0	0	1 (1.3%)	2 (2.5%)	1 (1.3%)	4 (1.0%)
156	0	0	0	0	0	0
158	0	0	0	0	0	0
168	0	0	1 (1.3%)	0	0	1 (0.3%)
169	0	0	0	0	0	0
170	14 (17.9%)	17 (22.7%)	16 (20.8%)	12 (15.2%)	12 (15.8%)	71 (18.4%)
174	21 (26.9%)	17 (22.7%)	27 (35.1%)	24 (30.4%)	16 (21.1%)	105 (27.3%)
175	2 (2.6%)	2 (2.7%)	1 (1.3%)	2 (2.5%)	0	7 (1.8%)
HCV geno/subtype 1a/other	37	35	39	37	30	178
Subjects with sequencing data	37	35	39	37	29	177
Polymorphism at positions of interest ^a	31 (83.8%)	28 (80.0%)	32 (82.1%)	30 (81.1%)	25 (86.2%)	146 (82.5%)
080	13 (35.1%)	9 (25.7%)	6 (15.4%)	7 (18.9%)	6 (20.7%)	41 (23.2%)
132	2 (5.4%)	1 (2.9%)	2 (5.1%)	0	0	5 (2.8%)
170	5 (13.5%)	1 (2.9%)	5 (12.8%)	2 (5.4%)	1 (3.4%)	14 (7.9%)
174	20 (54.1%)	15 (42.9%)	26 (66.7%)	23 (62.2%)	15 (51.7%)	99 (55.9%)
HCV geno/subtype 1b	41	40	38	42	47	208
Subjects with sequencing data	41	40	38	42	47	208
Polymorphism at positions of interest ^a	26 (63.4%)	28 (70.0%)	24 (63.2%)	22 (52.4%)	25 (53.2%)	125 (60.1%)
080	3 (7.3%)	1 (2.5%)	1 (2.6%)	2 (4.8%)	3 (6.4%)	10 (4.8%)
132	14 (34.1%)	13 (32.5%)	9 (23.7%)	10 (23.8%)	10 (21.3%)	56 (26.9%)
170	9 (22.0%)	16 (40.0%)	11 (28.9%)	10 (23.8%)	11 (23.4%)	57 (27.4%)
174	1 (2.4%)	2 (5.0%)	1 (2.6%)	1 (2.4%)	1 (2.1%)	6 (2.9%)

^a Any baseline polymorphism at NS3 positions 36, 41, 43, 54, 55, 80, 107, 122, 132, 138, 155, 156, 158, 168, 169, 170, 174 and/or 175 are considered. Polymorphisms were defined as changes from Con1 (AJ238799) and H77 (AF009606) for geno/subtype 1b and 1a/other, respectively.

^b Positions with a major difference (> 15%) in overall prevalence between genotype 1a and genotype 1b are shown.

Baseline substitutions were predominantly observed by the sponsor at NS3 amino acid positions 80, 122, 132, 170 and 174, and the substitutions that occurred at positions 132, 170, 174 and 175 were deemed to be polymorphic sites that substitutions detected at similar frequencies in baseline and later timepoint sequences (Table 5).

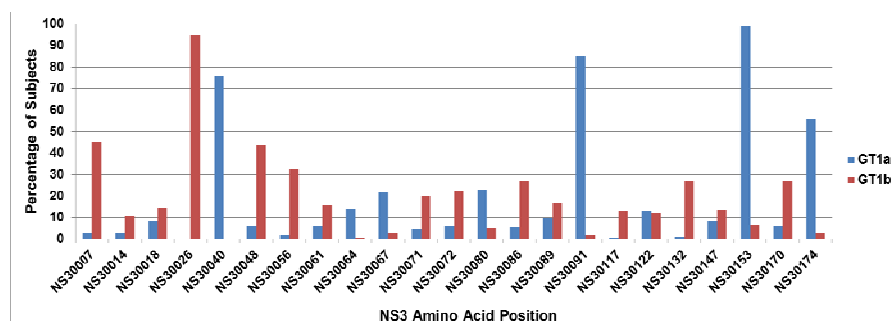


Figure 16. Baseline substitutions in the C205 censored population that differed from GT1a and GT1b reference sequences.

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DAVP looked at all sites and identified additional baseline substitutions (Figure 16), but these all occurred at positions identified by the sponsor to be polymorphic sites:

1. GT1a: 40, 64, 67, 80, 89, 91, 122, 153, 174
2. GT1b: 7, 14, 18, 26, 48, 56, 61, 71, 72, 86, 89, 117, 122, 132, 147, 170

Substitutions emerging from baseline to time of failure were also analyzed by the sponsor and DAVP. The sponsor came to this conclusion: *The presence of a Q80K polymorphism at baseline was associated with lower virologic response rates, mainly in the 75 mg TMC435 group. Most TMC435-treated subjects with viral breakthrough and viral relapse had emerging mutations at NS3 positions 80, 155 and/or 168, which generally conferred high-level resistance to TMC435 in vitro. In a substantial proportion of subjects with emerging mutations at time of failure, these were no longer detected at EOS (with a higher proportion in genotype 1b compared to genotype 1a infected subjects)* - Page 28, TMC435-C205 Antiviral Microbiology Report.

DAVP compared the amino acids detected at baseline to those detected at the timepoint closest to failure for each subject in the TMC435-C205 censored population to identify positions that were potentially associated with resistance. Given that the pivotal Phase 3 trials were conducted with a dose of 150 mg, the analysis was limited to subjects who received this dose. In subjects infected with HCV-GT1a, substitutions R155K (n=7), Q80K (n=4), and D168E/A (n=2) were most common (Table 6, Figure 17). For those infected with GT1b, resistance-associated substitutions occurred most frequently at D168V/E/A (n=6) (Table 6, Figure 18).

Table 6. Resistance-associated substitutions in TMC435-C205 among subjects who failed the 150 mg dose.

Major substitutions	NS3 Q80		NS3 R155	NS3 D168				NS3 A170			NS3 R155+NS3 D168
	K	R	K	E	V	A	A/V	I	V	T	K+E
All 150 mg ARMs	4	1	7	2	3	2	1	2	1	1	1
GT1a	4	0	7	1	0	1	0	0	0	0	1
GT1b	0	1	0	1	3	1	1	2	0	0	0
150 mg/12 Weeks	2	1	6	1	2	1	1	1	0	0	1
GT1a	2	0	6	1	0	1	1	0	0	0	1
GT1b	0	1	0	0	2	0	0	1	0	0	0
150 mg/24 Weeks	2	0	1	1	1	1	0	1	1	1	0
GT1a	2	0	1	1	0	0	0	0	0	1	0
GT1b	0	0	0	0	1	1	0	1	1	0	0

Comparison of failures who received different dose durations (12 weeks versus 24 weeks) of 150 mg of TMC435 revealed that each cohort had a different resistance profile (Figures 17 and 18). For GT1a, the different dose durations yielded these emergent substitutions:

1. 12 weeks: 36, 58, 80, 122, 155, 168
2. 24 weeks: 132, 155, 168, 170

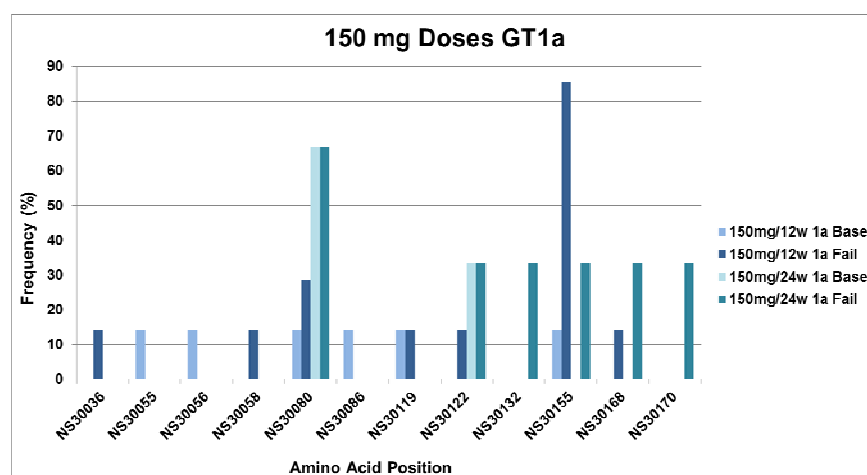


Figure 17. Resistance-associated emergent substitution positions for subjects infected with HCV GT1a who received 150 mg of TMC435 at durations of 12 vs. 24 weeks. Base, frequency of substitution at baseline in all failures in this arm; Fail, frequency of substitutions at timepoint closest to failure in all subjects in this arm.

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For GT1b, the different dose durations yielded these emergent substitutions:

1. 12 weeks: 39, 80, 168
2. 24 weeks: 168

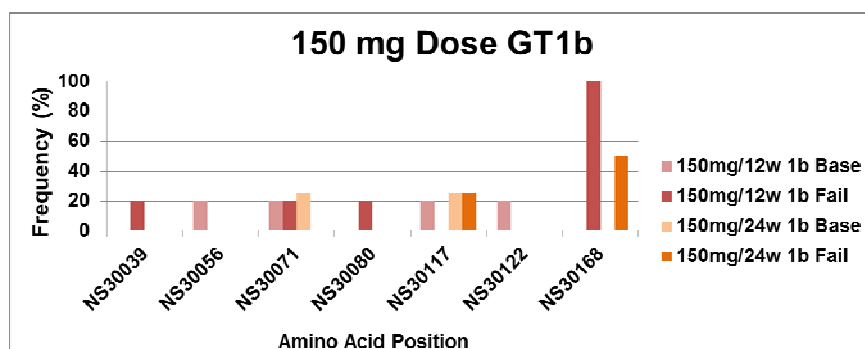


Figure 18. Resistance-associated emergent substitution positions for subjects infected with HCV GT1b who received 150 mg of TMC435 at durations of 12 vs. 24 weeks. Base, frequency of substitution at baseline in all failures in this arm; Fail, frequency of substitutions at timepoint closest to failure in all subjects in this arm.

Summary of Resistance Analysis based on Sanger Population Sequencing

The sponsor reported differences in emerging substitutions observed between GT1a (mainly R155K alone or in combination with other substitutions at positions 80 and/or 168) and GT1b (mainly D168V) infected subjects. The sponsor stated that most (83.3% [10/12]) subjects with baseline Q80K who experienced viral breakthrough or relapse also had additional emerging substitutions (mainly R155K) at the time of failure. The sponsor also stated that a substantial proportion of subjects with emerging substitutions at the time of failure had lost these resistance-associated substitutions at end of study (EOS). According to the sponsor, fewer subjects with GT1a/other (34.5%) compared to GT1b (80.0%) had wild type NS3 sequences at EOS. Furthermore, the sponsor concluded that D168V (mainly observed as emerging in GT1b) appeared to be less fit than R155K (mainly observed as emerging in GT1a). In 10 of 12 subjects with emerging D168V, a wild type D168 was observed at EOS while one subject had Q80R + D168E detected. By contrast, 9 of 13 subjects (5/7 without and 4/6 with baseline Q80K) with emerging R155K at the time of failure had R155K detected at EOS. Substitutions most commonly associated with resistance for TMC435-C205 were:

1. GT1a: 80, 155, and 168
2. GT1b: 168

Next Generation Sequence Analysis for TMC435-C205

In addition to the Sanger sequencing data presented for study TMC435-C205, the sponsor also conducted next generation sequencing (AKA deep sequencing) of the samples associated with failure. A total of 34 virological failures (Table 7) had samples that were sequenced using the Illumina platform, 23 of these subjects relapsed after the PR+DAA period, 10 subjects experienced virologic breakthrough during the PR+DAA and one subject was considered to be an unclassified failure. Failures were sequenced from each study arm (Table 7).

Table 7. Break down of samples that were subjected to deep sequence analysis by TMC435-C205 study arm.

Study	ARM	No. Failures (NGS)
C205	TMC435 150MG/ 12WKS	9
	TMC435 150MG/ 24WKS	6
	TMC435 75MG/ 12WKS	11
	TMC435 75MG/ 24WKS	8
Total		34

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The fastq sequences submitted by the sponsor were analyzed using the NGS data analysis pipeline described above ([NGS Data Analysis Pipeline](#)). Briefly, the reads for each sample were aligned to the HCV GT1a (H77) or HCV GT1b (Con1) references sequence for the NS3/4A gene. Variants were called using two algorithms, PVD75 and QbVD, and the variant tables were combined to generate Frequency tables showing all amino acid substitutions that occurred above a frequency $\geq 1\%$ for a given position within a given sample for each subject. Resistance Analysis tables were generated for each Frequency table for each variant detection algorithm using a threshold of 0.05, and the three resistance analysis tables were compared directly to determine how closely the three algorithms agreed with one another.

TMC435-C205 Variant Detection Algorithm Comparison

The Resistance Analysis tables were used to determine the frequencies of substitutions at each NS3/4A amino acid position based on the entire NGS population for all three algorithms used to identify variants (PVD75, QbVD, and the variant calls provided by the sponsor). Changes from the reference sequence were identified in the baseline samples and changes from baseline to later timepoints were assessed to determine the emergence of substitutions at each NS3/4A amino acid position among all of the treatment failures that were subjected to deep sequencing. This analysis provided an overview of the changes occurring in the population and showed that, in general, there was good agreement between the three variant detection methods (Figures 19 and 20).

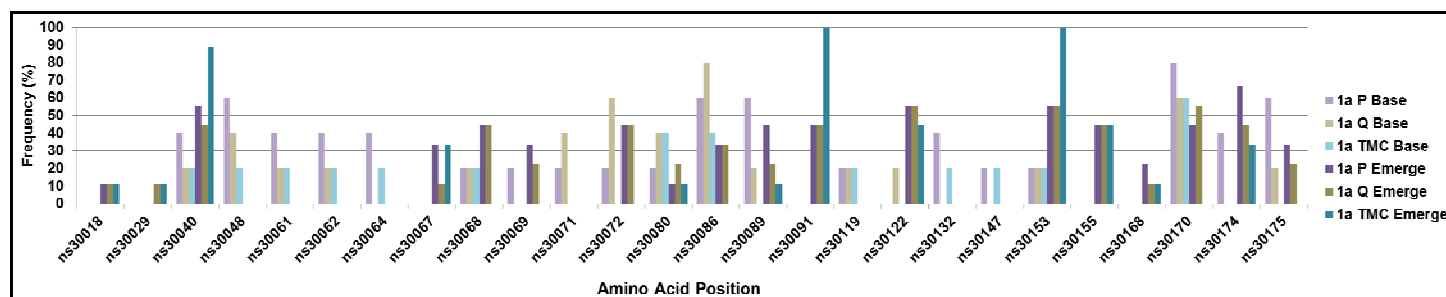


Figure 19. Comparison of variant detection algorithms in identifying the most frequent GT1a NS3 amino acid substitutions. Base, baseline differences from the reference sequence; Emerge, differences from baseline over time; P, PVD75; Q, QbVD; TMC, variant calls and frequencies reported by the sponsor.

Several emerging resistance-associated positions (positions showing an increase or decrease from baseline by at least two variant detection methods) were identified by NGS for GT1a in TMC435-C205, including substitutions at positions 18, 29, 67, 91, 155, 168, and 174 that were not detected at baseline but were detected at a frequency of ≥ 0.10 . For GT1b, using the same criteria, NS3 positions 80 and 170 were considered to be potentially resistance-associated positions that had emerging substitutions at or near the time of failure (Figure 20). However, additional positions were observed where a substitution was detected at baseline that was not detected at later timepoints (Figures 19 and 20).

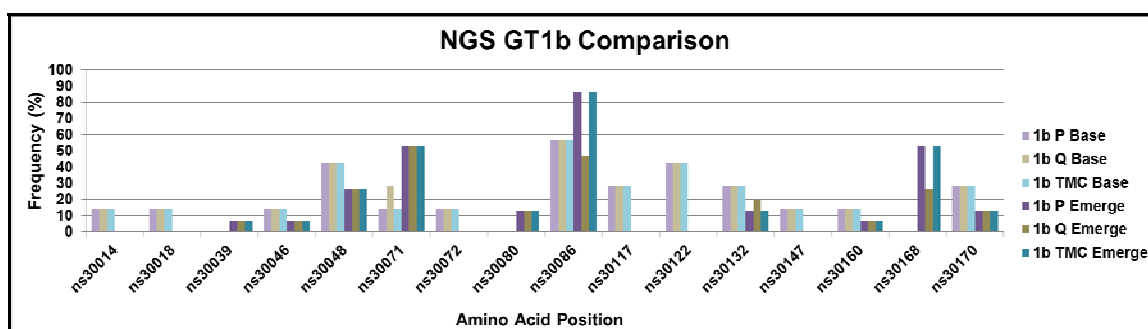


Figure 20. Comparison of variant detection algorithms in identifying the most frequent GT1b NS3 amino acid substitutions. Base, baseline differences from the reference sequence; Emerge, differences from baseline over time; P, PVD75; Q, QbVD; TMC, variant calls and frequencies reported by the sponsor.

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NGS Resistance Analysis for TMC435-C205

To further assess the population-based results generated using the Resistance Analysis tables, DAVP used the SUBS10+- filtering threshold to look at individual changes occurring at each position in the Frequency tables. SUBS10+- used the following criteria to identify substitutions of interest:

1. Identify all substitutions that were detected at a frequency of 0.10 or greater at a later timepoint but were not detected at baseline (<1% at baseline)
2. Ignore all substitutions that were detected with a total coverage of less than 1000

Using this approach, the Resistance Analysis tables were used to compare algorithms and identify population based positions of interest and the SUBS10+- threshold for analyzing the Frequency tables provided the exact substitutions of each individual subject that met this threshold. This provided a more robust prediction of substitutions that were likely to be associated with resistance.

The sponsor focused on the “long” and “short” lists of substitutions for determining sites of interest for resistance analysis by deep sequencing using the Illumina platform. DAVP used an unbiased approach, looking for resistance association for all observed substitutions. In general, the results obtained by NGS were similar to those obtained by Sanger population sequencing. However, the sponsor performed its NGS analysis by combining data for studies TMC435-C205 and -C206. The two phase 2 studies were first analyzed separately by DAVP, and then a meta-analysis was performed including data from both studies (see the meta-analysis data below).

For TMC435-C205, DAVP considered all substitutions to be potential emerging resistance-associated substitutions if they met these criteria: 1) met the SUBS10+- threshold, 2) were detected by 2-of-3 variant detection methods, and 3) occurred in 2 or more subjects. Potentially resistance-associated substitutions that arose in TMC435-C205 are shown in Figure 21. Sites of interest included:

1. GT1a: 80, 122, and 155
2. GT1b: 71, 122, 150, 168 and 170

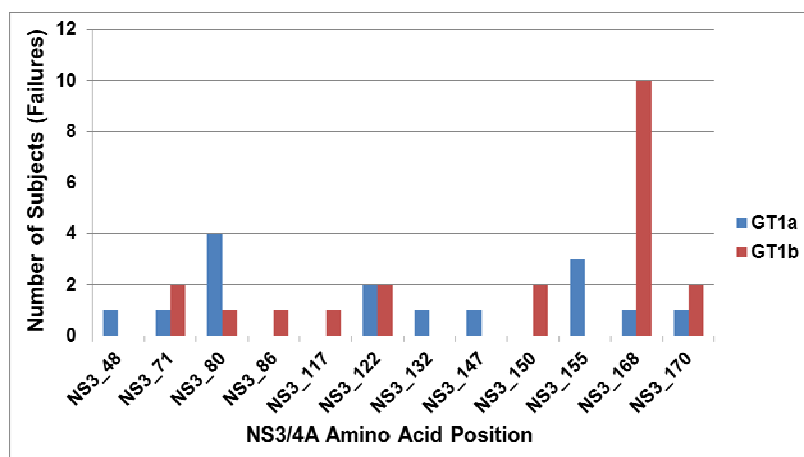


Figure 21. Emergent resistance-associated substitutions detected by SUBS10+- in TMC435-C205.

Some of the sites identified in the NGS analysis as emerging, were reported to be polymorphic by the sponsor. However, polymorphic sites have been known to confer resistance (Q80K is a known GT1a polymorphism), so all of these sites were considered to be potentially associated with resistance. This resulted in identifying roughly the same sites as were identified by population sequencing (Table 8). All substitutions identified by SUBS10+- are shown in the [appendix](#).

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Table 8. Resistance-associated substitutions that emerged by at least 10% frequency from baseline in study TMC435-C205 in at least two subjects.

SUBTYPE	NS3AA POS	SUBS10+-	Number	Ratio
1a	80	K80Q/Q80K/Q80R	4	2-1-1
	155	R155K	3	
	122	S122R/G122S	2	1-1
1b	168	D168V/H/A/E	10	5-3-1-1
	71	V71I	2	
	122	T122S	2	
	150	A150V/V150A	2	1-1
	170	I170V/V170I	2	1-1

SUB10+- -- substitutions that emerged or disappeared compared to baseline at a frequency $\geq 10\%$ (see text for complete details)

Ratio -- represents the number of each substitution listed in order

Of note, three subjects from TMC435-C205 were deemed to have no emerging substitutions at failure by population sequencing but were found to have emerging substitutions by NGS, although generally at low frequency.

Analysis of failures without emerging resistance by Sanger population sequencing

Three subjects in TMC435-C205 and three subjects in TMC435-C206 were treatment failures who had no emerging substitutions at sites on the sponsor's "long" list as detected by Sanger population sequencing but did have substitutions that emerged at sites on the "long" list as detected by NGS (Table 9). However, most of the emerging substitutions were detected at low frequency, and all three from the TMC435-C205 group were relapsers whose last timepoints may have been taken weeks after the resistant HCV population in the subject reverted to wild type.

Table 9. NGS results for subjects without emerging substitutions ("long list") at time of failure as detected by population sequencing (Table 12, page 27, TMC435-C205-C206-DS Antiviral Microbiology Report).

CRF ID, TMC435 Treatment	HCV Subtype, Response	Visit	HCV RNA (Log ₁₀ IU/mL)	PS Result	Frequency (%) of Substitutions at “Long List” NS3 Positions of Interest													
					Q80K	Q80L	S122G	S122N	S122T	R155K	D168E	D168H	V55I	V132I	I132L	V170I	V170T	N174S
205-0008 75mg/12Wks	1b Relapse	SCR WK48	6.2 5.7	V170I V170I												97.5 98.4		
205-0283 150mg/12Wks	1a Relapse	SCR WK36	6.5 6.0	V55I/V, R155K/R R155K						76.0 98.9			22.1				8.7	
205-0384 75mg/24Wks	1a Relapse	SCR WK60	6.6 6.0	Q80K Q80K	99.6 99.6			1.4	5.7									5.5
206-0456 150mg/12Wks	1b Relapse	SCR WK60 ^a	6.6 6.1	none none														
206-0009 100mg/24Wks	1a VBT	BL WK1	6.6 4.8	Q80K Q80K	98.7 99.7													
206-0569 150mg/12Wks	1b STOP	SCR WK24	7.5 6.9	V132I V132I		1.1		4.2	1.6					99.0 99.1				

^a For CRF ID 206-0456 no DS result was available at time of failure (Week 52). Therefore, the DS result obtained at Week 60 was analyzed instead.

Baseline polymorphisms at "short list" NS3 positions of interest are indicated with grey shading.

Frequencies of amino acid substitutions detected by both DS and PS are indicated in bold.

DS: deep sequencing; PS: population sequencing; Wks: weeks; BL: baseline; SCR: screening; VBT: viral breakthrough; STOP WK25: subject reached a virologic stopping rule; empty cell: frequency <1.0%

Interestingly, subject TMC435-C205-008 was infected with HCV GT1b and received a 75 mg dose of TMC435 for 12 weeks and achieved SVR12 (at Week 24, Follow-up Week 12) prior to relapse sometime before Week 36 or Follow-up Week 24 (this subject did not achieve SVR24). The sample that was subjected to Sanger Population Sequencing and NGS was taken at Week 48, a minimum of 12 weeks after relapse had occurred and when the viral titer had reached 5.6×10^5 HCV RNA copies/mL. Sanger population sequencing of the Week 48 sample did not detect any emerging substitutions; however, NGS sequencing identified known resistance-associated substitution D168H and this was detected by all three variant detection methods (Table 10).

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Table 10. Comparison of Sanger population sequencing versus NGS results for subject TMC435-C205-0008.

USUBJID	VISIT	SEQPL	NS3_026	NS3_071	NS3_089	NS3_147	NS3_167	NS3_168	NS3_170
TMC435-C205-0008	BASELINE	POPULATION	K	I/V	P/S	L/S	M/V		I
TMC435-C205-0008	WEEK 48	POPULATION	K		S				I
TMC435-C205-0008	BASELINE	NGS-QbVD	K	V	S				I
TMC435-C205-0008	WEEK 48	NGS-QbVD	K		S			H	I
TMC435-C205-0008	BASELINE	NGS-PVD75	K	V	S				I
TMC435-C205-0008	WEEK 48	NGS-PVD75	K		S			H	I
TMC435-C205-0008	BASELINE	NGS-JAN	K	V	S				I
TMC435-C205-0008	WEEK 48	NGS-JAN	K		S			H	I
Conservation at position			R(3.1/K)	I(84.7/V)	P(85.3/S)	S(88.7/L)	V(99.5)	D(99.5)	V(69.6/I)

SEQPL – Sequencing platform

Population – Sanger population sequencing

Conservation – reported by the sponsor, this box shows the amino acid present in the reference sequence followed by the percent conservation, followed by common substitutions observed

Importantly, the substitution identified by NGS sequencing at position D168 was detected at a frequency of ~30% (~30% of the more than 50,000 reads sequenced by NGS contained H, while the remaining ~70% contained D), indicating that Sanger sequencing was unable to detect this important substitution at this frequency level (Table 10). In addition, Sanger population sequencing detected mixed populations at positions S147 (L/S) and V167 (M/V) that were not detected by NGS. Of note, position S147 was reported to be 88.7% conserved with its most common substitution being an ‘S’, whereas V167 was reported to be 99.5% conserved by the sponsor. This result is surprising given that Sanger sequencing is much less sensitive and there is little evidence that there was a mixed viral population at a level that could be detected by Sanger sequencing (Table 11).

Table 11. Frequency tables comparing the different variant calls for TMC435-C205-008.

USUBJID	NGSPL	VISIT	NS3_AAPOS	TCOV	AAREF	AASUB	VCOV	AAREQ	Diff	SUBS29	VARDECT
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	4	43731	T	S	635	0.015			JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	13	47453	L	P	513	0.011			JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	26	53104	R	K	52717	0.993			JAN
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	26	70664	R	K	70382	0.996	0.996	R26K	JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	42	54259	S	F	618	0.011			JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	71	48536	I	V	29464	0.607		V71I	JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	72	48963	T	I	1439	0.029			JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	87	44921	A	V	668	0.015			JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	89	44621	P	S	33514	0.751			JAN
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	89	59799	P	S	58716	0.982	0.982	P89S	JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	95	43927	T	A	1085	0.025			JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	147	36483	S	L	444	0.012			JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	159	36695	C	Y	405	0.011			JAN
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	168	57474	D	H	17074	0.297	0.297	D168H	JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	170	41243	V	I	40222	0.975			JAN
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	170	59780	V	I	58821	0.984	0.984	V170I	JAN
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	4	11887	T	S	352	0.030			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	13	26424	L	P	409	0.016			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	26	45620	R	K	45432	0.996			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	26	64411	R	K	64380	1.000	1.000	R26K	QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	42	51194	S	F	571	0.011			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	71	44779	I	V	26193	0.585		V71I	QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	72	47051	T	I	1345	0.029			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	87	42806	A	V	631	0.015			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	89	39109	P	S	28980	0.741			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	89	53852	P	S	53490	0.993	0.993	P89S	QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	95	41510	T	A	977	0.024			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	104	29416	L	I	331	0.011			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	147	35602	S	L	407	0.011			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	159	33082	C	Y	381	0.012			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	168	54430	D	H	14676	0.270	0.270	D168H	QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	170	34266	V	I	33758	0.985			QbVD
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	170	53832	V	I	53620	0.996	0.996	V170I	QbVD
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	26	46038	R	K	45821	0.995			PVD75
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	26	64983	R	K	64930	0.999	0.999	R26K	PVD75
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	71	47475	I	V	28198	0.594		V71I	PVD75
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	89	41359	P	S	30591	0.740			PVD75
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	89	55734	P	S	55257	0.991	0.991	P89S	PVD75
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	168	55136	D	H	14904	0.270	0.270	D168H	PVD75
TMC435-C205-0008	ILLUMINA GAIIX	SCREENING	170	34993	V	I	34445	0.984			PVD75
TMC435-C205-0008	ILLUMINA GAIIX	WEEK 48	170	54346	V	I	54052	0.995	0.995	V170I	PVD75

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The next generation sequencing data for TMC435-C205-008 indicates that R26K, P89S, and V170I were polymorphisms that were detected at baseline and these substitutions remained present at roughly the same frequency at Week 48 (Table 11). Substitution V71I was not detected at baseline, but was detected at ~60% at Week 48, implying that this substitution could be linked to D168H, which was also not detected at baseline but emerged by Week 48. The presence of V71I at a greater than 2-fold higher frequency than D168H at Week 48 indicates that if these two substitutions were linked on the same genome, then reversion at site 168 from H to D may have been ongoing. Hypothetically, I71+H168 is less fit than I71+D168.

Resistance Analysis Conclusions for TMC435-C205

The sponsor reported that there was little evidence to support any additional resistance-associated substitutions than those that are known to confer resistance against TMC435. The NS3/4A amino acid positions known to be associated with resistance are:

1. GT1a: 80 and 155
2. GT1b: 168
3. Both: 122

All of these sites were identified by the sponsor and confirmed by FDA analysis as positions that gave rise to resistance-associated substitutions in study TMC435-C205. In addition, lower frequency sites of interest could potentially be associated with resistance, and these sites were reported by the sponsor and confirmed by DAVP. Using an emergence frequency threshold of 0.10 (SUBS10+-), DAVP identified the following sites in two or more subjects (bold represents additional sites), indicating a potential association to resistance:

1. GT1a: 80, 122, and 155
2. GT1b: **71**, 122, **150**, 168 and **170**

Of note, positions 71 (84.7% conservation), 150 (90.5% conservation) and 170 (69.6% conservation) were determined to be polymorphic sites by the sponsor.

Conclusion: The resistance-associated substitutions identified in TMC435-C205 analysis are:

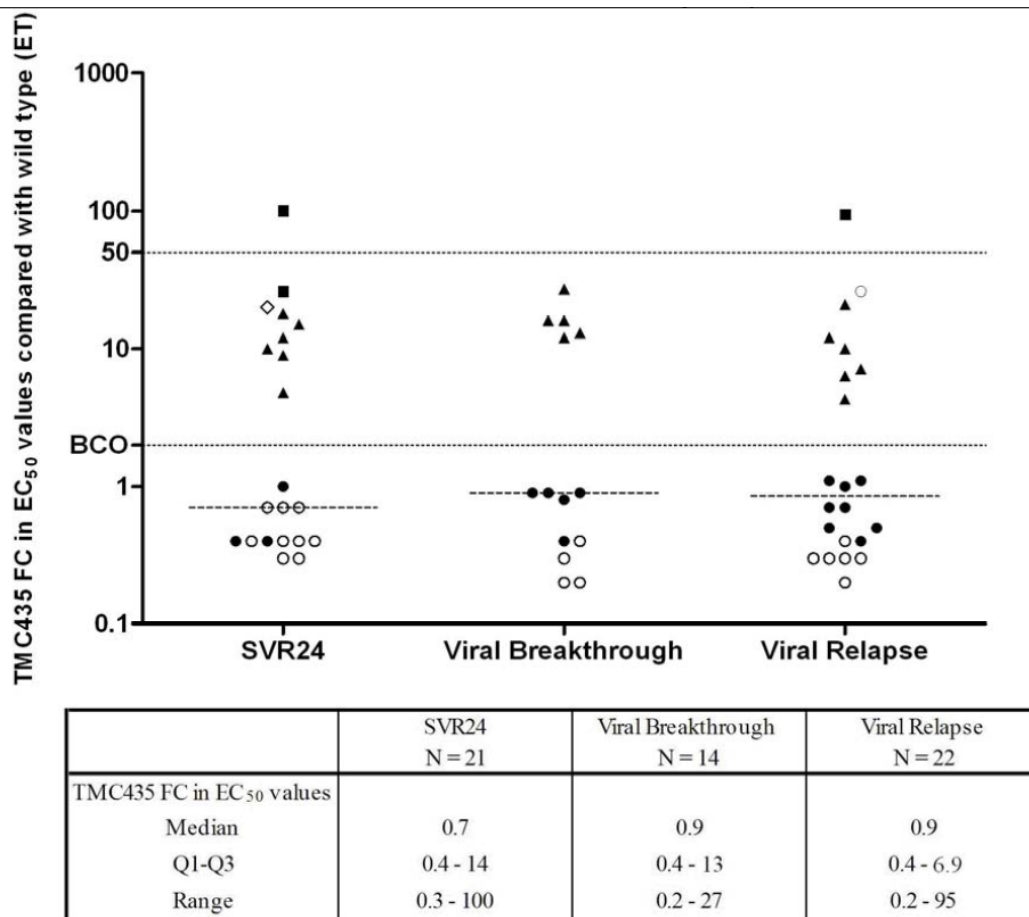
1. GT1a: 80, 122, and 155
2. GT1b: 122 and 168

Phenotypic Analysis Results for TMC435-C205

The sponsor performed phenotypic analysis using site-directed mutagenesis or chimeric replicons to test resistance-associated substitutions that arose during treatment with TMC435 in clinical trial C205. NS3 isolates were obtained at baseline for a subset of subjects who achieved SVR and at baseline, time of failure, and at the end of study for subjects with viral breakthrough or viral relapse. NS3 protease domains derived from these isolates were cloned into the GT1b replicon backbone and TMC435 activity against the chimeric replicon was assessed in a transient replicon assay and compared to the activity against the wild type replicon. Comparable median (Q1-Q3) TMC435 FC values were observed for baseline isolates from TMC435-treated subjects who experienced viral breakthrough (0.9 [0.4-13]), viral relapse (0.9 [0.4-6.9]), or achieved SVR24 (0.7 [0.4-14]) (Figure 22).

Given that DAVP uses phenotypic data to confirm an association, not to exclude one, these analyses were insufficient for determining resistance association and did not test individual substitutions that may be resistance-associated, including substitutions at positions 71, 150, and 170.

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FC: fold change in EC₅₀ values compared with wild type replicon (ET). BCO: biological cut-off. Dotted line: TMC435 FC of 2.0 (BCO) and 50 (to differentiate between low and high level resistance to TMC435²⁴). Dashed line: median TMC435 FC for each group. Filled labels: genotype 1a. Empty labels: genotype 1b. Squares: R155K polymorphisms. Diamond: Q80L in isolate and D168E in plasmid. Triangles: Q80K polymorphisms. Dotted lines: median FCs. Circles: No polymorphisms at positions 43, 80, 122, 155, 156, or 168 or polymorphisms Q80L, S122G, S122N, or S122T which do not reduce TMC435 activity as SDM (FC≤2.0).

Figure 22: Anti-HCV activity of TMC435 assessed in a transient replicon assay with chimeric GT1b replicons carrying NS3 protease domain sequences derived from clinical isolates obtained at baseline of TMC435-treated subjects by treatment outcome (Figure 5, page 53, TMC435-C205 Antiviral Microbiology Report).

REVIEW OF TMC435-C206

Summary of TMC435-C206

TMC435-C206 was a Phase IIb, randomized, 7-arm, double-blind, placebo-controlled study to compare the efficacy, tolerability, and safety of different regimens of TMC435 in combination with peginterferon α -2a and ribavirin (PegIFN/RBV) versus PegIFN/RBV alone in adult subjects with genotype 1 HCV infection who had failed at least one prior course of PegIFN/RBV therapy (Figure 23). The sponsor reported that in the overall population a larger proportion of subjects with SVR24 was observed across all TMC435 treatment groups (range 60.6% to 80.0%) compared to the placebo group (22.7%). Comparable SVR rates were observed between the different TMC435 doses (100 and 150 mg q.d.) and different TMC435 duration groups (i.e., 12, 24 or 48 weeks). A trend for higher SVR24 was observed in the prior null and partial responder population treated with TMC435 150 mg compared to 100 mg, while SVR24 rates were similar between the two TMC435 dose

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groups in prior relapsers. Moreover, a trend for higher SVR24 rates was noted with the 150 mg TMC435 dose group compared to the 100 mg dose group in most subgroups, including subjects with Metavir score F3-F4 and F4 and those infected with HCV GT1a.

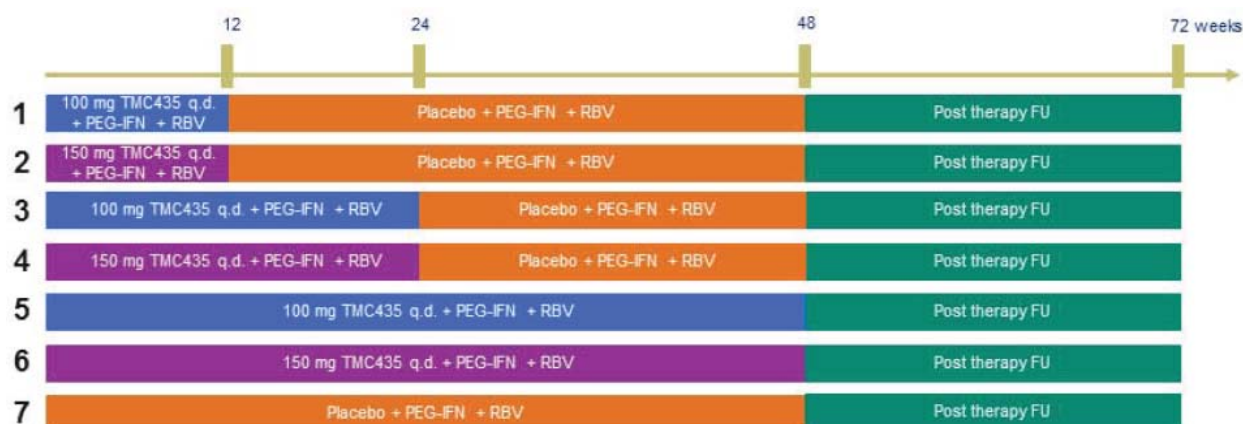


Figure 23. Schematic of TMC435-C206 (Figure 1, page 18, TMC435-C206 Antiviral Microbiology Report).

The virology datasets submitted by the sponsor for TMC435-C206 were analyzed to determine which subjects would be appropriate for resistance analysis. Data were submitted for 462 subjects, but 12 subjects were removed due to inadequate information for assessing resistance (no baseline sequences were available, the subject was on study drug for less than 2 weeks, the subject was lost to follow up while suppressed, etc.). A total of 450 subjects were included in the resistance analysis, including 185 subjects (41.1%) infected with HCV GT1a and 259 subjects (57.6%) infected with HCV GT1b (Table 12). Six additional subjects were included who were infected with a non-specified HCV GT1 (n=1), GT1d (n=2), GT1e (n=1), GT1e (n=1) or GT6e (n=1)(Table12).

Table 12. Characteristics of the censored population for TMC435-C206.

ARM	No. Subjects	GT1a	GT1b	GT1	GT1d	GT1E	GT1i	GT6p
PLACEBO	65	27	38	0	0	0	0	0
TMC435 100MG/ 12WKS	65	25	39	0	0	0	0	1
TMC435 100MG/ 24WKS	66	30	35	0	1	0	0	0
TMC435 100MG/ 48WKS	62	25	36	0	0	0	1	0
TMC435 150MG/ 12WKS	66	30	36	0	0	0	0	0
TMC435 150MG/ 24WKS	66	28	35	1	1	1	0	0
TMC435 150MG/ 48WKS	60	20	40	0	0	0	0	0
Totals	450	185	259	1	2	1	1	1

SVR12 rates in the censored population were nearly identical to SVR24 rates, and the SVR12 rates ranged from 62.9% to 70.8% for subjects receiving 100 mg of TMC435 and between 66.7% and 81.7% for subjects in the 150 mg dose cohorts compared to SVR12 rate of 23.1% in the placebo group (Figure 24, DAVP analysis).

The SVR12 rate was assessed in the censored population, comparing rates between HCV GT1a and GT1b and comparing those subjects who had Q80 at baseline (N=391) versus those that had a polymorphic residue at position 80 (n=59).

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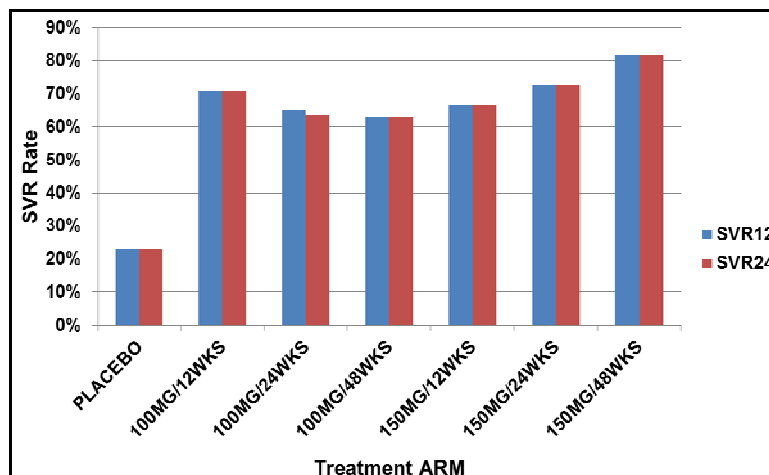


Figure 24. SVR12 and SVR24 rates for the censored population in TMC435-C206.

SVR12 rates ranged from 68-92% in subjects with GT1b, and these rates were lower in GT1a subjects who had rates between 52-68% compared to 18% for GT1a and 27% for GT1b in the placebo cohorts (Figure 25).

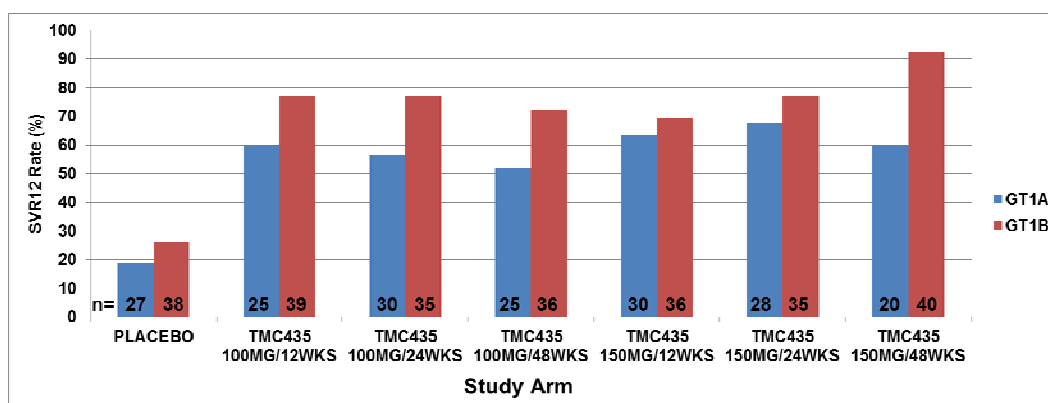


Figure 25. SVR12 rates by study arm for TMC435-C206.

Among those infected with HCV GT1a who had a polymorphic residue at NS3/4A position 80, the SVR12 rates were considerably lower, ranging from 0-62%, although the subgroups were small (Figure 26). In all cases, the SVR12 rate was lower for individuals who were infected with HCV GT1a that had a substitution at position 80.

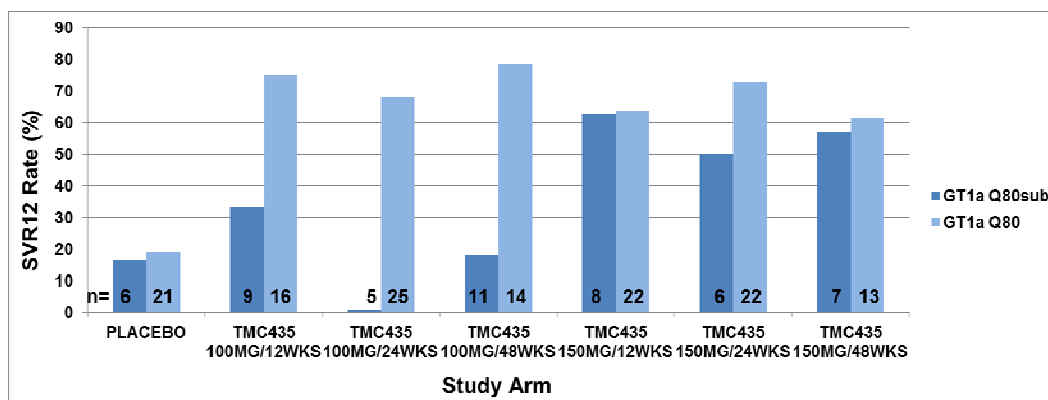


Figure 26. SVR12 rates in subjects infected with HCV GT1a with versus without a substitution at position 80.

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The presence of a substitution at NS3/4A position 80 reduced the SVR12 rates in all treatment arms of both phase 2 studies (TMC435-C205 and -C206) and the SVR rate was not significantly different from the placebo control (see the review of Clinical Virology Reviewer Damon Deming, Ph.D.).

TMC435-C206 Resistance Analysis with Sanger Population Sequencing

The sponsor provided resistance analysis datasets that were derived from both population sequencing using the Sanger sequencing method and from NGS using the Illumina platform. The methods used for RNA extraction, purification and preparation can be found in section [RNA Extraction and Preparation and HCV Genotyping](#) and the methods employed for [Sanger population sequencing for resistance analysis](#) can be found in the methods section of this review.

The sponsor focused on the sites listed in [Table 4](#) for conducting resistance analysis, first assessing the presence of substitutions at these sites at baseline and then to see if emerging substitutions appeared at these sites at or near the time of breakthrough or relapse. The sponsor's results for baseline resistance analysis are shown in Table 13. There was good agreement between the results reported by the sponsor and the independent assessment of baseline substitutions performed by DAVP (Figures 27).

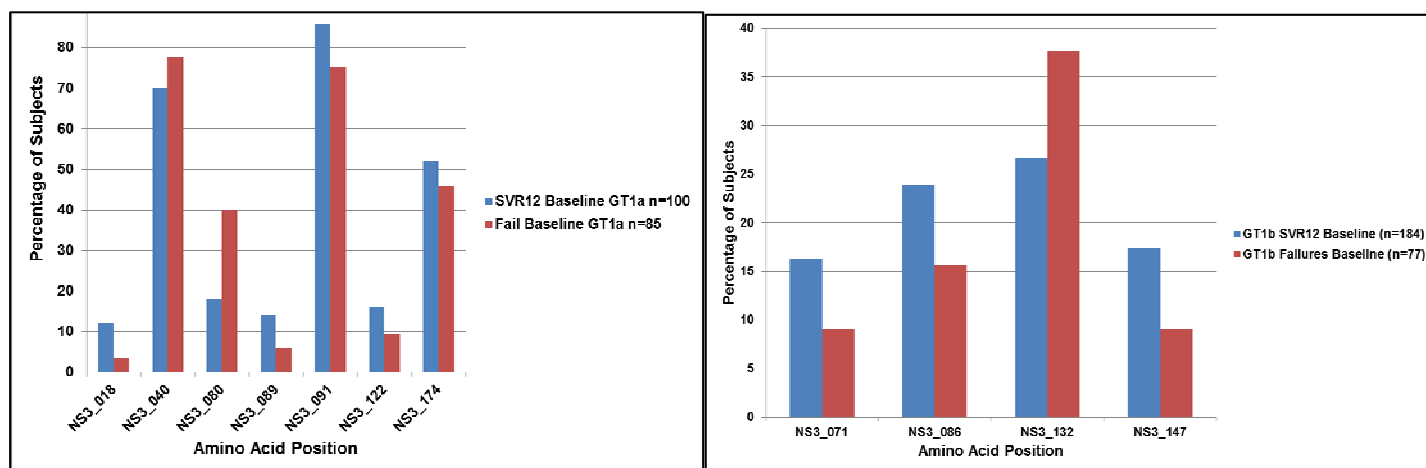


Figure 27. Baseline comparison between subjects who attained SVR12 and those that did not in TMC435-C206. GT1a is shown in the left panel and GT1b in the right panel.

The independent assessment of resistance at baseline performed by DAVP considered all amino acid positions without bias and was conducted by comparing baseline sequences from subjects who attained SVR12 compared to those that failed treatment based upon HCV GT (Figure 27). Only substitutions that were greater than 5% different were shown. These results showed that for GT1a, Q80 was the only site that appeared to be associated with lower SVR12 rates. In GT1b, substitutions at NS3/4A position 132 appeared to be detected at a higher frequency in treatment failures (~26% versus 37%). Interestingly, of the 80 subjects who had a substitution at position 132 at baseline, 51 achieved SVR12 for a rate of 63.75%. Of note, 75 of the 80 subjects with a substitution at I132 were infected with GT1b which had overall SVR12 rates between 68-92%, indicating that I132 may have an impact on efficacy. Substitutions at Q80 were the most frequent difference at baseline between subjects who achieved SVR12 and those who did not.

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Table 13. Baseline substitutions reported by the sponsor that corresponded to 18 sites of interest (Table 8, page 31, TMC435-C206 Antiviral Microbiology Report).

	TMC435 100 mg 12 Wks	TMC435 100 mg 24 Wks	TMC435 100 mg 48 Wks	TMC435 150 mg 12 Wks	TMC435 150 mg 24 Wks	TMC435 150 mg 48 Wks	Placebo	Total
Analysis set: ITT	66	65	66	66	68	65	66	462
All HCV geno/subtypes	66	65	66	66	68	65	66	462
Subjects with sequencing data	66	65	65	66	67	64	66	459
No polymorphism at positions of interest	19 (28.8%)	26 (40.0%)	25 (38.5%)	29 (43.9%)	21 (31.3%)	22 (34.4%)	22 (33.3%)	164 (35.7%)
Polymorphism at positions of interest	47 (71.2%)	39 (60.0%)	40 (61.5%)	37 (56.1%)	46 (68.7%)	42 (65.6%)	44 (66.7%)	295 (64.3%)
036	0	1 (1.5%)	2 (3.1%)	2 (3.0%)	2 (3.0%)	1 (1.6%)	1 (1.5%)	9 (2.0%)
041	1 (1.5%)	1 (1.5%)	0	0	0	0	1 (1.5%)	3 (0.7%)
043	0	0	0	0	0	0	0	0
054	1 (1.5%)	2 (3.1%)	0	0	1 (1.5%)	1 (1.6%)	3 (4.5%)	8 (1.7%)
055	1 (1.5%)	2 (3.1%)	0	0	1 (1.5%)	3 (4.7%)	4 (6.1%)	11 (2.4%)
080			11			12		61
	9 (13.6%)	6 (9.2%)	(16.9%)	8 (12.1%)	9 (13.4%)	(18.8%)	6 (9.1%)	(13.3%)
107	1 (1.5%)	0	0	1 (1.5%)	0	1 (1.6%)	1 (1.5%)	4 (0.9%)
122					10		8	49
	9 (13.6%)	5 (7.7%)	8 (12.3%)	3 (4.5%)	(14.9%)	6 (9.4%)	(12.1%)	(10.7%)
132	15 (22.7%)	14 (21.5%)	6 (9.2%)	16 (24.2%)	10 (14.9%)	7 (10.9%)	12 (18.2%)	80 (17.4%)
138	0	0	0	0	0	0	0	0
155	0	0	0	0	0	0	0	0
156	0	0	0	0	0	0	0	0
158	0	0	0	0	0	1 (1.6%)	1 (1.5%)	2 (0.4%)
168	0	1 (1.5%)	0	0	0	0	0	1 (0.2%)
169	0	0	0	0	0	0	0	0
170	10 (15.2%)	5 (7.7%)	(21.5%)	4 (6.1%)	(23.9%)	(17.2%)	(18.2%)	(15.7%)
174	18 (27.3%)	15 (23.1%)	13 (20.0%)	11 (16.7%)	22 (32.8%)	13 (20.3%)	13 (19.7%)	105 (22.9%)
175	2 (3.0%)	2 (3.1%)	1 (1.5%)	1 (1.5%)	2 (3.0%)	1 (1.6%)	0	9 (2.0%)
HCV geno/subtype 1a/other	27	30	27	30	33	24	27	198
Subjects with sequencing data	27	30	26	30	32	23	27	195
No polymorphism at positions of interest	4 (14.8%)	(36.7%)	3 (11.5%)	(40.0%)	5 (15.6%)	1 (4.3%)	(25.9%)	(22.1%)
Polymorphism at positions of interest*	23 (85.2%)	19 (63.3%)	23 (88.5%)	18 (60.0%)	27 (84.4%)	22 (95.7%)	20 (74.1%)	152 (77.9%)
080			11			10	6	56
	9 (33.3%)	5 (16.7%)	(42.3%)	8 (26.7%)	7 (21.9%)	(43.5%)	(22.2%)	(28.7%)
132	1 (3.7%)	1 (3.3%)	0	0	2 (6.3%)	0	0	4 (2.1%)
170	1 (3.7%)	1 (3.3%)	1 (3.8%)	0	5 (15.6%)	1 (4.3%)	1 (3.7%)	10 (5.1%)
174	16 (59.3%)	14 (46.7%)	13 (50.0%)	11 (36.7%)	20 (62.5%)	12 (52.2%)	12 (44.4%)	98 (50.3%)
HCV geno/subtype 1b	39	35	39	36	35	41	39	264
Subjects with sequencing data	39	35	39	36	35	41	39	264
	TMC435 100 mg 12 Wks	TMC435 100 mg 24 Wks	TMC435 100 mg 48 Wks	TMC435 150 mg 12 Wks	TMC435 150 mg 24 Wks	TMC435 150 mg 48 Wks	Placebo	Total
No polymorphism at positions of interest	15 (38.5%)	15 (42.9%)	22 (56.4%)	17 (47.2%)	16 (45.7%)	21 (51.2%)	15 (38.5%)	121 (45.8%)
Polymorphism at positions of interest*	24 (61.5%)	20 (57.1%)	17 (43.6%)	19 (52.8%)	19 (54.3%)	20 (48.8%)	24 (61.5%)	143 (54.2%)
080	0	1 (2.9%)	0	0	2 (5.7%)	2 (4.9%)	0	5 (1.9%)
132	14 (35.9%)	13 (37.1%)	6 (15.4%)	16 (44.4%)	8 (22.9%)	7 (17.1%)	12 (30.8%)	76 (28.8%)
170			13		11	10	11	62
	9 (23.1%)	4 (11.4%)	(33.3%)	4 (11.1%)	(31.4%)	(24.4%)	(28.2%)	(23.5%)
174	2 (5.1%)	1 (2.9%)	0	0	2 (5.7%)	1 (2.4%)	1 (2.6%)	7 (2.7%)

Any baseline polymorphism at NS3 Positions 36, 41, 43, 54, 55, 80, 107, 122, 132, 138, 155, 156, 158, 168, 169, 170, 174 and 175 are considered.

Polymorphisms are defined as changes from con1 (AJ238799) and H77 (AF009606) for HCV geno/subtype 1b and 1a/other respectively.

*Position are shown by geno/subtype for position for which the frequency differed between genotype 1a and 1b

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Substitutions emerging from baseline to time of failure were also analyzed by the sponsor and DAVP. The sponsor came to the following conclusions: *The proportion of subjects with polymorphisms at any of the 18 amino acid positions of interest were comparable in all 6 treatment arms ranging from 56.1% to 71.2%. The highest baseline polymorphism prevalence was these at positions 174 (22.9%), 132 (17.4%), 170 (15.7%), - 80 (13.3%), and 122 (10.7%). The prevalence at NS3 positions 132, 170 and 174 were mainly due to amino acid differences in the genotype 1a (H77) and 1b (Con 1) reference sequences at these positions. The polymorphisms present at NS3 positions 174, 170, and 132 did not reduce TMC435 activity when tested as SDM (FC≤2.0 fold, with exception of I132V: FC=2.3) in a transient replicon assay. Of the 61 (13.3%) subjects with polymorphisms at amino acid NS3 position 80 at baseline, 53 subjects had Q80K: 51 were infected with HCV genotype 1a and 2 subjects were infected with HCV genotype 1d. Q80K was present as a baseline polymorphism in 11.5% of the subjects overall and in 27.0% of genotype 1a infected subjects, and reduced TMC435 activity by 7.7-fold or 9.3-fold when tested as SDM in a genotype 1b or 1a replicon backbone, respectively. In addition 6 subjects had Q80L and 2 had Q80R at baseline. When tested as SDM in a transient replicon assay Q80L did not reduce TMC435 activity (TMC435 FC≤2), whereas Q80R reduced TMC435 activity by 13- or 6.9-fold when tested in a genotype 1a or 1b replicon backbone, respectively. Polymorphisms at NS3 amino acid position 122 were present in 49 (10.7%) subjects, and did not reduce TMC435 activity when tested as SDM (FC ≤ 2.0) in a transient replicon assay. Polymorphisms at NS3 positions 36, 41, 54, 55, 107, 158, 168, and 175 were observed at prevalences ranging from 0.2% to 2.0%. No polymorphisms were observed at NS3 amino acid positions 43, 138, 155, 156 and 169. - Page 2, TMC435-C206 Antiviral Microbiology Report. Nota bene: DAVP uses phenotypic assessments to confirm resistance associations but lack of a phenotype does not exclude a role in resistance.*

DAVP compared the amino acids detected at baseline to those detected at the timepoint closest to failure for each subject in the TMC435-C206 censored population to identify positions that changed and therefore, potentially associated with resistance. These analyses were not limited to the 18 sites identified by the sponsor, but the analysis was conducted on the arms receiving the 150 mg dose (this indication). In subjects infected with HCV-GT1a, substitutions at R155 (in >70% of subjects), Q80 (46% presence at baseline in subjects who failed, additional 6% acquired substitutions at this position during treatment), and substitutions at D168 (~38% of failures) were most common (Table 14, Figure 28). For those infected with GT1b, resistance-associated substitutions occurred most frequently at D168 (>90%) and Q80 (~22%) (Table 14, Figure 28).

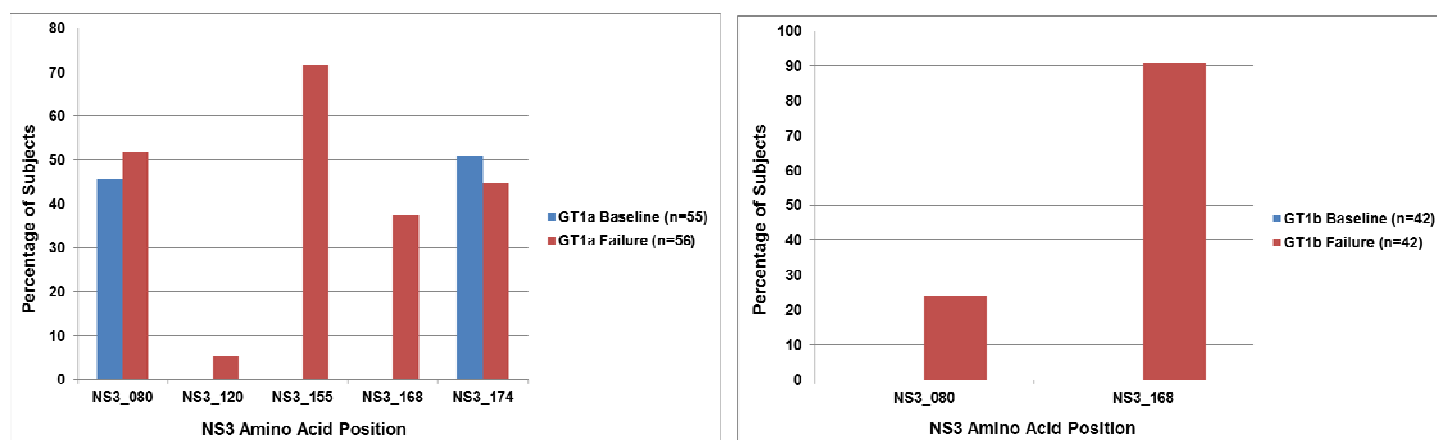


Figure 28. Most common emerging substitutions identified by Sanger population sequencing by HCV GT in TMC435-C206. GT1a, left panel; GT1b, right panel.

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Table 14. Most common resistance-associated substitutions for positions identified by Sanger population sequencing.

Major Substitutions	NS3 Q80			NS3 R155			NS3 D168							NS3 R155+NS3 D168				
	K	R	L	K	K/R	Q/R	V	E	D/E	D/V	D/H	A/V	A/D	K+E	K+D/E	Q/R+D/V	K/R+D/E	K/R+A/V
All 150 mg ARMS	9	3	1	18	3	1	14	7	3	2	1	1	1	2	2	1	1	1
GT1a	8	1	1	17	3	0	3	4	3	0	0	1	1	2	2	0	1	1
GT1b	1	2	0	0	0	1	11	3	0	2	1	0	0	0	0	1	0	0
TMC435 150MG/12WKS	4	2	0	5	1	0	5	4	1	1	0	0	1	0	1	0	0	0
GT1a	3	1	0	5	1	0	1	2	1	0	0	0	1	0	1	0	0	0
GT1b	1	1	0	0	0	0	4	2	0	1	0	0	0	0	0	0	0	0
TMC435 150MG/24WKS	2	1	0	7	0	1	9	2	0	1	0	0	0	1	0	1	0	0
GT1a	2	0	1	7	0	0	2	1	0	0	0	0	0	1	0	0	0	0
GT1b	0	1	0	0	0	1	7	1	0	1	0	0	0	0	0	1	0	0
TMC435 150MG/48WKS	3	0	0	6	2	0	0	1	2	0	1	1	0	1	1	0	1	1
GT1a	3	0	0	6	2	0	0	1	2	0	0	1	0	1	1	0	1	1
GT1b	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Summary of TMC435-C206 Resistance Analysis based on Sanger Population Sequencing

The predominant sites associated with resistance, as determined by Sanger Population sequencing were:

1. GT1a: 155, 80, and 168
2. GT1b: 80 and 168

These sites were reported by the sponsor and confirmed by the analysis performed by DAVP.

TMC435-C206 NGS Data Analysis

In addition to the Sanger sequencing data presented for study TMC435-C206, the sponsor also conducted next generation sequencing (AKA deep sequencing) of the samples associated with failure, using the same methods described in the [NGS methods](#) section above. A total of 147 subjects (Table 15) had samples that were sequenced using the Illumina platform, 63 subjects having attained SVR12, 31 of these subjects relapsed after the P/R+DAA period, 39 subjects experienced virologic breakthrough during the P/R+DAA, 10 subjects were non-responders and 4 subjects were considered to be unclassified failures. These subjects were selected from each study arm (Table 15).

Table 15. Characteristics of samples that were deep sequenced by TMC435-C206 study arm.

Study	Arm	No. Failure (NGS)
C206	TMC435 150MG/12WKS	24
	TMC435 150MG/24WKS	27
	TMC435 150MG/48WKS	24
	TMC435 150MG/12WKS	24
	TMC435 150MG/24WKS	24
	TMC435 150MG/48WKS	24
	Total	147

The fastq sequences submitted by the sponsor were analyzed using the [NGS data analysis pipeline](#) described above. Briefly, the reads for each sample were aligned to the HCV GT1a (H77) or HCV GT1b (Con1) references sequence for the NS3/4A gene. Variants were called using two algorithms, PVD75 and QbVD, and the variant tables were combined to generate Frequency tables showing all amino acid substitution that occurred above a frequency of 1% for a given position within a given sample for each subject. Resistance analysis tables were generated for each Frequency table for each variant detection algorithm using a threshold of 0.05, and the three resistance analysis tables were compared directly to determine how closely the three algorithms agreed with one another.

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TMC435-C206 Variant Detection Algorithm Comparison

To further assess the population-based results generated using the Resistance Analysis tables, DAVP used the SUBS10+- filtering threshold to look at individual changes occurring at each position in the Frequency tables. SUBS10+- used the following criteria to identify substitutions of interest:

1. Identify all substitutions that were detected at a frequency of 0.10 or greater at a later timepoint but were not detected at baseline (<1% at baseline)
2. Ignore all substitutions that were detected with a total coverage of less than 1000

Changes from the reference sequence were identified in the baseline samples and changes from baseline to later timepoints were assessed to determine emergence of substitutions at each NS3/4A amino acid position among all of the treatment failures. In general, and as shown in study TMC435-C205 (Figures 19 and 20), there was good agreement between the three variant detection methods (data not shown) and the most frequent changes from baseline.

NGS Resistance Analysis for TMC435-C206

The sponsor focused on the “long” and “short” lists of substitutions for determining sites of interest for resistance analysis by deep sequencing using the Illumina platform. As described previously, DAVP performed an unbiased resistance analysis on all substitutions observed. In general, the results obtained by NGS were similar to those obtained by population sequence. Of note, three subjects from TMC435-C206 were deemed to have no emerging substitutions at failure by Sanger population sequencing but were found to have emerging substitutions by NGS, although generally at low frequency. The sponsor performed its NGS analysis by combining data for studies C205 and C206. These studies were first analyzed separately by DAVP, and then a meta-analysis was performed including data from both studies (see the [meta-analysis data analysis section](#) below).

For TMC435-C206, DAVP considered all substitutions to be potential emerging resistance-associated substitutions if they met these criteria: 1) met the SUBS10+- threshold, 2) were detected by 2-of-3 variant detection methods (QbVD, PVD75 or variants detected by the sponsor), and 3) occurred in 2 or more subjects. Potentially resistance-associated substitutions that arose in TMC435-C206 are shown in Figure 29. Sites of interest included (positions not seen by Sanger population sequencing are in bold):

1. GT1a: 80, 155, **343, 344, 477, 534**, and **NS4A_029**
2. GT1b: **48**, 80, 168, **344, 383, 472**, and **574**

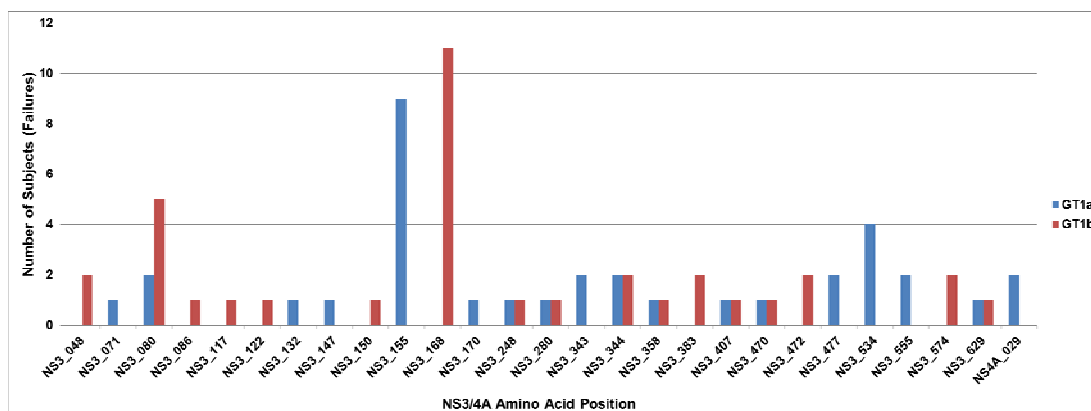


Figure 29. Emergent substitutions in TMC435-C206 as identified using the SUB10+- threshold.

Many of the sites identified in the NGS analysis as emerging, were sites reported to be polymorphic by the sponsor. However, polymorphic sites have been known to confer resistance (Q80K is a known GT1a

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polymorphism), so all of these sites were considered to be potentially associated with resistance. This resulted in identification of several potential resistance-associated substitutions for TMC435-C206 and confirmed the predominant substitutions that were identified by population sequencing (Table 16). In addition, several substitutions that are potentially associated with resistance were also observed (Table 16). All substitutions identified by SUBS10+- are shown in the TMC435-C206 Substitutions section in the [appendix](#).

Table 16. Resistance-associated substitutions that emerged by at least 10% frequency from baseline in study TMC435-C206.

SUBTYPE	NS3AA POS	SUBS10+-	Number	Ratio
1a	155	R155K	8	
	534	G534S/S534G	4	3-1
	80	Q80K/L	2	1-1
	343	S343N	2	
	344	T344I	2	
	477	T477A/A477T	2	1-1
	555	E555D/D555E	2	1-1
	NS4A_029	V660I/I660V	2	1-1
1b	168	D168V/D168E	10	7-3
	80	Q80R/Q80K	5	4-1
	48	I48V/V48I	2	1-1
	344	T344A/I344T	2	1-1
	383	G383S/S383G	2	1-1
	472	I472V	2	
	574	P574A/S	2	1-1

SUB10+- -- substitutions that emerged or disappeared compared to baseline at a frequency $\geq 10\%$

Ratio -- represents the number of each substitution listed in order

Resistance Analysis Conclusions for TMC435-C206

The predominant resistance-associated substitutions for TMC435-C206 occur at the following positions:

- GT1a: 80 and 155
- GT1b: 80 and 168

However, additional substitutions were identified in two or more subjects and these could represent resistance-associated substitutions. These include:

- GT1a: 343, 344, and 534
- GT1b: 472, and 574

Other substitutions were also observed; however, these were polymorphic sites that had more than one variant detected at baseline and substitutions that occurred were predictable. Therefore, these sites are likely to be natural variants. These sites include:

- GT1a: 477 (93.4% conservation), 444 (undetermined conservation), and NS4A_029 (66.2% conservation)
- GT1b: 48 (60.6% conservation), 344 (73.3% conservation), and 383 (65.1% conservation)

Phenotypic Analysis Results for TMC435-C206

The sponsor performed the same phenotypic analyses for TMC435-C206 that was performed for study TMC435-C205, and showed similar results. However, these results did not confirm or exclude resistance association with any substitutions.

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COMBINED RESISTANCE ANALYSIS

TMC435-C205 and TMC435-C206 NGS Data Meta-analysis

In addition to analyzing the substitutions associated with resistance in the two individual clinical trials (TMC435-C205 and -C206), resistance data were also combined to determine if trends observed in one study were found in the other and to identify lower frequency substitutions that may play a role in resistance. All substitutions were considered to be potential emerging resistance-associated substitutions if they met these criteria: 1) met the SUBS10+- threshold, 2) were detected by 2-of-3 variant detection methods, and 3) occurred in 2 or more subjects in either study.

In general, the same trends were observed when combining the resistance substitutions (Table 17). D168V/E was the predominant substitution for GT1b (n=20), and R155K was the predominant substitution associated with failure among GT1a (n=11). Emerging Q80K was associated with failure in both GT1a and GT1b (n=6 and n=6, respectively).

Table 17. Predominant resistance-associated substitutions identified in both phase 2 studies (TMC435-C205 and -C206) from the NGS data. This table does not show substitutions identified previously that were predicted to be natural variants of polymorphic sites.

SUBTYPE	NS3AA POS	SUBS10+-	Number	Ratio
1a	155	R155K	11	
	80	K80Q, Q80K/R/L	6	2-2-1-1
	534	G534S/S534G	4	3-1
	71	V71I/I71V	3	2-1
	343	S343N	2	
	344	T344I	2	
1b	168	D168V/E/H/A	20	12-4-3-1
	80	Q80R/Q80K	6	5-1
	48	I48V/V48I	3	2-1
	122	T122S/S122R	3	2-1
	150	A150V, V150A	3	2-1
	71	V71I	2	
	86	H86Q	2	
	472	I472V	2	
	574	P574A/S	2	1-1

Of note, several sites were identified that were already observed in the individual analyses of TMC435-C205 and -C206. However, H86Q was observed in two subjects infected with HCV GT1b, one in each of the two trials, indicating that this could be a potential resistance-associated substitution as it is a conserved amino acid.

Using the same criteria listed above, a meta-analysis was performed across all subjects regardless of HCV GT or subtype and this resulted in identifying an additional substitution that may be resistance-associated (Table 18). Substitution V629I was identified in two subjects and may be associated with resistance to TMC435.

Table 18. Meta-analysis of TMC435-C205 and -C206 across genotypes. Substitutions identified using SUB10+-.

NS3AA POS	SUBS10+-	Number	Ratio	ST Ratio (1a/1b)
168	D168V/E/H/A	21	13-4-3-1	1-20
80	Q80R/Q80K/K80Q/Q80L	12	6-3-2-1	6-6
122	S122R/T122S/G122S	5	2-2-1	2-3
71	V71I/I71V	4	3-1	2-2
344	T344I/T344A/I344T	4	2-1-1	2-2
170	I170V, V170I	3	2-1	1-2
629	V629I	2		1-1

ST Ratio, Subtype ratio – the ratio of subjects infected with different HCV GT1a or GT1b with the substitution

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SUMMARY AND CONCLUSIONS ON RESISTANCE TO TMC435

Table 19 identifies two potential resistance-associated substitutions that we recommend be phenotyped and added to the label for TMC435 (Table 19). These include the following substitutions: P574A/S and V629I.

Table 19. Resistance-associated substitutions of TMC435.

SUBTYPE	NS3AA POS	SUBS	Number	NGS	SANGER	Explanation	Conclusion
1a	80	Q80K/R/L	12	✓	✓	Known RAS	Known RAS
1a	132	I132V	2	✓	✓	Present in both directions, small n	Not likely a RAS
1a	155	R155K	21	✓	✓	Known RAS	Known RAS
1a	343	S343N	4	✓		Small n, 3-1 ratio, 98.2% conserved, found at baseline in multiple subjects	Not likely a RAS
1a	344	T344I	2	✓		Small n, one direction, 99.6% conserved, found at baseline in multiple subjects	Not likely a RAS
1a	534	G534S/S534G	4	✓		Polymorphic site, 3-2 ratio	Not likely a RAS
1b	71	V71I	4	✓		Polymorphic site, small n, known change	Not likely a RAS
1b	86	H86Q	2	✓		Q86P occurs, polymorphic site, H and Q observed at baseline	Not likely a RAS
1b	168	D168V/E/H/A	21	✓	✓	Known RAS	Known RAS
1b	170	I170V	2	✓	✓	Present in both directions, small n	Not likely a RAS
1b	472	I472V	2	✓		94.9% conserved, small n, all one direction	Not likely a RAS
1b	574	P574A/S	2	✓		98.4% conserved, novel substitutions, small n	Potential RAS
Both	122	S122R/T122S/G122S	5	✓		Known RAS	Known RAS
Both	629	V629I	2	✓		98.4% conserved, small n, one direction	Potential RAS

ADDITIONAL STUDIES

Frequency and emergence of R155K

In addition to resistance analysis, the NGS data was analyzed to determine how rapidly R155K arises in subjects who develop resistance against TMC435. This analysis was performed because R155K is a known resistance-associated substitution of other protease inhibitors, including boceprevir and telaprevir, two antiviral drugs that are currently approved for the treatment of HCV GT1a and 1b infection along with Peg/RBV. The concern was that rapid resistance against TMC435 could potentially render subjects resistant to the entire class of HCV protease inhibitors. (b) (4)

To assess the onset of R155K, the NGS data for TMC435-C206 were analyzed to determine the frequency of R155K at each timepoint for each subject who had this substitution emerge. There were 20 subjects who had NGS data submitted and who also had R155K arise during the course of treatment (Table 20).

Table 20. Subjects in TMC435- C206 with R155K for whom NGS data were submitted.

R155K	WEEK of STUDY															
Subject	0	1	2	3	4	6	8	12	16	20	24	28	36	48	52	72
0009	0.000	0.010	0.661	0.851	0.952	0.913						0.583	0.145	0.049		0.000
0042	0.000	0.000	0.889	0.928	0.937	0.874		0.980			0.992		0.989	0.976		0.897
0066	0.000	0.799														
0079	0.000														0.333	
0080	0.000				0.164					0.077						
0112	0.000															0.230
0118	0.000		0.428		0.279			0.437								
0125	0.000	0.530										0.019	0.132			
0148	0.000									0.963						0.232
0232	0.000		0.987	0.993	0.986	0.972	0.991		0.991		0.373		0.029			
0241	0.000							0.996								
0273	0.000		0.598													0.130
0274	0.000				0.557									0.493		
0322	0.000															0.994
0361	0.000															0.864
0403	0.000		0.699							0.985						0.020
0465	0.000		0.992		0.992			0.622								
0510	0.000							0.995			0.994		0.982			
0531	0.000			0.931												
0617	0.000															0.991

Timepoint 0 = baseline

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A total of 10 subjects (50% of those who had R155K emerge) had R155K at a frequency above 50% at or prior to Week 4, with 8 of these subjects (40% of those who had R155K emerge) exhibiting R155K frequencies greater than 50% by Week 2 (Table 20, Figure 30). This observation provides evidence that R155K emerges rapidly in a high percentage of subject (b) (4)

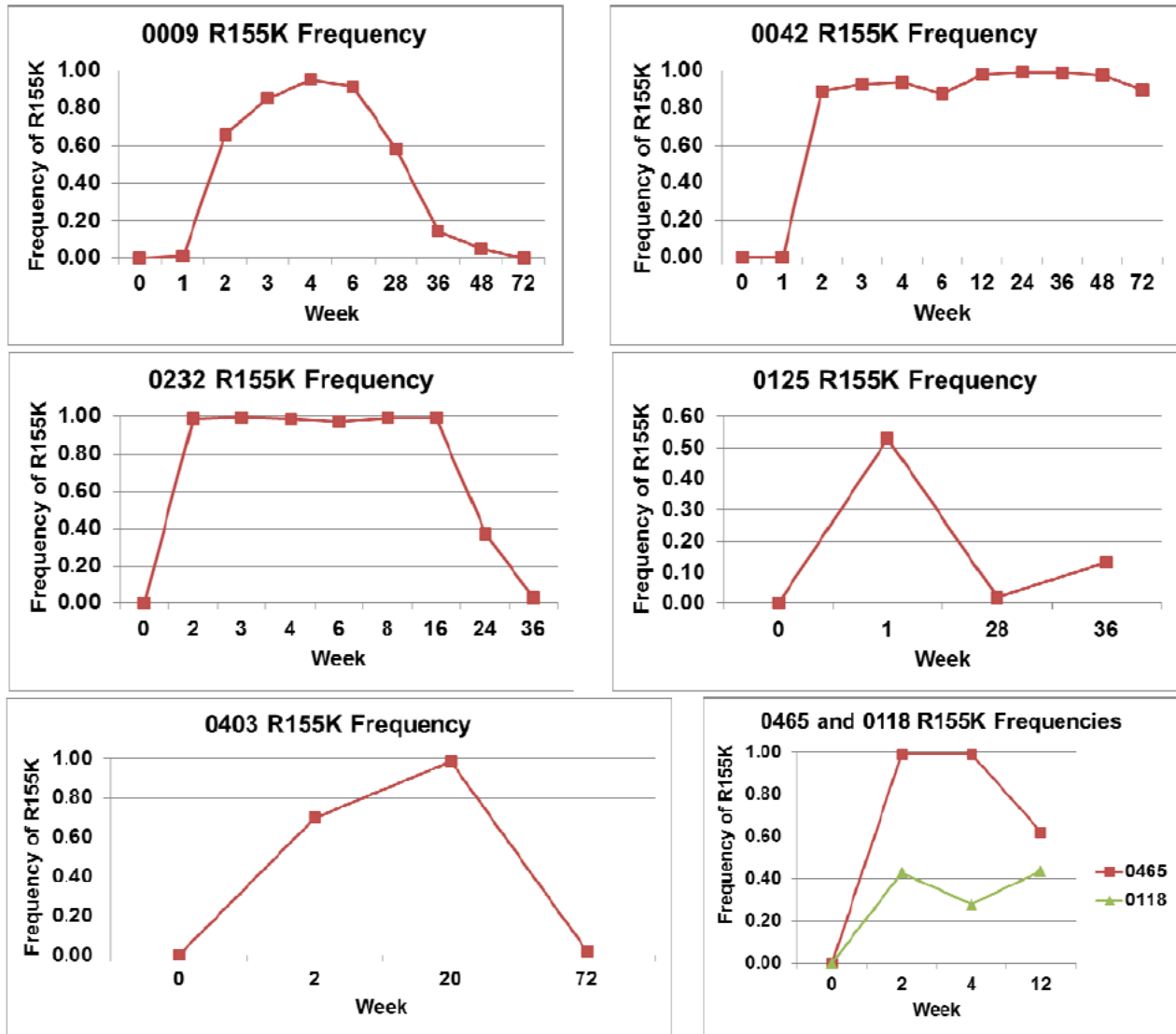


Figure 30. Frequency plots for seven subjects in TMC435-C206 who developed resistance to TMC435. Subject 0042: by Week 2, R155K was detected in 89% of NGS reads, TMC435 100MG/24WKS. Subject 009: by Week 2, R155K was detected in 66% of NGS reads, TMC435 100MG/48WKS. Subject 0232: by Week 2, R155K was detected in 98.7% of NGS reads, TMC435 150MG/48WKS. Subject 0465: by Week 2, R155K was detected in 99.2% of NGS reads, TMC435 100MG/48WKS. Subject 0118: by Week 2, R155K was detected in 43% of NGS reads, TMC435 150MG/12WKS. Subject 0403: by Week 2, R155K was detected in 69.9% of NGS reads, TMC435 100MG/24WKS. Subject 0125: by Week 1, R155K was detected in 53% of NGS reads, TMC435 100MG/48WKS.

Conclusion: R155K arose within 2-4 weeks in 50% of subjects in TMC435-C206 for whom NGS data was available. (b) (4)

In at least one subject, R155K persisted for 72 weeks, indicating that two weeks for therapy on TMC435 could knock out all protease inhibitors for at least one year for that subject.

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Homogeneity of Q80K

The presence of substitutions at position 80 was assessed by analyzing the NGS data to determine the frequency of substitutions at baseline and to evaluate how these frequencies changed over the course of treatment with TMC435. In the majority of subjects (41 of 65 subjects) from TMC435-C205 and C206 who had a substitution at position 80 at baseline it was detected at greater than 90% frequency and this frequency was maintained throughout the course of treatment. Among subjects who had an emergent substitution at position 80, 13 of 19 had frequencies greater than 65% and these were often maintained across timepoints (Table 21).

Table 21. Frequencies of Q80 substitutions in studies TMC435-C205 and C206.

USUBJID	NGSPL	VISIT	GT	NS3 AAPOS	TCOV	AAREF	AASUB	VCOV	AAFREQ	Status
TMC435-C205-0308	ILLUMINA GAlx	WEEK 36	1a	80	33233	Q	K	33010	0.993	Emerge
TMC435-C205-0412	ILLUMINA GAlx	WEEK 36	1a	80	28297	Q	R	27747	0.981	Emerge
TMC435-C205-0015	ILLUMINA GAlx	SCREENING	1a	80	51786	Q	K	51579	0.996	Present
TMC435-C205-0015	ILLUMINA GAlx	WEEK 48	1a	80	31398	Q	K	31088	0.99	
TMC435-C205-0096	ILLUMINA GAlx	SCREENING	1a	80	37750	Q	K	37674	0.998	Present
TMC435-C205-0096	ILLUMINA GAlx	WEEK 72	1a	80	41289	Q	K	40723	0.986	
TMC435-C205-0170	ILLUMINA GAlx	SCREENING	1a	80	50042	Q	K	49580	0.991	Present
TMC435-C205-0170	ILLUMINA GAlx	WEEK 36	1a	80	31567	Q	K	31346	0.993	
TMC435-C205-0223	ILLUMINA GAlx	SCREENING	1a	80	40083	Q	K	39791	0.993	Present
TMC435-C205-0223	ILLUMINA GAlx	WEEK 72	1a	80	44527	Q	K	44019	0.989	
TMC435-C205-0271	ILLUMINA GAlx	SCREENING	1a	80	41482	Q	R	482	0.012	
TMC435-C205-0361	ILLUMINA GAlx	SCREENING	1a	80	45574	Q	K	44956	0.986	Present
TMC435-C205-0361	ILLUMINA GAlx	WEEK 60	1a	80	31735	Q	K	31561	0.995	
TMC435-C205-0384	ILLUMINA GAlx	SCREENING	1a	80	33994	Q	K	33868	0.996	Present
TMC435-C205-0384	ILLUMINA GAlx	WEEK 60	1a	80	31315	Q	K	31179	0.996	
TMC435-C205-0395	ILLUMINA GAlx	SCREENING	1a	80	37298	Q	L	36882	0.989	Present
TMC435-C205-0395	ILLUMINA GAlx	WEEK 36	1a	80	35899	Q	L	35415	0.987	
TMC435-C205-0395	ILLUMINA GAlx	WEEK 72	1a	80	31802	Q	L	31251	0.983	Emerge
TMC435-C205-0478	ILLUMINA GAlx	SCREENING	1a	80	34105	Q	K	33769	0.99	Present
TMC435-C205-0478	ILLUMINA GAlx	WEEK 72	1a	80	31907	Q	K	31537	0.988	
TMC435-C205-0197	ILLUMINA GAlx	WEEK 12	1b	80	46516	Q	R	34837	0.749	Emerge
TMC435-C205-0197	ILLUMINA GAlx	WEEK 3	1b	80	32727	Q	R	1081	0.033	
TMC435-C205-0197	ILLUMINA GAlx	WEEK 12	1b	80	25087	Q	R	19395	0.773	
TMC435-C205-0308	ILLUMINA GAlx	WEEK 36	1a	80	33233	Q	K	33010	0.993	Emerge
TMC435-C205-0412	ILLUMINA GAlx	WEEK 36	1a	80	28297	Q	R	27747	0.981	Emerge
TMC435-C205-0015	ILLUMINA GAlx	SCREENING	1a	80	51786	Q	K	51579	0.996	Present
TMC435-C205-0015	ILLUMINA GAlx	WEEK 48	1a	80	31398	Q	K	31088	0.99	
TMC435-C206-0107	ILLUMINA GAlx	WEEK72	1b	80	57745	Q	K	56255	0.974	Emerge
TMC435-C206-0364	ILLUMINA GAlx	WEEK20	1b	80	40130	Q	R	11311	0.282	Emerge
TMC435-C206-0391	ILLUMINA GAlx	WEEK12	1b	80	50361	Q	R	49524	0.983	Emerge
TMC435-C206-0391	ILLUMINA GAlx	WEEK36	1b	80	57496	Q	R	12471	0.217	
TMC435-C206-0402	ILLUMINA GAlx	WEEK16	1b	80	69419	Q	R	63642	0.917	Emerge
TMC435-C206-0402	ILLUMINA GAlx	WEEK20	1b	80	30773	Q	R	29923	0.972	
TMC435-C206-0402	ILLUMINA GAlx	WEEK20	1b	80	37830	Q	R	36857	0.974	
TMC435-C206-0402	ILLUMINA GAlx	WEEK20	1b	80	39756	Q	R	38787	0.976	
TMC435-C206-0402	ILLUMINA GAlx	WEEK24	1b	80	38004	Q	R	37273	0.981	
TMC435-C206-0402	ILLUMINA GAlx	WEEK36	1b	80	62117	Q	R	35443	0.571	
TMC435-C206-0402	ILLUMINA GAlx	WEEK48	1b	80	42805	Q	K	19232	0.449	
TMC435-C206-0402	ILLUMINA GAlx	WEEK48	1b	80	42805	Q	R	18127	0.423	
TMC435-C206-0402	ILLUMINA GAlx	WEEK72	1b	80	53212	Q	K	29426	0.553	
TMC435-C206-0479	ILLUMINA GAlx	BASELINE	1b	80	54842	Q	L	54245	0.989	Disappear
TMC435-C206-0548	ILLUMINA GAlx	WEEK3	1b	80	45066	Q	R	9733	0.216	Emerge
TMC435-C206-0573	ILLUMINA GAlx	BASELINE	1b	80	57148	Q	L	41173	0.720	Disappear
TMC435-C206-0587	ILLUMINA GAlx	WEEK6	1b	80	46939	Q	R	31030	0.661	Emerge
TMC435-C206-0009	ILLUMINA GAlx	BASELINE	1a	80	49794	Q	K	49142	0.987	Present
TMC435-C206-0009	ILLUMINA GAlx	WEEK 1	1a	80	35262	Q	K	35143	0.997	
TMC435-C206-0009	ILLUMINA GAlx	WEEK 16	1a	80	27348	Q	K	27130	0.992	
TMC435-C206-0009	ILLUMINA GAlx	WEEK 2	1a	80	42607	Q	K	42240	0.991	
TMC435-C206-0009	ILLUMINA GAlx	WEEK 28	1a	80	81786	Q	K	80946	0.990	
TMC435-C206-0009	ILLUMINA GAlx	WEEK 3	1a	80	50033	Q	K	49658	0.993	
TMC435-C206-0009	ILLUMINA GAlx	WEEK 36	1a	80	46319	Q	K	45992	0.993	
TMC435-C206-0009	ILLUMINA GAlx	WEEK 4	1a	80	33085	Q	K	32904	0.995	
TMC435-C206-0009	ILLUMINA GAlx	WEEK 48	1a	80	46756	Q	K	46545	0.995	
TMC435-C206-0009	ILLUMINA GAlx	WEEK 72	1a	80	85753	Q	K	84592	0.986	
TMC435-C206-0010	ILLUMINA GAlx	BASELINE	1a	80	72043	Q	K	71039	0.986	Present*
TMC435-C206-0013	ILLUMINA GAlx	BASELINE	1a	80	60743	Q	K	59815	0.985	Present*
TMC435-C206-0021	ILLUMINA GAlx	BASELINE	1a	80	40163	Q	K	39647	0.987	Present*

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TMC435-C206-0028	ILLUMINA GAlx	DAY 3	1a	80	59814	Q	K	47824	0.800	Emerge then Disappear
TMC435-C206-0028	ILLUMINA GAlx	DAY 3	1a	80	59814	Q	R	11494	0.192	
TMC435-C206-0028	ILLUMINA GAlx	WEEK 72	1a	80	75399	Q	R	5686	0.075	
TMC435-C206-0033	ILLUMINA GAlx	BASELINE	1a	80	65826	Q	K	65034	0.988	Present*
TMC435-C206-0042	ILLUMINA GAlx	WEEK 12	1a	80	38179	Q	R	603	0.016	Emerge
TMC435-C206-0042	ILLUMINA GAlx	WEEK 12	1a	80	38179	Q	L	3037	0.080	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 2	1a	80	32868	Q	L	384	0.012	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 2	1a	80	32868	Q	R	407	0.012	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 2	1a	80	32868	Q	H	646	0.020	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 2	1a	80	32868	Q	K	807	0.025	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 24	1a	80	29564	Q	L	4531	0.153	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 3	1a	80	37837	Q	L	1479	0.039	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 36	1a	80	34544	Q	L	4921	0.142	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 4	1a	80	36458	Q	L	2048	0.056	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 48	1a	80	45492	Q	L	10185	0.224	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 6	1a	80	37800	Q	L	3285	0.087	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 72	1a	80	33292	Q	L	13304	0.400	
TMC435-C206-0042	ILLUMINA GAlx	WEEK 8	1a	80	38787	Q	L	2299	0.059	
TMC435-C206-0066	ILLUMINA GAlx	BASELINE	1a	80	92816	Q	K	91550	0.986	Present
TMC435-C206-0066	ILLUMINA GAlx	WEEK 1	1a	80	48655	Q	K	48322	0.993	
TMC435-C206-0066	ILLUMINA GAlx	WEEK 72	1a	80	33838	Q	K	33542	0.991	
TMC435-C206-0079	ILLUMINA GAlx	BASELINE	1a	80	49432	Q	K	49066	0.993	Present
TMC435-C206-0079	ILLUMINA GAlx	WEEK 52	1a	80	39792	Q	K	39648	0.996	
TMC435-C206-0079	ILLUMINA GAlx	WEEK 72	1a	80	39154	Q	K	39032	0.997	
TMC435-C206-0080	ILLUMINA GAlx	WEEK 20	1a	80	43614	Q	L	40420	0.927	Emerge
TMC435-C206-0080	ILLUMINA GAlx	WEEK 4	1a	80	50681	Q	L	6090	0.120	Emerge
TMC435-C206-0112	ILLUMINA GAlx	WEEK 52	1a	80	62735	Q	R	62019	0.989	Emerge
TMC435-C206-0118	ILLUMINA GAlx	WEEK 12	1a	80	41357	Q	R	14153	0.342	Emerge
TMC435-C206-0118	ILLUMINA GAlx	WEEK 12	1a	80	41357	Q	K	1093	0.026	
TMC435-C206-0118	ILLUMINA GAlx	WEEK 4	1a	80	57545	Q	R	4634	0.081	
TMC435-C206-0119	ILLUMINA GAlx	BASELINE	1a	80	92957	Q	K	90982	0.979	Present*
TMC435-C206-0148	ILLUMINA GAlx	BASELINE	1a	80	67982	Q	K	67669	0.995	Present
TMC435-C206-0148	ILLUMINA GAlx	WEEK 20	1a	80	43862	Q	K	43331	0.988	
TMC435-C206-0148	ILLUMINA GAlx	WEEK 72	1a	80	51250	Q	K	50684	0.989	
TMC435-C206-0170	ILLUMINA GAlx	BASELINE	1a	80	53311	Q	K	53004	0.994	Present*
TMC435-C206-0190	ILLUMINA GAlx	BASELINE	1a	80	48330	Q	K	1237	0.026	
TMC435-C206-0190	ILLUMINA GAlx	BASELINE	1a	80	48330	Q	L	8016	0.166	
TMC435-C206-0200	ILLUMINA GAlx	BASELINE	1a	80	48973	Q	K	48626	0.993	Present*
TMC435-C206-0204	ILLUMINA GAlx	SCREENING	1a	80	41950	Q	K	41749	0.995	Present*
TMC435-C206-0232	ILLUMINA GAlx	BASELINE	1a	80	44235	Q	K	44044	0.996	Present
TMC435-C206-0232	ILLUMINA GAlx	WEEK 16	1a	80	32322	Q	K	32210	0.997	
TMC435-C206-0232	ILLUMINA GAlx	WEEK 2	1a	80	26249	Q	K	26107	0.995	
TMC435-C206-0232	ILLUMINA GAlx	WEEK 24	1a	80	29425	Q	K	29316	0.996	
TMC435-C206-0232	ILLUMINA GAlx	WEEK 3	1a	80	35186	Q	K	35065	0.997	
TMC435-C206-0232	ILLUMINA GAlx	WEEK 36	1a	80	24127	Q	K	23890	0.990	
TMC435-C206-0232	ILLUMINA GAlx	WEEK 4	1a	80	28920	Q	K	28826	0.997	
TMC435-C206-0232	ILLUMINA GAlx	WEEK 52	1a	80	35064	Q	K	34935	0.996	
TMC435-C206-0232	ILLUMINA GAlx	WEEK 6	1a	80	35537	Q	K	35438	0.997	
TMC435-C206-0232	ILLUMINA GAlx	WEEK 72	1a	80	35860	Q	K	35631	0.994	
TMC435-C206-0232	ILLUMINA GAlx	WEEK 8	1a	80	21414	Q	K	21355	0.997	
TMC435-C206-0241	ILLUMINA GAlx	SCREENING	1a	80	37451	Q	K	36885	0.985	Present
TMC435-C206-0241	ILLUMINA GAlx	WEEK 12	1a	80	27699	Q	K	27608	0.997	
TMC435-C206-0241	ILLUMINA GAlx	WEEK 72	1a	80	36639	Q	K	36338	0.992	
TMC435-C206-0273	ILLUMINA GAlx	BASELINE	1a	80	52080	Q	L	13026	0.250	Emerge
TMC435-C206-0273	ILLUMINA GAlx	WEEK 2	1a	80	39627	Q	R	640	0.016	
TMC435-C206-0273	ILLUMINA GAlx	WEEK 2	1a	80	39627	Q	L	21575	0.544	
TMC435-C206-0273	ILLUMINA GAlx	WEEK 72	1a	80	37145	Q	L	36143	0.973	
TMC435-C206-0274	ILLUMINA GAlx	WEEK 4	1a	80	43908	Q	K	1261	0.029	
TMC435-C206-0274	ILLUMINA GAlx	WEEK 4	1a	80	43908	Q	R	2367	0.054	
TMC435-C206-0279	ILLUMINA GAlx	BASELINE	1a	80	55196	Q	K	54920	0.995	Present*
TMC435-C206-0309	ILLUMINA GAlx	BASELINE	1a	80	52048	Q	K	51784	0.995	Present*
TMC435-C206-0335	ILLUMINA GAlx	BASELINE	1a	80	48021	Q	K	47832	0.996	Present*
TMC435-C206-0360	ILLUMINA GAlx	BASELINE	1a	80	57502	Q	K	57269	0.996	Present*
TMC435-C206-0361	ILLUMINA GAlx	BASELINE	1a	80	56408	Q	K	55536	0.985	Present
TMC435-C206-0361	ILLUMINA GAlx	WEEK 72	1a	80	43194	Q	K	42728	0.989	
TMC435-C206-0365	ILLUMINA GAlx	BASELINE	1a	80	40920	Q	R	40270	0.984	
TMC435-C206-0365	ILLUMINA GAlx	DAY 3	1a	80	39211	Q	R	38729	0.988	Present
TMC435-C206-0383	ILLUMINA GAlx	SCREENING	1a	80	41259	Q	K	41133	0.997	Present
TMC435-C206-0383	ILLUMINA GAlx	WEEK 72	1a	80	38970	Q	K	38889	0.998	

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TMC435-C206-0403	ILLUMINA GAlx	WEEK 2	1a	80	51527	Q	R	20394	0.396	Emerge
TMC435-C206-0403	ILLUMINA GAlx	WEEK 2	1a	80	51527	Q	K	659	0.013	
TMC435-C206-0403	ILLUMINA GAlx	WEEK 2	1a	80	51527	Q	L	984	0.019	
TMC435-C206-0403	ILLUMINA GAlx	WEEK 20	1a	80	47669	Q	L	13932	0.292	
TMC435-C206-0403	ILLUMINA GAlx	WEEK 20	1a	80	47669	Q	R	11534	0.242	
TMC435-C206-0426	ILLUMINA GAlx	BASELINE	1a	80	54986	Q	K	54201	0.986	Present*
TMC435-C206-0459	ILLUMINA GAlx	BASELINE	1a	80	54829	Q	K	54497	0.994	Present*
TMC435-C206-0465	ILLUMINA GAlx	BASELINE	1a	80	40516	Q	K	40355	0.996	Present
TMC435-C206-0465	ILLUMINA GAlx	WEEK 12	1a	80	49631	Q	K	49383	0.995	
TMC435-C206-0465	ILLUMINA GAlx	WEEK 2	1a	80	45876	Q	K	45706	0.996	
TMC435-C206-0465	ILLUMINA GAlx	WEEK 4	1a	80	42896	Q	K	42676	0.995	
TMC435-C206-0465	ILLUMINA GAlx	WEEK 72	1a	80	46210	Q	K	45738	0.990	
TMC435-C206-0500	ILLUMINA GAlx	BASELINE	1a	80	30680	Q	K	30385	0.990	Present*
TMC435-C206-0510	ILLUMINA GAlx	BASELINE	1a	80	57295	Q	R	1769	0.031	Present
TMC435-C206-0531	ILLUMINA GAlx	BASELINE	1a	80	45005	Q	K	44861	0.997	
TMC435-C206-0531	ILLUMINA GAlx	WEEK 3	1a	80	36792	Q	K	36638	0.996	
TMC435-C206-0531	ILLUMINA GAlx	WEEK 72	1a	80	30597	Q	K	30271	0.989	
TMC435-C206-0533	ILLUMINA GAlx	BASELINE	1a	80	50830	Q	K	50223	0.988	Present*
TMC435-C206-0540	ILLUMINA GAlx	BASELINE	1a	80	56410	Q	K	55902	0.991	Present
TMC435-C206-0540	ILLUMINA GAlx	WEEK 72	1a	80	36807	Q	K	36434	0.990	
TMC435-C206-0545	ILLUMINA GAlx	BASELINE	1a	80	53996	Q	K	53698	0.994	Present*
TMC435-C206-0558	ILLUMINA GAlx	BASELINE	1a	80	63088	Q	K	62593	0.992	Present*
TMC435-C206-0567	ILLUMINA GAlx	BASELINE	1a	80	62421	Q	K	61654	0.988	Present*
TMC435-C206-0584	ILLUMINA GAlx	WEEK 48	1a	80	34597	Q	L	34315	0.992	Emerge
TMC435-C206-0584	ILLUMINA GAlx	WEEK 72	1a	80	24860	Q	L	17559	0.706	
TMC435-C206-0605	ILLUMINA GAlx	BASELINE	1a	80	55182	Q	K	54791	0.993	Present*

Present = detected at baseline and throughout the study

Present* = detected at baseline but no later timepoints were sequenced or those runs failed

Emerge = not detected or low frequency at baseline that increased over time

Disappear = detected at high frequency at baseline but not found at later timepoints

Gray = frequencies are too low (less than 0.20) to make a call

Comparison of GT1a and GT1b NS3 Proteases

Given that there are different resistance pathways associated with the different HCV GT1 subtypes, a comparison of the two proteases of subtypes 1a and 1b was conducted. Sequence alignment of the two peptide sequences of these protease proteins shows that there are 42 out of 632 sites that differ at the amino acid level between the two reference sequences (H77 for GT1a and Con1 for GT1b) (Figure 31).

To determine which amino acids are important for binding by TMC435, the ^{(b) (4)} structure of the GT1a protease Apo region in complex with TMC435 (PDB accession: 3KF2) was analyzed and the GT1a Apo region was compared to the GT1b protease Apo region (PDB accession: 4A1T) and to a third structure comprised of a GT1a protease Apo region for a protease with the Q80K substitution (PDB accession: 3RC5). The ^{(b) (4)} structures were comprised of the Apo region, which is approximately the first 180 amino acids of the NS3/4A gene, as this region contains the protease active site. GT1a structure 3KF2 was identical to the H77 reference sequence in the first 180 amino acids, whereas GT1b structure 4A1T differed at five sites to the GT1b reference Con1 sequence (Figure 32).

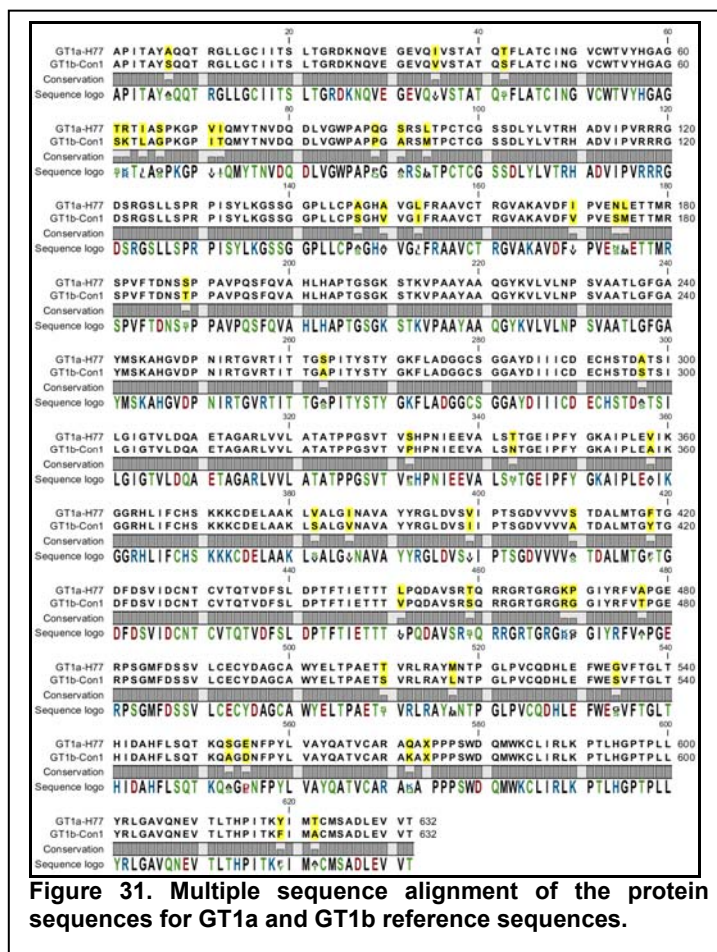


Figure 31. Multiple sequence alignment of the protein sequences for GT1a and GT1b reference sequences.

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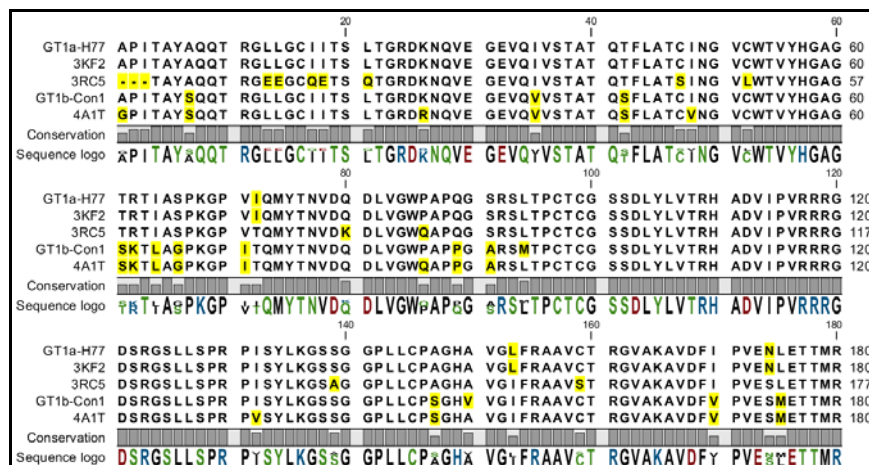


Figure 32. Multiple alignment of the amino acid sequences for the NS3/4A protease Apo domain of GT1a and GT1b (b) (4) structures and reference sequences.

Amino acids that were (b) (4) of TMC435 in the co-structure were identified because substitutions at these positions could potentially reduce or ablate TMC435 binding to the protease active site. A total of 33 amino acids were (b) (4) of TMC435, including all of amino acid positions that have been associated with resistance to TMC435 (Table 22).

Table 22. Amino acids from GT1a and GT1b NS3/4A protease Apo domain that are (b) (4) of TMC435 in the co-structure.

All unique AAs	Potential interaction with	H77 Ref	Conservation	Observed Subs	Con1	Conservation	Observed Subs
41	TMC435	Q	99.2	Q	Q	99.6	Q
42	TMC435	T	98.1	T	S	97.4	S
43	TMC435	F	100.0		F	100.0	
55	R155	V	94.8	V	V	99.1	V
56	TMC435	Y	99.3	Y	Y	68.3	F
57	TMC435, R155	H	100.0		H	100.0	
58	TMC435	G	99.6		G	99.6	
77	Q80/K80	N	100.0		N	99.0	
78	TMC435, Q80/K80	V	99.5		V	99.5	
79	TMC435, Q80/K80	D	99.2	D	D	100.0	D
80	TMC435, R155	Q	68.0	K	Q	96.4	Q
81	TMC435, R155, Q80/K80	D	100.0		D	100.0	
82	Q80/K80	L	99.9	L	L	99.9	L
122	R155, D168	S	88.8	G	S	85.8	T
123	D168	R	99.6	R	R	99.6	R
132	TMC435	I	98.7	I	V	70.9	I
135	TMC435	L	99.6	L	L	100.0	L
136	TMC435	K	99.9	K	K	99.8	K
137	TMC435	G	100.0		G	100.0	
138	TMC435	S	99.9		S	99.9	
139	TMC435, R155	S	99.8		S	100.0	
153	R155	L	1.9	I	I	95.3	I
154	TMC435, R155, D168	F	99.9		F	100.0	
155	TMC435, D168, Q80	R	99.3	R	R	100.0	
156	TMC435, R155, D168	A	100.0		A	100.0	
157	TMC435	A	100.0		A	100.0	
159	TMC435	C	100.0		C	99.8	
167	D168	V	99.5	V	V	99.5	V
168	TMC435, R155	D	99.6	D	D	99.5	D
169	R155	F	99.8	F	F	100.0	
170	R155, D168, Q80/K80	I	94.3	V	V	69.6	I
174	Q80/K80	N	52.4	G/S	S	95.0	S
178	Q80/K80	L	98.6	L	M	99.2	M

Yellow, sites that differ between subtypes 1a and 1b.

Bold, sites that interact with R155K

Comparing the two (b) (4) structures of the NS3/4a protease Apo domains of GT1a, including structure 3KF2 with Q at position 80 and structure 3SUD with K at position 80 revealed some important information regarding the impact of this substitution on the binding of TMC435 to the active site of the protease. In the wild type GT1a structure with Q at position 80, R155K hydrogen bonds with D168 and Q80, and TMC435 interacts with

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all three, forming a tight, non-covalent interaction that inhibits the activity of the protease (Figure 33, panels A and C). The Q80K substitution completely alters the TMC435 interaction with the protease, primarily because the positive charge of the lysine (K80) clashes with the positive charge of the arginine (R155), which forces both amino acids to adopt favorable rotameric positions. This forces R155 into the binding interface and K80 away from the binding interface, completely disrupting the hydrogen bonding network that stabilizes this region in the wild type structure (Figure 33, panels B and D). In this structural comparison, it appears that the presence of Q80K forces R155 into the binding interface, which would reduce or modify TMC435 binding to the NS3/4a protease.

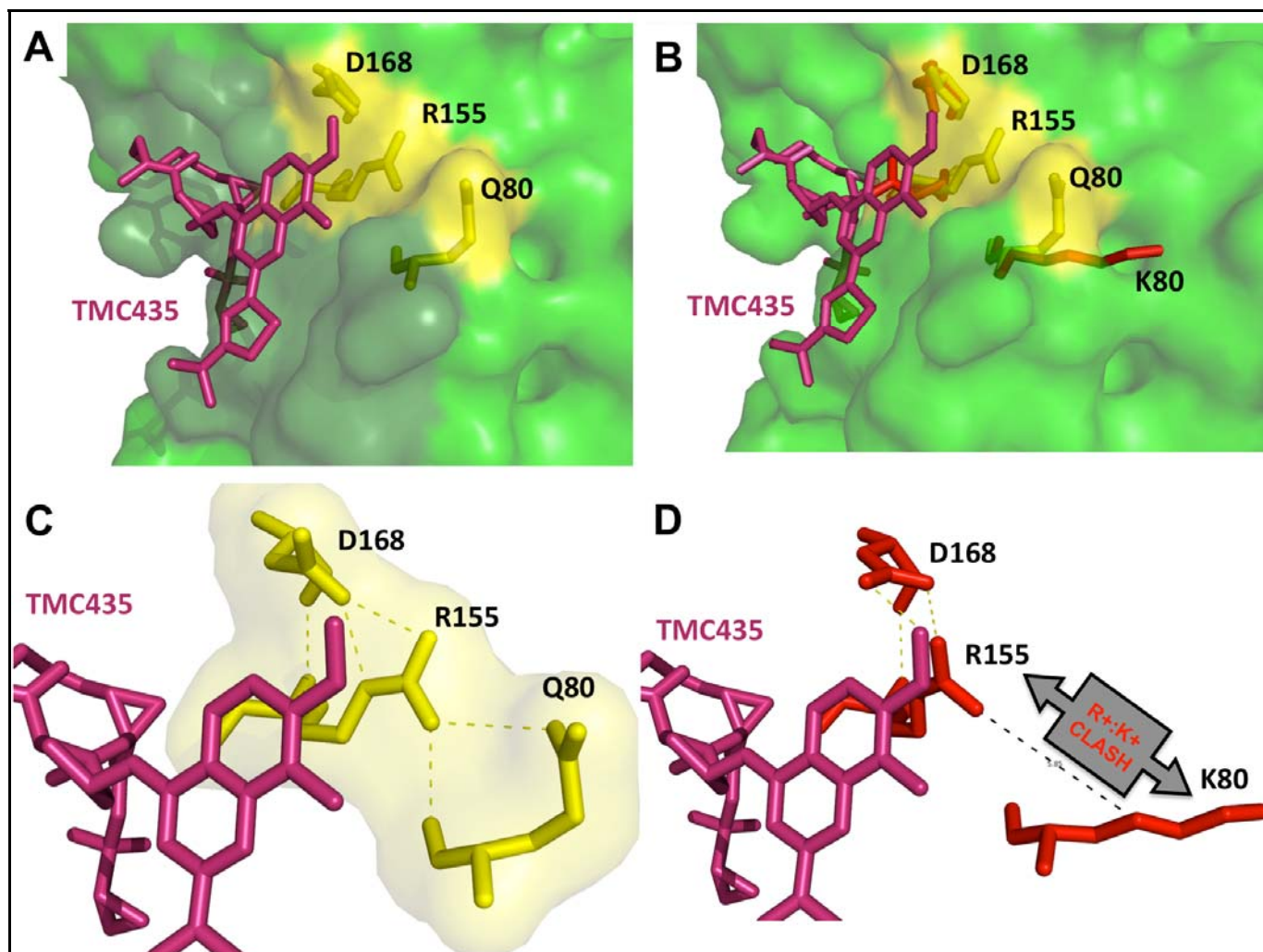


Figure 33. Structural comparison of TMC435 in complex with GT1a NS3/4A protease Apo domain. Pymol was used to compare the structures of two GT1a NS3/4A protease Apo domains, one with Q at position 80 (3KF2) and one with K at position 80 (3SUD), in the context of binding to TMC435. Panel A, TMC435 binding to GT1a NS3/4A protease with Q80. Panel B, TMC435 binding to GT1a NS3/4A protease with K80. Panel C, Q80 interacts with R155 to form a hydrogen bond favorable for binding to TMC435. Panel D, electrostatic repulsion between R155 and K80 forces rotameric positioning that is less favorable for TMC435 binding.

Taken together with the reduced SVR12 rates associated with subjects infected with HCV GT1a, which are at the same level as the placebo group, these observations indicate that the presence of Q80K in GT1a subjects provides resistance against TMC435. It is possible that TMC435 still binds to the active site of the protease, but at reduced levels or that additional compensatory amino acid substitutions can occur to accommodate the rotameric posturing of R155K; however, these lines of evidence indicate TMC435 is not effective in treating subject infected HCV GT1a if the virus contains Q80K at baseline.

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METHODS

RNA Extraction and Preparation and HCV Genotyping

Samples for HCV RNA quantification were taken at every study visit, including at screening (Week -6), baseline (Day 1), Days 3 and 7, and Weeks 2, 3, 4, 6, 8, 12, 16, 20, 24, 28, 36, 48, 52, 60, and/or 72. Samples were also collected from subjects who prematurely discontinued all treatment with TMC435 at withdrawal, at 4 weeks post-treatment, and every 12 weeks counting from baseline until Week 72. The samples were then processed in real time at a central laboratory (b) (4) using the Roche COBAS[®] Taqman[®] HCV/HPS v2.0 assay for the quantification of HCV RNA genotypes 1 through 6 in human serum or plasma. The High Pure System (HPS) Viral Nucleic Acid Kit was used for sample preparation and the COBAS[®] TaqMan 48 Analyzer was used for amplification and detection (for HCV genotype 1, the assay has a linear range from 25-300,000,000 IU/mL, a lower limit of quantification (LLOQ) of 25 IU/mL and a limit of detection of 15 IU/mL). According to the sponsor, HCV RNA levels below the LLOQ were reported as HCV RNA '< 25 IU/mL detectable' if traces of RNA were detected or '< 25 IU/mL undetectable' if no HCV RNA was detected. For data presentation, the HCV RNA results of '< 25 IU/mL HCV RNA detected' were set to 24 IU/mL and 'HCV RNA not detected' results were set to 9 IU/mL before log transformation.

Plasma samples taken at screening (Week -6) were analyzed to determine HCV genotype and subtype using the Trugene[®] assay to assess study eligibility and for randomization purposes. Plasma samples were also taken at baseline to determine HCV genotype and subtype for study analyses, using the sponsor's NS5B sequence based assay.

The Trugene HCV 5'NC genotyping assay was used to analyze a 183-base pairs (bp) segment of the HCV 5' non-coding region to determine the HCV genotype and subtype for each subject. HCV RNA was extracted and amplified using the Roche COBAS[®] Amplicor[™] HCV assay followed by CLIP[™] sequencing of the amplicon. The sequence obtained for each subject was compared to reference sequences using the OpenGene[®] DNA Sequencing software to assign the HCV genotype and subtype based on the best match with characterized isolates from the HCV 5'NC gene library v3.1.2.

In addition to the Trugene HCV 5'NC genotyping assay, an HCV NS5B sequence-based genotype and subtype assay was performed by the sponsor. This assay analyzed a known subtype-predictive 329bp segment in the HCV NS5B polymerase gene. According to the sponsor, the HCV RNA was extracted from plasma using the EasyMAG[®] platform and reagents (Biomérieux, Boxtel, The Netherlands). HCV RNA was then reverse transcribed and amplified using ABI 9700 thermal cyclers (Life Technologies, Carlsbad, CA) with the SuperScriptIII[®] One-Step RTPCR/Platinum[®]Taq High Fidelity kit (Invitrogen, Merelbeke, Belgium), followed by a nested PCR using the Expand[®] High Fidelity kit (Roche, Basel, Switzerland). PCR products of the correct length were purified using the QIAquick[®] 96 PCR BioRobot kit (Qiagen, Hilden, Germany). Sequencing reactions were performed in ABI 9700 thermal cyclers using Big Dye[®] Terminator Mix (Life Technologies, Carlsbad, CA) and reactions were purified using the DyeEx[®] 96 well plate kit (Qiagen, Hilden, Germany) before they were loaded on ABI3730XL analysers (Life Technologies, Carlsbad, CA) for standard Sanger sequencing.

Contig assembly and sequence analysis was performed using Seqscape[®] version 2.5 software (Life Technologies, Carlsbad, CA). The resulting 329bp sequences were analyzed with the GAM BLAST Subtyper module (software developed by Janssen Diagnostics), which uses a BLASTn-based algorithm to compare the sequences from the samples with a panel of reference sequences, obtained from the European HCV database (euHCVdb) and Los Alamos National Library (LANL) covering all 6 major genotypes and their respective subtypes. The GAM BLAST Subtyper software automatically assigned the genotype and subtype from the reference sequence with the highest BLAST similarity score to the sample.

Sanger Population Sequencing for Resistance Analysis

A population-based sequencing assay was performed by Janssen Diagnostics to determine the HCV NS3/4A gene sequence using standard Sanger sequencing. The sponsor applied a two-tier sequencing approach using

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DNA derived from HCV RNA extractions performed using the same methods described previously for the genotype and subtype assays. The first-line amplification and sequencing was subtype-specific using population-based sequencing of a 2055 bp sequence covering the entire HCV NS3/4A gene region. If the full NS3/4A sequence could not be determined, second-line subtype-specific amplification and sequencing protocols were used targeting the protease domain of NS3 (N-terminal 181 amino acid of NS3). For some samples, including those for which the NS3 protease sequence could not be determined or for samples that were non HCV GT1a/1b, a sequencing assay was performed using different primer sets for amplification and sequencing. The sequences obtained by Sanger sequencing were then compared to each other and HCV GT1a and GT1b reference sequences using a GAM alignment module developed by the sponsor (using a Hidden Markov Model to calculate the alignment). The reference sequences were obtained from the euHCVdb and LANL public databases. Sequence data were reported as amino acid (AA) changes from Con1 (GenBank accession number AJ238799) or H77 (GenBank accession number AF009606) for genotype 1b and 1a/others, respectively. Mixtures of AAs present at one position were reported with a "/". If 5 or more AAs per position were present it was indicated with an "X".

NGS methods (copied from pages 9-13 of the TMC435-C205-C206-DS Antiviral Microbiology Report)

The sponsor reported that they used several experimental measures to maximize the accuracy of the deep sequencing process. First, the number of analyzed HCV RNA copies was maximized by extracting 600 µl of plasma and eluting in a relatively small elution volume of 35 µl. In addition, to minimize the chance for selective amplification bias potentially introduced by low HCV RNA levels, the reverse transcriptase reaction was performed using random hexamer primers and PCR reactions were performed in triplicate. Finally, high fidelity RNA/DNA polymerases were used for all amplification reactions; and error correction was performed to differentiate "real" variants from sequencing errors.

RNA was isolated from 600 µl plasma using the NucliSENS easyMAG platform (bioMérieux) and eluted in 35 µl. Sixteen µl RNA was reverse transcribed to cDNA in a reaction volume of 30 µl, using random hexamer primers (Life Technologies) and AccuScript® High Fidelity Reverse Transcriptase (Agilent Technologies, Santa Clara, CA). An amplicon encompassing the NS3/4A region was created during a subtype-specific (1a/other or 1b) PCR followed by a nested PCR using KOD DNA polymerase (EMD Millipore - Merck, Darmstadt, Germany) in 3-fold, starting from 2 µl of cDNA per replicate. PCR replicates were pooled and amplicons were purified from agarose gel (QIAquick Gel Extraction Kit, Qiagen). Two hundred and fifty ng on average of DNA per sample was sheared to an average length of 200 bp using a Covaris® E210 system (Covaris, Woburn, MA). End repair, A-tailing, adaptor ligation, indexing and size selection of DNA fragments was performed using the SPRI®works Fragment Library System I (Beckman Coulter Genomics, Indianapolis, IN) with NEXTflex® DNA Barcodes (Bioo Scientific, Austin, TX). The library was enriched during a limited number of PCR cycles (NEXTflex DNA Sequencing Kit, Bioo Scientific). Barcoded samples were quantified in order to pool them at equimolar amounts, per lane (8) of a Genome Analyzer (GA) IIx System (Illumina, San Diego, CA). Prior to sequencing, clonal amplification of individual DNA fragments was performed using the cBot Cluster Generation System combined with the TruSeq® PE Cluster Kit version 2 (Illumina). One hundred forty seven cycles of paired-end sequencing was carried out on the Illumina GA IIx system (TruSeq SBS kit version 5, Illumina).

The Illumina analysis software package (CASAVA 1.8.2) was used to produce high quality sequence reads and consensus sequences per sample were constructed by iterative reference mapping of sequence reads. The sequence reads were assembled to their consensus sequence using the CLC Genomics Workbench (CLC bio, Muehlital, Germany). As a result, an average of 2.5 million sequence reads per sample (range 1-5 million) with a median read length of 70 bp were mapped, leading to an approximate depth of 44000 reads per sample and per position in NS3 (amino acid position 1-181). Frequencies of codon variants versus the universal reference (H77 for 1a/other and Con1 for 1b) were determined with a limit of detection of 1%. By means of additional error correction, codon frequencies $\geq 1\%$ that were only represented by reads in one sequence direction were considered "false positive".

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The rationale for selecting 1% as the lower limit of quantification of minority variants was based on careful consideration of various technical limitations at the level of sample preparation, sequencing and sequencing analysis. Deep sequencing of RNA viruses starts with reverse transcription of isolated RNA followed by PCR amplification of viral regions of interest. Despite the use of high fidelity enzymes to perform these steps, the low but inevitable error rate of these polymerases (10^{-6} – 10^{-7} mutations/bp/duplication) remains a source of error. In addition, the relative frequencies of viral variants can be disturbed by selective amplification bias, especially in low viral load isolates. To minimize the potential impact of PCR as a source of sequence errors, the amplification reactions were processed in parallel on independent replicates and subsequently pooled before sequencing.

Sequencing itself can also introduce substitution errors, insertions and/or deletions. The Illumina platform is typically prone to errors originating from systematic base calling biases. Most importantly, the 3' ends of Illumina sequences tend to have higher sequencing error rates compared to the 5' ends, and more single-base errors have been observed in the proximity of GGC motifs. Along with optimizing experimental lab conditions to minimize errors introduced during target amplification and sequencing, various data analysis tools have been developed to correct for these errors. These algorithms are however explorative and often designed for specific sequencing applications. For our analyses, bi-directionality (forward and reverse frequency) of identified variants was taken into account as a parameter to differentiate "real" variants from errors.

The lower limit of minority variant quantification is dependent on the median number of reads per sequence position (i.e. sequencing depth). Read coverage must be sufficiently high to maximize the number of reads in which a certain minority variant is observed. Sequencing sensitivity is however also dependent on the level of error introduced during sample preparation and sequencing. Analysis of clonal viral isolates with a known single sequence (i.e. plasmids) using Illumina and other platforms showed a high density of sequence errors at frequencies below 1%, supporting decreased reproducibility of these measurements. This high density of sequence variants at frequencies below 1% was also observed during deep sequencing of (more heterogeneous) clinical isolates. Setting the lower cut-off at 1% frequency resulted in the exclusion of all the observed errors in the clonal isolates, and was therefore used for deep sequencing analysis of clinical isolates using Illumina technology.

Linkage analysis was performed to assess whether 2 mutations detected by PS at NS3 positions of interest were present on the same viral genome. Paired-end sequencing analyzed each DNA strand from both ends and resulted in two reads of ± 70 bp for each DNA strand. The distance between these two reads was solely dependent on the length of the DNA strands and was determined by the shearing of the amplicons. Individual reads spanning one of two amino acid positions of interest were considered to originate from the same DNA strand if they carried the same flow cell XY-coordinates. Linkage frequencies were calculated by dividing the number of these paired reads carrying a specific combination of two substitutions by the total number of read pairs spanning both positions. PCR-mediated recombination has been suggested to cause artificial combinations of mutations in PCR products. The formation of recombinant amplicons can be caused by: degraded RNA template during extraction and storage, incomplete cDNA strand production by RT, or by premature PCR termination, resulting in incomplete DNA strands. The frequency of these recombinant forms has been estimated to represent up to 20% of the total amplicon pool. The sponsor used high quality RNA extraction methods and relatively long reverse transcription and PCR elongation times to minimize the formation of recombinant amplicons, but it cannot be excluded that some of the observed linked mutations were created by a PCR-mediated recombination event.

Phenotypic Analysis of Potential Resistance-associated Substitutions (copied from pages 21-22, TMC435 Antiviral Microbiology Report).

Plasma samples collected for viral sequencing at every study visit after screening¹ could be used for phenotypic analysis upon request by the sponsor's virologist. A phenotypic analysis assay was performed by Janssen Diagnostics. To determine the impact of single, double or triple mutations on TMC435 activity, site-directed mutations (SDMs) were

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inserted in the NS3 region of genotype 1a and 1b replicons at (b) (4). A plasmid encoding a genotype 1b subgenomic bicistronic replicon (pFK_i341_PILuc_NS3-3'_ET) with the poliovirus internal ribosome entry site (IRES) driven luciferase reporter and cell culture-adaptive mutations E1202G and T1280I in NS3 and K1846T in NS4B (provided by R. Bartenschlager) was used as genotype 1b backbone for site-directed mutagenesis. A plasmid encoding a genotype 1a subgenomic replicon (H77) with the poliovirus IRES driven luciferase reporter and cell culture-adaptive mutations P1496L in NS3, K1691R in NS4A, and F2080K and S2204I in NS5A (made in-house (b) (4)) was used as genotype 1a backbone (H77_L+I_high) for site-directed mutagenesis.

To determine the susceptibility of NS3 protease sequences derived from clinical isolates to HCV protease inhibitors, a chimeric replicon was generated. The genotype 1b pFK_i341_PILuc_NS3-3'_ET replicon sequence was modified. Two SacII restriction sites were introduced in NS3 and a replication-incompetent replicon shuttle vector was created by SacII digestion and excision of the parental NS3 protease sequence (AA 5-192). NS3 protease sequences comprising AA 5-192 amplified from clinical isolates were cloned into the replication-incompetent shuttle vector using the In-Fusion™ Advantage PCR Cloning Kit (Clontech, Mountain View, US)11. Site-directed mutant plasmids and chimeric replicon plasmids were linearized and transcribed into RNA. Subsequently, Huh7-Lunet cells were transfected and replication was quantified by measuring the enzymatic activity of the luciferase protein after 48 hours. The 50% effective concentration of TMC435 was determined against the chimeric replicon or SDM and compared with the wild type (ET) replicon to obtain fold change (FC) in EC_{50} values (FC value = EC_{50} mutant or chimeric replicon versus EC_{50} wild type).

Bioinformatics Programs Used For These Analyses

Pymol. Pymol was used (b) (4)

CLC Genomics Workbench. CLC Genomics Workbench was used to generate multiple sequence alignments for the NS3 protease sequence of HCV GT1a and GT1b amino acid sequences.

CONCLUSIONS

This submission was the first NDA that contained NGS data to support the approval of an antiviral drug. To ensure that the interpretation of the data was consistent between algorithms, DAVP conducted an independent analysis that largely agreed with the data submitted by the sponsor. DAVP used two independent variant detection algorithms to call variants and compare the results to those reported by the sponsor. There was good agreement between these analyses, and no major difference were noted.

The predominant resistance-associated substitutions observed in these analyses have been identified previously:

- GT1a: Q80K, R155K
- GT1b: D168V/E

However, additional resistance-associated substitutions were noted, including S122R/T122S, which is a known resistance-associated substitution of other NS3/4A protease inhibitors that have been approved for use in the United States.

In addition, two potential resistance-associated substitutions were observed: P574A/S and V629I. In general, these occurred at low frequency; however, these substitutions occurred at relatively conserved NS3/4A amino acid positions in two or more subjects. Therefore, we recommend that these substitutions be further evaluated as potential resistance-associated substitutions.

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The NGS data allowed us to independently evaluate two additional observations. First, the sponsor has proposed (b) (4) a treatment algorithm (b) (4)

Second, the NGS data allowed us to verify that Q80K, which is strongly associated with resistance among subjects with Q80K at baseline, is generally detected at high frequency in those subjects at baseline and this frequency does not change much over time. In addition, Q80K does emerge in subjects infected with HCV GT1a and 1b that has Q80 at baseline and this is also associated with failure, indicating that Q80K, which is a common polymorphism in GT1a (~ 30% of the US population infected with GT1a has this polymorphism) reduced the effectiveness of TMC435, particularly in subjects infected with this variant at baseline. In fact, the SVR rates among GT1a subjects with Q80K at baseline is comparable to placebo, with performance rates less than other approved NS3/4A protease inhibitors.

Based on the data observed in the review of this NDA, clinical virology recommends screening subjects prior to treating with TMC435, and only treating subjects infected with GT1b and GT1a with Q at position 80.

RECOMMENDATIONS

1. Two potential resistance-associated substitutions were observed: P574A/S and V629I. These occurred at low frequency; however, these substitutions occurred at relatively conserved NS3/4A amino acid positions in two or more subjects. Therefore, we recommend that these substitutions be further evaluated as potential resistance-associated substitutions as part of a post marketing agreement.

Eric F. Donaldson, Ph.D.
Clinical Virology Reviewer

CONCURRENCES

HFD-530/Clin Virol TL/J O'Rear

Date: _____

cc:
HFD-530/IND
HFD-530/Division File
HFD-530/RPM/Olagundoye

APPENDICES

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA# 205123 SDN 003 DATE REVIEWED: 08/10/13

Appendix 1: OVERALL RESISTANCE ANALYSIS BY DAVP USING 10% EMERGENCE CRITERIA

TMC435-C205

TMC435-C205	TMC435 150MG/12WKS	1a	TMC435-C205-0324	S122R	122	P/R+DAA BREAKTHROUGH	EMERGE	0.914
			TMC435-C205-0459	I132V	132	P/R+DAA BREAKTHROUGH	DIS	0.853
			TMC435-C205-0459	A147S	147	P/R+DAA BREAKTHROUGH	DIS	0.868
			TMC435-C205-0324	R155K	155	P/R+DAA BREAKTHROUGH	EMERGE	0.988
		1b	TMC435-C205-0079	V71I	71	P/R+DAA RELAPSER	DIS	0.112
			TMC435-C205-0197	Q80R	80	P/R+DAA BREAKTHROUGH	EMERGE	0.768
			TMC435-C205-0079	H117R	117	P/R+DAA RELAPSER	DIS	0.778
			TMC435-C205-0462	T122S	122	P/R+DAA RELAPSER	DIS	0.282
			TMC435-C205-0015	D168E	168	P/R+DAA BREAKTHROUGH	EMERGE	0.106
			TMC435-C205-0079	D168V	168	P/R+DAA RELAPSER	EMERGE	0.989
			TMC435-C205-0197	D168V	168	P/R+DAA BREAKTHROUGH	EMERGE	0.834
			TMC435-C205-0392	D168H	168	P/R+DAA RELAPSER	EMERGE	0.467
			TMC435-C205-0462	D168V	168	P/R+DAA RELAPSER	EMERGE	0.959
			TMC435-C205-0283	I170V	170	P/R+DAA RELAPSER	DIS	0.991
	TMC435 150MG/24WKS	1a	TMC435-C205-0308	Q80K	80	P/R+DAA RELAPSER	EMERGE	0.993
			TMC435-C205-0478	K80Q	80	P/R+DAA BREAKTHROUGH	DIS	0.967
		1b	TMC435-C205-0097	T122S	122	P/R+DAA RELAPSER	DIS	0.475
	TMC435 75MG/12WKS	1a	TMC435-C205-0412	V71I	71	P/R+DAA RELAPSER	DIS	0.913
			TMC435-C205-0412	Q80R	80	P/R+DAA RELAPSER	EMERGE	0.982
			TMC435-C205-0357	R155K	155	P/R+DAA RELAPSER	EMERGE	0.870
		1b	TMC435-C205-0008	V71I	71	P/R+DAA RELAPSER	DIS	0.589
			TMC435-C205-0326	V150A	150	P/R+DAA BREAKTHROUGH	DIS	0.277
			TMC435-C205-0008	D168H	168	P/R+DAA RELAPSER	EMERGE	0.279
			TMC435-C205-0054	D168V	168	P/R+DAA RELAPSER	EMERGE	0.205
	TMC435 75MG/24WKS	1a	TMC435-C205-0426	D168H	168	P/R+DAA RELAPSER	EMERGE	0.340
			TMC435-C205-0096	V170I	170	P/R+DAA BREAKTHROUGH	EMERGE	0.991
			TMC435-C205-0170	K80Q	80	P/R+DAA RELAPSER	DIS	0.335
		1b	TMC435-C205-0395	G122S	122	P/R+DAA RELAPSER	DIS	0.605
			TMC435-C205-0170	R155K	155	P/R+DAA RELAPSER	EMERGE	0.985
			TMC435-C205-0271	I48V	48	P/R+DAA RELAPSER	DIS	0.978
			TMC435-C205-0257	H86Q	86	P/R+DAA RELAPSER	DIS	0.131
			TMC435-C205-0119	A150V	150	P/R+DAA RELAPSER	EMERGE	0.118
			TMC435-C205-0257	D168V	168	P/R+DAA RELAPSER	EMERGE	0.963
			TMC435-C205-0286	D168A	168	P/R+DAA RELAPSER	EMERGE	0.667

NONRECAT, non-response category

SFLAG, Substitution flag;

DIS, substitution was detected at baseline and was the same as the reference sequence at later timepoints;

EMERGE, substitution was not detected at baseline but was detected at later timepoints

MEANFQ, mean frequency (calculated based on frequencies reported by 2 or 3 variant detection methods).

TMC435-C206

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA# 205123 SDN 003 DATE REVIEWED: 08/10/13

STUDYID	ARM	SUBTYPE	USUBJID	SUBS10+-	NS3 AAPOS	NONRECAT	SFLAG	MEANFQ		
TMC435-C206	TMC435 100MG/12WKS	1a	TMC435-C206-0361	R155K	155	P/R+DAA RELAPSER	EMERGE	0.776		
			TMC435-C206-0022	D168V	168	P/R+DAA RELAPSER	EMERGE	0.986		
			TMC435-C206-0361	G534S	534	P/R+DAA RELAPSER	DIS	0.996		
			TMC435-C206-0361	E555D	555	P/R+DAA RELAPSER	DIS	0.921		
		1b	TMC435-C206-0107	Q80K	80	P/R+DAA RELAPSER	EMERGE	0.974		
			TMC435-C206-0364	Q80R	80	P/R+DAA BREAKTHROUGH	EMERGE	0.277		
			TMC435-C206-0587	Q80R	80	P/R+DAA BREAKTHROUGH	EMERGE	0.652		
			TMC435-C206-0364	S122R	122	P/R+DAA BREAKTHROUGH	EMERGE	0.621		
			TMC435-C206-0107	D168E	168	P/R+DAA RELAPSER	EMERGE	0.994		
			TMC435-C206-0364	D168V	168	P/R+DAA BREAKTHROUGH	EMERGE	0.991		
			TMC435-C206-0548	D168V	168	P/R+DAA BREAKTHROUGH	EMERGE	0.740		
			TMC435-C206-0587	D168V	168	P/R+DAA BREAKTHROUGH	EMERGE	0.996		
			TMC435-C206-0587	S280P	280	P/R+DAA BREAKTHROUGH	EMERGE	0.306		
			TMC435-C206-0587	M470R	470	P/R+DAA BREAKTHROUGH	EMERGE	0.523		
			TMC435-C206-0107	I472V	472	P/R+DAA RELAPSER	EMERGE	0.872		
			TMC435-C206-0022	P574A	574	P/R+DAA RELAPSER	EMERGE	0.988		
			TMC435 100MG/24WKS	1a	TMC435-C206-0042	I71V	71	P/R+DAA BREAKTHROUGH	EMERGE	0.988
					TMC435-C206-0028	Q80K	80	P/R+DAA BREAKTHROUGH	EMERGE	0.785
					TMC435-C206-0042	R155K	155	P/R+DAA BREAKTHROUGH	EMERGE	0.983
					TMC435-C206-0322	R155K	155	P/R+DAA RELAPSER	EMERGE	0.988
	TMC435-C206-0403	R155K			155	P/R+DAA BREAKTHROUGH	EMERGE	0.824		
	TMC435-C206-0042	I248V			248	P/R+DAA BREAKTHROUGH	EMERGE	0.997		
	TMC435-C206-0042	S343N			343	P/R+DAA BREAKTHROUGH	EMERGE	0.618		
	TMC435-C206-0042	T344I			344	P/R+DAA BREAKTHROUGH	EMERGE	0.760		
	TMC435-C206-0042	I407V			407	P/R+DAA BREAKTHROUGH	EMERGE	0.998		
	TMC435-C206-0042	M470T			470	P/R+DAA BREAKTHROUGH	EMERGE	0.897		
	TMC435-C206-0042	V629I			629	P/R+DAA BREAKTHROUGH	EMERGE	0.507		
	1b	TMC435-C206-0402			Q80R	80	P/R+DAA BREAKTHROUGH	EMERGE	0.986	
		TMC435-C206-0402		D168E	168	P/R+DAA BREAKTHROUGH	EMERGE	0.991		
		TMC435-C206-0402		T344A	344	P/R+DAA BREAKTHROUGH	EMERGE	0.977		
		TMC435-C206-0402		I472V	472	P/R+DAA BREAKTHROUGH	EMERGE	0.992		
		TMC435 100MG/48WKS		1a	TMC435-C206-0066	R155K	155	P/R+DAA BREAKTHROUGH	EMERGE	0.786
					TMC435-C206-0531	R155K	155	P/R+DAA BREAKTHROUGH	EMERGE	0.959
					TMC435-C206-0066	S343N	343	P/R+DAA BREAKTHROUGH	EMERGE	0.375
					TMC435-C206-0066	T344I	344	P/R+DAA BREAKTHROUGH	EMERGE	0.401
	TMC435-C206-0383				V358T	358	P/R+DAA RELAPSER	DIS	0.890	
	TMC435-C206-0112		D555E		555	P/R+DAA RELAPSER	EMERGE	0.996		
	1b		TMC435-C206-0470	H86Q	86	P/R+DAA RELAPSER	DIS	0.301		
			TMC435-C206-0544	R117C	117	P/R+DAA BREAKTHROUGH	EMERGE	0.448		
			TMC435-C206-0544	A150V	150	P/R+DAA BREAKTHROUGH	EMERGE	0.330		
			TMC435-C206-0544	D168V	168	P/R+DAA BREAKTHROUGH	EMERGE	0.826		
			TMC435-C206-0575	D168V	168	P/R+DAA BREAKTHROUGH	EMERGE	0.926		
			TMC435-C206-0470	V248I	248	P/R+DAA RELAPSER	DIS	0.997		
	TMC435 150MG/12WKS	1a	TMC435-C206-0575	G383S	383	P/R+DAA BREAKTHROUGH	EMERGE	0.533		
			TMC435-C206-0470	P574S	574	P/R+DAA RELAPSER	EMERGE	0.789		
			TMC435-C206-0118	V132I	132	P/R+DAA BREAKTHROUGH	EMERGE	0.989		
			TMC435-C206-0118	S147A	147	P/R+DAA BREAKTHROUGH	EMERGE	0.987		
		1b	TMC435-C206-0118	R155K	155	P/R+DAA BREAKTHROUGH	EMERGE	0.670		
			TMC435-C206-0274	G534S	534	P/R+DAA BREAKTHROUGH	DIS	0.976		
			TMC435-C206-0094	I48V	48	P/R+DAA RELAPSER	DIS	0.988		
			TMC435-C206-0391	Q80R	80	P/R+DAA BREAKTHROUGH	EMERGE	0.989		
			TMC435-C206-0569	T358F	358	P/R+DAA NONRESPONDER	EMERGE	0.938		
			TMC435-C206-0456	S383G	383	P/R+DAA RELAPSER	DIS	0.958		
			TMC435-C206-0152	V407I	407	P/R+DAA RELAPSER	DIS	0.997		
			TMC435-C206-0391	D168E	168	P/R+DAA BREAKTHROUGH	EMERGE	0.925		
	TMC435 150MG/24WKS	1a	TMC435-C206-0584	Q80L	80	P/R+DAA BREAKTHROUGH	EMERGE	0.993		
			TMC435-C206-0584	A280S	280	P/R+DAA BREAKTHROUGH	DIS	0.989		
			TMC435-C206-0241	T477A	477	P/R+DAA BREAKTHROUGH	EMERGE	0.329		
			TMC435-C206-0273	A477T	477	P/R+DAA BREAKTHROUGH	DIS	0.974		
			TMC435-C206-0584	S534G	534	P/R+DAA BREAKTHROUGH	EMERGE	0.985		
			TMC435-C206-0273	V660I	660	P/R+DAA BREAKTHROUGH	DIS	0.450		
		1b	TMC435-C206-0137	V48I	48	P/R+DAA RELAPSER	EMERGE	0.989		
			TMC435-C206-0137	D168V	168	P/R+DAA RELAPSER	EMERGE	0.354		
			TMC435-C206-0495	D168V	168	P/R+DAA BREAKTHROUGH	EMERGE	0.972		
			TMC435-C206-0137	I344T	344	P/R+DAA RELAPSER	DIS	0.676		
			TMC435-C206-0137	V629I	629	P/R+DAA RELAPSER	EMERGE	0.967		
			TMC435 150MG/48WKS	1a	TMC435-C206-0510	R155K	155	P/R+DAA BREAKTHROUGH	EMERGE	0.953
	TMC435-C206-0617	I170V			170	P/R+DAA RELAPSER	DIS	0.706		
	TMC435-C206-0617	G534S			534	P/R+DAA RELAPSER	DIS	0.887		
	TMC435-C206-0232	I660V			660	P/R+DAA BREAKTHROUGH	EMERGE	0.266		

See Table for TMC435-C205 for legend

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/s/

ERIC F DONALDSON
08/28/2013

JULIAN J O REAR
08/28/2013

Product Quality Microbiology Review

8/13/2013

NDA: 205123

Drug Product Name

Proprietary:

Non-proprietary: Simeprevir

Review Number: 1

Dates of Submission(s) Covered by this Review

Submit	Received	Review Request	Assigned to Reviewer
3/28/2013	3/28/2013	5/24/2013	5/28/2013
7/24/2013	7/24/2013	N/A	N/A
8/09/2013	8/09/2013	N/A	N/A

Submission History (for 2nd Reviews or higher)

None

Applicant/Sponsor

Name: Janssen Research and Development

Address: 1125 Trenton-Harbourton Road, Titusville, NJ 08560

Representative: Michele Dias, Global Regulatory Affairs, 920 Route 202, Raritan, NJ 08869

Telephone: (908) 927-5048

Name of Reviewer: Steven P. Donald, M.S.

Conclusion: Recommended for Approval

Product Quality Microbiology Data Sheet

- A.
1. **TYPE OF SUBMISSION:** Original NDA
 2. **SUBMISSION PROVIDES FOR:** the manufacture and marketing of a new drug product.
 3. **MANUFACTURING SITE:**
Janssen Pharmaceutica NV 3002807337 Janssen Pharmaceuticaaan 3,
Geel B-2440, Belgium (Intermediate product)

Janssen Cilag SpA 3003164454 Via C. Janssen, Borgo San Michele,
Latina 04100, Italy (final drug product)
 4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** Capsule, oral, 150 mg
 5. **METHOD(S) OF STERILIZATION:** NA
 6. **PHARMACOLOGICAL CATEGORY:** Antiviral
- B. **SUPPORTING/RELATED DOCUMENTS:** None
- C. **REMARKS:** An information request was sent to the sponsor on 7/15/2013; a response was received on 7/24/2013. A subsequent information request was sent 8/1/2013; a response was received on 8/9/2013. All responses to information requests are reviewed herein.

filename: N205123r1.doc

Executive Summary

I. Recommendations

- A. Recommendation on Approvability** - Recommended for approval
- B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable** – N/A

II. Summary of Microbiology Assessments

- A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology** – The drug product is

(b) (4)

- B. Brief Description of Microbiology Deficiencies** – No product quality microbiology deficiencies were identified based upon the information provided.

- C. Assessment of Risk Due to Microbiology Deficiencies** – None

- D. Contains Potential Precedent Decision(s)**- ☐ Yes ☒ No

III. Administrative

- A. Reviewer's Signature** _____

Steven P. Donald, M.S.
Microbiology Reviewer

- B. Endorsement Block** _____

Stephen Langille, Ph.D.
Senior Microbiology Reviewer

- C. CC Block**
N/A

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/s/

STEVEN P DONALD
08/13/2013

STEPHEN E LANGILLE
08/13/2013

PRODUCT QUALITY MICROBIOLOGY FILING CHECKLIST

NDA Number: 205123

Applicant: Janssen Research
and Development

Letter Date: 3/28/2013

Drug Name: simeprevir

NDA Type: 505 (b)(1)

Stamp Date: 3/28/2013

The following are necessary to initiate a review of the NDA application:

	Content Parameter	Yes	No	Comments
1	Is the product quality microbiology information described in the NDA and organized in a manner to allow substantive review to begin? Is it legible, indexed, and/or paginated adequately?	X		The submission is in eCTD format
2	Has the applicant submitted an overall description of the manufacturing processes and microbiological controls used in the manufacture of the drug product?	X		See 3.2.3.P.3.3 and 3.2.P.3.4; microbiological purity has been monitored at release and during stability studies as reported in the Stability Data section. No test for microbiological purity is included in the specification.
3	Has the applicant submitted protocols and results of validation studies concerning microbiological control processes used in the manufacture of the drug product?		X	Validation studies are not presented but USP <61> and <62> are referenced in the stability summary.
4	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	
5	Has the applicant submitted preservative effectiveness studies (if applicable) and container-closure integrity studies?			N/A
6	Has the applicant submitted microbiological specifications for the drug product and a description of the test methods?	X		Reduced microbiological testing is proposed for product release but testing will remain on the stability protocol for the first three

	Content Parameter	Yes	No	Comments
				commercial batches. No Microbiological testing is proposed during the annual commitment to stability.
7	Has the applicant submitted the results of analytical method verification studies?		X	
8	Has the applicant submitted all special/critical studies/data requested during pre-submission meetings and/or discussions?			N/A
9	If sterile, are extended post-constitution and/or post-dilution hold times in the draft labeling supported by microbiological data?			N/A
10	Is this NDA fileable? If not, then describe why.	X		Reference to product release microbial limits testing methods (historical) will be requested

Additional Comments:

The applicant states that there is no microbial limits specification for product at release due to the (b) (4) manufacturing process, the state of control over the excipients and drug substance, the GMP status of the facility, the validated equipment cleaning procedures and manufacturing process. A detailed assessment of the drug product manufacturing process and controls was performed, including historical microbiological data for the drug substance, excipients, and finished product, as well as finished product (b) (4). The assessment did not identify any potential issues that would adversely impact the microbial bioburden level of the drug product and concluded that routine microbial purity testing at release is not required (See justification-of-specification.pdf, Section 2.2). Note that two different sites are proposed for the manufacturing the drug product; Geel, BE and Latina, It.

Stability (microbiological) data from 4 batches are presented, two 12-month studies and two 3-month studies, each using a different batch. All microbiological acceptance criteria are met per USP <61> and <62>. These data refer to product batches in (b) (4) bags (intermediate product) or capsule in bottles (final drug product). The bags were manufactured at the Geel, BE proposed manufacturing site and the capsules were manufactured at the Beerse, BE development site. Data are presented for the intermediate product manufactured at the Latina site and capsules manufactured at the Beerse site. All batches presented are within specification for microbial limits but only USP <111> is referenced for microbial limits testing. There is no mention of USP <61> and <62> for finished product testing, although it is assumed since stability testing references these methods.

Hard gelatin capsules are used in the manufacture of the drug product; the microbial limits testing on the stability program is noted. The excipient capsule does not have any microbiological specification.

Reviewing Microbiologist	Date
--------------------------	------

Microbiology Secondary Reviewer/Team Leader	Date
---	------

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/s/

STEVEN P DONALD
06/24/2013

STEPHEN E LANGILLE
06/24/2013

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 205123

Applicant: Janssen

Stamp Date: March 28, 2013

**Drug Name: TMC435
(Simeprevir)**

NDA Type: NME

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 <u>submitted</u> 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 <u>submitted</u> any required animal model studies necessary for approvability of the product based on the submitted draft labeling?			N/A
6	Has the applicant <u>submitted</u> all special/critical studies/data requested by the Division during pre-submission discussions?	X		
7	Has the applicant <u>submitted</u> the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?	X		Some confusion regarding multiple amino acid sequences for some isolates. Clarifying comment sent to sponsor.
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		Both types of methods were used. It appears that adequate supporting information and/or raw data were submitted for nonstandardized techniques (e.g., NGS).
9	Has the applicant <u>submitted</u> draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		
10	Has the applicant <u>submitted</u> 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	X		

File name: Virology Filing Checklist for a NDA or Supplement 010908

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
	references been included and cross-referenced in the 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 VIROLOGY SECTION OF THE APPLICATION FILEABLE? Yes

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

None, but the following comment was communicated to the sponsor on April 17, 2013:

Several isolates in the “GENOTYPE” databases for C208, C216, and HPC3007 contain two or three distinct amino acid sequence entries for the same time point. For example, Subject TMC435-C208-0007 has three rows devoted to the Week 28 isolate collected on 2011-10-24 at 7:15:00, M766235, and each row contains a distinct substitution pattern. Please clarify the significance of each row. If the multiple rows are independent RT-PCR amplifications or sequence runs of the same sample, please consolidate the substitution changes into a single row for each isolate.

Reviewing Virologist	Date
----------------------	------

Virology Team Leader	Date
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/s/

DAMON J DEMING
04/23/2013

JULIAN J O REAR
04/23/2013